

## Pollination strategy and reproductive success of *Gentiana pannonica* in a natural population

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### Abstract

The effects of different pollination treatments (open-pollination, cross-pollination without emasculation, cross-pollination with emasculation, manual self-pollination, spontaneous self-pollination) and year-to-year variation in reproductive success of *Gentiana pannonica* Scop. were investigated in a two year field experiment, which was carried out in one native population. The highest seed production was found in open-pollinated flowers in both years. It seems that the seed-set of open-pollinated flowers was not limited by a lack of pollinators. Conversely, the seed-set of manually selfed flowers was ca 48–66% lower than that of open-pollinated flowers, with large proportion of aborted seeds indicating an inbreeding depression. Herkogamy (spatial separation of stigma and stamens) together with a mild protandry prevented spontaneous selfing. The occurrence of pollinators (mainly bumblebees) was therefore essential for the successful pollination and development of viable seeds. Significant year-to-year variability was found only in the number of ovules per fruit, but the same trends in seed-set were observed in both years. No significant differences in ex situ and in situ germination of seeds resulting from different pollination treatments were found.

*Key words:* clonal plant, endangered species, herkogamy, inbreeding, pollination experiment

### INTRODUCTION

Due to expansion of forests in Holocene, many light-demanding species receded to the alpine grassland zone in high mountains, steep and rocky slopes, permanently wet locations and regularly disturbed sites. Since the Middle Ages, grassland species expanded their range due to increased human impact in all European mountain regions, when the timberline was lowered and large areas at lower elevations were deforested to create mountain hay meadows and pastures (LANG 1994). Many alpine species spread to new locations and people promoted the spreading of ornamental and medicinal plants. They planted some useful species in their gardens and near their settlements (HEGI 1975). Demographic changes in the second half of the 20<sup>th</sup> century caused the depopulation of many mountain regions in Europe. Grazing activities were reduced; many semi-natural grasslands were abandoned and allowed to undergo natural succession (RYBNÍČEK & RYBNÍČKOVÁ 1974). In the surrounding of settlements, some garden plants spread to countryside, a phenomenon reflected in several recent studies that discuss the natural occurrence of some mountain species in relatively isolated locations (ŠOUREK 1963, KOPECKÝ 1973, SLAVÍK 1997). The history of occurrence of each species is unique and it is often impossible to distinguish the native locations from introduced ones. Some rare species naturally occur in small populations as a result of fragmentation of their habitats during the Late Glacial and Holocene (SLATKIN 1987, BARRETT & KOHN 1991, STEH-

LIK et al. 2002). Other species, historically occurring in larger areas or in many small populations connected to each other (“new rare” in sensu HUENNEKEN 1991), dramatically decreased in the number of populations and population sizes due to changing management practices during the last decades.

Generally, habitat fragmentation together with isolation of recent populations are supposed to strongly affect the viability of populations (KÉRY et al. 2000, LUIJTEN et al. 2000, JACQUEMYN et al. 2001). Small isolated populations are more vulnerable to demographic, environmental and genetic stochasticity, because the maintenance of genetic variation in natural populations is usually considered to be important for ecological plasticity and evolutionary processes (CRUDEN & HERMANN-PARKER 1977, BARRETT & KOHN 1991, MENGES 1991). Vulnerability to fragmentation differs among species with different life histories (ELLSTRAND & ELAM 1993). A strong impact of fragmentation on fitness, measured as a reproductive success, was recorded in small populations of animal-pollinated, outcrossed, and self-compatible plant species (SCHEMSKE & LANDE 1985, CHARLESWORTH & CHARLESWORTH 1987, BARRETT & KOHN 1991, ELLSTRAND & ELAM 1993). Interactions with pollinators can be interrupted and, in particular, small patches of flowering plants may be unable to attract sufficient numbers of pollinators (KWAK 1988, KWAK et al. 1998). Small populations of rare species may become increasingly dependent on the presence of simultaneously flowering species, which may help to attract insect pollinators (OOSTERMEIJER et al. 1998). Furthermore, there may be a reduction of seed-set as a result of competition for pollinators (SPIRA & POLLAK 1986, KWAK & JENNERSTEN 1991).

