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Phenotypic plasticity and gene diversity in *Pistacia lentiscus* L. along environmental gradients in Israel

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Abstract The Mediterranean common shrub *Pistacia lentiscus* is distributed in a wide range of habitats along the climatic gradient in Israel. We studied the factors that may shape its morphological, physiological, and genetic differentiation. We examined the phenotypic and molecular genetic variability among and within the six Israeli populations as correlated with the local environmental conditions. The genetic structure of the shrub on the island of Cyprus was also examined. Plant morphological parameters correlated significantly with the local environmental conditions, especially with the annual precipitation and temperature. Gene diversity did not differ significantly among locations, and, hence, no differentiation among Israeli populations or between populations in Israel and Cyprus was found. The major part of the molecular variance (69%) was found within the populations, 22% of the variance was found between Israel and Cyprus and 9% among the populations within the region. Gene flow estimates among all the tested populations were high with no indication for the isolation by distance. We did not find any pattern of ecologically related genetic differentiation; hence, the morphological and physiological differences are probably due to phenotypic plasticity. It seems that the ability of *P. lentiscus* to express the different phenotypes in response to the varying conditions in the Mediterranean region is an adaptive trait in a species that is characterized by intensive gene flow.

Keywords Phenotypic plasticity · Gene diversity · RAPD · AFLP · Gene flow · *Pistacia lentiscus* · Israel · Cyprus

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Introduction

Long-lived plant species, like trees and shrubs, have to cope through their lifetime with a wide range of diverse environmental conditions. Bradshaw (1965) suggested that a variable environment within the life span of an organism should favor a flexible phenotype. Adaptations to heterogeneous conditions such as seasonal and annual climatic changes may include both genetic differentiation as well as expression of an alternative range of phenotypes, commonly known as "phenotypic plasticity" (West-Eberhard 1989; Scheiner 1993; Jump and Penuelas 2005). Consequently, natural selection may lead to genes that determine the complex character responses (Bradshaw 2006). In addition, a heterogenic habitat may favor genetic heterogeneity among populations; thus, both local adaptations as well as adaptations to novel environments may result in the subsequent genetic changes (Linhart and Grant 1996; Hufford and Mazer 2003; Jump and Penuelas 2005; de Jong 2005).

Specific varieties that represent genotypes adapted to local environmental conditions are referred to as "ecotypes" (Hufford and Mazer 2003). Spatial genetic differentiation (ecotype formations) along climate gradients have been documented for many species of trees and shrubs (Grant

and Mitton 1977; Mitton et al. 1977; Kelly et al. 2003; Gratani et al. 2003), suggesting that the genetic architecture of woody species may often be shaped by natural selection (Linhart and Grant 1996).

The ecologically relevant genetic differentiation presumably affects many loci, most of which have not been isolated to date. Commonly, the general genetic differentiation of marker loci is examined in order to identify the major forces shaping the genetic profile of populations (Kawecki and Ebert 2004). However, the recent advances in genetics have made it possible to simultaneously examine numerous loci and thus may contribute to increase the understanding of evolutionary processes that influencing variation across genomes and populations (Luikart et al. 2003). In the present study, we examined the factors that may shape morphological, physiological, and genetic differentiation in the Mediterranean common shrub *Pistacia lentiscus* L.

P. lentiscus (Anacardiaceae) is a typical thermophylic evergreen shrub distributed throughout the Mediterranean region of Europe, the Levant, and North Africa (Zohary 1952). In Israel, *P. lentiscus* grows in diverse habitats along a climatic gradient that varies in solar radiation, temperature, and precipitation. Using morphological and physiological (but not genetic) parameters, it was previously suggested that genetic, ecotypic differentiation enables *P. lentiscus* to grow successfully in highly contrasting habitats in Israel (Shaviv 1978). Shaviv (1978) described three distinct ecotypes (see Fig. 1): (1) Mt. Gilboa type that is adapted to the high temperatures and low humidity, (2) Mt. Carmel type that does not tolerate high temperatures and is capable of growing slowly throughout the year, and (3) coastal type that is tolerant to sea salt spray and has an extensive root system that improves water absorption in sandy soil. In the current study, we have returned to the

