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Pollination ecology and patch-dependent reproductive success of the rare perennial *Gentiana pneumonanthe* L.

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SUMMARY

The reproductive behaviour of *Gentiana pneumonanthe* L., a rare plant species in The Netherlands, was studied in a relatively large wet heathland population during summer 1989. The species co-occurred with the grass *Molinia caerulea* and co-flowered with *Erica tetralix* and *Calluna vulgaris*. The flowering period lasted from July to October, peaking in late August to late September. Flowers are protandrous. The species appeared to be self-compatible, but spontaneous self-pollination was strongly limited. Hand-crossing and hand-selfing resulted in the same amount of seed set as in natural pollination. Reproductive success was dramatically reduced late in the season. Pollination was achieved sternotribically by the species *Bombus pascuorum*, which visited the flowers for nectar. In the study area, three patch types were distinguished: co-dominated by *Erica*, by *Calluna* and *Erica*, and by *Molinia*, respectively. Frequency of visits to *Gentiana* was highest in the *Erica*-patch. However, this did not result in a higher seed set. In the *Molinia*-patch seed set was reduced. However, in the *Molinia*-patch the mean number of ovules was greater than in the others (as high as in plants raised indoors and in cross-pollinated plants). Fruits from the *Calluna*-patch had less ovules than those from the *Erica*-patch. We conclude that, in this remnant population, *Gentiana* is not pollination-limited. However, it is likely that in the *Molinia*-patch geitonogamy is frequent, which may lead to inbreeding depression. The greater number of ovules observed in *Molinia*-patches may reflect an excess of nutrients available there, relative to the *Calluna*-patch where there may be reduced water availability.

Key words: *Gentiana pneumonanthe*, pollination, patch-dependent reproduction, seed set, bumblebee.

INTRODUCTION

Rare plant species often occur in small populations, as a result of the fragmentation, destruction or deterioration of their natural habitats. In such small, remnant and often isolated populations, genetic structure is dominantly influenced by genetic drift, which results in loss of genetic variation and consequently increases the probability of local extinction (Barrett & Kohn, 1991; Ouborg, van Treuren & van Damme, 1991; van Treuren *et al.*,

1991; Raijmann *et al.*, 1994). These populations may also suffer from inbreeding depression (loss of fitness as an effect of increased inbreeding) and reduced levels of gene flow. Especially in animal-pollinated and predominantly outbreeding plant species, reduced population size and fragmentation of natural habitats may have dramatic effects on the reproductive output (Jannersten, 1988; Kwak, 1988; Kwak *et al.*, 1991; Petanidou, den Nijs & Ellis-Adam, 1991) and also, for plants with a mixed mating system, on the balance between selfing and outcrossing (Oostermeijer *et al.*, 1992). Because they may be unable to attract sufficient numbers of pollinators (Kwak, 1988), small populations of rare species may become increasingly dependent on the presence of simultaneously flowering species, that

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may help to attract insects (pollination facilitation). Furthermore, rare plants in small populations, may be especially prone to reduced seed set as a result of competition for pollinators (Mosquin, 1971; Kwak & Jennersten, 1991) or interspecific pollen transfer (Waser, 1978; Thomson, Andrews & Plowright, 1981; Rathcke, 1983; Campbell & Motten, 1985).

In this paper, we examine whether *Gentiana pneumonanthe* L., a rare perennial plant, is pollination-limited in a relic, but still large population, i.e. a remnant of a formerly large population. There is evidence that the reproductive success of this species is reduced in small populations (Petanidou *et al.*, 1991; Oostermeijer *et al.*, 1992). We hypothesize that in the relic population we studied (i) plants are likely to suffer reproductive loss because of their reduced effectiveness in attracting pollinators, and (ii) the co-occurrence of other species is of crucial importance in terms of competition or facilitation for pollination. In this context, we examine the effects of the presence of the co-flowering species *Erica tetralix* L., and *Calluna vulgaris* (L.) Hull on the reproductive success of *Gentiana pneumonanthe*, as well as the effect of the tall neighbouring grass *Molinia caerulea* (L.) Moench on pollinator visitation rates. *Molinia* is known to replace *Erica* and *Calluna* in areas of atmospheric acidification and eutrophication (Heil & Bruggink, 1987). Dominance of the species also inhibits recruitment in populations of *G. pneumonanthe* (Oostermeijer *et al.*, 1992; Oostermeijer, van 't Veer & den Nijs, 1994b). Since it is likely that the differential dominance of these three species in sites with *G. pneumonanthe* reflects the existing differences in water and nutrient availability (e.g. relatively wet and nutrient-poor: *Erica* dominant; relatively dry and nutrient-poor: *Calluna* dominant; and relatively wet and nutrient-rich: *Molinia* dominant), we also examine the hypothesis (iii) that these ecological factors affect the reproductive potential of *Gentiana* (i.e. the number of ovules and viable seeds).

