Offspring fitness in relation to population size and genetic variation in the rare perennial plant species Gentiana pneumonanthe (Gentianaceae)

Oostermeijer, J.G.B.; van Eijck, M.W.; den Nijs, J.C.M.

DOI
10.1007/BF00317317

Publication date
1994

Published in
Oecologia

Oecologia (1994) 97:289–296

ORIGINAL PAPER

J. G. B. Oostermeijer · M.W. van Eijck
J. C. M. den Nijs

Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae)

Received: 5 February 1993 / Accepted: 8 December 1993

Abstract Seeds were sampled from 19 populations of the rare *Gentiana pneumonanthe*, ranging in size from 5 to more than 50,000 flowering plants. An analysis was made of variation in a number of life-history characters in relation to population size and offspring heterozygosity (based on seven polymorphic isozyme loci). Life-history characters included seed weight, germination rate, proportion of seeds germinating, seedling mortality, seedling weight, adult weight, flower production per plant and proportion of plants flowering per family. Principal component analysis (PCA) reduced the dataset to three main fitness components. The first component was highly correlated with adult weight and flowering performance, the second with germination performance and the third component with seed and seedling weight and seedling mortality. The latter two components were considered as being maternally influenced, since these comprised life-history traits that were significantly correlated with seed weight. Multiple regression analysis showed that variation in the first fitness component was mainly associated with heterozygosity and not with population size, while the third fitness component was only correlated with population size and not with heterozygosity. The latter relationship appeared to be non-linear, which suggests a stronger loss of fitness in the smallest populations. The second (germination) component was neither correlated with population size nor with genetic variation. There was only a weak association between population size, heterozygosity and the population coefficients of variation for each life history character. Most correlation coefficients were negative, however, which suggests that there is more variation among progeny from smaller populations. We conclude that progeny from small populations of *Gentiana pneumonanthe* show reduced fitness and may be phenotypically more variable. One of the possible causes of the loss of fitness is a combination of unfavourable environmental circumstances for maternal plants in small populations and increased inbreeding. The higher phenotypic variation in small populations may also be a result of inbreeding, which can lead to deviation of individuals from the average phenotype through a loss of developmental stability.

Key words Conservation biology · Inbreeding
Life history · Phenotypic variation · Population viability

J. G. B. Oostermeijer (✉) · M.W. van Eijck · J. C. M. den Nijs
Hugo de Vries Laboratory, Department of Systematics, Evolution and Paleobiology, University of Amsterdam, Kruislaan 318,
1098 SM, Amsterdam, The Netherlands

Introduction

Due to the destruction, deterioration and fragmentation of their habitats, many species have recently been forced into small and isolated populations. Population genetic theory predicts that such populations will experience genetic erosion due to random genetic drift and inbreeding, and that isolation may prevent the amelioration of these effects by gene flow (Wright 1946; Barrett and Kohn 1991). A loss of genetic variation may reduce the ability of local populations to adapt to changes in the environment and the potential for evolutionary change (Frankel and Soulé 1981; Beardmore 1983). In small populations, especially of outcrossing species with historically large population sizes (Huenneke 1991), inbreeding depression can accompany increased selfing rates and increased mating between close relatives (Charlesworth and Charlesworth 1987; Lande and Schemske 1985). Significant relationships between population size or rarity and measures of genetic variation have been found in a number of rare plant species (Meagher et al. 1978; Moran and Hopper 1983; McClenaghan and Beauchamp 1986; Karron et al. 1988;

Billington 1991; Treuren et al. 1991). A study of *Gentiana pneumonanthe* has also revealed a significant positive relationship between allozyme variation and population size (Raijmann et al. in press).

Besides suffering from genetic erosion, small populations may also be more sensitive to environmental and demographic stochasticity (Menges 1991a). Since genetic, environmental and demographic processes interactively affect the population viability of rare species, studies incorporating all three disciplines are of special interest in conservation biology (Gilpin and Soulé 1986; Barrett and Kohn 1991; Menges 1991a).

Although the number of studies on rare plant species has been steadily increasing in the last decades (Barrett and Kohn 1991), there are still rather few data on fitness in relation to population size (Ouborg et al. 1991; Oostermeijer et al. 1992; Menges 1991b). Ouborg et al. (1991) demonstrated a positive relationship between population size and variation in fitness components for two rare perennial plant species. In general, this type of variation is better suited to ecological and demographic interpretation than variation in isozyme loci. When studying variation in life-history characters, one has to keep in mind that these are often under considerable maternal influence (Schaal 1984; Roach and Wulff 1987). It is therefore very interesting to investigate to what extent offspring fitness is affected by maternal carry-over and to what extent by genetic variation.

