

PERFORMANCE OF *Iris variegata* GENOTYPES IN DIFFERENT LIGHT CONDITIONS: FLOWERING PHENOLOGY AND REPRODUCTIVE OUTPUT

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We analyzed genetic variability and phenotypic plasticity of flowering pattern and reproductive success in 97 clonal genotypes of *Iris variegata* originating from contrasting light habitats in Deliblato Sands and expressed under different experimental light conditions. Rhizome segments were taken from each of these clones and transplanted in the experimental garden near the Institute for Biological Research in Belgrade. Significant differences between genotypes were found for two traits (start of flowering, average flowering time). Genotypes originating from open and understory habitats significantly differed for three traits (number of flowers, number of capsules/number of flowers, seed mass/capsule). Significant effect of light treatment were found for three traits (number of capsules/number of flowers, seed mass/capsule, average seed mass). Statistically significant correlations between explored traits were generally similar but also to some extent habitat and treatment specific. Comparing these results with research carried out on congeneric species we noted that there are similar responses for some traits, but also significant differences in some components of flowering and fruiting success.

Key words: genetic variability; flowering phenology *Iris variegata*; light environment; reproductive output

INTRODUCTION

The flowering behavior of higher plants, as almost all aspects of plant development, is modified by environmental signals (FITTER and HAY 2001, JENKS and HASEGAVA 2005, LAMBERS *et al* 2008). Light is the foremost of these environmental signals and can modify flowering in several different ways. Many plant physiological studies have shown that environmental light acts to regulate flowering through the three main variables - quality, quantity and duration (THOMAS

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2006), while phenological patterns can be also influenced by genetic variability and phylogeny (DAVIES *et al* 2013)

Plants grown under canopy shade conditions show a range of responses to the change in light quantity as well as in red/far-red ratio of the ambient light, i.e. light quality. These responses, known as the shade-avoidance syndrome, or near neighbor detection response, are characterized, among others, by changes in flowering phenology and reproductive output. However, these responses to changes in light quantity and quality are very variable and different for species inhabiting predominantly open in comparison to species inhabiting predominantly shaded habitats (TARASJEV 1997, MATTSON and ERWIN 2005, BARIŠIĆ *et al* 2006, MILJKOVIĆ 2009, TARASJEV *et al* 2009).

The individual's flowering pattern is defined by the timing or temporal distribution of flowering (start of flowering, average flowering period), the duration of flowering, as well as the number of flowers produced. The flowering pattern has a dramatic influence on reproductive success of plants, and therefore a shift in the flowering phenology in response to selection is likely to influence the expression of subsequent reproductive traits (number of fruits, or number and size of seeds). Flowering phenology leading to successful seed production depends on factors that may vary in space and time, such as pollination and/or pollen removal, availability of resources for fruit and seed ripening, and avoidance of seed predators (BRODY 1997). As a consequence, reproductive success depends not only on the mean time of onset of flowering (OLLERTON and LACK 1998), but also on the variation around this mean time (HENDRY and DAY 2005). The period during which flowering yields the greatest seed set may vary from year to year, depending on the availability of resources and pollinators, and on the abundance of seed predators and pathogens (PICO and RETANA 2001, MAHORO 2002).

In outcrossing plants, flowering patterns determine the outcome of selection by influencing the reproductive output and are directly linked to the plant establishment, persistence, spread, and abundance. Yet little is known about constraints on the timing, duration, number of flowers, fruiting or seed forming, and the selective forces that shape the evolution of these various patterns. Furthermore, temporal distribution, duration, number of flowers produced, number of fruits and seeds have often been examined in isolation from one another (MOLAU *et al* 2005, KAWAGOE and KUDOH 2010). These functionally related traits that constitute reproductive timing, including seasonal patterns of flower production, fruit maturation and seed dispersal are likely to be phenotypically integrated and interact with each other to shape the diversity of flowering and reproductive patterns observed in nature (PIGLIUCCI 2003, EDWARDS and WEINIG 2011). Identification of correlation patterns between reproductive traits that characterise a complex phenotype and its environmental variability (plasticity of correlation) is important for the selection of traits associated with fitness, since correlations can act as constraints in the formation of the optimal flowering phenotype (SCHLICHTING 1989, DONOHUE *et al* 2000, BAWA *et al* 2003). Such interactions between components of flowering pattern and reproductive output had seldom been explored in variable environmental conditions although it has long been known that the variability in light, temperature, water and other abiotic as well as biotic factors considerably influence expression of these traits (KELLY and LEVIN 2000, JENTSCH *et al* 2009, FORREST and MILLER-RUSHING 2010, MUNGUIA-ROSAS *et al* 2012, GALLOWAY and BURGESS 2012).

