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Intra-inflorescence variations in floral morphological and reproductive traits of *Iris sibirica* L.

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ABSTRACT Within inflorescences both temporal and spatial variations can be found in aspect of either attractive traits or reproductive organs. Morphological parameters of a flower depend on several factors like intra-plant position of the flower, other extrinsic and intrinsic factors, resulting in altered intra-inflorescence flower sizes or numbers, fruit set or seed set. In our study the sexual reproductive characteristics of Siberian Iris (Iris sibirica L.) were investigated, with special emphasis on how the floral morphological and reproductive traits vary spatially and temporally. We found that stalks typically had 2 levels and 5 flowers, and significant differences were found among individual flowers within the inflorescences. Flower diameter, sepaloid and petaloid tepal length and length of the filament significantly decreased with blooming order but none of the parameters showed significant correlation with the number of flowers per inflorescence. Pollen viability was above 90%, and did not dicrease with flowering order, which suggests that pollen production is independent of flower position. Parameters of female reproductive success such as ovule number and seed set per fruit also declined with flowering order, which was probably due to resource limitation within stalks or plants. Values of sex allocation (P/O ratios) refer to the type of breeding system of Iris sibirica. Acta Biol Szeged 54(2):103-110 (2010)

KEY WORDS

Iris sibirica L. cymose inflorescence flowering order pollen viability female reproductive success resource allocation

Numerous researchers have already confirmed that flowers vary in several morphological traits within inflorescences. In both racemose and cymose inflorescences, in which flowers often open sequentially, temporal and/or spatial variation can be found with respect to either attractive traits like the size of the perianth or to reproductive organs such as lenght of filaments or anthers (Brunet and Eckert 1998; Kliber and Eckert 2004; Ishii and Morinaga 2005; Zeng et al. 2008). Blooming order of racemose inflorescences generally go acropetally, from the base up to the top, and not only the flower size but also the number of pollen grains or ovules per flower, fruit set or seed set seem to decrease toward the apex (Brunet and Eckert 1998; Kudo and Molau 1999; Hiraga and Sakai 2007; Zeng et al. 2008; Zhao et al. 2008). However, plants with cymose inflorescences generally have the opposite order of anthesis, from the top downward to the base (basipetally), variation in flower size (diameter) occur to follow the same tendency like those of racemose inflorescences, *i. e.* decrease from early to late blooming flowers (Worley et al. 2000). Thus, it seems that morphological parameters of a flower depend not only on the intra-plant position of a flower, but probably on other extrinsic and intrinsic factors, as well. Intrinsic factors can be, e.g., resource allocation, competition among flowers or other architectural constraints, while

Accepted Dec 14, 2010 *Corresponding author. E-mail: szoszo@bio.u-szeged.hu pollen limitation, foliar and floral herbivory, climatic changes exert their influence externally, and these effects are often expressed as different flower sizes and numbers within an inflorescence or later as altered fruit or seed set (Wolfe 1992; Worley et al. 2000; Wesselingh and Arnold 2003; Harder and Barrett 2006; Kostrakiewicz 2006).

Although large flowers are more attractive for pollinators, higher floral display can be advantegous, too (Conner and Rush 1996; Worley et al. 2000). At the same time, if many flowers with smaller sizes are open at one time on a plant, the possibility of autogamous selfing (self-pollination) via geitonogamy increases which may reduce fitness of both flowers, individuals and the offspring, especially in the case of self-incompatible species (Eckert 2000; Worley et al. 2000; Ishii and Sakai 2001). Most of the studies conducted up to now have investigated racemose inflorescences (Kudo and Molau 1999; Mazer and Dawson 2001; Ishii and Sakai 2002; Hiraga and Sakai 2007; Zeng et al. 2008; Zhao et al. 2008), and we know relatively little about variations within cymose inflorescences. Since Siberian Iris (Iris sibirica L.) is protected in Hungary, and so there are not much data available about its sexual reproductive characteristics, we aimed to examine that (1) how floral morphological and reproductive traits of Iris sibirica L. vary among (inter-inflorescence level) and within inflorescences (intra-inflorescence level) and (2) what relationship occurs among floral traits and between these traits and the flowering sequence.