MATERIALS AND METHODS

Biology of the study species

Gentiana pneumonanthe (Gentianaceae), the marsh gentian, is a herbaceous perennial plant of wet heathlands and hay meadows of temperate Europe from the northern Balkans to Fennoscandia. An adult, reproductive plant may be up to 40 cm high, and bear 1–25 flowers each season. The flowers are actinomorphic and funnel-shaped. The corolla lobes are blue to mauve with a deep floral tube, the inner surface of which is greenish blue with a distinctive pattern of nectar guides.

In The Netherlands, a country at the margin of its distribution range, the marsh gentian used to be far more common and widespread before 1950, both in

numbers of localities and in population sizes, than it is today (Mennema, Quené-Boterbrood & Plate, 1985). This is mainly the result of loss of habitats and changes in land use. The only sites, where the species still occurs, are nature reserves containing wet heathlands and nutrient-poor, unfertilized moist hay meadows. Population size ranges from 5 to 5000 (exceptionally 100000) flowering individuals. Most of populations are relatively small and occur in isolated, fragmented habitats. In this country, the most important seed predators of *Gentiana pneumonanthe* in the wild are the larvae of a gall midge, possibly *Dasineura gentianae* Kieffer (Cecidomyiidae) (H. Roskam, personal communication), which are especially frequent in wet summers (J. C. M. den Nijs and J. G. B. Oostermeijer, personal observations).

Study site

The field work was carried out during summer 1989, in the wet heathland 'De Heidebloem' (3 km SE of Hilversum, The Netherlands). The population is a relict of an extensive complex of populations formerly existing in this region. The size of the population, at the time of this study, was estimated at about 2000 flowering individuals, spread over c. 0.5 ha. The site belongs to the Goois Nature Reserve Council, and was managed by sod-cutting and, more recently, by mowing every year, in order to reduce the dominance of grasses, in particular *Molinia caerulea*. Other dominant species are *Erica tetralix* and, on higher ground, *Calluna vulgaris*. Flowering of *Erica* and *Calluna* partly overlaps that of *Gentiana*. *Erica* begins flowering in June, about a month earlier than *Calluna*; their co-flowering continues until September. Much of the fieldwork was performed during two study periods, one during the peak flowering period from 22 August to 2 September, and another one late in the season from 3 to 14 October 1989.

Flowering and nectar characteristics

The number of open flowers was counted once a week from July to October in both 1988 and 1989, in a permanent plot (5 × 5 m) situated in the centre of the area with the highest density of *Gentiana*. Flower life span was measured on 48 flowers borne on different plants and in different positions on a plant. Flowers were marked at the bud stage during the peak flowering period, and their behaviour was monitored twice daily, until they withered. The nectar standing crop (i.e. the amount of nectar present in freely exposed flowers) was measured by inserting a 1 µl Drummond microcapillary into the corolla tube. Nectar was harvested from a number of open flowers of all stages, selected at random in the

centre of the population. Nectar concentration (expressed in % w/w sucrose) was measured with a pocket refractometer (Bellingham & Stanley, Tunbridge Wells).

Reproductive success after pollination treatments and in different patches

Fruit and seed set, after different pollination treatments were measured in both study periods, and in the near vicinity of the permanent plot. Self-compatibility and spontaneous self-pollination were studied on the same individuals ($n = 31$), by covering the plants with a metal cage with fine gauze to prevent insect visits. Flowers were marked at the bud stage with waterproof paint to be self-pollinated by hand during female stage ($n = 48$ at the flowering peak, hereafter referred to as n_{Aug} ; and $n = 10$ late in the season, hereafter referred to as n_{Oct}) or to remain untouched ($n_{\text{Aug}} = 88$, $n_{\text{Oct}} = 30$). Hand-pollination was performed using the flowers' own pollen. Outcrossing success was estimated on uncaged flowers. Freshly exposed stigmas were hand-crossed. The flowers were made and left uncovered until harvesting ($n_{\text{Aug}} = 38$, $n_{\text{Oct}} = 45$). All hand-pollinations were performed using single applications of abundant pollen.

Free or natural pollination success, and putative competition for pollination, was measured on a number of flowers, marked at bud stage and left uncaged to be visited by insects. Three types of mixed plots, all 5×5 m, were examined, namely *Gentiana* co-dominated (1) by *Erica*, (2) by *Calluna* and *Erica*, and (3) by *Molinia*. We will refer to them as the *Erica*-, *Calluna*-, and *Molinia*-plots, respectively. The *Erica*-plot represents the commonest vegetation type with a dense stand of *Gentiana* (46 plants bearing 65 flowers, along with 386 flowering stems of *Erica*). The *Calluna*-plot contained fewer *Gentiana* (21 plants with 36 flowers, together with 50 inflorescences of *Erica* and ± 500 of *Calluna*), whilst in the *Molinia*-plot only *Gentiana* flowers (83 plants, 109 flowers) were present.

In order to check whether the reproductive success in the field was resource-limited (by nutrients or water availability), parallel cross-pollinations were performed on control plants, from the same population, that had been raised from seeds in a greenhouse ($n = 118$ flowers on > 50 plotted plants). For each hand-crossing the pollen of at least two donors was used.

