Future prospects for the rare, late-flowering *Gentianella germanica* and *Gentianopsis ciliata* in Dutch nutrient-poor calcareous grasslands

J.G.B. Oostermeijer*, S.H. Luijten, A.C. Ellis-Adam, J.C.M. den Nijs

Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam PO Box 92064, NL-1090 GB Amsterdam, The Netherlands

Abstract

We discuss the population biology of two calcareous grassland gentians, *Gentianella germanica* and *Gentianopsis ciliata*, in relation to the habitat management currently practiced in The Netherlands. There, at the margin of their range, both species are rare. *Gentianella germanica* persists on six remaining locations, whereas *Gentianopsis ciliata*, with two populations, is nearly extinct. *Gentianella germanica* is a strict biennial, *Gentianopsis ciliata* an iteroparous perennial. Both species depend on insects for seed production and suffer from low insect visitation. Pollination experiments in one Dutch population demonstrated that *Gentianopsis ciliata* is self-compatible, but hardly sets any seed under natural conditions due to pollen limitation. The low reproductive success of both species is partly due to the low pollinator densities at their late flowering time, partly caused by the small population size of the gentians themselves, and partly a result of mowing too early. The latter has destroyed the seed crop of several subsequent years in one population of *Gentianopsis ciliata* and some of *Gentianella germanica*. In *Gentianella germanica*, the early mowing and low insect visitation seems to have resulted in selection of less herkogamous and consequently more autofertile individuals. The perspectives for *Gentianopsis ciliata* are currently extremely poor in The Netherlands. Under the present circumstances, extinction will most likely occur within 10–20 years. Population reinforcement (seeding, artificial cross-pollination with nearby populations) should be considered if we want to conserve this species. For both gentians, but also of other flagship species of nutrient-poor calcareous grasslands, the total grassland area needs to be enlarged and must constitute an interconnected network of reserves. The traditional management method, sheep grazing, is to be preferred over mowing, but only if the flock visits each grassland patch or reserve for only a short time of less than a day, and with intervals of more than 2 weeks between visits. If mowing is preferred for other reasons, it should be done rotationally, and not before October. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Nutrient-poor calcareous grasslands are scarce in The Netherlands. Nevertheless, they form an important habitat type as they harbour an enormous diversity of plants and insects. Along with the considerable fragmentation of calcareous grasslands, their natural scarcity in this country has contributed considerably to the positions of many of the characteristic species on red lists. At present, these species are nearly all confined to a handful of nature reserves, in which the nature conservation organizations struggle to prevent their extinction through traditional or non-traditional management

methods. The ecological research basis for such habitat management is quite solid (Willems, 1980; Verkaar and Schenkeveld, 1984; Van Tooren, 1988; Willems and Bobbink, 1990; Bobbink and Willems, 1993; Huber, 1994; Morris et al., 1994; Fischer et al., 1996; Willems and Van Nieuwstadt, 1996; Niemela and Baur, 1998; Schlapfer et al., 1998).

Recently, however, we have come to realize that there are additional problems associated with the management of rare species in nature reserves. In combination with their fragmentation, the often restricted size of many sites has resulted in small population sizes and a disruption of dispersal processes in the metapopulation (Fischer and Stöcklin, 1997). Small population sizes may increase the vulnerability of populations for environmental stochasticity and catastrophes (Menges, 1992) and may result in genetic erosion through genetic drift

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* Corresponding author. Tel.: +31-20-525-7817; fax: +31-20-525-7662.

E-mail address: oostermeijer@science.uva.nl (J.G.B. Oostermeijer).

and inbreeding if this is no longer compensated for by gene flow (Oostermeijer et al., 1996; Young et al., 1996; Oostermeijer, 1999). As a consequence of inbreeding depression, the demographic performance of species may deteriorate (Oostermeijer et al., 1994; Oostermeijer, 1996; Luijten et al., 2000; Oostermeijer, 2000). At least for animal-pollinated plant species, the reduction in seed production as a result of pollination limitation poses an additional problem (Lamont et al., 1993; Kennington and James, 1997; Groom, 1998; Oostermeijer, 2000).

Especially in The Netherlands, many species of nutrient-poor calcareous grasslands occur at the edge of their generally central European distributional range. This situation is naturally associated with habitat fragmentation and the genetic and demographic consequences described earlier (Levin and Clay, 1984; Thomas et al., 1994; Van Rossum et al., 1997; Lande, 1998; Siikamaki and Lammi, 1998). However, the degree of fragmentation has increased tremendously since the beginning of the twentieth century, by the abandonment of livestock herding and transhumance (Poschlod and Bonn, 1998) and by artificial fertilization of large grassland areas. Hence, the problems for this group of species have become more severe.

2. Aims of this paper

As the traditional management by sheep herding is often no longer a practical option when dealing with isolated fragments, the managers often have to use alternative methods, such as mowing and haymaking. This may present problems to some or many of the characteristic species, in the case that they are adapted to the more variable disturbance associated with grazing. In this paper, we want to evaluate the present management of calcareous grasslands in The Netherlands from the perspectives of two rare plant species which differ in life history, the biennial *Gentianella germanica* Willd. (Börner), and the perennial *Gentianopsis ciliata* (L.) Ma, which we have studied since 1988. Both species are highly characteristic of nutrient-poor calcareous grassland throughout their range, and are considered important flagship species for this habitat type. However, they both occur at the very margin of their central European distribution here (Fig. 1).

