

Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. *Oncocyclus* = the royal irises) along an ecogeographical gradient in Israel and the West Bank

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Abstract

Iris haynei and *I. atrofusca* are two closely related narrow endemics distributed vicariously along an ecogeographical north–south gradient in Israel and the West Bank. To obtain baseline information of the taxonomic status, conservation and population history of these taxa, we investigated patterns of phenotypic variation and the partitioning of genetic variation within and among populations using dominant random amplified polymorphic DNA (RAPD) markers. Multivariate (principal components analysis) and taxonomic distance analyses based on morphometric traits from eight populations revealed no unambiguous separation into two distinct groups. Results of genetic analyses for nine populations differed only slightly when either allele- or marker-based approaches were employed. Mean within-population diversity was high (0.258 for Nei's expected heterozygosity), but there was no significant relationship between genetic diversity and either population size or latitude. Although the range-wide estimate of G_{ST} (≈ 0.20) revealed relatively high differentiation among populations this value was inflated because of a small, but significant, component of molecular variance among regions viz. taxa ($\approx 5\%$). Limited long-distance dispersal capabilities in conjunction with a linearized habitat distribution are proposed to contribute to the approximate isolation by distance pattern observed. It also appears that extant populations are currently deviating from equilibrium conditions because of primary divergence of a formerly more widespread ancestral population. Given the absence of deep genetic and phenotypic subdivision among northern (*I. haynei*) vs. central/southern (*I. atrofusca*) populations, we argue for a revision of their species status. Nonetheless, we recommend conservation attention to these geographically differentiated segments as separate management units, which can be seen as an instructive example of incipient species formation.

Keywords: conservation genetics, *Iris*, morphometric traits, population history, primary divergence, RAPDs

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Introduction

The great floristic richness of many Mediterranean and (semi) arid habitats in the Near East (Zohary 1973; Zohary & Feinbrun-Dothan 1966–86) provides challenges for evolutionary biologists and conservation biologists alike. The Israel/West Bank region alone, with an area of only

20 700 km², harbours ≈ 2200 plant species of which 165 (7.5%) are endemic (Shmida 1984; Médail & Quézel 1997; Fragman *et al.* 1999). Determining the population genetic structure of narrow endemics in this region provides opportunities for understanding evolutionary processes viz. speciation over small spatial scales. For conservation purposes, genetic variability analyses can be used to provide baseline information for sampling strategies, reintroduction, translocations and genetic enhancement (reviewed by Woodruff 2001). However, despite the

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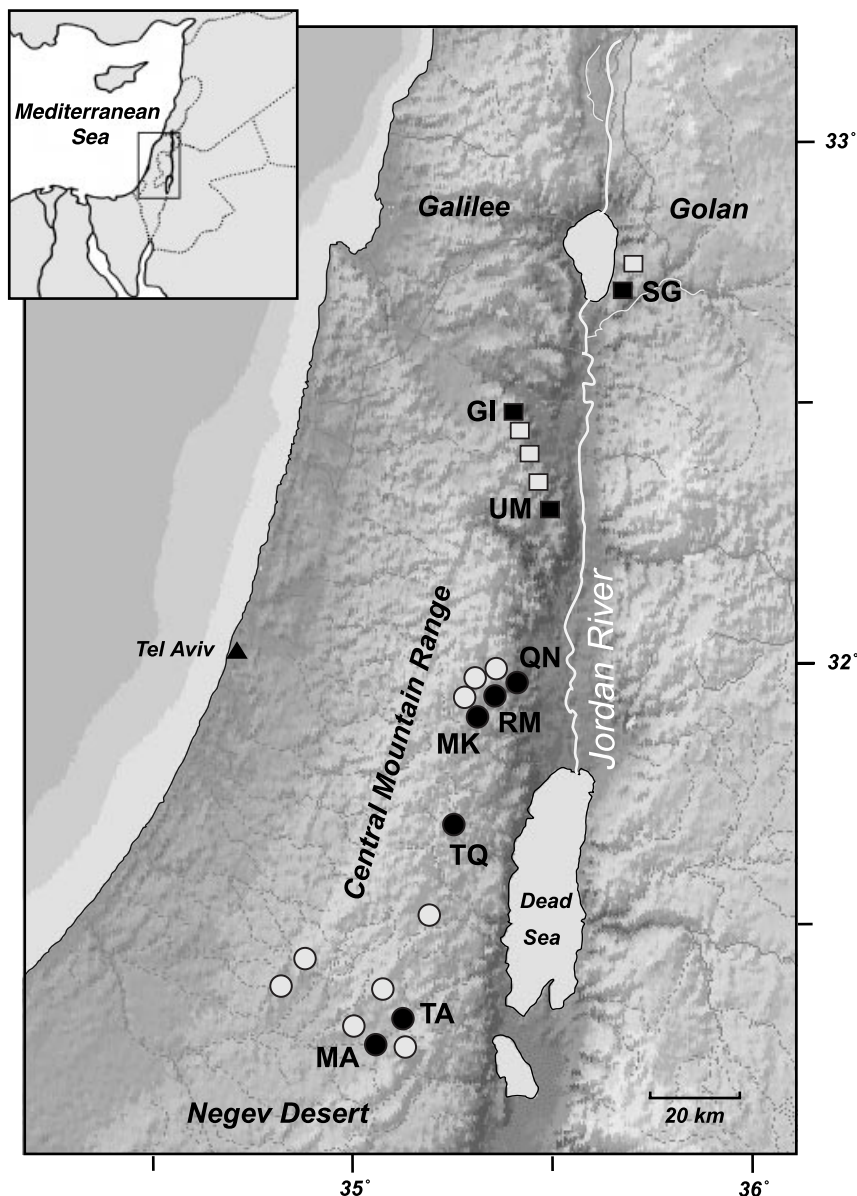


Fig. 1 Collection localities for *Iris haynei* (■) and *I. atrofusca* (●) in Israel and the West Bank (Palestinian Authority), together with known populations of the two taxa (□, ○) based on information stored at the Israel Plant Information Center (ROTEM), Hebrew University of Jerusalem. All populations sampled were analysed for both random amplified polymorphic DNA (RAPD) and morphometric variation, except population (MK) of *I. atrofusca* (only RAPDs). Refer to Table 1 for locality abbreviations and further details.

accepted importance of the Near East as one of the hot-spots of biodiversity in the Mediterranean basin (Auerbach & Shmida 1985; Médail & Quézel 1997; Myers *et al.* 2000), there have been few studies in this region which have analysed the genetic (sub)structure of natural plant populations other than those of wild cereals (e.g. *Aegilops*: Mendlinger & Zohary 1995; *Hordeum*: Nevo *et al.* 1979, 1998; Dawson *et al.* 1993; *Triticum*: Nevo *et al.* 1982; noncereals: *Alkana*: Wolff *et al.* 1997; *Senecio*: Comes & Abbott 1999).

It is well known that determinants of within- and between-population genetic variability vary widely between different plant taxa. Geographical range, population size, successional status and a host of other factors (life form, mode of reproduction, mating system, dispersal capabilities) are likely to affect allele distributions and gene flow within species ranges

(Hamrick & Godt 1990; Nybom & Bartish 2000). In addition, the history of a population (recent origin, occurrence of bottlenecks, expansion in range) may have significant effects on its population genetic structure (Utelli *et al.* 1999; Hannan & Orick 2000). As a consequence, conflicting patterns are expected in different taxa. However, endemic and/or narrowly distributed plant species tend to exhibit lower levels of within-population diversity than all other species or their widespread congeners (Hamrick & Godt 1990; Gitzendanner & Soltis 2000). Some of these differences may be due to population size *per se*, which is often smaller in endemic taxa (Ellstrand & Elam 1993). If correct, endemic taxa are likely to be more vulnerable to demographic variation, environmental stochasticity, and genetic factors that tend to drive small populations to extinction (Gilpin & Soulé 1986).

Table 1 Geographical locations, associated vegetation description, coordinates, altitude and rainfall information for the *Iris* populations sampled in this study. The number of plants (*N*) at each site analysed for morphological and RAPD variation are indicated. The first three populations were collected as *Iris haynei* (marked with an asterisk), the remaining six as *I. atrofusca*. Populations are ordered from north to south

Popn code	Locality	Lat. °N	Long. °E	Alt. (masl)	Annual rainfall (mm)†	N Morph.	N RAPDs
SG*	Southwestern Golan Heights, Hamat Gader road, semisteppe batha (climax)	32°43'	35°39'	250	450	36	15
GI*	Mt. Gilboa, xeromorphic shrub formation	32°27'	35°26'	450	420	36	15
UM*	Northeastern Samaria, Um Zuka, semisteppe batha (climax)	32°17'	35°30'	70	250	27	10
QN	Eastern Samaria, Qubet Najme, semisteppe batha	31°55'	35°23'	580	330	23	10
RM	Eastern Judea, Rimmonim, semisteppe	31°54'	35°22'	550	330	30	14
MK	Eastern Judea, Ma'alé Mikhmas, semisteppe batha	31°52'	35°19'	550	340	NA	14
TQ	Eastern Judea, Teqoa, semisteppe batha	31°41'	35°14'	620	350	30	15
TA	Northern Negev, Tel Arad, loess semidesert	31°17'	35°07'	535	230	30	14
MA	Northern Negev, Wadi Mar'it, loess semidesert	31°16'	35°04'	450	230	30	12

NA, not analysed; Lat., latitude; Long., longitude; Alt. altitude; Morph., morphology. †Rainfall data from Average Annual Rainfall (1951–80) Map, Israel Ministry of Transport, Meteorological Service.