The fruits were harvested approximately one month after they had been treated and/or marked. Recovery of the caged fruits was almost 100%, but many fruits of uncaged plants were infected by the gall midge mentioned above. As a standard procedure, reproductive success was assessed by counting the filled and empty, shrivelled seeds ($n = 30$,

when available) of the uninfected recovered fruits, under a dissecting microscope. Germination tests showed that *c.* 90% of filled seeds germinated, while empty, shrivelled seeds did not germinate at all. The first category was, therefore, considered viable, the second as unfertilized and/or aborted. The sum of the viable and aborted seeds was considered to represent the total initial number of ovules in the ovary. Seed set was the ratio of viable seeds to this initial number of ovules. A check on the initial number of ovules was also made by counting them in a sample of fresh, unpollinated flowers from the centre of the population ($n = 10$).

The possibility of anemophily was tested in the field (22 August), by using 23 glass slides covered with silicon oil. In the morning before the flowers opened, the slides were placed around and between the target plants at different heights (0–25 cm) and angles (0° – 90°). Slides were recovered after about 10 h, when the flowers had closed. Very few pollen grains of *Gentiana* (0–22) were found on the great majority of the slides (22 out of 23). However, on one exceptional slide there were 600 *Gentiana* pollen grains, in a clump mixed with *Erica/Calluna* grains, most probably deposited by a passing or colliding insect. Anemophily was not, therefore, considered further in the analysis of pollination ecology.

Flower visitors

The behaviour of flower-visiting insects was monitored four or five times a day for 15 min periods. To record insect visits, two observers were present. They noted the number of visitors entering and performing on the plot, and the number and the sequence of visits to different flowers. During the peak (22–24 August) and late flowering (4–5 October), observations were made in the permanent quadrat as well as on the three plots with different plant composition.

The pollination efficiency of the flower visitors was assessed in three different ways: (i) The proportion of heterospecific pollen grains in pollen loads of visitors was investigated in the centre of *Gentiana* population. After having visited a *Gentiana* flower, insects were captured, lightly anaesthetized by means of ethyl-acetate, and the pollen from the different body parts was removed separately by using small, sticky pieces of gel (Beattie, 1972). The pollen was counted under a light microscope and identified by using appropriate reference pollen from the area. (ii) Flowers were kept caged until they reached the female stage, and were then opened. The number of pollen grains deposited by a bumblebee worker on virginal stigmas of non-emasculated flowers ($n = 8$) of *Gentiana* was then counted. After a single bumblebee visit, each stigma was collected and stored (Beattie, 1972) for microscopical analysis. (iii) Seed set was measured in the plots of different

species composition, and the number of pollinator visits was recorded.

Data analysis

Differences between mean values were tested either by Student's *t* test or, if the data sets were not normally distributed, a Mann-Whitney U test. For *t* tests, the number of viable seeds was log-transformed and seed set and proportions in pollen loads were angular transformed. In all cases, mean values are given together with their standard error. Times of day are those of Central European Time. (Note the change from local summer to winter time during an investigation.)

RESULTS

Flower, anthesis and nectar characteristics

The population we studied started to flower in late July. The peak of flowering (with at least 50% of the

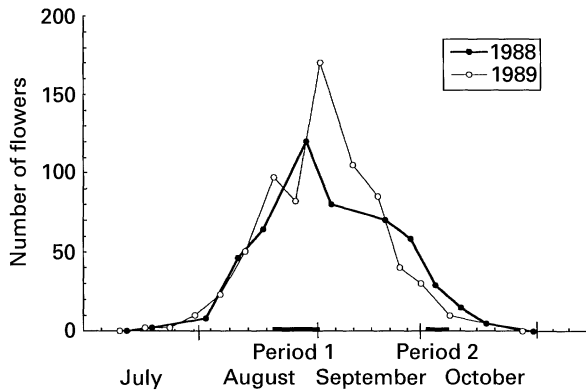


Figure 1. Flowering phenology of *Gentiana pneumonanthe* in the years 1988 and 1989. The number of open flowers in the permanent plot in the two study periods of summer 1989.

maximal number of flowers open) lasted from August 15 until the end of September (Fig. 1).

The flowers were strongly protandrous. During the peak of anthesis they remained open for 3.9 ± 0.8 ($n = 48$, range 2–6) d, and closed for 2.0 ± 0.6 d, in rainy and other inclement weather. Pollen exposure lasted 2.3 ± 0.9 (range 1–5) d. At the same time, the style was developing. When most of the pollen had been removed, the unfolding stigma exposed its receptive papillae. This 'true' female phase of anthesis lasted for only 1.3 ± 0.8 (ranging from 1 to 3) d. In this phase, once the flower had been hand-pollinated with an abundant quantity of pollen, it closed within several hours and did not open again. In the evening, and under inclement weather conditions, the flowers remained closed, otherwise they were open, *c.* 50% of the day, between 9.00 and 16.00 h. Relative humidity and temperature were significantly correlated with this daily opening pattern (T. Petanidou *et al.*, unpublished).

