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STAGES OF FLOWER BUD DEVELOPMENT IN *IRIS PUMILA* AND BETWEEN-HABITAT MORPHOLOGICAL DIFFERENCES

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Abstract - Previous studies revealed significant phenotypic plasticity and between-population differentiation in flower morphometric traits of *Iris pumila* in response to environmental variability between natural shade and exposed habitats. Since these habitats differed in flowering times as well, in this work we investigated at which stages of flower bud development differences between open and shaded habitats start to appear. Our analysis detected several groups of trait development patterns through the *I. pumila* bud development in two contrasting habitats, with stem length being the most suitable trait for application in further analyses of so-called "shade avoidance syndrome".

Key words: Phenotypic plasticity, between-population differentiation, "shade avoidance syndrome", flower morphometric traits, stage allometry, *Iris pumila*

INTRODUCTION

The evolution of adaptation depends not only on the genetic variation that leads to between-population differentiation for the traits in question, but also on their phenotypic plasticity (Schlichting and Pigliucci, 1998; Pigliucci, 2001; de Jong, 2005). Previous studies of *Iris pumila* flower morphometric traits in natural habitats detected significant environmental heteroscedascity (Tucić et al., 1990) and revealed both significant plastic responses as well as between-population differentiation between shaded and open habitats (Tarasjev et al., 2009). Plasticity and differentiation were mostly consistent with responses expected in the case of so called "shade avoidance syndrome" (Schmitt and Wulff, 1993; Von Wettberg, et al., 2008; McGoey and Stinchcombe, 2009; Tarasjev et al., 2009). On the other hand, the study of *I. pumila* phenology revealed similar significant phenotypic plasticity as well as population differentiation in the flowering time of populations occupying the same contrasting habitats (Tarasjev, 1997). The possible influence of flowering time on detected differences in flower morphometric traits in previous studies must also be examined. To approach this relationship, the ontogenic aspect must be covered since plasticity in plants can be the result of the direct response of a trait to environmental cues during development, or a passive result of changes in the plant growth rate due to various factors (Schlichting and Pigliucci, 1998; Wright and McConnaughay, 2002; Varin et al., 2009), with the phenological response influencing other traits being a significant one (Diggle, 1999; Forrest and Miller-Rushing, 2010). Previous studies of the flower buds in populations from open and shaded habitats, but performed at different time points (Barišić Klisarić et al., 2011), revealed that stem length was the only trait that showed higher values in shaded habitats for the very beginning. Most of the other traits had higher values in the bud stage in habitats that will flower earlier (open habitats) compared to habitats that have higher values for open flowers (shaded). To supplement the analysis of the flower's morphometric traits at the same time periods, we studied the morphometric traits in the same stages of flower bud development.

The goals of this study were to analyze the morphometric flower traits at different stages of flower ontogeny (stage allometry) on naturally growing clones of *I. pumila* occupying contrasting light habitats (open vs. shaded) in order to: analyze traits at different stages during the flower bud ontogenetic development and detect the stages at which the growth of certain flower parts occurs; analyze traits at different stages during the flower bud ontogenetic development and to detect the stages at which differences between habitats in the size of certain flower parts occur, and to compare the detected differences in the flower bud stages between clones from the two habitats with differences described in previous studies in which plants in the flowering stage were used.

MATERIALS AND METHODS

The dwarf bearded iris *Iris pumila* L. is a small monocot that occurs in the lowlands of Central and Eastern Europe (Mathew, 1981). *I. pumila* is perennial clonal plant, which spreads vegetatively through underground rhizomes. Therefore, the same genotypes can be studied over different years and sufficient numbers of replicas of the same genotype established in the natural population can be obtained for experimentation.

This study was conducted in the Deliblato Sands, a protected sandy area 40 km NE from Belgrade, Serbia (44°48'N, 20^0 58' E), on 64 naturally growing clones in two *I. pumila* habitats (47 from open habitats and 18 from shaded ones). In an open habitat the plants are exposed to direct sunlight compared to plants in shaded habitats (e.g. planted pine forest) created under anthropogenic influence. The contrasting habitats are located in the northwestern part of the Deliblato Sands and they differ in many ecological indices (Kojić et al., 1994), foremost in the quality and intensity of light. In the sand-steppe habitat, the radiation intensity is 666.1 μmol $m^{-2}s^{-1}$ and red/far red ratio (R/FR) is 1.16 ((E), estimated with a LiCor 1000 (D) datalogger and Li190SA and Skye SKR-110 sensors). In the forest site, clones of *I. pumila* grow in the vegetation shade (radiation intensity of 170.9 μmolm^{-2}s^{-1} and R/FR ratio of 0.96).