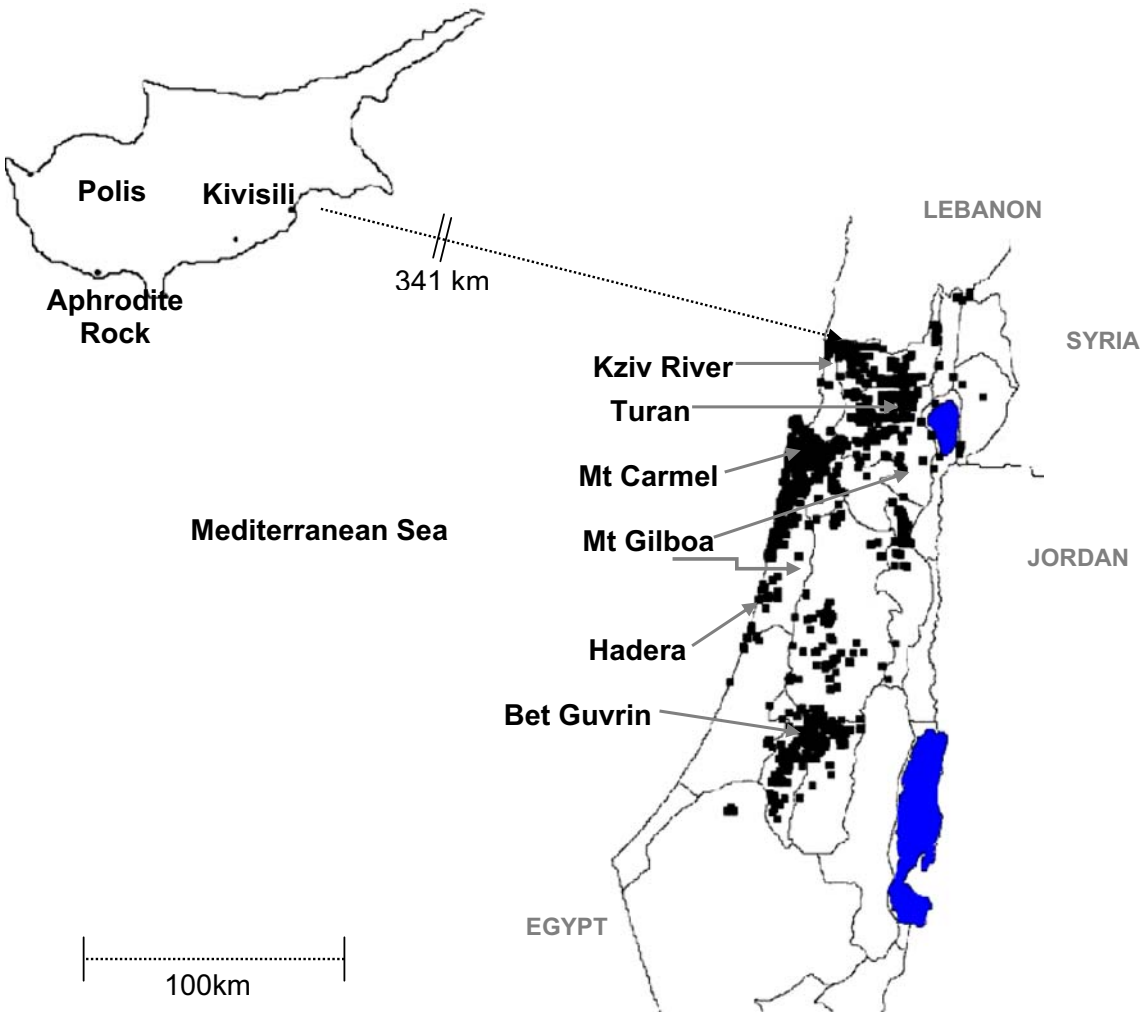

Fig. 1 The distribution map (*black dots*) of *P. lentiscus* and the sampling sites in Israel (*right*) and the sample sites in Cyprus (*left*). Mt. Carmel, Mt. Gilboa, and Hadera are similar to the populations examined by Shaviv (1978)

three of the *P. lentiscus* populations studied by Shaviv (1978) and added three more populations so as to fully cover the distribution range along the aridity gradient in Israel. Our aim is to study the genetic and phenotypic variability among and within the populations of *P. lentiscus* as correlated with the local environmental conditions. In addition we attempt to identify the relative roles of genetic ecotypic differentiation and phenotypic plasticity in the adaptation of *P. lentiscus* to the wide range of habitats along the climatic gradient in Israel. On a broader scale, genetic analysis of *P. lentiscus* samples in the various locations throughout the Mediterranean basin has also showed geographical differentiation (Barazani et al. 2003a). Thus, we compared the genetic diversity of the Israeli populations with the samples collected in the island of Cyprus.

