

## DEVELOPMENTAL STABILITY OF *IRIS PUMILA* FLOWER TRAITS: A COMMON GARDEN EXPERIMENT

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*Abstract* - *I. pumila* natural populations usually occur in two different habitat types: dune and forest. These *I. pumila* habitats differ in many abiotic environmental factors, but mostly in available light intensity and quality. The effects of different light intensity on the developmental stability of *I. pumila* floral traits were analyzed on clones taken from two different natural light habitat types that were raised in contrasting light treatments in experimental garden conditions (common garden experiment). As an indicator of developmental stability, we used two fluctuating asymmetry indices (FA1 and FA8a) of three bilateral symmetric traits of *I. pumila* flower (FW-fall width, SW-standard width and STW- style branch width). In addition, statistically significant treatment x population interaction was observed for style width. According to the presented results, the observed FA patterns of particular traits did not reflect the whole organism buffering capacity under the given environmental conditions.

*Key words:* Developmental stability, flower fluctuating asymmetry, local adaptation, common garden experiment, *Iris pumila*

### INTRODUCTION

Developmental stability is defined as the set of mechanisms that maintain a constant phenotype despite small random deviations (Debat and David, 2001), i.e. it is an organism's ability to maintain stable development despite environmental and genetic deviations (Rao et al., 2002). On the other hand, developmental instability represents morphological changes that arise as a response to deviations during the developmental process itself (Klingenberg and Nijhout, 1999; Klingenberg, 2003; Nijhout and Davidowitz, 2003; Van Dongen, 2006). Developmental instability is the result of cellular stochastic disturbance processes during development, such as developmental noise (Klingenberg, 2003) under specific environmental conditions (Polak, 2003). Random deviations in cellular developmental processes cause the differences between the homolog parts of an in-

dividual (Debat and David, 2001). Deviations from symmetrical traits (bilateral or radial) are a measure of developmental instability and are their phenotypic result (Klingenberg, 2003; Van Dongen, 2006). Any type of symmetry deviations could be considered as the developmental system's inability to maintain stable development despite random deviations (Van Dongen and Talloen, 2007).

Developmental instability at the individual and population levels can be estimated by the fluctuating asymmetry values of the analyzed bilaterally symmetrical traits of an individual. Fluctuating asymmetry (FA) is the result of small random deviations from symmetry of bilaterally symmetrical traits (Moller and Swaddle, 1997; Palmer and Strobeck, 2003). In the absence of directional symmetry or antisymmetry, the average value of the (R - L) difference of bilaterally symmetrical traits in some populations or

some group of individuals is expected to be equal to zero and to have a normal distribution (Palmer and Strobeck, 2003). The left and the right side of an organism or an organ are separate copies of the same structure. Body sides have the same genetic base, and during development they are influenced by the same environmental conditions. Detected asymmetry of organs or organ parts is the visual result of developmental noise that occurred during the process of development (Klingenberg, 2003).

Environmental factors, such as light intensity, drought, presence of herbivores, etc, mostly affect plant performance, as well as an organism's developmental stability (Puerta-Pinero et al., 2008, Tucić and Miljković, 2010). If organisms are exposed to less stressful environmental conditions their developmental stability is expected to increase and the amount of their fluctuating asymmetry is expected to decrease (Stige et al., 2004). Different light intensities induce differences in the morphological traits of *I. pumila* leaves and flowers among populations as a consequence of adaptation to local environmental conditions (Avramov et al., 2007; Tarasjev et al., 2009; Miljković, 2009), and it has previously been shown that other phenotypic responses (such as phenotypic plasticity to environmental variability) are distinct from the asymmetry of the flower parts in *Iris pumila* (Tarasjev, 1995; Pigliucci, 2001). *I. pumila* populations inhabit two contrasting light intensity habitats in the Deliblato Sands nature reserve. Dune populations live in exposed habitats with high light intensity, while forest populations occupy shaded habitats with low light intensity. As the stressful environments are supposed to enlarge the size of fluctuating asymmetry, the less stressful ones are supposed to reduce it (Stige et al., 2004). Therefore, we can expect that the populations already adapted to their natural habitats will perceive a contrasting habitat as more stressful and less optimal, and will have a less stable development compared to conditions that mimic their natural one. Exposure to suboptimal conditions should modify the pattern of fluctuating asymmetry as the measure of developmental stability, by increasing it. The aim of this study was to determine whether populations of *I. pumila* from different natural habitats

(exposed and shaded, i.e. growing in high and low light intensities, respectively) differed in the fluctuating asymmetry patterns of floral traits.

## MATERIAL AND METHODS

### *Study area and species*

*Iris pumila* (Iridaceae) is a rhizomatous perennial monocot naturally growing in the lowlands of central and southeastern Europe. In Serbia, this species is widely distributed in the Deliblato Sands, a sandy area situated about 50 km from Belgrade (44°47'39"N/ 21°20'00"E; 45°13'10"N / 21°26'08"E). *I. pumila* populations usually occur in two different habitat types: dune and forest. The first is covered with annual and perennial herbaceous plants and low shrubs while the latter consists of sandy areas covered with black pine (*Pinus nigra*), white pine (*Pinus silvestris*) and acacia (*Robinia pseudoacacia*) forests. These *I. pumila* habitats also differ in many abiotic environmental factors, but mostly in the available light intensity and quality (Kojić et al., 1994).

