Survival in the alpine landscape

Genetic, demographic and reproductive strategies of the rare monocarpic perennial *Campanula thyrsoides* in the Swiss Alps

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Chapter 1

General Introduction

The aim of this thesis

This thesis comprehensively studies the genetic, demographic and reproductive strategies of the long-lived monocarpic perennial *Campanula thyrsoides* to assure survival in the alpine environment. The work is part of the larger project “How patchy habitats and isolation affect plant life: genetic diversity, gene flow and mating systems” which forms the framework for the PhD investigations of Hafdís Hanna Ægisdóttir and the author under the supervision of PD Dr. Jürg Stöcklin. The main objective is to generate and combine knowledge from different aspects of the life-history of *C. thyrsoides* in order to assess the short- and long-term persistence potential of this rare species in Switzerland. This thesis therefore provides valuable information to understand why the species is rare, whether and how we need to establish appropriate conservation measures, and how susceptible *C. thyrsoides* is with respect to global change.

Survival or extinction?

The alpine environment is characterized by a pronounced topographical heterogeneity and a complex matrix of different abiotic conditions which creates a mosaic of niches and habitats for a large diversity of organisms. Apart from the spatial component, the Alpine is also a highly dynamic ecozone with a pronounced variability of ecological conditions in time, from diurnal and seasonal to geological time-scales. The important question to ask is: “How do species and populations maintain their presence and expand their range by colonizing new and suitable land?”. Generally, a plant has to invest in seed production or in vegetative propagules to be able to migrate. Migration occurs at different spatial scales and is realized either through alternative modes of dispersal, i.e. wind, water, gravity or animals, or through detachment from the mother plant once autonomy has been achieved. After successful establishment in a new site the best strategy is often to occupy this very space for as long as possible (space-holder strategy; Körner, 2003). There are numerous ways how this task can be realized and the alpine flora shows a wealth of clonal and non-clonal growth forms.

Persistence in space, however, always needs to consider persistence in time because both factors naturally operate together. Alpine plants are for the most part long-lived species and individual ramet longevity is often surpassed by genet longevity. As a

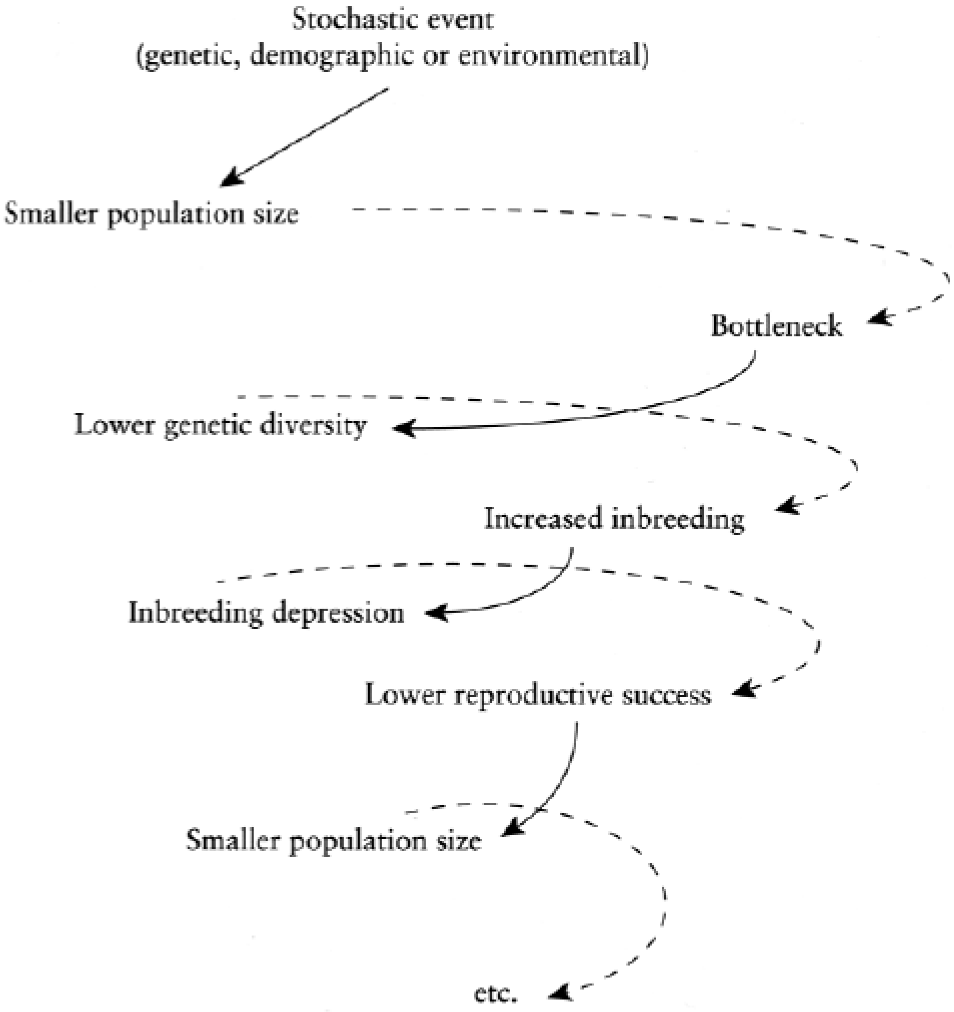

Figure 1. Extinction vortex of small populations (from Till-Bottraud and Gaudeul, 2002; after Gilpin and Soulé, 1986).

consequence of the temporal variability, numbers of annual and biennial species diminish drastically with increasing altitude because such populations cannot buffer successive reproductive loss (Körner, 2003). Even for long-lived plants it is advantageous to spread flowering over several seasons (polycarpy) and not to rely on a single reproductive event (monocarpy) (Young and Augspurger, 1991). There are of course always exceptions to the rule.

Persistence in time and space has also a genetic component and the molecular variability of a species will determine both its short- and long-term ability to respond to environmental fluctuation. This can be described in terms of a species' 'adaptive capacity' and 'evolutionary potential' to buffer change. It is widely accepted that genetic and demographic factors synergistically determine the dynamics of a plant population and that both factors have to be optimized to avoid extinction (Till-Bottraud and Gaudeul, 2002). Such complex interactions are best illustrated in the extreme form, namely the ‘extinction vortex of small populations’ (Figure 1; Gilpin and Soulé, 1986). This essentially negative feedback loop is initiated by a stochastic (catastrophic) event which leads to a substantial reduction in population size. Consequently, the gene pool is reduced, the probability for inbreeding depression increases, and a decreased seed set subsequently causes the population to further decline in size. Given the mosaic of habitats in the alpine environment plant populations are usually small, spatially isolated from each other and therefore more vulnerable to stochastic processes than plant populations in stable environmental conditions or of large sizes.