In small populations of short-lived species, pollen limitation, together with genetic deterioration, may cause a fast decline in population size, because the production of many viable seeds is crucial for survival of the population (FISCHER & MATTHIES 1998a). In long-lived species, the negative consequences of population size reduction and population isolation might not be obvious for a long time. Well-established adult individuals, especially of clonal plants, can survive for many years (TAMM 1972, HARPER 1977) even though the reproductive success may be affected much sooner than survival, resulting in the population becoming “senile” (sensu OOSTERMEIJER et al. 1994a).

An example of the latter situation is the long-lived clonal species *Gentiana pannonica* SCOP., an endangered species in the Bohemian Forest (=Šumava Mts., Böhmerwald). The populations of *G. pannonica* in the Bohemian Forest are isolated from each other. The sites differ in many biotic and abiotic characteristics. The species occurs both at natural sites and in semi-natural mountain grasslands. It is assumed that steep slopes in the cirques of glacial lakes in the Bohemian Forest were refuges for *G. pannonica* and other light-demanders during the Holocene, when woods covered the Bohemian Forest up to the highest elevations (above 1300 m a.s.l., CULEK 1996). Only a few refuges existed and they were isolated from each other (SKALICKÝ 1998). Deforestation of the landscape in this region started in the Middle Ages and the species spread into suitable semi-natural habitats (PROCHÁZKA & ŠTECH 2002). Additionally, the mountaineers promoted the spreading of *G. pannonica*. They probably planted the plants growing in the cirques of the Šumava glacial lakes close to their settlements. The utilization of this medicinal species was similar to *Gentiana lutea* in the Alps (ROSENBAUER 1996). The number of localities and population sizes of *G. pannonica* in the Bohemian Forest fluctuated during the 19<sup>th</sup> and 20<sup>th</sup> century (KRIST 1933, PROCHÁZKA & ŠTECH 2002). Before World War II, gentian roots were collected and used as medicine, for preparation of alcoholic drinks etc. Many locations were destroyed at that time but, simultaneously, some locations appeared because people probably planted the species. Later the post-war political and demographic changes in the region increased the number of *Gentiana* populations and the viability of their populations (PROCHÁZKA 1961, PROCHÁZKA & ŠTECH

2002). Today, semi-natural grasslands are the most frequent habitats of *G. pannonica* in the Bohemian Forest (PRACH et al. 1996, PROCHÁZKA & ŠTECH 2002). Many populations occur close to former home-steads and their origin is unclear. Vegetative propagation prevails at many sites and seedlings are rather rare.

In this study we investigated the pollination ecology, reproductive biology, and breeding system of a *G. pannonica* population in a semi-natural mountain grassland. The aim of our study was to determine the factors limiting the generative reproduction of the species. We tested the self-incompatibility and the pollination strategies of the species to evaluate the level of inbreeding depression and other limits of seed reproduction in a study population.

## MATERIALS AND METHODS

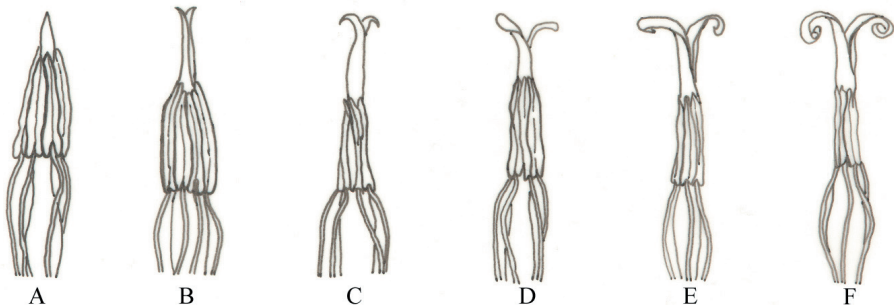
### Study species

*Gentiana pannonica* Scop. (Gentianaceae) is a long-lived hemicryptophyte, which spreads mainly by clonal growth due to belowground rhizomes and creates large polycormons (KLIMEŠ et al. 1997). Sexual reproduction by seeds is necessary for transport of genes over larger distances and for colonization of new sites.

The flowering stems emerging from the mature leaf rosettes can reach up to 150 cm. They carry one to four dense clusters of flowers, each consisting of 5–8 flowers. The calyx is campanulate with 5(–9) stripes. The funnel shaped corolla is 3.0–5.5 cm long with 5(–9) lobes and purple with reddish-black spots (TUTIN 1996, KIRSCHNER & KIRSCHNEROVÁ 2000). The herkogamous flowers are mainly pollinated by bumblebees (for more details about the development of the stigma and the stamens see Fig. 1). On average one ovary contains 200–300 seeds and seeds size is 5×3 mm.

