

Effects of outcrossing distance and selfing on fitness components in the rare *Gentiana pneumonanthe* (Gentianaceae)

J. GERARD B. OOSTERMEIJER, RUUD G. M. ALTENBURG and HANS C. M. DEN NIJS

Institute for Systematics and Population Biology, Hugo de Vries laboratory, University of Amsterdam, Kruislaan 318, 1098 SM, Amsterdam, The Netherlands

SUMMARY

Variation in offspring fitness as a result of different distances of pollen donors has been described for several plant species. In some of these, inbreeding depression after selfing or mating between close neighbours and outbreeding depression following crossing over large distances may lead to an intermediate crossing distance with optimal fitness. We studied the relationship between outcrossing distance and offspring fitness in a large population of the rare herbaceous perennial *Gentiana pneumonanthe*. The relative success of selfing and cross pollination with pollen from increasing distances from maternal plants, including an 'interpopulation' cross, was measured as seed set and seed weight, and in a consecutive glasshouse experiment as the proportion of seeds germinating, seedling weight, adult weight and total relative fitness. Regression analyses showed significant positive relationships between outcrossing distance and the fitness components seed set, seedling weight and adult weight. Additional statistics showed, however, that the observed significant regressions were mainly the result of a relatively low fitness following selfing (inbreeding depression) and a comparatively high performance of the interpopulation crosses (heterosis). No significant effects of within-population crossing distance were found when selfing and interpopulation crosses were omitted from the analyses. We therefore conclude that there is no substantial evidence for an effect of outcrossing distance on fitness components in the studied population of *Gentiana pneumonanthe*. The results are discussed in the context of conservation biology of rare plant species. Artificial interpopulation crossing may be a way to counteract the losses of genetic variation and fitness in small, isolated populations. In our opinion, however, this is only a feasible management option for very rare and endangered species.

Key-words: conservation biology, fitness, inbreeding depression, outbreeding depression, outcrossing distance, population genetic structure.

INTRODUCTION

In plants, inbreeding depression is mostly interpreted as the decline in the mean values of fitness characters as a result of selfing as compared to cross-pollination (Charlesworth & Charlesworth 1987; Waser 1993). Inbreeding may result in higher proportions of embryo abortion, while the produced offspring may show reduced germination, lower growth rates, higher mortality and a lower probability of flowering (Charlesworth & Charlesworth 1987; Dudash 1990; Barrett & Kohn 1991). The reduction in fitness of the more homozygous inbred offspring may be caused either by a higher fitness of heterozygotes (the 'overdominance' hypothesis) or by increased expression of recessive deleterious alleles (the 'partial dominance' hypothesis; Mitton 1989, 1993). An increase in homozygosity does not result only from complete selfing, however, but also from matings between individuals that are related (biparental inbreeding; Uyenoyama 1986). Because plants are sessile and generally have limited seed dispersal, it is likely that neighbouring individuals in populations will share a certain degree of kinship (Levin 1988). In such structured populations, mating between two neighbouring plants—which is a rule rather than an exception because of the short flight distances of many pollinators—may result in a considerable level of biparental inbreeding.

Within plant populations, local adaptation may also be observed on a small scale as a result of microgeographical differentiation in selection pressures (Turner *et al.* 1982). Outcrossing over longer distances—between plants from different micro-habitats—might lead to reduced fitness as a consequence of (i) the disruption of co-adapted gene-complexes, or (ii) the production of offspring that is intermediate and hence adapted to neither of the parental habitats (Templeton *et al.* 1986; Levin 1988; Schmitt & Gamble 1990; Waser 1993). This phenomenon has been described as 'outbreeding depression' (Templeton 1986).

In a number of plant species, an 'optimal intermediate outcrossing distance' has been demonstrated at which offspring fitness is highest because it is least affected by both inbreeding and outbreeding depression (Price & Waser 1979; Waser *et al.* 1987; McCall *et al.* 1991; Svensson 1988, 1990; Waser & Price 1989, 1991a,b). On the other hand, no evidence for an optimal intermediate outcrossing distance was found in several other studies, although in some cases an increase or decrease in fitness with increasing crossing distance was observed (Levin 1984; Koptur 1984; Harder *et al.* 1985; Galen *et al.* 1985; Fenster & Sork 1988; Sobrevila 1988; Dudash 1990).

In most papers, seed or fruit set was used as the only measure of fitness. Waser & Price (1989) argued that the relative performance of the offspring in later stages of the life cycle will give a better estimate of fitness, partly because the causes of differences in seed set are difficult to discern. These differences may result from variation in pollen tube growth (Fenster & Sork 1988; Cruzan 1990; Waser & Price 1991a), from (selective) abortion by the maternal plant, which may be related to the availability of resources at her microsite (Fenster 1988, 1991; Waser & Price 1991b), or they may be caused by early-acting lethal recessives that are expressed in (part of) the embryos (Seavey & Bawa 1986; Lyons *et al.* 1989).