Materials and methods

Sampled populations

P. lentiscus is a natural plant in Israel that survives fire and is not utilized by the local population. The shrub has no annual rings, thus, precise age can not be determined. Nonetheless, because of similar history and characteristics of the sampled population, we assume comparable age distribution within populations. Six natural populations representing the distribution range of *P. lentiscus* along Israel were sampled (Table 1 and Fig. 1). An additional three populations were sampled in Cyprus (Kivisili, Polis, and Aphrodite, Fig. 1) for the random amplified polymorphic DNA (RAPD) genetic analysis only. The maximum distance between the populations in Israel is 165 km and between Israel and Cyprus is 524 km.

Morphological and physiological measurements

In each Israeli population, ten shrubs were marked and the following traits recorded:

(i) Shrub morphology: the height, length, and width of all marked shrubs were measured during August and September, 2004. Shrub volume was calculated assuming a semi-spherical shape. The annual growth of ten randomly selected young branches was used as a measure for shrub growth.

(ii) Leaf morphology: total leaf area and number of leaflets parameters were chosen as indicators of adaptation to draught and terminal leaflet as an indicator for stress. Fifteen leaves were randomly selected from the middle part of the current-year shoots. Leaf area was measured using a digital leaf area meter (CI-202, CID Inc., Vancouver, WA, USA). The number of leaflets per leaf and the presence of a terminal leaflet were also recorded.

(iii) Predawn water potential was measured in the 3–6 randomly selected shoots of all marked shrubs in every Israeli site with Pressure Chamber Instrument Model 600.

Genetic analysis

The genetic analyses were based on the leaves collected from 10–20 plants of each of the Israeli populations and from the 27 plants collected in Cyprus (ten from Kivisili, seven from Polis, and ten from Aphrodite rock; see Fig. 1). DNA was extracted using DNeasy Plant mini Kit (Qiagen, Hilden, Germany) with minimal modifications.

RAPD analysis Forty-five random primers (ten bases long each) were screened for reliable and repeatable amplification products. Eight (5′-AAGCCTCGTC-3′, 5′-CCGCATC TAC-3′, 5′-CTCACCGTCC-3′, 5′-GTTGCGATCC-3′, 5′-CAAACGTCGG-3′, 5′-GAAGGCACTG-3′, 5′-GTCGCCGTCA-3′, 5′-ACTTCGCCAC-3′) were chosen for further population analysis. Polymerase chain reaction (PCR) (13 μl) included: denaturation at 94°C for 4 min; 44 cycles of 94°C—1 min, 35°C—1 min, and 72°C—2 min. Amplification products run in 1.5% agarose gels containing ethidium

Table 1 The sampled populations of *P. lentiscus* and their environmental characteristics, along the climatic gradient in Israel and their climatic conditions (Braun 2004; Atlas of Israel)

Population	Average temperature (°C) August	Average temperature (°C) January	Total yearly rain (mm)	Number of rainy days	Distance from sea (Km)	Elevation (m)	Rock and soil types
Kziv River	25	11	608	55	10	150	Terra Rosa, dolomite, chalk
Turan	26	11	530	52	31	200	Terra Rosa, dolomite, chalk
Mt. Carmel	26	13	697	59	4	450	Terra Rosa, dolomite, chalk
Mt. Gilboa	27	15	408	60	38	400	Rendzina, chalk
Hadera	27	13	540	44	2	50	Sand
Bet Guvrin	28	9	425	38	29	200	Rendzina, chalk

bromide (0.5 μg/ml). The different bands were recorded directly from the gels.

AFLP analysis The amplified fragment length polymorphism (AFLP) procedure applied followed Vos et al. (1995). High quality genomic DNA (~100 ng) was digested with a pair of restriction enzymes (*Eco*RI/*Mse*I) at 37°C for 3 h then ligated to the double stranded *Eco*RI (E-) and *Mse*I (M-) adaptors. The resulting fragments were preamplified with the nonselective primers, where the ligated adaptors serving as target sites for primer annealing. Three selective primer combinations were used for AFLP amplification as follows: E-ACC/M-GTAC, E-TCAG/M-CAC, and E-ACGG/M-GTAC (E- and M- representing the restriction site). Selective *Eco*RI (E-) primers were end-labeled with [γ-^{33}P]ATP. PCR reactions were carried out in a total volume of 13 μl. PCR amplification cycles started at the annealing temperature of 65°C, after which the annealing temperature was lowered by 0.7°C per cycle for 12 cycles (a touch down phase of 13 cycles) then followed 23 cycles at the annealing temperature of 56°C. The products were separated on a 6% denaturing polyacrylamide gels and exposed to Kodak BioMax film.