*Gentiana pannonica* is a mountain species that occurs in alpine grasslands, drier mountain meadows or in alluvium of streams and cirques of glacial lakes. The species is a typical East-alpine element with a discontinuous range of occurrence. The centre of its distribution is situated in the East Alps, where the species occurs in alpine and subalpine areas at an

**Fig. 1.** The development of reproductive organs of *Gentiana pannonica* (stigma, stamens) (© Hofhanzlová, 2004). A – A stigma is closed and stamens are not mature in the closed buds. The ring of stamens is not stuck on a carpel. B – Flowers are slightly protandrous with stamens producing pollen before stigma becomes receptive. Maturity of stamens and receptivity of the stigma can partly overlap. C – Stamens without majority of pollen grains. D – The stigma is receptive for couple of days. Usually, only few pollen grains remain on the stamens, which are strongly stuck on a carpel, when the stigma is receptive. The top position of stigma ensures collection of pollen grains from body of all bumblebees entering the flower. E, F – Non-pollinated stigma continues opening and the ends of stigma's dewlaps turn towards the stamens, thus creating possibility for self-pollination.



elevational range of 1300 to 2300 m a.s.l. (KRIST 1933, PROCHÁZKA 1961, HEGI 1975). Outside the Alps, *G. pannonica* occurs in the Bohemian Forest (Böhmerwald) in the border region of the Czech Republic, Germany and Austria. Most of the locations are situated in the Czech part of the mountains at elevations from 1000–1300 m a.s.l. (PROCHÁZKA 1999).

### Study site and population

Our field experiment was performed in 2000 and 2001. The study site was situated close to Horská Kvilda, a small village (1084 m a.s.l., 49°03' N, 13°33' E) in the Bohemian Forest, in the southern part of the Czech Republic. The study population is situated in the centre of the species' distribution in the Bohemian Forest. The short grasses *Nardus stricta* and *Avenella flexuosa* together with *Vaccinium uliginosum* and *Calluna vulgaris* dominate this dry and stony semi-natural grassland, which was created about 200 years ago when deforestation started around the first settlements.

*Gentiana pannonica* occurs on 9 ha, and the population size is about 400 leaf rosettes (or clumps) with about 300 flowering stems.

Monthly means of temperature and precipitation recorded at the Horská Kvilda meteorological station from 2000 and 2001 were used to compare differences in climatic conditions for the two experimental years.

### Pollination experiment and determination of reproductive success

The field experiment was performed in 2000 and 2001 to investigate year-to-year variability in reproductive success. In July 2000, we randomly chose 120 flowering stems and from each terminal cluster we randomly marked an individual flower. Each flower was submitted to one of four pollination treatments (30 flowers per treatment): (1) natural open-pollination, (2) manual cross-pollination with pollen from one flower of 30 donor plants from 5 km distant locality, (3) manual self-pollination with pollen from the same flower, and (4) spontaneous self-pollination. All treatments, except open-pollination, were performed on flowers of terminal clusters, which were covered with bags made from fine-mesh gauze to prevent insect visitation. The manual pollen transfers were performed with cotton sticks.

An analogous experiment with few changes was performed in July 2001. We selected individual flowers on 150 flowering stems, which were randomly subjected to five pollination treatments (30 flowers per treatment). In addition to the four treatments used in 2000 year, (5) a cross-pollination treatment with emasculation was added, to investigate the influence of spontaneous self-pollination on seed set of cross-pollinated flowers. In this treatment, a randomly chosen bud from the terminal cluster was marked and all stamens were removed with a fine forceps. Later, when the stigma was receptive, the flower was manually pollinated with pollen from the other locality. The same donor locality was used for the cross-pollination treatments in both years. This time, insect visitation was prevented using metal cages with fine-meshed gauze. The cages replaced the bags from the previous year, because the bags increased a probability of mould infection in rainy weather. The cages also helped to protect target stems from deer grazing.