Besides being of high evolutionary interest, knowledge of inbreeding and outbreeding depression in wild plant populations is also very important for the conservation of rare species (Barrett & Kohn 1991; Ellstrand & Elam 1993; Schamske *et al.* 1994). Small populations may suffer from loss of genetic variation and reduced fitness through inbreeding and genetic drift, resulting in an increased probability of extinction in the short term and a reduced potential for evolutionary adaptation in the longer term (Soulé

1980; Beardmore 1983; Ellstrand & Elam 1993). Artificial crossing or stimulation of natural outbreeding between populations has sometimes been suggested as a way to counteract these threats (Barrett & Kohn 1991; van Treuren *et al.* 1993; Ellstrand & Elam 1993). However, we need a much deeper understanding of the occurrence and mechanisms of outbreeding depression before it can be considered safe to use these measures in the conservation of rare species (Ellstrand 1993).

In this paper, the effects of the distance of the pollen donor (including selfing and outcrossing with pollen from a nearby population) on seed set and a number of fitness-related parameters measured in a glasshouse environment, are investigated for the rare perennial plant *Gentiana pneumonanthe* L. It has been shown that small populations of this species have reduced genetic variation (Raijmann *et al.* 1994) and that both this and deteriorated habitat conditions have resulted in reduced offspring fitness (Oostermeijer *et al.* 1994a). Outbreeding of genetically depauperate small populations with larger populations containing more genetic variation may be a way to restore their viability. Thus, besides being a contribution to the still scarce knowledge of the effects of outcrossing distance, this study hopes to provide more insight into the effects of outbreeding between populations for conservation purposes.

MATERIALS AND METHODS

Study species

Gentiana pneumonanthe is an iteroparous herb reproducing exclusively by seeds. An individual reproductive plant bears an average total of 2–3 (range 1 to >60) blue, protandrous flowers, which are mainly visited by bumblebees (in The Netherlands mostly *Bombus pascuorum* subsp. *romanioides* and *floralis*, *B. lucorum*, and *B. terrestris*; Petanidou *et al.* 1991, 1995). The species appears to be fully self-compatible, since no significant differences in seed set were observed between manual self- and cross pollinations (Petanidou *et al.* 1995). In the absence of pollinators, however, seed set is reduced drastically as a result of severe pollen limitation (Petanidou *et al.* 1995). Seed set is also significantly lower in small populations, probably as a result of limited pollinator visitation, which also leads to increased (spontaneous) selfing rates (Oostermeijer *et al.* 1992; Raijmann *et al.* 1994).

G. pneumonanthe prefers acidic, nutrient-poor sites with a fluctuating phreatic level: inundated in winter and wet to moist in summer. For recruitment, *G. pneumonanthe* depends on relatively open vegetation, such as paths created by cattle or man, or areas which are mown or where sods have been cut (Oostermeijer *et al.* 1994b). Throughout most of its range, the species is mostly confined to wet to moist heathlands and hay meadows. Since these habitat types are decreasing in both number and size, *G. pneumonanthe* has become a rare species in The Netherlands (Mennema *et al.* 1985) and also in other European countries (Great Britain, Clapham *et al.* 1987; Belgium, Van Rompaey & Delvosalle 1978; Germany, von Weihe 1972; Switzerland, E. Landolt, personal communication; Poland, Oleksyn & Reich 1994; Czech Republic, Z. V. Krenová, personal communication).

Study population and pollination experiments

The population of *Gentiana pneumonanthe* in which the experiment was conducted is situated in the central area of The Netherlands, near the city of Hilversum, in the 'Goois

Nature Reserve'. In this, for Dutch standards, large heathland population (>5000 flowering plants in 1992, but occupying an area of only $125 \times 60 \text{ m}^2$), 58 randomly chosen plants with two or more flowers were caged to exclude insect visitation. Although methodologically it would have been better if all pollination distances could have been performed on each individual, this would have required a selection of exceptionally large individuals from the population. It might be that these plants are rather old, or grow on favourable microsites, which may affect allocation to reproduction (Fenster 1991; Waser & Price 1991b). Since this would probably yield biased results that cannot be translated to the average individual in the population, it was decided to spread the pollination treatments over randomly selected plants. Hence, one or two (in one case three) flowers on each maternal plant were emasculated when they had just opened, by removing the still undehisced anthers with fine forceps. In total, 95 flowers were pollinated with pollen from donors growing on different distances, grouped in the following nine classes: selfed, 1–50 cm, 50–100 cm, 1–2 m, 2–5 m, 5–10 m, 10–20 m, >20 m and from another population of intermediate to small size (*c.* 100 flowering plants) at a distance of approx. 800 m (population 'Drinkput'). Per treatment, a total of 10–12 flowers was pollinated on randomly selected caged plants. All pollinations were performed from 12 to 19 August 1991. The mature fruits were collected on 17 September. Fruit set was 100%, i.e. all treated flowers produced a ripe fruit.