Genetic diversity is of course not determined by population size alone. There is still considerable debate about what determines genetic diversity and in more detail, what are the factors governing within-population diversity as opposed to among-population diversity (= genetic differentiation). The more general agreement is that long-lived, outcrossing, late successional plant species retain the greatest share of their variability within populations, while for annual, selfing and/or early successional taxa a high percentage of genetic diversity is found among populations (Hamrick and Godt, 1989; Nybom, 2004). An additional factor is the Quaternary history of a species because spatio-temporal isolation can lead to differentiation and speciation processes (Aguinagalde et al., 2005; Schönswetter et al., 2005).

At this point it is now of importance to rephrase and subdivide the initial question and ask: “How does *Campanula thyrsoides* maintain high genetic diversity even in small populations to avoid inbreeding depression, how does the Quaternary history and important life-history traits determine levels of genetic diversity, how are population growth and reproduction optimized in order to assure persistence in a variable environment, and how well adapted is the species to colonize new and open habitats?”

This doctoral thesis

For this doctoral thesis I have used a variety of modern and traditional approaches to follow the questions outlined above. The work starts at the genetic level applying neutral molecular markers to understand processes of gene flow at the population and landscape level, it further relies on sophisticated modelling and laboratory techniques to determine the key demographic factors that assure population persistence in time, uses classical pollination biological approaches to understand nature and dynamics of the breeding system, and provides a concise description of all aspects of the biology of *C. thyrsoides*.

Outline

Chapter 2, 3, 4 and 5 are written for publication in peer-reviewed scientific journals. For co-authorship and status of each publication see below.

Chapter 2	P. Kuss, A.R. Pluess, H.H. Ægisdóttir, J. Stöcklin **Spatial isolation and genetic differentiation in naturally fragmented alpine plant populations** *American Journal of Botany*, resubmitted after review

This chapter addresses the genetic diversity aspect of *C. thyrsoides* in the Swiss Alps and the potential role of natural landscape fragmentation in shaping genetic patterns. We hypothesize that spatial isolation has led to reduced genetic connectivity among populations which is reflected in a high level of population dissimilarity. Such a hypothesis however can only validly be tested in comparison with other species and within a standardized experimental framework. We therefore included our data set from *Epilobium fleischeri* and the raw data from a published study on *Geum reptans* (Pluess and Stöcklin, 2004) applying advanced computational routines to achieve maximum comparability of the three species. We especially focus on an ecological interpretation of the results by incorporating information on the Quaternary history of plant migration, population size and altitudinal location, as well as important life-history traits, e.g. dispersal potential and breeding system. The obtained genetic information on *Campanula thyrsoides* is an important contribution to our understanding of the long-term survival strategy of this species and relies in part on the breeding system study presented in **Chapter 4**.

Chapter 3 P. Kuss, M. Rees, H.H. Ægisdóttir, J. Stöcklin
Evolutionary demography of the long-lived monocarpic perennial *Campanula thyrsoides* in the Swiss Alps
Ecology, submitted

This manuscript investigates the demographic processes operating within populations of *C. thyrsoides* and how they are optimized to assure population persistence. We used a novel approach that combined permanent plot observations from a 3-year-census period with single-season herb chronology data to parameterize site-specific integral projection models (IPMs). This allowed us to investigate many aspects of the life-cycle of this long-lived monocarpic plant without the necessity of following the population for more than an average generation, i.e. 10 years. The computational framework of the IPMs made it further possible to avoid the methodological biases of traditional matrix models and to achieve an in depth analysis of the demographic buffer capacity of *C. thyrsoides* to the variability of the alpine environment. We additionally evaluated whether the species follows an evolutionary stable strategy and simulated how microsite limitation for seedling establishment may affect the population dynamics. Together with the genetic results presented in **Chapter 1** and the life-history information outlined in **Chapter 4** the demographic work also provides the necessary tools to adequately assess conservation priorities for this species.

Chapter 4 H.H. Ægisdóttir, D. Jespersen, P. Kuss, J. Stöcklin
No inbreeding depression in an outcrossing Alpine species: the breeding system of *Campanula thyrsoides*
Flora, accepted

In this article we focus on the reproductive strategy of *C. thyrsoides* exploring the consequences of spatial isolation on the species' breeding system. We conducted a classic pollination experiment in a common garden setting with plants from different seed-families originating from an isolated population in the Swiss Alps. In detail, we explore the rate of self-incompatibility in selfed and sister-crossed individuals and study the potential breakdown of this system with flowering age. The results are valuable basic information for the interpretation of the genetic patterns presented in **Chapter 1** and add to the overall understanding about population persistence in the variable alpine environment.

Chapter 5

P. Kuss, H.H. Ægisdóttir, J. Stöcklin
The biological flora of Central Europe: *Campanula thyrsoides*
Perspectives in Plant Ecology, Evolution and Systematics,
submitted

This chapter presents all existing information on *Campanula thyrsoides* in concise form of a monography. As data mining becomes an internationally more and more important aspect of science we hereby contribute to the overall understanding of this species, outline gaps in current knowledge and facilitate future research by providing valuable information on experimental failures or genetic marker unsuitability. The monography is therefore based on an intensive literature research of historic and new publications written in many languages, unpublished data and information from local experts around Europe, as well as numerous screening tests and smaller field experiments conducted by the authors which were necessary basic information for the focal investigations of this thesis.

In the **General Discussion** I incorporate the results of the individual chapters in a general discussion about the nature of rarity of *Campanula thyrsoides* and the rarity of the monocarpic life-history in the polycarpic Alpine environment. This chapter closes with an **Outlook** in which I outline future research questions.

In the **Conclusions** I summarize the main findings from the individual chapters of this thesis and make general conclusions with respect to conservation neccessities for this rare species in Switzerland.

