

Local adaptation in four *Iris* species tested in a common-garden experiment

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Local adaptation is a commonly observed result of natural selection acting in heterogeneous environment. Common-garden experiments are a method of detecting local adaptation, as well as studying phenotypic plasticity and gradients of traits. The present study aimed to analyse reaction norms of four closely-related *Iris* species of section *Oncocyclus* and to identify a role of environmentally-specific natural selection in their plastic responses. The plant vegetative and phenological, as well as performance traits were measured in a full factorial common-garden experiment with three levels of water amount and three soil types. We found a significant effect of species identity on all traits measured. Water amount and soil type affected many of the traits, but soil type did not affect the performance. There was no significant difference in the effect of water amount and soil type on performance as reflected by rhizome growth; in other words, there was no significant genotype \times environment interaction for performance. Plasticity levels and directions of response were also similar among the species. We conclude that phenotypic differences among species are of genetic origin, although no adaptive value was demonstrated for them at the time and life-stages ‘frame’ of this experiment. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society* 2009, 98, 267–277.

ADDITIONAL KEYWORDS: genotype by environment interaction – natural selection – *Oncocyclus* – phenotypic plasticity – reaction norm.

INTRODUCTION

The geographical range that a species occupies is the result of both selective and nonselective evolutionary forces. Local adaptation is a result of natural selection, which, by acting on genotypes in different environmental settings, creates adaptive genetic differentiation. Different populations then evolve different trait values, which provides them with a fitness advantage in their native environment, which is evident as a genotype \times environment interaction for fitness (Kawecki & Ebert, 2004).

However, local adaptation of populations or related species is not always the evolutionary outcome as a result of several factors that limit natural selection

(Barton & Partridge, 2000). Gene flow, which is related primarily to seed and pollen dispersal in plants, has a homogenizing effect on genetic variation, preventing the differentiation of populations (Lenormand, 2002; Goldberg & Lande, 2007). Another two factors comprise genetic drift and mutations, which can overwhelm differences in selection and confound the possibility of local adaptation, or even lead to foreign genotype advantage (Hereford & Winn, 2008).

There are other reasons for a lack of local adaptation. One is temporal variation in selection, which can favour a generalist with high phenotypic plasticity (Gomulkiewicz & Kirkpatrick, 1992). Therefore, local adaptation requires costs or limits on phenotypic plasticity: the cost of maintaining the genetic and cellular machinery necessary to be plastic (Scheiner, 1993). This provides a fitness advantage for a genotype in some, but not all environments, although these costs are difficult to demonstrate (DeWitt, Sih &

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Wilson, 1998; Van Kleunen & Fischer, 2001; Caruso, Maherali & Sherrard, 2006; Volis, 2009; but see also Bell & Galloway, 2008).

Another prerequisite for local adaptation is the presence of sufficient genetic variation on which selection can act (Houle, 1992). Models predict that the extent and structure of genetic variation within a species may affect the evolutionary trajectory and the equilibrium of its reaction norm (Gomulkiewicz & Kirkpatrick, 1992) and therefore the possibility of local adaptation to evolve.

Detecting local adaptation is performed experimentally by comparing the reaction norm (of performance or fitness) of genotypes across environments (Pigliucci, 2001). There are two main types of experiment (Kawecki & Ebert, 2004). One comprises a reciprocal transplant, which is conducted in the field, with each genotype being tested in its own native habitat and the native habitats of the other genotypes. Usually, this is the best option because all properties of the habitats are present, but it is not always feasible as a result of logistical and conservation reasons. The other is a common-garden experiment, in which some properties of the environment are recreated in the laboratory or greenhouse (or in the field; Bell & Galloway, 2008) and all the genotypes are tested in different treatments of these properties. On the one hand, the results obtained may not reflect the situation in reality if some important properties of the environment are missing. On the other hand, a common-garden experiment enables specific hypotheses about the environmental properties and process of adaptation to be tested. Therefore, these two experimental approaches can be viewed as complementary (Pigliucci, 2001; Volis, Mendlinger & Ward, 2002a; Volis, Mendlinger & Ward, 2002b; Byars, Papst & Hoffmann, 2007).

Common-garden experiments have been efficiently used in analysis of inter- and intraspecific phenotypic variation (e.g. studies of intraspecific variation: Schluchting & Levin, 1990; Volis *et al.*, 2002b; Rutter & Fenster, 2007; Bell & Galloway, 2008; Suzuki, 2008; studies of interspecific variation: Zangerl & Bazzaz, 1984; Macdonald, Chinnappa & Reid, 1988; Wright & Westoby, 1999; Ackerly *et al.*, 2000; Caruso *et al.*, 2006).

