

Population viability analysis of the rare perennial *Gentiana pneumonanthe* in Switzerland



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## Introduction

Nowadays increasing numbers of plant species become rare and endangered, which is caused by numerous reasons, such as habitat fragmentation or changes in ecological quality of sites. Populations of those species are often small and isolated, which has consequences for the fitness of the plants due to inbreeding, lack of gene flow and genetic drift (Oostermeijer *et al.* 1992). Nature conservation organisations are therefore trying to preserve large areas and corridors between sites to prevent extinction of species. In case of semi-natural habitats, such as can be found in many parts of Europe, the sites are managed in different ways; for example mowing in certain periods or grazing of cattle in parts of the reserves. But research is needed to assess the viability and structure of populations of rare plant species and then determine the right management strategy. Furthermore, population structure of rare species could be used as an indication of the ecological change of the site (Oostermeijer *et al.* 1994). Of course, these species can only be used as indicator if they react upon succession, which is an important factor in semi-natural habitats.

Of course, long-term studies would be the best way to get detailed information about populations. But those studies are very costly and time consuming. Another method is determining the relative proportions of the different 'life stages' or 'age stages' (Rabotnov 1985). Individuals are then placed in groups of about the same ontogenetic stage in the life cycle. It is a single-census method, which has been demonstrated to describe populations of perennials in a satisfying manner (Rabotnov 1985, Oostermeijer *et al.* 1994, Hegland *et al.* 2001).

In this study, demographic viability of populations of the rare perennial *Gentiana pneumonanthe*, situated near the lake of Neuchâtel and near the lake of Geneva in Switzerland, were investigated. Viable populations can be characterized, among other features, by sufficient reproductive success, ensuring no loss of population size. Therefore, the population structure was assessed to examine the number and proportion individuals of each age state present in the populations; this to make a distinction between different types of populations. A similar study was done by Oostermeijer *et al.* 1994 in the

Netherlands, in which there appeared to be three population types; 1) the invasive type, with high numbers of seedlings and juveniles, 2) the stable type, characterized by high numbers of flowering adults and also some seedlings and juveniles, and 3) the regressive type, which lacked the presence of seedlings and juveniles and consisted of only adult plants. However, management regimes can have an effect on the structure of populations (Hegland *et al.* 2001, Lennartsson and Oostermeijer 2001) and some suggestions for future management could be made after the analysis of the populations.

*Gentiana pneumonanthe* is an endangered species in Switzerland and has become increasingly rare throughout its range. The species often lives in small, isolated populations. Furthermore, because individuals can grow very old (more than 30 years) and the adult mortality rate is very low, populations may persist in closed vegetation for a long period. Even if the population is shown to be 'regressive' (Oostermeijer *et al.* 1992). Merely counting the number of flowering adults would not give a good impression of the viability of populations. For this reason Population Viability Analysis (PVA) is used, in which more specifically the threats for the population are identified, such as e.g. a change in environmental conditions or management (Burgman & Possingham 2000, Oostermeijer 2000). Frequently, demographic measurements are employed in PVA, leading to an assessment of the probability of extinction of that population. Population structure and environmental parameters in this study are tested with multiple regression analysis to see whether there are factors which currently reduce population viability. Dense and high vegetation and isolation of populations are thought to have a negative effect on the viability of the vegetation, which could be reflected by the number of 'regressive' populations found in the field.

A second part of this study concerned measuring the environmental maternal effects of the plants on the performance of their offspring. Parental environmental effects have been studied in several plant species (Schmid and Dolt 1994, Roach and Wulff 1987, Galloway 2001, Weiner *et al.* 1997, Kärkkäinen *et al.* 1999). Especially maternal effects have been subject of research in many papers, because they are thought to have a bigger impact on their offspring in comparison with the influence of the paternal parent (Roach and Wulff 1987, Helerum and Schaal 1996). The environment of the maternal parent will affect the quality and quantity of the provisions for the offspring and also the thickness of



the seed coat could be influenced (Mazer and Gorchov 1996). Furthermore, the endosperm genetic material and the cytoplasmic DNA are typically two-thirds of maternal origin and could reflect the maternal environment (Mazer and Gorchov 1996). The influence of the paternal parent is only prezygotic (Lacey 1996). However, paternal environment may influence the offspring by other, less direct mechanisms (Schmid and Dolt 1994). There is some evidence that pollen quality and quantity are affected by the environment of the paternal parent (Young and Stanton 1990), but overall it is very difficult to recognize paternal effects in the presence of maternal effects. To assess the influence of environment on the maternal plant and the effect of the maternal parent on their offspring, hypotheses were formulated and tested with a multiple regression analysis.

These hypotheses are formulated as follows:

- There should be a positive correlation between number of flowers of the maternal plant and the openness of the vegetation, because the plants receive more light and can spend more energy on their reproductive organs.
- Biomass of the maternal plant should be positively correlated to the openness of the vegetation, again because light can easily reach the plant and more energy can be spent on the growth of plant. Higher biomass of the maternal plants could also be expected in dense vegetation; competition for light is higher and the plant needs to be larger.
- The number of stems of the maternal plant is expected to be positively correlated with the openness of the vegetation; if more light can reach the lower parts of the vegetation and this can provide more energy to the maternal plant to produce more biomass, this is likely to be reflected in more stems. On the other hand, a maternal plant can also have more stems in denser vegetation. This could then be due to stronger competition pressure.
- A positive correlation between flower number of the maternal plant and their number of ovules and the natural seed set is expected, because flower number will

reflect a higher potential investment in reproduction and a higher attractivity to pollinators. Alternatively, seed set of plants that produce more ovules could also be lower, because pollen limitation occurs sooner, or because resource limitation decreases the number of ovules, which can develop fully into seeds.