Despite selection pressures acting on flowering and reproductive patterns, significant inter- and intra- population variability are demonstrated in many plant species (PILSON 2000, SOLA and EHRLÉN 2007, TARAYRE *et al* 2007, KLEUNEN 2007, TORANG *et al* 2010). Within-

population reproduction asynchrony could be due to relaxed selection on natural genetic variability and/or environmental heterogeneity.

Our research was conducted on *Iris variegata* L. (Hungarian, variegated or multicolored iris) a rhizomatous perennial clonal herb. In the presence of both sexual and vegetative reproduction, the relationship between flowering phenology and fitness becomes more complex (STOCKLIN and WINKLER 2004) On the other hand, vegetative reproduction allows experiments in which genetically identical individuals are grown in different environmental conditions. This makes *Iris variegata* a suitable object for detecting genetic and environmental variability in flowering and fruiting patterns.

In the field studies of flowering patterns, environmental and genetic differences are usually confounded, so conclusions can be misleading. One solution is to study flowering phenologies under controlled experimental conditions. In this study, we conducted a common-garden experiment in order to analyze flowering pattern and reproductive success in genotypes of *Iris variegata* originating from two habitats with contrasting light conditions that have been transplanted in two experimental light treatments. We address the following questions: a. Is there genetic variability in flowering pattern and reproductive success expressed under experimental conditions? b. Does the pattern of response to light treatments differ between genotypes originating from open and understory habitats? c. Are there statistically significant correlations between explored traits and do they depend on habitat and experimental conditions? d. Are patterns of flowering and seed forming in *Iris variegata* similar to patterns previously observed on congeneric *Iris pumila*?

MATERIALS AND METHODS

Study species

Iris variegata is native to the area stretching through central and southeastern Europe to Ukraine, and can be found in southwestern Germany, southern Czech Republic, Austria, southern Slovakia, Hungary, western Ukraine, Bulgaria, southern Romania and north Serbia. It is endangered and protected species in the Czech Republic and Slovakia. *I. variegata* is a rhizomatous perennial clonal herb with sturdy swordlike leaves and tall stems (up to 90 cm long) bearing two or more flowers. The scentless flowers appear in the early summer, mainly May and June. Each flower measures up to 5–7 cm. The standards (inner petals) are yellow, and the falls (outer petals) are white to pale yellow, with red to purple veins, sometimes fusing into a purple blotch. The style branches and the beard are yellowish.

This species is a good model system in evolutionary ecology research for several reasons. This is an open-pollinated species with large hermaphrodite flowers that are easy to pollinate and for which, due to its structure, pollination is easily controlled. Seeds are large and easy to manipulate. Reproduction is vegetative (rhizomes) that enables obtaining a larger number of replicas of the same clone (genotypes), and sexual (seeds) so that the controlled crossings may produce offspring with different degrees of kinship. *I. variegata* grows mostly in arid grassy and open forest habitats. Its natural habitats differ in many ecological indices, foremost in quality and intensity of light and can generally be divided into open habitats where the plants are exposed to full sunlight, and shaded habitats in the ground floor of the different forest stands with a reduced intensity and changed light quality.

Experimental design

I. variegata in Serbia appears in small and fragmented populations. Plant material for this experiment were collected in 2011, during the flowering phase of *Iris variegata*, when 97 clonal genotypes were chosen. These genotypes were inhabiting locations with contrasting light conditions in Deliblato Sands (Open and Shaded), protected sandy area 40 km NE from Belgrade, Serbia. Rhizome segments were taken from each of these clones and transplanted in the experimental garden near the Institute for Biological Research in Belgrade. Plants were allowed to acclimate during one growing season (2012). Experimental treatments utilized in this study differed in the amount of available light as well as in ratio of red light to far-red light (R/FR), resembling natural vegetative shadow. Difference in light treatments is achieved by setting the PVC shading net which leads to a reduction of light intensity, and decrease in R/FR ratio. Replicas of each clone were assigned to each of the four experimental blocks- two blocks with higher light intensity and higher red/far-red ratio (High treatment) and two blocks with lower light intensity and lower red/far-red ratio (Low treatment). These conditions mimic the light conditions in *I. variegata* natural habitats.