The relative success of each pollination treatment was determined by measuring the following five fitness-related parameters (see also Oostermeijer *et al.* 1994a):

- (i) seed set, i.e. the ratio of filled, viable seeds to the initial number of ovules;
- (ii) seed weight;
- (iii) proportion of seeds germinating;
- (iv) estimated weight 4 weeks after planting (seedling stage);
- (v) estimated weight 8 weeks after planting (adult stage).

Of all fruits, the number of viable and aborted seeds was counted under a dissecting microscope. Aborted seeds can readily be distinguished from viable seeds because they are smaller and obviously empty. The sum of the number of viable and aborted seeds was considered to equal the initial number of ovules present in the ovary. Per fruit, 100 viable seeds were selected at random and weighed per 100 on a microbalance. The seeds were then placed on wet filter paper in a Petri dish at 25°C and a day/night regime of 8/16 h. 52 days after the start of the experiment, when no additional germination occurred, the total proportion of germination was determined. 30 days after the experiment started, 28 randomly selected seedlings per family (if available) were planted in soaked peat pellets. After 4 weeks of growth, the above-ground dry weight of the seedlings was estimated using a non-destructive method. Of 20 seedlings per family (and hence 200–240 per pollination treatment), the length and width of the largest rosette leaf and the total number of leaves was determined. The product of these measurements is an estimation of total leaf area and thus, indirectly, of the above-ground biomass. Regression between the estimated seedling weight and the natural log of the actual dry weight was highly significant in earlier experiments ($R^2=0.771$, 87 d.f., $P \leq 0.0001$). The same measurements were repeated 8 weeks after planting. At this point, the plants varied between the immature and the (vegetative) adult stage (Oostermeijer *et al.* 1994b).

An estimate of the total fitness per fruit (maternal plant) was also calculated as follows: for each flower, the seed set resulting from the pollination treatment it received was multiplied by a fixed number of ovules, *viz.*, the total average over all sampled fruits (687). This yields a number of viable seeds per treatment that is independent of initial

Table 1. Matrix of Pearson's product-moment correlation coefficients ($N=93$) between the fitness parameters measured on offspring from maternal plants selfed or crossed with pollen from different distances. The data (means per maternal family) were transformed prior to analysis (see text). Correlation coefficients were tested for significance using Bonferroni criteria

	Seed set	Seed weight	Proportion germinating	Seedling weight
Seed weight	-0.292*			
Proportion germinating	-0.064NS	0.120NS		
Seedling weight	-0.277NS	0.420***	0.182NS	
Adult weight	-0.067NS	0.194NS	-0.068NS	0.504***

* $P \leq 0.05$, ** $P \leq 0.025$, *** $P \leq 0.01$, NS, not significant.

variation in the number of ovules. The number of viable seeds was then multiplied by the corresponding proportion of germination and the average adult weight, which yields an estimate of the total biomass that was produced per fruit as a result of the pollination treatment, standardized for an equal initial number of ovules in each fruit.

Data analyses

Prior to all statistical analyses, the data were transformed if necessary, to improve normality and homogeneity of variances. Seed set and proportion of seeds germinating were angular (arcsin-square root) transformed, seed weight and total relative fitness were ln-transformed, and estimated seedling- and adult weight were square-root transformed.

Regression analyses were performed to examine the effect of pollination distance on each of the fitness components. Instead of using the distance classes as ranked values with equal distance to each other, the distance halfway the class limits of each of the pollination distance classes was log ($x+1$)-transformed. These transformed class values were used in the regression analyses.

A second analysis was performed to test the differences in mean fitness between selfing, near within-population (0.01–1.0 m), intermediate within-population (1–5 m), far (>5 m), and interpopulation-crosses. Differences among these five groups were tested with ANOVAs with pollination treatment and maternal plant as main effects. Following significant ANOVA models, the differences between mean values per treatment were tested a posteriori using Tukey's HSD multiple comparison of means.

RESULTS

As expected, the measured life-history parameters were partly intercorrelated (Table 1). Seed set was negatively correlated with seed weight. This suggests that the maternal plant allocates more energy to each individual seed when only few seeds are maturing in a fruit. The positive correlation between seed weight and seedling size indicates that this higher investment in the seeds is beneficial for the offspring, and thus may compensate for the lower seed set to some extent. A similar maternal effect on performance in the early life stages was found in an earlier fitness study on *Gentiana pneumonanthe* (Oostermeijer *et al.* 1994a). However, in contrast to that study, seed

weight did not significantly affect germination performance. Seedling and adult weight were also significantly positively correlated, which agrees with our earlier observations as well (Oostermeijer *et al.* 1994a).