Mean nectar standing crop was highest in the morning, and declined in the course of the day [23–24 August; $0.17 \pm 0.04 \mu\text{l}$ at 9.30 h ($n = 31$); $0.08 \pm 0.02 \mu\text{l}$ at 14.00 h ($n = 33$), and $0.03 \pm 0.02 \mu\text{l}$ at 17.30 h ($n = 23$)]. The sugar concentration was generally low, ranging from 9 to 39%. Nectar standing crop declined dramatically late in the season, compared to the peak flowering ($0.10 \pm 0.02 \mu\text{l}$, $n_{\text{Aug}} = 87$, *vs.* $0.02 \pm 0.01 \mu\text{l}$, $n_{\text{Oct}} = 34$).

Constancy and efficiency of pollinators

Gentiana was visited predominantly by workers, occasionally by queens, and particularly late in the season by males of the bumblebee species *Bombus pascuorum* (Scopoli). This species exists in polymorphic populations in this region, composed of specimens with the phenotypes of both *B.p. floralis*

Table 1. Number (mean, SE and range) of pollen grains carried on different body parts of bumblebees captured on flight, after having visited a *Gentiana pneumonanthe* flower. Data of the flowering peak period were taken on 22–24 and 28 August between 0830–1500 h, and those of late season on 3–4 October 1989 between 1030–1300 h. C: *Calluna vulgaris*, E: *Erica tetralix*, M: *Molinia caerulea*

	Total number of pollen grains				Species	
	Mean	SE	Range	<i>n</i>	<i>Gentiana</i> (% of total)	Main other
Peak of flowering						
Head	336	97	142–676	6	91	C + E
Front legs	2843	1303	708–7735	5	97	C + E
Front thorax	2936	703	787–5885	7	98	C + E + M
Hind thorax	178	25	51–230	7	84	M
Front abdomen	1317	371	317–2560	7	97	C + E + M
Hind abdomen	204	24	118–223	4	90	C + E + M
All body parts	8011		2123–17309		93	C + E + M
Late flowering						
All body parts	18608	437	5465–37450	5	97	C + E

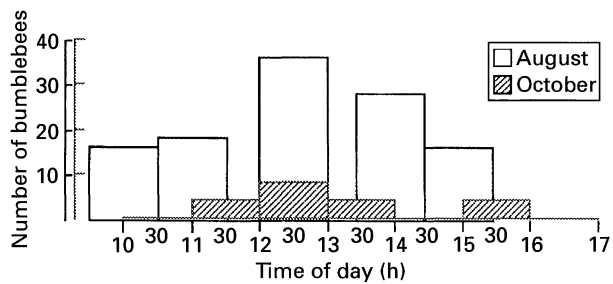


Figure 2. The number of bumblebees visiting the permanent plot in the course of the day during the flowering peak (22–24 August, observation time 930–1530 h), and during the late flowering period (4–5 October, observation time 10–17 h). Note the different observation time schedules in August and October. No bumblebees were observed from 10–11 h, 14–15 h and 16–17 h in the October period.

(Gmelin) and *B.p. romanioides* Krüger. *Bombus terrestris* (Linnaeus) was noticed only at a very low frequency, sometimes acting together with *Bombus lucorum* (Linnaeus) as a nectar robber. This was frequently observed during summer 1991. *Bombus pascuorum* visits the flowers for nectar, gathering it by moving around the ovary base. As a rule, the insects touch the reproductive organs sternotribically (Table 1). Direct pollen collecting was never witnessed. Nevertheless, large quantities of *Gentiana* pollen adhered on the bumblebee bodies, which were groomed and packed in the corbicula during the subsequent flight. The high mean total-body pollen load late in season may be due to the fact that mainly non-grooming males were active. Bumblebee visitors to *Gentiana* were numerous during peak flowering, but their number declined dramatically late in the season (Fig. 2).

Six hoverfly species, mainly males, visited *Gentiana*, but they paid far fewer visits than bees. These were *Episyrphus balteatus* (Degeer), *Eristalis tenax* (Linnaeus), *Helophilus pendulus* (Linnaeus), *Syrphus ribesii* (Linnaeus), and *Rhingia campestris* Meigen during the flowering peak and *Sericomyia silentis* (Harris), *Episyrphus balteatus*, and *Helophilus pendulus* late in the season. Hoverflies visited the flowers only for pollen, switching from the open anthers, early in anthesis, to the exposed stigmas later on. Compared to bees they were poorer pollen vectors, making a very limited contribution to the transfer of *Gentiana* pollen. During the peak period their average body load was 597 ± 284 pollen grains ($n = 6$, range 13–1628), late in the season 994 ± 437 ($n = 4$, range 60–1794).