Genetic data analysis

Amplification products were scored as discrete character states (present/absent) and transformed into band frequencies. DNA of the several samples (~5%) were amplified and run in duplicates to validate repeatability. The identities between the duplicated fingerprints were found to be higher than 98%. Samples that exhibited unclear band formations (<5% of all amplification products), suggesting contamination, were excluded from the analysis. Diversity values were based on a phenotype frequency (phenotypes being the band patterns produced by individual primer pairs). Data were analyzed by POPGENE software version 1.31 (Yeh et al. 1997) and Tools for Population Genetic Analyses (TFPGA) software version 1.3 (Miller 1997). These programs consider RAPD and AFLP bands as diploid-dominant markers, in which the estimated allele frequencies are based on the square root of the frequency of the null (recessive) genotype. Population differentiation was tested by exact tests (1,000 dememorization steps, ten batches, 2,000 permutations per batch; Raymond and Rousset 1995). The number of dispersants between any two populations in every generation (Nm_{est}) was calculated following Slatkin (1985). The significant relationship between the two distance matrices (Nm and geographical) were calculated by Mantel test using TFPGA software (with 999 times of randomly switch rows and columns of one of the matrices). Molecular analysis of variance (Nested analysis of molecular variance (AMOVA)) was conducted using GenAlEx (Peakall and Smouse 2006).

Results

Plant morphology

All tested parameters of plant morphology differed significantly among the Israeli populations. Because some parameters were intercorrelated, we discuss only representative data. The six populations differed significantly in shrub volume (Fig. 2a; $F_{5.94}$=4.117, p<0.002). One of the populations (Kziv) was eventually found to be highly grazed and was thus excluded from the analysis of plant volume. The largest plants were found on Mt. Carmel and the smallest on Mt. Gilboa. The populations differed in the number of leaflets (Fig. 2b; $F_{5.94}$=40.72, p<0.01); more leaflets were found in the southernmost population of Bet Guvrin and fewer leaflets were found on Mt. Carmel. As

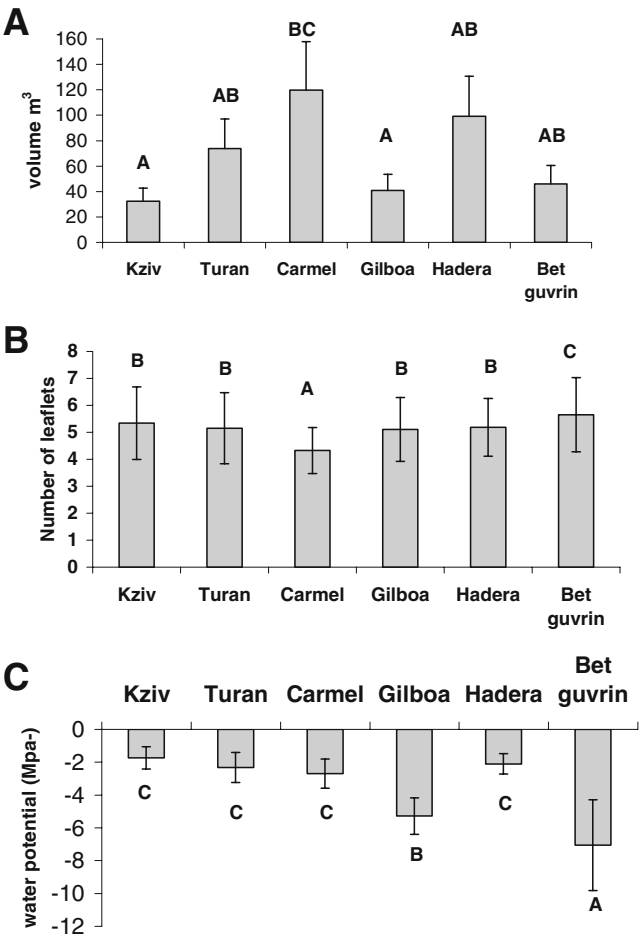

Fig. 2 Morphological characteristics (mean±SD) of *P. lentiscus* shrubs in Israel. *Columns with common letters* are not significantly different (Duncan Post-hoc test, P<0.05). **a** Shrub volume. **b** Number of leaflet. **c** Predawn water potential