Some narrow endemics have been shown to have strong genetic structure among populations despite being restricted to specialized ecosystems (Brauner *et al.* 1992; Travis *et al.* 1996). Limited pollen and/or seed dispersal, isolation in small, disjunct populations and regional habitat selection in response to different soil types or climatic conditions are just a few of the mechanisms by which population genetic structure can arise in species with narrow geographical range (Endler 1977; Levin 2000). Regional landscapes can also have marked effects on population genetic structure by limiting dispersal routes (Wolff *et al.* 1997; Comes & Abbott 1999). Clearly, if narrow endemic plant species have a practical dispersal limit, one would expect that even the restricted ranges of these species are still large enough for isolation by distance to play a role in population differentiation and incipient species formation. Also, recent computer simulations substantiate the claim that species with smaller range sizes should have higher speciation rates (Gavrilet *et al.* 2000).

To further understand processes underlying differentiation of narrow endemic plant taxa, we examined random amplified polymorphic DNA (RAPD) and phenotypic variation in two nominal species of *Iris* sect. *Oncocyclus* (Siemss.) Baker (the 'royal irises'), collected from populations scattered vicariously along an environmental aridity gradient in Israel and the West Bank. According to Feinbrun-Dothan (1986), *I. haynei* Baker primarily occurs on Mt. Gilboa (northern Samaria), and the east-facing slopes of the Upper Jordan Rift Valley (northeastern Samaria), but has also been reported as far north as the southwestern Golan Heights (Fragman *et al.* 1995; Fig. 1). Most of these populations receive mean annual rainfalls of ≈ 420 –450 mm (Table 1), except populations from the Upper

Jordan Valley, which suffer from rain shadow (≈ 250 mm/year). Further south, in the central and southern parts of the Central Mountain Range, *I. haynei* is replaced by *I. atrofusca* Baker, ranging as far south as the northern Negev desert [Israel Plant Information Center (ROTEM) database, A. Shmida, unpublished data]. Corresponding rainfall averages decrease from ≈ 350 to 230 mm/year, resulting in an overall aridity gradient across the entire range of the two taxa.

In Israel and the West Bank, *Iris* sect. *Oncocyclus* is represented by a rich endemic series of eight species replacing each other over short geographical distances (Avishai & Zohary 1980). *Iris haynei* and *I. atrofusca* are generally regarded as closely related (Avishai 1977; Avishai & Zohary 1980), or members of a single biological species (Dykes 1913; Shimshi 1979/80). They are best characterized by the sharing of large, purplish to brown or nearly black flowers, the possession of a strong flower scent, and mostly erect leaves that either reach the base of the spathe or overtop it (Feinbrun-Dothan 1986; O. Fragman, unpublished data). However, there is a suspicious lack of clear taxonomic distinctions between *I. haynei* and *I. atrofusca*, which are generally identified by suites of quantitative characters and grades of flower coloration (Feinbrun-Dothan 1986). Provisionally, therefore, we refer to these supposedly distinct groups of populations as the *haynei* and *atrofusca* 'morphotypes' throughout this study.

During recent years, approximately seven and 15 sites of occurrence have been monitored for *haynei* and *atrofusca*, respectively, thereby occupying no more than 54 (*haynei*) and 46 (*atrofusca*) quadrats of 1 km² (Fragman *et al.* 1995; ROTEM, A. Shmida, unpublished data; Fig. 1). Both morphotypes have the ability to reproduce by stout, compact

rhizomes; thus forming dense clumps (Feinbrun-Dothan 1986). Based on our own census data, population size varies from as few as 14 to up to 100 000 clumps or putative individuals (genets), albeit with a strong bias towards small population size (i.e. ≤ 300 clumps; Table 3). Several causes have been invoked that may have contributed to isolation and habitat fragmentation of these *Iris* populations over the past millennia, all concerning human-mediated perturbations such as agricultural development, ploughing and grazing (Shimshi 1979/80). However, only the advent of modern mechanized farming, along with intensified grazing pressure, but also urban development and afforestation have been implicated in posing major threats to *haynei* and *atrofusca* (Shimshi 1979/80; Fragman *et al.* 1995; Y. Sapir, unpublished data). For example, the number of *atrofusca* populations from the northern Negev has declined greatly over the past 30 years (ROTEM, A. Shmida, unpublished data), probably as a result of abandoning traditional farming (Shimshi 1979/80). At present, like all *Oncocyclus* species in Israel and the West Bank, *haynei* and *atrofusca* are protected by law (Fragman *et al.* 1999; Shmida *et al.* 1999). Their status has been determined as 'vulnerable' based on IUCN categories (IUCN 1994; Sapir *et al.* manuscript in preparation).

The *haynei* and *atrofusca* morphotypes represent a good test case for exploring the relationship between incipient species formation, natural history and ecogeographical structure. In addition, their effectively linearized distribution along an environmental gradient may reveal patterns of gene flow that are not readily apparent in less constrained landscapes. Because no population genetic information exists on the royal irises, the genetic variability analysis reported here will also help to propose conservation measures. Specifically, the major objectives were: (i) to clarify the pattern of phenotypic variation between the two morphotypes by detailed morphometric analysis; (ii) to evaluate levels of genetic diversity within populations of the two morphotypes; (iii) to quantify differentiation among populations and gene flow; and (iv) to assess genetic and morphological distances between populations in relation to their ecogeographical distances. Finally, we tried to explore whether the extant patterns of genetic variability observed allow inferences to be made about population history.

Materials and methods

The study system

Both morphotypes are herbaceous, polycarpic perennials found primarily in the Mediterranean–desert transition zone (*sensu* Danin & Plitmann 1987) of Israel and the West Bank. This bioclimatic zone of semisteppe communities extends, on various soils, as a narrow strip from the Golan

Heights, throughout the eastern slopes of the Central Mountain Range, towards the northern Negev. Associated vegetation includes *Retama raetam*, the grass *Hyparrhenia hirta* and scattered trees such as *Pistacia atlantica* and *Ziziphus spina-christi*. However, the type locality of *haynei* at Mt. Gilboa (GI) occurs in a *Ceratonia siliqua*/*Pistacia lentiscus* community under xeric Mediterranean conditions, whereas the most southerly populations of *Iris atrofusca* [e.g. Tel Arad (TA), Wadi Mar'it (MA)] grow under semidesert conditions in a segetal/*Achillea santolina* association on loessial soils.

Following Feinbrun-Dothan (1986), *atrofusca* differs from *haynei* in being in all parts smaller and in having a perianth that is as long as broad (opposed to longer than broad in *haynei*). In contrast to previous accounts (Dykes 1913), there are no consistent differences in flower colour between morphotypes. Detailed field studies indicate that there is considerable variation for flower colour (all shades of brown/purplish) within and among several *haynei* populations from the northern and central parts of the overall range [e.g. Gilboa (GI), Rimmonim (RM)], whereas the southern populations of *atrofusca* [e.g. Teqoa (TQ), Tel Arad (TA), Wadi Mar'it (MA)], and also the northernmost populations of *haynei* [e.g. South Golan (SG)] tend to be almost monomorphic for dark purple or nearly black flowers (Y. Sapir, unpublished data).

Plants survive the dry summer months via a persistent rhizome (see above), whereas the aerial leaves and stems disappear each year. Total life span is not known but can be > 20 years based on long-term field observations at the GI and TA locations (O. Fragman, personal observation). An individual is usually composed of several flowering stems, each bearing a large single flower (6–10 cm in diameter). Although offering no food reward in terms of nectar (Ivri & Izikovitz 1988; Shmida & Ivri 1996), these flowers provide an overnight shelter for male solitary bees (Shmida & Ivri 1996; Y. Sapir, unpublished data). Avishai & Zohary (1980) reported that *Oncocyclus* species are 'largely self-incompatible', implying that intra- or interflower selfing (geitonogamy) cannot be ruled out entirely. Plants flower from late February (in the Jordan Valley) to early April (Arad area), with each flower lasting for up to 5 days. Mature capsules release seeds from May onwards, with ≈ 30 –50 seeds set per capsule (Avishai & Zohary 1980; Y. Sapir, unpublished data). Interspecific greenhouse crosses indicate that all species of sect. *Oncocyclus*, all of which are diploid with $2n = 20$, produce fertile F1 hybrids (Avishai & Zohary 1977, 1980).