The measurement of relative fitness of individuals, defined as 'the relative number of offspring contributed to the next generation by particular individuals or genotypes' (Primack & Kang, 1989), should be performed over the whole life-cycle of a plant. This is impractical in many cases, especially when the species in question is perennial. Instead, it is common to measure performance traits, such as seed germination, vegetative growth, probability of flowering, seed production, and survival. This can be conducted in the

laboratory and the measurements obtained can be used as a surrogate for fitness in studies of adaptation, even though they need to be complemented by measuring the relationship of the performance components to fitness (Arnold, 1983). This relation of performance and fitness is eminently logical; however, it has never been fully evaluated (Primack & Kang, 1989).

In the present study, we present the results obtained in an experiment aiming to test local adaptation and plasticity in four *Oncocyclus Iris* species. These are endangered perennial herbs that are among the important plants for conservation in Israel (Sapir, Shmida & Fragman, 2003; Shmida & Pollak, 2007). Despite a growing knowledge of the *Oncocyclus* irises (e.g. morphological: Shimshi, 1980; Sapir *et al.*, 2002; cytological: Avishai, 1977; genetic variation: Arafeh *et al.*, 2002; pollination biology: Sapir, Shmida & Ne'eman, 2005; Sapir, Shmida & Ne'eman, 2006), their evolutionary history and the causes of their current discontinuous distribution remain to be studied. Sapir *et al.* (2002) studied morphological variation in 42 natural populations of nine *Iris* species of the section *Oncocyclus*. Most of the floral and vegetative characters showed directional change in 12 populations of three of these species that were distributed along the aridity gradient in Israel: *Iris atrofusca* (Baker), *Iris petrana* (Dinsmore; both are used in the present study as well), and *Iris haynei* (Baker). The directional change observed was in accordance with known adaptations to aridity (i.e. decrease in size and organ dimensions; increase in leaf curvature), which led to the hypothesis of natural selection shaping this variation, resulting in local adaptation. In the present study, we add to the information provided by the study of Sapir *et al.* (2002) in two respects. The first is testing for local adaptation by measurements of performance in an array of environmental conditions. The second is measuring the amount and direction of phenotypic plastic responses, possibly an adaptive trait as well. To accomplish this, we conducted a common-garden experiment with four *Iris* species and measured morphological, phenological, and performance traits in different combinations of three treatments for each of two environmental factors that we knew to vary in the irises natural habitats: water amount and soil type.

Our main question was: are soil types, water amount, or their combination, the major environmental factors responsible for species adaptation and their boundaries of distribution?

MATERIAL AND METHODS

STUDY SYSTEM

We studied local adaptation in four species of *Iris* section *Oncocyclus*. These are perennial rhizomatous

plants, with approximately 33 species (Rix, 1997) that are distributed throughout the Middle East. Eight species are recorded in Israel, and all are endemic, or sub-endemic to Israel and the neighboring countries. Species distribution is rarely sympatric, and usually allopatric. Species can grow in short geographic distances from each other. Population distribution within the species range is also discontinuous and consists of many populations with clearly recognizable boundaries (Sapir *et al.*, 2002).

Plants of the *Oncocylus* irises are clonal and create large patches of leaf fans (ramets) that are connected by the rhizome to form a genet. Each ramet can produce only one flower. Plants are completely self-incompatible, and successful pollination requires the mediation of insect pollinators, which are night-sheltering solitary male bees (Sapir *et al.*, 2005).

In the present study, we used four species from the coastal plain and the south of Israel: *Iris atropurpurea* (Dinsmore), *I. atrofusca*, *Iris mariae* (W. Barbey) and *I. petrana*. A morphological study showed that there is a broad overlap in trait values among these species (Sapir *et al.*, 2002). On the other hand, the species grow in different environmental conditions, with respect to amount of rainfall and soil type. *Iris atropurpurea* grows on sandy soils in the coastal plains, in areas with an annual precipitation in the range 400–600 mm; *I. atrofusca* grows on loess and rendzina in areas with an annual precipitation in the range 200–300 mm; *I. mariae* grows on stabilized desert sand dunes in areas with an annual precipitation in the range 100–200 mm; and *I. petrana* grows on a mixture of loess and sand in areas with an annual precipitation of 100 mm.

COMMON-GARDEN EXPERIMENT

Plants of the four species were obtained from habitats that were about to be destroyed, from a single population per species. For example, *I. mariae* plants were dug out from an area that became a potato field. A total of 612 plants were used in this experiment. Each rhizome was weighted and planted singly in 3-litre pots in one of the three soil types: loess, sand, or rendzina, receiving one of the three water treatments: 100, 200, or 300 mm of water (17 plants for each species/soil/water combination). The experimental design was full-factorial, where factors are species, soil types, and the amount of water. The pots were placed on tables inside a net house in the Institutes for Applied Research, Beer-Sheva. Plants of all combinations of species and soil types were randomly placed on each of nine tables, and each table assigned randomly to one of the three water treatments.