The stems remained covered with the bags or cages from July to September until the fruits were harvested. All fruits were carefully collected into paper bags, and the seeds were separated immediately after arrival to the laboratory. The viable (relatively large and obviously full) seeds were distinguished from aborted (small and obviously empty) seeds and ovules, counted and weighed. Mouldy fruits enabled accurate distinguishing of capsule formation, but the counting of seeds could not be performed. Reproductive success was deter-

mined by counting the viable seeds present in each fruit. The number and weight of unfertilized ovules and aborted seeds were also estimated. Seed set was calculated by dividing the total number of viable seeds per fruit by the total number of ovules (both viable and aborted seeds and ovules) per fruit.

### **Germination of seeds**

We tested the germination probability of seeds obtained from the different pollination treatments. We used the seeds from the 2001 season for two germination experiments.

The first experiment was started in autumn 2001 when the seeds were sown in the field experimental plots immediately after ripening. These experimental plots were situated in the typical habitat close to population of *G. pannonica*, but the natural input of additional seeds was excluded. Forty seeds from each of open-pollinated and cross-pollinated (non-emasculated) flowers and thirty seeds from each of cross-pollinated (emasculated) and manually self-pollinated flowers were sown in 10×10 cm plots from which the sod was removed. The experiment was designed as randomised complete blocks with thirty replications for the open-pollinated and the non-emasculated cross-pollinated treatments, and twenty replications for the emasculated cross-pollinated and the manually self-pollinated treatments. Germination of the seeds from the spontaneously self-pollinated flowers was not tested because of the low number of viable seeds. The number of seedlings was recorded monthly from May to September 2002.

The second germination experiment was established in autumn 2002. Seeds from 2001, which were stored at 4°C, were used for this experiment. The experiment, with the same design as in the previous year, was performed in the experimental garden in České Budějovice and regularly irrigated. The number of seedlings was recorded monthly from May to September 2003.

Total germination rate in both experiments was calculated by dividing the total number of seeds sown per plot by the total number of seedlings per plot.

### **Statistical analysis**

Statistical analyses of the data were completed using STATISTICA 6.0 (ANONYMUS 2001). Differences in the number of seeds and seed set under different treatments were tested by One-way analysis of variances (ANOVA) followed by the Tukey-Kramer HSD test. Effects of the pollination treatments and years on the number of seeds and seed set were analysed by Two-way ANOVA. Year-to-year differences were tested only among the four treatments (open-pollination, cross-pollination with stamen, manual selfing, spontaneous selfing). The cross-pollination treatment with emasculatation was excluded from the analysis, because this treatment was carried out only in the 2001 season.

Differences in total germination rate of seeds under different treatments were tested by one-way ANOVA followed by the Tukey-Kramer HSD test. The numbers of viable seeds were log-transformed ( $y = \log(x + 1)$ ) and seed-set and the total germination rate were arcsin-transformed ( $y = \arcsin \sqrt{x}$ ) to normalize distributions and homogenize variances.

Nomenclature follows ROTHMALER (2000).

## **RESULTS**

### **Reproductive success under different treatments**

We found significant differences among different pollination treatments in the number of viable seeds per fruit, seed set, and weight of ovules in both seasons. The open-pollinated

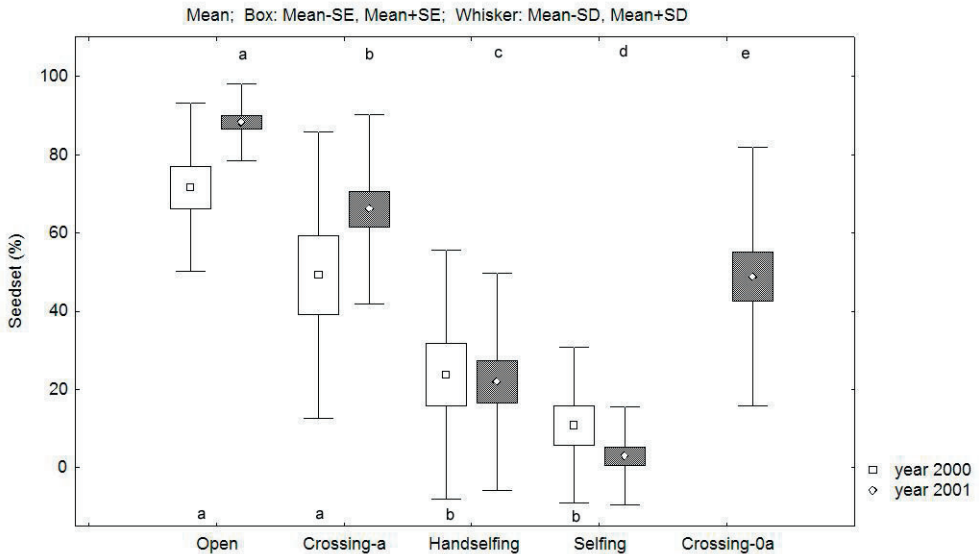
and cross-pollinated flowers had significantly higher seed set than the manually and spontaneously self-pollinated flowers in both years (2000:  $F_{df(3, 57)} = 14.87$ ,  $P < 0.001$ , 2001:  $F_{df(4, 138)} = 66.24$ ,  $P < 0.001$ , respectively). The highest seed set was found in fruits of the open-pollination treatment (Fig. 2). The cross-pollination treatments (non-emasculated and emasculated) differed from each other. The seed set of the manually self-pollinated flowers was significantly higher than that of the spontaneously self-pollinated flowers, but only in 2001 (Fig. 2). Only a few viable and well-developed seeds ripened in the spontaneously self-pollinated flowers.