Morphometric analysis

Altogether eight populations of *haynei* (three) and *atrofusca* (five), chosen to cover most of the geographical range of the two morphotypes (Fig. 1; Table 1), were surveyed for phenotypic variation. During spring 1998, measurements

Table 2 Morphological characters analysed in populations of *Iris haynei* and *I. atrofusca*, together with character coefficients (loadings) for the first two principal components of individual plant values (see Fig. 2). Note that 'fall' and 'standard' refer to the outer and inner petals, respectively. 'Signal patch' refers to the guide mark on the outer petals

No.	Character	Code	Description/remarks	PC1	PC2
1.	Flower height (cm)	FH	Measured from base of fall to apex of standard	0.831	0.447
2.	Flower diameter	FD	Measured at the height of the pollination tunnel	0.751	0.223
3.	Flower shape	FS	Calculated as FD/FH	-0.413	-0.392
4.	Flower surface (cm ²)	FSU	Calculated as FD × FH	0.853	0.398
5.	Fall width (cm)	FAW	Measured in its broadest place	0.831	0.337
6.	Standard width (cm)	STW	Measured in its broadest place	0.848	0.272
7.	Signal patch length (cm)	SPL	Measured in its broadest place	0.662	-0.108
8.	Signal patch width (cm)	SPW	Measured in its broadest place	0.753	0.156
9.	Signal patch surface (cm ²)	SPS	Calculated as SPL × SPW	0.767	0.027
10.	Leaf arch	LA	Classified as erect (1), semicurved (2), and curved (3)	-0.333	-0.370
11.	Leaf width (cm)	LW	Measured at the point of deflection from stem	0.668	-0.472
12.	Leaf height (cm)	LH	From soil-surface to highest point (which could be the peak of the leaf curve)	0.778	-0.530
13.	Stem height (cm)	STH	From soil-surface to base of fall	0.752	-0.536
14.	Stem gap	STG	(STH-LH)/STH; gap between leaf and flower in relation to stem height	-0.075	0.098
15.	Flower height/stem height	FH/STH	—	-0.468	0.766

were taken directly in the field at the peak of flowering of each population. Individuals within populations were chosen randomly and at widely spaced intervals, so as to minimize chances of sampling clones. In total, 242 plants were measured, with sample sizes for a given population varying from 23 to 36 individuals (Table 1). For each individual, 15 characters were recorded on a single flowering stem chosen at random within a clump. These characters are listed in detail in Table 2. Nine of the characters were descriptors of floral morphology, whereas three described leaf size and shape (measured on the second basal leaf departing from the stem). Leaf shape, as the only categorical trait, was classified as erect (1), semicurved (2) or curved (3). The remaining characters were stem height and descriptors of stem/flower architecture. Unfortunately, two variables (LH, STG) could not be recorded in population TQ because of high grazing pressure of goats, which had fed on the leaves of most genets during the sampling period. Population means of morphometric values are available on request from the corresponding author.

For morphometric data analysis, all variables were standardized by subtracting the character mean of the total sample from each individual value, and then dividing by the character standard deviation, using NTSYS-PC, Version 2.02 g (Rohlf 1998). Principal components analysis (PCA) was then performed on the correlation matrix of standardized variables and the component scores of each individual were projected in two dimensions. In addition, taxonomic distances between populations were derived from standardized population means, and sub-

jected to unweighted pair-group analyses (UPGMA) using NTSYS-PC.

RAPD analysis

During spring 2000, all populations analysed for morphological variation were resampled for DNA analysis, in addition to a single population of *atrofusca* from the Judean Mountains (Ma'alé Mikhmas, MK; Fig. 1). In general, leaf material from 15 plants per population was sampled, except for populations QN and UM (10 plants each). Leaf material was collected from putative genets at $\approx$ 5-m intervals or, in smaller populations, wherever plants occurred beyond. Material was silica-dried and stored at room temperature. For comparative analysis, we also collected five individuals of the light-coloured *I. lortetii* Barbey from a single locality near Beit Dajan (12 km west of Nablus/Samaria). *I. lortetii* is clearly not a member of the *haynei*/*atrofusca* aggregate of *Oncocylus* species as defined by Avishai & Zohary (1980). In total, 124 individuals (including *I. lortetii*) were finally available for RAPD analysis because six individuals failed to amplify (Table 1).

Total genomic DNA was extracted and cleaned with the DNeasy™ (Qiagen, Hilden, Germany) extraction kit. DNA concentrations were determined by spectrophotometry with a GeneQuant RNA/DNA calculator (Pharmacia, Uppsala, Sweden). The complete survey was performed using 10 arbitrary 10-mer primers: OPI-15, OPI-16, OPI-20 and OPJ-14 (Operon Technologies Inc., Alameda, CA, USA), and C-4, C-9, C-10, C-12, D-20 and M-4 (Carl Roth GmbH & Co., Karlsruhe, Germany); these were chosen for

the clarity and variability of fragments after primer trials with one DNA sample each from six populations, and RAPD amplifications with 53 primers (Series I and J, Operon Technologies; Series C, D and M, Roth). The 10 primers chosen produced fragments in a scorable size range from 390 to 1500 bp. Separate test amplifications with the initial samples and each chosen primer confirmed that RAPD fragments were reproducible.

Amplifications were carried out in 20- μ L volumes, containing 1 μ L template DNA ($\approx$ 12.5 ng); 14.0 μ L deionized sterile water; 1 μ L 50 mM MgCl_2 ; 2 μ L 10 $\times$ BioThermTM buffer [GeneCraft, Münster, Germany; 670 mM Tris-HCl, pH 8.8; 160 mM $(\text{NH}_4)_2\text{SO}_4$; 0.1% Tween]; 0.2 μ L of a 20-mM dNTP solution in equimolar ratio; 1.6 μ L of primer at 5 pmol/ μ L; and 1 unit (0.2 μ L) BioThermTM polymerase (GeneCraft). Polymerase chain reactions (PCR) were performed in a thermal cycler (PTC-100, MJ Research Inc.) programmed for 3 min at 94 °C, followed by 40 cycles of 30 s at 36 °C, 1 min at 72 °C and 20 s at 94 °C. Following the 40 cycles, the reactions were given an additional step of 72 °C (8 min) for final elongation. Samples were kept at 4 °C until analysis. PCR products were electrophoresed on 1.4% agarose gels in 0.5 $\times$ TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.0), visualized with ethidium bromide and photographed under UV light.

Statistical analyses of RAPD patterns were based on the following assumptions: (i) RAPD fragments behave as diploid, dominant markers with alleles being either present (amplified) or absent (nonamplified); (ii) comigrating fragments represent homologous loci; and (iii) polymorphic loci are inherited in a nuclear (Mendelian) fashion. Given the lack of information on the heterozygosity of populations, two alternative methods of analysis were employed to explore the amount of relative bias induced by the dominance problem. First, we assumed that populations are in Hardy-Weinberg equilibrium (HWE) in which case allele frequencies were estimated based on the square root of the frequency of the null (recessive) allele (hereafter, allele-frequency based estimates). Second, analyses were performed on the basis of presence/absence of RAPD fragments (hereafter, marker-frequency based estimates); this essentially equals the assumption of complete inbreeding for many generations ($F_{IS} = 1$) and Hardy-Weinberg disequilibrium. All calculations were performed exclusively on polymorphic markers (i.e. polymorphic across the whole data set).

The following estimates of mean-within population diversity were calculated with the assistance of TFPGA Version 1.3 (Miller 1997) and POPGENE Version 1.31 (Yeh *et al.* 1997): (i) the percentage of polymorphic loci out of all polymorphic loci ($P\%$); (ii) Nei's (1978) unbiased expected heterozygosity (H_E); and (iii) Shannon's index of phenotypic diversity (I). In contrast to H_E , this latter index does not rely on HWE (Lewontin 1972; Bussell 1999). Estimates of H_E and I were obtained by averaging across loci.

To evaluate interpopulation differentiation, a Monte Carlo approximation of Fisher's exact ($R \times C$) test was performed on marker frequencies of each locus between all pairs of populations (Raymond & Rousset 1995; Miller 1997). By assuming that loci are independent of each other, Fisher's combined probability test was employed as a global test over loci to determine the overall significance (Sokal & Rohlf 1995). Both tests were performed with TFPGA.

Estimates of genetic differentiation between populations were calculated as Nei's (1972) estimator G_{ST} using POPGENE. For two alleles at a locus, as applicable in RAPD analysis, G_{ST} is identical to Wright's F_{ST} (Nyblom & Bartish 2000). Corresponding estimates of gene flow ($N_e m$), i.e. the average effective number of migrants exchanged between populations each generation, were based on the inverse relationship between G_{ST} and $N_e m$ for nuclear genes [$N_e m = (1 - G_{ST})/4 G_{ST}$; Wright 1951]. To test for isolation by distance (Slatkin 1993), pairwise $\log_{10}$ -transformed values of $N_e m$ among populations were linearly regressed against their geographical (= aerial map) distances (in km). Significance of regression slopes was evaluated by Mantel's test (Mantel 1967) with 10 000 random permutations using TFPGA. Because very similar results were obtained by using either marker or allele frequencies in the above calculations, we largely focus on estimates based on allele frequencies, unless stated otherwise.

Estimates of genetic distance between populations (after Nei 1978) were calculated from allele and marker frequencies, respectively, and subjected to UPGMA clustering using TFPGA. Bootstrap values were obtained by resampling with replacement over loci (10 000 replicates). The *I. lortetii* population served as outgroup. Significance of various associations between genetic, taxonomic, geographical and environmental ('aridity') distances between populations of *haynei/atrofusca* was tested by pairwise Mantel tests. 'Aridity distance' was defined as the difference in mean annual rainfall among pairs of populations (see Table 1).