Several measures were taken to make the amounts and timing of watering for each treatment as close as

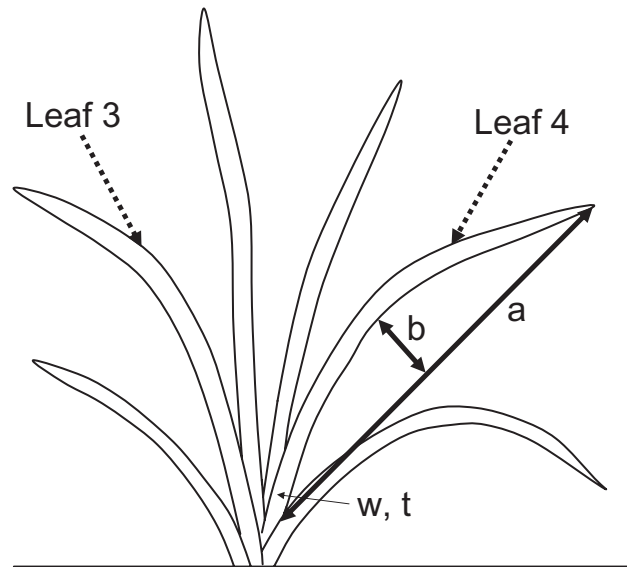


Figure 1. Vegetative measurements that were taken on one leaf fan: *a*, length; *b*, curvature; *w*, width; *t*, thickness. Curvature used in the analysis was calculated as the ratio b/a .

possible to those occurring naturally throughout the year. In each pot, there was a dripper with 2 litre h^{-1} flow rate for additional irrigation in the 200-mm and 300-mm treatments. To achieve reduction of rainfall for the 100-mm treatment, we mounted a transparent plastic cover approximately 1 m above the table. These plastic sheets were unfolded in the approximate middle of each rain event, and folded back immediately after the rain ended, to ensure that its potential effects other than decreased rain were minimal. Two rain gauges were placed inside the net house, on one of the 200-mm and on one of the 100-mm tables. Using these measures, the plants received 90, 188, and 296 mm at the end of the season, which we refer to as 100, 200 and 300 mm for simplicity.

Rhizomes were planted in September 2007. Starting from the middle of November, plants were recorded for having a leaf fan, or not, every 2 weeks. Between the middle of February and the middle of March, vegetative traits were measured on plants that had at least six leaves. Five hundred and twenty-two plants (85.3%) reached this stage and were measured. On each plant, the measurements of leaf length, thickness, width, and curvature were taken on the third and fourth mature leaves (Fig. 1). If more than one leaf fan was present, the largest one was measured. For the analysis, we used the average of the two measurements per trait per plant. The number of leaf fans was counted for all plants on two consecutive days in March. During the flowering

season, which lasted from the end of February to the beginning of April, plants were surveyed daily and floral longevity was determined for each flower that emerged. When all the aboveground biomass was dry (middle of June 2008), rhizomes were dug out and final weight was recorded.

In the present study, we used rhizome growth during the season and the flowering probability of the plant as performance components approximating (through a direct positive relationship) plant fitness. These performance measures have been used as indicators of fitness in numerous studies (Johnston *et al.*, 2001; Willi, Van Buskirk & Fischer, 2005). Trade-offs between growth and reproduction in plants are well known; therefore, it is important to measure both. We are aware of two assumptions of this type of fitness measure. First, we assume that the performance traits are correlated with fitness. Second, for practical reasons, we assume that other parts of the life cycle, such as seed production and germination, are not critical for genotype by environment interaction of these species.

Phenotypic variation in a common-garden experiment may have several causes: genetic, environmental, and maternal effects. The genetic effect is treated in the present study as the genetic origin of a plant (i.e. the species). Maternal effects can cause bias in the results, according to the environmental conditions that the plants experienced in previous generations (Roach & Wulff, 1987). We control for maternal effect of rhizome initial weight by using it as a covariate (see Statistical analysis). We assume that other environmental effects of the original natural microhabitat can be ignored as a result of the random representation of plants from each population and their random assignment to treatments.

STATISTICAL ANALYSIS

We used three-way analysis of variance (ANOVA) with a full factorial model to test the effects of the species, water amount, soil type and, most importantly, their interaction, on the five vegetative traits. A significant species \times environment interaction term indicates different plastic responses of the species (analogous to a $G \times E$ interaction; Schlichting, 1986). To improve normality, leaf curvature, which is a ratio of curvature to length, was arcsin(square-root) transformed and the start and end weights of the rhizomes were log transformed.

We used analysis of covariance (ANCOVA) to control the effect of start weight on final weight when testing for the effect of the manipulated environment (water, soil) and the effect of genetic identity (species) on biomass accumulation of the rhizome.

Differences in survivorship rate (%) between the 36 treatments and probability of flowering among nine

treatments for each species were compared using chi-squared test. The time elapsed until leaf emergence and flower appearance for each plant were analysed by Cox proportional hazards regression, with species, water, and soil type as independent variables. Plants that did not develop leaves or did not flower in the end of the time interval were treated as censored, meaning they were included in the analysis as cases where the event analysed (leaf emergence or flowering) has not occurred by the end of the time period.