Detailed descriptions of the parts of the *I. pumila* flower, as well as their graphical representation, are given in Tarasjev et al. (2009). During the beginning of the flowering season, buds were collected from selected clones in four stages of flower bud development (two buds per stage/clone combination). Up to two opened flowers per clone were also collected. The analyzed stages are presented in Fig. 1, and these are: *1. Hidden bud stage* (H) with no visible flower parts, but the bud is palpable between the leaves; *2. Spathe stage* (S) where two spathes are visible outside the leaf "pocket", but no other parts of the flower bud are visible yet; *3. Colored bud stage* (C) where the colored parts of perianth are visible outside the leaf "pocket" between the spathes; *4. Final bud stage* (B) – the flower has not yet opened, but most of the perianth parts are already outside the spathes, and 5. *Open flower stage* (F).

A total of 65 clones (47 from open and 18 from shaded habitats) were analyzed with up to two samples per each stage on each clone resulting in a total of 423 analyzed buds and flowers. All measurements of the vegetative and floral parts were made to the nearest millimeter using fresh material. The following traits were utilized in this analysis: length of the first spathe (LIS), length of the second spathe (LIIS), stem length (LS), ovary length (OL), ovary width (OW), tube length (TL), tube radius (TR), fall length (FL), fall width (FW), standard length (SL), standard width (SW), stigma length (SML), crest length (CL), crest width (CW), stamen length (STL) and anther length (AL). Morphometric data were transformed to natural logarithms as the appropriate transformation determined by the method given by Box et al. (1978) and the SAS program given by Fernandez (1992). Statistical analyses were carried

