

## Reproductive biology of the rare biennial *Gentianella germanica* compared with other gentians of different life history

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### SUMMARY

We tested reproductive biology and pollination limitation of the rare *Gentianella germanica* in two large populations in The Netherlands, at the margin of its distribution area. *Gentianella germanica* is self-fertile, but pollinators are essential for the transport of pollen to the stigmas. In caged and untouched flowers the mean seed set was reduced to less than 30%. However, the ability of auto-deposition of pollen on the stigma varied between individuals (0–90%). The reduced seed set after hand-selfing in one population indicates some inbreeding effects on ovule or seed abortion, but in the other population no inbreeding depression was observed. This population had an overall lower seed set and seed number per fruit. Despite the favourable nutrient conditions, and higher number of ovules per flower in this population, there was apparently a limit to the number of seeds that could be matured per fruit. There was no evidence for pollination limitation in either population. A comparison of autofertility and ovule production per flower with several other gentian species differing in life history confirmed the hypothesis that the annual and most gentians are selfers and the perennials predominant outcrossers. Hence, particularly the perennial gentian species risk reductions of reproductive success and inbreeding depression owing to habitat fragmentation and pollination limitation. In contrast to other biennials, *G. germanica* was more similar to the perennial species, because of its poor autofertility. The possible role of herkogamy and dichogamy in the varying ability of individuals to self-pollinate spontaneously is discussed, and will be studied in the near future.

*Key-words:* autofertility, conservation, Gentianaceae, life history, marginal population, reproductive success.

### INTRODUCTION

Plant species demonstrate a remarkable diversity in life history strategies which are often closely related to the type of habitat in which they are found (Grime 1979). In many cases, a relationship is also found between a plant's life history strategy and its reproductive biology (Salisbury 1942). Annuals are typical for open and disturbed habitats, whereas biennials are characteristically species of intermediately disturbed, and generally open vegetation (Harper 1977; Grime 1979; Van der Meijden *et al.* 1992).

These short-lived species are often highly dependent on a seed bank (Leck *et al.* 1989) and produce many small seeds per fruit (i.e. they have a comparatively large 'brood size', Wiens 1984). Many short-lived plants are self-fertile and capable of spontaneous self-pollination which reduces failures in reproductive success due to (periodical) scarcity of pollinators and assures a high seed set (Levin 1972). In contrast to annuals and biennials, polycarpic perennials are mostly found in more closed and stable communities. Although self-fertility is common in perennials, reproductive assurance is generally not necessary since a high annual seed production is considered to be of less importance (Stebbins 1950). This is especially true for species that have vegetative reproduction (Eriksson 1993; Cook 1993).

In this paper, we restrict ourselves to a group of plant species occurring in nutrient-poor habitats: grasslands, hay meadows, moist heatlands, wet dune slacks and calcareous and montane grasslands. Many taxa characteristic of these habitats are declining or becoming extinct, because agricultural practices changed, the landscape became more fragmented, and ecological conditions deteriorated (Mennema *et al.* 1985; Lennartsson & Svensson 1996; Fischer & Stöcklin 1997). Fragmentation and isolation of populations also affect genetic variation, gene flow and reproductive success (Ellstrand & Elam 1993; Oostermeijer *et al.* 1994; Young *et al.* 1996). In small and isolated populations, reproductive output may be reduced because of low visitation rates of pollinators (Kwak 1988; Kwak *et al.* 1991). As a consequence, plants are forced to self-pollinate. In predominantly outbreeding plant species, reproductive success and offspring fitness decreases due to inbreeding depression (Oostermeijer *et al.* 1994). Short-lived species that are predominantly selfers will suffer less from inbreeding depression. In these species, repeated selfing has already purged deleterious alleles from the population (Charlesworth & Charlesworth 1987).

Members of the family Gentianaceae are plant species which are expected to be affected by these changes. In this paper we consider gentians belonging to the closely related genera *Gentiana* and *Gentianella*, which differ in life history. Many gentians have become rather rare throughout most of their distribution area, but mainly in the western and northern parts of Europe (Pritchard 1972; Mennema *et al.* 1985; Lennartsson & Svensson 1996). Populations of most species have declined or have become extinct, and many species are now placed on the Red Data Lists of several European countries (Korneck & Sukopp 1988; Weeda *et al.* 1990; Landolt 1991; Ingelög *et al.* 1993; Olivier *et al.* 1995).