In the Dutch nutrient-poor calcareous grasslands, the late flowering time of both species seems to conflict with the current management, which struggles to meet the demands of a variety of flagship species, amongst which there are also rare orchids, butterflies and other invertebrates (see this volume). Our aims are, firstly, to provide insight in the behaviour of gentian populations in response to management, and secondly, to give suggestions to improve the current management. To meet these aims, we review the recent literature on both species and in addition present new results of some pollination experiments on *Gentianopsis ciliata* to provide information on its breeding system and on the occurrence of pollen limitation during its late flowering period.

In the following, we will first describe the biology of the two species (Section 3), and subsequently present the results of the pollination experiments on *Gentianopsis ciliata* (Section 4). In Section 5 we discuss the specific problems of both species in relation to habitat fragmentation and habitat management, followed by an assessment of the future perspectives for each species in The Netherlands (Section 6). In the latter section, we incorporate suggestions for population reinforcement and changes in the habitat management.

3. Population biology of the two gentian species

3.1. Gentianella germanica

Gentianella germanica is a short-lived semelparous species characteristic of the typical calcareous grassland community Gentiano-Koelerietum (Willems, 1982). The species has shown a decline parallel to that of its habitat throughout its range (Landolt, 1991; Korneck et al., 1996) and is classified as "vulnerable" on the recent proposition for the Dutch Red List of vascular plants (Van der Meijden et al., 2000). Recently, it has received considerable research attention (Verkaar and Schenkeveld, 1984; Van Tooren et al., 1987; Fischer, 1996; Fischer and Matthies, 1997, 1998a,b,c; Fischer et al., 1997; Luijten et al., 1998, 1999; Wagner and Mitterhofer, 1998). The distribution area is shown in Fig. 1. In The Netherlands, there are—to our knowledge—currently six populations, five in nature reserves, managed by the State Forestry Servic (Kunderberg, Wrakelberg, Gerendal Laamheide, Gerendal experimental slope and Wylrer Akkers) and one on private land (Eys).

The seeds germinate in the spring of the first year and form a vegetative rosette. In the second year, the surviving rosettes develop into a flowering plant of 5–100 cm tall, bearing 1 to >150 flowers from late August until early October (Verkaar and Schenkeveld, 1984; Fischer and Matthies, 1998a; Luijten et al., 1998). The lilac flowers have a long corolla tube with a rim of fringes at the throat, typical for the genus *Gentianella* (Pritchard and Tutin, 1972; Lennartsson, 1997; Petanidou et al., 1998; Luijten et al., 1999). The flowers are visited by bumblebees, solitary bees, syrphid flies and noctuid moths (Fischer and Matthies, 1997; Luijten et al., 1998). Like other *Gentianella* species, such as *Gentianella campestris* (Lennartsson, 1997), *Gentianella germanica* shows variable herkogamy, meaning that the relative position of the anthers and the stigma surface within a flower varies considerably between plants in a
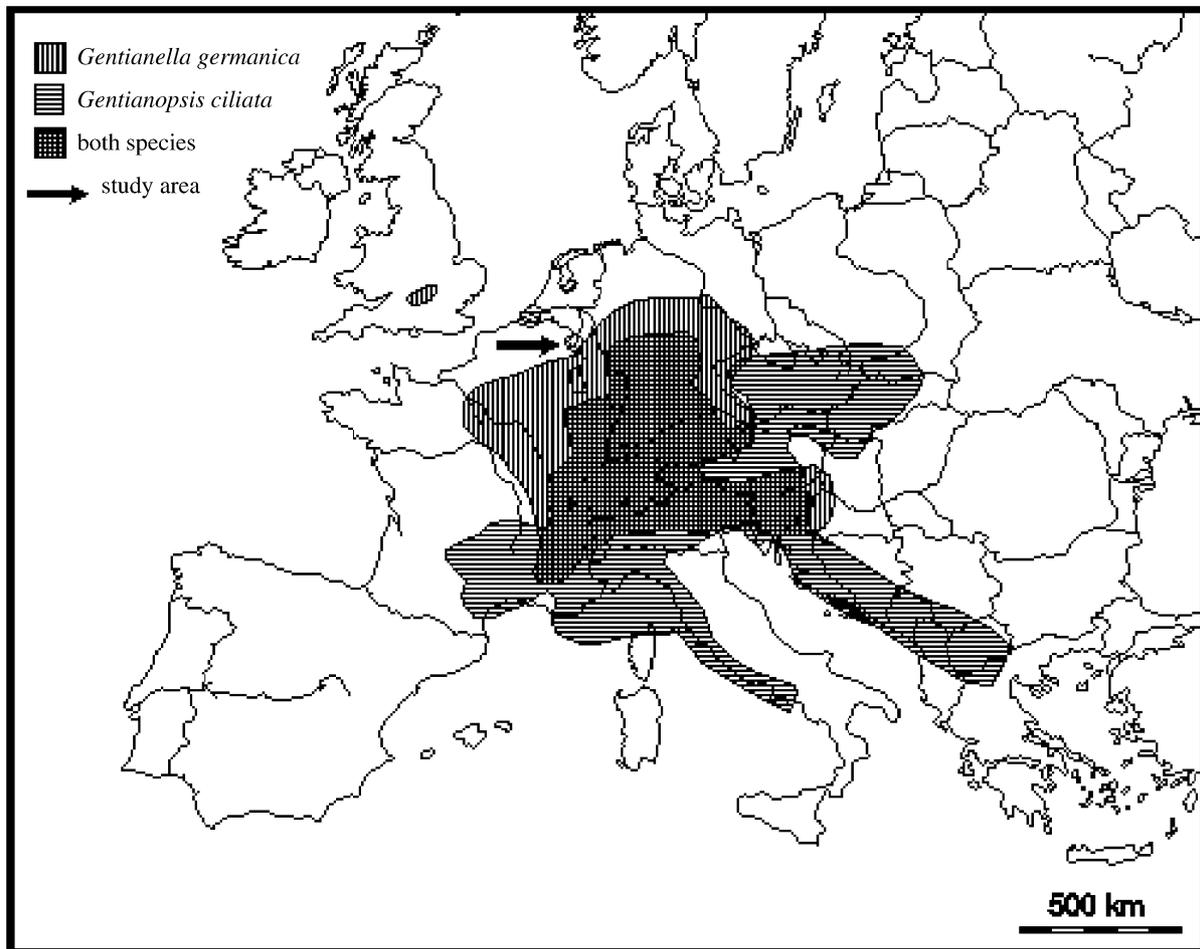
Fig. 1. Map of Europe, showing the distribution area of *Gentianella germanica* and *Gentianopsis ciliata* and indicating the position of the South-Limburg study area in The Netherlands, which is their only location in this country.