Studies in which (variation in) fitness is related to both population size and the level of genetic variation are still very scarce. Indications of reduced fitness in small and genetically eroded populations of *Salvia pratensis* and *Scabiosa columbaria* have recently been found by Ouborg (1993) and van Treuren (1993).

This paper deals with the association between population size, isozyme heterozygosity, and a number of life history parameters of the rare plant species *Gentiana pneumonanthe*, investigating whether progeny from small populations show less variation and reduced fitness. In a previous paper, we demonstrated reduced weight of seedlings from a small population as compared to those from a large, nearby population (Oostermeijer et al. 1992). Because variation among small and isolated populations is most probably high, the comparison of only few populations increases the risk of false conclusions. Therefore we studied 19 populations, ranging in size from 5 to more than 50,000 flowering individuals.

Materials and methods

Study species

Gentiana pneumonanthe L. (Gentianaceae) is a herbaceous, long-lived perennial that, in The Netherlands, occurs in wet to moist heathlands and unmanured hay meadows. The plant reproduces exclusively by means of its very small seeds, which are normally produced in large quantities (400–1000 seeds/fruit). In large populations, *Gentiana pneumonanthe* is predominantly outcrossing (Raijmann et al. in press). The strongly protandrous flowers are pollinated by bumblebees. The species is entirely self-compatible; bumblebees are necessary for sufficient pollination, since after spontaneous selfing seedset is reduced from c. 85% to 30% (Petanidou et al. 1991).

In The Netherlands, as in other European countries, the distribution area of *Gentiana pneumonanthe* has decreased enormously in the last decades (Mennema et al. 1985). This is mainly due to reclamation of its habitat for agricultural use, a general lowering of the groundwater table, excessive use of fertilizers, and cessation of former small-scale management practices, such as mowing and haymaking, sod-cutting and extensive grazing. Recently, atmospheric acidification and eutrophication of the soil pose additional threats (Heil and Diemont 1983).

As with many species, not only has the number of populations been reduced considerably, but also the population size, while the degree of isolation has increased. Because there are many very

Table 1 Name, location and size [number of individuals (*Nfl*) flowering in 1991] of the populations studied, and average offspring heterozygosity (±1 SE) for seven variable isozyme loci (see text)

Population	Nature reserve	Municipality, province	Size (Nfl)	Average offspring heterozygosity
LOCHEM III	Grote Veld	Vorden, Gelderland	5	0.524±0.048
LANGE VEN	Gooi Nature Reserve	Hilversum, North Holland	9	0.229±0.053
LOCHEM II	Grote Veld	Vorden, Gelderland	12	0.314±0.070
ANSERDENNEN	Dwingelderveld National Park	Ansen, Drenthe	15	0.357±0.071
HOUTBEEK	Kootwijk Forestry	Stroe, Gelderland	30	0.200±0.049
TERHORSTERZAND	Terhorst	Beilen, Drenthe	50	0.254±0.021
PESSE	Spaarbankbos	Pesse, Drenthe	75	0.314±0.067
DRINKPUT	Gooi Nature Reserve	Hilversum, North Holland	75	0.317±0.062
KRALO 'A'	Dwingelderveld National Park	Kralo, Drenthe	100	0.257±0.056
KRALO 'Postweg'	Dwingelderveld National Park	Kralo, Drenthe	125	0.444±0.044
HOLTINGERZAND	Holtingerveld	Havelte, Drenthe	150	0.471±0.064
ZUIDERHEIDE	Gooi Nature Reserve	Hilversum, North Holland	150	0.339±0.038
ZEEGSE DUINEN	'Drenthse A' Nature Reserve	Zeegse, Drenthe	175	0.329±0.043
BRUGGINK	De Velhorst	Lochem, Gelderland	250	0.476±0.066
ANSEN	Dwingelderveld National Park	Ansen, Drenthe	500	0.243±0.060
ASSEL	Kootwijk Forestry	Kootwijk, Gelderland	500	0.443±0.044
LEEMPUTTEN	Staverden	Ermelo, Gelderland	800	0.321±0.080
HEIDEBLOEM	Gooi Nature Reserve	Hilversum, North Holland	500	0.304±0.083
DE DEELEN	'De Deelen' Nature Reserve	Heerenveen, Friesland	50000	0.476±0.025

small populations, but also still large (viable?) populations to serve as a basis for comparison, *Gentiana pneumonanthe* is especially suitable for studying the effects of reduced population size.

Studied populations and sampling procedure

Random samples of ripe fruits (one per maternal plant) were taken in 19 populations, ranging from 5 to more than 50,000 flowering individuals. Data on names, locations and size of the populations are given in Table 1.