The differences in visitation rates (Fig. 3c–d) indicate that the insects preferred *Gentiana* flowers to these of the more numerous *Calluna* and *Erica*. This implies that a single flower in the population may receive a considerable number of bumblebee visits (i.e. at least 3, Fig. 3). Their preference for *Gentiana* could also be deduced from the observations on closed flowers in the morning and evening

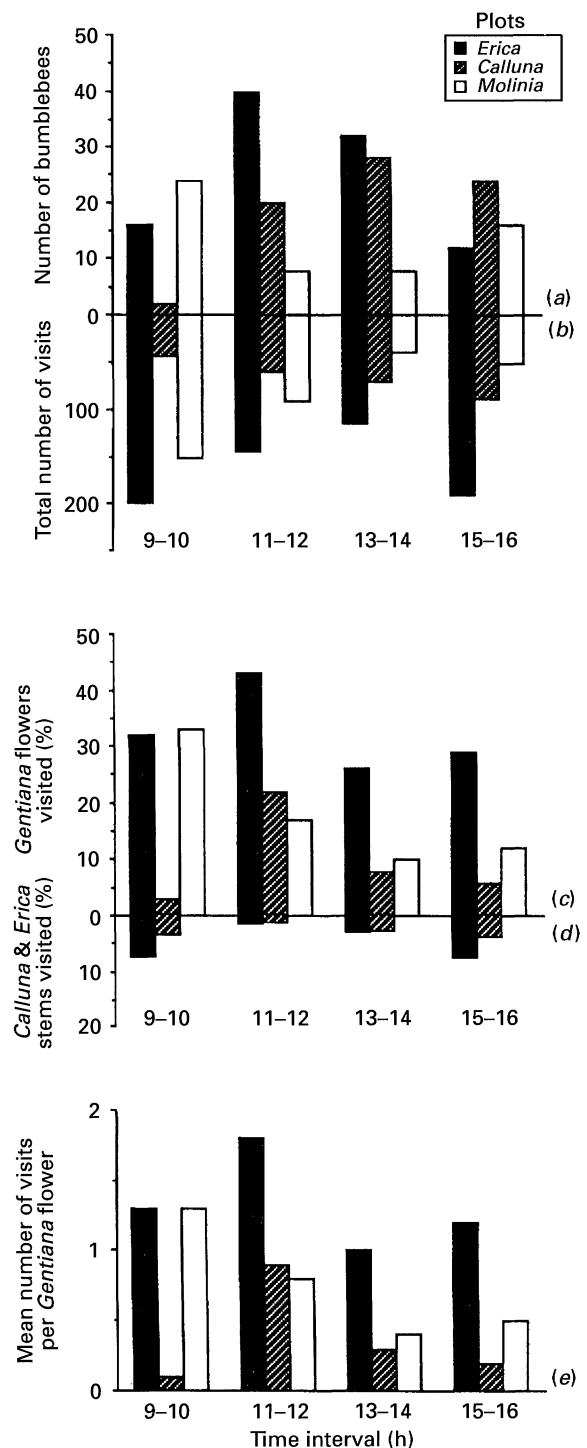


Figure 3. Data on daily bumblebee visitation to each of the three mixed plots with different species composition during the flowering peak (22, 24, and 28 August 1989). (a) number of bumblebees, (b) total number of visits to the three flowering species, (c) percentage *Gentiana pneumonanthe* flowers visited of the total available, (d) percentage visited flowering stems of *Erica tetralix* and *Calluna vulgaris* of the total available, (e) mean number of visits per available *Gentiana* flower. The maximum number of open flowers of *Gentiana*, and the number of flowering stems of *Erica* and *Calluna* in each plot, respectively, are: *Erica*-plot 65G, 386E, 0C, *Calluna*-plot 36G, 50E, 500C, and *Molinia caerulea*-plot 109G, 0E, 0C.

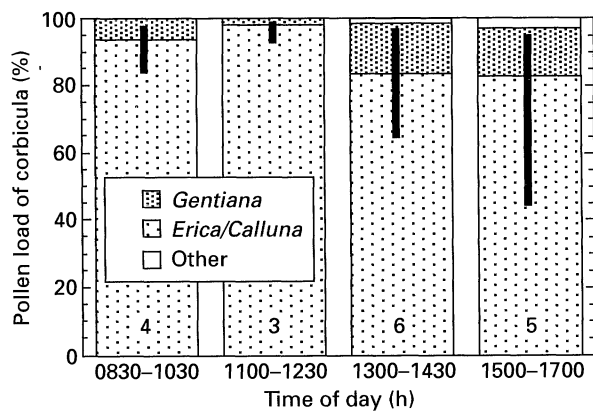


Figure 4. Pollen composition (% pollen grains per plant species) of the corbicula loads of the bumblebees visiting *Gentiana pneumonanthe* throughout the day. Black, vertical bars give the range for *Gentiana* pollen over the samples examined; per time interval the number of corbiculae studied is given. The samples were taken on 22–25, and 28 August 1989.

hours. Bumblebees pushed apart the corolla lobes forcing their way in.