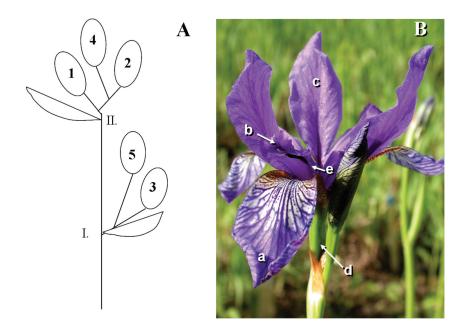


Figure 1. Schematic achitecture of a 2-level 5-flowered inflorescence (A) and a single flower (B) of *Iris sibirica* L. Roman numbers signify the levels, arabic numbers refer to flowering order within the inflorescence (A). Main parts of a flower are sepaloid tepals (a), petaloid style (b), petaloid tepals (c), ovary (d) and stamens (e).

Materials and Methods

Study species and study site

Iris sibirica L. or Siberian Iris belongs to the Iridaceae family (subfamily Iridoideae, tribe Irideae, subgenus Limniris; Goldblatt et al. 1998; Goldblatt and Manning 2008) and is native to central, eastern Europe and northern Asia. This perennial plant whose shoots grow even to 120 cm bears long narrow leaves and monochasial cymose inflorescences called rhipidium with 1-7 bluish lilac actinomorphic flowers (Goldblatt et al. 1998; Goldblatt and Manning 2008). In Hungary it is tipically found in moorlands and swamps (Molinio-Juncatea, Simon 2000) and is capable of both generative and vegetative reproduction. In its perfect flowers the tepals are arranged in 2 whorls of 3 with only 3 stamens inserted at the base of the outer tepals. The ovary is inferior positioned and is composed of 3 carpels, the short style continues in 3 petaloid style branches which are flattened and each has a rhomboid stigmatic lobe for pollen reception (Fig. 1A-B).

This stigma surface is dry with unicellular papillae (Heslop-Harrison and Shivanna 1977; Goldblatt et al. 1998) it becomes receptive within 1-2 days after flower dehiscence but only for pollen grains from other flowers. Stamens are shorter at the beginnig of blooming and anthers open distally in the buds before anthesis since the members of the *Iris* genus are protandrous. Due to temporal (protandry) and spatial separation of the anthers and the stigmatic surface (herkogamy), and having a strong self-incompatibility system, the possibility of self-pollination is excluded (Goldblatt et al. 1998).

Our investigations were carried out in an *ex situ* population of about 200 individuals in the Botanical Garden of University of Szeged, Hungary and 30 plants were randomly selected representing the population. Observations of this study group were conducted in May of 2005.

Observations on floral traits

We marked 2 flowering stalks with similar numbers and the spatial arrangement of the flowers on each individual of the 30 selected plants. We recorded the position of each flower within the inflorescence according to its blooming schedule (flowering order). Various morphological traits were measured on freshly opened flowers of one of the inflorescences, without removing them. We registered the following parameters: diameter of flowers, length and width of outer (sepaloid) tepals, length and width of inner (petaloid) tepals, length of filaments and anthers. In all, 122 flowers were involved in the measurements.

Male reproductive success variation

In order to estimate the male reproductive success, the other inflorescence was used to assess pollen yield and viability by removing only one anther from each bud and putting it into Eppendorf tubes filled with Alexander's pollen stain (Alexander 1969). Later, the anthers were chopped up within the tubes using a dissecting needle and the samples were cooked for 10 min in boiling water. Then number of aborted (green) and mature (purple red) pollen grains was determined using

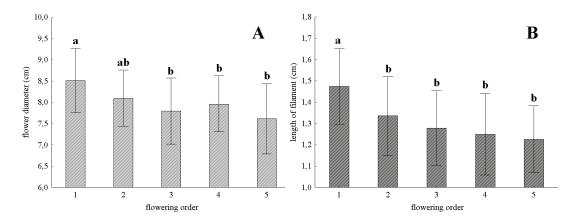


Figure 2. Flower diameter (A), filament length (B), sepaloid (C) and petaloid (D) tepal length of flowers at various positions according to flowering order within inflorescences of *Iris sibirica* L. Bars are mean values \pm SD. Different letters refer to significant differences among groups at p<0.05.

a haemocytometer. We calculated pollen load (total pollen number) per anther and pollen viability as follows:

Total pollen number per anther= mature pollen number + aborted pollen number

Pollen viability (%) = mature pollen number / total pollen number x 100.