Depending on the size of the population five to ten fruits were sampled at random. A decision was made not to increase the number of fruits in the larger populations above ten to avoid too large a difference in sample size. In total, 166 fruits (maternal families) were sampled.

Per fruit, 100 filled (non-aborted) seeds were randomly selected and weighed together on a microbalance. The seeds were then placed on wet filter paper in a petri dish at 25° C and a light regime of 12 h day/12 h night. Germinated seeds (seeds with emerged radicle) were counted daily to obtain data on the germination rate. This is expressed as the slope of the regression line through the germination curve from day 5 to day 10 after the start of the experiment. After 30 days, when no additional germination occurred, the total number of seedlings was determined per dish (proportion of seedlings germinating). In a considerable number of petri dishes, seedlings had turned brown and died without any apparent cause. The number of these dead seedlings was also counted at the end of the experiment (seedling mortality). Of the remaining seedlings, a random sample of, if possible, 14 individuals per family was planted in soaked peat pellets, potted after 4 weeks of growth and allowed to grow under our standard greenhouse conditions until most individuals reached the flowering stage (this being after a total of 8 months). The seedlings were arranged in a randomized order to avoid possible location effects in the greenhouse. After 4 weeks of growth, the aboveground weight of each seedling was estimated using a non-destructive method. This estimation is based on the product of the total number of leaves per rosette (minus the cotyledons), the length and the width of the largest leaf present at that moment. Regression between estimated seedling weight and the natural logarithm of the actual dry weight was highly significant in earlier experiments ($r^2=0.771$, $df=87$, $P\leq 0.0001$).

The size of the offspring at the adult stage was estimated after 8 months of growth, when the following parameters were measured: (1) the total number of leaves, (2) the length and width of the largest stem leaf and (3) the total number of flowers produced at the moment (including fruits, excluding flower buds). In this way, also the percentage of flowering plants per maternal family and per population could be calculated. From measurements 1 and 2 the estimated adult weight was calculated in the same way as the estimated seedling weight (see above).

Per population, leaves were also taken from a subsample of ten progeny at the end of the experiment and used for isozyme electrophoresis. Preparation of the leaf material, gel electrophoresis and enzyme staining were performed as described by Raijmann et al. (in press). The subsamples were only screened for the seven loci (on six enzymes) that were polymorphic in a larger sample of Dutch populations (in which 18 loci were studied). The enzymes, loci and their EC numbers are: aspartate aminotransferase (2.6.1.1, loci AAT-I and III), uridinephosphoglucose-pyrophosphorylase (2.7.7.9, UGPP-II), phosphoglucomutase (5.4.2.2, PGM-I), NADH dehydrogenase (1.6.99.3, NADH.DH), malate dehydrogenase (1.1.1.37, MDH) and 6-phosphogluconate dehydrogenase (1.1.1.44, 6PGDH-II). A more detailed analysis of the relationship between the level of isozyme heterozygosity and individual fitness will be presented elsewhere (Oostermeijer et al. in prep).

Data analysis

Pearson's product-moment correlation coefficients were calculated among the measured life-history characters in the total dataset. Since the parameters were partly significantly intercorrelated, principal component analysis was used to identify uncorrelated linear combinations of the original variables. The relationship between the original parameters (means per family) and the (varimax rotated) PCA scores was examined by means of the rotated component loadings. The association between population size, isozyme heterozygosity and the calculated PCA scores was examined by single and multiple regression analysis. Multiple regression was performed on two different datasets, one with scores per maternal plant ($n=166$) and one with the average component score per population ($n=19$) to reduce the possible bias resulting from the variation in sample size among large and small populations.

To examine the relationships between population size, heterozygosity and phenotypic variation, Pearson's correlation coefficients were calculated. As measures of phenotypic variation, we used the population coefficients of variation of each original life-history character ($n=19$).

Where necessary, the data were transformed prior to statistical analysis (Sokal and Rohlf 1981). The data on population size, seed weight, estimated seedling weight, estimated adult weight and the number of flowers per plant were natural log (ln)transformed, and seedling mortality, proportion of seeds germinating and proportion of flowering progeny were angular transformed.

For all statistical analyses, the computer package SYSTAT 5.2 (Wilkinson 1989) was used.