Of the five fitness components, seed set, seedling weight, and adult weight showed a significant positive regression with crossing distance, although the proportion of variance that was explained by the models was rather small (*c.* 5–7%: Table 2, Fig. 1a–e). When examining the data and the graphs more closely, it seems that the significant regressions are mostly determined by a low mean fitness following selfing and a relatively high fitness after interpopulation crossing (Fig. 1a–e). When selfing is omitted from the regression analysis, only the relationship between crossing distance and seed set remains (marginally) significant (Table 2). When the interpopulation cross is omitted, only the correlation between distance and seedling weight is still significant. The relationship between distance and seed set is no longer significant, and the regression between distance and adult weight becomes marginally significant ($0.05 < P < 0.10$, Table 2). When both classes were simultaneously omitted from the analyses, all significant effects of crossing distance disappeared (Table 2). Together, these results indicate that indeed most of the significant regressions between crossing distance and the fitness components resulted from inbreeding depression and to a lesser degree from heterosis. Seed set was apparently mostly affected by heterosis, and seedling and adult weight by inbreeding depression.

This result was only to some degree confirmed by the second analysis of differences in mean fitness between five treatment classes (using ANOVA, see Methods section). Differences between pollination treatments were not significant for seed set ($F_{[4, 33]} = 0.939$, $P = 0.445$), seed weight ($F_{[4, 33]} = 0.212$, $P = 0.931$), germination percentage ($F_{[4, 33]} = 0.987$, $P = 0.419$), and total relative fitness ($F_{[4, 33]} = 1.005$, $P = 0.419$). Maternal plants had a significant effect on offspring performance in all of these cases, though ($F_{[55, 33]} > 1.72$, $P \leq 0.05$). For seedling weight and adult weight, however, the pollination treatment had a more significant effect on offspring performance than the maternal plant (seedling weight: treatment effect $F_{[4, 33]} = 4.058$, $P = 0.009$; maternal plant effect $F_{[55, 33]} = 1.352$, $P < 0.05$; adult weight: treatment effect $F_{[4, 33]} = 4.342$, $P = 0.007$; maternal plant effect ($F_{[55, 33]} = 1.399$, $P > 0.05$). Tukey's HSD multiple comparison of means showed that for these two fitness components, offspring from selfing had a significantly lower mean ($P \leq 0.05$) than all other treatments. No significant differences were found between the means of the various outcrossing treatments. Thus, the results of the second analysis showed a significant inbreeding depression of plant size, but no significant heterosis was detected.

DISCUSSION

In general, the studies of the effects of outcrossing distance that have been performed thus far have yielded contradictory results (for a recent review, see Waser 1993). Most of the published papers indicate that the crosses over intermediate distances (usually 3–5 m) result in the highest fitness (Waser *et al.* 1987; Waser & Price 1983, 1989, 1991a; Schemske & Pautler 1984; Coles & Fowler 1976; Sobrevila 1988; Svensson 1988, 1990; McCall *et al.* 1991). Some studies, however, have demonstrated higher fitness of the long-distance crosses (Koptur 1984; Levin 1984; Galen *et al.* 1985; Harder *et al.* 1985; Zimmermann & Pyke 1988; Redmond *et al.* 1989), while in a few others the shorter

Table 2. Results of regression analyses of pollination distance ($\log(x+1)$ -transformed) on various fitness components in *Gentiana pneumonanthe*: (a) with selfing and interpopulation crosses included in the analysis; (b) with selfing omitted; (c) with the interpopulation crosses omitted; and (d) with both selfing and interpopulation crosses omitted. R^2 is the proportion of total variance explained by the regression models, F is the F -ratio of the ANOVA testing the significance of the regression model, and P is the probability of this F -ratio for $\nu_1 = 1$, and $\nu_2 = 91$ (model a), $\nu_2 = 80$ (models b and c), or $\nu_2 = 69$ (model d). For graphic presentation of the regressions, see Fig. 1

Variable	(a) Total regression			(b) Without selfing			(c) Without interpopulation cross			(d) Without selfing and interpopulation cross		
	R^2	F	P	R^2	F	P	R^2	F	P	R^2	F	P
Seed set	0.046	4.426	0.038	0.043	3.561	0.063	0.029	2.408	0.125	0.025	1.796	0.185
Seed weight	0.009	0.844	0.361	0.007	0.593	0.444	0.011	0.854	0.358	0.011	0.744	0.391
Germination (%)	0.002	0.178	0.674	0.001	0.006	0.940	0.004	0.328	0.568	0.000	0.010	0.920
Seedling weight	0.047	4.520	0.036	0.005	0.377	0.541	0.037	3.091	0.083	0.000	0.000	0.993
Adult weight	0.071	6.916	0.010	0.007	0.529	0.469	0.063	5.339	0.023	0.001	0.103	0.749
Relative fitness	0.021	1.992	0.162	0.019	1.569	0.214	0.009	0.739	0.393	0.005	0.372	0.544