The main study species in this paper is the strict biennial *Gentianella germanica* (Willd.) Börner, a rare plant in The Netherlands. The species occurs in a few populations, which are found only in the south-eastern part of the province of South-Limburg (Mennema *et al.* 1985). These populations are on the north-western border of its distribution area (Hulten & Fries 1986). It can be expected that ecological conditions at a species' border are less favourable than in the centre. Therefore, marginal populations are often smaller and more isolated, and suitable pollinators might be scarce (Levin & Clay 1984). Such conditions may induce selfing and lead to higher autofertility (Lloyd 1992; Jarne & Charlesworth 1993). Possibly as a consequence of the inbreeding and genetic drift associated with this, genetic variation of border populations is often reduced compared to central ones (Brussard 1984; Hoffmann & Blows 1994). Fischer & Matthies (1997) have studied reproductive biology of a population of *G. germanica* in Switzerland, in the core of the species' distribution. They found, among other things, that the species depends on pollinators for a good seed production and, as a result, is

sensitive to pollination limitation. Hence, given the rarity and marginal position of *G. germanica* in The Netherlands, it is interesting to investigate whether the species (1) has a different reproductive biology, e.g. is a better selfer, than in the centre of its range and (2) experiences pollination limitation. Also, since after this study we will have data on the reproductive biology of several gentian species, we are able to investigate the relationship between life history and the degree of autofertility within this taxonomic group. With this comparison, we can test the hypothesis that short-lived, annual and biennial species show higher autofertility and seed-ovule ratios than long-lived perennials (Wiens 1984; Lloyd 1992; Molau 1993).

## MATERIALS AND METHODS

### *Description of the study species*

*Gentianella germanica* germinates in early spring (March to May), forms a rosette during the first year and flowers late (August–September) in the second growing season (Verkaar & Schenkeveld 1984). Plants vary in size from rather small (<10 cm) individuals bearing few (1–5) flowers to robust specimens (>50 cm) with many flowers (>100). The pinkish flower is typical of the genus *Gentianella*. In the throat of the flower there is a characteristic rim of erect, whitish fringes. The function of these fringes is not well understood. It may be that they either protect the flower entrance from small, nectar robbing insects (Ricca 1870; Müller 1881), or prevent the dilution of the nectar by rain (Schultz 1988). The species has protandrous (Ricca 1870), nectar-producing flowers which are mainly visited by bumblebees in our study populations (personal observations). Fischer & Matthies (1997) found that small bees and Diptera were the main pollinators in Switzerland.

### *Study populations*

*Gentianella germanica* was studied in two large populations, located in calcareous grasslands in the southernmost part of The Netherlands. The populations (each >5000 flowering plants in the study season) are, respectively, situated in the State Nature Reserves 'Wrakelberg' and 'Gerendal' (hereafter called populations Wrakelberg and Gerendal). The Wrakelberg Nature reserve is facing south with a slope of c. 16°. Since c. 1945, the vegetation is mown annually, late in the season when all plant species have set fruit. The Gerendal Nature reserve faces NW with an inclination of 20°. The site was fertilized until 1967. Thereafter it was grazed by sheep and/or was mown annually, after the growing season (Willems 1980).

### *Pollination experiments*

The reproductive biology of *G. germanica* was studied in summer 1991 by means of different pollination treatments. To prevent insects from visiting the flowers, metal cages covered with fine-meshed gauze were placed over more than 40 plants in each population. Per caged plant, three flowers were used. One flower of each caged plant was left untouched, to investigate the ability for spontaneous self-pollination. The second flower was pollinated by hand using either pollen from the same flower or another flower from the same plant. The third flower was pollinated with outcross pollen from another plant in the same population. Hand pollinations were effected by

brushing a freshly dehiscent anther which was removed from a self or outcross flower with fine forceps against the receptive stigma. Prior to the outcrossing treatment, flowers were emasculated just before the flower was about to open, by removing all undehiscent anthers with fine forceps. We observed no effects of emasculation of anthers on the development of the flower (cf. Fischer & Matthies 1997). Reproductive success of open-pollinated flowers was investigated on a separate group of 40 uncaged plants to which visitors had free access. All flowers receiving different pollination treatments were colour-coded on the calyx with a small dot of acrylic paint.

Three weeks after the pollination treatments, cages were removed and mature fruits were collected. Reproductive success was determined as follows. With a brush, the fruits were emptied into a Petri dish and examined under a dissecting microscope. Viable (relatively large and obviously filled) seeds could be readily distinguished from aborted (shriveled and obviously empty) ovules. The sum of these was considered to be equal to the total initial number of ovules. Seed set was calculated by dividing the total number of viable seeds per fruit by the total number of ovules of the same fruit.

### *Statistical analysis*

All data were tested for normality and heteroscedasticity. When the data did not fulfil the assumptions of parametrical statistics, and transformation did not improve this, non-parametric tests were used. To investigate if variation in reproductive success resulted from the different pollination treatments, either parametric or non-parametric (Kruskal–Wallis) ANOVAS were performed. Differences between the mean reproductive success of the various pollination treatments were tested by Tukey's HSD multiple comparison of means following parametric ANOVA or by separate Mann–Whitney *U*-tests after Kruskal–Wallis (Sokal & Rohlf 1981).