Results

Morphometric analysis

The PCA of 242 plants of *Iris haynei/atrofusca* from eight populations failed to discriminate two separate groupings (Fig. 2). The first principal component provided the best overall discrimination among individuals by accounting for 47.45% of the total variance, whereas the second and third components explained much less (15.40 and 10.09%, respectively). Plants of *haynei* tended to have high scores along the first principal axis (PC1), whereas most of the *atrofusca* individuals had lower scores. In fact, variance analysis of the pooled scores of each morphotype plotted

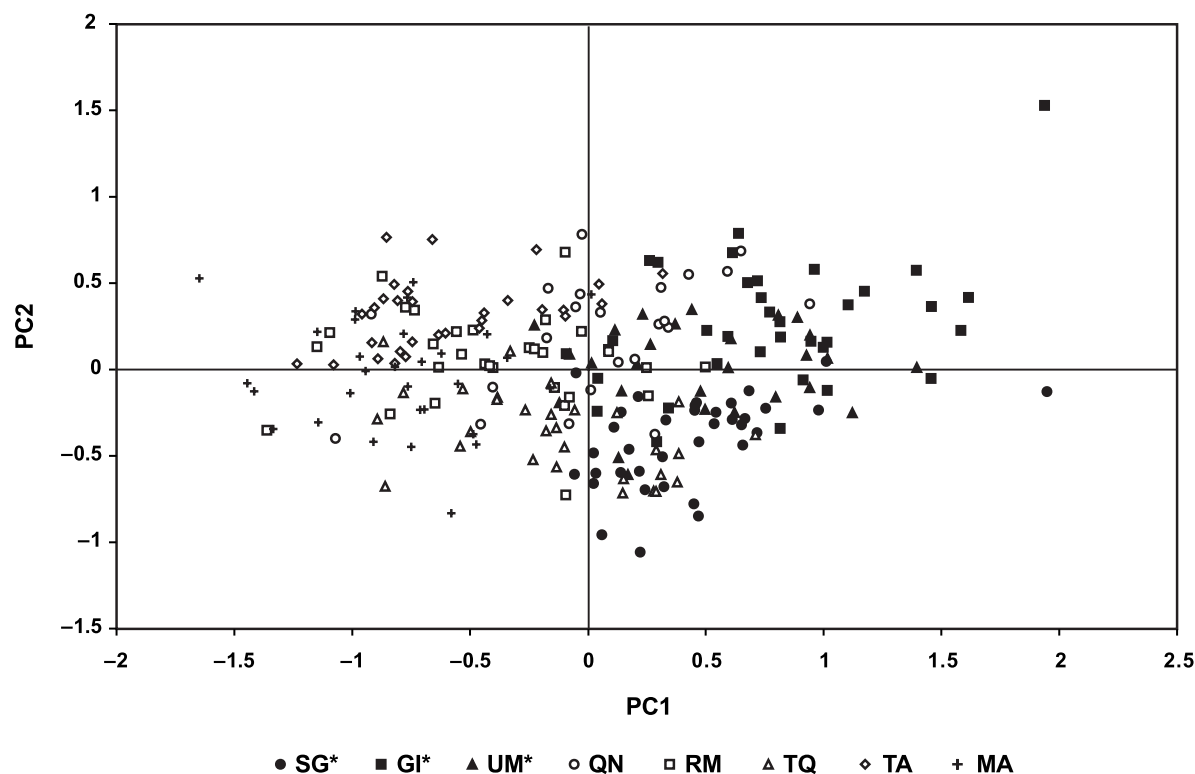


Fig. 2 Principal components analysis of 242 plants of *Iris haynei* (dark symbols) and *I. atrofusca* (open symbols and crosses) based on 15 morphological characters. Refer to Table 1 for population abbreviations.

on PC1 indicated a highly significant difference (ANOVA, $F_{1,240} = 232.26$, $P < 0.001$).

Examination of character coefficients (Table 2) revealed that many of the original variables were strongly and positively correlated with PC1, including (i) flower size and shape (FH, FD, FSU, FAW, STW); (ii) size and shape of the guide mark ('signal patch') on the outer petals (SPL, SPW, SPS); (iii) leaf size (LH, LW); and (iv) stem height (STH). Thus, by accurately extending previous taxonomical reports (Feinbrun-Dothan 1986), the PCA confirmed that plants of *atrofusca* from central/southern, and increasingly arid environments tended to be smaller in all floral and vegetative parts. However, other traits of supposedly taxonomical importance, such as flower shape (FS) and leaf curvature (LA), exerted an only minor influence on PC1 (total sample standardized coefficients ≤ 0.4 ; Table 2).

By contrast, the UPGMA analysis on average taxonomic distances among populations produced two separate clusters that were entirely congruent with the taxonomic status of *haynei* and *atrofusca* (Fig. 3). Significantly, the *haynei*-like population SG from the South Golan (cf. Fragman *et al.* 1995) was clearly assigned to *haynei* rather than *atrofusca*, as suggested by previous accounts (Feinbrun-Dothan 1986). From this phenogram it was further evident that populations of *haynei* and *atrofusca* were entirely grouped according to their geographical origin. Corroborating

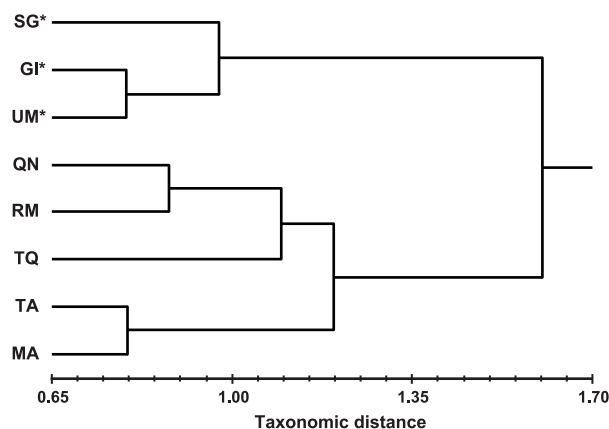


Fig. 3 UPGMA phenogram based on average taxonomic distances between populations of *Iris haynei* (marked with an asterisk) and *I. atrofusca*, illustrating overall morphological relationships. Refer to Table 1 for population abbreviations.

this, Mantel's test showed that the taxonomic distances between these populations were strongly associated with their geographical distances ($r_M = 0.773$; $P = 0.005$), and, ambiguously, with environmental (aridity) distances also ($r_M = 0.257$; $P = 0.057$).

In summary, and particularly in view of the results of the individual-based PCA analysis, we contend that there is no

Table 3 Genetic diversity indices and approximate population size of the nine populations of *Iris haynei* (marked with an asterisk) and *I. atrofusca* studied. Note that only 14 putative individuals were found at the UM site in 2000 compared to 27 individuals in 1998

Population	Population size	Percentage of polymorphic loci ($P\%$)	Nei's expected heterozygosity (H_E)	Shannon's index (I)
SG*	500	79.55	0.266	0.414
GI*	100 000	71.21	0.257	0.382
UM*	14 (27)	62.12	0.242	0.342
QN	300	72.73	0.251	0.374
RM	10 000	83.33	0.274	0.413
MK	15	76.52	0.255	0.388
TQ	200	75.00	0.262	0.391
TA	240	72.73	0.259	0.400
MA	100	75.00	0.253	0.390
Mean		74.24	0.258	0.388

evidence for an unambiguous separation of *haynei* and *atrofusca* into two distinct groups with clearly different features in floral and vegetative characters. However, these morphometric data strongly indicate an ecogeographical trend of population differentiation along the north–south gradient, whereby phenotypic divergence is *relatively* pronounced among northern (*haynei*) vs. central/southern (*atrofusca*) populations as summarized by the UPGMA phenogram.

RAPD patterns and polymorphism

For the entire data set of 124 individuals, the 10 RAPD primers used amplified 141 scorable fragments (putative loci), 137 of which were polymorphic. Excluding *I. lortetii* (outgroup), the number of polymorphic fragments for the nine populations of *haynei*/*atrofusca* studied decreased to 132, with 8–17 polymorphic fragments generated per primer. All individuals tested produced different RAPD profiles, indicating that clonal effects will not affect the level of genetic variation detected in this study. Full data matrices (including/excluding the outgroup) are available on request from the corresponding author.

In general, genetic divergence among populations of *haynei*/*atrofusca* was due mainly to differences in allele frequencies rather than the fixation of population- or taxon-specific alleles. Nonetheless, allele/marker frequencies differed substantially between the two morphotypes at three loci, namely *OPJ-14*₇₅₀, *C-4*₁₃₀₀ and *OPI-16*₅₃₀ (designated by the primer used and the molecular mass of the amplified fragment in base pairs; data not shown).

Intrapopulation genetic diversity

Percentage of polymorphic loci ($P\%$) and the Nei (H_E) and Shannon (I) indices for each population were significantly correlated (Table 3, $P\%$ vs. H_E : $r = 0.89$; H_E vs. I : $r = 0.91$; all $P < 0.001$). Based on 95% confidence intervals, populations South Golan (SG) and Rimmonim (RM) contained significantly

higher than average diversities (H_E), whereas diversity within population Um Zuka (UM) was significantly lower. No significant relationship was detected by regressing H_E on either decimal-transformed latitude ($df = 8$, $r^2 = 0.0001$, $P = 0.935$) or the logarithm of approximate population size ($df = 8$, $r^2 = 0.255$, $P = 0.166$). Although there was a positive relationship between H_E and the number of analysed plants per population ($df = 8$, $r^2 = 0.534$, $P = 0.025$), this was entirely due to population UM in which small population size (14 putative individuals in 2000) limited possible sampling. Thus, after excluding UM, this relationship proved to be nonsignificant ($df = 7$, $r^2 = 0.319$, $P = 0.144$).