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Chapter 2

Spatial isolation and genetic differentiation in naturally fragmented alpine plant populations

Patrick Kuss, Andrea R. Pluess, Hafdís Hanna Ægisdóttir, Jürg Stöcklin

Summary

The effect of landscape fragmentation on the genetic diversity and adaptive potential of plant populations is a major issue in conservation biology. Here, we investigate molecular patterns of three Alpine plants and ask whether spatial isolation has led to high levels of population differentiation, increasing over distance, and to a decrease of within-population variability. For all species we found a significant isolation-by-distance relationship but only a moderately high differentiation among populations (Φ_{st}: 14.8%, 16.8%, 22.7%, respectively). Within-population diversity was not reduced in comparison to lowland species (H_e: 0.19-0.21, P_p: 62-75%) and even small populations with less than 50 reproductive individuals contained high levels of genetic diversity. We further found no indication that a high long-distance dispersal potential enhances genetic connectivity among populations. Gene flow seems to have a strong stochastic component causing large dissimilarity between population pairs irrespective of the spatial distance. Our results suggest that other life-history traits, especially the breeding system, may play an important role in genetic diversity partitioning. We conclude that spatial isolation in the alpine environment has a strong influence on population relatedness but that a number of factors can considerably influence the strength of this relationship.

Key words: *Campanula thyrsoides*; *Epilobium fleischeri*; *Geum reptans*; isolation by distance; life-history traits; molecular diversity; RAPDs; Swiss Alps.

Introduction

The effect of landscape fragmentation on the genetic diversity of plant populations is a major issue in conservation biology (Young et al., 1996; Frankham et al., 2002). It is important to predict a species' extinction risk as a result of habitat loss and impeded genetic connectivity between populations in order to establish applicable protection measures (Gilpin and Soulé, 1986). This is particularly true in the rapidly changing modern landscape that is shaped by anthropogenic resource exploration such as agricultural practices, deforestation or infrastructure building (e.g. Groom and Schumaker, 1993; Fischer and Stöcklin, 1997). By contrast, natural fragmentation is a characteristic feature of the alpine environment and has played a key role in the evolution of species (Körner, 2003). Alpine plants are organized into local populations of different sizes, highly structured in space and with a high capacity for extended local persistence due to perennity and/or clonality (Bliss, 1971; Körner, 2003). On the other hand, colonization of new sites is a slow and irregular process which largely depends on rare long-distance dispersal events (Austrheim and Eriksson, 2001). While a growing number of studies have evaluated the genetic consequences of habitat fragmentation in the lowlands (Bartish et al., 1999; Buza et al., 2000; Rosquist and Prentice, 2000; Bacles et al., 2004; Pluess and Stöcklin, 2004a; Galeuchet et al., 2005; Hensen et al., 2005), the effect of spatial isolation on alpine species is poorly known. In the light of global warming alpine plants are particularly vulnerable to rapid change (Pauli et al., 2003) and it is therefore important to estimate their adaptive potential.

In general, habitat fragmentation and the resulting decline in population size can have a multitude of effects, such as erosion of genetic variation, increased random genetic drift or elevetated inbreeding, which can all enhance the risk of extinction (e.g. Gilpin and Soulé, 1986; Young et al., 1996; Frankham and Ralls, 1998). An intuitive consequence of spatial isolation is a reduced genetic connectivity between populations which leads to stronger dissimilarity of population pairs with increasing distances, generally referred to as 'isolation-by-distance' (IBD, Wright, 1943). However, the magnitude and significance of IBD patterns is often considered to be a result of a number of additional factors, such as physical barriers, dispersal ability, effective population size, maximim geographic sampling distance or time since colonization (e.g. Garnier et al., 2004; Crispo and Hendry, 2005) so that general predictions are difficult to make. Further, irrespective of habitat fragmentation, there is still considerable uncertainty about the relative influence of specific environmental constraints, the Quaternary history and life-history traits on the genetic diversity of a given species. Since molecular markers have different resolutions and modes of inheritance (Lowe et al., 2004) they tend to emphasize different factors. In this respect, reviews of nuclear marker studies of predominantly lowland species showed that long-lived, outcrossing, late successional plant species retain the greatest share of their genetic variability within populations, while for annual, selfing and/or early successional taxa, a high percentage of genetic diversity is found among populations (Hamrick and Godt, 1989; Nybom and Bartish, 2000; Nybom, 2004). Similar results have been shown for alpine species (Till-Bottraud and Gaudeul, 2002) but, with only a few studies available, an effect of harsh alpine habitats cannot be ruled out. On the other hand, a meta-analysis of cpDNA studies found little influence of life-history traits on genetic diversity, but evidence for glaciation-derived patterns (Aguinagalde et al., 2005).

Here, we study and compare genetic diversity and differentiation of three representative alpine perennial plant species. Given the complex interactions involved in the creation of molecular patterns as outlined above, we took particular care to standardize as many parameters as possible in order to minimize known biases (Nybom and Bartish, 2000; Lowe et al., 2004). We standardized the number of populations, individuals, RAPD primers as well as loci for each species and further restricted the data analysis to the same maximum geographic distance within a single prominent area of post-glacial migration. We also decided to re-analize results of a previous study on the alpine *Geum reptans* (Pluess and Stöcklin, 2004b) with two new investigations on *Epilobium fleischeri* and *Campanula thyrsoides*. Thus, in this common framework we can considerably improve the comparability of individual patterns. Our objective is to elucidate the effect of natural fragmentation on the genetic diversity of alpine plant species in which spatial isolation can be assumed to have existed for centuries or millenia. In particular (1) we expect genetic population differentiation to be high and significantly increasing with increasing distances. Since our study species differ particularly with respect to long-distance seed dispersal, (2) we expect relatively lower genetic differentiation and the least pronounced isolation-by-distance pattern for species with morphological adaptations to seed dispersal compared to plants lacking those functional structures. (3) We further investigate levels of within-population diversity and expect a significant decrease of diversity with decreasing population sizes.

Table 1. Life-history traits of three Alpine plant species.