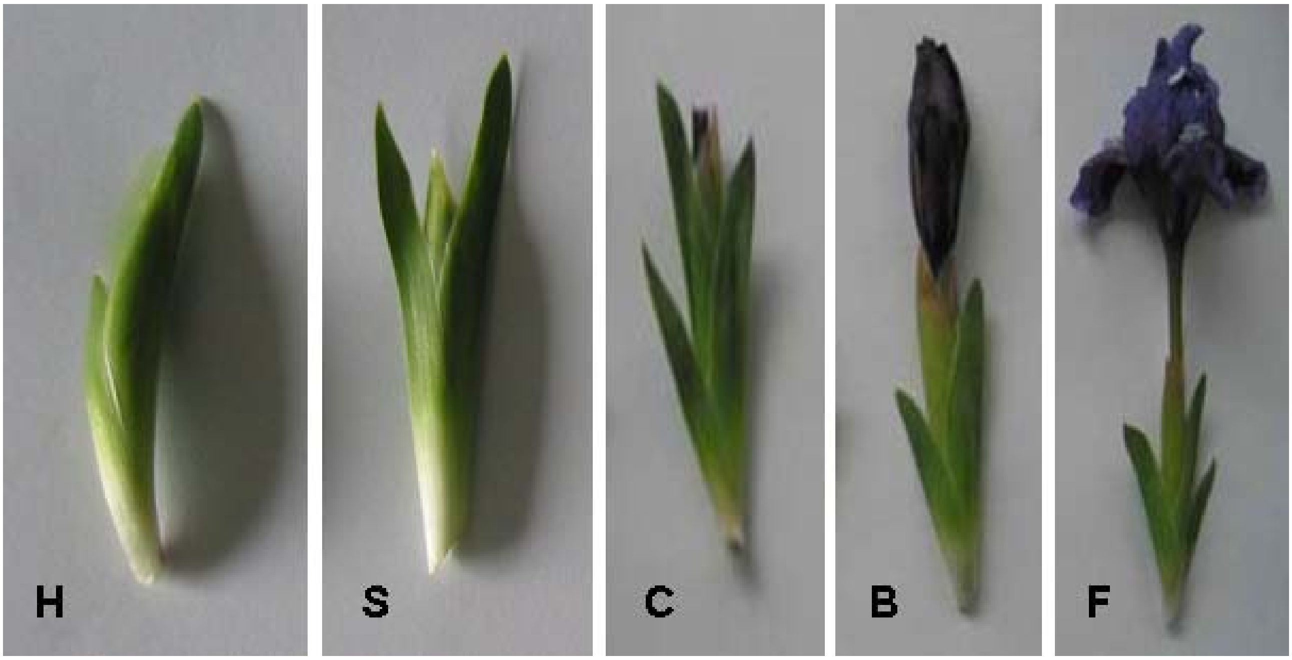

Fig. 1. Analyzed bud stages in development of *I.pumila* flower: *Hidden bud stage* (H), *Spathes stage* (S), *Colored bud stage* (C), *Final bud stage* (B) and *Open flower stage* (F). For description of each stage see Matherial and methods section

out on clone means by ANOVA with Habitat and Stage as the main factors. For pairwise comparisons between stages, the Scheffe test was used. All statistical testing was done by SAS program (SAS 1989) PROC MEANS and PROC GLM procedures.

RESULTS AND DISCUSSION

Several studies of the morphology of the flowering dwarf bearded iris *Iris pumila L.* have previously been conducted on plants and populations of this perennial monocot from open and shaded localities in Natural Protected Reserve of Deliblato Sands, and the results of comparison of plants occupying open and shaded habitats were mostly consistent with the responses expected in the case of so called "shade avoidance syndrome" (Tarasjev, 2009).

The results of this study are presented in Fig. 2 for the outer parts of the *I. pumila* blossom and in Fig. 3 for the perianth parts. Analysis indicated that most of the development occurred in the earlier stages of bud development compared to the later ones. No differences between B and F stages were detected in this study for all traits and both habitats, and significant differences between C and B stages were detected only for stem length and tube length, both in the open habitat. However, a large number of significant differences between the C and F stages (Fig 2 and 3) indicate that a certain amount of growth occurs in the later stages, too. However, growth was more profound in the earlier stages of flower bud development, with all but one analyzed trait showing significant differences between the H and S stages in both habitats, while 11 out of 16 analyzed traits showed significant differences between the S and C stages. The lengths of the first and second spathes, as well as anther length, finished their development in S stage.

Regarding the differences between habitats, they were significant for only two traits (stem and ovary lengths) in the earliest stage of flower bud development. The number of traits with significant between-habitat differences gradually increased in the later stages: five traits showed significant between-habitat