- I expect seed set to be negatively correlated with seed weight. Plants have a limited supply of energy and all viable seeds receive a proportion of this energy. If a fruit bears a relatively high number of seeds, each individual seed will have a relatively low weight. The alternative, seed set positively correlated with seed weight, can only be explained if the plants have an unlimited supply of resources.
- In case of resource limitation or pollen limitation, the number of flowers should be positively correlated with seed weight, because the seed set in that situation is low and weight of each individual seed can increase. A negative correlation can be expected in populations with no resource limitation or pollen limitation and therefore also a high seed set.
- Biomass of the offspring is expected to be positively correlated with seed weight. High seed weight should be an indication for more availability of resources to provide to the offspring. Negative correlation between biomass of the offspring and seed weight can, in this study, not be explained by differences in resource availability and a genetical cause is suggested as an explanation.
- A positive correlation between number of stems of the offspring and seed weight is expected, because high seed weight indicates more energy in the seeds to provide to the seedling for growth. Again a negative correlation between biomass of the offspring and seed weight cannot be explained by resource limitation in the seedlings; genetic effects of the maternal plant are suggested.
- I also expect a positive correlation between offspring biomass and their number of stems. Seedlings with a high biomass have a high amount of resources available for further development. Alternatively, a negative correlation between biomass of the offspring and their number of stems is again not caused by a resource limitation and therefore some other, maybe genetic effect, is suggested.
- Biomass of the offspring and their number of buds are expected to be positively correlated. A high amount of resources stored in large seedlings can provide

energy for reproduction and thus for development of buds. Resource limitation could not cause a negative correlation in this case and again a genetic effect is suggested.

# Material and Methods

## *Study species*

*Gentiana pneumonanthe* is an herbaceous, iteroparous perennial, which reproduces only by seeds. The species overwinters as a rosette of overwintering stems with small, scale-like leaves. It is a long-lived species, which is characteristic for wet heatlands, unmanured grasslands and hay meadows.

The blue, protandrous flowers of *Gentiana pneumonanthe* are mainly visited by bumblebees (Petanidou *et al.* 1995). A reproductive adult can have 1 to about 25 flowers in one season and the plants can grow up to 40-45 cm tall.

*Gentiana pneumonanthe* was a relatively common species in a large part of Europe. The distribution area extends from southern Scandinavia to Northern Spain and Portugal and from Great Britain into Siberia (Meusel *et al.* 1978). But the destruction of its main habitat as a result of excessive use of fertilisers, drainage of the ground water and changes in management caused *Gentiana pneumonanthe* to become an endangered plant species throughout its range (Oostermeijer *et al.* 1994).

## *Determination of age-stages*

Individuals of *Gentiana pneumonanthe* cannot be determined by their actual age and therefore the plants were assessed according to their ontogenetical stage or 'age state' in the life cycle (Rabotnov 1969). A demographic study on the species has revealed six different age states for the marsh gentian (Oostermeijer *et al.* 1994), which are defined as follows (figure 2):

- a) Seeds: individuals in dormant state on or directly below the soil surface.
- b) Seedlings: individuals developed directly after germination of seeds. Plants have cotyledons and often also one or two leaf-pairs, but no stem or overwintering shoot.

- c) Juveniles: individuals have one or sometimes more stems and/or overwintering shoots. They still have cotyledons and the other leaves are thinner and more ovally shaped then the ones of the adult plants.
- d) Vegetative adults: plants without cotyledons and one or more flowerless stems. The leaves are thick and mostly revolute.
- e) Generative adults: individuals with one or more flowering stems. Leaves are the same as those of vegetative adults.
- f) Dormants: plants in dormant state without visible parts aboveground.

The seed- and dormant 'age state' are not included in the analysis, because it concerns a single-census method. In long-term research these states could be included; using permanent plots and assessing the number of age states each year can make a good estimate made of the dormant and seeds in the plot.

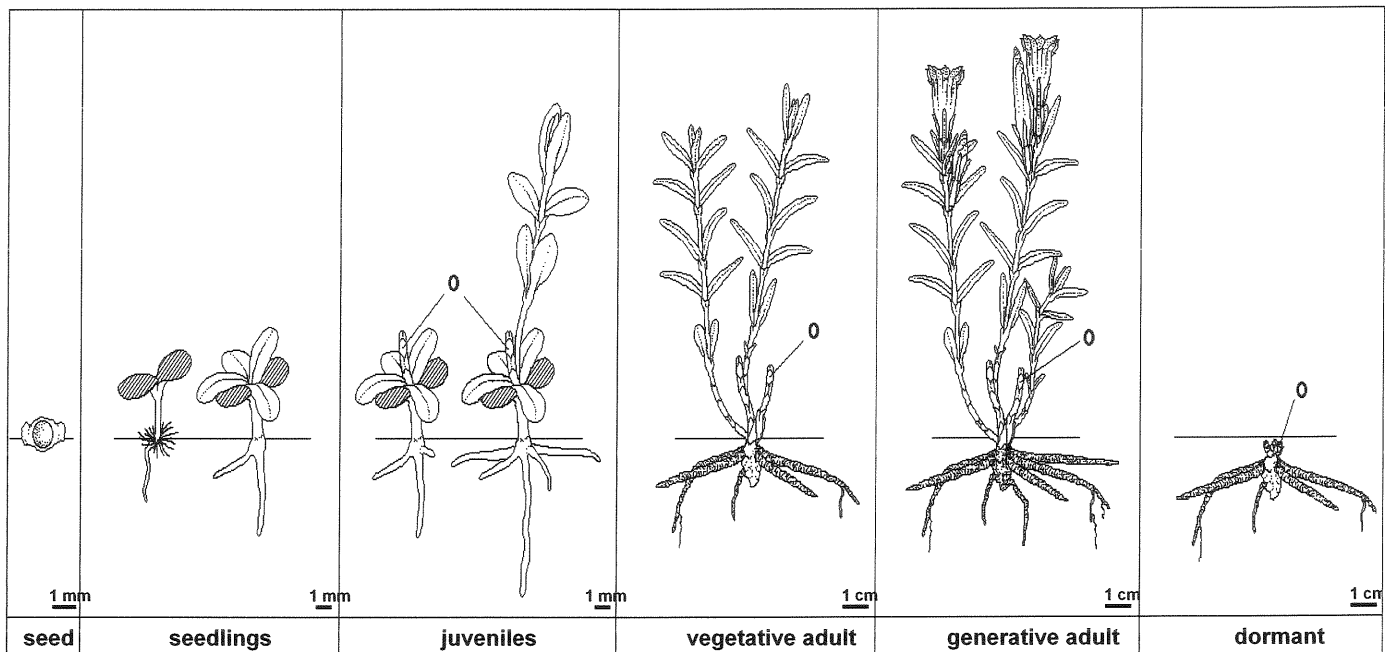


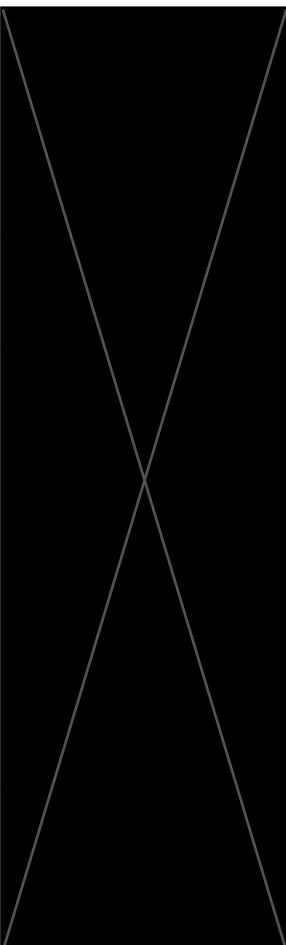
Figure 2: Life stages of *Gentiana pneumonanthe*. (Oostermeijer *et al.* 1994)