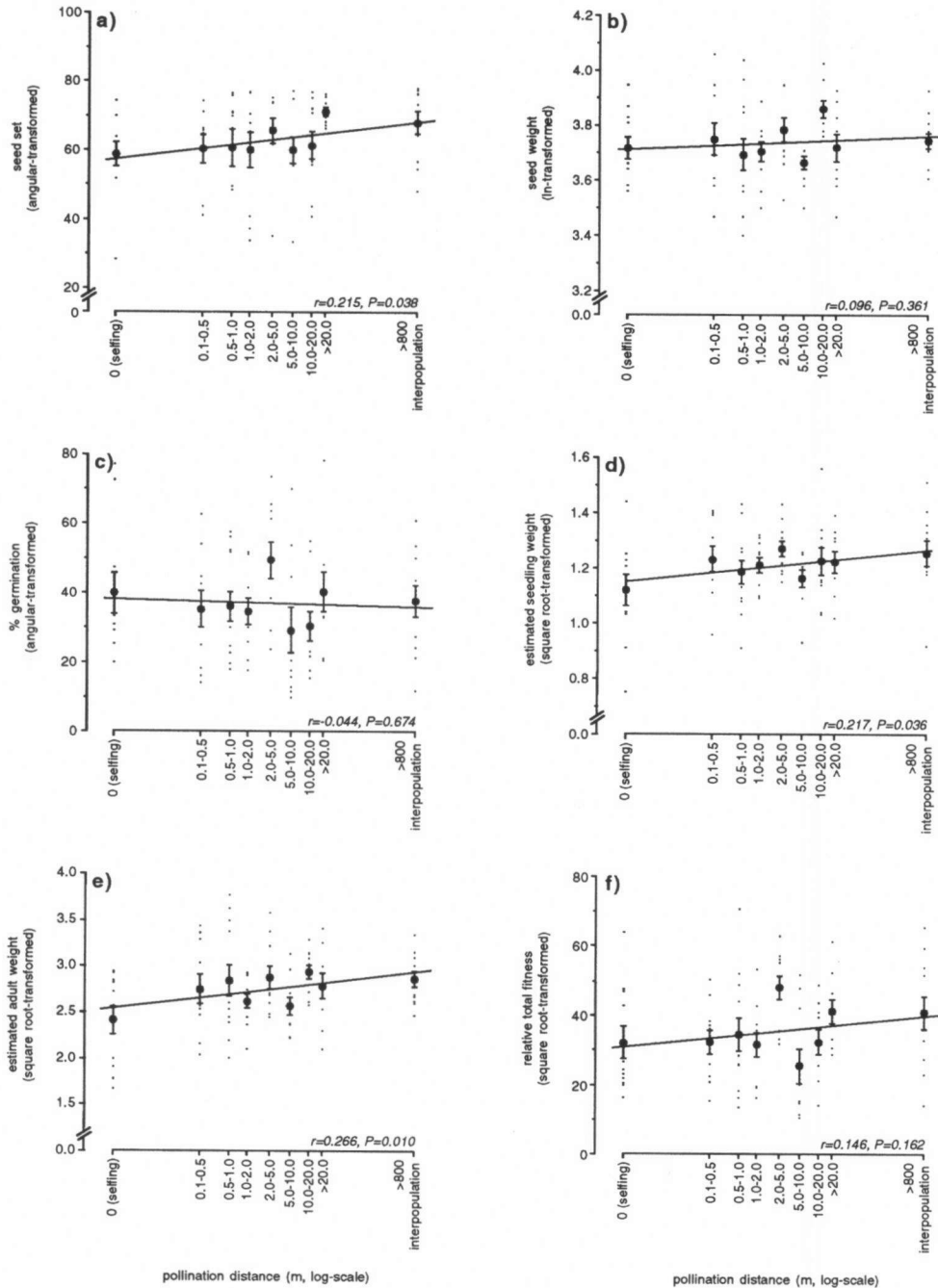


Fig. 1. Relationship between pollination distance (on a logarithmic scale, see text) and the measured fitness components in *Gentiana pneumonanthe*: (a) seed set; (b) seed weight; (c) proportion of seeds germinating; (d) estimated seedling weight; (e) estimated adult weight. The small dots represent the mean values of the maternal families, the larger dots with error bars represent the mean ± 1 standard error per pollination distance class. In the lower right hand of each graph, the Pearson's product-moment correlation coefficient (r , calculated on the family means) is given, together with its probability P . For extended regression statistics, see Table 2.

crossing distances performed better than the crosses over a long distance (Ritland & Ganders 1987; Whitkus 1988).

This study describes the effects of outcrossing distance on the performance from seed set of maternal plants up to the adult size of the offspring in *Gentiana pneumonanthe*. The regression analyses revealed several significant positive relationships between outcrossing distance, seed set and offspring fitness. Additional analyses of the data showed, however, that these significant positive regressions were mainly the result of a relatively low seed set and offspring fitness after selfing and a higher fitness after outcrossing with another, nearby population. When these treatments were omitted from the analyses, no significant effects of distance of the pollen donor were detected within the study population. Hence, we conclude that our observations are the result of inbreeding depression and heterosis (Charlesworth & Charlesworth 1987; Lande & Schemske 1985). Inbreeding depression seemed to affect mainly seedling and adult size, while heterosis appeared to be the main explanation for the observed regression between distance and seed set. No differences in mean seed set (nor any of the other fitness parameters) were observed between any of the outcrossing treatments, however, so even the effect of heterosis appears to be only weak.