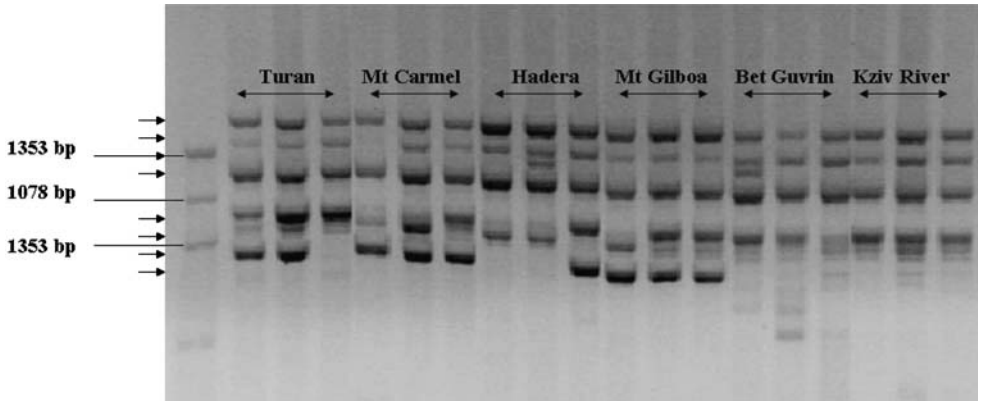

Fig. 3 RAPD fingerprint amplification product of *P. lentiscus* genomic DNA from the six Israeli populations using the primer 5′-AAGCCTCGTC-3′. The *left lane* indicate the fragment size. *Arrows* point to the respective loci

expected, the highest values of predawn water potential were found in the northern (mesic) populations and the lowest in the southern (xeric) most population of Bet Guvrin (Fig. 2c; $F_{5.54}$=41.005, p<0.001). Water potential was negatively correlated with the maximum temperature in August (summer's hottest month) (r_p=−0.947, p=0.004) and had a significant relationship to the annual growth (r^2= 0.723, $F_{5.54}$=10.877, p<0.05).

Plant morphological parameters were correlated with the local environmental conditions (Table 1), primarily with the annual precipitation and annual average temperature. The shrubs on Mt. Carmel that undergo higher annual rainfall and moderate temperatures had a high predawn water potential. These shrubs were larger with a low number of leaflets compared with those in the arid sites of Mt. Gilboa and Bet Guvrin (Fig. 2). Terminal leaflets (an indication for deviation from leaf symmetry and thus stress) occurred in approximately 18% of the tested leafs. Their presence was negatively correlated with the annual rainfall (r_p=−0.843, p=0.035). Thus, the frequency of leafs with terminal leaflet was higher in dryer and warmer habitats. Linear regression indicated that 67% of the variation in the number of terminal leaflets is explained by rainfall (F=8.23, p<0.05). Plant volume was also highly correlated with the annual rainfall (r_p=0.972, p<0.05). The number of rainy days and total annual rainfall had high influence on the plant water potential, annual growth rate, and plant volume.

Genetic analysis

One hundred twenty six shrubs, originating from six populations from Israel, and an additional 27 individuals from the three populations from Cyprus were genetically analyzed. The eight RAPD primers revealed 70 loci (Fig. 3) and the three primer combinations, E-ACC/M-GTAC, E-TCAG/M-CAC, and E-ACGG/M-GTAC, revealed 42, ten, and six loci, respectively, yielding the additional 58 putative loci (bands). Levels of polymorphism and gene diversity for each population are summarized in Table 2. Most loci, 57 (81.4%), were found to be polymorphic at the stringent 95% criterion in the RAPD analysis (Israel and Cyprus) and 55.2% were polymorphic in the AFLP analysis (Israel). There was no genetic diversification or any gradient changes in genetic variability within any of the

Table 2 Gene diversity (unbiased heterozygosity (He)), and percent polymorphic loci (*P*; 95% criterion) of the sampled *P. lentiscus* populations in Israel and Cyprus