Among-population differentiation

Based on the Monte Carlo approximation of Fisher's exact test, most population pairs were significantly differentiated on the basis of marker frequencies ($P \leq 0.002$ in 32 of 36 cases). Only weak or nonsignificant differentiation was observed between various populations in close proximity, including Rimmonim RM and Qubet Najme QN (separated by 2 km; $P = 0.031$), Rimmonim RM and Mikhmas MK (6 km; $P = 0.490$), and Tel Arad TA and Wadi Mar'it MA (5 km; $P = 0.628$). Rather unexpectedly, there was no significant differentiation between the geographically distant populations Rimmonim RM and Tel Arad TA (72 km; $P = 0.359$).

The overall G_{ST} value based on allele frequencies was 0.18, corresponding to an $N_e m$ value of 1.14. Corresponding estimates based on marker frequencies resulted in only a slight increase/decrease ($G_{ST} = 0.21$; $N_e m = 0.94$). In fact, pairwise G_{ST} values among populations, as calculated on either allele or marker frequencies, were highly correlated ($r = 0.97$; $P < 0.001$), with a mean absolute difference of only 0.02 (range: 0.0032–0.03). This suggests a very low sensitivity of G_{ST} and gene flow estimates to assumptions about F_{IS} and Hardy–Weinberg conditions in the populations under study.

Hierarchical analyses

In order to gain more insights into how much of the genetic variance within the *haynei/atrofusca* data set can be attributed to differences among morphotypes (viz. regions), hierarchical *F*-statistics were calculated based on G_{ST} using POPGENE. These analyses showed that only a minor part of genetic variation was found among morphotypes (allele-frequency-based: 4.4%), and that there was considerably less genetic variation partitioned among populations within morphotypes (14.1%) than within populations (81.5%). Analysis of molecular variance (AMOVA, Excoffier *et al.* 1992), with calculations conducted on entire RAPD fragment profiles (Schneider *et al.* 1997), resulted in comparable *F*-fixation indices ($F_{CT} = 0.051$, $F_{SC} = 0.136$, $1 - F_{ST} = 0.820$), all of which were significant at the 0.1% level (after performing 10 000 replications). Thus, despite the low magnitude of the morphotype-to-total component, there are differences in allele frequencies between northern (*haynei*) and central/southern (*atrofusca*) populations that contribute significantly to the genetic variance across the data set.

Patterns of isolation by distance

The regression of $\log(N_m)$ on $\log(\text{km})$ for all pairwise comparisons among the nine *haynei/atrofusca* populations indicated a nonsignificant ($P = 0.086$) pattern of isolation by distance (allele frequency-based: $y = 0.462 - 0.085x$, $r^2 = 0.08$, $P = 0.086$). Observations that largely contributed to a less than perfect fit involved one instance with lower than expected levels of gene flow between proximate sites (UM/QN), and one comparison with relatively high levels of gene flow between distant sites (RM/TA). Removal of those comparisons from the data set resulted in a weak but significant ($y = 0.478 - 0.094x$, $r^2 = 0.13$, $P = 0.037$) isolation by distance pattern. Similar relationships were detected when N_m values were derived from marker frequencies (data not shown).

Genetic distance analysis

The UPGMA phenograms based on Nei's (1978) unbiased genetic distances between all pairwise combinations of populations (including *I. lortetii*) revealed different patterns of relationships with the two alternative ways of analysis employed. Based on allele frequencies, *haynei* and *atrofusca* appeared to constitute two different units, with two major clusters corresponding strictly to their taxonomic status (Fig. 4a). Moreover, the way populations of *haynei* and *atrofusca* tended to cluster was independent of their geographical origin, and there was no significant association between genetic and geographical distances as shown by Mantel's test ($r_M = 0.242$; $P = 0.163$). In contrast,

by calculating genetic distances on marker frequencies, there was no genetic subdivision among designated populations of *haynei* and *atrofusca* that corresponded to their taxonomy (Fig. 4b). Instead, the majority of these populations tended to cluster (at higher genetic distances) according to geography, despite a lack of significant association between genetic and geographical distance ($r_M = 0.311$; $P = 0.097$). RAPD divergence among populations of *haynei/atrofusca* was either weakly or significantly associated with their taxonomic distances, depending on the genetic distance estimate employed (allele-frequency based: $r_M = 0.289$, $P = 0.054$; marker-frequency based: $r_M = 0.347$, $P = 0.035$). No association was found between RAPD divergence and environmental (aridity) distance (allele-frequency based: $r_M = 0.007$, $P = 0.487$; marker-frequency based: $r_M = -0.015$, $P = 0.348$).

Overall, and despite the low bootstrap values generally observed, both phenograms supported the distinctness of *haynei/atrofusca* when compared with *I. lortetii*, and the genetic coherence of a subset of central-southern populations (RM, MK, TQ, TA, MA) that always were part of a terminal cluster (Figs 4a,b).

Discussion

Intrapopulational genetic diversity

In the nine *Iris haynei/atrofusca* populations studied for RAPD variation the mean within-population diversity was 0.388 for Shannon's index (*I*) and 0.258 for Nei's expected heterozygosity (H_E), and the mean proportion of loci polymorphic ($P\%$) was 0.742. A recent review of the RAPD literature (Nybom & Bartish 2000) indicates that *haynei/atrofusca* exhibit higher levels of intrapopulational diversity than those commonly found in other plant species (mean 0.214 ± 0.177 SD based on various estimates of H). Despite the wide range of values reported, the H_E estimate for *haynei/atrofusca* is higher than the mean values for monocots (0.191) and geographically restricted species (0.191) but is similar to the average values reported for long-lived perennials (0.242) and outcrossers (0.260; Nybom & Bartish 2000). With respect to previous isozyme analyses performed in *Iris*, the level of genetic diversity for *haynei/atrofusca* is comparable with that found in the widespread and self-compatible *I. cristata* ($H_E = 0.231$; Hannan & Orick 2000) but is generally higher than in four outbred species of *Iris* sect. *Iris* (mean $H_O = 0.199$; Tusic *et al.* 1984). In summary, the level of within-population RAPD diversity maintained by *haynei/atrofusca* is somewhat surprising, particularly if one considers their narrow geographical distribution. However, unexpectedly high levels of RAPD diversity have also been reported in the allogamous *Allium aaseae*, another monocot species with life history and geographical traits similar to the *Iris* taxa studied here ($H = 0.274$, $P\% = 0.522\text{--}0.672$; Smith & Pham

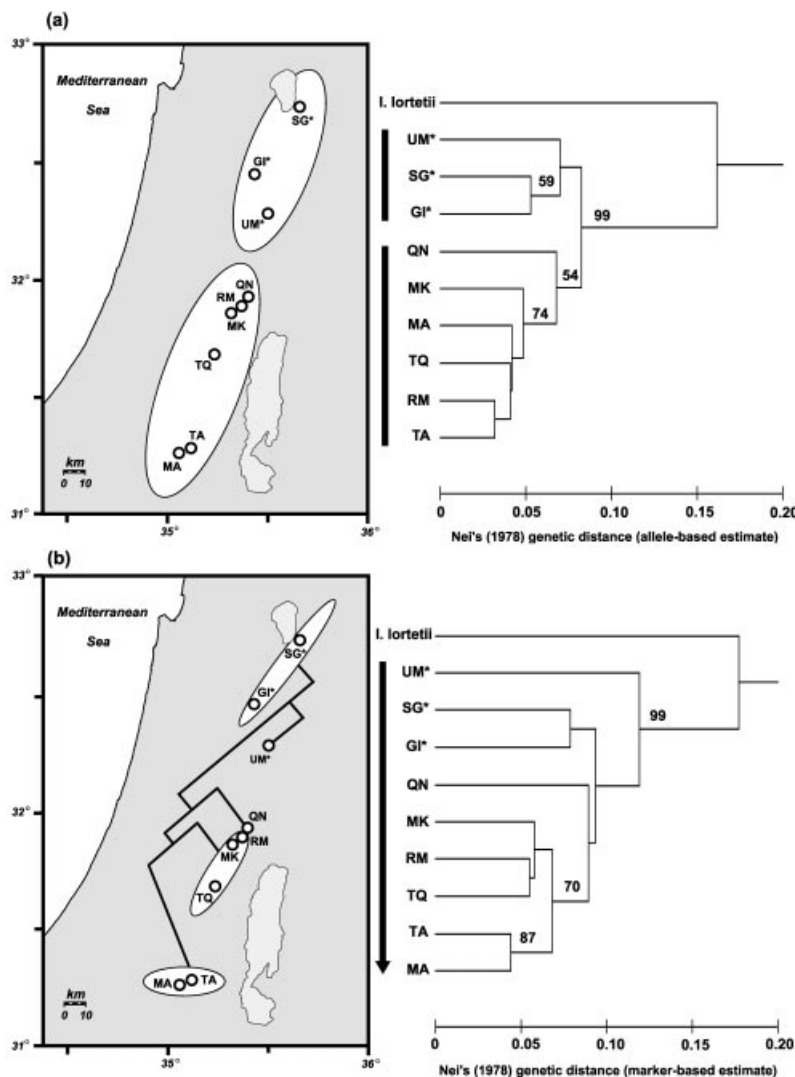


Fig. 4 Two alternative UPGMA phenograms based on Nei's (1978) unbiased genetic distances for nine populations of *Iris haynei* (marked with asterisks) and *I. atrofusca*, plus the outgroup population (*I. lortetii*), with per cent bootstrap replication scores > 50% indicated. Distances were calculated based on (a) allele or (b) marker frequencies. Major patterns of genetic relationships among populations, as inferred from both analyses, are superimposed on the distribution map of populations (see Table 1 for locality abbreviations).