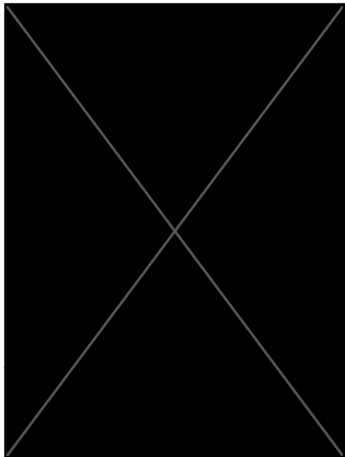
## Population viability analysis

12 populations of *Gentiana pneumonanthe* in Switzerland were investigated by means of 1m<sup>2</sup> plots. In 11 populations 10 plots were analysed; one population was too small and in this population only 6 plots were examined. 4 populations were situated near the lake of Neuchâtel, 7 near the lake of Geneva and 1 near Bern (Figure 1a and b & Table 1).

Measurements within these plots consisted of estimations of cover percentages of bryophytes, litter, grasses, scrubs, trees, water and bare soil. Also the species composition and the average and total height of the vegetation were assessed. Then the number of individuals of *G. pneumonanthe* in each age state was counted.

**Table1: Populations of *Gentiana pneumonanthe* in Switzerland. \* Pollen-addition experiments. \*\* No pollen addition.**

City or Village	Site name	Altitude ca.	Coord. Latitude	Coord. Longitude	Status	Number of flowering plants
Font	Font*	432 m			Nature reserve	ca. 150
Champ Martin	Champ Martin**	431 m			?	ca. 20
Cudrefin	Cudrefin A*	430 m			Nature reserve	ca. 100
Cudrefin	Cudrefin B	431 m			Nature reserve	ca. 10
La Rippe	La Rippe*	580 m			Nature reserve	ca. 150
Chésérèx	La Tropaz A*	635-660 m			Nature reserve	ca. 200
Chésérèx	La Tropaz B*	635-660 m			Nature reserve	ca. 50
Trélex	Au Chevry**	560 m			Nature reserve	ca. 150

Mollens	Le Parc**	730 m		?	< 50
Pampigny	Les Monod	670 m		Nature	ca. 15
Villeneuve	Les	373 m		reserve	
	Grangettes			Nature	< 50
Allmen- dingen	Bern*	520 m		reserve	ca. 50

### *Analysis of maternal environmental effects*

In six populations of *G. pneumonanthe* (see table 1) pollen addition experiments were performed. 15 individuals with at least 2 flowers were selected randomly in each population. Each individual was characterised by assessing the number of leaf pairs, number of flowers/buds and number of stems. Furthermore, average and total height was noted and also cover percentages of bare soil, litter, bryophytes, grasses, scrubs, trees, water and surrounding vegetation were estimated in plots of 30 \* 30 cm<sup>2</sup> surrounding the plants.

After environmental measurements had been made, anthers were removed from one of the flowers of an individual and transported to a preselected maternal plant. Pollination was done by rubbing the anthers on the stigma of the individual. The flower with the removed anthers received pollen from another maternal plant. Except for removal of the anthers from the flowers, no other precautions were taken to prevent self- or additional cross-pollination. Distances between pollen donor and acceptor were not measured.

About 4 to 5 weeks after the pollen addition, fruits were collected. They were opened and the aborted and viable seeds were counted under a dissection microscope. Aborted (shriveled) seeds are distinctly different from (clearly filled) viable seeds. After counting, a batch of 100 randomly chosen viable seeds was weighed on a microbalance.

Populations Champs Martin, Au Chevy and Le Parc (see table 1) only provided fruits from maternal plants, which were naturally pollinated. Populations Champs Martin and Le Parc were too small to pollinate a statistically sufficient number of plants. Population Au Chevy was announced to be mown and therefore no pollination was performed here.

In January 2002, seeds of every maternal family, which means seeds from every fruit sampled, were placed in Petri-dishes on wet filter paper and placed in the greenhouse. After five days no germination occurred and fungus started to develop, so it was decided to add a solution of 300 PPM gibberellic acid to the seeds. The seeds germinated about three days after adding the solution. For some maternal families containing a lot of fungus new Petri-dishes with batches of around 100 seeds were prepared. Again, the same amount of the aforementioned solution of gibberellic acid was added.

In the period from the 25<sup>th</sup> of January to the 7<sup>th</sup> of February 2002, 35 seedlings, if possible, of each maternal family were planted in soaked peat pellets. 20 plants, if available, of these 35 seedlings of a maternal family were then measured and repotted in the period from the 19<sup>th</sup> of March to the 18<sup>th</sup> of April 2002. Of each plant the number of leaves (without the cotyledons), length of the longest leaf and width of the longest leaf were assessed.

The plants were measured a second time in the period from the 13<sup>th</sup> of May to the 3<sup>rd</sup> of June 2002, whereby the number of stems, the number of buds and the height of the plants was assessed. The data was gathered in a way to assure that each plant was approximately of the same age at the moment of measurement.

### *Data analysis*

Data consisting of population samples, 10 plots per population, were used in a multiple regression analysis (Sokal and Rohlf 1981) to examine relationships between the relative amount of each age state and vegetation parameters. Age state structure was determined per population; the data of the age states was angular transformed and the mean of each age state in each population was determined. The means of the environmental parameters per population were angular transformed in case of proportions; total height and average height were log transformed. In a search for significantly different population types, the population structure data was used in a K-means clustering (Hartigan 1975), which clusters samples in a preselected number of groups. This method maximises the between-relative and within-group variation. Again all age states were angular transformed.



Seed set was tested with a paired T-test to determine whether there was a significant difference between the two treatments, hand-pollination and natural pollination, which were performed on the same maternal plant.

An ANOVA was used to examine if seed weight differed among populations and treatments. To satisfy the statistical requirements of ANOVA, the data was log transformed.