Female reproductive success variation

In July, all fruits of inflorescence pairs of each marked plant were collected to count the number of mature and aborted seeds. Almost 150 mature but not opened capsules were harvested. To represent the variation in female reproductive success, the number of ovules per flower and seed set per each fruit were calculated as follows:

Ovule number per flower = mature seed number + aborted seed number

Seed set per fruit = mature seed number / ovule number.

Variation in floral sex allocation

Intra-inflorescence variation of floral sex allocation was quantified by calculating pollen/ovule (P/O) ratio. We used the data (pollen load per anther and ovule number per flower) from 103 flowers. Ovule number per flower was calculated as written above.

Data analysis

Statistical analysis was carried out using STATISTICA 5.0. and 8.0 softwares. One-way ANOVA was applied to test variability of parameters, Newman-Keuls post hoc test was used to evaluate the significance of differences. To determine the relationship between flowering order and floral traits, and between fruit set and flowering order or flower position (at which level within inflorescence) a non-parametric analysis of correlation (Spearman's Rank Order Correlation) was executed. Data are given in mean values ± standard devia-

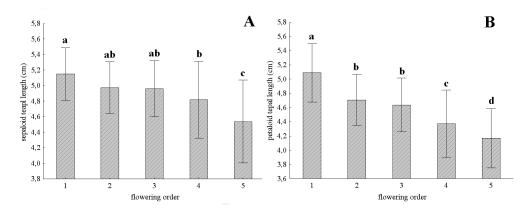


Figure 3. Sepaloid (A) and petaloid (B) tepal length of flowers at various positions according to flowering order within inflorescences of Iris sibirica L. Bars are mean values ± SD. Different letters refer to significant differences among groups at p<0.05.

Table 1. Variability of floral morphological parameters of Iris
sibirica at inter- and intra-inflorescence levels.

Parameters	Source of variance [#]	F	Compo- nents of variance	Vari- ance %
Flower diameter	inter-infl	F 29.91= 2.206**	0.141	23
	intra-infl	29, 91 2.200	0.466	77
Sepaloid tepal length	inter-infl	F 29 91= 1.715*	0.031	15
	intra-infl	25, 51	0.173	85
Sepaloid tepal width	inter-infl	F _{29.91} = 3.361***	0.031	37
	intra-infl	,	0.054	63
Petaloid tepal length	inter-infl	F 29, 91= 1.462	0.026	10
	intra-infl		0.230	90
Petaloid tepal width	inter-infl	F 29, 91 = 2.987***	0.022	33
	intra-infl		0.044	67
Length of filament	inter-infl	F 29, 91 = 2.076**	0.008	21
	intra-infl		0.029	79
Length of anther	inter-infl	F 29, 91= 3.641***	0.006	40
	intra-infl		0.010	60

#inter-infl = among inflorescences, intra-infl = within inflorescences *p<0.05; **p<0.01; ***p<0.001</pre>

Table 2. Mean \pm SD, minimum and maximum values of various floral morphological parameters.

Parameters	Ν	$Mean\pmSD$	Min.	Max.
Flower diameter (cm)	115	7.98 ± 0.78	5.10	10.00
Sepaloid tepal				
length (cm)	115	4.88 ± 0.46	3.10	5.70
width (cm)	115	2.32 ± 0.29	1.40	3.00
Petaloid tepal				
length (cm)	115	4.58 ± 0.50	3.20	5.80
width (cm)	115	1.59 ± 0.23	0.80	2.10
Length of filament (cm)	114	1.31 ± 0.19	0.90	1.80
Length of anther (cm)	114	1.10 ± 0.13	0.90	1.60

tion (SD). Significant differences in ANOVA analysis were established at p<0.05. Levels of significance were marked with * p<0.05, ** p<0.01 and *** p<0.001.