A drawback of the present experiment with *Gentiana pneumonanthe* is that the assessment of offspring fitness was performed in the glasshouse. This might lower any effects of outbreeding depression if this is mainly based on selective genotype-environment interactions (Mitchell-Olds 1986; Waser 1993) and less on the breakdown of coadapted gene-complexes (Shields 1982; Templeton 1986). The few studies presenting data that support an optimal intermediate outcrossing distance are based on estimates of long-term offspring fitness under field conditions (Waser & Price 1989, 1991a). Moreover, if there was a genetical cause for outbreeding depression, theory predicts that this would be expressed most strongly in the F_2 and later generations (Templeton 1986), which was not tested in this study. However, a genetic mechanism of outbreeding depression could be expected in small and isolated populations, where random genetic drift combined with local selection may have caused sufficient differentiation of the genomes (Hedrick 1984; Templeton 1986). After the experiments, it appeared that both the large population 'Heidebloem' (which went through a bottleneck during the 1970s) and the comparatively small population 'Drinkput' are genetically depauperate, with a relatively low allozyme polymorphism and heterozygosity (Oostermeijer *et al.* 1994a; Raijmann *et al.* 1994). This may have caused the slight heterosis effect observed in the offspring. This agrees with Van Treuren *et al.* (1993), who also observed heterosis instead of outbreeding depression after interpopulation crosses with the rare *Scabiosa columbaria*.

Preliminary results of recent reciprocal transplant experiments with *Gentiana pneumonanthe* in the same area between population 'Heidebloem' and another small population, 'Lange Ven' suggest that local adaptation is not very pronounced. Performance of plants at their 'home' site is similar to that at the 'alien' site, although this may have been overruled by strong differences in quality between the two sites (J. G. B. Oostermeijer and K. Vijverberg, unpublished data). On the basis of these results, no outbreeding depression should be observed, which conforms to our present findings.

We therefore conclude that there is no evidence for the existence of an optimal intermediate outcrossing distance in this population of *Gentiana pneumonanthe*. If we

find mainly heterosis instead of outbreeding depression in small, genetically depauperate populations, as this and other experiments (Van Treuren *et al.* 1993) suggest, manual interpopulation-crossing may be a useful measure to counteract the loss of population viability by genetic erosion. However, in our opinion, this 'genetical management' would only be a feasible option for a limited number of very rare species threatened by extinction even when ecological management is demographically favourable. In most cases, we are dealing with large numbers of formerly common species that are now starting to suffer from isolation and small population sizes. The best management for these species is enlarging the area of suitable habitat (increasing population sizes) and improving the connectivity between these habitats in the landscape. One method of achieving the latter is to create road verges (corridors) which are richer in flowering plant species, so they become more suitable for a more natural exchange of pollen by pollinating insects.

ACKNOWLEDGEMENTS

The authors would like to thank A. Altenburg, E. de Boer, M. van den Bovenkamp, S. Luijten, S. Ooms and B. Oosterbaan for their support in the field, Prof. K. Bachmann, Dr P. Klinkhamer and an anonymous referee for their critical comments, and E. Anink, J. Bakker and J. van Bennekom for their help in the glasshouse. Our special thanks go to the people from the 'Stichting Goois Natuurreservaat' for their interest in the field and for the opportunity to perform experiments with the *Gentiana* populations in their reserve.

REFERENCES

- Barrett S.C.H. & Kohn, J.R. (1991): Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A. & Holsinger, K.E. (eds): *Genetics and Conservation of Rare Plants*. 3–30. Oxford University Press, New York.
- Beardmore, J.A. (1983): Extinction, survival, and genetic variation. In: Schoenewald-Cox, C.M., Chambers, S.M., MacBryde, B. & Thomas, L. (eds): *Genetics and Conservation*. Benjamin-Cummings, Menlo Park.
- Charlesworth, D. & Charlesworth, B. (1987): Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**: 237–268.
- Clapham, A.R., Tutin, T.G. & Moore, D.M. (1987): *Flora of the British Isles*. Cambridge.
- Coles, J.F. & Fowler, D.P. (1976): Inbreeding in neighboring trees in two white spruce populations. *Silvae. Genet.* **25**: 29–34.
- Cruzan, M.B. (1990): Pollen donor interactions during pollen tube growth in *Erythronium grandiflorum*. *Am. J. Bot.* **77**: 116–122.
- Dudash, M.R. (1990): Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* **44**: 1129–1239.
- Ellstrand, N.C. (1993): Gene flow by pollen: implications for plant conservation genetics. *Oikos* **63**: 77–86.
- Ellstrand, N.C. & Elam, D.R. (1993): Population genetic consequences of small population size: implications for plant conservation. *Annu. Rev. Ecol. Syst.* **24**: 217–242.
- Fenster, C.B. & Sork, V.L. (1988): Effect of crossing distance and male parent on in vivo pollen tube growth in *Chamaecrista fasciculata*. *Am. J. Bot.* **75**: 1898–1903.
- Fenster, C.B. (1988): *Gene flow and population differentiation in Chamaecrista fasciculata (Leguminosae)*. Doctorate Thesis. University of Chicago.
- Fenster, C.B. (1991): Gene flow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. *Evolution* **45**: 398–409.
- Galen, C., Plowright, R.C. & Thomson, J.D. (1985): Floral biology and regulation of seed set in the lily, *Clintonia borealis*. *Am. J. Bot.* **72**: 1544–1552.
- Harder, L.D., Thomson, J.D., Cruzan, M.B. & Unnasch, R.S. (1985): Sexual reproduction and variation in floral morphology in an ephemeral