Multiple regression analysis (Sokal and Rohlf 1981) was then used to test hypotheses on relationships between vegetation structure and the characteristics of the maternal plants and the relation between the characteristics of the maternal plant and their offspring. The vegetation structure parameters were reduced by a Principal Component Analysis (PCA) to three composite variables. A varimax rotation was performed to obtain a better distribution of every value in the data set on one of the PCA-axes. To assure that the residuals of the multiple regression were normally distributed, the data was transformed; proportional data was angular transformed and absolute data was log transformed.

All data was analysed using the computer programs SYSTAT (Wilkinson 1989) and the SPSS 10.0 for MacIntosh statistical package.

# Results

## Population viability analyses

### *Population types and structure*

The K-means clustering revealed two groups of population types. Other numbers for clustering groups, for example three or four, showed an insufficient number of samples per group. The age state structure of the cluster groups is shown in Figure 3. A reverse transformation of the data was made to give a better impression of the situation in Figure 3.

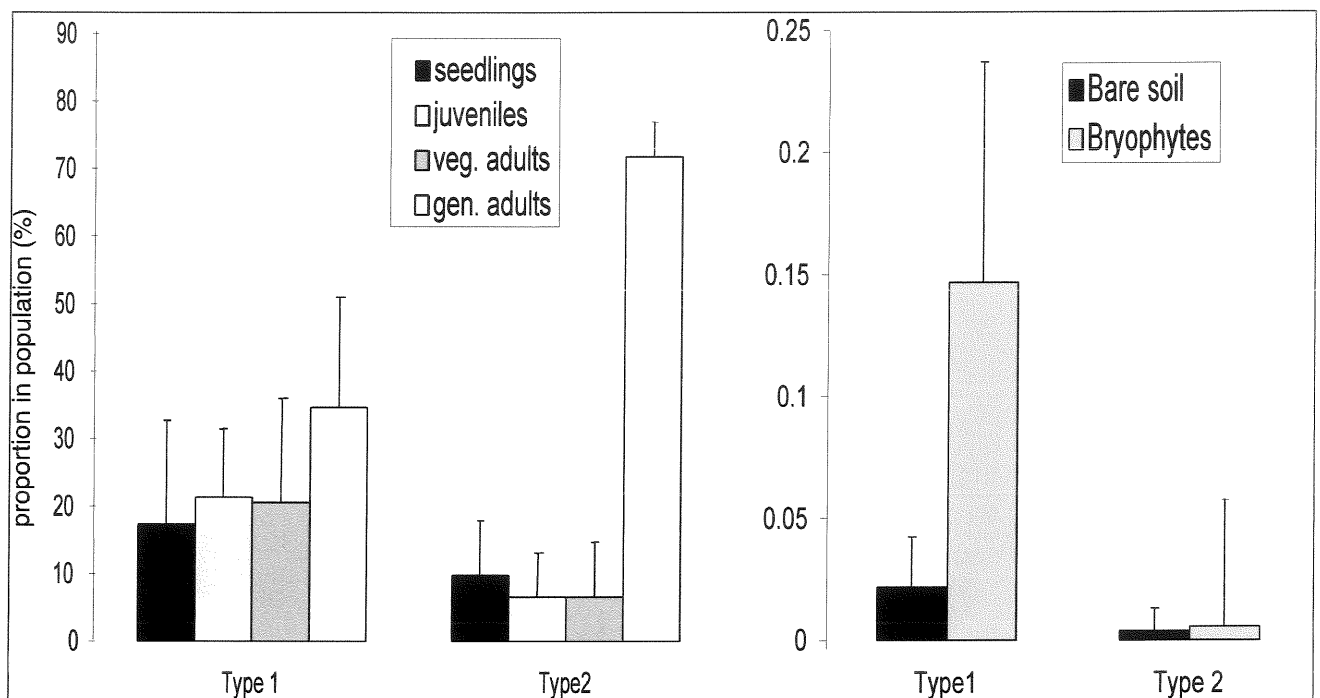
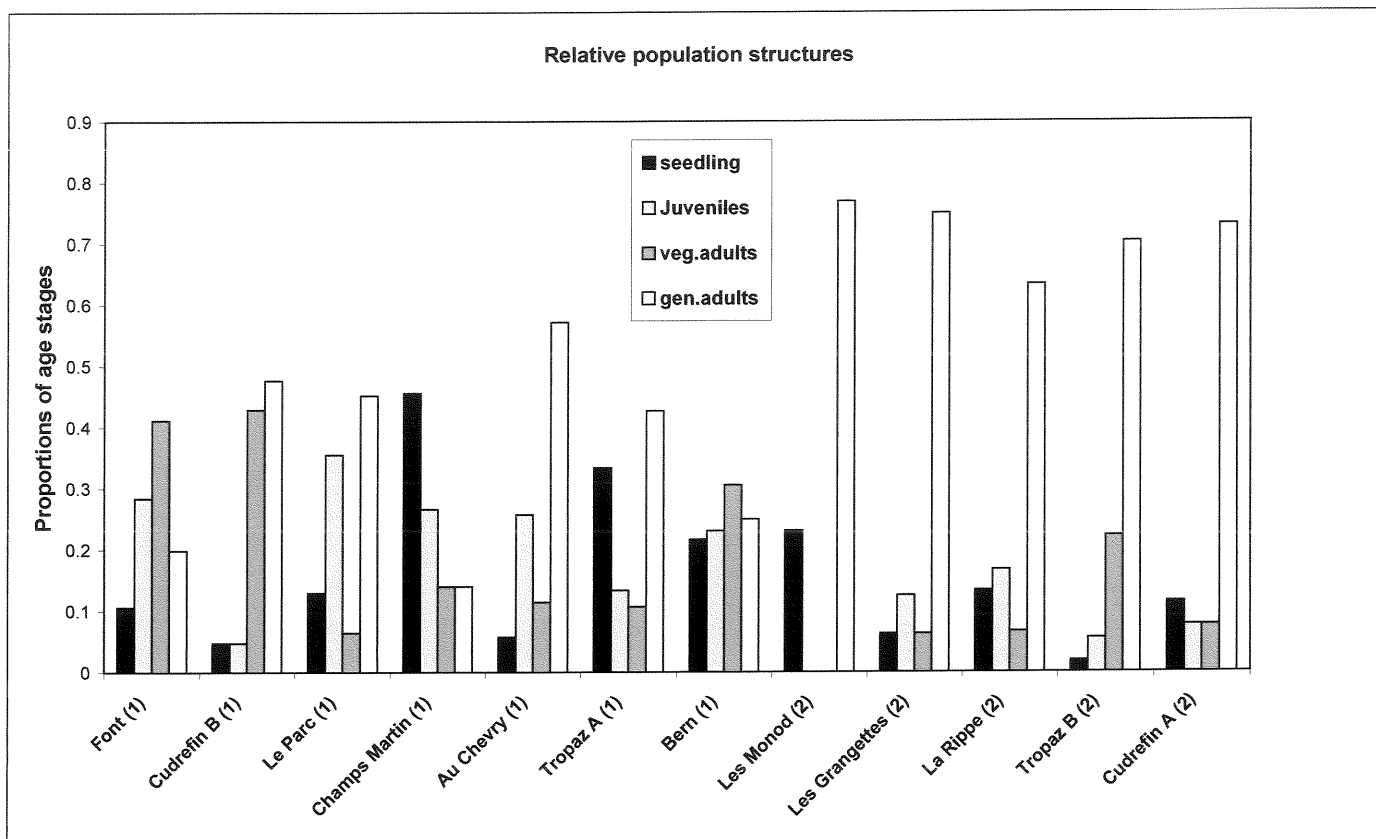