Flowering and reproductive output were recorded during the flowering season in May and June of 2013. Flowering pattern was described by four traits: start of flowering (# of days from the first flower occurrence in the whole experiment, SF), duration of flowering (# of days from the first to the last flower occurrence in the single pot, DF), number of flowers (# of flowers in the pot, NF), and average flowering time (mean flowering time for all flowers in the pot, AFT). Pollination success was measured by five traits: number of capsules (# of capsules in the pot, NC), the ratio of the number of capsules to the number of flowers (NC/NF), number of seeds per capsule (# of seeds, NSC), seed mass per capsule (g, SMC) and average seed mass per capsule per pot (g, ASM).

Statistical analysis

The descriptive statistical analysis of the data obtained in this experiment was carried out using the MEANS procedure of the SAS statistical package (PROC MEANS, SAS INSTITUTE 2011) for each of the analyzed characteristics. Norms of reaction for nine traits are presented using mean values of genotypes in each of the experimental light treatments (High and Low) and from both habitats (Open and Shaded). To compare the distribution of the number of flower stalks in genotypes originating from the two habitats we used the Chi-square statistic in FREQ procedure (SAS INSTITUTE 2011)

To investigate the effect of different genotypes origins (type of habitat) on phenotypic variation of the flowering phenology and fruiting success of *I. variegata* manifested in the two experimental light treatments, we used two-way mixed analysis of variance (ANOVA), in which the type of habitat and light treatments were treated as fixed factors (two distinct type of habitat- Open and Shaded; and two specific light treatments- High and Low), and genotypes nested within habitat as a random factor (selected genotypes are random samples of all genotypes). For this analysis, we applied the GLM procedure of the SAS statistical package (SAS INSTITUTE, 2011). To achieve normality and homoscedasticity, the data set was analyzed by the method described by BOX et al (1978) and SAS programme given by FERNANDEZ (1992) and appropriate transformations were applied. The mixed-model analyses of variance (proc GLM, SAS INSTITUTE, 2011) were computed for each trait in order to evaluate the following sources of phenotypic variation: light treatment (phenotypic plasticity), habitat (genetic variation between genotypes

from different habitats), clone (genetic variation within habitat), treatment by habitat interaction (variation for plasticity between habitats), treatment by clone interaction (genetic variation for plasticity between genotypes in response to light treatment), block (microenvironmental variation).

Phenotypic correlations between pairs of traits within each light treatment were calculated by the CORR procedure (SAS Institute 2011). Ratio of the number of capsules to the number of flowers was not included in correlation analysis due to potential problem with “spurious correlations” (JACKSON and SOMERS 1991). Pearson’s coefficient of correlations were calculated separately for genotypes originating from Open and Shaded habitat. We used a formula of COHEN *et al* (2003) to compare correlation coefficients of the same pair of traits between light treatments for each habitat separately.

RESULTS

In this study on *Iris variegata* we detected several statistically significant Treatment, Habitat and Clone-within-habitat effects (Table 1) on analyzed traits, while some of effects on particular traits closely approached chosen level of significance of 0.05 (including some of the interaction effects) which indicate that they should also be given attention in further studies.

Table 1. Results of mixed-model analyses of variance performed on nine traits of *Iris variegata* genotypes originating from two natural habitats and observed under two light treatments.

Source of variation	df	Start of flowering (number of days)		Duration of flowering (number of days)		Average flowering time (number of days)		Number of flowers	
		F	P	F	P	F	P	F	P
Treatment	1	2.47	0.1210	3.20	0.0782	0.20	0.6555	3.47	0.0672
Block	1	0.01	0.9247	2.22	0.1397	0.23	0.6339	1.04	0.3112
Habitat	1	0.17	0.6804	2.66	0.1064	1.32	0.2529	19.85	0.0001
Clone(habitat)	89	1.79	0.0070	1.25	0.1701	1.94	0.0026	1.19	0.2304
Treat*Habitat	1	0.00	0.9842	1.61	0.2074	0.05	0.8219	1.09	0.2988
Treat*Clon(hab)	65	1.22	0.1886	3.90	0.0521	1.37	0.0800	0.94	0.5976
Error	249								