In spite of their preference, bumblebees were not constant visitors to *Gentiana* flowers. In the *Erica*-plot, 54% of the bumblebee visits recorded were paid to *Gentiana*, but only 67% of these were followed by a second visit to the same species. In the *Calluna*-plot these figures were even lower: here *Gentiana* received only 21% of the bumblebee visits, and only 50% of these were consecutive. Apparently, constancy is less pronounced when the number of heterospecific flowers in the neighbourhood increases. Bumblebees were more numerous and constant to *Gentiana* flowers in the *Erica*-plot than in the *Calluna*-plot. In the *Erica*-plot, *Gentiana* received the highest amount of visits per flower (Fig. 3e). In the *Molinia*-plot, the bumblebees were constant by necessity, but their visitation rate, with respect to both total numbers of visitors and visits, was comparatively low and, surprisingly, highest early in the morning.

Data on pollen composition of both body and corbicula loads (Table 1, Fig. 4), show very high percentages of *Gentiana* pollen, suggesting a much higher preference for *Gentiana* than can be concluded from the observations on visits (Figs 2 and 3). The high percentage of *Gentiana* pollen in the bumblebee corbicula loads dropped in the afternoon, although not significantly (Mann–Whitney tests, $P > 0.05$).

The visit of a single bumblebee worker to a virginal stigma of unemasculated flowers of *Gentiana* resulted in the deposition of 8146 ± 1291 of pollen grains ($n = 8$; sternotribic). Pollen from other sources than *Gentiana*, namely from *Calluna* and *Erica*, was detected in only very small quantity (namely 0.05–1.1%).

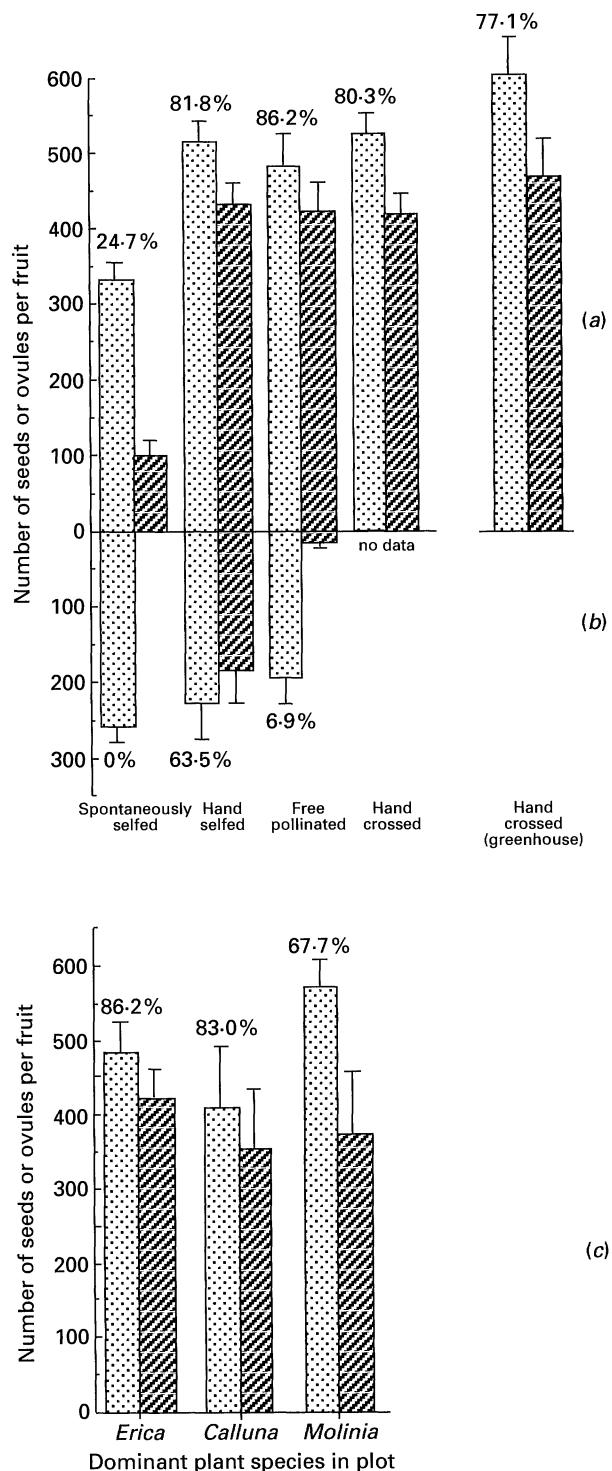


Figure 5. Reproductive success of *Gentiana pneumonanthe* pollinated in (a) August and (b) October, in comparison with that of cross-pollination conducted on plants grown in the greenhouse (a, apart). Free-pollinated fruits were sampled from the *Erica tetralix*-dominated plot. (c) Comparative results of the reproductive success of free-pollinated *Gentiana* flowers in the three plots dominated by *Erica tetralix*, *Calluna vulgaris* and *Molinia caerulea*, respectively, during August. Columns represent the mean total number of ovules (the sum of viable and aborted seeds) (□), and the mean number of viable seeds per fruit (▨). The seed set (as %) is given on top of each pair of columns. Error bars represent 1 SE.