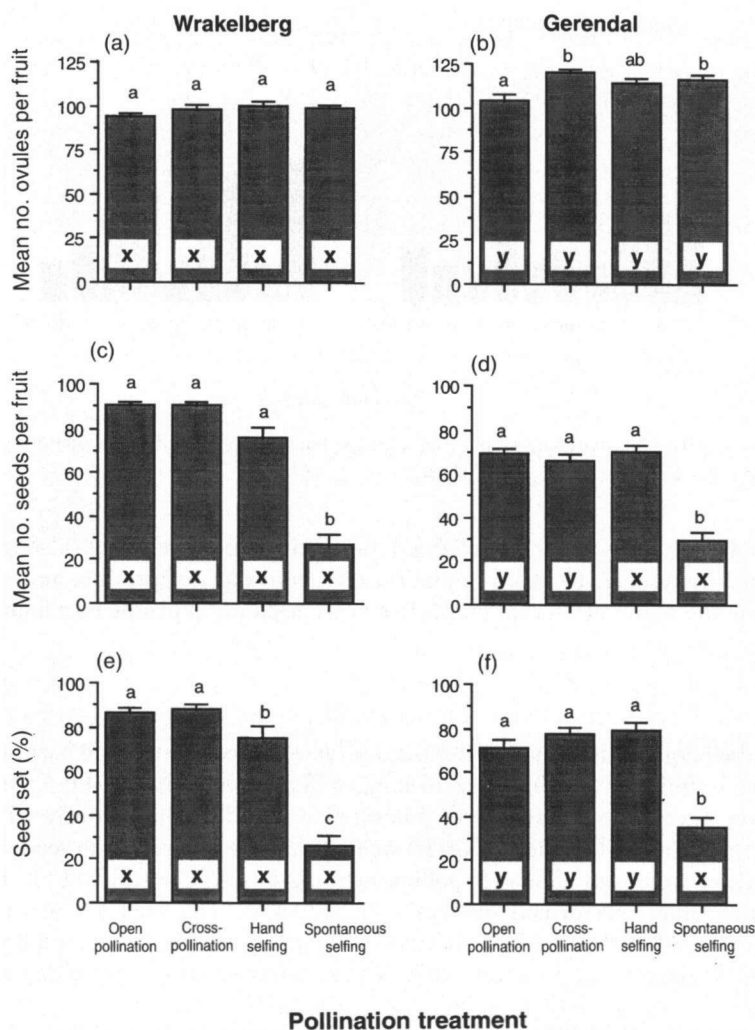
## RESULTS

The recovery of fruits was nearly 100%, suggesting that no significant fruit abortion occurred as a result of the pollination treatments. Therefore, the analyses presented in the following sections are based on the results from all treated flowers.

### *Number of ovules*

A clear difference in the number of ovules was found between the two study populations. Plants in the Gerendal population produced significantly more ovules per flower. Average ovule production was 114 in the Gerendal and 98 in the Wrakelberg population (Fig. 1a,b).

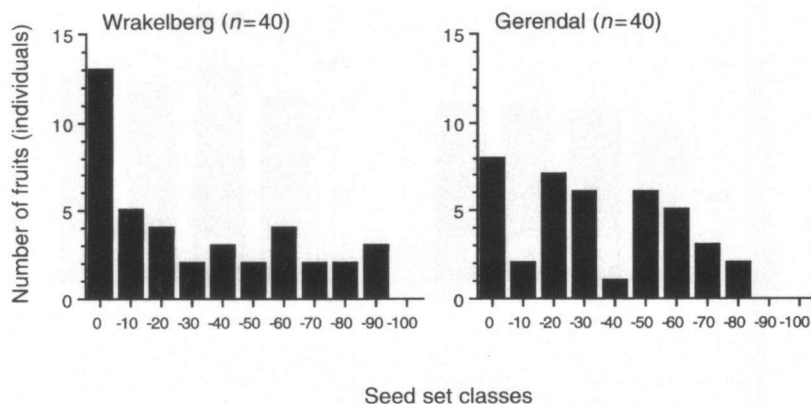
In the Wrakelberg population, no significant differences between pollination treatments were found in the number of ovules (Fig. 1a). However, this was not the case in population Gerendal (Fig. 1b). In open-pollinated flowers the mean number of ovules per fruit (104) was significantly less in comparison to hand outcrossed (120), and spontaneously selfed flowers (116) but not compared to hand-selfed flowers (114). Within the caged treatments, we observed no significant variation in the number of ovules.