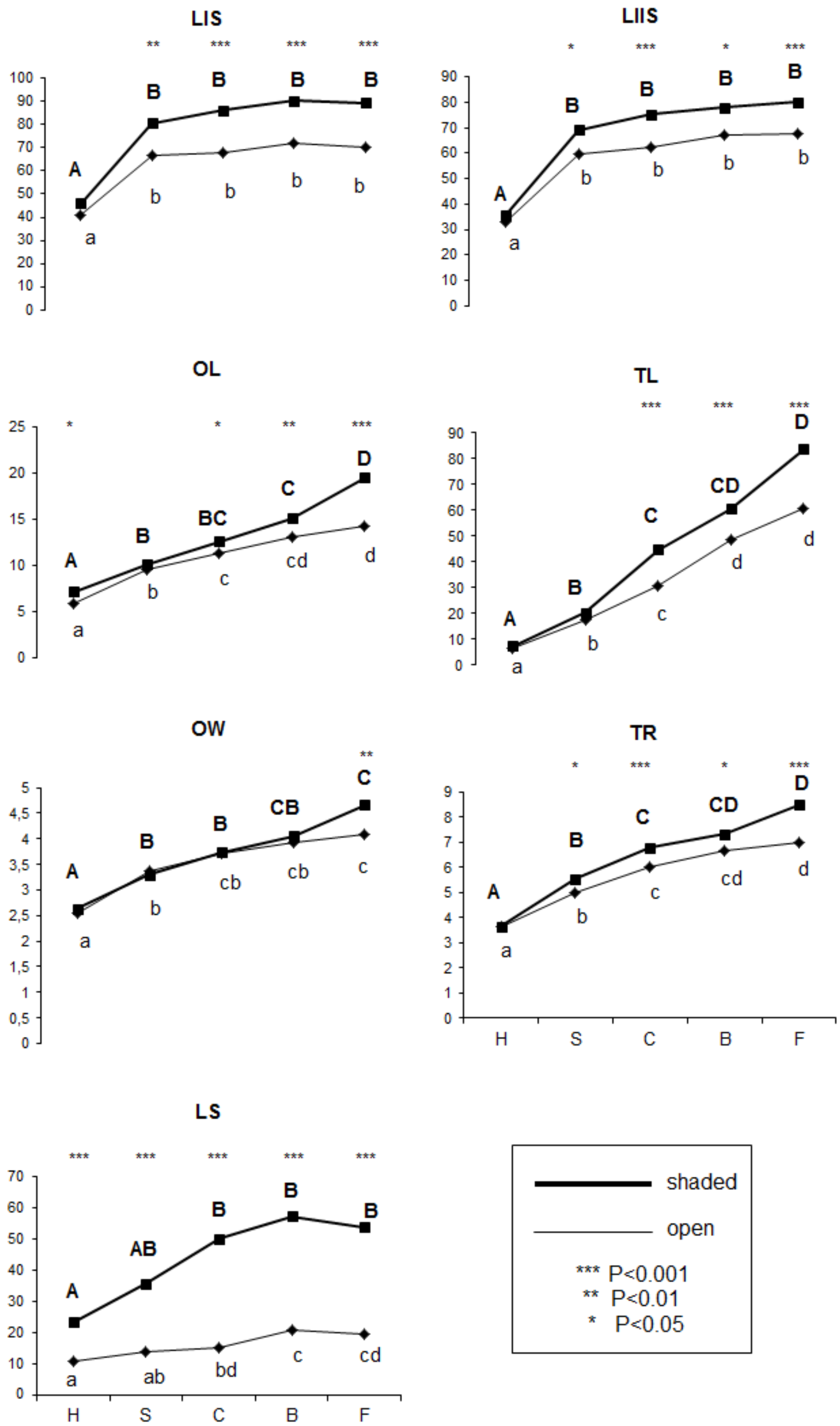

Fig. 2. Morhometric change through developmental stages of outer parts of *I.pumila* blossom in two habitats (open and shaded one). Developmental stages within each habitat that share the same letter (capital letters for shaded habitat, small letters for open habitat) do not differ significantly on the basis of Scheffe test. Siginificant differences between habitats for particular stage is indicated by asterisks. For character abbreviations see Matherial and methods section.