Reproductive success

Fruit set was 100% in all cases examined, but seed set differed (Fig. 5a–c). During the flowering peak, there was no difference in the mean number of ovules and the mean number and percentage of viable seeds between hand- and freely-pollinated flowers (all treatments, $t < 1.6$, $P > 0.10$). Also, between hand cross- and self-pollination, no differences were observed in seed and ovule production (number of ovules: $t[58] = 0.288$, $P > 0.10$; number of viable seeds: $t[58] = 0.337$, $P > 0.10$; seed set: $t[58] = 0.660$, $P > 0.10$). There was, however, a strong and very significant reduction both in the number of ovules and viable seeds and in seed set after spontaneous selfing, in comparison to hand-selfing (number of ovules: $t[60] = 5.104$, $P \leq 0.01$; number of viable seeds: $t[60] = 5.954$, $P \leq 0.01$; seed set: $t[60] = 9.793$, $P \leq 0.01$).

Comparison of the mean reproductive investment (in terms of the initial numbers of ovules produced per flower) of freely-pollinated flowers in the plots with different species composition (Fig. 5c), showed no significant difference between the *Calluna*- and the *Erica*-plot, but in the *Molinia*-plot the mean number of ovules estimated from the fruit tended to be higher than in the *Calluna*-plot ($t[10] = 1.809$, $P \leq 0.10$). On the other hand, plants in the *Molinia*-plot had a lower mean seed set ($t[26] = 1.755$, $P \leq 0.05$) than those in the other plots. However, there was no difference between the plots as far as the absolute number of viable seeds is concerned (*Molinia-Erica*: $t[26] = 0.877$, $P > 0.10$; *Molinia-Calluna*: $t[10] = 0.180$, $P > 0.40$).

Late in the season, the number of ovules in free-pollination, and in all the other experimental pollinations, was significantly less than in the peak (for all treatments, $1.572 \leq t \leq 7.668$, $0.01 < P \leq 0.025$). Self-pollination by hand yielded a relatively high number of viable seeds, although much less than during the flowering peak ($t[37] = 3.124$, $P < 0.005$). The success of free-pollination dropped dramatically ($t[50] = 13.859$, $P \leq 0.01$), while spontaneous self-pollination yielded no seeds at all.

Cross-pollination in the greenhouse (Fig. 5a) resulted in the highest number of viable seeds obtained during this study (470.7 ± 45.5), but there was no statistically significant difference between this and the hand-pollinations performed in the field (t -tests, $P > 0.05$). The number of ovules in flowers produced in the greenhouse was also the highest observed (606.7 ± 49.0) and was significantly higher than the mean values observed in both the *Calluna*- and the *Erica*-plots ($t[33] = 1.792$, $P \leq 0.05$, and $t[49] = 1.823$, $P \leq 0.05$, respectively). However, the number of ovules produced in the greenhouse was neither different from the mean number of ovules counted in the *Molinia*-plot ($t[14] = 0.335$, $P > 0.10$) nor from that in virgin flowers collected in

the field ($t[37] = 0.117$, $P > 0.40$). The number of ovules in the latter sample was also significantly higher than the ovule number estimated in ripe fruits from the *Calluna*- ($t[14] = 3.012$, $P \leq 0.01$), and the *Erica*-plot ($t[30] = 2.049$, $P < 0.025$), but again there was no difference with the *Molinia*-plot ($t[14] = 1.238$, $P > 0.10$).

DISCUSSION

Mating system of Gentiana

Gentiana pneumonanthe is a strongly protandrous self-compatible species, but as it is neither wind- nor sufficiently spontaneously self-pollinated, it requires flower visitors for its reproduction (*cf.* Fig. 5). In the case of *Gentiana*, at the study site, only *Bombus pascuorum* need be considered. It visits *Gentiana* mainly for nectar, according to the available reward (i.e. majoring when nectar standing crop is high and minoring when nectar is little). Hoverflies are not only poor pollinators, but according to our observations, they are pollen thieves, licking pollen from the stigmas. According to Knuth (1898) other species of bee visitors to *Gentiana* flowers used to be more numerous in northern Europe. One of these was *Bombus muscorum* Fabricius, which was observed in our study area by Hugo de Vries (1875), but this species is not now found in the area.

The fact that reproductive success is strongly reduced late in the season certainly reflects the unfavourable physiological and environmental conditions for the flowers to develop properly during that period (e.g. reduced temperature and light). The reduction of the nectar production may demonstrate this, but also the low numbers of pollinators during this period (Figs 2, 5b) are responsible for the dramatical reduction in reproductive success.

Reproductive behaviour in the patches dominated by Erica, Calluna and Molinia

Absence of pollinator and pollen limitation. In none of the studied patches was *Gentiana* found to suffer from reduced seed set (Fig. 5a, c, etc), as has been observed in smaller populations of this species elsewhere (Petanidou *et al.*, 1991; Oostermeijer *et al.*, 1992), and for other rare plant species (Jennersten, 1988; Kwak, 1988; Kwak & Jennersten, 1991; Kwak *et al.*, 1991). This implies first that the population is apparently large enough to ensure its reproductive success by attracting sufficient numbers of visitors, and, secondly, that the same is true for all three patch types despite the differences in visitation rate we observed (Fig. 3). Thirdly, the bumblebee inconstancy we detected, resulting in interspecific pollen transfer between the locally co-occurring species, has no impact on the reproductive success of *Gentiana* (Table 1, Fig. 4, etc). This may partly be attributed to different flower morphologies and

partly to the differential characteristics of the pollen grains, which are sticky and purely entomophilous in *Gentiana*, but dry and somewhat anemophilous in *Erica* and *Calluna* (Kugler, 1970; Faegri & van der Pijl, 1979). Bumblebee-borne pollen of *Erica* and *Calluna* is more likely to be detected during the afternoon, when *Gentiana* pollen may be less sticky, while that of *Erica* and *Calluna* is better dispersed, because of the increased temperature and reduced relative air humidity.