Within each habitat, the mean light intensity was measured under clear sky conditions at ground level near the sampled clones, between 10.00 and 12.00 AM. The amount of photosynthetically active radiation (PAR) was measured with a point quantum sensor (Li-190SA, Li-Cor, Inc., Lincoln, NE, USA). The red to far-red ratio (R:FR) was recorded with an R:FR ratio sensor (Skye SKR-110; Skye Instruments Ltd., Powys, UK). The average instantaneous radiation intensity ( $\pm$  standard error) for the open dune habitat was PAR  $1173.1 \pm 29.99 \mu\text{mol m}^{-2} \text{s}^{-1}$ , CV (%) 8.48, and the ratio of R:FR light  $1.025 \pm 0.0057$ , CV (%) 1.85. For the shaded forest understory the mean PAR was  $128.36 \pm 18.27 \mu\text{mol m}^{-2} \text{s}^{-1}$ , CV (%) 30.12, and the R:FR ratio,  $0.78 \pm 0.02$ , CV (%) 14.80.

The experimental populations of *I. pumila* consisted of families derived from exposed and shaded habitats of the Deliblato Sands that were obtained in 1995 by crossbreeding of the chosen clones' flowers within the habitat. Circle-shaped clones are polymorphic for flower color and each can be considered

a unique genotype (Tucić et al., 1988) since flower color diversity is determined by segregation at several gene loci. Seedlings were planted in the plant growth chamber at the Institute for Biological Research under controlled light and temperature conditions (16 h light, 8 h darkness; 26°C and 21°C, respectively).

After six months, the plants were transferred to the garden of the Institute for Biological Research. Twelve replicates of each family were randomly exposed to the high and low light intensity treatments. The plants maintained in high light conditions were exposed to full sunlight, while those kept in low light were shaded with a green plastic grid. The intensity and quality of the light in the low light treatment was: PAR  $620.32 \pm 6.22 \mu\text{mol m}^{-2} \text{s}^{-1}$ , CV(%) 3.88; R : FR  $1.030 \pm 0.001$ , CV(%) 2.90; in the high light intensity, the PAR was  $1330.57 \pm 16.42 \mu\text{mol m}^{-2} \text{s}^{-1}$  CV(%) 5.66, and R : FR  $1.051 \pm 0.001$  CV(%) 2.28. Each treatment consisted of two replicated blocks. Plant position within a block was randomized.

During the blooming phase of *Iris pumila* in April 1994, two to three flowers (depending on clone size) were collected from each clone (genotype). We conserved each fresh flower separately in 50% alcohol, and stored them at room temperature until dissection. The sample for analysis consisted of a total of 307 flowers, of which 210 (12 families) were taken from plants produced by the crossing of clones from the Dune population, and 97 flowers (6 families) were taken from plants produced by the crossing of clones from the Forest population.

The flowers of *I. pumila* consist of three repeated floral organ parts: the inner petals (referred to as “standards”), the outer petal-like sepals (referred to as “falls”), the female sex organs – the style with stigma on the top, and the male sex organs – the stamens (the anther + filament). This is an actinomorphic or radially symmetrical form of flower because it exhibits multiple planes of symmetry (Neal et al., 1998; Kalisz et al., 2006).

The flower organ traits measured for fluctuating asymmetry analysis were: fall width (FW), standard

(SW) and style branch width (STW) (Fig. 1). Each floral organ (fall, standard and style) was cut at the perianth base and flattened over a glass plate embedded in glycerol to preserve the original size. Floral digital images were taken with a Hewlett Packard Jet 3400C scanner at 600 dpi resolution. Measurements were performed with UTHSCSA Image Tools, Version 3.0 (San Antonio, Texas) image analysis software. On each part of a given floral organ, we measured the distance from the midrib to the right and left margins at the widest point (Fig. 1). The author performed all measurements twice in order to estimate measurement error. The floral asymmetry was calculated as the difference in the width between the right (R) and left (L) sides (in mm) of each of the three repeated floral organ parts. The unsigned asymmetry  $|R - L|$  was calculated as the average of three floral organ parts per flower, obtained in the two series of measurements.

#### Statistical analysis

Preliminary analyses were performed to examine departures from fluctuating asymmetry (Palmer and Strobeck, 2003; Tucić and Miljković, 2010). Aberrant measurements in two separate analyses of the same sample that appeared to be either ME or FA outliers were removed using Grubb's test (Sokal, 1995). To assess whether the between-sides differences due to FA were greater than those due to ME, two-way mixed model ANOVA was used (with side the fixed and the individual the random factor). As a measure of developmental precision, FA should be ideal (mean 0 and normal distribution of R – L variation). Deviations from ideal FA include directional asymmetry (DA) and departures from normality. To test for the presence of DA we used a one-sample t-test (Swaddle et al., 1994), as well as two-way ANOVA, while for antisymmetry checking Shapiro-Wilk's test was used. In order to find a connection between obtained fluctuating asymmetry and trait, size nonparametric correlation significance tests (Spearman's, Kendal's and linear regression analysis) were performed (Palmer, 1994). In this study, fluctuating asymmetry analyses were done according to Palmer and Strobeck (2003).

We calculated two indices of fluctuating asymmetry: FA1, size incorporated asymmetry, the mean absolute (unsigned) difference between the right and the left side of a bilaterally symmetrical trait ( $|R-L|$ ); FA8a, size scaled value of asymmetry, the mean absolute difference between sides on log-transformed the right and left side values ( $|\log(R/L)|$ ). Five-way mixed model ANOVA of FA1, FA8a, (Levene's test) was used to test for differences in FA between traits, treatments, blocks (nested in treatment), populations, and families (nested in the populations). In this analysis, the treatment and population effects were considered fixed, while the family, block and trait effects were considered random. Comparison of fluctuating asymmetry floral trait indices (FA1<sub>FW</sub>, FA1<sub>SW</sub>, FA1<sub>STW</sub>, FA8a<sub>FW</sub>, FA8a<sub>SW</sub>, FA8a<sub>STW</sub>) between the treatments, blocks (nested in treatments), populations and families (nested in the populations) was carried out by four-way ANOVA. All statistical analysis was performed using appropriate procedures of the SAS 9.1 package (SAS Institute, 2002).

