Fig. 2: Ontogenetic stages in populations of *Platanthera bifolia* in Murmansk Region, Russia (MU) and in The Netherlands (NL); j = juveniles, im = immatures, v = vegetatives, g = generatives, and s = seniles
Zusammenfassung


Die Fitness von Populationen der zwei Orchideenarten Coeloglossum viride und Platanthera bifolia wird in zwei verschiedenen Klimazonen untersucht, einmal bei Murmansk (MU) nördlich des Polarkreises, zum anderen in den Niederlanden (NL) in der atlantischen Zone Westeuropas. Die Populationsfitness wird anhand verschiedener morphologischer Merkmale der Pflanzenindividuen indirekt geschätzt.


Insgesamt ergeben sich für beide untersuchten Arten deutliche Unterschiede zwischen den beiden 3200 km entfernten Klimabezirken sowohl hinsichtlich ihrer Wachstumsleistung als auch ihrer Populationscharakteristik.

1. Introduction

Plant morphology and population characteristics may differ within the same species in relation to different climatic conditions within its distribution area. Intraspecific variation as adaptation to dissimilar climatic features has been studied in ephemerals (ARONSON et al. 1990), short-lived perennials (REINARTZ 1984) and long-lived tree species (WHITE 1971), however this kind of research is rather uncommon. Studies in which population characteristics of long-lived herbaceous species are related to differences in climatic conditions are even rarer (WILLEMS & ELLERS 1996).

We investigated intraspecific variation in both plant morphology and fitness parameters in two orchid species, Coeloglossum viride (L.) Hartm. and Platanthera bifolia (L.). Rich. (hereafter referred to as C. viride and P. bifolia) in two different climatic regions, viz. in Murmansk Region north of the Arctic Circle in Russia and in The Netherlands in the Atlantic Region of Western Europe.

Population fitness has been defined as the number of offspring that reach reproductive maturity (HARPER 1977, LLOYD 1987). However, it is almost impossible to measure this criterion exactly, especially in wind dispersed species like orchids. Therefore, we used as an estimate for population fitness the number of flowers per plant, the percentage of successfully pollinated flowers and the number of seeds per capsule (WDNN & GROSS 1993). The duration of the juvenile phase can also be considered a factor affecting population fitness: a short juvenile stage speeds up the timing of maturity and this is an indirect advantage for seed production in a population (WILLEMS & ELLERS 1996).

In this study we focus on morphology, leaf size and total leaf area of individual plants of the two orchid species and the possible consequences for generative reproduction in both regions. Moreover, the duration of the ontogenetic stages as well as the seed production of both orchid species are compared between both regions. In the present paper we addressed the following questions, i: are there relevant morphological differences in individual plant performance and ii: are population characteristics different between the regions, situated some 3200 km from each other?
2. Material and Methods

For a detailed description of the study sites and the species involved in this research, see the previous paper on the phenological aspects of the populations (Blinova et al. 2003).

Populations of two orchid species, the short-lived *C. viride* and the long-lived *P. bifolia*, were monitored over two successive years, viz. 1998 and 1999. The study is based on data from individual plants registered in permanently marked plots, which made it possible to return to individually marked plants. In each population a number of vegetative and generative adult plants were checked each year in order to collect data on morphometrical parameters. The following characteristics were measured: height of the flowering stalk, length of inflorescence, number of flowers and fruits, number of leaves and stem bracts, number of leaf ribs and the number of seeds per capsule.

The length and the width of every green leaf were measured in order to measure total leaf area. Total leaf area (TLA) was calculated using the following formula:

\[ TLA = Co \times (W1 \times L1 + W2 \times L2 + \ldots + Wn \times Ln), \text{ (cm}^2) \]

*Co* is a coefficient, determined by measuring the real leaf area of randomly collected leaves (n=20), using a leaf area meter Li-3100 (Li-Cor, inc. Lincoln, Nebraska, USA). The coefficient equaled 0.75 for *C. viride* and 0.62 for *P. bifolia*. Leave sizes: *W* – width of a leaf (cm), and *L* – length of a leaf (cm).

Leaf ribs are counted on the adaxial side of the largest green leaf. This parameter is considered to be a reliable assessment to distinguish the ontogenetic stages related to plant age (Vakhrameeva & Denissova 1990, Tatarenko 1991, Kulikov 1995, Fardeeva 1997), which avoids digging them up, forbidden by law in many countries, in order to analyze their underground structure for information on age.