Figure 3: Age state structure of the two population types of *Gentiana pneumonanthe* as revealed by the K-means clustering and the corresponding vegetation structure. Error bars represent standard deviations.

The 12 Swiss populations of *Gentiana pneumonanthe* can be categorized according to the clusters 1 and 2 (Table 2). The population structure of each of the populations is shown in Figure 4.

**Table 2: Cluster selection for each population of *Gentiana pneumonanthe*.**

Population	Cluster number
Font	1
Cudrefin B	1
Le Parc	1
Champ Martin	1
Au Chevy	1
Tropaz A	1
Cudrefin A	1
Bern	1
Les Monod	2
Les Grangettes	2
La Rippe	2
Tropaz B	2



**Figure 4: Population structure of 12 populations of *Gentiana pneumonanthe*. The age states are given in proportions of the total of plants in one population.**

It can be seen that in every population seedlings as well as older generative plants were present (Figure 4). This is an indication for the occurrence of regeneration in the populations. There were young plants present of the previous year(s) and there were generative adults, which can produce seeds every year.

#### *Relationships between age states and vegetation structure*

All of the age states, except generative adults with two and with three or more stems, showed a positive correlation with the cover of bryophytes. Vegetative adults also were positively correlated with bare soil. Furthermore, seedlings and juveniles showed a negative correlation with grasses, and seedlings were also negatively correlated with shrubs. Total vegetation height and the cover of litter were negatively correlated with generative adults with one stem and generative adults with three or more stems, respectively (Table 3).

**Table 3: Multiple regression models with age states as dependent- and vegetation structure parameters as independent variables. Age states and vegetation structure parameters were log transformed before the analysis, except total height and average height (angular transformed).**

<b>Dependant variable</b>	<b>Independent variable</b>	<b>Standardized coefficients Beta</b>	<b>Adjusted R<sup>2</sup></b>	<b>Significance model</b>
<b>Seedlings</b>	Bryophytes	0.435	0.376	<0.001
	Grasses	-0.423		
	Shrubs	-0.270		
<b>Juveniles</b>	Bryophytes	0.488	0.333	<0.001
	Water	-0.275		
	Grasses	-0.183		
<b>Veg. adults</b>	Bryophytes	0.440	0.372	<0.001
	Bare soil	0.295		
<b>Gen. adults 1 stem</b>	Total height	-0.298	0.124	0.001
	Water	-0.255		
	Bryophytes	0.208		
<b>Gen. adults 2 stems</b>	No significant model	-	-	-
<b>Gen. adults 3 stems or more</b>	Litter	-0.216	0.038	0.023

The multiple regression models for the younger age states have relatively high  $R^2$ -values. This indicates that the vegetation structure explains a relatively high proportion (>30%) of the variation in abundance of these life stages. For the adult life stages, vegetation structure explains very little (<15%) of the observed variation in abundance.

### **Analysis of maternal effects**

#### *Seed set and seed weight*

Only four populations had enough individuals with fruits of both treatments. These populations were analysed to determine whether treatments had an effect on the seed set. A significant difference would indicate the presence of pollen limitation in a population. This difference is observed in two of the four populations. Bern displays a mean difference of 34 percent ( $P < 0.05$ ). The mean difference between the treatments in Cudrefin A is 19 percent, but this difference has a  $P$  value  $< 0.1$ .

The seed weight was measured of the four populations and then tested with an ANOVA to see if there is a difference between populations and treatments (Table 4). A significant difference in seed weight was found between populations and between treatments. However, the interaction of population and treatment was not significant which indicates that population-treatment combinations do not influence the seed weight.

**Table 4: ANOVA of seed weights (log transformed).**

Source	df	Mean square	Sig.
POP	3	.262	.000
TREAT	1	9.456E-02	.002
POP* TREAT	3	4.968E-02	.640

Population Cudrefin A has the highest seed weight for both treatments, whereas Tropaz B has the lowest seed weight (Table 5). It can also be noticed that the naturally pollinated maternal families have an overall higher seed weight (Table 5).

**Table 5: Mean seed weight ( $\pm$  SD) per population and treatment. Na= naturally pollinated, EX=pollen addition**

Population	NA	EX
Bern	4.2331E-05 ( $\pm$ 2.9353E-05)	3.2355E-05 ( $\pm$ 2.1096E-05)
Cudrefin A	4.4098E-05 ( $\pm$ 2.9011E-05)	3.8636E-05 ( $\pm$ 2.6812E-05)
La Tropaz A	3.2204E-05 ( $\pm$ 1.1216E-05)	2.52E-05 ( $\pm$ 1.4057E-05)
La Tropaz B	2.1308E-05 ( $\pm$ 2.1285E-05)	2.0867E-05 ( $\pm$ 1.8087E-05)

#### *Relationships between vegetation structure, maternal plants and their offspring*

The vegetation structure parameters were reduced by a PCA to three composite variables (Table 6). PCA axis 1 is a variable which explains how open the vegetation is, PCA axis 2 is a variable for vegetation height and PCA axis 3 is a variable that represents tree- and shrub cover.