Source of variation	df	Number of capsules		No. of capsules/ No. of flowers		Number of seeds/ capsule		Seed mass/capsule (g)		Average seed mass (g)	
		F	P	F	P	F	P	F	P	F	P
Treatment	1	1.07	0.3054	11.20	0.0016	3.99	0.0512	9.75	0.0030	7.68	0.0079
Block	1	0.20	0.6580	0.56	0.4586	0.70	0.4067	0.42	0.5215	0.03	0.8679
Habitat	1	2.71	0.1036	6.85	0.0105	0.83	0.3643	5.16	0.0256	3.23	0.0756
Clone(habitat)	89	0.73	0.9037	0.99	0.5327	1.29	0.1662	0.97	0.5601	1.46	0.0599
Treat*Habitat	1	0.41	0.5225	3.17	0.0798	0.00	0.9563	0.31	0.5799	0.15	0.7032
Treat*Clon(hab)	65	1.29	0.1725	0.88	0.6771	0.93	0.6071	1.07	0.4012	1.14	0.3135
Error	249										

Statistically significant effects of both treatments (i.e. phenotypic plasticity) and habitats (genetic variability) were obtained for capsule to flower ratio ($p=0.0016$ and $p=0.0105$, respectively) and seed mass per capsule ($p=0.0030$ and $p=0.0256$, respectively) (Table 1). The

ratios were higher in plants from the Open habitat (Figure 1). Genotypes had lower capsule to flower ratio in Low light treatment compared to High light treatment, as well as lower ratio in genotypes from Shaded habitat and there is indication that this difference (slope of norm of reaction, Figure 1) can be greater in High light treatment (significance level of 0.08, Table 1). Seed mass per capsule was higher in plants growing in Low light treatment regardless of their origin (Figure 1). Genotypes originating from Shaded habitat had higher seed mass per capsule in both light treatments (0.37g vs. 0.33g and 0.49g vs. 0.43g).

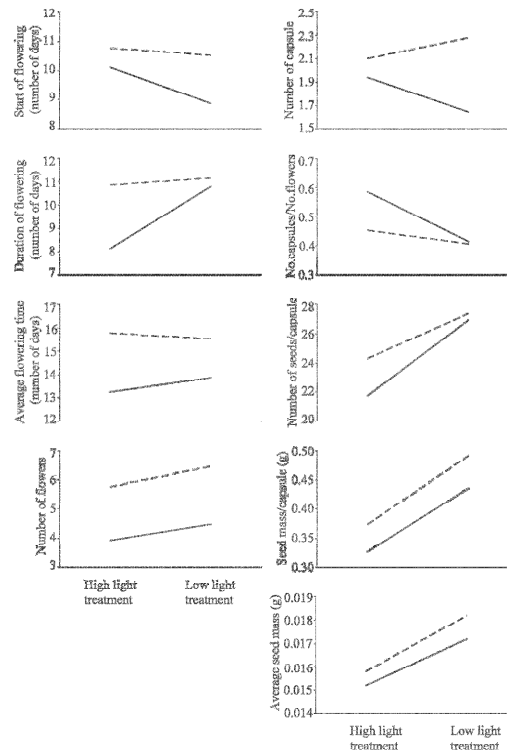


Figure 1. Mean reaction norms for nine traits of *Iris variegata* genotypes originating from the Open habitat (full lines) and the Shaded habitat (dashed lines) grown in light treatments (the High and the Low ones).

For average seed mass only Treatment effect was significant at $p < 0.05$ level with higher values in Low light treatment (Figure 1). However, for this trait effects of Habitat and Clone within habitat closely approached significance levels ($p < 0.08$ and $p < 0.06$, respectively) which

indicates the need for further investigation of genetic variability of this trait both on within and between habitat levels. Treatment effect was extremely close to chosen significance level of 0.05 for number of seeds per capsule ($p=0.0512$) indicating that there can be a greater number of seeds in low light treatment (Figure 1).

I. variegata genotypes originating from Shaded habitat exhibited higher number of flowers comparing to plants from Open habitat (Figure 1) and this difference was statistically significant in ANOVA (effect of Habitat, $p<0.0001$, Table 1). Treatment effect was close to significance ($p=0.0672$) indicating the possible higher number of flowers in Low light treatment (Figure 1), as well as for duration of flowering ($p=0.0782$).