- vernal lily, *Erythronium americanum*. *Oecologia* **67**: 286–291.
- Hedrick, P.W. (1984): Is there an inbreeding optimum? *Zoo. Biol.* **3**: 167–169.
- Koptur, S. (1984): Outcrossing and pollinator limitation of seed set: breeding systems of neotropical *Inga* trees (Fabaceae: Mimosoideae). *Evolution* **38**: 1130–1143.
- Lande, R. & Schemske, D.W. (1985): The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**: 24–40.
- Levin, D.A. (1984): Inbreeding depression and proximity-dependent crossing success in *Phlox drummondii*. *Evolution* **38**: 116–127.
- Levin, D.A. (1988): Local differentiation and the breeding structure of plant populations. In: Gottlieb, L.D. and Jain, S.K. (eds) *Plant Evolutionary Biology*. 305–329. Chapman & Hall, New York.
- Lyons, E.E., Waser, N.M., Price, M.V., Antonovics, J. & Motten, A.F. (1989): Sources of variation in plant reproductive success and implications for concepts of sexual selection. *Am. Nat.* **134**: 409–433.
- McCall, C., Mitchell-Olds, T. & Waller, D.M. (1991): Distance between mates affects seedling characters in a population of *Impatiens capensis* (Balsaminaceae). *Am. J. Bot.* **78**: 964–970.
- Mennema, J., Quené-Boterenbrood, A.J. & Plate, C.L. (1985): *Atlas van de Nederlandse Flora*, deel 2. Bohn, Scheltema en Holkema, Utrecht.
- Mitchell-Olds, T. (1986): Quantitative genetics of survival and growth in *Impatiens capensis*. *Evolution* **40**: 107–116.
- Mitton, J.B. (1989): Physiological and demographic variation associated with allozyme variation. In: Soltis, D. & Soltis, P. (eds) *Isozymes in Plant Biology*. 127–145. Dioscorides Press, Oregon.
- Mitton, J.B. (1993): Theory and data pertinent to the relationship between heterozygosity and fitness. In: Thornhill, N.W. (ed.) *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*. 17–41. University of Chicago Press, Chicago.
- Oleksyn, J. & Reich, P.B. (1994): Pollution, habitat destruction, and biodiversity in Poland. *Cons. Biol.* **8**: 943–960.
- Oostermeijer, J.G.B., Nijs, J.C.M. den, Raijmann, L.E.L. & Menken, S.B.J. (1992): Population biology and management of the marsh gentia (*Gentiana pneumonanthe* L.), a rare species in The Netherlands. *Bot. J. Linn. Soc.* **108**: 117–130.
- Oostermeijer, J.G.B., Eijck, M.W. van & Nijs, J.C.M. den (1994a): Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). *Oecologia* **97**: 289–296.
- Oostermeijer, J.G.B., Veer R. van't & Nijs, J.C.M. den (1994b): Population structure of the long-lived perennial *Gentiana pneumonanthe* in relation to vegetation and management. *J. Appl. Ecol.* **31**: 428–438.
- Petanidou, T., Nijs, J.C.M. den & Ellis-Adam, A.C. (1991): Comparative pollination ecology of two rare Dutch *Gentiana* species, in relation to population size. *Acta Hort.* **288**: 306–312.
- Petanidou, T., Nijs, J.C.M. den, Oostermeijer, J.G.B. & Ellis-Adam, A.C. (1995): Pollination ecology and patch-dependent reproductive success of the rare perennial *Gentiana pneumonanthe* L. *New Phytol.* **129**: 155–163.
- Price, M.V. & Waser, N.M. (1979): Pollen dispersal and optimal outcrossing in *Delphinium nelsonii*. *Nature* **277**: 294–296.
- Raijmann, L.E.L., Leeuwen, N.C. van, Kersten, R., Oostermeijer, J.G.B., Nijs, J.C.M. den & Menken, S.B.J. (1994): Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. *Cons. Biol.* **8**: 1014–1026.
- Redmond, A.M., Robbins, L.E. & Travis, J. (1989): The effects of pollination distance on seed set in three populations of *Amianthium muscaetoxicum* (Liliaceae). *Oecologia* **79**: 260–264.
- Ritland, K. & Ganders, F.R. (1987): Variation in the mating system of *Bidens menziesii* (Asteraceae) in relation to population substructure. *Heredity* **55**: 235–244.
- Rompae, E. van & Delvosalle, L. (1978): *Atlas van de Belgische en Luxemburgse Flora, Pteridofyten en Spermatofyten*. Meise, Belgium.
- Schemske, D.W. & Pautler, L.P. (1984): The effects of pollen composition on fitness components in a neotropical herb. *Oecologia* **62**: 31–36.
- Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M. & Bishop, J.G. (1994): Evaluating approaches to the conservation of rare and endangered plants. *Ecology* **75**: 584–606.
- Schmitt, J. & Gamble, S.E. (1990): The effect of distance from the parental site on offspring performance and inbreeding depression in *Impatiens capensis*: a test of the adaptation hypothesis. *Evolution* **44**: 2022–2030.
- Seavey, S.R. & Bawa, K.S. (1986): Late-acting self-incompatibility in angiosperms. *Bot. Rev.* **52**: 195–219.
- Shields, W.M. (1982): *Philopatry, Inbreeding, and the Evolution of Sex*. State University of New York Press, Albany.
- Sobrevila, C. (1988): Effects of distance between pollen donor and pollen recipient in fitness components in *Espeletia schultzii*. *Am. J. Bot.* **75**: 701–724.