To compare the amount of plasticity and the direction of reaction in the four species, we summarized the variation in the five vegetative traits using discriminant analysis. The analysis was performed twice: once with groups being combinations of species and water treatments and once with groups being combinations of species and soil type treatments. The results obtained are the distances between population centroids in a canonical variable plot, where the axes are the first two canonical variables and the points are the canonical variable scores.

ANOVA, ANCOVA, chi-square tests, discriminant analysis and Cox proportional hazards regression analysis were performed using STATISTICA (StatSoft Inc., 2004).

Although start weight can affect the final weight of the rhizome, it can also affect the number of leaf fans and leaf traits, which, in turn, might affect the final weight as well. To test the importance of such multiple and indirect effects, we used structure equation modelling and path analysis (Li, 1975; Shipley, 2000). By this method, we were able to test for hierarchical effects of rhizome weight and vegetative properties on performance, expressed as the final rhizome weight. We used values from the first axis from principal component analysis (PCA) on leaf traits (explaining 55.1% of the variation) to reduce dimensions for the model. PCA was conducted with STATISTICA, and path analysis was conducted using the sem package in R (Fox, 2006; R Development Core Team, 2008). Model validation was tested using maximum-likelihood chi square for the deviation of the hypothetical structure of the model from the observed covariance matrix.

RESULTS

TREATMENT EFFECT ON MORPHOLOGY, PHENOLOGY, AND PERFORMANCE

Overall survivorship of the rhizomes was very high: only four rhizomes (0.65%) did not survive. Another five rhizomes (0.82%) were dormant (i.e. did not grow leaf fans but were not dead).

Most of the plants (97.7% in all treatments combined) produced leaf fans before 13 February. The

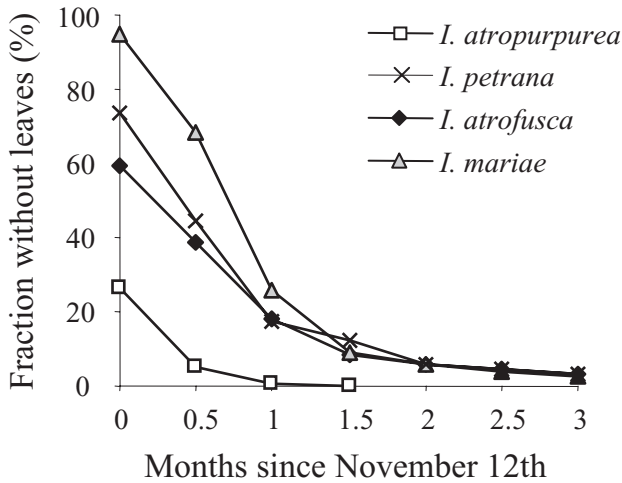


Figure 2. Time of leaf emergence, expressed as the percentage of plants without leaves as a function of time, in four *Iris* species.

timing of leaf emergence (vegetative growth) was significantly different between species in the Cox proportional hazards regression (Wald statistic = 19.7, $P < 0.001$; Fig. 2) but the effects of water amount and soil type were not significant (Wald statistic = 0.9 and 1.9, respectively, $P > 0.05$ in both cases).

The genetic origin (i.e. species identity) significantly affected all vegetative traits measured (Table 1). Water amount and soil type affected four out of the five traits. The direction of water level effect was positive on all four traits (data not shown). There were similar reaction norms among species, as indicated by very few significant interactions involving species (for leaf width: species \times soil; for number of leaf fans: species \times water effects).

The phenology (i.e. time of flowering) differed among the four species based on the results of Cox proportional hazards regression (Wald statistic = 3.9, $P < 0.05$). Interestingly, the order in which the species flower does not correspond to the distribution of the natural populations along the aridity gradient: *I. atropurpurea*, the most northern species from the Mediterranean climate, and *I. petrana*, the most southern species, were the first to flower (Fig. 3A). The effect of water amount was also significant (Wald statistic = 14.3, $P < 0.001$), with a positive effect of water amount on probability and the onset of flowering (Fig. 3B). The effect of soil type was not significant (Wald statistic = 0.4, $P > 0.05$).

