ABSTRACT: Magnitude and variation in leaf plasticity were quantified in two Iris pumila (L.) populations from habitats of contrasting light conditions (open dune vs wood understory) at three light intensities (high – 110, medium – 65, and low – 29 μmol m⁻² s⁻¹). Siblings developed from hand-pollinated seeds from 13 and 15 clonal genotypes in an open and a shaded population, respectively, raised in a growth-room were scored for morphological (leaf number, leaf area, specific leaf area), anatomical (stomatal density, leaf thickness, vascular bundle number, sclerenchyma and cuticle widths) and biochemical (chlorophyll content, chlorophyll a:b ratio) traits. Morphological traits in general and SLA (projected leaf area per unit leaf dry mass) in particular were more sensitive to variation in light conditions than any other examined leaf attribute, indicating their key importance for maximizing light-energy interception at low irradiance. Regardless of the population origin, the average plasticity (percentage trait change between two successive treatments) of morphological traits declined with decreasing irradiance, opposite to anatomical traits, particularly leaf thickness, which increased parallel to light intensity decrease. Mean plasticity variation (across-family CV) changed with light level, ranking in the following order morphological < anatomical < biochemical. A higher degree of leaf plasticity to irradiance in the shaded comparing to the open population could be the adaptive outcome of different selective history they have encountered within their natural habitats.

KEY WORDS: environmental heterogeneity, Iris pumila, irradiance gradient, leaf traits, phenotypic plasticity, population divergence

1. INTRODUCTION

As photosynthetic but sessile organisms, plants have evolved a remarkable capability to adopt the varying demands of a heterogeneous radiation environment by generating appropriate morphological, physiological and biochemical responses (Bradshaw 1965, Sultan 1995, 2003, Schlichting and Pigliucci 1998). Because photosynthesis – and thus plant growth – directly depends on the amount of available radiant energy, and since leaves are essentially energy-gaining plant organs, light-evoked developmental modifications are most notable at the foliage level (Björkman 1981, Fitter and Hay 1981, Bazzaz 1996). Substantial progress has been made in recent decades in both illuminating the ecological significance of a particular plastic response relative to other plasticities, as well as in characterizing leaf plasticity in terms of the amount and
the pattern. In many plant species, adaptive plastic responses to high irradiance were found to be dominated by adjustments in leaf physiology, which contrasts with adaptations to the low light, where the alterations in foliar morphology, especially in specific leaf area (SLA, projected leaf area per unit leaf dry mass), were far more important in determining relative growth rate (RGR, the increase in plant mass per unit of mass present and per unit of time) than biochemical modifications (Björkman 1981, Hikosaka and Terashima 1996, Niinemets and Tenhunen 1997, Niinemets et al. 1998, Evans and Poorter 2001, Shipley 2000). Poorter and van der Werf (1998) provided the evidence that, in herbaceous plants, the SLA is large, displaying a high interspecific variation at low irradiance, but small and similar across species at a high irradiance. An inverse trend was observed for net assimilation rate (NAR, biomass increase per unit leaf area and time) – a physiological component of RGR (Evans and Poorter 2001, Shipley 2000).

Although the qualitative responses of different leaf traits to light intensity are relatively well explained, it is still insufficiently known at what position along a light gradient they are exhibiting the greatest plasticity. Large plasticity in leaf traits is considered to be particularly important for survival and reproduction in the wild, where individual plants commonly face a wide range of irradiances during their growth and development (Poorter 2001, Shipley 2000).


Accordingly, a plant taxon that experiences highly variable environmental conditions should exhibit a higher amount of phenotypic plasticity compared to that inhabiting more homogeneous environments. The main conclusion emerging from these studies (applicable at both population and species level) is that different selective histories may promote evolutionary divergence of plant taxa with regard to the magnitude of phenotypic changes (Roff 1992, Linhart and Grant 1996, Lortie and Aarssen 1996, Van Buskirk 2002, Griffith and Sultan 2005).

In the present work, we explored variation in phenotypic expression of different leaf attributes (morphological, anatomical and biochemical) in 6-month-old Iris pumila seedlings exposed to three irradiance levels (approx. 110, 65 and 30 μmol m–2 s–1 photosynthetically active radiation) in a growth room. Plants were developed from hand-pollinated seeds produced by distinct clonal genotypes growing in two natural habitats that differed in the ambient light conditions: one, experiencing full sunlight at an open dune site, and the other, under reduced light intensity in a pine understory. It has been empirically found that the Iris genotypes are able to respond adaptively to the actual light conditions of their native environments, as well as, that at a given irradiance, the magnitude of plastic responses can differ among conspecific populations from distinct light habitats (Tucić and Avramović 1996, Pemac and Tucić 1998, Tucić et al. 1998, 1999).