The seed mass per fruit (weight of all seeds and ovules in ovary) was strongly correlated with the number of viable seeds, which were much heavier than the aborted ones. The highest seed mass per fruit was recorded in the open-pollinated and cross-pollinated flowers (2000:  $F_{df(3, 57)} = 5.95$ ,  $P = 0.001$ , 2001:  $F_{df(4, 138)} = 25.93$ ,  $P < 0.001$ ). No significant difference was found between manual self-pollination and cross-pollination (emasculated) treatments in 2001. The lowest seed mass was recorded in the spontaneously self-pollinated flowers (Fig. 3).

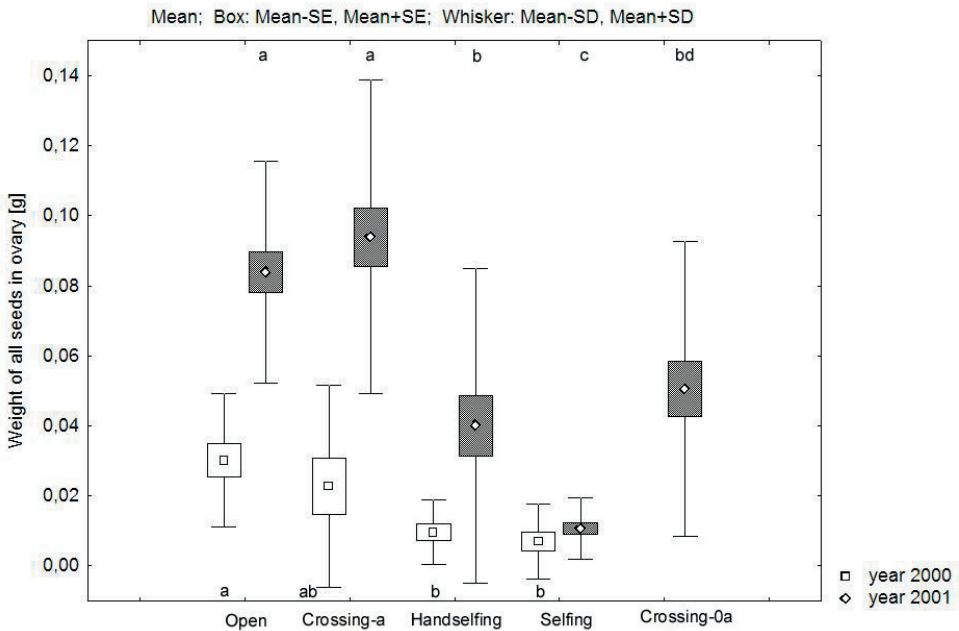
### Comparison of the two seasons

The number of ovules significantly differed between seasons ( $F_{df(1, 3, 168)} = 83.06$ ,  $P < 0.001$ , Table 1), with much higher number of ovules per fruit recorded in 2001 than in 2000 (Fig. 4). Nevertheless similar pattern of all pollination treatments was observed in both seasons (Fig. 2).