**Table 6: Principal Component Analysis. Varimax rotation with Kaiser Normalization.**

	Component	Component	Component
	1	2	3
Bare soil	<b>0.903</b>	-0.147	-0.029
Grasses	<b>-0.854</b>	0.175	-0.205
Litter	<b>-0.771</b>	-0.363	0.008
Bryophytes	<b>0.729</b>	0.319	-0.038
Average Height	0.099	<b>0.868</b>	-0.018
Total Height	0.335	<b>0.759</b>	0.018
Water	0.094	<b>-0.339</b>	0.007
Trees	0.010	-0.081	<b>0.922</b>
Shrubs	0.058	0.069	<b>0.919</b>

Multiple regression analysis was performed to test the hypotheses mentioned in the introduction about relationships between vegetation structure, maternal plant characteristics and their offspring (Table 7a and 7b).

**Table 7a: Summary of multiple regression analysis. Relationships between vegetation structure and characteristics of the maternal plants. EX= hand-pollinated populations NA= naturally pollinated populations. \* No significant correlations found.**

Treatment	Dependent variable	Independent variable	Standardized coefficient Beta	Adjusted R-square	Significance
Both	Flowers parent	PCA axis 2	0.405	0.176	0.000
		PCA axis 1	0.140		
Both	Seed set	Flowers parent	-0.288	0.072	0.006
NA*	Seed set	Flowers parent	-	-	-
EX	Seed set	Flowers parent	-0.432	0.168	0.002
Both	Ovules	Flowers parent	0.270	0.062	0.011
NA *	Ovules	Flowers parent	-	-	-
EX	Ovules	Flowers parent	0.518	0.250	0.001
Both	Stems parent	PCA axis 2	0.329	0.097	0.002
Both	Leaf pairs parent	PCA axis 2	0.297	0.077	0.006
Both	Seed weight	Seed set	-0.666	0.436	0.000
NA	Seed weight	Seed set	-0.396	0.135	0.010
EX	Seed weight	Seed set	-0.816	0.658	0.000
Both	Flowers parent	Seed weight	0.564	0.310	0.000
NA	Flowers parent	Seed weight	0.541	0.276	0.000
EX	Flowers parent	Seed weight	0.625	0.376	0.000

**Table 7b: Summary of multiple regression analysis. Relationships between the characteristics of the maternal plant and their offspring. EX= hand-pollinated populations NA= naturally pollinated populations. \*No significant correlations found.**

Treatment	Dependent variable	Independent variable	Standardized coefficient Beta	Adjusted R-square	Significance
Both	Biomass seedlings	Seed weight	-0.365	0.120	0.002
NA	Biomass seedlings	Seed weight	-0.560	0.291	0.001
EX*	Biomass seedlings	Seed weight	-	-	-
Both	Stems offspring	Seed weight	-0.317	0.087	0.007
NA	Stems offspring	Seed weight	-0.417	0.150	0.011
EX*	Stems offspring	Seed weight	-	-	-
Both	Stems offspring	Biomass seedlings	0.262	0.057	0.017
NA	Stems offspring	Biomass seedlings	0.354	0.107	0.012
EX*	Stems offspring	Biomass seedlings	-	-	-
Both	Buds offspring	Biomass seedlings	0.422	0.168	0.000
NA	Buds offspring	Biomass seedlings	0.495	0.229	0.000
EX*	Buds offspring	Biomass seedlings	-	-	-

The results of the tests are as follows (in the same order as the hypotheses posed in the introduction):

- The number of flowers of the maternal parent is positively correlated with PCA axis 2 and 1.



- The variable for biomass of the maternal plant is not significantly correlated with PCA axis 1. PCA axis 2 is positively correlated with leaf pairs parent.
- The number of stems per maternal plant is not significantly correlated with PCA axis 1 (variable for vegetation openness). However, the number of stems of the maternal plant is positively correlated with the height of vegetation (PCA axis 2, Table 7a).
- Flowers parent is positively correlated with ovules and negatively with seed set (Table 7a). This regression is significant only for the hand-pollinated part of the maternal families (EX, Table 7a).
- Seed set is negatively correlated with seed weight. Also in the hand-pollinated and naturally pollinated maternal families separately this correlation tested negative.
- The number of flowers of the maternal plant is positively correlated with seed weight.
- Seed weight is negatively correlated with biomass seedlings. The regression is only significant for the naturally pollinated, and not for the hand-pollinated flowers.
- Seed weight is negatively correlated with the number of stems of the seedlings. Again the regression is only significant for naturally pollinated flowers.
- The correlation between seedling biomass and stems offspring is significantly positive. Only naturally pollinated maternal families show a significant regression.
- The biomass of the seedlings is positively correlated with buds offspring. The regression is again only significant for the naturally pollinated maternal families.

# Discussion

## Demographic viability of Swiss populations of *G. pneumonanthe*

The K-means clustering resulted in two different population types, which can be compared with the population types of Oostermeijer *et al.* (1994). Both types are similar to the ‘stable’ type as described by Oostermeijer *et al.* (1994) (Figure 5). Type 2 can be seen as ‘stable’, but with a tendency to become a ‘regressive’ population type.