Here we tested the two hypotheses: (1) adaptation to low irradiance in shade-tolerant plants involves changes in both leaf morphology (more specifically LA and/or SLA) and foliar chemistry (nitrogen allocation between protein pools), with increasing plasticity in LA/SLA being more important in maximizing potential photosynthetic gain than increasing the chlorophyll content per unit leaf area (Chl μg cm–2), and (2) populations exposed to highly variable environmental conditions should exhibit high levels of phenotypic plasticity, in general.
2. MATERIAL AND METHODS

2.1. Study species

*Iris pumila* L. (Iridaceae) is a perennial clonal herb widely distributed in the lowlands of Central and Southeastern Europe. In its native habitats the species forms round-shaped clones which are polymorphic for flower color. Each of the flower color variants commonly found in the populations can be considered as a unique clonal genotype (Tucić et al. 1988). Stable coexistence of multiple colour genotypes in the extant populations of *I. pumila* could be ascribed to a combination of fluctuating temperatures giving rise to environmental heterogeneity and differential attractiveness of various petal colour morphs to pollinating insects (Tucić et al. 1989).

2.2. Study area

For the present study, we selected two natural populations of *I. pumila* that experience ecologically distinct conditions in the Deliblato Sands, a sandy area situated about 50 km northeast of Belgrade (44°48’N, 20°58’E), in Serbia. The “Dune” population was growing in full sun along the top and slope of a dune, where it coexisted with annual and perennial herbs and low shrubs. The “Wood” population inhabited the understorey of a *Pinus nigra* stand situated approx. 2.5 km far from the “Dune” population. The mean values for daily photosynthetically active radiation (PAR) in these populations, taken by a point quantum sensor (LI-190SA, LI-COR, Inc., Lincoln, USA) on 24 September 1998, between 10:00 a.m. and noon under clear sky conditions, were 505.06 μmol m–2 s–1 in the high, ~ 65 μmol m–2 s–1 in the medium, and ~ 29 μmol m–2 s–1 in the low light treatment, as recorded by a point quantum sensor (LI-190SA, LI-COR, Inc., Lincoln, USA). The illumination above the plants was provided by a set of four Philips TLD 36-W/33 fluorescent tubes. The created light treatments were within the range of light levels recorded in natural *I. pumila* populations (Tucić and Avramov 1996). The potted plants were placed in preassigned positions on a shelf in a randomized complete block design in a climate-controlled growth-room. Four blocks were established and each of them divided into three plots. Each plot was randomly assigned to one of the three light levels. Within each light level, every family was represented by 4 to 8 individuals, depending on seed availability. Plants were regularly top-watered to full soil capacity and fertilized every two weeks with 10 ml of full strength Hoagland’s solution. The ambient temperature was kept at 21/16 °C day/night, with a 16 h photoperiod. To minimize the position effects, the pots were rotated twice a week. Six months after the germination (approx. duration of a growing season), the following leaf traits were recorded on each plant: leaf number (*LN*), leaf area (*LA*; in cm2), specific leaf area (*SLA*; projected leaf area per dry mass, in cm2 g–1); stomata density (*SD*; in no. cm–2), leaf thickness (*LT*; in μm), vascular bundle number (*VBN*), sclerenchyma width (*SW*; in μm), cuticle width (*CW*; in μm), total chlorophyll...
content (ChlT; in μg cm$^{-2}$), and the ratio of chlorophyll $a$ to chlorophyll $b$ (Chla/b).

To allow seedlings to reach adulthood (for further investigation), individual leaf area was estimated non-destructively as the product of the longest leaf length and width, measured with a digital caliper. Specific leaf area was determined as the ratio of individual leaf surface area and dry leaf biomass (oven-dried for 48 h at 60°C). All anatomical analyses were done using the upper half of the last fully developed leaf. Stomata density was estimated by a micro-relief method (Pazourek 1970). For each leaf, stomata number was calculated in 20 randomly chosen microscope fields (0.159 mm$^2$ at 40× magnification) on each microscope slide. Leaf anatomical measurements were taken with an Olympus (Vanox) microscope, using 50-μm-thick leaf sections embedded in glycerol and placed on microscope slides.