1996). A similar pattern is seen in other nonmonocot species restricted in range (e.g. *Cardamine*: Nolan *et al.* 1996; *Erodium*: Martín *et al.* 1997).

Intrapopulation substructuring or pooling of subpopulations that differ in genetic composition may have contributed to the unexpectedly high intrapopulation variation found in *haynei/atrofusca*. However, because calculations were based on nearly all samples present within a population (UM, MK), or samples spaced at ≈ 5 -m intervals, it is unlikely that high genetic diversity is due to sampling artefacts. The observation that no identical RAPD phenotypes were found under this sampling scheme suggests limited clonal spread by means of rhizomes and that most separate pieces (ramets) of a given genotype (genet) are restricted to this spatial scale. Nonetheless, these observations demand more detailed investigations of the spatial association of genotypes within populations.

We did not find a significant relationship between genetic diversity and the logarithm of population size, and

only the small Um Zuka (UM) population from the Upper Jordan Valley stood out as a genetically depauperate population (see also Table 3). From this result, relatively small populations, such as MK, MA and QN, appear to maintain (nearly) as high levels of diversity as larger populations. Similar patterns were observed, e.g. in *Brassica oleracea* (Lannér-Herrera *et al.* 1996) and *Gypsophila fastigiata* (Prentice & White 1988), whereas in several other instances, a positive relationship between these variables was found (Ellstrand & Elam 1993; Fischer & Matthies 1998). Thus, contrary to theoretical expectations (Wright 1931), there are no guidelines about small population size and reduced levels of genetic diversity in plants (see also Levin 2000; further references therein).

The maintenance of genetic diversity, once produced, within populations of *haynei/atrofusca*, might be promoted by their perennial life form (with overlapping generations) and a rhizomatous growth habit. In the short run, these characteristics may protect these populations from loss of

genetic variability due to demographic–stochastic perturbations and associated genetic drift (Persson *et al.* 1998; Arnold 2000). Furthermore, as the estimates of intrapopulation diversity reported here would classify *haynei/atrofusca* as highly outcrossed, this would further promote genetic diversity within populations. From a historical perspective, these data are also suggestive of a long-term stability of these *Iris* populations, i.e. the absence of repeated episodes of local population extinction and recolonization.

Among-population genetic differentiation

According to the Monte Carlo approximation of Fisher's exact test, most populations of *haynei/atrofusca* differed significantly from each other, suggesting that long-term isolation and/or habitat fragmentation may have influenced genetic processes (Young *et al.* 1996). However, we must be careful with this statistical analysis because, for diploid/dominant data sets, this approximation can only be performed on marker frequencies (Miller 1997), and thus may lead to an overestimate of population differentiation. Nonetheless, G_{ST} values (and associated estimates of $N_e m$) differed only slightly when either allele or marker-based approaches were employed. Such a finding that population genetic structure as calculated from dominant markers is relatively insensitive to assumptions about HWE, heterozygosity, and levels of inbreeding (F_{IS}) within populations provides confidence in our estimates. Similar results were recently reported for both RAPD and (dominant) AFLP markers (Gaudeul *et al.* 2000; Vucetich *et al.* 2001).

Our range-wide estimates of G_{ST} indicate that a relatively large amount ($\approx 20\%$) of the genetic diversity observed in the nine *haynei/atrofusca* populations is apportioned among populations [Nei (1978) classified $G_{ST} < 0.05$ low, $0.05–0.15$ medium, > 0.15 high]. This also implies that there is only low to moderate gene flow among populations ($N_e m \approx 1$), according to the criteria outlined by Slatkin (1993). In context, this G_{ST} (and gene flow) estimate is of the same order of magnitude frequently reported in both the isozyme and RAPD literature for plant species that are insect-pollinated and outcrossing (Hamrick & Godt 1990; Nybom & Bartish 2000). However, a small, but statistically significant, amount of RAPD variation ($\approx 5\%$) was attributable to regional (or 'specific') differences among northern (*haynei*) vs. central–southern (*atrofusca*) populations, as measured by hierarchical F -statistics and AMOVA. Therefore, the range-wide G_{ST} value reported here is likely to be an overestimate. In fact, without the influence of regional/taxon substructure, $\approx 15\%$ of the variation was due to differences among populations. This translates into an effective level of gene flow ($N_e m \approx 1.42$) above the level ($N_e m \approx 1$) needed to prevent populations from diverging purely by genetic drift (Wright 1931; Slatkin 1985). Moreover, pairwise comparisons of $N_e m$ values among populations (total

range: 1.04–3.76) also suggest that the number of migrants exchanged every generation gives considerable scope for counteracting drift. We note that such a progressive increase in $N_e m$ at smaller spatial scales is expected when contributions to gene flow by regional patterns of genetic differentiation are eliminated (Husband & Barrett 1995).

When considering the overall distribution of *haynei/atrofusca* in Israel and the West Bank, there was no significant relationship between $\log(N_e m)$ and $\log(\text{km})$. This relationship, however, became significant after a small number of population comparisons (i.e. UM–QN; RM–TA) had been removed from the data set. Overall, we suggest that these data are in agreement with an *approximate* isolation by distance (or stepping stone) model with gene flow being largely restricted to adjacent populations (Wright 1943; Kimura & Weiss 1964). From a metapopulation perspective, the populations of *haynei/atrofusca* can be seen as forming a network of interconnected demes linked by moderate levels of gene flow. However, it is difficult to decide whether these demes either approach or deviate from equilibrium between genetic drift and gene flow (Slatkin 1993; see also below).

Notably, the mean geographical distance between populations of *haynei/atrofusca* is only 68.5 km, ranging from 2 to ≈ 170 km (Fig. 1). Finding approximate isolation by distance on such a relatively small geographical range scale is likely due to a conjunction of limited long-distance dispersal capabilities of our study system and its linear habitat distribution. Based on extensive field observations (Y. Sapir, unpublished data), *haynei* and *atrofusca* are almost exclusively pollinated by male solitary bees of the genera *Eucera* and *Synhalonia* (Apoidea: Anthophoridae/Eucerini) which visit the nectarless flowers shortly before sunset in order to find overnight shelter. By contrast, observations during daytime revealed only very rare and episodic visits (Y. Sapir, unpublished data). Therefore, gene flow via pollen is expected to be highly restricted by low pollinator visitation rates, and further limited by the distance between populations. Seed dispersal is probably similarly restricted as the dark, thick-coated seeds ($\approx 0.045–0.075$ g; Y. Sapir, unpublished data) are dispersed mainly by gravity or, owing to the presence of a large, creamy-white elaiosome, by ants. As a result, contemporary gene flow is probably limited to nearest neighbouring populations, and this is further supported by our data. In only one instance long-distance gene exchange may (have) occurred, i.e. between populations at Rimmonim (RM) and Tel Arad (TA), separated by 72 km. This hypothesis is supported by both their lack of significant differences in marker frequencies (see Results), and their *greater* than expected $\log N_e m$ value, as inferred from the isolation by distance analysis (data not shown). Hence, long-distance dispersal is not sufficiently common to prevent isolation by distance from being observed.

The close association of *haynei/atrofusca* populations with a narrow strip of steppic (Mediterranean–desert) vegetation along the eastern slopes of the Central Mountain Range may further promote a pattern of gene flow which is best described by an isolation by distance model. Similar migration patterns, albeit at lower altitudes along the Rift Valley System have recently been observed in *Senecio glaucus* (Comes & Abbott 1999), an annual species with mainly wind-dispersed seed. Overall, this emphasizes the possible role of the Central Mountain Range/Rift Valley topography in restricting the availability of routes for gene dispersal in plant populations, independent of their ecological preferences and/or dispersal capabilities. At the same time, these findings underline the importance of this linearized regional landscape in serving as a major corridor for gene exchange.

Historical considerations

Today, the vast majority of the ≈ 65 described binomials of *Iris* sect. *Oncocyclus* is widely distributed throughout the Fertile Crescent of southwestern Asia (Avishai 1979; Avishai & Zohary 1980). Although nothing is known about the phylogenetic relationships and historical biogeography of these taxa, there is still reason to believe that populations of *haynei* and *atrofusca* in Israel and the West Bank may have colonized their actual distribution range quite recently. Zohary (1973) proposed that southwest Asian steppe forest species could have expanded into the Near East during the hot and dry interpluvial cycles of the Pleistocene (Horowitz 1979). Among these invading species, Zohary (1973) also included the Irano-Turanian elements *Pistacia* and *Retama*, both of which are closely associated with *haynei* and *atrofusca* (see Material and Methods). Moreover, during the Holocene, when human activities of perturbation increased in the Near East, this pattern of Irano-Turanian immigration may have intensified (Blondel & Aronson 1999).