population (Luijten et al., 1999). A significant relationship between this herkogamy and the ability to set seed without pollinator visitation [autogamous seed set, or 'autofertility' sensu Lloyd and Schoen (1992)] has been shown both for *Gentianella campestris* (Lennartsson et al., 2000) and *Gentianella germanica* (Luijten et al., 1999). A high heritability of this trait has been demonstrated for *Gentianella campestris* (Lennartsson et al., 2000).

Flowers of *Gentianella germanica* produce between 60 and 100 ovules, potentially yielding 50–85 viable seeds (Fischer and Matthies, 1997; Luijten et al., 1998, 1999; Wagner and Mitterhofer, 1998). Recent data suggest that the seed bank is persistent (Fischer and Matthies, 1998b).

Gentianella germanica is a poor competitor (Verkaar et al., 1983; Fischer et al., 1997). Seedling establishment is more likely in an open vegetation with reduced competition (Fischer and Matthies, 1998b). Hence, it does not tolerate a closing of the vegetation caused by abandonment of grazing or mowing or after (atmospheric) eutrophication.

3.2. Gentianopsis ciliata

This species is usually named *Gentianella ciliata* (L.) Borckh. It is, however, very unlike the characteristic species of the genus *Gentianella*, such as *Gentianella germanica*. The fringes of the blue flower, leading to the common name 'fringed gentian', are not positioned in a rim at the throat of the corolla tube, as in *Gentianella*, but along the margins of the four lobes. In addition to differences in seed morphology, nectary structure and position and general plant architecture, there is clear molecular evidence to place the species in the genus *Gentianopsis* Ma (Gielly and Taberlet, 1996). Hence, we will use this name here.

Like *Gentianella germanica*, *Gentianopsis ciliata* is highly characteristic of the Gentiano-Koelerietum community (Willems, 1982). The species has declined considerably throughout its central European range, although (much) less than *Gentianella germanica* (Landolt, 1991; Korneck et al., 1996). The decline has probably mainly been a direct result of the fragmentation of its characteristic habitat. In The Netherlands,

Gentianopsis ciliata has always been rare, with only five to six populations in the southern part of the country at the start of the 20th century (Mennema et al., 1984). At present, it is found only on two sites, and has been classified as "Critically Endangered" on the recent proposition for the Dutch Red List of vascular plants (Van der Meijden et al., 2000).

One population occurs in the nature reserve Kunderberg, managed by the State Forestry Service. The other is found on private land, in a garden managed as calcareous grassland (population Eys). On the latter site, a nutrient-poor calcareous grassland has successfully been restored by mowing and hay removal since the early 1980s. *Gentianopsis ciliata* established in this site spontaneously in 1984, although it is not known whether this occurred from seed or from dormant plants already present. Since 1988, we have attempted to monitor the population by annual mapping of all flowering individuals. Because of their grass-like appearance, juveniles and adult vegetative plants are extremely difficult to detect in dense vegetation, so it was impossible to monitor these stages accurately.

At both Dutch *Gentianopsis ciliata* sites, *Gentianella germanica* is found as well, but both species do not occur in the same vegetation patches. At the Kunderberg site, *Gentianopsis ciliata* grows on patches with very short and open vegetation and *Gentianella germanica* in higher, albeit still open, vegetation. In the Eys site, however, *Gentianopsis ciliata* grows in higher vegetation on a more shadowed patch than *Gentianella germanica*. In the German Eifel region, *Gentianopsis ciliata* also seems to occur in rougher vegetation than *Gentianella germanica*.

Contrary to statements in many local floras, *Gentianopsis ciliata* is not semelparous and short-lived, but an iteroparous perennial. Individual plants are difficult to monitor, because they may reappear at a distance from the previous year's position (personal observations). This is probably related to the formation of lateral buds on underground stems (Kutschera et al., 1992, Fig. 2). In addition, seedlings have never been found in any of the Dutch locations, most likely because they are very small and difficult to identify. The thin and inconspicuous shoots of the adult plants emerge in April–May. Flowering occurs late in the season, from late August until the end of September or, occasionally, even until mid October. In The Dutch sites, plants usually have only one, and occasionally two flowers. In the German Eifel, however, we have observed sturdier plants with 4–5 flowering stems and 4–10 flowers. Until now, nothing is known about the reproductive success of this species. The blue flowers are relatively large and seem adapted for visitation by bumblebees. Compared with the species of *Gentiana* and *Gentianella* that occur in The Netherlands, the stigma lobes are quite large. Seeds or seedlings of the species have not been recorded
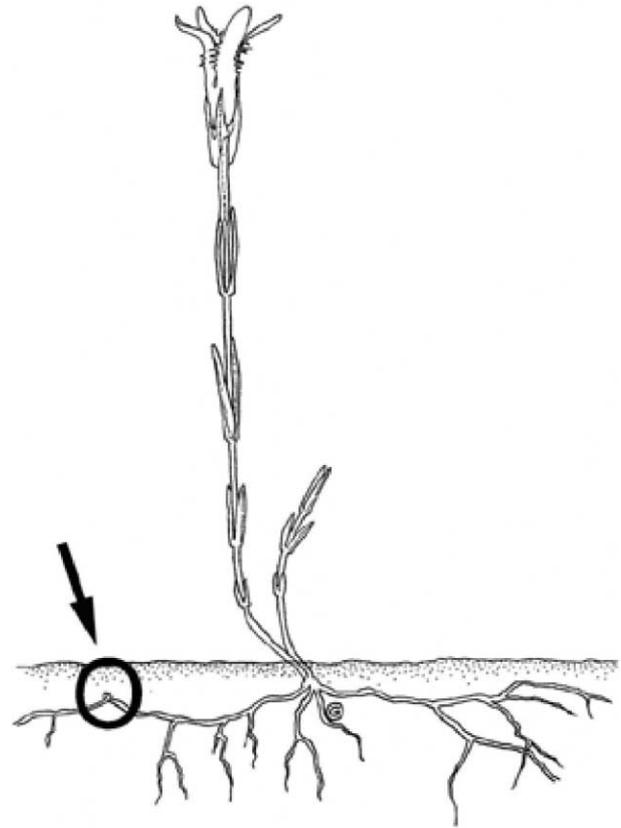