Chlorophyll content was estimated spectrophotometrically (Hiscox and Israelstam 1979), using a Shimadzu UV-160 spectrophotometer.

2.4. Statistical analysis

All analyses were done using 591 survived seedlings belonging to 28 full-sib families. The magnitude of phenotypic plasticity of a trait was estimated for each family in the two pairs of light-treatments (high and medium, and medium and low) by computing an index of phenotypic plasticity, $P_{AB}$ (Cheplick 1995):

$$P_{AB} = \left[ (\overline{X}_A - \overline{X}_B) / \overline{X}_A \right] \times 100$$

where

$P_{AB}$ is the percentage of change in a trait from the A (higher) to the B (lower) treatment;

$\overline{X}_A$ is the mean value for a family under the A (higher) light level;

$\overline{X}_B$ is the mean value for a family under the B (lower) light level.

The mean phenotypic plasticity ($P$) across families was computed between the high- and medium-light treatments ($P_{HA}$), and between the medium- and low-light treatments ($P_{ML}$).

The Wilcoxon two-sample test was employed for comparing the mean plasticities among individual traits, the trait categories, and the populations. The magnitude of variation across families in phenotypic plasticity was quantified by calculating a coefficient of variation (CV%), $\left[ \text{standard deviation/mean } P_{AB} \times 100 \right]$. To determine whether the two traits had the same level of variation among families in plastic response to irradiance, as well as whether plasticity variation of the same trait differs across populations, a variance ratio ($F_{S}$) test of the difference between two variances of the logarithms of the data was employed (Zar 1984).

3. RESULTS

The ambient light conditions experienced by the I. pumila seedlings markedly affected all aspects of their leaf phenotype: morphology, anatomy and biochemistry (Fig. 1). Regardless of the population origin, the mean values of morphological traits (leaf number, LN; leaf area, LA; and specific leaf area, SLA) increased gradually with the reduction in light availability, in contrast to anatomical traits (stomata density, SD; leaf thickness, LW, vascular bundle number, VBN; sclerenchyma width, SW; and cuticle width, CW) which tended toward the lower means at lower light intensities (Fig. 1). Of the two biochemical traits measured, plasticity in total chlorophyll content per area (ChlT) also took the form of co-gradient variation, while chlorophyll $a/b$ ratio (Chla/b) changed in the opposite direction to variation in irradiance level (Fig. 1).