**Fig. 1.** (a–f) Reproductive output for different pollination treatments in *Gentianella germanica* in populations Wrakelberg and Gerendal. Represented are ovule number (a and b), the number of seeds per fruit (c and d) and seed set (e and f). Differences *within* populations are given above the columns. Differences *between* populations but within the same pollination treatment are given within the columns. Columns that have no letter in common are significantly different ( $P \leq 0.05$ ).

### *Number of seeds per fruit*

In both populations, no differences in the number of seeds per fruit were found between open-pollinated, hand-outcrossed and hand-selfed flowers (Fig. 1c,d). In caged, unpollinated flowers, the number of seeds per fruit was reduced significantly in both populations, to 35 seeds in population Gerendal and to 26 seeds in Wrakelberg. This spontaneous seed production per fruit was statistically the same in both populations. A difference was found between the two populations in the number of seeds per fruit for open pollination and hand-outcrossing (Fig. 1c,d). In population Gerendal, the number of seeds per fruit was significantly lower in most treatments, except for the



**Fig. 2.** Frequency distribution of seed set per fruit after spontaneous selfing in two populations of *Gentianella germanica*.

hand-selfing treatment, after which seed production per fruit was also reduced in population Wrakelberg. Hence, population Gerendal did not produce more seeds per fruit, despite the observation that plants from this population produced a higher number of ovules.

#### *Seed set*

In the Wrakelberg population (Fig. 1e), seed set in open-pollinated and hand-outcrossed flowers was significantly higher than in hand-selfed flowers (90.6%, 90.5% and 75.3%, respectively), and approximately three times higher than in spontaneously selfed flowers (26.6%). In population Gerendal (Fig. 1f) we observed no differences in seed set between open, hand-outcross and hand-self-pollinations (68.8%, 65.6% and 69.6%). Here, also, spontaneous selfing performed distinctly low (29.4%). The seed set of open, hand-outcrossed and self-pollinated flowers was significantly lower in the Gerendal population than in the Wrakelberg population, while seed set after spontaneous selfing was similar (Fig. 1e,f).

A more detailed description of seed set after spontaneous selfing in individual flowers is presented in the form of frequency distributions of individuals over seed set classes (Fig. 2). In both populations quite a number of individuals, namely 32.5% in the Wrakelberg and 20% in the Gerendal population, completely failed to produce seeds when pollinators were excluded. Among the other individuals, seed set varied from 1 to 90%.

## DISCUSSION

#### *Reproductive success*

Both Dutch populations of *G. germanica* showed a high number of ovules (98 and 114) compared to the average of 71 observed in a Swiss population by Fischer & Matthies (1997).

The comparatively high ovule production of population Gerendal might be explained by the higher nutrient level at this site, observed by Willems (1980). Plants in the Gerendal could apparently allocate more energy to reproduction, resulting in larger

plants with more flowers and more ovules per flower. However, seed set was maximally c. 70% of the large number of ovules, whereas the comparatively high seed set of c. 90% on the Wrakelberg concerned a smaller ovule number. It cannot be assumed that pollen limitation is responsible for this lower seed set in Gerendal since, in the hand pollinations, pollen was applied onto the stigmas in high quantities (i.e. more pollen grains than ovules were available). One explanation might be that large plants with many flowers exhibit a lower proportional allocation to seed maturation per flower than smaller plants (from population Wrakelberg). In *Lupinus texensis*, Helenurm & Schaal (1996) found that the number and size of inflorescences and the amount of ovules per flower were positively correlated with the level of nutrients. In this species, fertilization of ovules was not affected by nutrient treatments, but abortion of developing fruits and seeds was resource-limited. Similar high ovule numbers and low seed/ovule ratios were found in large plants of *Amsinckia grandiflora* (Pavlik *et al.* 1993) and *Asclepias tuberosa* (Wyatt 1980).

In both populations, the number of seeds per fruit after hand selfing was as high as in cross- and open-pollinated flowers. However, seed set after hand-selfing in the Wrakelberg population was significantly reduced. This suggests a slight inbreeding depression of seed set which did not lead to a significantly lower seed number per fruit. Selfing may lead to the expression of recessive alleles that lead to abortion of a fraction of the seeds (Charlesworth & Charlesworth 1987; Waser & Price 1991). In the Gerendal population no indication for inbreeding was found. The higher natural abortion of ovules in this population might have affected the detection level for seed abortion as a consequence of inbreeding.

Fischer & Matthies (1997) found no inbreeding depression for the number of seeds per fruit and seed mass in the Swiss *G. germanica* population. They did find inbreeding later in the life cycle, however. Similar results were obtained for the perennials *Gentiana cruciata* and *Gentiana pneumonanthe* (Petanidou *et al.* 1995a; 1995b; Oostermeijer *et al.* 1994). In the perennial *Gentiana newberryi*, however, Spira & Pollak (1986) found significant inbreeding depression of fruit set, although the authors attributed this to weak self-incompatibility.