- Soulé, M.E. (1980): Thresholds for survival: maintaining fitness and evolutionary potential. In: Soulé, M.E. & Wilcox, B.A. (eds) *Conservation Biology: An Evolutionary-Ecological Perspective*. 151–169. Sinauer Ass., Sunderland.
- Svensson, L. (1988): Inbreeding, crossing and variation in stamen number in *Scleranthus annuus* (Caryophyllaceae), a selfing annual. *Evol. Trends Plants* 2: 31–37.
- Svensson, L. (1990): Distance-dependent regulation of stamen number in crosses of *Scleranthus annuus* (Caryophyllaceae) from a discontinuous population. *Am. J. Bot.* 77: 889–896.
- Templeton, A.R. (1986): Coadaptation and outbreeding depression. In: Soulé, M.E. (ed.) *Conservation Biology: The Science of Scarcity and Diversity*. 105–116. Sinauer, Sunderland.
- Templeton, A.R., Hemmer, H., Mace, G., Seal, U.S., Shields, W.M. & Woodruff, D.S. (1986): Local adaptation, coadaptation and population boundaries. *Zool. Biol* 5: 115–125.
- Treuren, R. van Bijlsma, R., Ouborg, N.J. & Delden, W. van (1993): The significance of genetic erosion in the process of extinction. III. Inbreeding depression and heterosis effects due to selfing and outcrossing in *Scabiosa columbaria*. *Evolution* 74: 1669–1680.
- Turner, M.E., Stephens, J.C. & Anderson, W.W. (1982): Homozygosity and patch structure in plant populations as a result of nearest-neighbor pollination. *Proc. Nat. Acad. Sci. USA* 79: 203–207.
- Uyenoyama, M.K. (1986): Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. *Evolution* 40: 388–404.
- Waser, N.M. & Price, M.V. (1983): Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. In: Jones, C.E. & Little, R.J. (eds) *Handbook of Experimental Pollination Biology*. 341–359. Van Nostrand Reinhold, New York.
- Waser, N.M. & Price, M.V. (1989): Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. *Evolution* 43: 1097–1109.
- Waser, N.M. & Price, M.V. (1991a): Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes and seed set. *Ecology* 38: 116–127.
- Waser, N.M. & Price, M.V. (1991b): Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *Am. J. Bot.* 78: 1036–1043.
- Waser, N.M., Price, M.V., Montalvo, A.M. & Gray, R.N. (1987): Female mate choice in a perennial herbaceous wildflower, *Delphinium nelsonii*. *Evol. Trends Plants* 1: 29–33.
- Waser, N.M. (1993): Population structure, optimal outbreeding, and assortative mating in Angiosperms. In: Thornhill, N.W. (ed.) *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*. 173–199. University of Chicago Press, Chicago.
- Weihe, K. von. (1972): *Garcke, Illustrierte Flora von Deutschland und angrenzende Gebiete*. Parey, Berlin.
- Whitkus, R. (1988): Experimental hybridizations among chromosome races of *Carex pachystachya* and the related species *C. macloviana* and *C. preslii* (Cyperaceae). *Syst. Bot.* 13: 146–153.
- Zimmerman, M. & Pyke, G.H. (1988): Pollination ecology of Christmas bells (*Blanfordia nobilis*): effects of pollen quantity and source on seed set. *Aust. J. Ecol.* 13: 93–99.