Apart from the general difference in flowering percentage among species (Fig. 3A) and an overall increase in percentage with increasing water amount (Fig. 3B), there were no consistent trends among

Table 1. *F*-ratios and significance of species, soil, water and their interactions effects on vegetative traits (d.f. error = 486) and rhizome end weight (d.f. error = 575)

Trait	Source of variation							
	Rhizome start weight†	Species (d.f. = 3)	Water (d.f. = 2)	Soil (d.f. = 2)	Species \times Water (d.f. = 6)	Species \times Soil (d.f. = 6)	Soil \times Water (d.f. = 4)	Species \times Soil \times Water (d.f. = 12)
Leaf width		119.8***	22.2***	20.5***	1.7 ^{NS}	2.6*	2.2 ^{NS}	1.3 ^{NS}
Leaf thickness		16.6***	31.2***	11.1***	1.1 ^{NS}	1.9 ^{NS}	1.5 ^{NS}	1.2 ^{NS}
Leaf length		213.8***	2.4 ^{NS}	7.6***	1.9 ^{NS}	2.1 ^{NS}	1.2 ^{NS}	0.9 ^{NS}
Leaf curvature‡		295.5***	9.1***	0.4 ^{NS}	1.7 ^{NS}	1.0 ^{NS}	0.9 ^{NS}	0.5 ^{NS}
Number of leaf fans		25.1***	24.3***	5.3**	2.4*	1.2 ^{NS}	3.1*	0.5 ^{NS}
Rhizome end weight‡	158.8***	25.9***	211.3***	1.9 ^{NS}	1.0 ^{NS}	1.2 ^{NS}	7.7***	0.5 ^{NS}

†Data were log-transformed.

‡Data were arcsin(square-root) transformed.

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

NS, not significant.

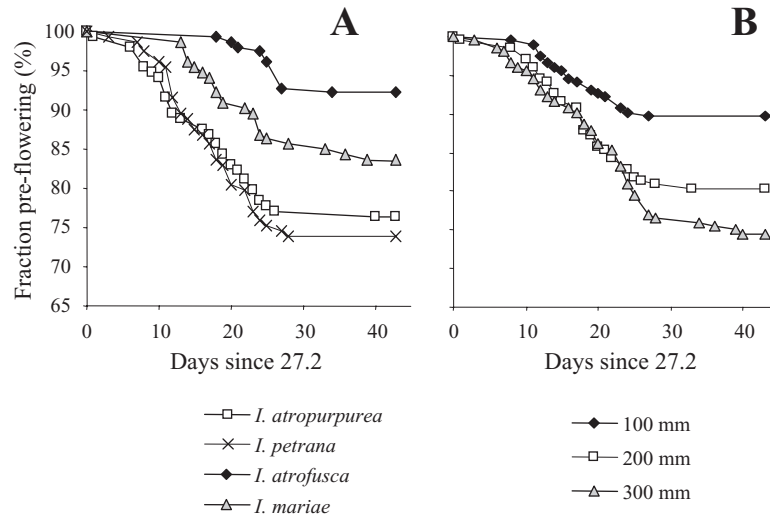


Figure 3. Phenology of four *Iris* species. A, time of flowering for the four species, expressed as the percentage of plants without flowers as a function of time, for the four species. B, time of flowering for the three water amounts.

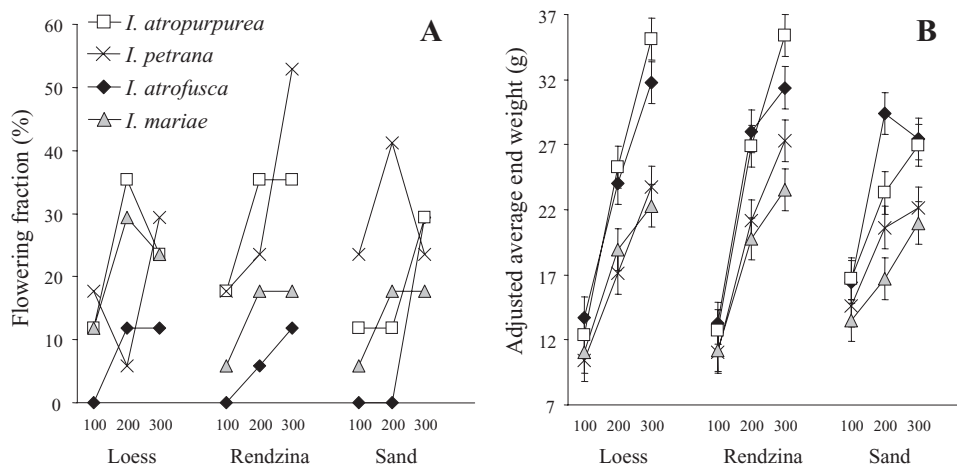


Figure 4. Reaction norms of the percentage of flowering (A) and the adjusted rhizome final weight (B), in four *Iris* species. Note that lines between points do not indicate continuous variation. Bars (in B) represent one standard error.

species in the reaction norms with respect to the percentage of flowering across treatments (Fig. 4A). The chi-square test showed that the fraction of plants that set flowers does not significantly differ across the nine combinations of water and soil treatments for three of the species. *Iris atrofusca* was an exception, showing a significant difference with respect to flowering percentage among the nine treatments ($\chi^2 = 17.9$, $P < 0.05$). Because of this lack of reaction in flowering probability, we cannot compare it among species.

All species flowered in all treatments, except for *I. atrofusca*, which did not flower in any of the soils with 100-mm treatments and only in one 200-mm treatment (on sand; Fig. 4A). Overall, 18.5% of the plants flowered.