Population	RAPD			AFLP		RAPD+AFLP	
	N	He	*P* (%)	He	*P* (%)	He	*P* (%)
Kziv River	10	0.19	49.2	0.09	19.6	0.15	35.3
Turan	10	0.19	50.8	0.05	10.7	0.16	40.5
Mt. Carmel	16	0.24	57.1	0.08	19.6	0.20	50.0
Mt. Gilboa	20	0.27	66.7	0.06	12.5	0.17	41.4
Hadera	20	0.26	61.9	0.07	16.1	0.17	42.2
Bet Guvrin	20	0.22	57.1	0.08	17.9	0.16	40.5
Overall Israel	126	0.28	78.3	0.10	25	0.20	55.2
Polis	7	0.25	60.0				
Kivisili	10	0.25	63.6				
Aphrodite Rock	10	0.19	53.3				
Overall	153	0.27	82.5				

Table 3 Pair-wise unbiased genetic identities (Nei 1978; above diagonal) and genetic distances (below diagonal) among the *P. lentiscus* populations in Israel (resulted from RAPD and AFLP)

	Kziv River	Turan	Mt. Carmel	Mt. Gilboa	Hadera	Bet Guvrin
Kziv River	–	0.969	0.960	0.964	0.973	0.981
Turan	0.032	–	0.970	0.964	0.971	0.968
Mt. Carmel	0.041	0.03	–	0.981	0.972	0.971
Mt. Gilboa	0.036	0.037	0.019	–	0.982	0.977
Hadera	0.027	0.03	0.029	0.019	–	0.982
Bet Guvrin	0.019	0.032	0.029	0.023	0.019	–

populations. Polymorphism levels and gene diversity did not differ significantly among the locations (P=0.79). The genetic identities (0.972±0.007; mean±SD) and distances (0.028±0.007) between *P. lentiscus* populations in Israel are summarized in Table 3. Pairwise analysis (exact tests, Raymond and Rousset 1995) showed no differentiation between populations (p=1.000). The genetic distance between Israel and Cyprus population was a bit higher than the genetic distance among Israeli populations (0.2023± 0.019). However, a Kruskal Wallis analysis showed no significant differentiation between Israel and Cyprus (χ^2= 4.689, DF=8, P=0.79). The resulted dendogram is presented in Fig. 4a.

The estimated numbers of dispersants in every generation (Nm) are summarized in the upper diagonal of Table 4. Nm estimated values for any pair of populations is larger than one, suggesting recurrent gene flow between populations within Israel as well as between Israel and Cyprus. A Mantel test indicated no correlation between genetic and geographical distances (r=−0.2038, p=0.831, Z=22,941).

AMOVA analysis (Peakall and Smouse 2006) revealed that, within Israel, the major part of the molecular variance (90%) was found within populations (Φ_{PT}=0.099, p= 0.001) and only 10% of the variance is found among populations. A similar result was found for the RAPD analysis including also the Cyprus populations, where the major part of the molecular variance (69%) was found within the populations (Φ_{PT}=0.312, p=0.001; Fig. 4b); 22% of the molecular variance was found between Israel and Cyprus (Φ_{RT}=0.225, p=0.001) and only 9% among populations within the region (Φ_{PR}=0.113, p=0.001).

Discussion

Phenotypic plasticity and ecotypic variation

Sessile organisms may encounter spatio–temporal variation in the environmental conditions during their lifetime. Adaptation of long-lived plants to such local environmental heterogeneity can be attained by the phenotypic plasticity. In addition, in patchy landscapes with distinct ecology, local adaptation of specific ecotypes may also be an evident. The distinction between phenotypic plasticity and local adaptation of ecotype is based primarily upon genetic analysis and transplantation experiments. In the reciprocal transplantation experiments of three grassland outbreeding perennials, Joshi et al. (2001) showed a consistent home advantage and a decreasing selection advantage with the distance across Europe. Jump and Penuelas (2005) review numerous cases of microgeographical genetic differentiation of plant species correlated with the climatic factors of temperature and water availability. Distinct morphological