## RESULTS

Preliminary analysis tests were computed for each population and each floral trait (both ME outliers and FA outliers were removed by applying Grubb's test). According to the results of the Shapiro-Wilk's test, the distribution of the differences between the right and left sides of traits (R-L) showed normal distribution (all ranges of W value were 0.974 - 0.993; with  $P > 0.05$ ). Moreover, the normal frequency distribution of the signed  $R - L$  was confirmed by the skewness and kurtosis statistics tests (all  $p > 0.118$  for skewness and all  $p > 0.120$  for kurtosis). A one-sample t-test revealed that the mean values of (R-L) for each trait did not differ significantly from zero (all  $P > 0.05$ ). The observed results indicated the absence of antisymmetry and directional asymmetry. Likewise, the absence of floral directional asymmetry was confirmed by a two-way mixed model ANOVA (with sides as the fixed factor and individuals as the random factor), where the probability for the Side effects (test for DA) was found to be greater than 0.05 for all floral traits within all the populations studied (except for SW in the high light conditions) (Table 1).

The highly significant effects of the other sources of variation, individuals (I) and side x individual interactions (S x I) indicated that the size of the analyzed floral traits differed among individual flowers (I) and that the estimated FA was greater than ME (S x I) (all  $p < 0.0001$ ). The results of the three nonparametric tests (Spearman's, Kendall's and linear regression) showed no dependence on observed FA for each of the analyzed traits with the size of trait  $(R+L)/2$  (all  $P > 0.05$ ).

These results (high repeatability in combination with low ME) clearly suggest that the estimated indices of FA can be used as a reliable measure of floral developmental instability in natural populations of *I. pumila*.

The results of a five-way ANOVA for FA differences (FA1 and FA8a) between treatments, blocks, populations, families and among multiple traits are presented in Table 2. Statistically significant differences were observed for trait and family when FA1 was used as a measure of floral DI. Conversely, in the case where FA8a was the measure of developmental instability, the ANOVA revealed the significant effect of traits (indicating that FA varied among traits) and treatment x population interactions. According to Scheffe's test, FA1 for STW appeared to differ significantly (having the smallest value) from both of its two counterparts, FW and SW, while for the FA8a index, SW had the smallest value in relation to FW and STW. In four-way ANOVA for each of the three *I. pumila* floral traits separately, the statistically significant family effect was obtained for FA1<sub>SW</sub> and FA8a<sub>SW</sub>, while for FA1<sub>STW</sub> and FA8a<sub>STW</sub> only treatment x population interactions were statistically significant (Table 3).

Variable levels of fluctuating asymmetry were observed for different traits and populations. The mean values (with the standard errors) of FA1 and FA8a indices estimated for the three floral traits, FW, SW and STW, for *I. pumila* populations occupying exposed and shaded habitats, are presented in Fig. 2. For both analyzed populations, the level of the FA1 index appeared to be the smallest for STW in

**Table 1.** Two-way mixed model ANOVA for three floral traits (FW, SW and STW) in the *Iris pumila* plants derived from crossing within populations inhabiting the natural exposed (Dune) and shaded (Wood) habitats, grown in two treatments (High and Low light intensity).

|                      | Side                          |        | Individual |       |          | Side x Individual |       |          | Error   |                        |
|----------------------|-------------------------------|--------|------------|-------|----------|-------------------|-------|----------|---------|------------------------|
|                      | (df=1)MS (*10 <sup>-2</sup> ) | F      | df         | MS    | F        | df                | MS    | F        | df      | MS(*10 <sup>-4</sup> ) |
| Dune population      |                               |        |            |       |          |                   |       |          |         |                        |
| High light intensity |                               |        |            |       |          |                   |       |          |         |                        |
| FW                   | 0.071                         | 0.19   | 105        | 0.082 | 22.42*** | 105               | 0.004 | 34.84*** | 424     | 0.106                  |
| SW                   | 1.244                         | 4.48*  | 105        | 0.147 | 52.78*** | 105               | 0.003 | 50.98*** | 424     | 0.050                  |
| STW                  | 0.590                         | 1.23   | 105        | 0.061 | 12.67*** | 105               | 0.005 | 24.49*** | 424     | 0.196                  |
| Low light intensity  |                               |        |            |       |          |                   |       |          |         |                        |
| FW                   | 0.001                         | 0.00   | 103        | 0.105 | 26.05*** | 103               | 0.004 | 46.94*** | 416     | 0.080                  |
| SW                   | 0.343                         | 1.56   | 103        | 0.135 | 61.45*** | 103               | 0.002 | 46.66*** | 416     | 0.047                  |
| STW                  | 0.106                         | 0.33   | 103        | 0.103 | 31.86*** | 103               | 0.003 | 17.44*** | 416     | 0.186                  |
| Wood population      |                               |        |            |       |          |                   |       |          |         |                        |
| High light intensity |                               |        |            |       |          |                   |       |          |         |                        |
| FW                   | 0.045                         | 0.15   | 48/48      | 0.092 | 29.93*** | 48/48             | 0.003 | 37.62*** | 196/196 | 0.080                  |
| SW                   | 1.905                         | 9.09** | 48         | 0.096 | 45.56*** | 48                | 0.002 | 51.96*** | 196     | 0.040                  |
| STW                  | 0.499                         | 2.22   |            | 0.039 | 17.21*** |                   | 0.002 | 12.77*** |         | 0.200                  |
| Low light intensity  |                               |        |            |       |          |                   |       |          |         |                        |
| FW                   | 0.1620                        | 0.35   | 47         | 0.106 | 22.85*** | 47                | 0.005 | 72.51*** | 192     | 0.006                  |
| SW                   | 0.0390                        | 0.11   | 47         | 0.141 | 38.00*** | 47                | 0.004 | 84.42*** | 192     | 0.044                  |
| STW                  | 0.0320                        | 0.07   | 47         | 0.079 | 16.56*** | 47                | 0.005 | 29.99*** | 192     | 0.159                  |

\* P&lt;0.05, \*\* P&lt;0.01, \*\*\* P&lt;0.001.

comparison with the two other traits in both light treatments. The patterns of fluctuating asymmetry expressed by the FA1 index were different between treatments of high and low intensity for the three bilaterally symmetric traits (FW, SW and STW). For the populations originally from the exposed natural habitat, the high light intensity was stressful for STW (according the values for both indices FA1 and FA8a (Fig. 2); for fall and standard width the same patterns of fluctuating asymmetry were observed. The pattern of fluctuating asymmetry for style branch width was the opposite for the individuals derived from

the shaded natural habitat, with less developmental instability of floral traits observed in the treatments with low light intensity (according to both indices of asymmetry) (Fig 2.).