The final weight of the rhizome was affected by the genetic origin (species) and water amount (positive effect of the latter; Fig. 4B), when start weight was controlled as a covariate (Table 1). A significant interaction was found for the effect of soil and water, but none of the other possible interactions were found to be significant. This suggests that the reaction norms for rhizome final weight are similar among species (Fig. 4B). The Mediterranean and semi-arid species, *I. atropurpurea* and *I. atrofusca*, had a higher relative growth rate, compared to the two desert species, *I. mariae* and *I. petrana* (Fig. 4B). Note that the final rhizome weight is presented as adjusted values, which are calculated from ANCOVA as the final weight expected if all species had the same start weight.

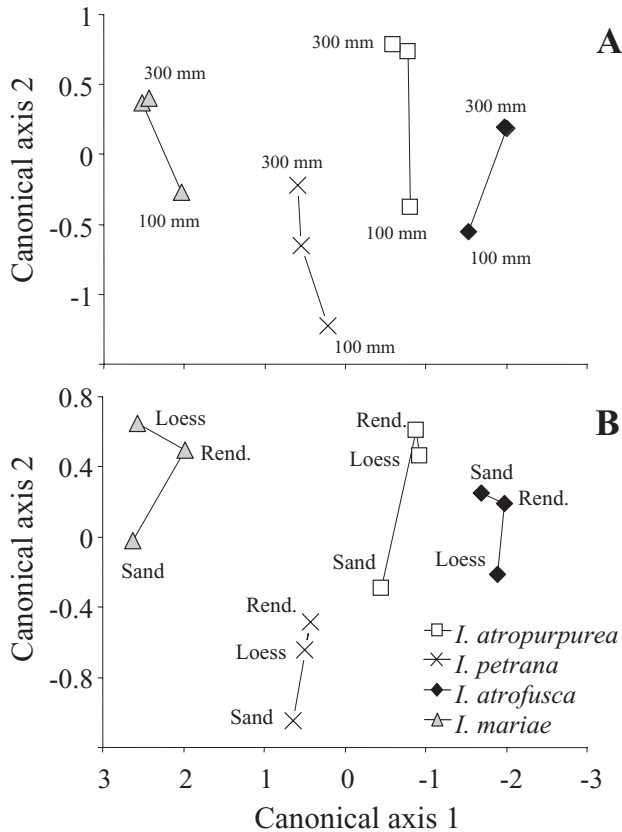


Figure 5. Response to amount of water (A) and to soil type (B), in four *Iris* species. Note that lines between points do not indicate continuous variation.

PLASTICITY AND DIRECTION OF RESPONSE OF VEGETATIVE TRAITS

Discriminant analysis summarizes the variation of five vegetative traits in different species under different treatments. The distances between the treatments in the discriminant analysis graphs demonstrate the amount of plasticity and the direction of response in a two-dimensional space (Volis *et al.*, 2002b).

Leaf width, thickness, and length contribute the most to the discriminating functions of species and treatments; however, all five traits had a highly significant contribution ($P < 0.001$). The species are obviously different from one another in vegetative traits, although the amount of their plasticity is similar (Fig. 5). When we compare water amount treatments (Fig. 5A), the pattern is also similar: the direction of response is similar for the four species (the direction is from the bottom points, which represent 100-mm treatments, upwards up to 200- and 300-mm treatments). As for soil types, the direction of response is similar for *I. petrana* and *I. atropurpurea*, but is different for the other two species (Fig. 5B).

MULTIPLE AND INDIRECT EFFECTS

All path models (Table 2) did not significantly deviate from the observed data ($P > 0.1$ in all models); thus, all models were valid. Examples for path models are shown in Fig. 6. Direct effects, defined as significant path coefficients, were found to be always positive. Although most models showed a significant positive effect of initial weight on final weight, the models for *I. atropurpurea* appear to have less significant direct effects with respect to the number of leaf fans and leaf traits. In addition, no significant indirect effects were found in all treatment combinations in *I. atropurpurea*, except for the indirect effect of initial weight through its effect on leaf traits. This was the only model where this indirect effect was significant (i.e. both path coefficients were significant) and, interestingly, this effect was negative.

DISCUSSION

The four studied *Iris* species differed in morphology and phenology under common-garden conditions. The vegetative traits, rhizome growth and flowering probability differed among species, indicating that the differences among species are mostly a result of genetic origin and are less induced by the environment.

However, there was no evidence for a pattern of local adaptation in these species for the two performance traits that we measured (i.e. rhizome growth and flowering probability). The reaction norms of these traits showed either a similar response for all species (rhizome growth) or no response at all (flowering). There is no evidence that the amount of water and soil types (in the range that we tested) are environmental factors limiting the distribution of these species.