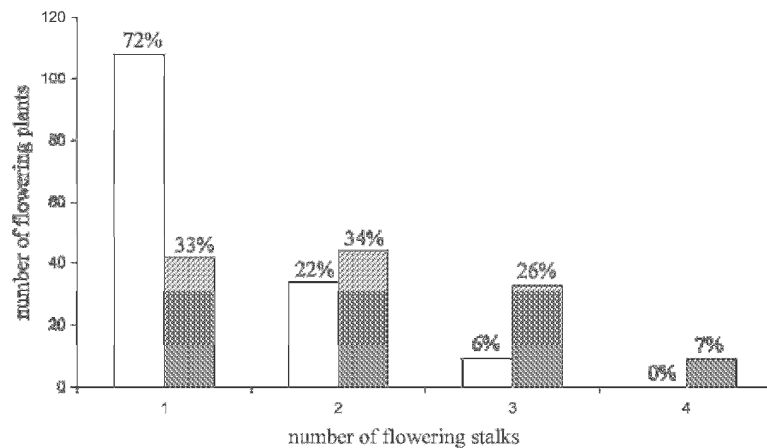


Figure 2. Frequency of *I. variegata* plants with one, two, three or four flowering stalks in genotypes originating from Open habitat (blank columns) and genotypes originating from Shaded habitat (cross-hatched columns).

ANOVA for day of first flower emergence and average flowering time revealed statistically significant differences between genotypes of *I. variegata* (significant genetic variability within habitats, $p=0.0070$ and $p=0.0026$, respectively; Table 1) while Treatment x Clone(Habitat) interactions were close to chosen significance level for duration of flowering ($p=0.0521$) and average flowering time ($p=0.08$).

Total number of flowering plants in experiment was two hundred and seventy nine. Slightly higher percentage of flowering plants was observed within High light treatment (54% vs. 46%). Number of flowering branches differed between genotypes originating from two habitats (Figure 2). Frequency of *I. variegata* plants with one flowering stalk were much higher for genotypes from Open habitat (72%) than for plants originating from Shaded habitat (33%). On the contrary, frequency of pots with two or three flowering stalks were much higher for genotypes originating from Shaded habitat (34 vs. 22% and 26 vs. 6%). Seven percent of genotypes from the Shaded habitat developed four flowering stalks in contrast to the plants originating from the Open habitat where there was not any (Figure 2). Chi-Square statistic revealed significant difference in

frequency distribution of number of flowering stalks between genotypes originating from Open and Shaded habitats ($p < 0.0001$).

Pearson's correlation coefficients between eight traits are presented in Figure 3. Analyzed *I. variegata* traits can be divided into two groups: the first group were the flowering phenology traits and the second group were the reproductive success traits. Phenotypic correlations between traits of *I. variegata* were influenced by environmental conditions and place of origin. Number of significant correlations were almost the same across all treatments and habitats (10 or 11).