## DISCUSSION

In natural habitats, abiotic factors show a large heterogeneity that directly influences organisms. The effect of a single abiotic factor is hard to detect because it interacts with other abiotic and biotic factors. Variability of environmental conditions in ex-

**Table 2.** Five-way mixed model ANOVA for FA1 and FA8a indices for *Iris pumila* flower.

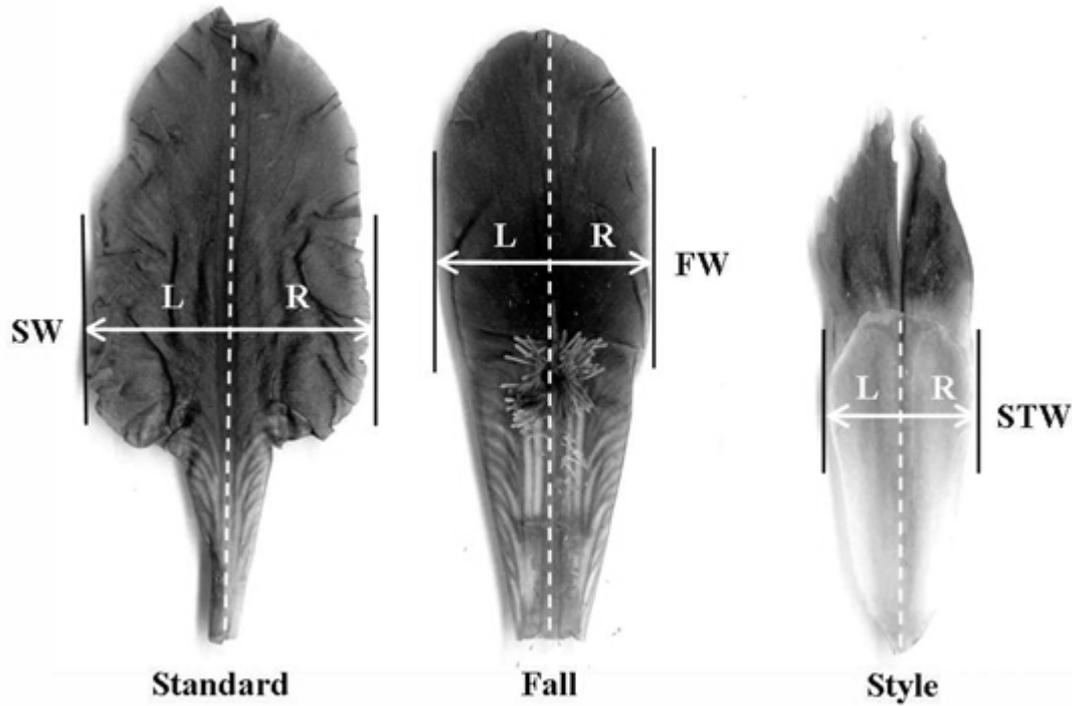
| Source of variation | df  | FA1    |         | FA8a   |        |
|---------------------|-----|--------|---------|--------|--------|
|                     |     | MS     | F       | MS     | F      |
| Trait               | 2   | 1.4260 | 81.42** | 0.0048 | 22.02* |
| Treatment (T)       | 1   | 0.1754 | 0.92    | 0.0022 | 0.33   |
| Population (P)      | 1   | 0.0215 | 0.10    | 0.0000 | 0.00   |
| Block B (T)         | 2   | 0.0118 | 0.24    | 0.0002 | 0.20   |
| Family (P)          | 16  | 0.0694 | 3.23**  | 0.0009 | 1.84   |
| Trait x T           | 2   | 0.0320 | 1.70    | 0.0004 | 0.35   |
| Trait x P           | 2   | 0.0178 | 1.00    | 0.0002 | 0.22   |
| Trait x Tret x Pop  | 2   | 0.0200 | 0.35    | 0.0010 | 1.16   |
| TxP                 | 1   | 0.1869 | 41.81   | 0.0062 | 9.06   |
| TxF(P)              | 16  | 0.0215 | 0.43    | 0.0005 | 0.58   |
| Error               | 893 | 0.0500 |         | 0.0009 |        |

\*P&lt;0.05; \*\*P&lt;0.01; \*\*\*P&lt;0.001

**Table 3.** Results of four-way mixed model ANOVA which used for testing differences in fluctuating asymmetry indices FA1 and FA8a of three *Iris pumila* flower traits: FW (fall width), SW (standard width) and STW (style branch width), with treatment (high and low light intensity in experimental growing conditions) and population (natural habitats: dune and forest) as fixed factor of source of variation, while block and family defined as a random factor.