Results

Variations in floral morphological and reproductive traits at inter- and intrainflorescence levels

Individuals of *Iris sibirica* produced typically 10-15 flowering shoots while two plants had extremely high number (nearly 60) of inflorescences. The inflorescences investigated bore 4-7 flowers but most of the stalks (65%) had 5 flowers (Fig. 1A). Duration of flowering of the stalks took 4-12 days, and that of 5-flowered stalks lasted for 9 days, on average.

The analysis of the results of morphological traits exhibited significant differences among stalks in flower diameter, length and width of sepaloid (outer) tepal, width of petaloid **Table 3.** Variability of floral morphological parameters of *Iris*sibirica among flower groups based on order of blooming.

Parameters	F
Flower diameter	F _{4, 109} = 4.863**
Sepaloid tepal length	F _{4, 109} = 6.032***
Sepaloid tepal width	F _{4, 109} = 1.725
Petaloid tepal length	F 4, 109= 15.543***
Petaloid tepal width	F _{4, 109} = 1.568
Length of filament	F _{4, 109} = 6.511***
Length of anther	F _{4, 109} = 2.878*

*p<0.05; **p<0.01; ***p<0.001

(inner) tepal, length of filament and anther (Fig. 1B; Table 1).

At the same time, 80-90% of total variance occured at intra-inflorescence level in flower diamater, sepaloid and petaloid tepal length, length of filament and anther, namely the inflorescences were more similar to each other than the flowers within the stalks. Therefore, we made groups according to flowering order. Since there was nothing but a few stalks that possessed 6 or 7 flowers, we took into account only 5-flowered inflorescences.

We took into account the data of almost 115 flowers, mean \pm SD, minimum and maximum values are shown in Table 2.

After further analysis of variance significant differences were found among flowers within the stalks but only in flower diameter, sepaloid and petaloid tepal length and filament length (Table 3).

Multiple comparison of flower groups exhibited significant differences (Fig. 2. A-D). The diameter of the flowers that opened first was nealy 8 cm on average (Table 2), while the 3rd, 4th and 5th flowers were significantly smaller (Fig. 2A). Analysis of correlation also confirmed that flower diameter decreases with blooming order (r=-0.30, p<0.001; Table 4). Moreover, significantly positive correlation was found between flower diameter and the lengths of both tepals or filament length. Neither the flower diameter nor any other parameters showed significant correlation with flower number per inflorescence (Table 4). Length of filament similarly attenuated within stalks from early to late blooming flowers (r=-0.39, p<0.001; Table 4), first flowers had remarkably longer filaments than those opening later (Fig. 2B).

Both types of tepals that are considered to be the most attractive parts of the flowers, decreased in size by blooming schedule supported by the results of analysis of correlation (Table 4). Generally, second and third flowers were similar in size but there were significant differences, e.g., between the 1st and the 5th flowers (Figs. 3A and 3B.)

Male reproductive success variation

In order to determine the male reproductive success, pollen

Table 4. Relationships among various floral morphological parameters, flowering order and flower number per inflorescence. Data shown are results of Spearman's Rank Correlation, level of significance are *p<0.05; **p<0.01; ***p<0.001.

Parameters	Flower diam- eter	Sepaloid tepal length	Sepaloid tepal width	Petaloid tepal length	Petaloid tepal width	Length of fila- ment	Length of anther
Flower diameter		0.56***	0.14	0.52***	0.20*	0.35***	0.16
Sepaloid tepal length			-0.02	0.53***	0.26**	0.37***	0.21*
Sepaloid tepal width				-0.04	0.32***	-0.17	-0.10
Petaloid tepal length					0.36***	0.52***	0.36***
Petaloid tepal width						0.02	0.25**
Length of filament							0.37***
Flowering order	-0.30***	-0.38***	0.18	-0.63***	-0.28**	-0.39***	-0.27**
Flower number per stalk	-0.01	0.06	-0.06	-0.09	-0.16	0.02	0.16

yield and viability was assessed by collecting anthers of 141 flowers in all, namely 25-30 flowers at each position within the stalks. We found high variability in the case of total pollen load per anther (coefficient of variance, CV= 35-40%) but there was no variation among the inflorescences ($F_{(29, 111)}$ = 1.519; p= 0.063) or among the flowers within the stalks ($F_{(3, 136)}$ = 0.211; p= 0.932; Fig. 4). Total pollen load per anther was 17,262 ± 6,005, and that of all flowers at various flowering positions were above 16,000, on average.