Fig. 2. Drawing of *Gentianopsis ciliata*, showing the underground root and stem system, with a new vegetation point (see arrow) underground (after Kutschera et al., 1992).

in any seed pool study (Thompson et al., 1997), so we assume that there is no persistent seed pool.

4. Pollination experiments on *Gentianopsis ciliata*

4.1. Methods

In 1998 and 1999, two experiments were performed in the Eys population of *Gentianopsis ciliata*. Owing to the very small population size, only very few plants were available for experimentation. In 1998, there were nine flowering individuals that had flowers in a suitable condition for manipulation of pollination. Four of these plants were placed in a wire cage covered with fine-meshed gauze to prevent insect access (manual pollination treatment), and five other plants were left open (natural pollination treatment). All flowers that were in bud stage or had their stigma still closed were marked individually with acrylic paint, using a different colour for each treatment. Two of the four caged plants were pollinated with their own pollen (self-pollination treatment) and the two remaining were cross-pollinated with pollen from any of the other plants that had pollen available (cross-pollination treatment). Owing to the

scarcity of flowers, only one flower could be treated on each plant.

In 1999, there were 11 flowering plants in the population which were used for an additional experiment on pollen limitation. Of the 10 plants with flowers suitable for experimentation, we marked the flowers which were in a more or less similar stage. Half of these were left to be naturally pollinated. We manually pollinated the other half with cross-pollen from two other plants of the group of flowering individuals, but did not cage them. The manual pollinations can thus be seen as a pollen supplementation treatment.

Fruits were left to ripen until October–November, and were harvested just prior to opening. No fruits were lost to herbivores. The contents of each fruit were examined under a dissecting microscope. Viable (filled) seeds could be readily distinguished from empty, non-viable ones. As there were very many seeds per fruit, we used a grid cell configuration under a petri-dish, over which the seeds were dispersed with a small brush and subsequently counted. Seed set was calculated as the ratio of viable seeds over the sum of viable and empty seeds (= equal to the initial ovule number).

Because of the low sample sizes and model residuals deviating from normality, we had to use Kruskal–Wallis nonparametric tests to compare the treatments. Of course, owing to the very small sample sizes, the risk of type I and II errors is considerable. However, the over-all differences between the treatments were so large that the main research questions can be answered.

4.2. Results

From the first pollination experiment in 1998, it appeared that there was no significant difference in seed set and ovule and seed production per fruit between manual cross- and self-pollination ($P = 1.0$ for all comparisons), although especially for this contrast ($n = 2$ for each treatment) the statistical power was very low (Fig. 3a). The median seed set after manual pollinations was at 47% quite low. This may have been caused by low pollen quality, as in freshly opening anthers the pollen-colour was light yellow, but in flowers of more than one day old it became reddish. Flowers left open to natural pollination levels had an extremely low median seed set ($< 1\%$) and produced only a few filled seeds. The differences with the manual pollination treatments were significant for the number of ovules ($\chi^2 = 6.0$, $P = 0.014$), the number of viable seeds ($\chi^2 = 6.2$, $P = 0.013$) and seed set ($\chi^2 = 6.3$, $P = 0.012$).