The numbers of seeds in randomly chosen capsules are counted using a binocular microscope (20 x) after releasing the seeds in a Petri dish with a thin layer of glycerol over a grid of 1 x 1cm. The number of seeds within a grid are counted at three to five different places and an average was taken and multiplied by the total number of grids with seeds.


All recorded shoots of *C. viride* and *P. bifolia* were examined and placed into the following classes or ontogenetic stages (Blinova 1998):

**Juvenile (j).** Individuals having small shoots with 1 or 2 leaves up to 3-4.5 cm in length and 3-6 mm in width, with 1-3 leaf ribs. Concerning *P. bifolia* in MU, most individual plants have only one green leaf.

**Immature (im).** Individuals intermediate between juveniles and adults, having shoots with 2 leaves with 4-6 leaf ribs;

**Vegetative (v).** Individuals including a mixed group with temporary non-flowering shoots and post-immature individuals having shoots of 2 or 3 leaves with 6-8 leaf ribs;

**Generative (g).** Flowering individuals having shoots of 2 or 4 leaves, with 8-16 leaf ribs and many flowers in the inflorescence;

**Senile (s).** These individuals were never found in our populations because many root-tuber orchids die after generative stage without passing through senile stage (Vakhrameeva & Denissova 1990), and therefore not relevant in this study.

For statistical analyses the data sets of 1998 and 1999 have been pooled together. Average means with standard errors for measurements conducted on generative and vegetative individuals were given. Each measurement corresponded to the populations of each species in 1998 and 1999. To compare two sites, *t*-test was used, *p*-value smaller than 0.05 indicated statistical mean difference.

We followed the Flora Europaea for plant names (Tutin et al. 1964-1980), in spite of the recent developments in plant taxonomy based on DNA sequences, and carried out on species of the Orchidaceae, too.

3. Results

3.1. Morphometric parameters

*a. C. viride*

Vegetative plants of *C. viride* differ in both regions with respect to the number of leaves, total leaf area and the number of leaf ribs per leaf (Table 1). The generative plants show almost a similar tendency concerning the total leaf area.
and the number of leaf ribs, except in the number of leaves which is equal in both areas, MU and NL.

Fitness parameters differ between both regions in terms of height of the flowering stalk, length of inflorescence, fruit number and fruit/flower percentage. The number of flowers along the flowering stalk and the number of seeds per capsule did not differ significantly among both regions. The total seed production as a result of much higher pollination rate however, was highest in the NL population, in spite of a relatively lower investment in terms of plant tissue in the flowering stalk and inflorescence.

b. *P. bifolia*

The diversity between vegetative plants belonging to this species in both regions is the number of leaves and leaf ribs. There is a statistically insignificant tendency of a larger total leaf area in NL (Table 2). These plant performance parameters are clearly different in the generative plants among both regions, i.e. number of leaves, total leaf area, leaf rib number and stem bracts are higher in NL. The flowering stalk height is similar on both regions. The number of flowers and fruits are higher in NL, whereas there is no difference in the fruit/flower ratio. The seed number per capsule is about double the number in NL compared to MU.

In conclusion: reproductive output in both species, and hence population fitness, is highest in NL, however achieved in a different way, viz. in *C. viride* in NL mainly as a result of an approximately four times higher pollination rate, and in *P. bifolia* by a two times higher seed number per capsule.

3.2. Ontogenetic structure of the populations

a. *C. viride*

The ontogenetic spectra of both populations show a dramatic difference in both regions (Fig. 1). In MU the spectrum consists of all four different life stages present in the population with a distinct predominance of vegetative plants (ca. 50%). In NL, the spectrum is incomplete, because immature individuals are missing in the population. Generative individuals however, dominate significantly in NL (ca. 60%), where there are twice more compared to the other life stages. The population size is very similar in both sites.

b. *P. bifolia*

The ontogenetic spectra of the populations of *P. bifolia* differ even more distinct among both regions (Fig. 2). All diverse life stages are represented in the spectrum from the MU populations with rather equal rate of the majority of ontogenetic groups, except for the immature plants. In NL, the spectrum is very poor, representing only two life stages, viz. the vegetative and generative groups of plants. The absolute number of individuals in the NL population is very low compared to the plant number in MU.