3.1. Within-population plasticity

The average level of phenotypic plasticity ($P$) to irradiance by the I. pumila leaves depended highly on both trait category and growth light conditions (Table 1). Relative to the position on the light gradient applied, the mean morphological plasticity appeared to be significantly greater at the upper- than at the lower-part of the gradient in both I. pumila populations, as was the average anatomical plasticity, but only in the "Dune" population (Table 2). Conversely, mean biochemical plasticity varied in the opposite
Fig. 1. Mean values (± SE) of 10 *Iris pumila* leaf trait expressions at high-, medium-, and low light intensities for the “Dune” (open bars) and “Wood” (closed bars) populations.
Table 1. Mean phenotypic plasticity ($P$), equation (1), and coefficient of variation (CV%) across families in plastic response to irradiance of 10 *Iris pumila* leaf traits in the “Dune” and “Wood” populations. $P_{HM}$ = mean phenotypic plasticity between the high and medium light treatments. $P_{ML}$ = mean phenotypic plasticity between the medium and low light treatments.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Dune</th>
<th>Wood</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$P_{HM}$</td>
<td>$P_{ML}$</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>CV%</td>
</tr>
<tr>
<td>Morphological:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf number</td>
<td>14.6</td>
<td>58</td>
</tr>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>38.7</td>
<td>38</td>
</tr>
<tr>
<td>Specific leaf area (cm$^2$g$^{-1}$)</td>
<td>29.8</td>
<td>37</td>
</tr>
<tr>
<td>Mean</td>
<td>27.7</td>
<td>44</td>
</tr>
<tr>
<td>Anatomical:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal density (no. cm$^{-2}$)</td>
<td>20.9</td>
<td>34</td>
</tr>
<tr>
<td>Leaf thickness (μm)</td>
<td>5.8</td>
<td>92</td>
</tr>
<tr>
<td>Vascular bundle number</td>
<td>8.7</td>
<td>58</td>
</tr>
<tr>
<td>Sclerenchyma width (μm)</td>
<td>6.0</td>
<td>81</td>
</tr>
<tr>
<td>Cuticle width (μm)</td>
<td>14.4</td>
<td>51</td>
</tr>
<tr>
<td>Mean</td>
<td>11.2</td>
<td>63</td>
</tr>
<tr>
<td>Biochemical:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll content (μg cm$^{-2}$)</td>
<td>7.4</td>
<td>106</td>
</tr>
<tr>
<td>Chlorophyll $a:b$ ratio</td>
<td>12.2</td>
<td>71</td>
</tr>
<tr>
<td>Mean</td>
<td>9.8</td>
<td>89</td>
</tr>
<tr>
<td>Grand mean</td>
<td>16.2</td>
<td>65</td>
</tr>
</tbody>
</table>
Table 2. Significance of difference in the mean phenotypic plasticity ($P_{\text{Mean}}$), equation (1), and coefficient of variation ($P_{\text{CV}}$) across families in plastic responses of 10 *Iris pumila* leaf traits, as expressed in the "Dune" and the "Wood" populations separately, between the two irradiance ranges: from the high- to medium- ($P_{\text{HM}}$) and from the medium- to low-light ($P_{\text{ML}}$) levels, as well as between the "Dune" and the "Wood" populations, within each irradiance range, respectively. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ms (marginal significance) = 0.05 < $P < 0.10$, ns = non-significant.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Irradiance ranges: $P_{\text{HM}}$ vs $P_{\text{ML}}$</th>
<th>Populations: Dune vs Wood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dune $P_{\text{Mean}}$ $P_{\text{CV}}$</td>
<td>Wood $P_{\text{Mean}}$ $P_{\text{CV}}$</td>
</tr>
<tr>
<td>Morphological:</td>
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<td></td>
</tr>
<tr>
<td>Leaf number</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
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<tr>
<td>Leaf area (cm$^2$)</td>
<td>*** $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>* $P_{\text{HM}}$ ms $P_{\text{ML}}$</td>
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<tr>
<td>Specific leaf area (cm$^2$ g$^{-1}$)</td>
<td>** $P_{\text{HM}}$ * $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Mean</td>
<td>*** $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>* $P_{\text{HM}}$ * $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Anatomical:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal density (no. cm$^{-2}$)</td>
<td>** $P_{\text{HM}}$ ** $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
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<tr>
<td>Leaf thickness (μm)</td>
<td>ns $P_{\text{HM}}$ ** $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
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<tr>
<td>Vascular bundle number</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
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<td>Sclerenchyma width (μm)</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Cuticle width (μm)</td>
<td>* $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Mean</td>
<td>** $P_{\text{HM}}$ ms $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Biochemical:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll content (μg cm$^{-2}$)</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns * $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Chlorophyll a/b ratio</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
<tr>
<td>Mean</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
<td>ns $P_{\text{HM}}$ ns $P_{\text{ML}}$</td>
</tr>
</tbody>
</table>
direction to the change in irradiance level, but this variation was not significant in both of the two populations (Table 2). Generally, the average morphological plasticity was significantly higher than anatomical plasticity, while the mean anatomical plasticity exceeded the magnitude of the biochemical phenotypic responses (Tables 1 and 3). When the percentage change in all leaf traits was compared in different photic environments (i.e., from the medium to low light treatment), the index of plasticity for specific leaf area had the greatest value comparing to any of the 10 leaf traits examined ($P_{ML}$: 18% in the “Dune”, and 22% in the “Wood” population; Table 1). The only exception was the magnitude of plasticity for \( Chla/b \) in the “Dune” population ($P_{ML}$ = 20%). Under higher irradiance (that is, from the high to medium light treatment), the two morphological traits, \( LA \) and \( SLA \), displayed the greatest plasticity means in both \( I. \textit{pumila} \) populations (Table 1). Interestingly, while in the “Wood” population, only two morphological traits, \( LN \) and \( LA \), differed significantly in the average plastic response expressed at the alternative positions on the irradiance gradient, in the “Dune” population, two morphological traits (\( LA \) and \( SLA \)), and two anatomical traits (\( SD \) and \( CW \)) exhibited a significantly greater plasticity mean at the upper than at the lower half of the gradient (Table 2). The mean plasticity for two biochemical traits, \( ChlT \) and \( Chla/b \), varied in the opposite direction to the change in irradiance level (Table 1), but insignificantly in any of the two populations (Table 2).