| Source of variation | df  | FA1 <sub>FW</sub>      |      | FA1 <sub>SW</sub>     |        | FA1 <sub>SBW</sub>    |          |
|---------------------|-----|------------------------|------|-----------------------|--------|-----------------------|----------|
|                     |     | MS                     | F    | MS                    | F      | MS                    | F        |
| Treatment (T)       | 1   | 0.17                   | 6.00 | 0.01                  | 0.79   | 0.05                  | 0.21     |
| Population (P)      | 1   | 0.02                   | 0.28 | 0.01                  | 0.08   | 0.00                  | 0.18     |
| Block B (T)         | 2   | 0.01                   | 0.59 | 0.06                  | 0.82   | 0.00                  | 0.01     |
| Family (P)          | 16  | 0.05                   | 1.20 | 0.11                  | 2.65*  | 0.03                  | 2.26     |
| Tret x P            | 1   | 0.03                   | 0.66 | 0.01                  | 0.29   | 0.21                  | 13.13*** |
| Tret x F(P)         | 16  | 0.04                   | 0.83 | 0.04                  | 0.58   | 0.01                  | 0.48     |
| Error               | 269 | 0.05                   |      | 0.07                  |        | 0.03                  |          |
|                     | df  | FA8a <sub>FW</sub>     |      | FA8a <sub>SW</sub>    |        | FA8a <sub>SBW</sub>   |          |
|                     |     | MS (x10 <sup>2</sup> ) | F    | MS(x10 <sup>2</sup> ) | F      | MS(x10 <sup>2</sup> ) | F        |
| Treatment (T)       | 1   | 0.11                   | 1.7  | 0.00                  | 0.00   | 0.20                  | 0.24     |
| Population (P)      | 1   | 0.00                   | 0.05 | 0.00                  | 0.04   | 0.00                  | 0.00     |
| Block B (T)         | 2   | 0.03                   | 0.39 | 0.02                  | 0.22   | 0.03                  | 0.34     |
| Family (P)          | 16  | 0.08                   | 1.10 | 0.11                  | 3.50** | 0.13                  | 2.25     |
| Tret x P            | 1   | 0.06                   | 0.82 | 0.05                  | 2.05   | 0.80                  | 11.85*** |
| Tret x F(P)         | 16  | 0.08                   | 0.88 | 0.03                  | 0.41   | 0.06                  | 0.57     |
| Error               | 269 | 0.09                   |      | 0.07                  |        | 0.10                  |          |

\*P&lt;0.05; \*\*P&lt;0.01; \*\*\*P&lt;0.001

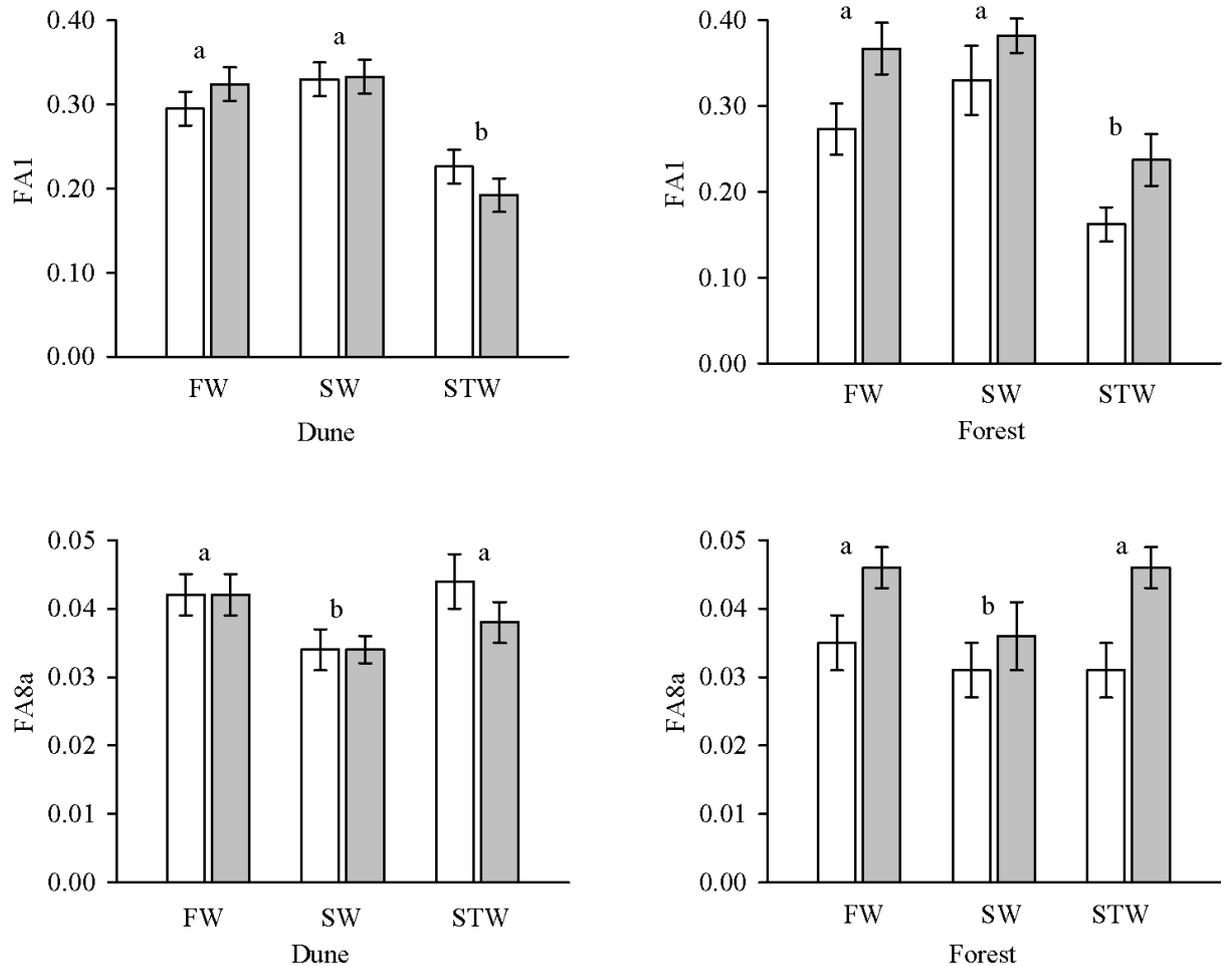


**Fig. 1.** Floral organs traits of *Iris pumila* and the measured left (L) and right (R) side of: fall width (FW), standard (SW) and style branch width (STW).

perimental settings is usually reduced to one or two factors under study (Parsons, 1990). Better insight into a stressor's action on the developmental stability of the analyzed traits can be obtained by comparison of the stressor's influence on developmental stability under different experimental conditions with those populations that are stable in their natural habitats (Moller and Pomiankowski, 1993; Badyaev, 1998). In recent studies, a common garden experiment was efficiently used in the analyses of intra- and inter-specific phenotypic variation (of morphological and physiological divergences (Dorman et al., 2009). The intraspecific variation of the developmental stability of floral organ traits in *I. pumila* plants was explored in this study.