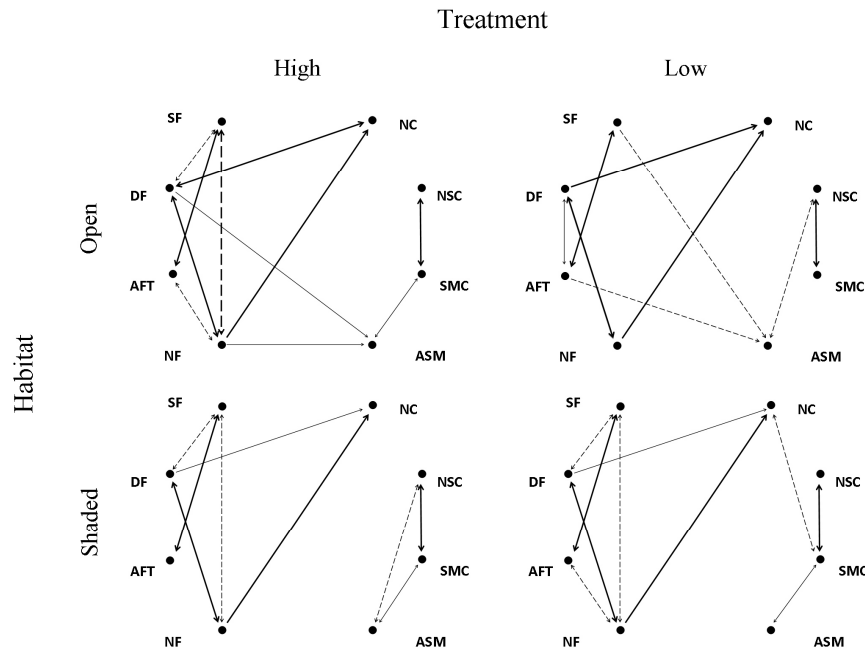


Figure 3. Changes in correlation structure across light treatments (High vs. Low) as well as habitat of origin (Open vs. Shaded) for a genotypes of *Iris variegata* from Deliblato Sands, Serbia. Solid lines indicate significant ($P < 0.05$) positive Pearson correlations; dashed lines indicate significant negative correlations. Thick lines indicate correlations strength above 0.50; thin lines indicate correlations strength below 0.50. For trait abbreviation see Material and Methods.

In the first group of traits, start of flowering and the average flowering time which were positively correlated among themselves were negatively correlated with the length of flowering and the number of flowers (the latter two traits were positively correlated).

For plants originated from Open habitat, start of flowering and average flowering time were significantly negatively correlated with reproductive output only in Low light treatment, and positive correlation with reproductive output was detected for the duration of flowering and number of flowers only in High light treatment.

In the second group (reproductive traits) seed mass/capsule positively affected number of seeds and average seed mass. There were trade-offs between seed number and average seed mass (negative correlations between these traits in plants originating from Open habitat and planted in Low light treatment as well as in plants originating from Shaded habitat and planted in High light treatment).

The differences between correlation coefficients between two light treatments for each pair of traits were tested separately for Open habitat and Shaded habitat. Significant difference between correlations for genotypes from Shaded habitat was observed for only one pair of traits (start of flowering and average flowering time). For *I. variegata* plants from Open habitat number of significant differences were much higher (start of flowering and average flowering time; start of flowering and number of flowers; duration of flowering and average flowering time; average flowering time and number of flowers; average flowering time and number of seeds /capsule; seed mass/capsule and average seed mass; number of seeds /capsule and average seed mass).

DISCUSSION

Effects of different intensity and quality of light on various reproductive traits were detected previously on many plant species (SEDDIGH and JOLIFF 1994, STEINGER *et al* 2003, QUINET *et al* 2004, MATTSON and ERWIN 2005, THOMAS 2006, JOHNSON 2007). During the previous research, on the related species- *Iris pumila* L., inhabiting the same territory but to some degree different habitats than *Iris variegata*, it was observed that the quality and quantity of light significantly affect the flowering and seedling characteristics (TARASJEV 1997, TARASJEV *et al* 2009, BARIŠIĆ *et al* 2012). Because *I. pumila* mostly occurs at sun-exposed, open sites, it can be classified as a shade-avoiding species, unlike *I. variegata* that inhabits almost equally often sun-exposed and understory sites. These previous data were used to compare phenotypic responses of two related *Iris* species.

Start of flowering in *I. variegata* was not significantly different in two light treatments. The same pattern was observed in research of AHMED *et al* (1993) on *Vigna unguiculata* where they manipulated red to far-red ratio. On the other hand, HALLIDAY *et al* (1994) revealed that lowering of red to far-red ratio accelerates flowering in *Arabidopsis thaliana*. Data concerning light intensity are diverse, too. BROWNSEY *et al* (2014) concluded that photoperiod, not light intensity, is the primary signal for the reproductive initiation. Increasing light intensity reduced days to the first flower occurrence in *Limnanthes alba* (SEDDIGH and JOLIFF 1994), *Sinapis arvensis* (STEINGER 2003) and *Plukenetia volubilis* (CAI 2011). TARASJEV (1997) stated that *I. pumila* clones growing in more exposed sites tend to flower earlier. Differences between genotypes originating from distinct habitats persist even in transplanted plants of this species. These distinctions between two species could be the consequence of *I. pumila* being an early-spring flowering species and different selective pressures could act at early summer when *I. variegata* is flowering.