Consistent with such a scenario of unidirectional range expansion, one of the UPGMA phenograms shows a striking pattern of sequential relationships among populations from north to south (Fig. 4b, marker-frequency based approach). A direct interpretation would be that the northern populations (*haynei*) are likely the source populations from which the central/southern (*atrofusca*) populations descended. Given restricted seed dispersal (see above), colonization might have involved successive, but rare, long-distance dispersal events and subsequent foundations beyond a compact wave of advance rather than a massive migration wave. Also, time might have been insufficient for a strong pattern of isolation by distance to arise, with populations currently approaching, rather than deviating from, equilibrium. Regardless, under a neutral model, the expansion scenario predicts a reduction in genetic diversity with increasing distance from the source (i.e. the

north), as migrating populations are affected by repeated stochastic events at the advancing edge (Ibrahim *et al.* 1996; Le Corre *et al.* 1997). However, the genetic diversity analysis provides no evidence that these populations passed through severe bottlenecks because high levels of diversity were found in nearly all the populations studied.

An alternative, and probably more likely, explanation to the approximate isolation by distance pattern is that populations are currently deviating from an equilibrium due to the build-up of a hierarchical structure (Slatkin 1993; Husband & Barrett 1995). Northern and central/southern populations tend to form two separate clusters based on the UPGMA phenogram calculated from allele frequencies (Fig. 4a), and this is strikingly concordant with the overall pattern of population clustering based on taxonomic distances (Fig. 3). Although not in conflict with a more ancient immigration scenario (see above), it seems likely that the two groups of populations are currently diverging from each other via habitat fragmentation of a single and widespread ancestral population. The small interregional/taxon component in the AMOVA ($\approx 5\%$) and the near lack of alleles 'specific' for each group (at 3 of 132 loci examined) may attest to the initial state of this process. In support of this hypothesis, the average intercluster genetic distance (Nei 1978) between northern vs. central/southern populations is relatively small (allele-based estimate: 0.087 ± 0.021 SD; marker-based estimate: 0.110 ± 0.021 SD), and typical for conspecific populations of flowering plant species (Crawford 1983). We therefore conclude that *haynei/atrofusca* provide an example of primary intergradation along the Central Mountain Range, i.e. having diverged within a continuous population, rather than two 'species' forming a secondary contact zone in this region after divergence in allopatry.

Further support for a primary divergence scenario comes from two key populations at the *haynei/atrofusca* range boundary, i.e. Um Zuka UM (*haynei*) and Qubet Najme QN (*atrofusca*) (Fig. 1). Rather than exhibiting high levels of diversity and/or excessive intermorph gene flow, as might be expected under a secondary contact hypothesis (Alexandrino *et al.* 2000), these populations: (i) contain average (QN) or significantly lower than average (UM) levels of RAPD diversity, as measured by H_E (Table 3); and (ii) exchange the smallest number of migrants ($N_e m \approx 1$), when compared with the rest of the populations. Accordingly, it is here, i.e. across a region ≈ 42 km wide and from which there are no records of *haynei* or *atrofusca* (Fig. 1), that the evolutionary consequences of limited gene exchange will be most pronounced. Unfortunately, the factors responsible for this limited gene exchange remain unclear. The presence of a deep, narrow and steep-sided valley (Nahal Tirza/Wadi Fari'a) in the area separating UM and QN might play a role in acting as a topographic barrier to gene flow, but also the small size of the UM

population *per se*, in conjunction with its low altitude (70 m), could be important in affecting gene flow properties. Despite the apparent homogeneity of the UM–QN environment in terms of overall vegetation structure (semisteppe), rainfall parameters (250 vs. 330 mm/year) and soil type (grey redzina on chalk and marl), there is a rather pronounced transition in morphological traits between these populations as suggested by the UPGMA phenogram (Fig. 3). However, as inferred gene dispersal is not great in the UM–QN area, as registered by RAPD markers, it is tempting to speculate that only low levels of selection on phenotypic fitness traits are needed to produce a fairly narrow (primary) hybrid zone in this area (Hewitt 1988).

Rather than considering a limited part of the *haynei*/*atrofusca* range, morphological relationships between populations are significantly correlated with geographical distance, and, ambiguously, with aridity also. Taken together, our RAPD and morphometric data may indicate that: (i) primary divergence between *atrofusca* and *haynei* is, in large part, induced by restricted gene flow in the UM–QN area; whereas (ii) natural selection by geographically varying environmental conditions is driving floral and vegetative evolution of the two morphotypes at the range-wide scale. This latter hypothesis, however, has to be treated with caution as patterns of seemingly adaptive differentiation might also reflect phenotypic plasticity. To address this issue, we are currently investigating the relative contribution of genetically determined variation to the phenotypic differences between *haynei* and *atrofusca* populations with reciprocal transplantations of clones to different environments (Y. Sapir, Jerusalem University; O. Fragman, Mainz University).

Conservation implications

This is the first report of the partitioning of genetic variability within and between populations of royal irises. High genetic diversity was found within populations of *haynei*/*atrofusca*, despite their disjunct distribution and often small population size. Under the presumption that RAPD variability detected within populations is an indirect indicator of overall genetic variability, most of these *Iris* populations seem to obtain a fairly comfortable status with respect to long-term viability. However, if populations have only recently experienced a large reduction in size (perhaps due to intensified grazing pressure), then the effects of isolation, genetic drift and elevated inbreeding may not have become manifested in contemporary levels of genetic diversity (Levin 2000). As a consequence, more detailed field, experimental and genetic work is required to assess the performance of those populations that are currently very small and thus prone to extinction (i.e. UM, MK, MA). Here, it would be of value to

carefully monitor reproductive success and pollination levels in relation to population size, along with reliable estimates of within-population structure and inbreeding (F_{IS}), derived from codominant markers.

The absence of deep population genetic subdivision and the evidence for primary intergradation among northern (*haynei*) vs. central/southern (*atrofusca*) populations would argue for a revision of the traditionally held view of these royal irises as fully diverged 'species'. Although our morphometric data substantiate this claim, they nonetheless support the existence of two geographically differentiated sets of populations, which then could be viewed as subspecies, still warranting conservation attention as separate management units (Moritz 1994). For example, with the population genetic structure reported herein, a suitable strategy for sampling, propagation and reintroduction may be formulated when *ex situ* conservation measures are required. However, conservation measures, which intend to increase gene exchange across the UM–QN range boundary or among geographically distant populations (e.g. via field transplantation or human-assisted pollination), should be avoided. This is to approximate natural and evolutionary processes in a network of interconnected demes of royal irises, which are likely adapted to an environmental aridity gradient, and which can be seen as an instructive example of incipient species formation.

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References

- Alexandrino J, Froufe E, Arntzen JW, Ferrand N (2000) Genetic subdivision, glacial refugia and postglacial recolonization in the golden-striped salamander, *Chioglossa lusitanica* (Amphibia: Urodela). *Molecular Ecology*, **9**, 771–781.
- Arnold M (2000) Anderson's paradigm: Louisiana irises and the study of evolutionary phenomena. *Molecular Ecology*, **9**, 1687–1698.
- Auerbach M, Shmida A (1985) Harmony among endemic littoral plants and adjacent floras in Israel. *Journal of Biogeography*, **12**, 175–187.
- Avishai M (1977) *Species relationships and cytogenetic affinities in section Oncocyclus of the genus Iris*. PhD Thesis, The Hebrew University of Jerusalem, Israel.
- Avishai M (1979) *Irises in Eretz Israel*. Society for the Protection of Nature in Israel, Tel Aviv (in Hebrew).