### *Autofertility*

Although the species is self-compatible, seed production is pollinator-dependent and seed set was reduced to less than 30% in caged and unpollinated flowers. This agrees with the results on *G. germanica* in Switzerland by Fischer & Matthies (1997). Thus, *G. germanica* does not show the expected tendency towards higher autofertility and increased pollinator independency on the margin of its distribution area. Also, both studied Dutch populations did not exhibit pollination limitation, despite the marginal position, the fragmentation and the reduced quality of the chalk grassland habitat in The Netherlands. It must be noted, however, that both populations comprised a considerable number of flowering plants in the study year. Poor management, in the form of complete mowing or intense grazing during the flowering period, has drastically reduced the population size of population Wrakelberg in the past 5 years.

In our pollinator exclusion experiments, we found much variation in seed set between individuals within a population. Apparently, some individuals have a better autodeposition of pollen on the stigma than others. This may result from two mechanisms; either the flowers are less dichogamous, which will result in increased pollen

viability during receptivity of the stigma (Webb & Lloyd 1986), or herkogamy is less pronounced, i.e. anthers and stigma are positioned closer to each other (Lloyd & Webb 1986). Recent field observations indicate that the latter of these explanations may be true. In some plants the stigma appears to protrude visibly above the level of the anthers, while in others the stigma is positioned at the same or below this level. Variation in the spatial arrangement of anthers and stigma has been also described for other *Gentiana* and *Gentianella* species (Müller 1881; Webb & Pearson 1993). Lennartsson (1997) demonstrated heritable variation for herkogamy within populations of *Gentianella campestris* in Sweden. Currently, experiments are being conducted to study the importance of herkogamy for the reproductive biology of *G. germanica*.

#### *A comparison of autofertility among gentians with different life history*

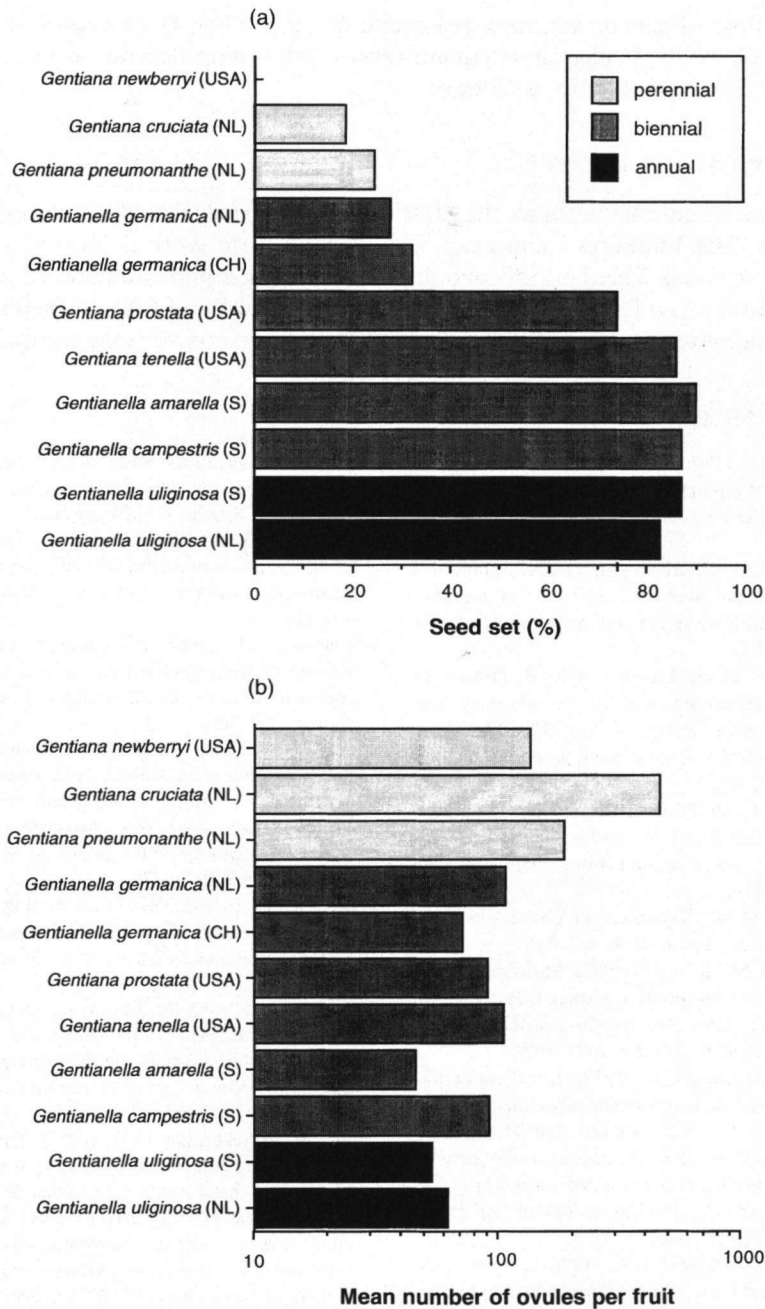
A comparison of the autofertility for several gentian species differing in life history is given in Fig. 3a. As expected, the annual *G. uliginosa* has a high seed production in the absence of pollinators (Petanidou *et al.* 1998). Similar results for the same species were found in Swedish populations (Lennartsson 1997). The biennial species *G. amarella* and *G. campestris* (Lennartsson 1997), and the alpine biennials *G. tenella* and *G. prostrata* (Spira & Pollak 1986) also have a full seed set in the absence of pollinators. In all of these species, pollinator visitation is generally not necessary to ensure high seed set. These results are in agreement with the theory that annuals have a higher ability to self-pollinate (Wiens 1984; Lloyd 1992). The ovule/seed ratios in these species are more indicative of inbreeders than of outbreeding species (Wiens 1984; Molau 1993).