Species	Habitat[a]	Altitudinal range (m)[a]	Breeding system	Seeds/pop (Mio)	Dispersal (%>1km)[g]	Pollen flow (m)	Clonality	Ramet age (yrs)
Epilobium fleischeri	Glacier forelands, river bank	1000 - 2700	Mixed[b]	4.5[e]	Wind 0.5	Insects 30[h]	Rhizomatous[b]	30[e]
Geum reptans	Glacier forelands, blockfields	1950 - 3500	Outcrossing[c]	10[f]	Wind 0.005[f]	Insects 30[f]	Stolons[f]	30[f]
Campanula thyrsoides	Alpine meadows, pastures	1300 - 2800	Outcrossing[d]	1.5[d]	Wind 0.001	Insects 39[d]	Non-clonal[a]	3-16[i]

[a] Hegi (1995), [b] Theurillat (1979) [c] Rusterholz et al. (1993), [d] Ægisdóttir (in press), [e] Stöcklin and Bäumler (1996) , [f] Pluess and Stöcklin (2004b), [g] Tackenberg (Uni Regensburg, Germany, personal comm.), [h] Stöcklin (unpublished), [i] Hänger (unpublished)

Methods

The plants species

Epilobium fleischeri Hochst. (Onagraceae), *Geum reptans* L. (Rosaceae), and *Campanula thyrsoides* L. (Campanulaceae) are widespread alpine plant species native to the European Alps and, partly, to adjacent mountain ranges in the East (Carpatians and Dinarians) and North-West (Jura; see Hegi, 1995). Throughout their ranges, plants are rare but locally abundant with population sizes ranging from a few hundred to over 50000 individuals. *E. fleischeri* and *G. reptans* are characeristic plants of glacier forelands appearing within few years after ice retreat. *C. thyrsoides* is found in mesic alpine meadows on calcareous soil. The species investigated differ with respect to several important life-history traits that potentially and differentially influence genetic-diversity partitioning within and among populations (Table 1). Life-history information are cumulated from a number of literature sources or from additional unpublished experiments and observations made by the authors. Data for long-distance seed dispersal was generated with the software PAPPUS implementing an alpine wind data set and the terminal velocity of the seeds (Tackenberg, 2003). Pollen flow observations are minimum distance estimates derived from flower to flower fluorescent powder transport by mainly bumble bees (*E. fleischeri, C. thyrsoides*) or flies (*G. reptans*) as described in Pluess and Stöcklin (2004b). We conducted pollinator exclosure and manual crossing experiments for the species to estimate individual self-compatibility as well as seed set under different pollination events. Ramet age estimates stem from herb

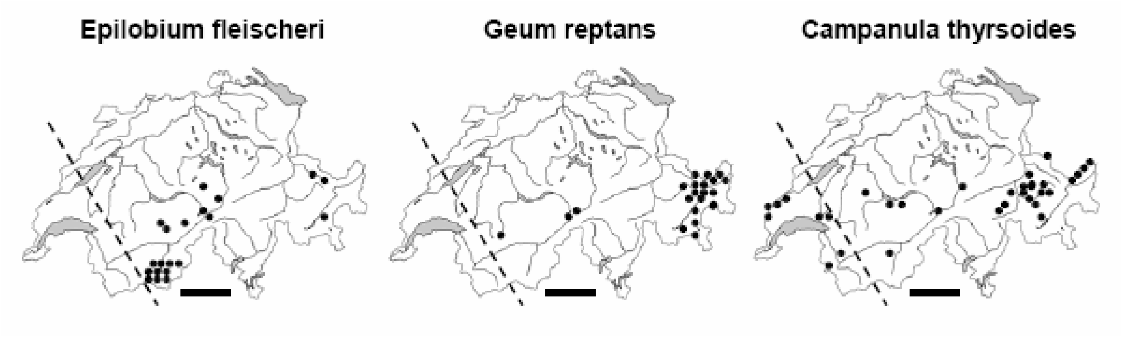

Figure 1. Geographic distribution of the studied populations of *Epilobium fleischeri*, *Geum reptans* and *Campanula thyrsoides* in the Swiss Alps and the Jura Mountains. Dashed line represents approximate border line between two glacial refugia (Schönswetter et al., 2005). Bar: 50 km.

chronology studies of roots with a representative number of individuals as presented in Dietz and Ullmann (1998). It would be desirable to have information on the potential genet age in the clonal species *E. fleischeri* and *G. reptans*, but investigations are still missing.

Sampling design

For all three species we sampled a minimum of 20 individuals per population and a minimum of 20 sites spread over the Swiss Alps. Leaf material from random individuals within a population were sampled, dried with silica gel and stored at room temperature until analysis. To avoid resampling the same clone in *E. fleischeri* and *G. reptans* a minimum distance of 4 m was chosen. Care was taken to cover the same altitudinal range and a similar geographic pattern wherever practical. In the case of *C. thyrsoides* we extended the sampling to additional populations in order to test the robustness of genetic pattern through randomization procedures (see below). Location of sampling sites and population descriptions are summarized in Fig. 1 and Appendix 1 (see Supplemental Data accomanying the online version of this article).

Molecular marker suitability

We tested two molecular methods that appeared promising for the application in all three study species, i.e. allozymes, RAPD. For *E. fleischeri* and *C. thyrsoides* we screened 22 and 18 isozyme-systems respectively on cellulose acetate gels in different electrophoresis buffer systems (TG, CAAPM, C). No polymorphisms were detected despite this large quantity of enzyme systems, including enzymes that have been successfully used in other *Epilobium,* i.e. MDH, PGI (Brian Husband, University of Guelph, Canada, pers. comm.), and *Campanula* species, i.e. AAT, GPI, IDH, MDH, 6PGDH, TPI (Ægisdóttir, 2003). Our results suggest that for both species most isozymes are fixed.

RAPD-markers

In the case of *E. fleischeri* and *G. reptans* we homogenized 20 mg silica dried leaf tissue (Retsch MM2, Retsch GmbH & Co KG, Haan, Germany) and extracted DNA with a DNeasy plant mini kit (Qiagen GmbH, Hilten, Germany). DNA concentrations were measured by fluorimetry (Turner design, Sunnyvale, California, USA) with PicoGreen dsDNA quantitation reagent (Molecular Probes Inc., Eugene, California, USA). For *C. thyrsoides* we milled 10 mg silica dried leaf tissue (Retsch MM300) and extracted DNA with a DNeasy Plant 96 Kit (Qiagen). We modified the manufacturers protocol by adding 25 mg polyvinylpyrrolidone (Fluka, Buchs, Switzerland) to each sample in the first extraction steps in order to remove polyphenols that may interfere with PCR amplification. DNA quantification was done spectrophotometrically using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, Delaware, USA). After an initial screening of up to 60 decamer primers we restricted the final analysis to 5 primers for each species (Kit A, K and P, Operon Technologies Inc., Alameda, California, USA and M-6 Microsynth, Balgach, Switzerland). We attempted to use the same 5 primers for all species but PCR products could not be obtained with this prerequisite. Therefore, we selected the primers with the highest number of reproducible polymorphic bands: *E. fleischeri* (OPA-8 [GTG ACG TAG G]; OPA-9 [GGG TAA CGC C]; OPA-12 [TCG GCG ATA G]; OPA-15 [TTC CGA ACC C]; OPP-12 [AAG GGC GAG T];), *G. reptans* (M06 [GTG GGC TGA C]; OPP-8 [ACA TCG CCC A]; OPP-9 [GTG GTC CGC A]; OPP-17 [TGA CCC GCC T]; OPP-19 [GGG AAG GAC A]), *C. thyrsoides* (OPA-7 [GAA ACG GGT G];OPA-11 [CAA TCG CCG T]; OPA-13 [CAG CAC CCA C];OPA-19 [CAA ACG TCG G]; OPP-3 [CTG ATA CGC C]). Amplifications were carried out in 25-µL reaction mixture containing 3 ng of template DNA, 100 µM dNTPs, 0.2 µmol/L primer, 1 × Taq Polymerase Buffer (*E. fleischeri* and