- Avishai M, Zohary D (1977) Chromosomes in the *Oncocylus* irises. *Botanical Gazette*, **138**, 502–511.
- Avishai M, Zohary D (1980) Genetic affinities among the *Oncocylus* irises. *Botanical Gazette*, **141**, 107–115.
- Blondel J, Aronson J (1999) *Biology and Wildlife of the Mediterranean Region*. Oxford University Press, Oxford.
- Brauner S, Crawford DJ, Stuessy TF (1992) Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. *American Journal of Botany*, **79**, 1436–1439.
- Bussell JD (1999) The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea*. *Molecular Ecology*, **8**, 775–789.
- Comes HP, Abbott RJ (1999) Population genetic structure and gene flow across arid versus mesic environments: a comparative study of two parapatric *Senecio* species from the Near East. *Evolution*, **53**, 36–54.
- Crawford DJ (1983) Phylogenetic and systematic inferences from electrophoretic studies. In: *Isozymes in Plant Genetics and Breeding* (eds Tanksley SD, Orton TJ), pp. 257–287. Elsevier, Amsterdam.
- Danin A, Plitmann U (1987) Revision of plant geographical territories of Israel and Sinai. *Plant Systematics and Evolution*, **150**, 43–53.
- Dawson IK, Chalmers KJ, Waugh R, Powell W (1993) Detection and analysis of genetic variation in *Hordeum spontaneum*: populations from Israel using RAPD markers. *Molecular Ecology*, **2**, 151–159.
- Dykes WR (1913) *The Genus Iris*. Dover Publications, New York.
- Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics*, **24**, 217–242.
- Endler J (1977) *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton, NJ.
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **86**, 991–1000.
- Feinbrun-Dothan N (1986) Flora Palaestina, 4, *Iris* L. In: *Flora Palaestina* (eds Zohary M, Feinbrun-Dothan N), pp. 114–127. Israel Academy of Sciences and Humanities, Jerusalem.
- Fischer M, Matthies D (1998) RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica*. *American Journal of Botany*, **85**, 811–819.
- Fragman O, Plitmann U, Heller D, Shmida A (1999) *Checklist and Ecological Data Base of the Flora of Israel and its Surroundings*. Mifalot 'Yeffe No' & The Middle East Nature Conservation Association, Jerusalem (in Hebrew).
- Fragman O, Shamir Z, Rabinowitz A, Leschner H, Shmida A (1995) *The Rare Plants of the Acco Plain and Mt. Gilboa – Survey Guide*. Rotem (N.R.A. & S.P.N.I.), Jerusalem (in Hebrew).
- Gaudeul M, Taberlet P, Till-Bottraud I (2000) Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology*, **9**, 1625–1637.
- Gavrilet S, Li H, Vose MD (2000) Patterns of parapatric speciation. *Evolution*, **54**, 1126–1134.
- Gilpin ME, Soule ME (1986) Minimum viable populations: processes of species extinction. In: *Conservation Biology: The Science of Scarcity and Diversity* (ed. Soule ME), pp. 19–34. Sinauer, Sunderland, MA.
- Gitzendanner MA, Soltis PS (2000) Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany*, **87**, 783–792.
- Hamrick JL, Godt MJW (1990) Allozyme diversity in plant species. In: *Plant Population Genetics, Breeding, and Genetic Resources* (eds Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 43–63. Sinauer, Sunderland, MA.
- Hannan GL, Orick MW (2000) Isozyme diversity in *Iris cristata* and the threatened glacial endemic *I. lacustris* (Iridaceae). *American Journal of Botany*, **87**, 293–301.
- Hewitt G (1988) Hybrid zones – natural laboratories for evolutionary studies. *Trends in Ecology and Evolution*, **3**, 158–167.
- Horowitz A (1979) *The Quaternary of Israel*. Academic Press, New York.
- Husband BC, Barrett SCH (1995) Estimates of gene flow in *Eichhornia paniculata* (Pontederiaceae): effects of range substructure. *Heredity*, **75**, 549–560.
- Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.
- IUCN (1994) *IUCN Red List Categories: Prepared by the IUCN Species Survival Commission*. Island Press, Washington, DC.
- Ivri Y, Izikovitz D (1988) The glorious of the royal irises – observations in *Iris lortetii* pollination. *Teva-Va'arets*, **30**, 5–9 (in Hebrew).
- Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.
- Lannér-Herrera C, Gustafsson M, Fält AS, Bryngelsson T (1996) Diversity in natural populations of wild *Brassica oleracea* as estimated by isozyme and RAPD analysis. *Genetic Resources and Crop Evolution*, **43**, 13–23.
- Le Corre V, Machon N, Petit RJ, Kremer A (1997) Colonization with long-distance seed dispersal and genetic structure of maternally inherited genes in forest trees: a simulation study. *Genetical Research, Cambridge*, **69**, 117–125.
- Levin DA (2000) *The Origin, Expansion, and Demise of Plant Species*. Oxford University Press, New York.
- Lewontin RC (1972) The apportionment of human diversity. *Evolutionary Biology*, **6**, 381–394.
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- Martin C, González-Benito ME, Iriondo JM (1997) Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. & Izco. *Molecular Ecology*, **6**, 813–820.
- Médail F, Quézel P (1997) Hot-spots analysis for conservation of plant biodiversity in the Mediterranean basin. *Annals of the Missouri Botanical Garden*, **84**, 112–127.
- Mendlinger S, Zohary D (1995) The extent and structure of genetic variation in species of the Sitopsis group of *Aegilops*. *Heredity*, **74**, 616–627.
- Miller MP (1997) *Tools for Population Genetic Analyses (TFPGA), Version 1.3*. Department of Biological Sciences, Northern Arizona University, Flagstaff.
- Moritz C (1994) Defining 'evolutionary significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 583–590.
- Nei M (1972) Genetic distance between populations. *American Naturalist*, **106**, 283–292.
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

- Nevo E, Baum B, Beiles A, Johnson DA (1998) Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. *Genetic Resources and Crop Evolution*, **45**, 151–159.
- Nevo E, Golenberg E, Beiles A, Brown AHD, Zohary D (1982) Genetic diversity and environmental associations of wild wheat, *Triticum dicoccoides*, in Israel. *Theoretical and Applied Genetics*, **62**, 241–254.
- Nevo E, Zohary D, Brown AHD, Haber M (1979) Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Israel. *Evolution*, **33**, 815–833.
- Nolan MF, Skotnicki ML, Gibbs AJ (1996) RAPD variation in populations of *Cardamine liliacina* (Brassicaceae). *Australian Systematic Botany*, **9**, 291–299.
- Nyblom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 93–164.
- Persson HA, Lundquist K, Nyblom H (1998) RAPD analysis of genetic variation within and among populations of Turk's-cap lily (*Lilium martagon* L.). *Hereditas*, **128**, 213–220.
- Prentice HC, White RJ (1988) Variability, population size and isolation: the structuring of diversity in Öland *Gypsophila fastigiata*. *Acta Oecologica/Oecologica Plantarum*, **9**, 19–29.
- Raymond ML, Rousset F (1995) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.
- Rohlf FJ (1998) *NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System, Version 2.02g*. Applied Biostatistics Inc, Exeter Software, Setauket, NY.
- Schneider S, Kueffer JM, Roessli D, Excoffier L (1997) *ARLEQUIN, Version 1.1: A Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Shimshi D (1979/80) Two ecotypes of *Iris atrofusca* Bak. and their relations to man-modified habitats. *Israel Journal of Botany*, **28**, 80–86.
- Shmida A (1984) Endemism in the flora of Israel. *Botanische Jahrbücher für Systematik*, **104**, 537–567.
- Shmida A, Fragman O, Nathan R, Shamir Z (1999) The red plants of Israel: a proposed update to the list of protected wild plants of Israel. *Ecology and Environment*, **4**, 251–165 (in Hebrew).
- Shmida A, Ivri Y (1996) Royal irises: lodging for male bees. *Teva-Hadvarim*, **14**, 32–49 (in Hebrew).
- Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.
- Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.
- Smith JF, Pham TV (1996) Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). *American Journal of Botany*, **83**, 717–726.
- Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edn. W.H. Freeman, New York.
- Travis SE, Maschinski J, Keim P (1996) An analysis of genetic variation in *Astragalus cremonophyllax* var. *cremonophyllax*, a critically endangered plant, using AFLP markers. *Molecular Ecology*, **5**, 735–745.
- Tucic B, Rak-Soltes E, Tucic N (1984) Isozyme variability in the *Iris* species. *Arhiv Bioloskih Nauka, Beograd*, **36**, 1–12.
- Utelli AB, Roy BA, Baltisberger M (1999) History can be more important than 'pollination syndrome' in determining the genetic structure of plant populations: the case of *Aconitum lycoctonum* (Ranunculaceae). *Heredity*, **82**, 574–584.
- Vucetich LM, Vucetich JA, Joshi CP, Waite TA, Peterson RO (2001) Genetic (RAPD) diversity in *Peromyscus maniculatus* populations in a naturally fragmented landscape. *Molecular Ecology*, **10**, 35–40.
- Wolff K, El-Akkad S, Abbott RJ (1999) Population substructure in *Alkana orientalis* (Boraginaceae) in the Sinai Desert, in relation to its pollinator behaviour. *Molecular Ecology*, **6**, 365–372.
- Woodruff DS (2001) Populations, species and conservation genetics. In: *Encyclopedia of Biodiversity*, Vol. 4 (ed. Levin SA), pp. 811–829. Academic Press, London.
- Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.
- Wright S (1943) Isolation by distance. *Genetics*, **28**, 114–138.
- Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.
- Yeh F, Yang RC, Boyle T *et al.* (1997) *POPGENE. The User-Friendly Shareware for Population Genetic Analysis*. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton.
- Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation in plants. *Trends in Ecology and Evolution*, **11**, 413–418.
- Zohary M (1973) *Geobotanical Foundations of the Middle East*. Gustav Fischer Verlag, Stuttgart.
- Zohary M, Feinbrun-Dothan N (1966–86) *Flora Palaestina*. 4 Volumes. Israel Academy of Sciences and Humanities, Jerusalem.

This study was conducted as part of a collaborative project reflecting the interests of the authors to gain a better understanding of evolutionary relationships, genetic variation, and reproductive biology in royal irises. Rami Arafeh, a PhD candidate, conducted the RAPD survey during his stay at Mainz University. The morphometric study forms part of Yuval Sapir's doctoral work, which in particular focuses on the pollination biology of the royal irises. Ori Fragman is a botanist interested in the ecology and evolution of bulbous plants and the construction of databases for the eastern Mediterranean flora. Professor Avi Shmida's research programme focuses on pollination ecology, plant conservation, and the Israel Flora Ecological Data Base (ROTEM). Professor Naim Iraki is involved in the application of molecular approaches to the study of plant pathogens and the determination of wild plant collections for mass propagation via tissue culture. Dr Hans Peter Comes is a research scientist interested in the molecular biogeography and phylogeography of Mediterranean and European high mountain plants.