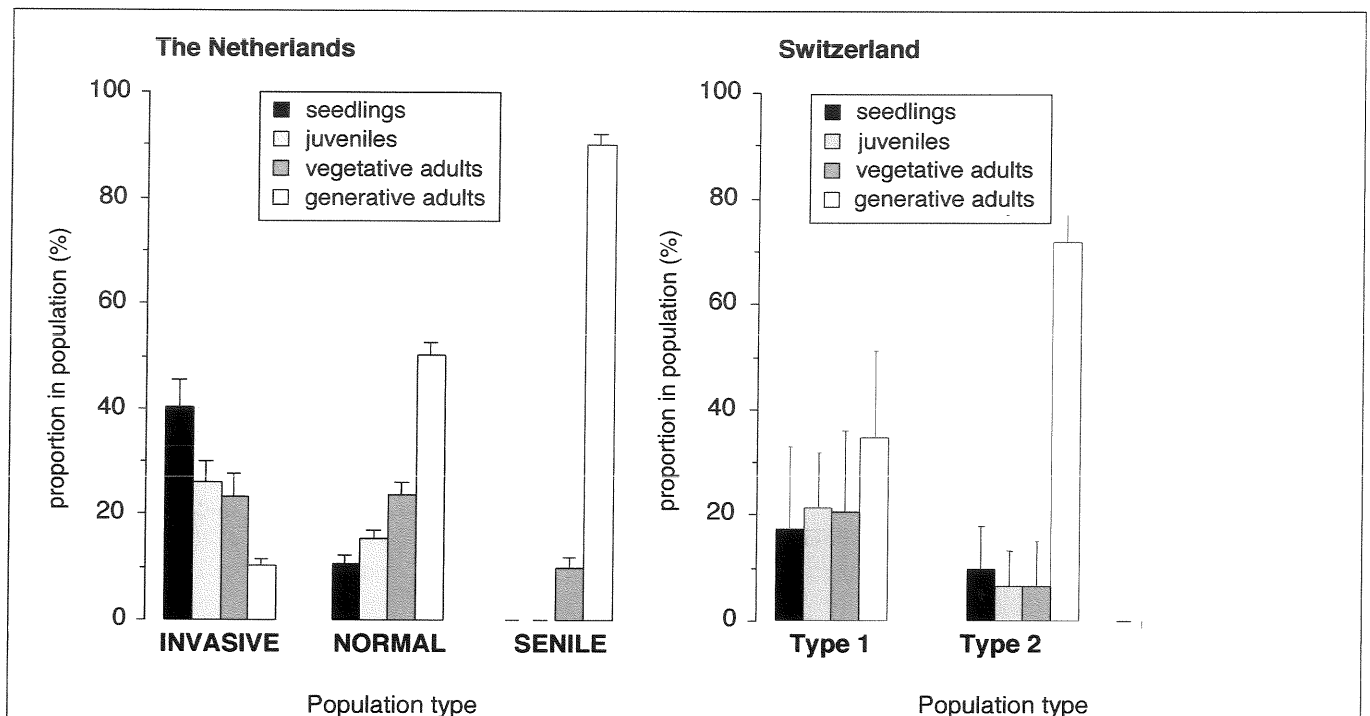


Figure 5: Population types in The Netherlands and in Switzerland.

The corresponding vegetation structure, as shown in Figure 3, illustrates that bryophyte cover facilitates a good environment for the younger age states (seedlings, juveniles and vegetative adults). This is also concluded from Table 3; bryophyte cover is an explanatory variable in all younger age states. Population type 1 shows a high proportion of these younger age states and therefore a corresponding, high proportion of bryophytes. In type 2, the proportion of the younger age states is lower and the proportion of bryophyte cover is also low. This result is in contrast to what was found by Oostermeijer *et al.* (1994); here bryophyte layer prevented the germination and establishment of seedlings. Population types in the Netherlands display a different corresponding vegetation structure as

shown in Figure 6 (Oostermeijer *et al.* 1994). In this case, percentage of bare soil has a more significant influence on the population structure than in Switzerland. In Switzerland, generally the habitat of *Gentiana pneumonanthe* is a hay meadow, whereas in the Netherlands the plant can also be found in wet heathlands.

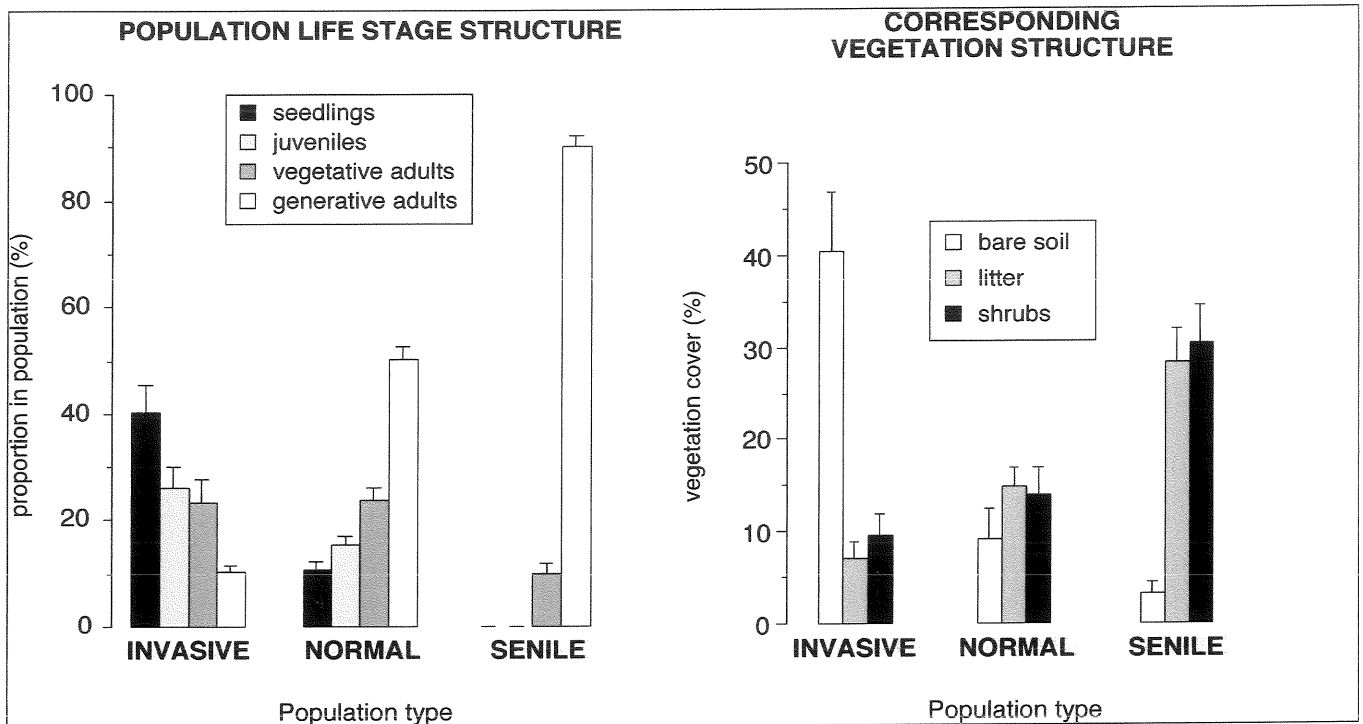


Figure 6: Population life stage structure with corresponding vegetation structure in The Netherlands (Oostermeijer *et al.* 1994).

The structure of the different populations showed that seedlings as well as older plants are present in each population. It is therefore concluded that all populations are demographically viable, because they have regeneration potential. The multiple regressions give some supplementary information about the relationships between the abundance of different age states and the vegetation structure. Especially for the young plants (seedlings, juveniles and vegetative adults), the variables describing the vegetation structure explain a significant part of the observed variation, which suggests that they are more sensitive to environmental change than the generative adults. So even if the populations are demographically viable now, they should be handled with caution. This sensitivity of young age states should be considered, when nature conservation agencies, private land-owners or the government plan to change management in those populations. However, Higgins *et al.* 2000 argue that the storage of reproduction potential across generations should also be considered in conservation of rare plant populations. *Gentiana pneumonanthe* could exhibit the storage effect, because the species is long-lived

and can therefore survive environmental variability. To research the effect of this storage in detail, long-term studies are suggested, for example, to follow individual plants over time.