C. thyrsoides: Qiagen, Hilten, Germany; *G. reptans*: Amersham Pharmacia Biotech, Piscataway, New Jersey, USA), additional 0.5 mmol/L $MgCl_2$ for the primers OPA-12, OPP-17, OPP-19, M-6, additional 1.5 mmol/L $MgCl_2$ for the primers OPA-8, OPA-15, and 1 U Taq DNA Polymerase (*E. fleischeri* and *C. thyrsoides*: Qiagen, Hilten, Germany; *G. reptans*: Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). To assure consistency in the PCRs we kept aliquots of a single master mix per two primers for all samples only adding primer, Taq Polymerase, and DNA before PCR. All PCRs were performed in the same thermal cycler (PTC-100, MJ Research, Inc., Watertown, Massachusetts, USA) programmed for 60 s at 93°C to denature the DNA followed by 34 cycles of 30 s at 92°C, 30 s at 37°C and 90 s at 72°C. Final extension lasted for 5 min at 72°C. Samples were kept at 4°C until further analysis. The PCR products were separated on 1.6 % agarose gels (Sea Kem LE agarose, BMA, Rockland, Maryland, USA) in 1 × TAE (Tris/Acetat/EDTA) buffer in an electrical field (170 mV). Depending on the RAPD primer gels were run between 1.75 and 2.5 h and stained with ethidium bromide for 20 min. We visualized the banding pattern under UV light and scored the presence and absence of bands within an estimated fragment length range of 450 to 2000 bp from digital images (AlphaDigiDoc and AlphaEaseFC software, Alpha Innotech Corp., San Leandro, California, USA).

To assure reproducibility and assess genotyping errors (Bonin et al., 2004), we repeated amplification at timely intervals with 12 initial screening samples, i.e. three individuals from each of four distinct populations. This was also repeatedly done for randomly chosen individuals. All amplifications contained blind samples (no DNA) or foreign DNA from different plant species (*Campanula barbata, Senecio incanus, Hypocheris uniflora*). Monomorphic bands served as references for genotype errors within and between amplification. The main source of genotyping error were 'ghost bands', faint bands that could not be scored unambiguously. In most cases repeated amplification of these individuals revealed a present band. If doubts remained, this locus was discarded totally. We further simulated the effect of artificially introduced random errors in the data sets and found no significant difference in genetic indices with artificial error rates up to 7% (data not shown). Thus, potential misclassification of RAPD bands is counterbalanced by a high number of populations and individuals therein. The final presence/absence data matrix contained for *E. fleischeri*: 400 individuals from 20 populations, for *G. reptans*: 386 individuals from 20 populations, and for *C. thyrsoides*: 736 individuals from 32 populations (Appendix 1).

Statistical analysis

In order to achieve a comparable framework for the statistical analysis, we adjusted our data sets in several consecutive steps to avoid biased results. First, we restricted the presence/absence matrix to bands whose observed frequencies were less than 1 - (3/*N*) where *N* is the mean number of sampled individuals per population (Lynch and Milligan, 1994). Second, in an initial analysis we visualized the molecular indices with the software 'Barrier' (Manni et al., 2004) in order to detect patterns of molecular contrast that geographically coincide with borders of proposed post-glacial migration areas (Schönswetter et al., 2005). In such a case we restricted the analysis to populations within the same area avoiding transborder effects. Third, for the calculation of diversity and differentiation measures that are valid for interspecies comparisons, we matched the number of populations, individuals, and loci for each species through multiple random reductions of the parameters (100 subsamples) similar to the approach of Leberg (2002). Even though the information on the heterozygosity of populations was lacking, we assume that Hardy-Weinberg equilibrium was not violated. Pollination experiments in *G. reptans* and *C. thyrsoides* showed that both species are obligatory outbreeders with low seed set after self-pollination and no subsequent germination (Rusterholz et al., 1993; Ægisdóttir et al., 2006). *E. fleischeri* is known to be largely outbreeding but having the potential for selfing (Theurillat, 1979, Stöcklin, unpublished). For this species, repeated calculations with varying F_{is} from 0 to 1 at 0.25 step intervals increased AMOVA-derived Φ_{st}-values but maximum increase was less than 1%. All statistical analyses (Aeschimann et al., 2005) were restricted to polymorphic bands and all computing was performed in 'R' (Ihaka and Gentleman, 1996) using the R-libraries 'ade4' (Thioulouse et al., 1997), 'vegan' (Dixon, 2003), 'smatr' (Warton et al., 2005) and self-written code.

Two commonly used indices of molecular diversity within populations were calculated: (1) Nei's expected heterozygosity H_{e} (Nei, 1978), and (2) the percentage of polymorphic bands (P_{p}). To quantify the variation of molecular diversity among populations, we calculated the coefficient of variation (CV) for H_{e} and P_{p} and compared species specific indices with univariate ANOVA and pairwise t-tests. For each species we assessed the correlation of H_{e} and P_{p} using Pearson correlation statistics. The relation between population sizes and molecular indices was calculated as a nonparametric Spearman's Rho (r_{s}) correlation. Moreover, the relation of altitude and molecular diversity was assessed as a linear regression (data only for *G. reptans* and *C. thyrsoides* available). Population differentiation, or among-population diversity, was calculated using AMOVA-derived fixation index Φ_{st} (Analysis of MOlecular VAriance, Excoffier et al., 1992). The species specific variance of Φ_{st}-values, obtained from multiple random reduction subsampling (see above), were then compared with a univariate ANOVA.

To test for isolation by distance (Slatkin, 1987), we applied Mantel test statistics correlating the genetic distance matrix (pairwise Φ_{st} values) and the geographic distance matrix (Euclidean square distances). Significance levels were obtained after performing 10100 and 10000 random permutations for the pairwise genetic distances (Φ_{st}) and the Mantel test respectively. We used standardized major axis regression (SMA) to quantify the pattern of linear covariation (Rousset, 1997) and compared species-specific regression slopes using one-sample tests with bootstrapping (n = 10000) over independent population pairs as implemented in ‘smatr’ (Warton et al., 2005).