Although *G. germanica* has comparable numbers of ovules to the annuals (Fig. 3b), seed production in this strict biennial is reduced by more than 60% when pollinators are excluded (Fig. 3a). In this respect, our study species behaves more like the perennial gentians *G. cruciata* (Petanidou *et al.* 1995a), *G. pneumonanthe* (Petanidou *et al.* 1995b), *G. newberryi* (Spira & Pollak 1986) and *G. saponaria* (Windus & Snow 1993). In these species, both herkogamy and protandry are pronounced, and appear to reduce spontaneous self-pollination efficiently. Polycarpic perennials such as these do not rely on a single reproductive burst, and therefore suffer less from an occasional year with pollination failure. Under normal conditions in large populations, the demographic risks of inbreeding avoidance are therefore smaller for species with this life history.

#### CONCLUDING REMARKS

As many gentian species are rare, and populations are small and threatened with (local) extinction (Pritchard 1972; Mennema *et al.* 1985), the observed differences in reproductive biology are important for their conservation. It is likely that most annual (gentian) species will hardly suffer from small population size in the form of increased risks of reduced reproductive success and inbreeding depression (Lande & Schemske 1985; Charlesworth *et al.* 1990). They are already highly adapted to selfing, so that genetic risks are probably less important than environmental stochasticity. However, for the perennials, and apparently also for at least one biennial species, reductions in population size pose a threat. Small populations of these species may be forced to rely on self-pollination, as pollinators can no longer find them (Jennersten 1988). This will lead to reduced seed set (Kwak 1988; Oostermeijer 1996), higher selfing rates (Raijmann *et al.* 1994; Ortega Olivencia *et al.* 1995) and increased inbreeding depression with the





**Fig. 3.** Comparison of autofertility (a) and mean number of ovules per fruit (b) for a number of *Gentiana* and *Gentianella* species differing in life history. Data modified from: Spira & Pollak 1986; Petanidou *et al.* 1995a; 1995b; Lennartsson 1997; Fischer & Matthies 1997; Petanidou *et al.* 1998). □, perennial; ■, biennial; ■, annual.

associated loss of genetic variation (Ellstrand & Elam 1993; Oostermeijer *et al.* 1994; Young *et al.* 1996). Under these circumstances, inbreeding depression may be more important than reproductive assurance.