Table 2 Pearson's product-moment correlation coefficients among the measured life-history characters, population size and average offspring heterozygosity

	Ln (pop. size)	Average heterozygosity	Seed weight	Germination rate	Proportion germinating	Seedling mortality	Seedling weight	Adult weight	# Flowers/plant
Seed weight	0.152*	0.082							
Germination rate	−0.004	0.067	0.048						
Proportion seeds germinating	−0.062	−0.060	0.163*	0.610***					
Seedling mortality	−0.176**	−0.100	−0.251***	0.007	0.053				
Seedling weight	0.263***	0.262***	0.351***	0.086	0.083	−0.323***			
Adult weight	0.241***	0.162**	0.112	0.097	0.040	−0.141	0.384***		
Number of flowers/plant	0.251***	0.321***	0.013	0.012	−0.113	−0.067	0.344***	0.358***	
Proportion plants flowering/family	0.184**	0.298***	0.011	−0.018	−0.123	−0.024	0.298***	0.292***	0.897**

* $P\leq 0.05$, ** $P\leq 0.025$, *** $P\leq 0.001$, $n=166$

Table 3 Principal component loadings of the measured life-history characters (after varimax rotation). Loadings given in boldface show the highest correlation between original values and principal component scores

Variable	ROTATED COMPONENT LOADINGS		
	PC1	PC2	PC3
Flowers/plant	**0.948**	−0.062	0.004
Proportion of plants flowering	**0.930**	−0.085	−0.045
Adult weight	**0.527**	0.132	0.328
Proportion of germination	−0.090	**0.892**	0.065
Germination rate	0.062	**0.884**	−0.009
Seedling mortality	−0.001	0.140	**−0.732**
Seed weight	−0.043	0.128	**0.729**
Seedling weight	0.430	0.107	**0.637**
Variance explained by rotated components	2.241 (28.02%)	1.654 (20.68%)	1.634 (20.43%)

Results

Relationships among life-history characters and determination of main fitness components

Correlation coefficients among the measured life-history characters are presented in Table 2. It is obvious that several of the parameters are significantly and sometimes highly intercorrelated. Up to the seedling stage, there appears to be a significant correlation with seed weight, which suggests that maternal carry-over effects are only present in early stages of the life cycle.

PCA reduced the total set of partly intercorrelated variables to three uncorrelated principal components. The (varimax rotated) component loadings per original variable and the proportion of total variance explained by the three components are given in Table 3. Together, the three principal components explain 69.1% of the total variation.

The first main fitness component appears to be correlated with a combination of adult weight, number of flowers per plant and the proportion of plants flowering. As described above, these are parameters that are probably no longer maternally affected, since they are not correlated with seed weight. Germination rate and the proportion of seeds germinating have the highest loadings for the second fitness component. The last component is correlated with seed weight, seedling weight and seedling mortality. Based on the correlation of germination, seedling weight and seedling mortality with seed weight (Table 2), we can conclude that both the second and third fitness components are still (at least partly) influenced by maternal carry-over.

Relationship between population size, heterozygosity and fitness components

Mean offspring heterozygosity per population (for the seven polymorphic loci) is given in Table 1. Ln population size was not significantly associated with the level of heterozygosity of the progeny ($r^2=0.032$, $F=0.557$, $P=0.466$). If the smallest population, which had the highest heterozygosity and therefore deviated strongly from the overall pattern, was omitted from the analysis, regression with ln population size was marginally significant ($r^2=0.183$, $F=3.579$, $P=0.077$).

From single regression analysis, it appears that the first fitness component is significantly related to both untransformed population size ($r^2=0.057$, $F=9.871$, $P\leq 0.002$) and ln population size ($r^2=0.061$, $F=10.651$, $P\leq 0.001$). Heterozygosity, however, explains a larger proportion of the variation in this fitness component ($r^2=0.107$, $F=19.713$, $P\leq 0.001$). The third main fitness component is significantly correlated only with ln population size ($r^2=0.058$, $F=10.107$, $P\leq 0.002$) and not with untransformed population size ($r^2=0.003$, $F=0.420$, $P=0.518$) and only marginally with isozyme heterozygosity ($r^2=0.018$, $F=2.979$, $P=0.086$). The second fitness component was neither associated with untransformed population size ($r^2=0.003$, $F=0.521$, $P=0.471$), ln population size ($r^2=0.001$, $F=0.218$, $P=0.641$) or heterozygosity ($r^2=0.000$, $F=0.000$, $P=0.987$).

Multiple regression analysis allows an evaluation of the relative importance of population size and isozyme heterozygosity (Table 4). The first fitness component appeared to be significantly correlated only with progeny heterozygosity and only marginally significant ($P<0.10$) with ln population size. Strikingly, the reverse was true for the third fitness component, that appeared to be only correlated with ln population size and not with heterozygosity. As expected, there was no significant multiple regression model for the second fitness component.

There were no large differences between the analyses on maternal plants and those on population means, except for a reduced level of significance (Table 4). Apparently, the differences in sample size, inevitable in studies on small and large populations, did not strongly affect the outcome of the analyses.