Further, we calculated an UPGMA cluster analysis (Unweighted Pair Group Method with Arithmetic Mean) of pairwise Nei’s unbiased genetic distances (Nei, 1978) to test for spatial separation, and displayed the results as dendrograms. Stable clusters were indicated (*) according to the 50% majority rule (Lowe et al., 2004) after bootstrapping of 10000 replicates.

Results

The adjustment of the presence/absence matrices following Lynch and Milligan (1994) resulted in 52 of 64 polymorphic loci in *E. fleischeri*, 49 of 51 in *G. reptans*, and 47 of 53 in *C. thyrsoides.* None of the scored bands were fixed at the population level. Matching RAPD-phenotypes were found twice for *E. fleischeri* originating from two distinct populations of the Scaletta glacier forefield and restricting the data set to 398 instead of 400 phenotypes. Similar phenotype matches occurred in two populations of *G. reptans* sampled in different glacier forefields (384 instead of 386 phenotypes). In *C. thyrsoides* all 736 phenotypes were different. For inter-species comparisons, we excluded those 8 populations of *C. thyrsoides* that belong to a separate area of post-

Table 2. Molecular diversity and differentiation indices of three alpine plant species. H_e: Nei's genetic diversity; P_P: percentage of polymorphic loci; Φ_{st}: AMOVA-derived fixation index Φ_{st}, standardized using multiple random reductions; IBD: Isolation by distance; $p\Phi_{st}$: pairwise Φ_{st}; *pgeo*: pairwise geographic distance.

	Genetic diversity within populations		Genetic diversity among populations	
	H_e	P_p	Φ_{st} (%)	IBD
Epilobium fleischeri	mean = 0.19 range = 0.13 - 0.22 SE = 0.006 CV = 11.8 %	mean = 74.8 range = 59.6 - 86.5 SE = 1.4 CV = 7.5 %	mean = 22.7 range = 20.1 – 24.9 SE = 0.098 CV = 4.3 %	R = 0.57 P < 0.001 $p\Phi_{st}$ = 4.7 – 44.4 % *pgeo* = 0.4 – 191.2 km
Geum reptans	mean = 0.21 range = 0.16 - 0.24 SE = 0.004 CV = 7.7 %	mean = 70.1 range = 48.0 - 80.0 SE = 1.7 CV = 9.3 %	mean = 14.8 range = 13.9 – 15.4 SE = 0.028 CV = 1.9 %	R = 0.81 P < 0.001 $p\Phi_{st}$ = 1.9 – 44.9 % *pgeo* = 0.2 – 208.1 km
Campanula thyrsoides	mean = 0.20 range = 0.18 - 0.22 SE = 0.003 CV = 6 %	mean = 61.84 range = 53.2 - 76.6 SE = 1.3 CV = 8.4 %	mean = 16.8 range = 16.3 – 17.3 SE = 0.036 CV = 2.1 %	R = 0.32 P = 0.007 $p\Phi_{st}$ = 2.3 – 29.3 % *pgeo* = 0.3 – 235.6 km

glacial migration (Appendix 1: Populations 1-8). Final calculations were then based on 47 loci (randomly chosen for *E. fleischeri* and *G. reptans*), 20 populations for each species (randomly chosen for *C. thyrsoides*), and 20 individuals per population.

Molecular diversity within populations

Mean genetic diversities, H_e, were similar in all species (*E. fleischer*: $H_e = 0.19$, *G. reptans*: $H_e = 0.21$; *C. thyrsoides*: $H_e = 0.20$) but significantly higher for *G. reptans* ($P < 0.05$) compared to the other two species. No difference was detected between *E. fleischeri* and *C. thyrsoides* ($P = 0.069$). The percentage of polymorphic loci, P_p, was significantly different between all three species ($P < 0.05$) with *E. fleischeri* showing highest ($P_p = 74.8$), *G. reptans* intermediate ($P_p = 70.1$) and *C. thyrsoides* lowest ($P_p = 61.8$) levels of polymorphism. Summary statistics for species specific diversity indices are presented in Tab. 2; the population specific indices are listed in Appendix 1. In all three species H_e and P_p were positively correlated (*E. fleischeri*: *cor* = 0.74, $P < 0.001$; *G. reptans*: *cor* = 0.70, $P < 0.001$, *C. thyrsoides*: *cor* = 0.46, $P < 0.01$). In general, within-population measures of *G. reptans* based on 47 loci (this study) were similar or identical to the results based on 49 loci (Pluess and Stöcklin, 2004b). Furthermore, we detected no influence of population size on the molecular diversity of *G. reptans*

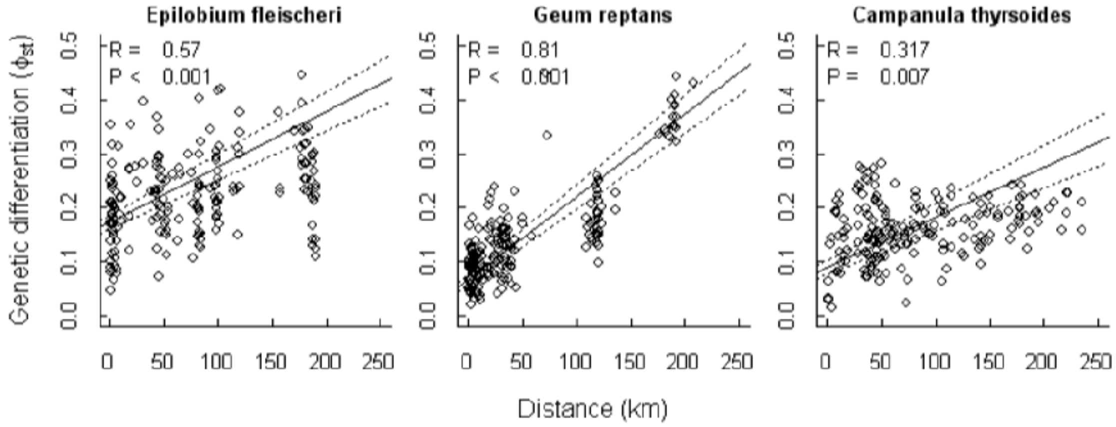

Figure 2. Matrix correlation of genetic (pairwise Φ_{st} values) and geographic distances. Solid line: standardized major axis (SMA) regression; dotted lines: SMA 95 % confidence interval.