Data concerning variations in flowering duration are numerous and generally are environment- and species- specific (LACEY *et al* 2003, TARAIRE *et al* 2007). Differences between

light treatments in duration of flowering in *I.variegata* closely approached chosen significance level ($p=0.052$). Direction of differences was similar to the one detected on *Limnanthes alba* by SEDDIGH and JOLIFF (1994) who had found significant shortening of flowering duration with increasing photosynthetic active radiation. Plants growing in more variable environmental conditions (more exposed habitat in our case) are expected to express greater phenotypic plasticity (BAZZAZ 1979, LORTIE and AARSSEN 1996) but we failed to detect significant treatment x habitat interactions in *I.variegata*. In congeneric *I.pumila* differences for average flowering time were statistically significant (genotypes from Open habitat and all clones in High light treatment flowered earlier) (TARASJEV 1997) but we failed to detect those differences in *I.variegata*. Number of flowers was significantly higher in *I.variegata* genotypes originating from Shaded habitat (but higher number of flowering stalks was recorded in genotypes from Open habitat). Between-habitat differences in flower number were similar in both treatments, while difference between treatments was very close to chosen significance level ($p=0.067$). Contrary to that, in *I.pumila* plants significantly higher flower number was observed in Hillock (open) habitat and population (TARASJEV *et al* 2009).

The absence of significant differences between light treatments for number of capsules was observed in *I.variegata* as well as in rhizomatous perennial *Smilacina japonica* (TAKASHI and KUDO 2009). In related *I.pumila* number of capsules was higher in the open site (TARASJEV *et al* 2009). In *I.variegata*, as well as in *I.pumila*, proportion of fruited flowers were significantly higher in plants growing in High light treatment (TARASJEV *et al* 2009). Significant differences were observed between genotypes from different habitat types in *I.variegata* but not in *I.pumila*. Number of seeds per capsule in *I.variegata* and in *I.pumila* was not significantly different between light treatments, but in *I.variegata* it closely approached significance level ($p=0.051$) marking the tendency for having more seeds per capsule in Low light treatment. This is different from studies of STEINGER *et al* (2003) where *Sinapis arvensis* formed 76% fewer seeds in Low light environment.

Significantly higher seed mass per capsule and average seed mass in Low light treatment in *I.variegata* was expected since seed mass is an especially important component of shade tolerance of many species (PORTER and ROSE 2005, but see GALLOWAY 2001). Seed size is one of the most important traits influencing the early phases of the plant's life cycle, including germination, emergence, growth, and survival of seedlings (QUERO *et al* 2007). Flowering and fruiting requires an input of energy and nutrients. Therefore, resource abundance and a plant's ability to assimilate and allocate these resources may influence these phenological patterns. It is obvious that our Low experimental treatment (low light intensity and low R/FR ratio) does not affect these phenology traits only in a passive way (by limiting resources) since in this treatment we obtained significantly higher total seed mass with lack of differences in total seed number. *I.pumila* plants originating from shaded habitat had larger seeds that germinate earlier and produce heavier seedlings (AVRAMOV 2008). The similar advantage of possessing heavier seeds could exist in *I.variegata* but this remains to be confirmed.

Significant differences between light treatments were observed only for three traits in *I.variegata* and all were related to reproductive output. Significant genotypic variability was observed for two traits related to flowering phenology. These results may suggest that there is a little genetic variability within habitats especially for reproductive pattern and that local light environments have a more pronounced impact in some of these traits.

There are a few of studies where number of flowering stalks were compared in different light conditions. In most of those works shading significantly reduced the number of flowering branches, or had no effect (DAWIS *et al* 1995, ENDELS *et al* 2005). It is interesting that beside our analysis on *I. variegata*, only NAUMBURG *et al* (2001) found significant increase in number of flowering stalks on *Festuca arizonica*, species with low shade tolerance, as response to shading.