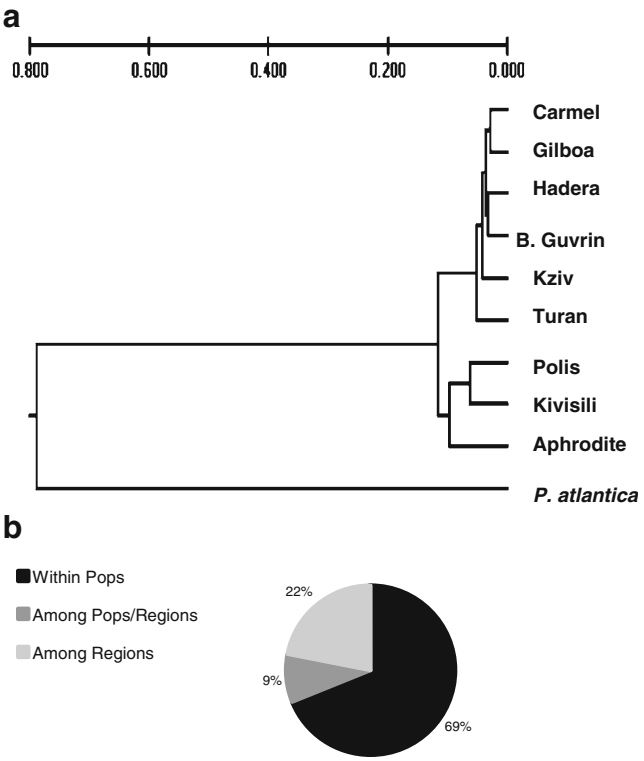

Fig. 4 Genetic differentiation among *P. lentiscus* populations. **a** Dendogram based on Nei's (1978) unbiased genetic distance among the populations in Israel and Cyprus as determined by RAPD analysis. **b** The results of molecular analysis of variance (Nested AMOVA) partitioned among regions, among populations, and within the populations

Table 4 Gene flow estimates (Nm) (Slatkin 1985) among the *P. lentiscus* populations in Israel and Cyprus

	Kziv River	Mt. Carmel	Turan	Mt. Gilboa	Hadera	Bet Guvrin	Kivissili	Aphrodite	Polis
Kziv River		1.23	3.60	3.66	3.25	1.88	3.85	4.14	3.73
Mt. Carmel	27		8.40	1.18	1.22	1.18	1.24	1.21	1.22
Turan	35	27		11.43	6.56	9.67	4.66	3.58	5.22
Mt. Gilboa	55	45	32		3.42	4.38	4.55	3.99	4.00
Hadera	66	33	55	42		3.28	3.39	3.30	3.32
Bet Guvrin	165	145	154	132	121		1.82	1.79	1.86
Kivissili	282	329	330	397	404	478		4.99	2.22
Aphrodite	291	344	341	396	402	464	111		1.49
Polis	341	392	391	452	459	524	87	76	

Above the diagonal were the Nm values; below the diagonal were the distances in Km.

and genetic differences were also found in *P. vera* between two populations in Turkmenistan (Barazani et al. 2003b).

P. lentiscus has a wide distribution in variable habitats. Shrubs growing in these different habitats are characterized by distinct morphologies. We found considerable differences in morphological and ecological parameters across the Israeli population (Fig. 2). For example, shrub size is strongly associated with annual precipitation increases water availability. We addressed the question of whether these morphological (and physiological) differences reflecting locally adapted genetic varieties (i.e., ecotypes) or, rather, the ability of a single genotype to exhibit a range of alternative phenotypes (i.e., phenotypic plasticity)?

In a greenhouse experiment, Shaviv (1978) transplanted seeds of *P. lentiscus* that were collected from the three populations in Israel differing in their ecological conditions. The phenotype of the seedlings retained some unique characteristics, suggesting that they represent distinct ecotypes (Shaviv 1978). The current genetic analysis (Table 3 and Fig. 4), that is based on the populations sampled by Shaviv (1978; Mt. Carmel, Mt. Gilboa, and Hadera) and an additional three populations revealed high genetic similarity among all populations. The majority of the genetic variance (90%, see below) was found within the populations. Unlike Shaviv (1978), we did not find any pattern of ecological-related genetic differentiation or verification for any type of local adaptation that would support the existence of distinct ecotypes of *P. lentiscus* in Israel. It seems that the significant morphological and physiological differences found between the tested populations resulted merely from phenotypic plasticity.

It also possible that the AFLP and RAPD analyses were not sensitive enough to detect genetic differentiations among populations. However, these methods were useful in the recognition of genetic structure among *P. lentiscus* population across the Mediterranean basin (Barazani et al. 2003a), between the Iberian Peninsula and North Africa (Werner et al. 2002) and between the Israel and Cyprus (Fig. 4a).