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## REFERENCES

- Brussard, P.F. (1984): Geographic patterns and environmental gradients: the central-marginal model in *Drosophila* revisited. *Annu. Rev. Ecol. Syst.* **15**: 25–64.
- Charlesworth, B., Charlesworth, D. & Morgan, M.T. (1990): Genetic load and estimates of mutation rates in highly inbred plant populations. *Nature* **347**: 380–383.
- Charlesworth, D. & Charlesworth, B. (1987): Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**: 237–268.
- Cook, R.E. (1993): Clonal plant populations. *Am. Scient.* **71**: 244–253.
- Ellstrand, N.C. & Elam, D.R. (1993): Population genetic consequences of small population size: implication for plant conservation. *Annu. Rev. Ecol. Syst.* **24**: 217–242.
- Eriksson, O. (1993): Dynamics of genetics in clonal plants. *Trends Ecol. Evol.* **8**: 313–316.
- Fischer, M. & Matthies, D. (1997): Mating structure, inbreeding depression and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). *Am. J. Bot.* **82**: 1685–1692.
- Fischer, M. & Stöcklin, J. (1997): Local extinctions of plants in remnants of extensively used calcareous grasslands 1950–1985. *Conserv. Biol.* **11**: 727–737.
- Grime, J.P. (1979): *Plant Strategies and Vegetation Processes*. Wiley and Sons, Chichester.
- Harper, J.L. (1977): *Population Biology of Plants*. Academic Press, London.
- Helenurm, K. & Schaal, B.A. (1996): Genetic load, nutrient limitation, and seed production in *Lupinus texensis* (Fabaceae). *Am. J. Bot.* **83**: 1585–1595.
- Hoffmann, A.A. & Blows, M.W. (1994): Species borders: ecological and evolutionary perspectives. *Trends Ecol. Evol.* **9**: 223–227.
- Hulten, E. & Fries, M. (1986): *Atlas of North European Vascular Plants North of the Tropic of Cancer*. Koeltz Scientific Books, Königstein, Germany.
- Ingelög, T., Andersson, R. & Tjernberg, M. (1993): *Red Data Book of the Baltic Region. Part 1. List of threatened vascular plants and vertebrates*. Swedish Threatened Species Unit, Uppsala.
- Jarne, P. & Charlesworth, D. (1993): The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu. Rev. Ecol. Syst.* **24**: 441–466.
- Jennersten, O. (1988): Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conserv. Biol.* **2**: 359–366.
- Korneck, D. & Sukopp, H. (1988): Rote Liste der in der Bundesrepublik Deutschland ausgestorbenen, verschollenen und gefährdeten Farn- und Blütenpflanzen und ihre Auswertung für den Arten- und Biotopschutz. *Schriften Reihe für Vegetationskunde* **19**: 1–210.
- Kwak, M.M. (1988): Pollination ecology and seed set in the rare annual species. *Melampyrum arvense* L. (Scrophulariaceae). *Acta Bot. Neerl.* **37**: 153–163.
- Kwak, M.M., Van der Brand, C., Kremer, P. & Boerrigter, E.J.M. (1991): Visitation, flight distances and seed set in populations of the rare species *Phyteuma nigrum* (Campunulaceae). *Acta Hort.* **288**: 303–307.
- Lande, R. & Schemske, D.W. (1985): The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**: 24–40.
- Landolt, E. (1991): *Gefährdung Farn- und Blütenpflanzen in der Schweiz mit gesamtschweizerischen und regionalen roten Listen*. Bundesamt für Umwelt, Wald und Landschaft (BUWAL), Bern.
- Leck, M.A., Parker, V.T. & Simpson, R.L. (1989): *Ecology of Soil Seed Banks*. Academic Press, London.
- Lennartsson, T. (1997): *Demography, Reproductive Biology and Adaptive Traits in Gentianella campestris and G. amarella*. Doctoral Thesis, Swedish University of Agricultural Sciences.
- Lennartsson, T. & Svensson, R. (1996): Patterns in