The results demonstrate that *Gentiana pneumonanthe* in Switzerland prefers open vegetation types with a certain cover of bryophytes. Therefore, management should focus on keeping the vegetation structure open if *Gentiana pneumonanthe* is a target species in the conservation program.

Pollen limitation is a problem for small, isolated populations. Most populations in Switzerland consist of less than 100 flowering plants; to be able to survive environmental stochasticity and genetic effects such as inbreeding at least 100 (preferably more than 500) flowering plants are needed. Therefore, these populations of *Gentiana pneumonanthe* can be affected by the problem of pollen limitation, although this species is self-compatible. There was a significant difference among pollination treatments, which suggests pollen limitation, for two of the Swiss populations. Population Bern is a very small and isolated population and it is likely that this population does not attract sufficient numbers of pollinators to have optimal seed set. However, Petanidou *et al.* 1995 showed that reduced seed set and seed production could also be explained by high proportions of heterospecific pollen. They observed the number of flower visits by pollinators and argued that, in that specific case, low visitation rates were not likely to be the reason of pollen limitation. For the Swiss populations no data is available on visitation rates of pollinators and therefore a good reason for the pollen limitation is lacking. Nevertheless, the surrounding vegetation contained some co-flowering species such as *Mentha aquatica* (own observation), which could result in interference of other pollen (e.g. heterospecific pollen) with *Gentiana* pollen on the stigma of *Gentiana pneumonanthe*.

### **Environmental effects on maternal plants**

Seed weight shows significant differences between populations and between treatments. The difference between populations could be explained by a difference in nutrient availability. Unfortunately, soil samples have not been analyzed.

Treatment differences in seed weight are elucidated by the number of ovules, which might be pollinated, and the seed set. The naturally pollinated maternal families had a number of ovules similar to the hand-pollinated maternal families, but not all ovules were fertilized (overall seed set was lower than in hand-pollinated maternal families) and therefore the energy per seed is more, resulting in a higher seed weight in naturally pollinated maternal families.

Plants with more flowers are correlated with a relatively low seed set in the hand-pollinated treatment (naturally pollinated treatment did not show a correlation), but they produce relatively similar numbers

of ovules as plants of the naturally pollinated treatment. Thus, even though the hand-pollinated maternal plants do not experience pollen limitation and can fertilize all ovules, plants with more flowers (and therefore more ovules) show some abortion of seeds. Plants of hand-pollinated treatments were probably able to abort seeds without risking loss of reproduction potential. In other words, if the plant has no restriction in pollen and resource availability the 'bad' seeds (e.g. inbred) could be aborted without severely decreasing the seed production. But the relatively low seed set in the hand-pollinated maternal families could of course also be caused by a simple shortage of resources to provide energy for all the fertilized ovules. In addition, in case of nutrient deficiency and/or pollen limitation, Petanidou *et al.* (1995) argued that the plant can partly resorb the non-fertilized ovules to nourish the developing embryos. Again, the soil samples are not analysed and a clear reason for this result can not be given.

The environment of the maternal plant can have an indirect effect on the offspring. The characteristics of the maternal plant are affected by the openness and height of the vegetation. When vegetation is more open, the plants receive more light and can therefore produce more biomass and reproductive features (flowers and ovules). More flowers are expected to result in relatively higher seed set, but this is not the case as is shown above. Naturally pollinated fruits did not experience any difference in seed set, when more flowers were present. And hand-pollinated fruits on plants with more flowers even had a relatively low seed set.

### **Maternal effects on offspring performance**

Heavier seeds (thus the maternal plant has invested more energy per seed) should have enough energy to result in seedlings with an, on average, higher biomass. But this does not fit with the results from the populations. The seed weight seems to have no significant impact on seedling biomass in the hand-pollinated group. But the naturally pollinated group showed seedlings with lower biomass related with heavier seeds. All seedlings were grown under the same conditions, therefore differences in nutrients or environment can be ruled out. This result could indicate a genetic effect of the maternal plant. In populations with small numbers of individuals the chance to be pollinated by a relative is extremely high. Additionally, in populations in larger areas and widely distributed individuals, individuals situated close to each other are likely to be related. A negative effect of inbreeding can be expected. Another negative effect could be expected if, due to widely distributed individuals and few pollinators, self-pollination occurs (Allee-effect). Further research on this subject is recommended.

The number of stems of the offspring in the naturally pollinated group was negatively correlated with seed weight. In these cases low biomass of the seedlings due to low seed weight explains this result, because the seedling does not have enough energy (provided by the seed) to produce more stems. The biomass of the seedlings will have an effect on the further development of the plants. And this result can be brought back to some kind of (genetic) effect of the maternal plant on the offspring (in this case: an effect on the seed weight).

## General conclusions

To summarize, the 12 populations of *Gentiana pneumonanthe* in Switzerland can be categorized in two types, 'type 1' and 'type 2', which are both similar to the 'stable' type mentioned by Oostermeijer *et al.* 1994. All populations seem to be viable, but they are sensitive to environmental changes. Especially the younger age states should be considered, when management regimes are to be changed. As mentioned before most population are small (less than 100 flowering plants) and this can result in genetic and environmental problems such as inbreeding and Allee-effects. Oostermeijer *et al.* 1992 argue that management practices leading to a more open vegetation structure (and ensuring the presence of some seed-producing individuals), will be favourable for regeneration of *Gentiana pneumonanthe*. In the Swiss populations, management practices should also focus on open vegetation structures. Furthermore, in the case of isolated populations (like the Bern population) small strips of more open vegetations between habitat patches of *Gentiana pneumonanthe* (and also the same holds for other rare plant species) can help pollinators to find those populations and consequently prevent inbreeding. A patchy mowing system, like performed in population Cudrefin, is preferred; thus mowing a patch once every two or three years and leaving the patch next to it untouched. This will leave enough seed-producing individuals in the population and the seedlings will be able to survive and persist in the population.

There are some effects of the environment of the maternal plant on their offspring. These effects are indirect; they act on the maternal plant and this in turn has an impact on the offspring. Some results of the multiple regression can not be explained by environmental maternal effects and further research on the genetic effects of maternal plants on their offspring is suggested.

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## Appendix I



**Population Bern**



**Population Champ Martin**





**Population Cudrefin**



**Population Le Parc**