Variation in the mean plasticity across families (CV) appeared to be trait- and environment-specific, as well. For example, the average level of plastic variation was apparently lower for the leaf morphological traits than for both anatomical and biochemical traits (mean CV: 52% vs 66% and 83%, respectively). In the “Dune” population, plasticity to irradiance in the average CV values for all three trait categories displayed a counter-gradient direction, while in the “Wood” population, it took the form of counter-gradient variation for morphological, but co-gradient variation for both anatomical and biochemical traits (Table 1). Although the magnitude of variation in plasticity among families changed with irradiance for the same trait category, a statistically significant difference in the mean CV values between the alternative gradient positions was revealed only for the anatomical leaf traits in the “Dune” population, and the morphological leaf traits in the “Wood” population (Table 2).

Averaging all leaf traits across the populations and the positions on the irradiance gradient, it appeared that relative to any of the 10 leaf traits examined, the minimum variation among families in phenotypic plasticity displayed \( SLA \) (mean CV = 45%), and the maximum \( ChlT \) (mean CV = 97%). A similar trend was observed for the mean plastic variation of leaf traits within each of the two \( I. \textit{pumila} \) populations (Table 1). Although the mean CV values in phenotypic plasticity were lower from the high- to medium- than from the medium- to low-light treatment for the majority of studied traits, their estimated values differed significantly only for \( SLA \), \( SD \) and \( LT \) in the “Dune” population, and for \( LN \), \( LA \) and \( ChlT \) in the “Wood” population (Table 2).

### 3.2. Between-population plasticity

The mean percentage change in both morphological and anatomical leaf traits from the high- to medium-light treatment tended to be lower in the “Dune” than in the “Wood” population, but did not differ significantly between the two populations (Table 2). However, under low-light availability, that is, from the medium- to low-irradiance treatment, the difference in plastic responses between the two populations was significant for anatomical traits, and a marginally significant for morphological traits (Table 2). By contrast, light-induced change in the mean biochemical plasticity exhibited a co-gradient direction, tending to be lower in the “Wood” than in the “Dune” population in general (Table 3).

The average plastic variation among families (mean CV) appeared to be trait-specific but similar in magnitude between the “Dune” and the “Wood” population for any of the three trait categories examined (Table 2). Regarding individual leaf traits, the coefficients of variation in plasticity for \( LA \), \( SD \) and \( VBN \)
displayed a co-gradient fluctuation in the “Wood” population, and a counter-gradient fluctuation in the “Dune” population. Their magnitudes differed significantly (SD and VBN) or marginally significantly (LA) between the two populations only from the high- to medium-light treatment (Table 2). By contrast, the level of across-family variation in both the LT and CW plasticity changed in the opposite directions with irradiance (decreased in the “Dune”, and increased in the “Wood” population), and differed significantly between the two populations from the medium- to low-light treatment only (Table 2). Of the two biochemical traits examined, the CV in plasticity for ChlT was generally greater in the “Dune” than in the “Wood” population, and its magnitude appeared to be significantly different between the two populations from the high- to medium-light treatment (Table 2).