In 1999, a very low natural seed set (median 1%) was observed again in the Eys population, and manual pollen supplementation resulted in a median seed set of 81%, differing significantly from natural pollination (number of viable seeds: $\chi^2 = 5.04$, $P = 0.025$; seed set: $\chi^2 = 3.78$, $P = 0.052$). Both results were comparable with
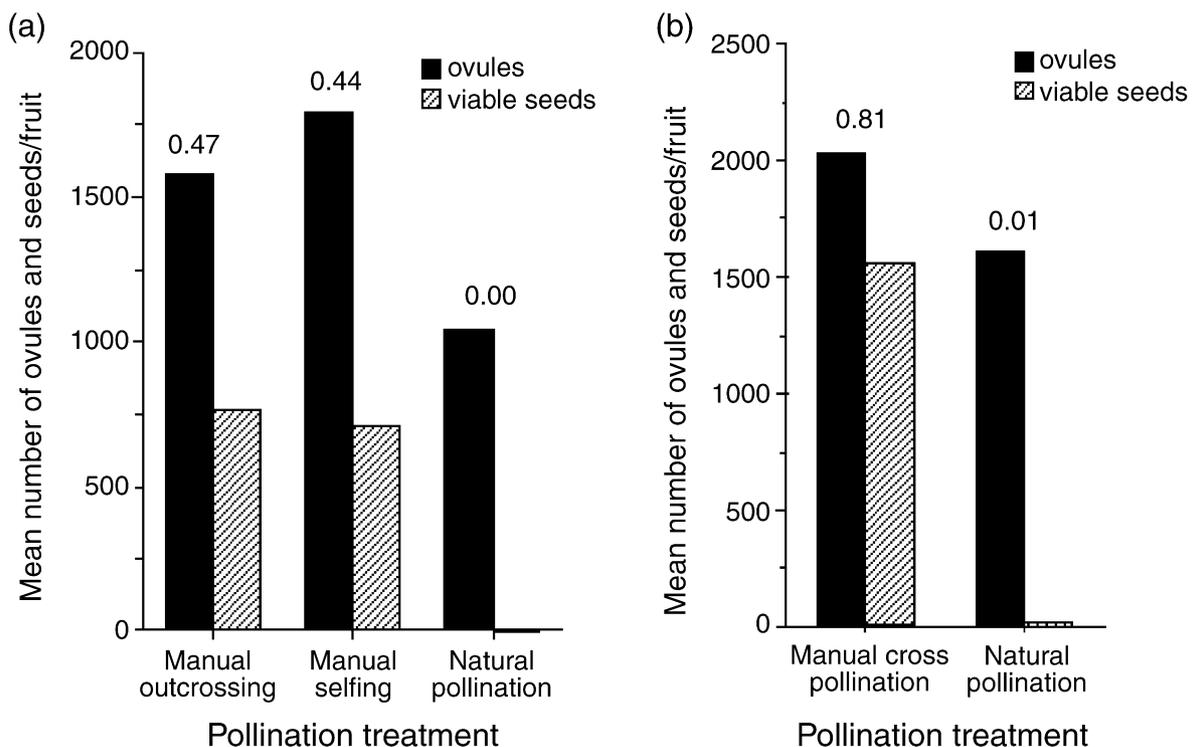

Fig. 3. Median ovule production and number of viable seeds per fruit of *Gentianopsis ciliata* after (a) cross-, self- and natural pollination in 1998, (b) manual cross pollination and natural pollination in 1999. The median seed set (viable seed:ovule ratio) per treatment is given above each pair of columns.

the level observed in 1998, but the number of ovules differed significantly between years for both manual ($\chi^2 = 3.68$, $P = 0.055$) and natural ($\chi^2 = 6.0$, $P = 0.014$) pollinations (Fig. 3b). In 1999, the number of ovules produced in naturally pollinated flowers was significantly lower ($\chi^2 = 5.5$, $P = 0.019$). The latter effect has been observed before in pollination experiments on gentians (Petanidou et al., 1995a,b, 1998; Luijten et al., 1999). It has often been attributed to the caging of manually pollinated flowers, but cages were not used in 1999, ruling out this possibility. Resorption of unfertilized ovules has been mentioned as an explanation in *Gentiana pneumonanthe* (Petanidou et al., 1995a).

5. Problems for the studied gentian species

5.1. Population size

In the present situation in The Netherlands, a reduction in the number of individuals per population perhaps forms less of a threat to the short-lived *Gentianella germanica*, which has a persistent soil seed pool, than to the perennial *Gentianopsis ciliata* which has not. The former species has populations varying from 100 to 150 flowering plants (Eys and Gerendal experimental slope) to more than 100,000 flowering plants in some years in the Wrakelberg and Kunderberg reserves. The populations in the Gerendal and Wylrer Akkers reserves are intermediate, with ca. 2000 individuals. However, in spite of the generally large numbers, we have witnessed dramatic reductions in population size in several reserves in the period 1994–1998 due to early mowing or sheep grazing. In the Wrakelberg reserve, only five plants were counted in 1996 and in many other years, 90% of the population was mown in the middle of the flowering period, end of August to early September. In the Kunderberg reserve, sheep grazing occurred as early as end of July to early August in recent years, reducing the population to three flowering plants in 1998. Postponing this grazing regime till after the fruiting of the gentians in 1999 led to an immediate increase in the number of flowering plants to 3000. Apparently, the long-lived soil seed bank of this species (Fischer and Matthies, 1998b) still functions as as a buffer against fluctuations in the above-ground population size. A number of years in a row with low seed input may, however, severely deplete this important seed pool.

The size of the only "wild" population of *Gentianopsis ciliata* in The Netherlands, in the Kunderberg reserve, is difficult to establish. Our first visit to the reserve in 1988 revealed a total of 133 flowering individuals. This relatively high number was never counted again in any of the subsequent years, despite the fact that we carefully mapped the plants with a triangular measurement to two fixed points in 1988 so they could be relocated.

During a census in 1992 we still found 36 flowering plants, but this number had declined to 19 in 1993, after which we found no plants at the site until 1999, when we counted five flowering plants and two vegetative shoots. Surprisingly, a total of 55 individuals was counted in 2000 (Fig. 4). Whether the recovery concerns adults that survived in the vegetative or underground state or new recruits from seed cannot be established. The very low seed production observed suggests the former rather than the latter. An observation on the plants on the Kunderberg that may be important is that during very hot and dry summers, most of the flowering stems desiccated at a young stage and produced no flowers and fruits. Apparently, the species is very sensitive to drought. It has been suggested earlier that this sensitivity has restricted the species to the eastern part of the province of South-Limburg, where there is relatively more precipitation (Mennema et al., 1984). It is clear that such a sensitivity would seriously increase the probability of extinction of the marginal populations.