The amount and direction of plastic responses in vegetative traits across environments, which is another possible type of adaptation, did not differ among these species. Possibly, these species did not develop fine-tuned responses to the environment as a result of the temporal variation in conditions among seasons, which is high in this region: coefficient of variation in annual precipitation of approximately 25–30% in the coastal plain and approximately 40% in central Negev desert (Goldreich, 1995). This could select for a broad niche size of the plants.

Clines in traits among populations are often suggested to be a result of selection caused by a cline of environmental conditions (Schlichting & Levin, 1990). Precipitation regimes in the natural habitats of the four studied species represent an environmental (aridity) cline. However, we did not detect a clear cline in vegetative growth (Fig. 2) or phenology (Fig. 3). Although morphological variation in the *Oncoclytus* irises was found to be clinal in natural

Table 2. Effects of initial rhizome weight, number of leaf fans and the principal component (PC) value of leaf traits on final rhizome weight in four *Iris* species in three soil and three water treatments, as obtained from path analyses. Direct effects are the standardized path coefficients leading directly from each factor to the response variable (rhizome final weight). Indirect effects are the multiplication of partial path coefficients leading from the initial weight of the rhizome, via one of the other factors. Values in bold denote path coefficients that are significantly ($P < 0.05$) different from zero. For examples of path models, see Fig. 6.

Species	Soil	Water	No. fans	PC leaves	Start weight (direct)	Start weight (indirect via no. fans)	Start weight (indirect via PC leaves)
<i>Iris atrofusca</i>	Sand	100	0.51	0.73	0.09	0.37	-0.15
		200	0.26	0.69	0.37	0.18	0.08
		300	0.46	0.08	0.51	0.05	0.01
	Loess	100	0.23	0.39	0.63	0.08	0.11
		200	0.46	0.53	0.02	0.27	0.11
		300	0.29	0.51	0.56	0.03	0.20
	Rendzina	100	0.20	0.58	0.47	0.03	0.15
		200	0.59	0.41	0.19	0.33	-0.16
		300	0.43	0.45	0.02	0.12	-0.03
<i>Iris atropurpurea</i>	Sand	100	0.10	0.48	0.62	0.07	-0.20
		200	0.17	0.13	0.33	0	0.02
		300	-0.11	0.31	0.71	-0.04	-0.13
	Loess	100	0.40	0.57	0.33	0.06	0.03
		200	0.32	0.17	0.46	0.03	0.03
		300	-0.002	-0.14	0.42	0	-0.04
	Rendzina	100	0.32	0.67	0.30	0.04	0.24
		200	0.38	0.3	0.30	0.06	-0.03
		300	0.30	0.23	0.60	0.02	0.01
<i>Iris mariae</i>	Sand	100	0.63	0.24	0.57	0.13	0.02
		200	0.84	-0.12	0.17	0.67	-0.04
		300	0.26	0.10	0.71	0.13	0.02
	Loess	100	-0.12	0.02	0.86	-0.06	-0.01
		200	0.41	-0.27	0.31	0.23	-0.20
		300	0.55	0.20	0.35	0.31	0.06
	Rendzina	100	0.53	0.03	0.60	0.22	-0.01
		200	0.65	0.32	0.46	0.22	-0.05
		300	0.49	0.21	0.43	0.20	0.05
<i>Iris petrana</i>	Sand	100	0.30	0.42	0.64	0.10	0.12
		200	0.29	0.04	0.68	0.01	-0.01
		300	0.56	0.16	0.28	0.01	0
	Loess	100	0.02	0.44	0.40	0.01	0.19
		200	0.39	0.26	0.67	0.07	0.10
		300	0.48	0.59	0.50	-0.03	-0.04
	Rendzina	100	0.19	0.57	0.71	0.08	-0.18
		200	0.52	0.49	0.06	0.26	0.21
		300	0.62	0.30	-0.09	0.16	-0.04

populations (Sapir *et al.*, 2002), we did not find clear evidence for it to be adaptive: there was no cline exactly corresponding to the environmental gradient in any of the measured traits and no performance advantage of each species in conditions more resembling its natural habitat.

Gene flow between populations of different *Iris* species in Israel is most likely limited or not existing,

even in the few places where two species grow in proximity (Arafah *et al.*, 2002). Therefore, we suggest that the lack of local adaptation observed in the present study is either because it is absent, and then the differences between the species are a result of random processes such as the founder effect and genetic drift, or because it is expressed in longer time scales and/or through interaction with other environ-