From the multiple regression analysis, it appears that the third fitness component is only significantly associated with population size, so the single regression models for this parameter are appropriate for further evaluation. There was a difference between the regression

Table 4 Results of multiple regression analyses on the three main fitness components, with population size and heterozygosity as independent variables. Standard partial regression coefficients and their statistics are given for an analysis on values per maternal family ($n=166$) on the left, and for analysis on mean values per population ($n=19$) on the right. At the head of each table, the squared multiple regression coefficient (r^2), together with the F- and P-value of the accompanying ANOVA are given

PCA scores for component 1 (adult size and flowering)

	Maternal plants $n=166$, $r^2=0.123$, $F=11.454$, P0.001			Population means $n=19$, $r^2=0.325$, $F=3.875$, $P=0.043$		
Variable	β	t_β	P (2-tailed)	β	t_β	P (2-tailed)
Ln (population size)	0.138	1.720	0.087	0.137	0.655	0.522
HETEROZYGOSITY	0.272	3.402	0.001	0.530	2.540	0.022

PCA scores for component 2 (germination)

	Maternal plants $n=166$, $r^2=0.002$, $F=0.133$, $P=0.875$			Population means $n=19$, $r^2=0.015$, $F=0.125$, $P=0.883$		
Variable	β	t_β	P (2-tailed)	β	t_β	P (2-tailed)
Ln (population size)	−0.044	−0.516	0.607	−0.077	−0.304	0.765
HETEROZYGOSITY	0.019	0.222	0.825	−0.085	−0.337	0.740

PCA scores for component 3 (seed and seedling weight and mortality)

	Maternal plants $n=166$, $r^2=0.060$, $F=5.172$, $P=0.007$			Population means $n=19$, $r^2=0.229$, $F=2.372$, $P=0.125$		
Variable	β	t_β	P (2-tailed)	β	t_β	P (2-tailed)
Ln (population size)	0.223	2.693	0.008	0.410	1.832	0.086
HETEROZYGOSITY	0.044	0.531	0.596	0.175	0.782	0.446

models that used untransformed population size or ln population size as independent variable (see above). Only the latter model was statistically significant, which means that the observed relationship is non-linear. The reduction in the maternally affected fitness component apparently is stronger below a certain threshold of population size ($n \approx 100$ flowering plants).

Relationships between population size, heterozygosity and coefficients of variation

There appeared to be few significant correlations between the population coefficient of variation (CV) values per parameter and population size (Table 5). The CV of seedling mortality was significantly positively correlated and the CV of the proportion of seeds germinating showed a negative correlation coefficient, which was marginally significant. However, nearly all correlation coefficients were negative, indicating that in most cases the small populations had relatively higher CV values than the large (Table 5). In an extended analysis, to enable comparison with the results of Ouborg et al. (1991), populations were given a rank number based on an increasing CV for each parameter. The CV rank numbers were then averaged over all parameters per population and correlated with ln-transformed population size and heterozygosity by Kendall's rank correlation. This procedure, presented at the bottom of Table 5, also yielded negative values of, but neither differed significantly from zero.

Table 5 Pearson's product-moment correlation coefficients between ln population size, isozyme heterozygosity and population coefficients of variation (CV values). At the bottom of the table, the Kendall's rank correlation coefficient (τ) with the average CV rank per population is given (see text)

	Ln(popsize)	heterozygosity
CV sdwt	−0.380	0.329
CV germ rate	−0.247	−0.330
CV prop germ	−0.416(*)	0.260
CV sdl mort	0.574***	−0.277
CV sdl wt	0.144	−0.287
CV ad wt	−0.092	0.075
CV fl/pl	−0.052	−0.453*
CV prop fl	−0.067	−0.598***
CV rank	−0.222	−0.261

(*) $0.05 \leqq P \leqq 0.10$, * $P \leqq 0.05$, *** $P \leqq 0.01$, $n=19$

Five out of eight correlation coefficients of population CV values with heterozygosity were again negative, and two were significantly different from zero (Table 5). Kendall's value of the average CV rank with heterozygosity is also negative, but again not significant.

Discussion

The data presented in this paper clearly show that in the rare species *Gentiana pneumonanthe*, population size and genetic variation are strongly positively correlated

with offspring fitness. One of the most interesting results is the difference we found between the different fitness parameters in their correlation with these population characteristics.

The maternally affected life-history characters, represented by seed weight, seedling weight, and seedling mortality, were most strongly correlated with population size and only marginally with genetic variation. Firstly, this may suggest that the deteriorated environmental circumstances which the maternal plants in small populations encounter affects the performance of their offspring negatively. In several other studies it has been shown that environmental stress on maternal plants is carried over to the progeny, especially in the first stages of their development (Schaal 1984; Roach and Wulff 1987). Secondly, the potential for maternal plants in small populations to invest in offspring may also be reduced by inbreeding depression, or because they are, just by chance, a poorly performing sample of survivors from the previous larger population. This last phenomenon, also known as demographic stochasticity, is a possible threat to very small populations (Menges 1991a).