The level of gene diversity

Genetic diversity is expected to increase under stressful conditions and environmental heterogeneity (Nevo et al. 1984; Linhart and Grant 1996; Nevo 2001). The eastern Mediterranean region is characterized by both temperature and precipitation stresses. In Israel, environmental heterogeneity is intensified in both west to east and north to south gradients. Two populations in this study (Mt. Gilboa in the east and Bet Guvrin in the south) grow under the relatively stressful conditions of annual rain and temperature compared with the relatively moderate conditions of Mt. Carmel (Fig. 1; Table 1). Nonetheless, the later population exhibits a higher level of gene diversity, although the differences between the populations are not significant (Table 2).

Environmental heterogeneity may create barriers to gene flow and consequently lead to spatial differentiation between populations (Linhart and Grant 1996). However, the genetic similarity among the Israeli populations is quite high (I=0.96–0.98; Table 3) and 90% of the molecular variance is found within the populations. A similar pattern of genetic similarity was found among Spanish populations of *P. lentiscus* (Werner et al. 2002), suggesting high gene flow among populations.

The genetic similarity between the Israeli and Cyprus populations was slightly lower than between the Israeli populations (I>0.8), indicating minor spatial differentiation between the mainland and island populations. AMOVA analysis revealed that only 22% of the variance is found among Israel and Cyprus, while 69% of the variance is found within the populations and 9% among populations within the regions (Fig. 4b). Barazani et al. (2003a) reported that the genetic similarity between population collected in Israel, Spain, and Tunisia was even higher than the similarity between Israel and Cyprus, suggesting recurrent gene flow among populations within the Mediterranean basin.

Spatial differentiation among populations and regions is affected by the amount of gene flow which can be a

homogenizing force that may even prevail over strong selection (Linhart and Grant 1996). *P. lentiscus* is wind-pollinated and its seeds dispersed by birds, thus, there may be extensive gene flow even among distant populations. Seeds are produced during the fall and 90% are eaten by eight different bird species that may result in the long distance seed dispersal within Israel (Izhaki and Safriel 1985). About 3% of the seeds are taken by transient migrating birds during their flight to the south; thus, seed dispersal from northern region (Cyprus for example) to Israel is also probable. Indeed, gene flow (Nm) estimates between all tested population within Israel as well as between Cyprus and Israel are high (Table 4), with no indication for isolation by distance.

Phenotypic plasticity and gene diversity

The genetic basis of plants adaptive responses to varying environment is still an open question. The genetic mechanism of plasticity should consist of genes that determine character means and character response (Bradshaw 2006). Thus, the genetic mechanism may comprise of quantitative trait loci with environmentally based allelic sensitivity as well as regulatory loci that are expressed in a differential manner across the environments (Via et al. 1995). Both types of genetic mechanisms suggest multilocus characteristics. The impact of short-term inherited epigenetic imprinting adds further complexity to the genetic mechanism. This study tested general genomic diversity as marker and estimator for the genetic variation also of the phenotypic plasticity loci.

Adaptive phenotypic responses can evolve only under the background of sufficient genetic variability (Via et al. 1995; van Kleunen and Fischer 2005). However, plants that show high levels of phenotypic plasticity are repeatedly reported as expressing low levels of gene diversity (reviewed in Linhart and Grant 1996). The level of gene diversity found for *P. lentiscus* populations in Israel and Cyprus (He=0.15–0.28; Table 2) is in the range found for dominant markers in other tree populations such as oaks (0.17–0.29; Mariette et al. 2002; Coart et al. 2002; Yakovlev and Kleinschmidt 2002) and pines (0.17–0.29; Peng et al. 2003; Kandedmir et al. 2004). Thus, the reported propensity of reduced gene diversity associated with the apparent phenotypic plasticity in plants is not evident in *P. lentiscus* populations, which exhibit high degree of morphological and physiological plasticity as well as high level of gene diversity.

Ecotypic local adaptation is promoted by low gene flow, moderate selection against intermediate genotypes, and strong selection against genotypes adapted to other habitats (Kawecki and Ebert 2004). *P. lentiscus* has a wide distribution in various habitats around the Mediterranean region. Adaptive plasticity can expand environmental tolerances and contribute to a wide distribution (Sultan 2005). It seems that the ability of *P. lentiscus* to change its phenotype in response to the varying conditions in the Mediterranean region allow plants to thrive in different environments in species that is characterized by intensive gene flow.

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