(H_e: $r_s = -0.02$, $P = 0.95$; P_p: $r_s = -0.11$, $P = 0.64$) or *C. thyrsoides* (H_e: $r_s = 0.29$, $P = 0.22$; P_p: $r_s = 0.04$, $P = 0.88$). Population sizes for *E. fleischeri* were not estimated in the field and thus, could not be included. Molecular diversity was not related to altitude, except for a single significant increase of P_p with increasing altitude in *E. fleischeri* (*E. fleischeri*: H_e: $r^2 = 0.25$, $P = 0.1$; P_p: $r^2 = 0.19$, $P = 0.03$, *G. reptans*: H_e: $r^2 = 0.1$, $P = 0.1$; P_p: $r^2 = 0.01$, $P = 0.3$; *C. thyrsoides:* H_e: $r^2 = -0.04$, $P = 0.66$; P_p: $r^2 = -0.05$, $P = 0.86$).

Spatial differentiation

Among-population diversity indices were significantly different among the three species ($P < 0.001$) with *E. fleischeri* showing highest population differentiation ($\Phi_{st} = 22.7$), and *G. reptans* ($\Phi_{st} = 14.8$) and *C. tyrsoides* ($\Phi_{st} = 16.8$) lower differentation levels (for summary statistics see Table 2). In *E. fleischeri* all pairwise Φ_{st}-values were significantly different, while one population pair in each of *G. reptans* or *C. thyrsoides* was genetically not differentiated, although separated geographically by over 2 km (SCE, GR1) or 4 km (SCM, FTA), respectively.

For all three species we found a significant isolation by distance (IBD) pattern as calculated with Mantel test statistics (Fig. 2, Table 2; *E. fleischeri*: $R = 0.57$, $P < 0.001$; *G. reptans*: $R = 0.81$, $P < 0.001$; *C. thyrsoides*: $R = 0.32$, $P = 0.007$). The slope of the standardized major axis regression lines (SMA) was significantly steeper in *G. reptans*

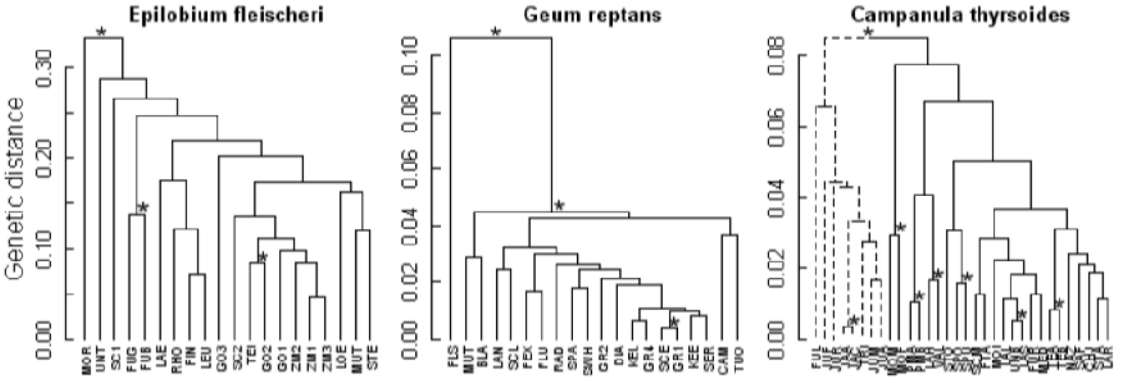

Figure 3. Dendrograms of the UPGMA cluster analysis based on Nei's (1978) unbiased measure of genetic distance (* indicate bootstrap values larger than 50%, based on 10 000 permutations). Dashed clusters represent populations belonging to a different glacial refugia.

compared to the other two species (each $P < 0.001$), while no difference was found between *E. fleischeri* and *C. thyrsoides* ($P = 0.12$). The equations for the (SMA) including the slope and intercept specific standard errors are described as follows: *E. fleischeri*: y = 0.01729 (0.0062) + 1.025 10^{-03} (6.159 10^{-05}) x; *G. reptans*: y = 0.06563 (0.00512) + 1.536 10^{-03} (6.512 10^{-05}) x; *C. thyrsoides*: 0.08874 (0.00671) + 9.34 10^{-04} (6.502 10^{-05}) x, $R = 0.089$.

UPGMA cluster analysis (Fig.3) and application of Monmonier's algorithm (results not shown) for *E. fleischeri* and *G. reptans* revealed no geographic patterns of genetic differentiation that coincide with the proposed areas of post-glacial migration in the western Alps (Schönswetter et al, 2005). In both species, all population pairs were significantly differentiated but stable dendrogram clusters were only present for a single geographically isolated population each (MOR and FLS, respectively). Even populations in close vicinity did not consistently group together. In *C. thyrsoides*, with both methods we detected a clear seperation of populations located in western Switzerland from those in central and eastern regions with the north-south running Aosta-Rhône-Valley as the geographic border. Within the two main UPGMA clusters, stable branches were mostly formed by population pairs separated by distances below 2 km. Nevertheless, two population pairs with distances of 49 and 73 km from each other formed stable ties (LAH, VAL, and UNB, LAS, respectively). All population pairs were significantly differentiated.

For *C. thyrsoides* we could further evaluate the effect of post-glacial migration on population differentation by repeating the analyses with the whole data set of 24+8 populations (Fig. 3). A high proportion of variability was explained by genetic differences between the two groups of populations (Φ_{ct} = 10.3%) and the total genetic variability among populations amounted to 27.2% (Φ_{st}). Further, we found a significant IBD pattern within the 8 western populations ($R = 0.34$, $P = 0.03$), the 24 central/eastern populations ($R = 0.32$, $P < 0.001$) as well as for the total of 32 populations ($R = 0.53$, $P < 0.001$). The slopes of the SMA lines among the three regions were all significantly different ($P < 0.001$), steepest for western populations (n = 8), intermediate for the central/eastern ones (n = 24), and lowest for all populations (n = 32).