From 1994 to 1998, the Kunderberg site was usually grazed by a flock of sheep in the middle of the flowering period of *Gentianopsis ciliata*, in August and early September. The grazing converted the vegetation into a 1–3 cm tall sward, leaving little opportunity for flowering of *Gentianopsis ciliata*. The early grazing is the best explanation for the absence of flowering individuals during these years. During the same period, the small numbers of *Gentianopsis ciliata* in the private calcareous grassland "garden" in Eys, which is mown annually after the plants have set fruit, remained more stable, from 10 putative genets (plants with multiple stems originating from a single point were considered ramets from a single genet) in 1989, nine in 1990, five in 1991, eight in 1992,
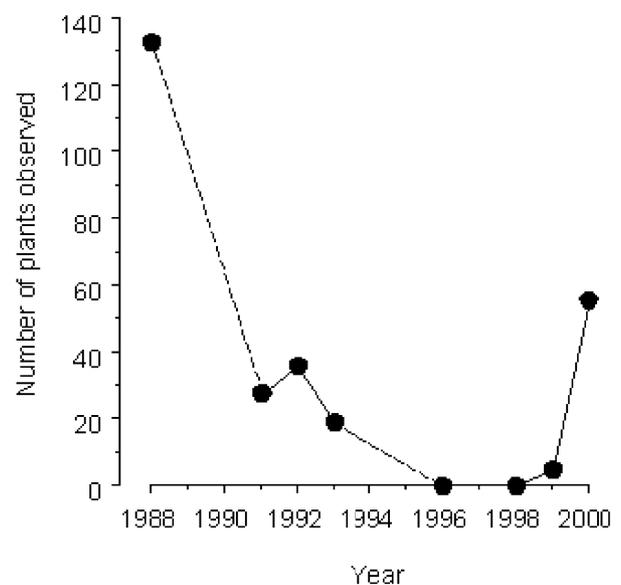

Fig. 4. Population dynamics of *Gentianopsis ciliata* in the Kunderberg reserve from 1988 to 2000. Dashed lines represent interpolations, because data of intermediate years were not available.

nine in 1997, nine in 1998, 11 in 1999 and eight in 2000. This relative stability does not necessarily mean that we found the same individuals again and again during those years: the positions of the plants shifted so much from year to year that we hardly ever found the same plant on exactly the same position in the next year. Sometimes we could guess that an individual had shifted by 5–15 cm from its previous year's position, but we were never sure. Together with the fact that seedlings and vegetative plants are nearly impossible to find, this habit makes the species highly unsuitable for demographic studies. Nevertheless, our observations on the Dutch populations suggest that individuals of *Gentianopsis ciliata* can become at least 10 years old.

5.2. Loss of genetic variation

As soon as populations become small and isolated, they are exposed to the risks of drift and inbreeding (Barrett and Kohn, 1991). This fragmentation and isolation has clearly happened to some populations of *Gentianella germanica* and certainly to *Gentianopsis ciliata* (see earlier). As we could not resolve allozyme variation for *Gentianopsis ciliata*, we have no data on the amount of genetic variation in this species in The Netherlands compared to more centrally located populations. Based on general trends, the very small sizes nor the marginal position of the Dutch populations do not promise much good in this respect, however (Hoffmann and Blows, 1994).

In The Netherlands, we have found very little genetic variation in both small and large populations of *Gentianella germanica* using allozymes (Luijten, 1992). Fischer and Matthies (1998c) found a significant positive relationship between population size and variation in RAPD-markers in the same species in Switzerland, in the center of its distribution. Based on these results, it may be expected that the marginal and isolated populations of *Gentianella germanica* in The Netherlands are all genetically depauperate. That this may have repercussions for demographic performance has also been suggested by the results of Fischer and Matthies (1998a). Significant relationships between genetic variation and offspring fitness have been found also in the heathland perennials *Gentiana pneumonanthe* (Oostermeijer et al., 1994, 1995) and *Arnica montana* (Luijten, 2001).

5.3. Reproductive biology

Assuming the same strong heritability of herkogamy in *Gentianella germanica* as in *Gentianella campestris* (Lennartsson et al., 2000; Luijten et al., 1999), there was a considerable genetic variation for this trait in the populations we studied in 1992 (Luijten et al., 1998, 1999). This variation has declined considerably in recent

years, possibly as a result of the management-induced population bottlenecks (Luijten et al., 1999).

It is very interesting that in the Wrakelberg population, mown during the peak of flowering for several years in a row (1993–1998), the average herkogamy was such that stigmas usually protruded above the anthers in early years, whereas they were predominantly positioned under the level of the anthers in recent years (Luijten et al., 1999). At the same time, the ability to self-pollinate spontaneously (negatively related to the level of herkogamy) increased in the population. In other words, the population seems to have changed from being generally oriented towards cross-pollination to predominantly selfing (Luijten et al., 1999). Not only was the management highly unfavourable during the period of our observations (1992–1998), but also the numbers of insects we saw visiting the species were extremely low. The latter may of course also have been related to the general scarcity of nectar and pollen sources after early mowing.

Because of the trend towards higher autofertility, the mentioned *Gentianella germanica* population bottlenecks have not reduced the absolute seed quantities per plant so much as the seed quality, as the selfing rate has increased. Furthermore, the reduction in the number of reproductive plants in the population has undoubtedly lowered the input of seeds into the seed pool. For a biennial species, this may have a strongly negative effect on the population viability (Fischer and Matthies, 1998a,b), especially when the unfavourable management is continued.