4. DISCUSSION

4.1. Magnitude of plasticity in light-responsive leaf traits

When the magnitude of foliage responses to irradiance in *I. pumila* was quantified in term of an index of phenotypic plasticity (P, Cheplick 1995), it appeared that leaf morphological traits in general, and specific leaf area (SLA) in particular, were more sensitive to variation in growth light conditions than any of the remaining examined leaf attributes. Shade-evoked increase in the leaf area per unit biomass allocated in leaves (SLA) is thought to be one of the universal aspects of developmental plasticity in plants (Fitter and Hay 1981, Sultan 2003). Since under conditions of low light availability the photosynthetic capacity per unit of leaf area is severely reduced, plant growth can be only sustained by a compensatory increase in the amount of photosynthetically active surface area for light capture (Fitter and Hay 1981, Poorter and van der Werf 1998, Shipley 2000). However, higher SLA under suboptimal irradiance levels implies not only an increased light-catching leaf area, but also a number of anatomically- and cell-based adjustments that enhance the light-harvesting efficiency of leaf tissue under those growth limits. Thus, as by definition, SLA is the inverse of the product of leaf lamina thickness (LT) and leaf dry mass (tissue) concentration (Witkowski and Lamont 1991, Shipley et al. 2005), it is obvious that modifications in leaf anatomy in concert with specific biochemical change attributes contribute to the
realized SLA values within a given light habitat (Meziane and Shipley 2001, Shipley et al. 2005). The present study provides the evidence that the average plasticity of individual morphological traits declined in general with decreasing growth irradiance in both I. pumila populations, in contrast to underlying anatomical plasticity, particularly leaf thickness, which tended toward greater values parallel to light supply decrease. Since the percentage change in the average SLA plasticity appeared to be much higher than in LT (41% vs 8% in the “Dune” population, and 28% vs 11% in the “Wood” population, for SLA vs LT, respectively), it seems reasonable to believe that variation in the other SLA component – that is, leaf tissue density (not measured in this study) have contributed simultaneously to high response flexibility of this trait. Leaf anatomical adjustments to very low irradiance in I. pumila were accompanied by a reduction in both stomata density (Fig. 1) and the number of epidermal cells per unit of leaf area (B. Tucić and S. Avramov, unpublished data), suggesting that the observed increase in SLA could be causally related to a relative enlargement in the size of leaf cells that became more swollen with water or alternatively due to some biochemical changes that took place within these cells (Shipley 2000, Shipley et al. 2005). Regardless of the population origin, the mean plasticity to irradiance of all morphological (and some anatomical) leaf traits markedly decreased with decreasing light intensity (Table 1), which indicates that the extremely low light conditions created in this experiment might have imposed metabolic limits on the leaf cell division and/or expansion influencing in this way leaf size, or that there is a biomechanical threshold to producing extremely thin, high SLA leaves in I. pumila (Sultan and Bazzaz 1993, Evans and Poorter 2001). The low irradiance treatment was particularly limiting because there were no occasional sunflackes as would occur within their naturally shaded habitats.

Along with leaf morphological changes, adaptation to low irradiance may also involve alterations in foliar biochemistry such as reallocation of leaf nitrogen from soluble proteins into pigment-protein complexes. There are numerous reports showing that in many plant taxa leaf chlorophyll concentration increases with decreasing irradiance (Niinemets 1997, Pindato et al. 1997, Niinemets et al. 1998, Cao 1999, Frak et al. 2001, Mommer et al. 2005). Because light-energy absorbance scales directly with chlorophyll content per unit leaf area and because leaf thickness decreases with decreasing irradiance, a shade-induced increase in leaf chlorophyll content is believed to be necessary to enhance the quantum yield of photosynthesis for an incident light (Niinemets et al. 1998). In our study, the changing light conditions did not alter total content of leaf chlorophyll per area in I. pumila, but did change relative amount of the pigment-protein complexes, as evidenced by a slight shift in the mean $Chl_a/b$ ratio across light treatments (Fig. 1). Although there was a general increase in both the average magnitude of plasticity and between-family variation in plastic responses to decreasing irradiance in both chlorophyll traits, a statistically significant difference was observed only for the CV value in plasticity of ChlT in the “Wood” population (Table 1). The observed results clearly indicate that acclimation to low light by the I. pumila seedlings was dominated by plastic changes in leaf morphology, which maximizes the amount of photosynthetic tissue per unit of leaf area rather than by plasticity in foliar biochemistry. Thus, variation in the photosynthetically active leaf surface area per biomass would be a major determinant of functional variation in shade-tolerance among natural I. pumila populations. As the plasticity means for leaf size in populations from ecologically distinct light habitats differed significantly only at very low irradiance, this indicates that the principal difference between these populations is not their response flexibility to light availability in general but “the capacity for appropriate plastic response to the particular challenge of low light intensity” (Sultan 2003).