4. Discussion

This study clearly shows the existence of intraspecific variation in both a short- and long-lived orchid species in two different parts of their distribution area. This variation is not parallel among the species. A crucial factor like the photosynthetic processes, as a function of the total leaf area, is larger in the flowering individuals in MU compared to the NL population of *C. viride* (Table 1). However, the opposite holds for the leaf area of *P. bifolia* plants, which is lower in MU (Table 2). This might be due to the phenomenon that the majority of the individuals of *P. bifolia* in MU has only one leaf ("unifolia") (BLINCOVA 1995). Whether this characteristic might influence the flowering frequency in this species negatively compared to the two-leaved plants, can only be unraveled by long-lasting and yearly monitoring of individual plants in a *P. bifolia* population (WILLEMS 2002).

It is a well-known feature that an individual orchid plant has to develop a given threshold size of the leaf area during a number of growing seasons before first flowering is possible (WELLS & COX 1989, WILLEMS 1982). The leaf area of vegetative plants of *C. viride* in MU (8.9 cm²) is nearer to the average leaf area of the flowering individuals in NL population (9.9 cm²), rather than to this in the flowering plants in MU (12.3 cm²) (Table 1). This does not appear to be the case in the populations of *P. bifolia*, in which the differences in leaf area among vegetative and generative plants are more pronounced, however not statistically significant in the vegetative plants (Table 2).

The investment in generative tissue, like the flowering stalk, is significantly lower in *C. viride* in NL, whereas the number of flowers does not differ that much between both areas. This means a lower investment in generative tissue per flower in NL, too. Pollination rate is more than four times higher in the NL population compared to the plants in MU. However, the percentage of successfully pollinated flowers in *C. viride* in NL (c. 17%) is rather low in comparison to data obtained in another short-lived orchid species, viz. c. 84% in *Ophrys sphegodes* (HUTCHINGS 1989). The pollination rate in MU was
It is remarkable that the numbers of seeds per capsule do not differ significantly in both areas in C. viride. The higher average seed production per plant of C. viride in NL, and hence the higher population fitness, largely depends on a higher pollination rate caused by an external vector, viz. the flower visiting insects, mainly Hymenoptera ssp (Van der Cingel 1995). The low pollination rate of C. viride in both regions might be the result of a low degree of interspecific competition ability for pollinators by this low-stature species with its inconspicuous greenish flowers (Willem & Lahinen 1997). This competition is probably stronger in the pine forests of the north taiga in MU than in the species-rich, semi-natural grassland in NL.

In P. bifolia, the average flowering stalk height in the MU and NL populations do not contrast, whereas the number of flowers per inflorescence significantly differs (Table 2). However, the pollination rate is similar and, compared to C. viride, is very high, viz. 58 and 55 % for respectively the populations in NL and MU. This might be the result of the diversity in pollinators among both species. Pollination in C. viride is the result of visual appeal, whereas P. bifolia attracts the insects, mainly long-tongued night-moths, by scent during the night, which is likely to be more effective by reducing intraspecific competition (Van der Cingel 1995). Moreover, the flowering stalk of P. bifolia is in both regions much higher than observed in C. viride, and therefore better accessible for pollinators (Table 2). The higher population fitness in the NL population is the result of more than double the number of seeds per capsule. It is surprising that the number of seeds produced in the short-lived C. viride is much lower compared to the long-lived P. bifolia in both regions (cf. Hutchings 1989, Willem 1994).

The ontogenetic analysis of the population of C. viride shows a predominance of the vegetative over generative plants in MU, whereas the opposite holds for the population in NL. Both ontogenetic spectra are the result of only two years of data collecting. This means that no solid conclusions can be drawn with respect to the long-term population fitness. However, it can be argued that the populations of C. viride in both regions are not immediately threatened with extinction given these life phase spectra. Almost all stages are present in this species of which it is known that it is an extremely short-lived orchid in NL, with an average age of the individuals of only three years (Willem & Melser 1998).