There are numerous evidences that the evolution of a phenotype is affected by correlations among traits (SCHLICHTING 1989, PIGLIUCCI *et al* 1995, BAWA *et al* 2003, AVRAMOV *et al* 2007, BOLMGREN and COWAN 2008). Similar number of correlations in both treatments and in *I. variegata* plants from both habitats indicates a similar level of integration and potentially similar degree of selection pressures. Some trait relations show the same pattern in all treatments and habitats. For example, significant positive correlations between duration of flowering and the number of flowers and the number of capsules, as well as between seed mass and seed number per capsule were extremely stable despite changes in light availability and despite the origin of plants (habitat). Although the seed number *vs.* seed size trade-off had firm theoretical foundation (SMITH and FRETWELL 1974, LLOYD 1987), to our knowledge, it had not often been subjected to this kind of analysis (see JAKOBSSON and ERIKSSON 2000). In *I. variegata* there is a trade-off between seed mass and seed number (significant negative correlations) in clones originating from Open habitat and growing in Low light treatment as well as in clones originating from Shaded habitat and growing in High light treatment. Some correlations were environment or/and habitat-specific. For example, there were trade-offs between start of flowering and duration of flowering and number of flowers in three of four combinations of habitat-environment. Only in *I. variegata* plants from Open habitat growing in Low light treatment these correlations were not significant. Unlike our research, SCHMITT (1983) did not obtain significant correlations and SEDDIGH and JOLIFF (1994) obtained significant but positive correlations between these traits.

We detected variability in structures of multivariate flowering and fruiting phenotypes, and that variability was affected by environmental conditions (light environments). Similar effects of environments were observed in few other research (SEDDIGH and JOLIFF 1994, GALLOWAY and BURGESS 2012). Larger number of significant differences in correlations between two light treatments for *I. variegata* plants originating from Open habitat indicate lower stability in variance-covariance matrices. These findings demonstrate that the analyzed traits can be selected and generally evolve in different ways in different environments and populations, with low genetic variability as a potentially main constraint. Therefore, to understand potential life cycle consequences of the evolution of multivariate flowering phenotype, correlated responses to selection must be studied in ecologically relevant habitats and included more conspecific populations.

Comparing the results of research carried out at two congeneric species (*I. variegata* and *I. pumila*), we noted that there are similar responses for some traits, but also significant differences in some components of flowering and fruiting success. These differences could originate from various causes and it stresses the importance of the comparative approach. While *I. pumila* as a species occupying predominantly open habitats showed many responses that are expected in "Shade avoidance syndrome" (TARASJEV 1997, TARASJEV *et al* 2009) those responses were often lacking in *I. variegata*. These differences between *I. variegata* and *I. pumila* are also to some extent dissimilar to situation of phylogenetic conservatism in flowering phenology observed for a number of species in work of DAVIES *et al* (2013).

Although addressing complex biological issues such as the evolution of flowering phenology and phenotypic integration requires a larger number of studies to incorporate all relevant causal factors, present results allowed us to derive some important conclusions, as well as aid us in outlining follow-up studies concerning, for example, the joint effects of several environmental factors, complex relationship between flowering phenology and fitness in clonal species, between-generation effects and other related issues.

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**ODGOVOR GENOTIPOVA *Iris variegata* NA RAZLIČITE SVETLOSNE USLOVE:
FENOLOGIJA CVETANJA I REPRODUKTIVNI USPEH**

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Izvod

U ovom radu analizirali smo genetičku varijabilnost i fenotipsku plastičnost osobina cvetanja i reproduktivnog uspeha kod 97 genotipova *Iris variegata* koji nastanjuju različita svetlosna staništa u Deliblatskoj Peščari. Delovi rizoma ovih genotipova su presađeni u eksperimentalnu površinu pored Instituta za biološka istraživanja u Beogradu i gajeni u dva eksperimentalna svetlosna tretmana. Statistički značajne razlike između genotipova su uočene za dve osobine: početak cvetanja i prosečno vreme cvetanja. Genotipovi *I. variegata* koji potiču iz dva različita staništa u Deliblatskoj Peščari se razlikuje za tri osobine: broj cvetova, odnos broja čaura i broja cvetova i mase semena po čauri. Značajan efekat svetlosnog tretmana je takođe uočen kod tri osobine: odnos broja čaura i broja cvetova, mase semena po čauri i prosečne mase semena. Fenotipske korelacije između ovih osobina cvetanja i plodonošenja su bile generalno slične ali su pokazivale i zavisnost od svetlosnog tretmana kao i od porekla genotipova (tipa staništa). Poredeći rezultate ovog istraživanja sa podacima dobijenim na srodnoj vrsti uočeno je da neke osobine pokazuju slične odgovore ali da takođe postoje značajne razlike u nekim od osobina koje opisuju cvetanje i plodonošenje.

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