**Fig. 2.** The seed set of the differently pollinated flowers of *Gentiana pannonica* in both seasons (2000, 2001). Back-transformed data to the original scale are used for graphical presentation. Pollination treatments: Open = open-pollination, Crossing-a = cross-pollination without emasculatation, Handselfing = hand self-pollination, Selfing = spontaneous self-pollination. Letters on the bottom indicate the results of the Tukey-Kramer HSD test for 2000 year; letters on the top indicate the results of the Tukey-Kramer HSD test for 2001 year; treatments labeled with the same letter did not differ significantly ( $p > 0.05$ ).



**Fig. 3.** Weight of all seeds in ovary from differently pollinated flowers of *Gentiana pannonica* in both seasons (2000, 2001) Back- transformed data to the original scale are presented. Pollination treatments: Open = open-pollination, Crossing-a = cross-pollination without emasculation, Handselfing = hand self-pollination, Selfing = spontaneous self-pollination. Letters on the bottom indicate the results of the Tukey-Kramer HSD test for 2000 year; letters on top the indicate the results of the Tukey-Kramer HSD test for 2001 year; treatments labeled with the same letter do not differ significantly ( $p > 0.05$ ).



**Table 1.** Two-way analyses of variance (ANOVA) on the effects of years (2000 and 2001) and the pollination treatment (natural open-pollination, manual cross-pollination, manual self-pollination with pollen from the same flower and spontaneous self-pollination) on the number of all ovules, seeds set and weight of the seeds. The significant P-values with  $p < 0.05$  are indicated in bold.

	df	Number of all ovules in ovary		Seed set		Weight of the seeds in ovary	
		F	P	F	P	F	P
<b>Year</b>	1	83.06	<0.001	1.55	0.215	69.47	<0.001
<b>Pollination treatment</b>	3	0.54	0.654	88.61	<0.001	27.03	<0.001
<b>Poll. treatment × year</b>	3	0.21	0.891	3.28	0.023	9.23	<0.001

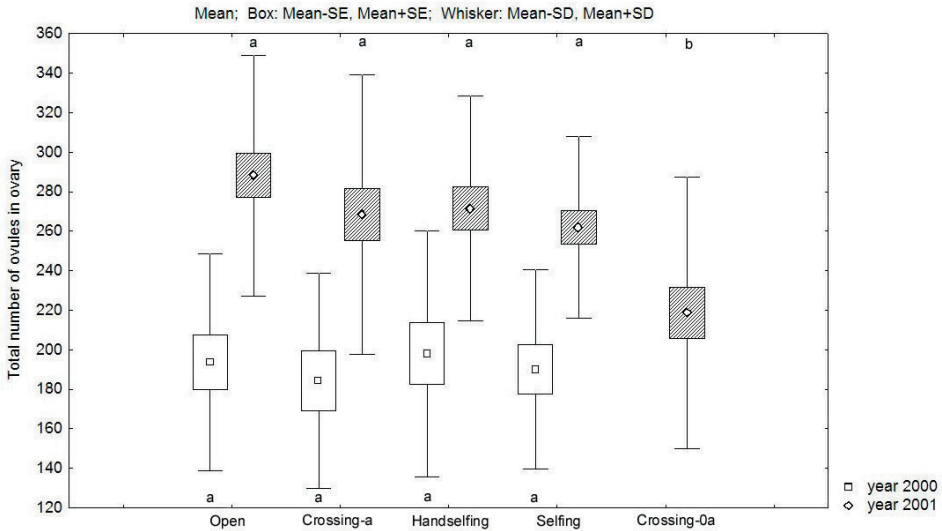
The seeds mass significantly differed between years and also among pollination types (Table 1). The number of seeds per fruit and seeds mass were lower in 2000 season than in 2001. In both years, the weight of seeds from the spontaneously self-pollinated flowers was similarly low (Fig. 3).

### Germination of the seeds from different pollination treatments

In the first germination experiment carried out in 2001, the mean ( $\pm$ S.E.) germination rate was 27.8%  $\pm$  1.7. No significant differences were found among the different pollination types ( $F_{df(3, 96)} = 1.51$ ,  $p = 0.216$ ).

**Fig. 4.** The number of all ovules (viable, aborted seeds and unfertilized ovules) in ovary of differently pollinated flowers of *Gentiana pannonica* in both seasons (2000, 2001). Back-transformed data to the original scale are used for graphical presentation in the graphs. Pollination treatments: Open = open-pollination, Crossing-a = cross-pollination without emasculation, Handselfing = hand self-pollination, Selfing = spontaneous self-pollination.