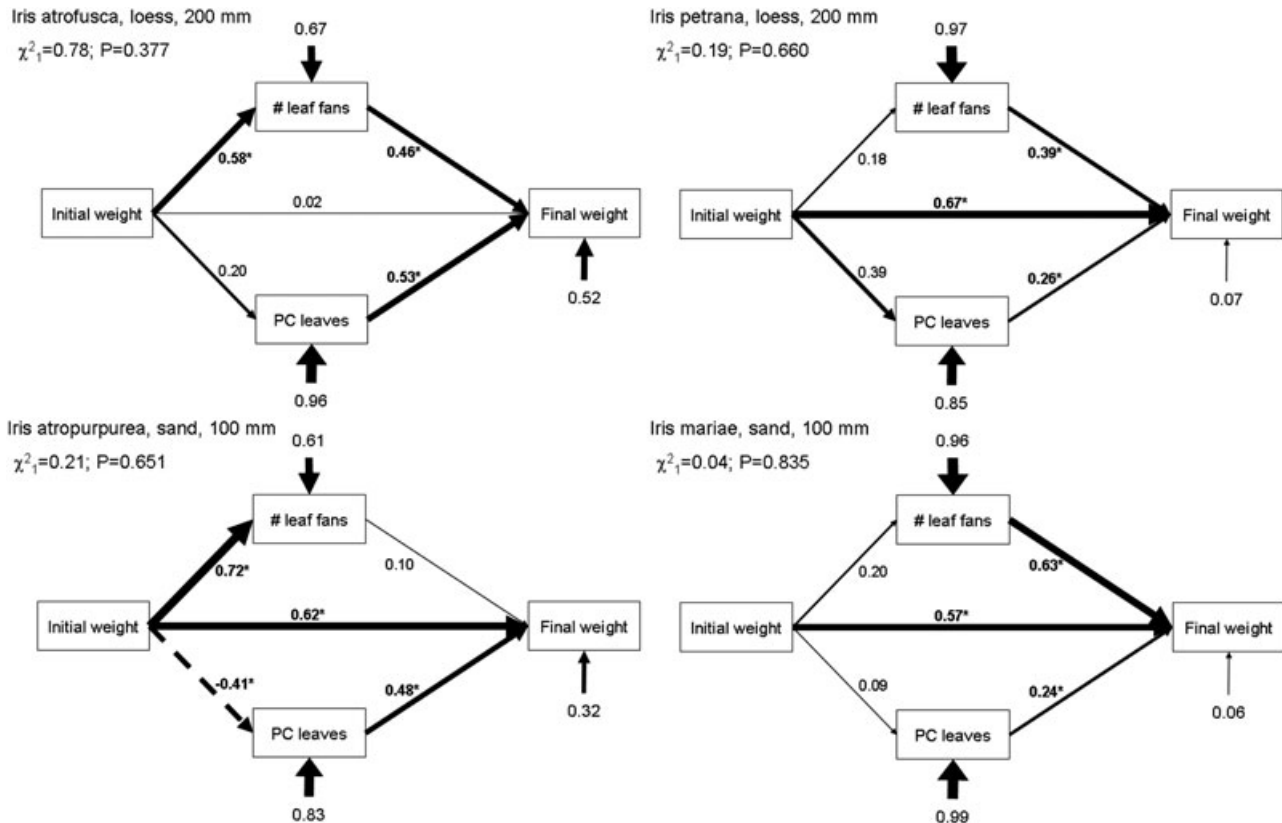


Figure 6. Examples of path models analysed for the effect of rhizome weight and leaf traits on final rhizome weight in four *Iris* species. Positive effects are indicated by solid arrows and negative effects are indicated by dashed arrows. Numbers attached to each arrow are standardized path coefficients, where significant coefficients are shown in bold and with an asterisk. Arrows width is proportional to the size of the effect. Short arrows leading to variables indicated by the unexplained variance. PC, principal component.

mental conditions or biotic factors that were not represented in our experiment. Another possibility is that adaptation is expressed at other life-history stages, such as germination and seed production, which were not tested in the present study.

In general, genetic diversity among similar species is either adaptive (i.e. created by natural selection) or random (i.e. created by genetic drift). Detecting local adaptation is one possible method for distinguishing between these two types of variation and reveals whether differences in trait values have an adaptive significance or not. The advantage of the common-garden method for doing this, as stated in the Introduction, is the ability to test specific environmental factors and thus their role in adaptation. The disadvantage is that we have to know which factors to test because local adaptation can never be ruled out when finite number of factors are tested. The variation itself may serve as a clue, such as the clinal variation in plant size along the aridity gradient found by Sapir *et al.* (2002). Other nonclinal types of variation, such

as onset of flowering, may be more complex and related to a set of environmental factors that is difficult to simulate in an experiment.

To summarize, in the present study, we tested for a local adaptation hypothesis concerning morphological/phenological divergence in four *Iris* species. These species do not show spontaneous hybridization and grow in distinct environmental conditions, which supposedly could favour local adaptation. However, this was not found in a common-garden experiment that tested the effects of presumed major environmental determinants of iris fitness: precipitation amount and soil type. The responses of the plants to soil type and water amount treatments did not correspond to those expected under local adaptation. There are two possible reasons for this: (1) limits to natural selection that constrain local adaptation (genetic drift; weak or variable selection) and/or (2) expression of adaptation in settings beyond those used in the present study (e.g. other environmental variables, different life stages, larger time scale).

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