For the long-lived *Gentiana pneumonanthe*, however, it is not very likely that there has been a history of inbreeding. Since there is no turnover of individuals in small populations (Oostermeijer et al. 1992), accumulation of inbreeding is very unlikely. Also, in a sample of 25 Dutch populations, a clear relationship beween population size and heterozygosity of the standing population was not found, although a significant trend towards a decrease in the proportion of polymorphic loci with declining population size was observed (Oostermeijer et al. 1992; Raijmann et al. in press). In this study also, there is only a trend towards decreasing heterozygosity of the offspring with reduced population size. This trend will be mostly the result of the relatively high selfing rates in small populations (Oostermeijer et al. 1992; Raijmann et al. in press).

There are also reasons to suppose that the plants in small populations are not just a random sample of the former large population. When population size declines in *G. pneumonanthe*, the adult generative individuals remain present for a long time as 'regressive' populations (Oostermeijer et al. 1992, in press). Since these flowering plants are probably 'proven survivors' and have been selected out of several cohorts of seedlings, they may be expected to have a relatively high vitality, and may thus be for instance more heterozygous than the plants that remained vegetative and died (cf. Schaal and Levin 1976; Mitton 1989; Raijmann et al. in press).

Considering all this, it is most likely that the reduction in maternally affected fitness in small populations is due to environmental stress on the remaining plants. From a previous study, it has emerged that small, 'regressive' populations of *Gentiana pneumonanthe* are mostly found in areas where the vegetation structure is very dense, often dominated by *Molinia caerulea* (Oostermeijer et al. in press). It has also been found that the nutrient availability in such areas is higher as a result of atmospheric deposition and increased mineralization due to a lowering of the phreatic level (Heil and Diemont 1983). The maternal plants are therefore probably subject to stress from low water availability and strong competition for light and resources. On the other hand, they might also have an excess of nutrients available to invest in their offspring.

Another factor that may affect the allocation of resources to seeds is the proportion of ovules that is fertilized following pollination. In a large population of *Gentiana pneumonanthe*, we found a negative correlation between seed set and seed weight, suggesting that as more ovules are fertilized, less energy is allocated to each ripening seed (Oostermeijer et al. unpublished data). However, seed set is significantly reduced in small populations of the species (probably because of reduced visitation from pollinators, Oostermeijer et al. 1992) which would result in heavier seeds. Since we observed the opposite in the present study (Table 2), there must be some counteracting factor that reduces seed size.

Another main fitness component comprises a set of parameters that are no longer influenced by maternal carry-over effects, e.g. adult weight, number of flowers per plant and proportion of offspring flowering. Several studies have found a significant positive relationship between plant size or weight and fitness or reproductive output (Solbrig 1981; Stanton 1984; Waller 1984; Mazer 1987; Ouborg 1993). In this study, this fitness component was significantly related to offspring heterozygosity and not to population size as such. Such positive relationships between heterozygosity and individual plant performance, mostly expressed in terms of size or weight, have frequently been found in plants, although in most cases for conifers (Mitton and Grant 1984; Mitton 1989; Wolff and Haeck 1990).

The significant relationship with heterozygosity and not with population size suggests that inbreeding depression is the most likely cause for the reduction in the fitness component that is not maternally affected. This is in agreement with the increased probability of self-fertilization that we observed in small populations of *G. pneumonanthe* (Oostermeijer et al. 1992; Raijmann et al. in press).

Although seed weight is reduced in small populations and seed weight is correlated with the proportion of seeds germinating, no correlation between population size or heterozygosity and germination performance was found for *Gentiana*. Also in *Salvia pratensis*, no correlation between population size and germination percentage was observed (Ouborg 1993). In both rare and widespread *Astragalus* species, inbreeding did not reduce but rather increased the proportion of seeds germinating (Karron 1989). In the rare prairie perennial *Silene regia*, however, a significant increase of germination with population size was observed (Menges 1991b).