The Tukey-Kramer HSD test showed no significant differences between treatments in each year.



In the second experiment with one-year-stored seeds, the rate of germination was extremely low (mean  $\pm$  S.E. =  $1.8\% \pm 0.3$ ). The different pollination treatments did not significantly differ in their germination effort ( $F_{df(3, 95)} = 1.3$ ,  $p = 0.279$ ), even though we found slightly higher germination of seeds from the cross-pollination treatment (mean  $\pm$  S.E. =  $2.5\% \pm 0.6$ ).

### The development of reproductive organs of *Gentiana pannonica*

Our observations on the morphology and development of the reproductive organs of *G. pannonica* showed that there is a strong herkogamy and slight dichogamy, indicative of an inbreeding-avoidance mechanism (Fig. 1). Pollen grains are carried out from ripened stamens during few hours in sunny days. Pollen remains in stamens when the flower is closed due to bad weather. Pollinators (mainly bumblebees, *personal observation*) carried out ripen pollen grains from stamens. Maturity of stamens and receptivity of the stigma can overlap.

## DISCUSSION

Although the manual self-pollination treatment showed that *G. pannonica* is self-compatible, the high seed production of open-pollinated flowers compared to the very low seed set of bagged and unmanipulated flowers showed the important role of pollinators. Together with herkogamy, dichogamy also helps to prevent an incidence of self-pollination. Although the flowers of *G. pannonica* are less dichogamous than flowers of other insect-pollinated gentians (i.e. *G. pneumonanthe* – PETANIDOU et al. 1995a; *G. cruciata* – PETANIDOU et al. 1995b; *G. lutea* – KÉRY et al. 2000; *G. newberryi* – SPIRA & POLLAK 1986) some degree of protandry was observed.

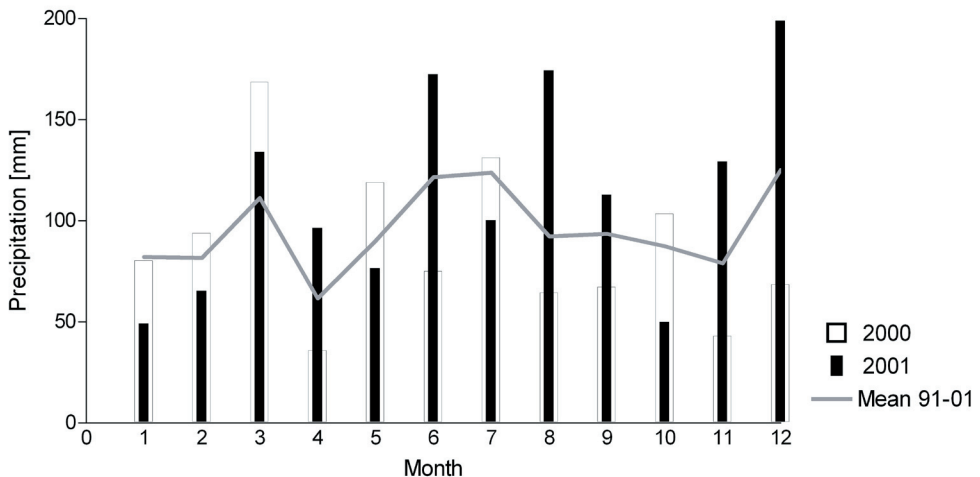


In the studied population, the seed set of open-pollinated flowers was the highest (Fig. 2), suggesting that the population was not pollen or pollinator limited. Hence, this population is apparently large enough to ensure its reproductive success by attracting sufficient numbers of visitors. Similarly, the highest seed production for *Swertia perennis* was reported in open-pollinated flowers (LIENERT & FISCHER 2004). Nevertheless, different results were recorded in small populations of several rare species, where the absence of pollinators or other causes of pollen limitation reduced reproductive success (SPIRA & POLLAK 1986, KWAK 1988, KWAK & JENNERSTEN 1991, FISCHER & MATTHIES 1997, KWAK et al. 1998, OOSTERMEIJER et al. 1998).