Fluctuating asymmetry differences between traits were determined (statistically significant effect of the

trait for both indices of FA) (Table 2). The amount of FA1 index of asymmetry for STW was lower in relation to the two floral traits for both populations (Figs. 2 A, B) The differences between treatments were not recorded in multi-trait FA analysis (Table 2), or in separate trait analysis (Table 3). The patterns of FA as a measure of developmental stability of *I. pumila* floral traits were specific for both populations derived from exposed and shaded habitats (but not a statistically significant population effect) (Table 2, 3). The genetic differentiation of populations within the treatments was revealed to be a statistical significant effect in treatment x population interaction for both indices FA1 and FA8a as an indicator of developmental stability of style branch width (Table 3). The genetically based variation was observed in the developmental stability expressed by the FA1 index (statistical significant effect of family) (Table 2), but



**Fig. 2.** Mean value ( $\pm$  SE) of the FA1 and FA8a indices for three floral traits (fall width – FW, standard width – SW and style branch width – STW) at the high (open bars) and low (closed bars) light intensity treatments, in the *Iris pumila* plants from the dune and forest natural habitats. The bars sharing the same letter do not differ significantly at the 0.05 probability level according to the results of the Scheffe's test.

in analyses for separate traits, genetic variation in developmental stability was observed only for SW (for both employed indices) (Table 3).

The treatment of low light intensity induced developmental instability in the style branch width for the flowers derived from shaded natural habitats (Fig. 2), while the high light intensity treatment induced developmental instability for flowers derived from the exposed natural habitat (Fig 2). In the separate analysis for floral traits, only  $FA_{STW}$  showed a population variation in response to different environments

(statistical significant effect of treatment and population interaction (Table 3)). Contrary to the expectation that the values of FA would increase with the stressor intensity (Palmer and Strobeck, 1986; Leary and Allendorf, 1989), significantly lower values of asymmetry in more stressful environmental conditions were also observed in natural populations (Tan-Kristanto et al., 2003).

The statistically significant effects of family were recorded for both FA indices of standard width, which indicated a genetic variability in develop-

mental stability. For example, Wilsey and Saloniemi (1999) failed to detect among-family variation in leaf FA of *Betula pubescens* ssp. *tortuosa*, which suggested that there was no genetic variance for developmental stability in mountain birch. The genotypes showed differences in developmental stability in different environmental conditions (Milligan et al., 2008).

The amount on FA also depended on the analyzed traits (Parsons 1990; Bennett and Hoffman, 1998). The results of multi-way ANOVA confirmed trait differences in the FA for both indices FA1 and FA8a (Table 2). The results of several earlier studies confirmed that different plant traits exhibit different patterns of developmental stability (Roy and Stanton, 1999; Hoffman and Woods, 2001). The differences between the developmental stability of a trait have been reported for many plant taxa, including the flowers and leaves of *Nicotiana tabacum* (Sakai and Shimamoto, 1965) and *Clarkia tembloriensis* (Sherry and Lord 1996a; 1996b), the leaves of *Quercus pyrenaica* seedlings (Puerta-Piñero et al., 2008), leaves of *Lythrum salicaria* and *Penthorum sedoides* (Milligan et al., 2008), cotyledons and flowers of *Brassica campestris* (Evans and Marshall, 1996) and cotyledons, flowers and fruits of *Sinapsis arvensis* (Roy and Stanton, 1999). Studies of different floral trait developmental stability have reported that developmental stability in reproductive traits was no more sensitive than it was in vegetative traits (Wilsey et al., 1998; Hochwender and Fritz, 1999; Perfectti and Camacho, 1999; Pélabon et al., 2004). The relationship between environmental stressors and developmental stability is not obvious. Several studies reported an increase in FA with environmental stress (Midgley et al., 1998; Hódar, 2002; Veličković, 2010), while others failed to find any statistical association (Bjorksten et al., 2000; Veličković and Perišić, 2006). In addition, stressful environmental conditions may influence different trait FA differently. In wild mustard *Sinapsis arvensis*, for example, stressful environmental conditions induced an increase in the FA of all traits analyzed; however, petal symmetry was only mildly influenced, while leaf and fruit symmetry appeared to be under a strong stress impact (Roy and Stanton, 1999).

The developmental stability of flower organs is essential for plants because it directly influences the fitness of each individual (Aldos et al., 2001, Aldos et al., 2002). We report that the fall (as a landing platform for the pollinator of *I. pumila* flowers) and standard (as the part of the flower which attracts insects) did not show differences in fluctuating asymmetry patterns in experimental contrasting light conditions, either for the populations derived from exposed habitats of those derived from shaded habitats. The flower size, shape and asymmetry of floral organs are visual stimuli for pollinators, which is one of the reasons of its lower sensibility to stress environmental conditions (Moller and Eriksson, 1994; Moller, 1995; Evans and Marshall, 1996; Moller and Shykoff, 1999; Aldos et al., 2001; Brock and Weining, 2007).