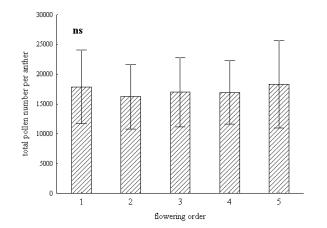
Pollen viability showed low variability (CV= 9-12%), pollen grains showed very high rate of longevity ($91\pm9\%$), which did not descrease with flowering order (Fig. 5). These results suggest that polllen yield and pollen viability is not influenced by the position of the flowers in the blooming schedule, and that probably there was no pollen discount during anthesis.

Female reproductive success variation

The inflorescences observed produced generally 3 ± 1 capsules; 60% of the 1st flowers developed to fruits, while 65% and

70% of the 2nd and 3rd flowers of the investigated stalks set fruit, respectively. Fruit to flower ratios (fruit set) of the 4th and 5th flowers were quite low with 40%. To evaluate female reproductive success nearly 150 capsules were collected and the number of mature and aborted seeds was registered, then ovule number and seed set per fruit were calculated. Remarkable differences were seen among flowers by blooming order with respect to the ovule number ($F_{(4, 143)} = 6.431$; p< 0.001). Flowers that opened first had 98 ± 27 ovules, that of the 2nd and the 3rd flowers had nearly 90, while late-opening (the 4th and the 5th) flowers had significantly lower values, namely 82 ± 19 and 70 ± 15, respectively (Fig. 6).

Capsules collected comprised of 55 ± 18 mature seeds, and mature seed/ovule ratio (seed set) was $60 \pm 13\%$, on average. In case of both seed number per fruit ($F_{(4, 142)} = 7.267$; p< 0.001) and seed set ($F_{(4, 142)} = 5.456$; p< 0.001) we found significant differences among flower groups. Fruits developed from the 5th flowers had significantly fewer mature seeds than those from flowers that opened earlier (Fig. 7A) and the tendency was similar in seed set (Fig. 7B).



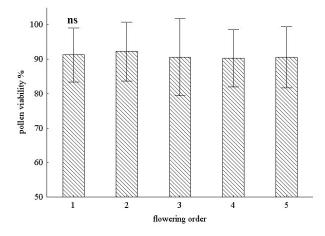


Figure 4. Total pollen number per anther at various positions within inflorescences of *Iris sibirica* L. Bars are mean values \pm SD, ns= not significant differences among groups.

Figure 5. Pollen viability at various positions within inflorescences of *Iris sibirica* L. Bars are mean values \pm SD, ns= not significant differences among groups.

Szőllősi et al.

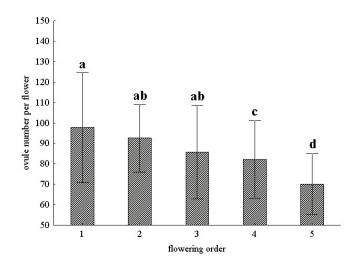


Figure 6. Ovule number per flower (mean \pm SD) in flowering sequence within inflorescences of *Iris sibirica* L. Letters refer to significant differences among groups at p< 0.05.

Both mature seed number per fruit, ovule number per flower and seed set showed a significantly negative relationship with flowering order (r= -0.36, r= -0.37 and r= -0.30; p<0.001). At the same time, we found remarkably positive correlation between ovule number and seed set (r= 0.65, p<0.001).

Floral sex allocation

Mean values of floral sex allocation expressed as P/O ratio ranged from 580 to 960 on average, and exhibited significant differences only between the 2nd and 5th flowers (Fig. 8). This parameter seemed to be strongly variable (CV ranged from 28 to 72%) and slightly increased with blooming order (r= 0.25, p<0.05).