In *Gentianopsis ciliata*, the seed production is even more limited than in *Gentianella germanica*. The pollination experiment clearly shows that the species is fully self-compatible, and that natural pollination in this population resulted in an extremely low seed set in both study years. Apparently, the species suffers from pollination limitation, which may be attributed to either the small population size (causing the Allee-effect), a scarce nectar or pollen reward, or the late flowering time, associated with low pollinator numbers or even absence of the right pollinator species. The latter two explanations seem not very likely, though, because numerous *Bombus pascuorum* were visiting *Scabiosa columbaria* at the Eys site during the flowering period of *Gentianopsis ciliata*. This bumblebee species is a frequent visitor of other gentian species in The Netherlands (Petanidou et al., 1995a, 1995b, 1998; Luijten et al., 1999; we observed a single bumblebee that was clearly attracted to the flowers of *Gentianopsis ciliata* but rejected them for some unknown reason.)

Pollinator visits to *Gentianopsis ciliata* have been observed (M.M. Kéry personal communication) in a very large population in the Swiss Jura, but even in this population fruit set seemed highly variable among individual plants (personal observation). Despite the very

low natural seed set in the only studied population in The Netherlands, the species produces very many (1000–2000) ovules per flower, so that quite a lot of viable seeds are produced once successful pollination has taken place. The high potential seed production, the tiny seed size and the iteroparous long-lived life cycle suggest that seedling recruitment is generally difficult and depends on the combination of favourable recruitment conditions with high reproduction in the previous year. Unfortunately, the cryptic life of the seedlings precludes any information on suitable conditions for germination and establishment. We have sown the seeds from our pollination experiments in the Eys population in fixed quadrats of varying vegetation structure, but so far we have seen no emergence of seedlings, nor of any adult vegetative or flowering plants.

6. Future prospects

6.1. Population viability of the two gentian species and specific measures to restore it

Based on the problems described above, we can conclude that there is no viable metapopulation of *Gentianella germanica* in The Netherlands (regarded in the longest term of 100–100 years). At present, the six populations in the country are much too limited, although some are still large and individually viable populations. Hence, much work needs to be done to increase the size and stability of the remaining populations, to facilitate establishment of new populations and to increase the connectivity between populations, before there can be a viable metapopulation.

The few large, viable populations may serve as sources for (re)colonization when future restoration of calcareous grassland is undertaken. However, our observations also indicate that even the viability of such large populations may be undermined by poor habitat management, so that special attention from the reserve managers in this respect is definitely needed (see later).

The question is whether we need specific measures to alleviate any effects of genetic erosion or inbreeding in the Dutch populations. The data of Fischer and Matthies (1998 a etc.) suggest that measures to boost the demographic performance of small, inbred populations could be beneficial. However, the same authors also found that plant performance of *Gentianella germanica* in the greenhouse and experimental garden declined after hybridization with plants from another population (Fischer and Matthies, 1997), possibly as a consequence of outbreeding depression (disruption of coadapted gene complexes). Also, germination of seeds of the species was better in home than in alien sites (Fischer and Matthies, 1998b). In this light, any attempts to counter genetic erosion, for instance by

reinforcing small populations by adding seed from larger, viable ones, seem risky. However, if the experiment by Fischer and Matthies (1997) had been performed under more stressful field conditions, the positive effects of the increased heterozygosity might have outweighed the deleterious effects of fragmented gene complexes (Armbruster et al., 1997; Fenster and Galloway, 2000). In general, there is still little empirical evidence demonstrating that outbreeding depression is indeed a serious risk, but good evidence for the opposite is equally scarce (Dudash and Fenster, 2000). Given the presence of some large viable source populations, the main message for *Gentianella germanica* is that we should better wait before undertaking mitigative measures against genetic erosion and instead focus on management to ensure better habitat quality, large habitat and population size, and good connectivity between habitat patches.

In contrast to *Gentianella germanica*, the future prospects of *Gentianopsis ciliata* in The Netherlands are much worse. Both remaining populations are extremely small, suggesting that the current situation for this species is truly marginal. The reproduction of the species, especially, seems to be restricted by its sensitivity to summer drought and the scarcity of pollinators during the late flowering period. Any other demographic bottlenecks, such as limited seedling recruitment, are possibly obscured by the cryptic nature of the early life cycle stages. This makes any evaluation of the response of this species to management changes extremely difficult. It is clear, however, that the recent management of the Kunderberg population has not been optimal, to say the least. Even though the species is likely to disappear as a result of purely natural causes, we should ensure that its eventual extinction is not accelerated or even caused by wrongly timed human disturbances. Given the immediate risk of extinction of *Gentianopsis ciliata* in The Netherlands, a decision has to be made whether attempts should be made to preserve the species and what these attempts should be. If it is decided that the marginal Dutch populations should be conserved, we strongly suggest reinforcing the two remaining populations by introducing genetic material from nearby populations in the German Eifel region and not wait until more information becomes available on the risks of outbreeding depression. As stated before, it is very likely that most of the genetic variation has been lost though genetic drift. It is also clear that there are no chances of restoring a more natural gene exchange with other populations in the near future, as these are located too far away. Hence, the best options for reinforcement seem to be either introduction of seeds from the closest large and viable population or artificial cross pollination with pollen from such a site (or both simultaneously). If conditions for recruitment are favourable, we expect that these measures will increase genetic variation and reduce possible inbreeding depression, and that selection of the

best-performing genotypes from the enlarged gene pool will alleviate any outbreeding depression (Armbruster et al., 1997; Van Groenendael et al., 1998; Bowles et al., 1998).