The estimated different patterns in the FA of *I. pumila* floral traits disenable a general relationship between their developmental stability and environmental light conditions. Namely, a statistical significant treatment and population interaction for both employed indices of fluctuating asymmetry was observed only for the style branch width, which confirmed the differences in developmental stability among populations in high and low light experimental treatments. The buffering capacity against stochastic perturbations for style branch width (in treatments with low and high light intensity), depended on the populations' natural environment, while for the other two floral traits it did not. In the study of the *I. pumila* floral organ trait developmental stability in two natural light habitats, a greater developmental stability in the plants from the forest habitat with low light intensity surroundings was reported (Tucić and Miljković, 2010).

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## REFERENCES

- Aldos, C. L., Navarro, T., Escos, J., Cabezudo, B., and J. M. Emlen (2001). Translational and fluctuating asymmetry as tools to detect stress in stress-adapted and non-adapted plants. *Int J Plant Sci* **162**, 607-616.

- Alados, C. L., Giner, M. L., Dehesa, L., Escos, J., Barroso, F. G., Emlen, J. M., and D. C. Freeman (2002). Developmental instability and fitness in *Periploca laevigata* experiencing grazing disturbance. *Int J Plant Sci* **163**(6), 969-978.
- Avramov, S., Pemac, D., and B. Tucić (2007). Phenotypic plasticity in response to an irradiance gradient in *Iris pumila*: adaptive value and evolutionary constraints. *Plan. Ecol.* **190**(2), 275-290.
- Badyaev, A. V. (1998). Environmental stress and developmental stability in dentition of Yellowstone grizzly bears. *Behav. Ecol.* **9**, 339-344.
- Bennett, D. M. and A. A. Hoffman (1998). Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). *J. Anim. Ecol.* **67** (4), 580-591.
- Bjorksten, T., David, A. P. and A. Pomiankowski (2000). Fluctuating asymmetry of sexual and non-sexual traits in stalk-eyed flies: a poor indicator of developmental stress and genetic quality. *J. Evol. Biol.* **13**, 89-97.
- Brock, M. T., and C. Weinnig (2007). Plasticity and environment-specific covariances: an investigation of floral – vegetative and within flower correlations. *Evol.* **61**(12), 2913-2924.
- Debat, V. and P. David (2001). Mapping phenotypes: canalization, plasticity and developmental stability. *T. Ecol. and Evol.* **16**(10), 555-561.
- Dorman, M., Sapir, Y. and S. Volis (2009). Local adaptation in four *Iris* species tested in common-garden experiment. *Biol. J. Linn. Soc.* **98**, 267-277.
- Evans, A. and S. M. Marshall (1996). Developmental instability in *Brassica campestris* (Cruciferae): fluctuating asymmetry of foliar and floral traits. *J. Evol. Biol.* **9**, 717-736.
- Hochwender, C. G., and R. S. Fritz (1999). Fluctuating asymmetry in *Salix hybrid* system: the importance of genetic versus environmental causes. *Evol.* **53**, 408-416.
- Hoffmann, A. A., and R. E. Woods (2001). Trait variability and stress: canalisation developmental stability and the need for a broad approach. *Ecol. Lett.* **4**, 97-101.
- Kalisz, S., Ree, H. R., and D. R. Sargent (2006). Linking floral symmetry genes to breeding system evolution. *T. Pl. Sci.* **11**, 568-573.
- Klingenberg, C. P., and H. F. Nijhout (1999). Genetics of fluctuating asymmetry: a developmental model of developmental instability. *Evol.* **53**, 358-375.
- Klingenberg, C. P. (2003) A developmental perspective on developmental instability: theory, models, and mechanisms pp. 14-34; Developmental instability as a research tool: using patterns of fluctuating asymmetry to infer the develop the origins of morphological integration. pp. 427-442. In: M. Polak, (Ed.) *Developmental Instability: Causes and Consequences*, Oxford University Press, Oxford.
- Kojić, M., Popović, R., and B. Karadžić, (1994). Ecological analysis of the Deliblato Sands vegetation. - In: *The Deliblato Sands - Proc. VI, Pančevo in Serbian with abstract in English*.
- Leary, R.F., and F.W. Allendorf (1989). Fluctuating asymmetry as an indicator of stress in conservation biology. *T. Ecol. Evol.* **4**, 214-217.
- Miljković, D. (2009). The influence of abiotic stressors on the development stability and morphological variability of *Iris pumila* in natural and experimental populations [dissertation]. Belgrade (Serbia). Faculty of Biology. University of Belgrade. 202p.
- Milligan, J. R., Krebs, R. A., and T. K. Mal (2008). Separating developmental and environmental effects on fluctuating asymmetry in *Lythrum salicaria* and *Penthorum sedoides*. *Int J Plant Sci* **169**(5), 625-630.
- Møller, A. P., and A. Pomiankowski (1993). Fluctuating asymmetry and sexual selection. *Genetica* **89**, 267-279.
- Møller, A.P., and M. Eriksson (1994). Patterns of fluctuating asymmetry in flowers: implications for sexual selection in plants. *J. Evol. Biol.* **7**, 97-113.
- Møller, A. P. (1995). Bumblebee preference for symmetrical flowers. *Proceedings of the National Academy of Sciences of the USA* **92**, 2288-2292.
- Møller, A. P., and J. P. Swaddle (1997). *Asymmetry, Developmental stability, and Evolution*. Oxford University Press, Oxford.
- Møller, A. P., and J. A. Shykoff (1999). Morphological developmental stability in plants: Patterns and causes. *Int J Plant Sci* **160**, S135-S146.
- Neal, P. R., Amonts, D., and M. Giurta (1998). Symmetry and its role in plant-pollinator systems: terminology, distribution, and hypothesis. *An. Rev. Ecol. Syst.* **29**, 345-373.
- Nijhout, H. F., and G. Davidowitz (2003). Developmental perspectives on phenotypic variation: canalization, and fluctuating asymmetry. In: *Developmental Instability: Causes and Consequences* (M. Polak, ed.), pp. 3-13. Oxford University Press, Oxford.
- Palmer, A. R., and C. Strobeck (1986). Fluctuating asymmetry: measurement, analysis, patterns. *An. Rev. Ecol. and Syst.* **17**, 391-421.
- Palmer, A. R. (1994) Fluctuating asymmetry analyses: a primer, in: T.A Markow, (Ed.), *Developmental stability: Its Origins and Evolutionary Implications*. Kluwer, Dordrecht, 335-364.