The reduced seed set in the *Molinia*-plot may be explained by the higher number of ovules produced per flower, perhaps requiring more visits per flower than in the other plots (Fig. 5c). In fact, the average *Gentiana* flower in this plot is likely to receive, at a maximum, as many visits as a flower in the *Calluna*-plot (Fig. 3). This may result in a slight pollen limitation. A similar phenomenon was observed in *Gentianella germanica*, where plants growing in a relatively nutrient-rich soil also produced more ovules and had lower seed set than plants in another population growing on a poorer soil (Luijten, 1992). Furthermore, as a result of the relative isolation of the *Gentiana* plants, the same number of visits is paid by fewer bumblebee individuals, which repeatedly visit the flowers, especially when these are fully open (Fig. 3). This increases the probability of geitonogamy and may therefore reduce offspring fitness through inbreeding depression (Oostermeijer, van Eijck & den Nijs, 1994a). In this respect, the increasing dominance of *Molinia caerulea* in many heathlands (Heil & Diemont, 1983) not only inhibits seedling recruitment of *Gentiana pneumonanthe* (Oostermeijer *et al.*, 1994b), but also poses a threat to the number and quality of the offspring (Oostermeijer *et al.*, 1994a).

Changes in the initial number of ovules. An important, and complicating, aspect of the reproductive ecology in our study is the number of ovules per *Gentiana* flower. In the field, this number is higher in the *Molinia*- than in the *Calluna*-plot. Also, ripe fruits on plants growing in the *Erica*- and the *Calluna*-plot (but not on those from the *Molinia*-plot) have a lower estimated number of ovules than plants grown under favourable (relatively nutrient-rich) conditions in the greenhouse. Furthermore, the initial number of ovules in fresh, unfertilized *Gentiana* flowers is significantly higher than the number of ovules estimated from the ripe fruits developing in the *Calluna*- and the *Erica*-plots, but the number is the same in the *Molinia*-plot.

The observed differences in reproductive output of *Gentiana* in the three patches are important in the light of the deterioration of its habitats. It has been demonstrated that both the lowering of the groundwater table and the rise in soil nitrogen, due to atmospheric deposition, increase the competitiveness of *Molinia caerulea* in heathland vegetation with

Erica tetralix (Heil & Diemont, 1983; Berendse & Aerts, 1984; Heil & Bruggink, 1987). It may be expected, therefore, that patches of vegetation dominated by *Molinia* might have a higher nutrient content, in comparison to patches dominated by *Erica* or *Calluna*. Indeed, soil analyses from another site in The Netherlands (at Deelense Veld in the National Park Hoge Veluwe) have revealed, that *Molinia*-dominated soil samples had higher nitrogen content in the form of nitrite and ammonium than the *Erica*-dominated patches ($P < 0.0001$; Oostermeijer, Borst, & Fehse, unpublished data). *Calluna* generally occupies the drier parts of heathlands; patches dominated by *Molinia* and *Erica* are wetter. A higher nutrient status may result in *G. pneumonanthe* developing more ovules in the *Molinia*-plot, while in drier parts dominated by *Calluna* reduction in ovule numbers may occur (Fig. 5c). If nutrient or water availability is limiting, it is not unlikely that unfertilized ovules may be resorbed (cf. Fenster, 1991; Waser & Price, 1991). After the ripening of the fruit, the resorbed ovules may then be too small to recognize, leading to an underestimation of the number of aborted seeds. If nutrients are available in quantity (as seems possible in the *Molinia*-plot), resorption of unfertilized ovules may not occur. The fact that covered and untouched flowers in the *Erica*-plot have a significantly lower number of ovules also supports this hypothesis, because in these fruits there are many unfertilized ovules to be resorbed (Fig. 5a). In this respect, it is also very interesting that in large populations of *Gentiana pneumonanthe*, there is a negative correlation between the number of viable seeds per fruit and seed weight (Oostermeijer *et al.*, 1992). Recently, it has been shown that especially in small populations, maternal effects such as those described above have very significant negative effects on the performance of offspring in early stages of the life cycle (e.g. seed and seedling weight), while inbreeding as a result of increased geitonogamy and autogamy reduces performance in later stages, such as adult weight and flowering (Oostermeijer *et al.*, 1994a). From our results it is clear that a knowledge of pollination ecology is very important in understanding the changes that occur in small populations of rare and endangered plant species.

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