4.2. Population differentiation in the magnitude of plasticity to irradiance

The hypothesis that adaptive plasticity evolves under natural selection imposed by
heterogeneous environmental conditions predicts that populations and/or species that experience relatively variable environments should exhibit a greater magnitude of phenotypic plasticity (Roff 1992, Linhart and Grant 1996, Lortie and Aarssen 1996, Van Buskirk 2002, Sultan and Spencer 2002, Griffith and Sultan 2005). This prediction was tested by comparing leaf morphogenetic responses to changing irradiance level in two *I. pumila* populations from contrasting light habitat. The irradiance gradient created in the study evoked similar direction of plastic responses in each of the examined leaf traits, irrespective of the population origin, indicating that such responses might be quite general (Fig. 1). Conversely, the average magnitude of plasticity appeared to be higher in the “Wood” than in the “Dune” population, but exclusively between the medium and low light treatments. The observed population divergence in foliage plasticity of *I. pumila* could be interpreted as the adaptive outcome of different selection history they have experienced within their natural habitats. At open dune sites - such as those inhabited by the “Dune” population - herbaceous plants of low stature experience horizontally more homogeneous patchy light conditions, compared to plants from a woodland understory which are exposed to a spatially and temporally highly unstable light climate near the forest-floor (Dong 1995). Relative to an exposed dune site, *I. pumila* plants from a forest understory respond to shading by producing larger leaves, a phenotypic response that is commonly thought to be functionally advantageous in shady environments. A phenotypic selection gradient analysis using a synthetic *I. pumila* population of known genotypes transplanted in two natural habitats with contrasting light conditions documented that the observed variation in leaf size associated with light availability was comparable to the estimated phenotypic selection gradients, which detected stronger natural selection favoring larger leaves in the woodland than in the open dune habitat (Tucić et al. 1998). It has been theoretically shown that any kind of temporal heterogeneity in the environment selects for more plastic genotypes but temporal variance within generations plays a more important role than that between generations (Lynch and Gabriel 1987). Spatial heterogeneity may also select for a higher degree of plasticity, but only when it operates in conjunction with the within-generation temporal heterogeneity (Lynch and Gabriel 1987). Since under *Pinus* canopy – a shady habitat of *I. pumila*, long periods of diffuse light are interrupted unpredictably by sunflacks and small gaps, this great temporal heterogeneity in the light availability (together with existing spatial variance) may have selected for more plastic genotypes there, promoting in this way evolutionary divergence between the “Dune” and the “Wood” population.

Population differentiation between plants from open and shaded habitats in response to light availability has been suggested for a number of plant species (Bain and Attridge 1988, Schmitt 1993, Dudley and Schmitt 1995, Donohue et al. 2000, Balaguer et al. 2001). Natural populations of *Impatiens capensis* Meerb., an herbaceous annual from open and woodland habitats, differed genetically in sensitivity to irradiance in a variety of traits including axillary meristem allocation to branches (Schmitt 1993, Dudley and Schmitt 1995, Donohue et al. 2000). Relatively greater plastic responses to growth irradiance in the exposed population than in the shaded one were interpreted as local adaptation to fine scale heterogeneity in this light environment caused by density variation (Schmitt 1993, Dudley and Schmitt 1995, Donohue et al. 2000). Population divergence in the plastic responses to the ambient light conditions was also observed in *Quercus occifera* L. (Balaguer et al. 2001), an evergreen oak, which encounters light environments of contrasted heterogeneity in the Mediterranean Basin like relict ocean forests, garrigue landscape and exposed rock outcrops. When averaged over several light-responsive traits, the magnitude of plasticity in *Q. coccifera* populations ranked in a way corresponding to their habitat heterogeneity; that is, forest> garrigue> rock. In the study on shade tolerance plasticity in response to neutral vs green shade cues in *Polygonum* species of contrasting ecological breadth – *P. persicaria* L., occurring in an extremely broad range of different habitats and *P. hydropiper* L., restricted to consistently high light
sites – it has been found that the magnitude and direction of plasticity to neutral shade in these species correlated with their ecological distribution (Griffith and Sultan 2005). This suggests that a stronger shade-tolerance responses (increased leaf allocation and leaf area ratio) to reduced irradiance level than to reduced the $R:FR$ ratio expressed by *P. persicaria* could be causally related to their widespread distribution with respect to light conditions (Griffith and Sultan 2005).

In conclusion, given that low light availability inevitably reduces photosynthetic rate per unit leaf area, developmental plasticity in leaf morphology, especially SLA, can maximize the amount of photosynthetically active surface area for light interception, allowing in this way sustainable plant growth despite limited light conditions. The obtained results corroborate the hypothesis that temporal environmental heterogeneity in the light conditions within generations (in conjunction with spatial heterogeneity) can select for a higher degree of plasticity in functionally important leaf attributes. The populations’ differences in *I. pumila* with respect to the magnitude of plasticity in leaf responses to variation in light intensity are consistent with the hypothesis of adaptive divergence in plasticity.

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5. REFERENCES


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