In our studied population, the cross-pollination treatment showed a higher proportion of viable seeds (seed-set) than the manual self-pollination treatment (Fig. 2). The higher seed abortion in manually self-pollinated flowers could be the result of inbreeding depression, where the deleterious effects are often expressed during seed development (CHARLESWORTH & CHARLESWORTH 1987, BARRETT & KOHN 1991, ELLSTRAND & ELAM 1993). On the other hand, differences between germination of seeds of different pollination treatments were not significant and the negative consequences of inbreeding depression seemed to be significant only in early life history stages. The abortion of developing seeds already removes a considerable number of inbred offsprings and the remaining viable seeds are thus less inbred. The reduced fecundity expressed in lower seed set of naturally pollinated flowers has been also found in small populations of a perennial gentian, *Gentiana lutea* (KÉRY et al. 2000). Selfing plays an important role in *G. cruciata* (PETANIDOU et al. 1995b) and *G. pneumonanthe* (OOSTERMEIJER et al. 1995), where inbreeding depression was significantly documented only in the performance of offspring. Based on these examples, the reduction of seed set seems to be typical for long-lived clonal species such as *G. pannonica* and *G. lutea* (KÉRY et al. 2000), but lower seed set following manual selfpollination was also found in the short-lived, non-clonal *Gentianella germanica* (LUIJTEN et al. 1998). WIENS (1984) indicated that a relatively high percentage of ovule and seed abortion is a frequently observed phenomenon in perennials, and it is argued that this results mainly from a higher genetic load of outcrossing perennials in comparison with selfing annuals. For example, the same reproductive success of manually selfed and cross-pollinated flowers was observed in annual, biennial and short-lived species such as *Gentiana tenella*, *G. prostrata*, and *Gentianella germanica* (SPIRA & POLLAK 1986, FISCHER & MATTHIES 1998b, LUIJTEN et al. 1998). This supports the generally accepted theory that the persistence of populations of annual and short-lived species requires regular reproduction and seedling establishment, whereas the persistence of well-established individuals and vegetative reproduction plays an important role in the survival of perennial species (SPIRA & POLLAK 1986, EISTO et al. 2000).

Large differences among years were observed in the number of ovules per fruit. This is most likely a result of limitation of a vital resource (LLOYD 1980). In 2000, there were drought periods in June and August 2000, which may have had a negative effect on the number of inflorescences per plant as well as on the number of ovules per fruit in comparison to the more humid summer months in 2001 (Fig. 5). The pattern of seed set in particular pollination treatments was similar in both years of experiment. Only seeds mass differed between both years and pollination treatments. The fruits of the selfed flowers contained mainly aborted seeds and ovules. The seeds mass was dependent both on the amount and quality of the viable seeds. The lower number of seeds in ovary did not result in increased size and weight of seeds as reported for other species (KÉRY et al. 2000). Interestingly, the seed set of cross-pollinated flowers with emasculation was lower than that of cross-pollinated flowers without emasculation. The reason for this is unclear, but we assume that the emasculation might have had a negative impact on flower/fruit development. Some of the fruits in this treatment were ripen sooner than other fruits and this caused a minute loss of seeds during

**Fig. 5.** The monthly averages of precipitation in 2000 and 2001 years together with ten year averages (1991–2001) are displayed. The data were acquired from the meteorological station in Horská Kvilda.



the fruits collection.

The results of our manipulated field experiment helped to answer questions about the effects of different pollination types and year-to-year variation on reproductive success of *G. pannonica*. We studied the pollination strategy of the species in a natural population located in an isolated area of the species distribution range. The seed set of the open-pollinated flowers was not reduced and we suppose this population does not suffer pollen limitation. The production of only a few low quality seeds open-pollinated flowers was observed by ŠOUREK (1963) who cultivated few plants from a small isolated population.

The future research should address the questions related to the reproductive success and genetic variability of populations with different size, as the genetic drift and consequences of inbreeding play important roles in the performance of small populations of gentian species (OOSTERMEIJER et al. 1994b, LUIJTEN et al. 1998).

**Acknowledgements.** The research was supported by the Grant Agency of the Czech Academy of Sciences (junior grant GA AV ČR 6141901), FRVŠ 1281/2002, MŠMT 1K03011, and the Mattoni Award. We would like to thank to M. Šmilauerová for critical comments on the earlier drafts of this manuscript and K. Edwards for correcting English. We are grateful also to I. Matějková, L. Ekrt, and D. Zývalová for their help with field work.

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Received: 22 January 2007

Accepted: 22 April 2007