Discussion

Up to now most of the investigations have been conducted on reproductive characteristics and the success of racemose inflorescences, therefore, in this study we aimed to assess variations in floral morphological and reproductive parameters among and within the cymous inflorescences of a protected species, *Iris sibirica*. Since the results of ANOVA analysis showed higher dissimilarities among the flowers within the same inflorescence, we established flower groups in accordance with flowering order, as was done in former studies of species with racemose inflorescence (Ishii and Sakai 2002; Hiraga and Sakai 2007; Zeng et al. 2008; Zhao et al. 2008).

Variation in size of morphological and reproductive organs

Flowers within stalks differed from each other in terms of the diameter and length of both tepals and of the filament. These parameters declined in blooming order (Figs. 2A-B and 3A-B, Table 4), from early-opening to late-opening flowers like in the case of mass or height of perianth in acropetally opening racemose Narthecium asiaticum (Ishii and Sakai 2002), Corydalis remota var. lineariloba (Zeng et al. 2008), and Aconitum gynandrum (Zhao et al. 2008) or flower size in basipetally blooming cymose Aquilegia caerulea (Brunet and Eckert 1998) and Narcissus dubius (Worley et al. 2000). This decline of the attractive floral parts mentioned is probably due to resource limitation and/or competition within stalk and not to the architecture effects of the inflorescence, namely the distal flowers that opened first were significantly larger, while the flowers opening later were closer to the resources in photosynthetic leaves or rhizomes, but were much smaller (Zeng et al. 2008). Moreover, larger size might be more attractive and therefore an advantage for the first flowers when

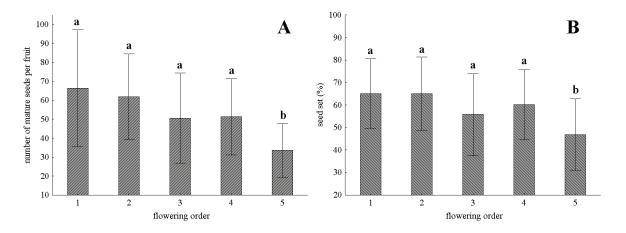
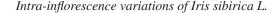


Figure 7. Mean values (±SD) of seed number per fruit (A) and seed set (B) within inflorescences of *Iris sibirica* L. in flowering order. Letters refer to significant differences among groups at p< 0.05.



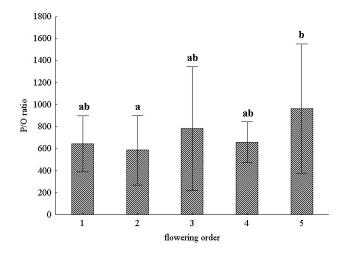


Figure 8. Mean values (\pm SD) of pollen/ovule ratio (P/O ratio) within inflorescences of *Iris sibirica* L. in flowering order. Different letters indicate significant differences among groups at p< 0.05.

a relatively few pollinators visit the plants due to low display size. Later on, when the floral display enhances and more pollinators arrive, the likelihood of successful pollination of the smaller flowers increases (Worley et al. 2000; Kennedy and Elle 2008).

Male reproductive success

Although at high variability, pollen load per anther exhibited abundance and stability during the blooming period with >90% pollen viability regardless of the position of the flower (on which level within a stalk) or of flowering order (Fig. 4 and 5), suggesting that neither pollen yield nor pollen viability might be a limiting factor in successful fruit set and seed set. Wesselingh and Arnold (2003) found that even if the amount pollen grains deposited on stigma of *Iris fulva* flowers was variable, pollen load did not affect seed production.

Variations in female reproductive success

We found a decline in fruit set according to blooming order, which was probably due to resource limitation within stalks or plants (Charlesworth and Morgan 1991; Stöcklin 1997). At the same time, Wesselingh and Arnold (2003) noticed that fruit set of *Iris fulva* with similar blooming order depended on flower position (i. e. at which level) rather than flowering order. Similarly, Buide (2004) observed a reduction in fruit set from the primary (firstly opened) flowers of cymose *Silene acutifolia* to the later flowers at secondary or tertiary positions. Nonetheless, an earlier study of Brunet (1996) showed that in the case of cymose *Aquilegia caerulea* both fruit and seed set declined significantly from the early flowers to the later ones.