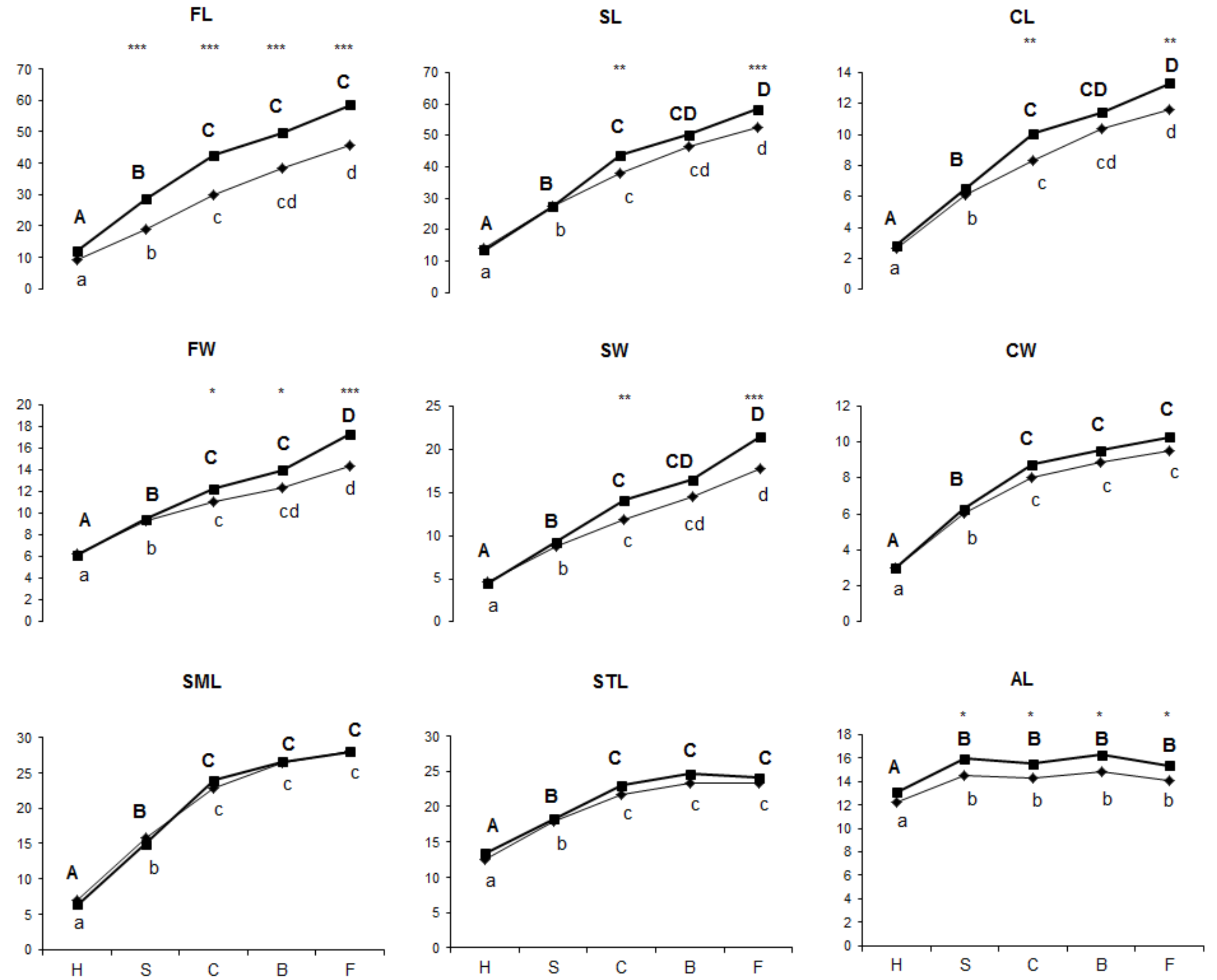

Fig. 3. Morhometrical change through developmental stages of perianth parts of *I.pumila* blossom in two habitats (open and shaded one). Developmental stages within each habitat that share the same letter (capital letters for shaded habitat, small letters for open habitat) do not differ significantly on the basis of Scheffe test. Siginificant differences between habitats for particular stage is indicated by asterisks For character abbreviations see Matherial and methods section.

differences in the S stage, eight traits in the C and B stages, and twelve traits in the open flower stage (F). No differences between habitats were detected for FL CW, SML and STL.

On the basis of these findings, the analyzed traits can be divided into several groups and one representative trait for each group can be utilized in further studies. The first group consists of traits that showed no differences between habitats and ended their development in the C stage (FL, CW, SML and STL). This group could be excluded from further analyses of shade avoidance syndrome of *Iris pumila*. Ovary width (OW) showed a continuous pattern of growth but with differences that were detectable only in the open flower stage with no differences in the bud stages, which was similar to the previous group. The other group consists of traits that start to appear between-habitat differences from the C stage (TL, SL, CL FW and SW) but continue to grow through the last two stages. The third group begins to diverge in the S stage (LIS, LIIS, TR, AL) with only the tube radius (TR) continuing to grow further through the later stages.

A special case of between-habitat difference is the length of the stem (LS). In previous analysis of flower bud morphometric traits in different time periods (Barišić Klisarić, 2011), the LS was the only trait that showed divergence between habitats in all time periods, and the direction of this difference was the same as in analyses of *I. pumila* flowers (Tarasjev, 2009). In this study, it was one of only two traits (together with ovary length) that showed between-habitat differences from the earliest bud stage. However, while the difference for OL was small and even undetectable in the following S stage, the LS was two times larger in the shaded habitat compared to the open one in the "Hidden bud" (H) stage. This size difference increases threefold in later stages. This makes stem length the most usable trait in further studies of flower morphological differences between open and shaded habitats that might by accompanied by selected representative traits from two other groups. The reduction of the number of traits that is suggested by the results of this study can therefore enable larger and more extensive experiments compared to those already performed on the *I. pumila* flower morphometric traits (Tucić et al., 1990; Tarasjev, 1994; Tarasjev, 1995; Tarasjev et al., 2006; Tarasjev et al., 2009).

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