6.2. Suggestions for changes in habitat management

In the traditional situation, nutrient-poor calcareous grasslands formed a more or less continuous habitat that was regularly grazed by a sheep herd. The dung was gathered in a sheepfold at night. Today, these grasslands form isolated fragments in a matrix of mainly agricultural land which are either grazed by a sheep herd for a period during late summer or early autumn, or they are mown annually at some time at the end of the season, after which the hay is removed. During the sheep grazing, the dung is deposited in the reserves, which may be a possible source of eutrophication. Another, perhaps more important, difference with the traditional management is that the major disturbance takes place within a short time-frame, and over the entire fragment (i.e. the reserve). In other words, the vegetation is grazed or cut short within one day or at most a few days. Hence, the timing of the disturbance becomes very important, because there is no place of refuge for sensitive species. Even though the majority of the species at a site may have already fruited and disappeared aboveground, the remainder may seriously suffer from a wrong timing. It is clear especially that species that are active until late in the season are currently at risk. Partly as a strategy to prevent *Brachypodium pinnatum* dominance (Bobbink and Willems, 1993), the mowing of calcareous grasslands is carried out too early, during the peak flowering of the late flagship gentian species. This destroys most of the seed crop (Van Tooren et al., 1987), which is very detrimental, especially to the short-lived plants (Poschlod and Jackel, 1993; Fischer and Matthies, 1998b). Furthermore, the non-rotational cutting of entire reserves allows no escape of canopy-inhabiting invertebrates in undisturbed parts with a higher vegetation (Morris and Rispin, 1988; Morris, 1990; Gibson et al., 1992).

It has been suggested that early mowing to counter *Brachypodium pinnatum*-dominance has no detrimental effects on the reproduction of *Gentianella germanica*, because the observed significantly reduced regrowth and flowering was compensated for by the flowering of very small second-year rosettes which otherwise would have remained vegetative (Van Tooren et al., 1987). Overcompensation, i.e. the production of a higher number of flowers after cutting or grazing, which has been observed in *Gentianella campestris* (Lennartsson et al., 1997, 1998), was not observed in *Gentianella germanica* (Van Tooren et al., 1987). However, by early mowing (1) the early seed crop is destroyed, which probably had a higher outcrossing rate [and thus higher offspring

fitness, see Oostermeijer et al. (1994) and Fischer and Matthies (1997)] than the late flowers, because of higher insect activity, and (2) the reproduction of individuals that had the poorest growth of the cohort is favored. Both types of selection will have detrimental effects on the viability of the population, and our observations (Luijten et al., 1998, 1999) suggest that this has indeed happened. Hence, the remarks made by Van Tooren et al. (1987) that late-flowering, short-lived species such as *Gentianella germanica* are very sensitive to the wrong mowing time, were quite to the point.

On the basis of our findings on the ecology of *Gentianella germanica* and *Gentianopsis ciliata*, we can formulate management suggestions to increase the population viability of these late-flowering species:

1. Individual management of remaining sites
   1.1. Mowing: mow only after 90% of the seed shedding of the latest species has taken place (in practice, this will be around mid-October). Remove the hay 1 or 2 days after mowing, or later when allowed by dry weather conditions. Mowing should be done rotationally (see later) in order to allow survival of invertebrates that overwinter in the vegetation canopy.
   1.2. Mowing: if earlier mowing (August–September) is indicated because of increasing dominance of *Brachypodium pinnatum*, mow rotationally, leaving at least 25–30% of the total area unmown. It has to be made sure that similarly large parts of the populations of the late-flowering species are spared, and that different parts are spared each year.
   1.3. Grazing: graze individual sites intermittently through the season, with a relatively small sheep flock of 20–30 animals, guided by a shepherd. Visits of the flock to a site should each be shorter than a day, with intervals between visits of more than 2 weeks. In between visits to calcareous grassland sites, the sheep should be kept in restoration sites (see later), where most of the standing crop has to be removed in the early stages of restoration, or in production grasslands with a higher phytomass. Preferably, the dung is to be collected in a sheepfold, to avoid eutrophication of existing reserves and to speed up removal of nutrients from the restoration sites.
2. Enlargement and restoration of the regional calcareous grassland system
   2.1. The remaining calcareous grassland reserves should be embedded in a larger regional system of semi-natural grasslands, served by the same sheep herd or (in large regions) by more than one herd.

2.2. In order to achieve this, farmers owning grasslands or fields have to be bought out and private land has to be acquired. It is important that an interconnected series of grasslands is created.

2.3. The calcareous grassland reserves have to be grazed according to the suggestions given in (1.3). The main grazing intensity of the sheep flock has to be placed on the grasslands to be restored, so that these can be developed into nutrient-poor calcareous grassland within a time-frame of ca. 10 years. Colonization with characteristic calcareous grassland species can take place in and on the fur of the sheep migrating from the reserves to the new grasslands (Fischer et al., 1996).

In principle, the management suggestions given above for the two gentian species do not conflict with the demands of most other characteristic inhabitants of nutrient-poor calcareous grasslands. We expect that intermittant grazing of short duration and at an intermediate to low intensity will eventually lead to a more varied vegetation structure (Dennis et al., 1997, 1998). The population densities of some plant and invertebrate species that are favoured by a very short vegetation height may be reduced, whereas the densities of species of higher vegetation will increase (Warren, 1993; Dennis et al., 1998; Wettstein and Schmid, 1999; Morris, 2000). The same is expected to occur when mowing is performed later in the season and rotationally (Wettstein and Schmid, 1999). To keep track of the changes, a sound monitoring program will have to be installed that follows the population fluctuations of some early and late-flowering indicator plants (including for instance *Gentianella germanica* and *O. militaris*, but also *Brachypodium pinnatum*) and some easily counted invertebrates, such as the butterflies *Erynnis tages, Cupido minimus* and *Thecla betulae*.

In conclusion: a number of adjustments to the management in combination with careful monitoring of their effects will not only ensure the survival of the late-flowering gentian species, but will at the same time increase the species diversity of the Dutch calcareous grassland ecosystem.

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