Only a weak correlation between population size and phenotypic variation in the greenhouse was found for *Gentiana* in this study. Ouborg et al. (1991) report a

significant positive correlation between population size and morphological variation (which was expressed as an average rank, based on the CV values per parameter) for the rare perennial plants *Salvia pratensis* and *Scabiosa columbaria* in The Netherlands. It is therefore interesting that for *Gentiana*, most CV values per population, as well as the similarly computed CV rank, showed negative instead of positive correlation coefficients with population size. This implies that phenotypic variation was higher instead of lower in small populations, and thus contrasts strongly with the results on *Salvia* and *Scabiosa*. The question then arises whether genetic erosion in small populations should also result in reduced phenotypic variation. For *Gentiana*, significant negative relationships between coefficients of variation in fitness components and heterozygosity were also observed. These data correspond with the general finding that reduced heterozygosity does not only lower individual fitness, but may also increase the amount of variation among progeny since 'developmental homeostasis' is disturbed in homozygotes (Mitton and Grant 1984; Mitton 1989). Other studies have found that genetic variance increased rather than decreased after founder events or population bottlenecks (Goodnight 1988; Bryant et al. 1986; Carson 1990). Small populations can therefore also be expected to show increased instead of reduced variation in fitness components among their progeny, which may be an explanation for the negative correlation coefficients between most of the coefficients of variation and population size that were found in this study.

The non-linear relationship of the maternally affected fitness component with population size (Table 4) implies that the reduction in this component of offspring fitness is strongest in the smallest populations (approx. 100 flowering individuals). The same was observed for germination percentage of the rare *Silene regia* (Menges 1991b). The data may support the hypothesis that there is a certain minimum viable population (MVP) size for *Gentiana pneumonanthe* below which a reduction in fitness is detectable. From the results of this study alone, however, it cannot be concluded that this MVP is approximately 100 flowering individuals. All experiments described here were performed in a controlled environment, which makes extrapolation to a natural situation difficult. For instance, inbreeding depression is often more severe in natural environments (Dudash 1990; Barett and Kohn 1991) and maternal plants can also perform significantly different under different circumstances (Schmitt et al. 1992). Additional field experiments are therefore needed (and conducted at present) to conclude anything about the consequences of the observed results for demography.

It can be concluded that there is a very significant fitness decline in small populations of *Gentiana pneumonanthe* that is partly caused by environmental stress on maternal plants and partly by inbreeding. Whether or not the observed reduction in fitness has consequences for the probability of extinction of small populations remains to be studied, however. As stated above, many small populations of *Gentiana pneumonanthe* have a 'regressive' structure, i.e. they do not show any seedling recruitment (which is mainly due to a closed vegetation structure) and consist only of adult individuals which are the survivors of formerly large populations (Oostermeijer et al. 1992, in press). Since these regressive populations are declining gradually and will probably go extinct if the vegetation structure does not change, genetic erosion and inbreeding depression seem at present less important for their survival. Genetic factors become important again after suitable ecological circumstances have been restored by management practices: the reduced fitness of the progeny of remnant small and regressive populations, as described in this paper, may mean that regeneration to a new, viable population will never occur.

Acknowledgements The authors wish to thank A. K. Masselink (Agricultural University Wageningen) for showing us many of the studied populations, J. Bakker and E. Anink for their care for the plants in the greenhouse, S. Menken, K. Bachmann, L. Raijmann and an anonymous reviewer for their useful comments on the manuscript. Many thanks also go out to the students of the 1992 course in Evolutionary Genetics, who enthousiastically and thoroughly measured the adult plants.

References

Barrett SCH, Kohn JR (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE (eds) Genetics and conservation of rare plants. Oxford University Press, New York, pp 3–30

Beardmore JA (1983) Extinction, survival and genetic variation. In: Schonewald-Cox CM, Chambers SM, Macbryde B, Thomas WL (eds) Genetics and conservation, a reference for managing wild animal and plant populations. Benjamin-Cummings, London, pp 125–151

Billington HL (1991) Effect of population size on genetic variation in a dioecious conifer. Conserv Biol 5:115–119

Bryant EH, McCommas SA, Combs LM (1986) The effects of an experimental bottleneck upon quantitative genetic variation in the housefly. Genetics 114:1191–1211

Carson HL (1990) Increased genetic variation after a population bottleneck. Trends Ecol Evol 5:228–230

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Dudash MR (1990) Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. Evolution 44:1129–1139

Frankel OH, Soulé ME (1981) Conservation and evolution. Cambridge University Press, Cambridge

Gilpin ME, Soulé ME (1986) Minimum viable populations: processes of species extinction. In: Soulé ME (ed) Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, pp 19–34

Goodnight (1988) Epistasis and the effect of founder events on the additive genetic variance. Evolution 42:441–452

Heil GW, Diemont WH (1983) Raised nutrient levels change heathland into grassland. Vegetatio 53:113–120