- Palmer, A. R., and C. Strobeck (2003). Fluctuating asymmetry analysis revisited, in: M. Polak, (Ed.) *Developmental Instability: Causes and Consequences*, Oxford University Press, Oxford, pp. 279-319.
- Parsons, P. A. (1990). Fluctuating asymmetry: an epigenetic measure of stress. *Biol. Rev.* **65**, 131-145.
- Pélabon, C., Hensen, T. F., Carlson, M. L. and W. S. Ambruster (2004). Variational and genetic properties of developmental stability in *Dalechampia scandens*. *Evol.* **58**, 50-514.
- Perfectti, F., and J. P. M. Camacho (1999). Analysis of genotypic differences in developmental stability in *Annona cherimola*. *Evol.* **53**(5), 1396-1405.
- Pigliucci, M. (2001). *Phenotypic plasticity: beyond nature and nurture*. Johns Hopkins Univ. Press, Baltimore, MD.
- Polak, M. E., (2003). *Developmental Instability: Causes and Consequences* In: M. Polak, (Ed.), Oxford University Press, Oxford.
- Puerta-Piñero, C., Gomez J. M., and J. A. Hódar (2008). Shade and herbivory induce fluctuating asymmetry in a Mediterranean oak. *International J. of Pl. Scien.* **169**(5):631-635.
- Rao, G. Y., Andersson, S., and B. Widén (2002). Flower and cotyledon asymmetry in *Brassica cretica*: genetic variation and relationships with fitness. *Evol.* **56**, 690-698.
- Roy, B. A., and M. L. Stanton (1999). Asymmetry of wild mustard, *Sinapsis arvensis* (*Brassicaceae*), in response to severe physiological stresses. *J. Evol. Biol.* **12**, 440-449.
- Sakai, K. I. and Y. Shimamoto (1965). *Developmental instability in leaves and flowers of Nicotiana tabacum*. *Genetics* **51**, 801-813.
- SAS Institute, SAS/STAT user's guide, version 9.1.3, SAS Institute Inc., Cary, N.C., 2003.
- Sherry, R. A., and E. M. Lord (1996a). Developmental stability in leaves of *Clarkia tembloriensis* (*Onagraceae*) as related to population out crossing rates and heterozygosity. *Evol.* **50**, 80-91.
- Sherry, R. A., and E. M. Lord (1996b). Developmental stability in flowers of *Clarkia tembloriensis* (*Onagraceae*). *J. of Evol. Biol.* **9**, 911-930.
- Sokal, R. R., and F. J. Rohlf (1995) *Biometry*. Freeman Press, San Francisco,
- Stige, L. C., Hessen, D. O., and L. A. Vollestad (2004). Severe food stress has no detectable impact on developmental instability in *Daphnia magna*. *Oikos* **107**, 519-530.
- Tan-Kristanto, A., Hoffman, A., Woods, R., Batterham, P., Cobbett, C. and C. Sinclair (2003). Translational asymmetry as a sensitive indicator of cadmium stress in plants: a laboratory test with wild-type and mutant *Arabidopsis thaliana*. *N. Phyt.* **159**, 471-477.
- Tarasjev, A., Barišić Klisarić, N., Stojković, B., and S. Avramov (2009). Phenotypic plasticity and between population differentiation in *Iris pumila* transplants between native open and anthropogenic shade habitats. *Russian Journal of Genetics* **45** (8), 944-952.
- Tarasjev, A. (1995). Relationship between phenotypic plasticity and developmental instability in *Iris pumila* L. *Russ.J. Genet.* **31**(12), 1655-1663.
- Tucić, B., Milojković, S., Vujčić, S. and A. Tarasjev (1988). Clonal diversity and dispersion in *Iris pumila*. *Acta Oecologica. Oecologica plantarum* **9**, 211-219.
- Tucić, B., and D. Miljković (2010) Fluctuating asymmetry of floral organ traits in natural populations of *Iris pumila* from contrasting light habitats. *Pl. Spec. Biol.* **25**, 173-184.
- Van Dongen, S. (2006). Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. *J. Evol. Biol.* **19**, 1727-1743.
- Van Dongen, S., and W. Talloen (2007). Phenotypic and genetic variations and correlations in multitrail developmental instability: a multivariate Bayesian model applied to speckled wood butterfly (*Pararge aegeria*) wing measurements. *Gen.Res. Camb.* **89**, 155-163
- Veličković, M., and S. Perišić (2006). Leaf fluctuating asymmetry of common plantain as an indicator of habitat quality. *Pl. Biosys.* **140** (2), 138-145.
- Veličković, M. (2010) Reduced developmental stability in *Tilia cordata* leaves: effects of disturbed environment. *Period. Biolog.* **112** (3), 273-281.
- Wilsey, B. J., Haukioja, E., Koricheva, J., and M. Sulkinoja (1998). Leaf fluctuating asymmetry increases with hybridization and elevation in tree-line birches. *Ecol.* **79**, 2092-2099.
- Wilsey, B.J., and I. Saloniemi (1999). Leaf fluctuating asymmetry in tree-line mountain birches, *Betula pubescens* ssp. *tortuosa*: genetic or environmentally influenced? *Oikos* **87**, 341-345.