Further results suggested that ovule number declined form early-opening to late-opening flowers in cymose inflorescences of Iris sibirica similarly to racemose inflorescences such as Clarkia unguiculata (Mazer and Dawson 2001), Lobelia sessiliflora (Hiraga and Sakai 2007) or Corydalis remota var. lineariloba (Zeng et al. 2008). Moreover, Guitián et al. (2004) found in racemose Polygonatum odoratum that both ovule number and pollen load per flower significantly varied with the position, *i. e.* decline of these parameters from the bottom to the top was observed. In the case of Iris sibirica, female function in biomass sex allocation probably depended on flower position in accordance with blooming order, while the mean number of pollen grains was constant and therefore seemed to be independent of flower position. Namely, within the stalks during anthesis plants allocated relatively fewer resources to female function in late-blooming flowers than in those opening earlier, as it had been observed in numerous protandrous but mainly racemose species, e.g. Lobelia sessiliflora (Hiraga and Sakai 2007), and many studies confirmed that one of the most common causes of either fruit or seed abortion might be resource limitation within plants (Charlesworth and Morgan 1991; Stöcklin 1997).

Both the mean number of seeds and seed set (mean seed/ ovule ratio) also decreased with flowering sequence like in the case of protandrous and racemose Aconitum gymnandrum (Zhao et al. 2008), Lobelia sessiliflora (Hiraga and Sakai 2007) and Corydalis ambigua (Kudo et al. 2001), as well as those of cymose Aquilegia canadensis (Kliber and Eckert 2004). Three main hypotheses have been put forward to explain these intra-inflorescence variations: pollen limitation, resource limitation and architectural effect (Zeng et al. 2008). Since pollen production was abundant and pollen viability was nearly 100%, the pollen limitation hypothesis can be excluded. Nevertheless, declines of the flower size (diameter, tepals), ovule and mature seed number accross the flowering sequence suggest that female reproductive success if Iris sibirica L. might be limited by resources available and/ or structure of the inflorescence, as it was observed in both racemous and cymose species mentioned above.

Floral sex allocation

Relatively high values of sex allocation (about 700, regardless of the flower position) refer to the type of breeding system of *Iris sibirica*, namely facultative xenogamy or obligate xenogamy, while Wang et al. (2004) reported lower P/O ratios for obligate xenogamy (between 184 and 540) in various species of Zingiberaceae; therefore we may expect higher P/O ratio compared to autogamous species (Cruden 1977, Queller1984, Vasek and Weng 1988).

We found a moderate increase in P/O by flowering order due to nearly constant level of pollen yield but decreasing tendency in ovule number, which was similar to *Corydalis remota* var. *lineariloba* (Zeng et al. 2008) where flowers opened first showed lower P/O ratio compared to later ones. Mazer and Dawson (2001) also detected increasing P/O

Szőllősi et al.

ratios from the basal to the top flowers within racemose inflorescences of *Clarkia unguiculata*, which seemed to be significantly affected by the flower position. Moreover, in the case of the racemose *Narthecium asiaticum* the P/O ratio significantly increased from lower to upper flowers due to the approximately constant level of pollen production but a decrease in the ovule number in the order of blooming (Ishii and Sakai 2002).

At the same time, within cymose and protandrous inflorescences of *Aquilegia caerulea* the P/O ratio also increased according to flowering sequence because the ovule number per flower declined but pollen yield remained constant (Brunet 1996, revised by Zhao et al. 2008).

Conclusions

We have a relatively few data on the reproductive characteristics of Iridaceae species, especially the protected Siberian Iris. Our results show that variations in floral morphological traits and female reproductive success are probably due to intra-inflorescence rescource allocation and/or limitation. In order to find out whether other structural parameters within inflorescence (e.g. number of levels), and phenological traits such as number of opened flowers per plant affect the reproductive success of *Iris sibirica*, further investigations are needed.

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