Huenneke LF (1991) Ecological implications of genetic variation in plant populations. In: Falk DA, Holsinger KE (eds) Genet-

ics and conservation of rare plants. Oxford University Press, New York, pp 31–44
Karron JD (1989) Breeding systems and levels of inbreeding depression in geographically restricted and widespread species of *Astragalus* (Fabaceae). Am J Bot 76:331–340
Karron JD, Linhart YB, Chaulk CA, Robertson CA (1988) The genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). Am J Bot 75:1114–1119
Lande R, Schemske DW (1985) The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24–40
Mazer SJ (1987) The quantitative genetics of life history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed weight variation. Am Nat 130:891–914
McClenaghan LR, Beauchamp AC (1986) Low genic differentiation among isolated populations of the Californian fan palm (*Washingtonia filifera*). Evolution 40:315–322
Meagher TR, Antonovics J, Primack R (1978) Experimental ecological genetics in *Plantago*. III. Genetic variation and demography in relation to survival of *Plantago cordata*, a rare species. Biol Conserv 14:243–257
Menges ES (1991a) The application of minimum viable population theory to plants. In: Falk DA, Holsinger KE (eds) Genetics and conservation of rare plants. Oxford University Press, New York, pp 450–61
Menges ES (1991b) Seed germination percentage increases with population size in a fragmented prairie species. Conserv Biol 5:158–164
Mennema J, Quené-Boterenbrood AJ, Plate CL (1985) Atlas van de Nederlandse Flora, Vol II. Bohn, Scheltema and Holkema, Utrecht
Mitton JB (1989) Physiological and demographic variation associated with allozyme variation. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Chapman and Hall, London, pp 127–145
Mitton JB, Grant MC (1984) Associations among protein heterozygosity, growth rate and developmental homeostasis. Annu Rev Ecol Syst 15:479–499
Moran GF, Hopper SD (1983) Genetic diversity and the insular population structure of the rare granite rock species, *Eucalyptus caesia* Benth. Aust J Bot 31:161–172
Oostermeijer JGB, Nijs JCM den, Raijmann LEL, Menken SBJ (1992) Population biology and management of the marsh gentian (*Gentiana pneumonanthe* L.), a rare species in The Netherlands. Bot J Linn Soc 108:117–130
Oostermeijer JGB, Veer R van 't, Nijs JCM den (in press) Population structure of the rare, long-lived perennial *Gentiana pneumonanthe* in relation to vegetation and management in The Netherlands. J Appl Ecol
Ouborg NJ (1993) On the relative contribution of genetic erosion to the chance of population extinction. Ph D thesis, University of Utrecht, The Netherlands
Ouborg NJ, Treuren R van, Damme JMM van (1991) The significance of genetic erosion in the process of extinction. II. Morphological variation and fitness components in populations of varying size of *Salvia pratensis* L. and *Scabiosa columbaria* L. Oecologia 86:359–367
Petanidou T, Nijs JCM den, Ellis-Adam AC (1991) Comparative pollination ecology of two rare Dutch *Gentiana* species in relation to population size. Acta Hortic 288:306–312
Raijmann LEL, Leeuwen NC van, Kersten R, Oostermeijer JGB, Nijs JCM den, Menken SBJ (in press) Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. Conserv Biol
Roach DA, Wulff RD (1987) Maternal effects in plants. Annu Rev Ecol Syst 18:209–235
Schaal BA (1984) Life history variation, natural selection and maternal effects in plant populations. In: Dirzo R, Sarukhán J (eds) Perspectives on plant population biology. Sinauer, Sunderland, pp 188–206
Schaal BA, Levin DA (1976) The demographic genetics of *Liatris cylindracea*. Am Nat 110:191–206
Schmitt J, Niles J, Wulff RD (1992) Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. Am Nat 139:451–466
Sokal RS, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research, 2nd edn. Freeman, San Francisco
Solbrig OT (1981) Studies on the population biology of the genus *Viola*. II. The effect of plant size on fitness in *Viola sororia*. Evolution 35:1080–1093
Stanton ML (1984) Seed variation in wild radish: effect of seed size on components of seedling and adult fitness. Ecology 65:1105–1112
Treuren R van (1993) The significance of genetic erosion for the extinction of locally endangered plant populations. Ph D thesis, University of Groningen, The Netherlands
Treuren R van, Bijlsma R, Ouborg NJ, Delden W van (1991) The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. Heredity 66:181–189
Waller DM (1984) Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. Evolution 38:427–440
Wilkinson (1989) SYSTAT: the system for statistics. Systat Inc, Evanston
Wolff K, Haeck J (1990) Genetic analysis of ecologically relevant morphological variability in *Plantago lanceolata* L. VI. The relation between allozyme heterozygosity and some fitness components. J Evol Biol 3:243–255
Wright S (1946) Isolation by distance under diverse systems of mating. Genetics 31:39–59