The ontogenetic structures of both populations of P. bifolia show a sharp contrast. In MU all stages are represented in the spectrum and all with fairly high numbers of individuals, though the immature stage shows the lowest number. The spectrum indicates a well-established and healthy population. This is on the contrary to the population in NL, in which only two stages, the vegetative and the generative ones, remain in only very low numbers of individuals. This clearly indicates the limited size of this severely threatened Red List species in NL (Heukels & Van der Meijden 1990). The position in the Berghofweide N. R. concerning P. bifolia is very vulnerable, not only due to the small population size, however, especially since the juvenile and immature stages are completely absent. This is likely the result of the applied management, viz. mowing of the vegetation and removal of the hay in late July followed by aftermath grazing by sheep in late autumn or early winter (Willem & Dorland 2000). Although P. bifolia flowers at this site already in the second half of June (Kreutz 1992), seed formation takes several months, and seed release happens not earlier than the second half of September, approximately three months after flowering. This implies that no seed production is realized in this population from 1980 onwards, when the present management has been introduced. This management favored the majority of the rare and endangered plant species at this site, with the exception of P. bifolia. It is evident that no recruitment could be realized by the absence of viable seeds, from 1980 onwards, the first year of the present management. It is likely that the few remaining individuals, which are subject of this study, originate previous to 1980, which implies that P. bifolia can be considered a long-lived orchid species.

Based on these results, the responsible nature conservational authority adjusted the management and avoided mowing of a yearly shifting plots (size 10 x 10m) with flowering plants of P. bifolia. Whether this small-scale management proves to be fruitful for the population can only be answered by continued monitoring of the population during a sufficient number of years. It is not yet known how many years the under ground protocorm development of this species will last (Rasmussen & Whigham 1998). This stage is known to be very variable among orchid species, and may last from 3 to 11 years (Wells 1981, Willem 1982, Vanhecke 1991). Therefore, it is unknown how many years it will take before an expected positive effect of this small-scale management will be observed at the Berghofweide site.

It has become evident from the present studies on the intraspecific variation in orchid populations in two different climatic regions, that in the North the seasonal timing of the life cycle is of decisive importance for the fate of the
orchid populations involved. The adjustment of the phenological phases to the short growing period in the Arctic Circle is obvious. The several phases are very short compared to those in the Atlantic region, where species are present above ground only during a part of the growing period (Blinova et al. 2000X).

Therefore, it is likely that stochastic climatic factors may have a greater impact in northern orchid populations compared to those in Western Europe. Since orchid species, like C. viride and P. bifolia, are found growing in nearly natural vegetation in the North, their habitats in Western Europe are mainly semi-natural vegetation (Ellenberg 1988, Willems 2001). This implies that appropriate site management is of decisive importance for their future preservation in Western Europe, whereas habitat preservation is a necessary prerequisite in the North in order to safeguard populations of indigenous orchids.

Within the framework of studies on the effects of global climatic change on flora and fauna studies like the ones presented here, are indispensable in order to establish changes in population fitness in an early stage.

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Author's address:
Dr. Ilona Blinova
Polar-Alpine Botanical Garden-Institute
184256 Kirovsk 6, Murmansk Region
RUSSIA
e-mail: ilona@aprec.ru

Dr. Jozef H. Willems
Plant Ecology Department
Utrecht University
Sorbonnelaan 16
NL-3508 TB Utrecht
The Netherlands

Drs. Karin Tromp
Department of Geobiology
Landscape Ecology group
Utrecht University
Sorbonnelaan 16
NL-3508 TB Utrecht
The Netherlands

Appendices:
Table 1, 2: p 340.
Figures 1, 2: p 341-342.
Table 1. Morphometrical parameters of generative and vegetative individuals in two orchid populations of *Coeloglossum viride*: in Murmansk Region, Russia (MU) and in The Netherlands (NL) over two years, 1998-1999. Statistically significant differences between sites are shown as p-values, n = sample set.

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<th>stem bracts</th>
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Table 2. Morphometrical parameters of generative and vegetative individuals in two orchid populations of *Platanthera bifolia*: in Murmansk Region, Russia (MU) and in The Netherlands (NL) over two years, 1998-1999. Statistically significant differences between sites are shown as p-values, n = sample set.

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Fig. 1: Ontogenetic stages in populations of *Coeloglossum viride*: 1 = juveniles, 2 = immatures, 3 = vegetatives, 4 = generatives, and 5 = seeds.