- the decline of three species of *Gentianella* (Gentianaceae) in Sweden, illustrating the deterioration of semi-natural grasslands. *Acta Univ. Upsal. Symb. Bot. Upsal.* 31: 170–180.
- Levin, D.A. (1972): Competition for pollinator service: a stimulus for the evolution of autogamy. *Evolution* 26: 668–674.
- Levin, D.A. & Clay, K. (1984): Dynamics of synthetic *Phlox drummondii* populations at the species margin. *Am. J. Bot.* 71: 1040–1050.
- Lloyd, D.G. & Webb, C.J. (1986): The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *N.Z. J. Bot.* 24: 135–162.
- Lloyd, D.G. (1992): Self- and cross-fertilization in plants. II. The selection of selfing. *Int. J. Plant Sci.* 153: 370–380.
- Mennema, J., Quené-Boerenbrood, A.J. & Plate, A.J. (1985): *Atlas van de Nederlandse Flora 2: zeldzame en vrij zeldzame planten*. Bohn, Scheltema and Holkema, Utrecht.
- Molau, U. (1993): Reproductive ecology of the three Nordic *Pinguicula* species (Lentibulariaceae). *Nord. J. Bot.* 13: 149–159.
- Müller, H. (1881): *Alpenblumen, ihre Befruchtung durch Insekten und ihre Anpassungen an dieselben*. Wilhelm Engelmann, Leipzig.
- Olivier, L., Galland, J.-P. & Maurin, H. (1995): *Livre Rouge de la Flore Menacée de France. Tome 1: espèces prioritaire*. Collection Patrimoine Naturels. Série Patrimoine génétique, Volume No. 20. Muséum National d'Histoire Naturelle, Paris.
- Oostermeijer, J.G.B. (1996): *Population Viability of the Rare Gentiana pneumonanthe: the relative importance of demography, genetics and reproductive biology*. Doctoral Thesis, University of Amsterdam.
- Oostermeijer, J.G.B., Van Eijck, M.W. & Den Nijs, J.C.M. (1994): Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). *Oecologia* 97: 289–296.
- Ortega Olivencia, A., Carrasco Claver, J.P., Devesa Alcaraz, J.A. (1995): Floral and reproductive biology of *Drosophyllum lusitanicum* (L.) Link (Droseraceae). *Biol. J. Linn. Soc.* 118: 331–351.
- Pavlik, B.M., Nickrent, D.L. & Howald, A.M. (1993): The recovery of an endangered plant. I. Creating a new population of *Amsinckia grandiflora*. *Conserv. Biol.* 7: 510–526.
- Petanidou, T., Den Nijs, J.C.M. & Oostermeijer, J.G.B. (1995a): Pollination ecology and constraints on seed set of the rare perennial *Gentiana cruciata* L. in The Netherlands. *Acta Bot. Neerl.* 44: 55–74.
- Petanidou, T., Den Nijs, J.C.M. & Oostermeijer, J.G.B. (1995b): Pollination ecology and patch-dependent reproductive success of the rare *Gentiana pneumonanthe* L. *New Phytol.* 129: 155–163.
- Petanidou, T., Ellis-Adam, A.C., Den Nijs, J.C.M. & Oostermeijer, J.G.B. (1998): Pollination ecology and mating system of *Gentianella uliginosa*, a rare annual of the Dutch dunes. *Nord. J. Bot.* (in press).
- Pritchard, N.M. (1972): Where have all the Gentians gone? *Trans. Bot. Soc. Edinb.* 41: 279–291.
- Raijmann, L.E.L., Van Leeuwen, N.C., Kersten, R., Oostermeijer, J.G.B., Den Nijs, J.C.M. & Menken, S.B.J. (1994): Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Conserv. Biol.* 8: 1014–1025.
- Ricca, L. (1870): Osservazioni sulla fecondazione incrociata dei vegetali alpini e subalpini fatte nelle Alpi della somma Val Camonica (1). *Atti Soc. Ital. Sci. Nat.* 13: 254–263.
- Salisbury, E.J. (1942): *The Reproductive Capacity of Plants*. Bell, London.
- Schultz, A. (1888): Bestäubungseinrichtungen und Geschlechtsverteilung bei den Pflanzen. *Bibliotheca Bot.* 10: 1–103.
- Sokal, R.R. & Rohlf, F.J. (1981): *Biometry: the principles and practice of statistics in biological research*. W.H. Freeman, San Francisco, California.
- Spira, T.P. & Pollak, O.D. (1986): Comparative reproductive biology of alpine biennial and perennial Gentians (*Gentiana: Gentianaceae*) in California. *Am. J. Bot.* 73: 39–47.
- Stebbins, G.L. (1950): *Variation in Plants*. Columbia University Press, New York.
- Van der Meijden, E., Klinkhamer, P.G.L., De Jong, T.J. & Van Wijk, C.A.M. (1992): Metapopulation dynamics of biennial plants: how to exploit temporary habitats. *Acta Bot. Neerl.* 41: 249–270.
- Waser, N.M. & Price, M.V. (1991): Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *Am. J. Bot.* 78: 1036–1043.
- Webb, C.J. & Lloyd, D.G. (1986): The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. *N.Z. J. Bot.* 24: 163–178.
- Webb, C.J. & Pearson, P.E. (1993): The evolution of approach herkogamy from protandry in New Zealand *Gentiana* (Gentianaceae). *Plant Syst. Evol.* 186: 187–191.
- Weeda, E.J., Van der Meijden, R. & Bakker, P.A. (1990): Floron Red Data List 1990. Red Data List of the extinct, endangered and vulnerable plants in the Netherlands in the period 1980–1990. *Gorteria* 16: 1–26.
- Wiens, D. (1984): Ovule survivorship, brood size, life history, breeding system, and reproductive success in plants. *Oecologia* 64: 47–53.
- Willems, J.H. (1980): An experimental approach of species diversity and above-ground biomass in

- chalk grassland. *Proc. Konink. Nederl. Akad. Wetensch. Ser. C* **83**: 279–306.
- Windus, J.L. & Snow, A.A. (1993): Fruit set and seed predation in an Ohio population of *Gentiana saponaria*. *Am. Midl. Nat.* **129**: 346–351.
- Wyatt, R. (1980): The reproductive biology of *Asclepias tuberosa*: I. Flower number, arrangement, and fruit-set. *New Phytol.* **85**: 119–131.
- Young, A., Boyle, T. & Brown, T. (1996): The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* **11**: 413–418.