Discussion

Spatial isolation and genetic differentiation

In all three species we found a significant and positive IBD pattern which supports our hypothesis that genetic connectivity among populations decreases with increasing spatial distance as a result of natural fragmentation. At a distance of less than 200 km population pairs in all species were highly differentiated with maximum Φ_{st}-values ranging from 29% (*C. thyrsoides*) to 44% (*E. fleischeri*, *G. reptans*). In addition, Mantel plots of each species showed a considerable amount of scatter demonstrating a large variability of genetic differentiation at a given distance. This is most pronounced for *E. fleischeri* where even at a distance of less than 5 km Φ_{st}-values ranged from 5 to 35%. Such a high variability suggests that genetic connectivity between populations has a strong stochastic component at all spatial scales and that the populations are not in gene flow/drift equilibrium (Hutchison and Templeton, 1999). Apart from genetic drift, founder events during post-glacial colonization and/or bottlenecks due to demographic stochastisity may contribute to the large variability in pairwise Φ_{st}-values encountered.

For *C. thyrsoides* we found that populations belong to two different areas of post-glacial migration, so that we decided to standardize the data for among-species comparisons. However, analysing the complete data set of *C. thyrsoides* allows us to shortly evaluate, first, the potential effect of the Quaternary history on molecular diversity patterns and second, the bias introduced to those patterns when the effect of the Quaternary is not acknowledged. The two groups of populations are significantly differentiated with 10.3% (Φ_{ct}) of the genetic diversity partitioned between the groups. Hence, gene flow between the groups has not been strong enough during the last centuries or millenia as to mask the effect of isolation in different periglacial refugia. Within each group we found a significant IBD pattern suggesting that recent gene flow is also impeded (see above). Trans-border analysis now shows an increase of Φ_{st}-values from 16.8% (20 populations) to 27.2% (32 populations), an increasing Mantel correlation from $R = 0.32$ to $R = 0.53$, but a decreasing slope of the SMA regression line. These results clearly support our decision to standardize the species' data sets and provide indication that genetic diversity patterns in the alpine are not shaped by natural fragmentation alone.

Still, it is important to ask whether IBD is a characteristic feature of alpine plant populations, and whether IBD is more pronounced under alpine compared to lowland conditions. The few studies focussing on alpine plants find inconsistent IBD relationships and the significance of an IBD pattern to be dependent on population sub-grouping or, as discussed above, on geographic scale and post-glacial migration history. For example, in *Eryngium alpestris* no significant IBD was found at a geographic distance of 250 km (Gaudeul et al., 2000). In contrast, when subdivisions of 2 of these 14 populations were acknowledged the overall IBD was significantly positive. For the subdivided populations within individual valleys, a positive IBD was only found at distances up to 0.2 or 2 km. Such an effect of population subdivision was not found in any of our study species (data not shown). In a study on *Rumex nivalis*, IBD was only significant within a single large region of Switzerland (the same glacial refugia investigated in the present study), but the correlation was non-significant when populations from larger distances were included (Stehlik, 2002). Moreover, a significant IBD could be found in *Hypericum nummularium* for populations in the Alps, but IBD was lacking in the Pyrenees (Gaudeul, 2006). As for alpine species, no clear indication of the causes governing an IBD pattern or its magnitude is visible for lowland species of fragmented or continous populations. Presence or absence of an IBD is, again, explained by a multitude of potentially important factors, such as time since colonization (Jacquemyn et al., 2004), general rarity (Dittbrenner et al., 2005), breeding system (Irwin, 2001), dispersal potential (Coleman and Abbott, 2003), ocean currents (Bond et al., 2005) or maximum geographic distance (Hilfiker et al., 2004; Moyle, 2006). In the present study, we standardized a number of the above mentioned factors that potentially influence IBD and we will discuss the relative role of specific life-history traits further down. It can however be said, that in the current absence of standardized comparative studies or meta-analyses with a larg number of species, there is no ample evidence that alpine species behave differently than lowland species or that natural fragmentation enhances IBD.

Spatial isolation and within-population diversity

Our three species have similar values of mean genetic diversity (H_e = 0.19 to 0.21) which are in concordance with other alpine species such as *Eryngium alpestris* (H_e = 0.20, Gaudeul et al., 2000) and *Trollius europeus* (H_e = 0.22, Despres et al., 2002) or the rather wide range of H_e found in many other alpine or lowland species (see Till-Bottraud and Gaudeul, 2002; Nybom, 2004). The results for mean polymorphic loci are much more diverging but nevertheless high and show a considerable amount of variance (P_p = 62 - 75, Table 2). It has to be born in mind that measures of H_e are considered less sensitive to detect consequencs of isolation and population bottlenecks than alternatives such as allelic diversity (Amos and Balmford, 2001). Therefore, we would expect to find an effect of fragmentation and an effect of declining population sizes with measures of P_p, rather than H_e. Particularly in species with a short generation time low levels of P_p should be found (e.g. Young et al., 1996; Till-Bottraud and Gaudeul, 2002). Our data does show the lowest levels of P_p in the relatively short-lived *C. thyrsoides* as compared to the long-lived, clonal *E. fleischeri* or *G. reptans*. However, levels of P_p in *C. thyrsoides* are still high and we do not find a significant correlation between population size and P_p even though a number of populations are comprised of less than 100 reproductive individuals. This result suggests that even small populations of *C. thyrsoides* with only 45 flowering individuals are large enough to maintain high levels of genetic diversity and, as has been shown recently, do not need to suffer from inbreeding depression (Ægisdóttir et al., 2006). Genetic diversity was also not related to altitude so that the adaptive potential to buffer consequences of global warming is likely to be similar in all populations, irrespective of their altitudinal position. The single significant increase of P_p in *E. fleischeri* with increasing altitude shows a poor correlation and can be shown to be an artefact of primer selection. In this one case, the significance of the correlation was highly influenced by only five loci of a single primer. Simulations with the complete data set of 89 polymorphic loci of 10 primers showed not significant difference of P_p in relation to altitude. In general, we have no indication that natural fragmentation has led to lasting consequence on within-population diversity, given the high values of H_e and P_p in all three species and the absence of a population size effect.

Genetic differentiation and life-history traits

The strength of IBD is indicated by the slope of the SMA line which was significantly higher in *G. reptans* and not different between *E. fleischeri* and *C. thyrsoides*. From our assumption that a high long-distance dispersal (LDD) potential reduces the genetic difference among populations, we expected a less pronounced IBD patter for *E. fleischeri* than for the other species. *E. fleischeri*'s LDD potential was modelled to be by a factor 100 - 500 higher than *G. reptans* and *C. thyrsoides* (Table 1). However, our data does not distinguish between good and poor dispersers regarding IBD or mean Φ_{st} in a plausible way. Other life-history traits or a combination thereof may be more influential on genetic similarity of populations than seed dispersal alone. LDD by pollen is unlikely since all of our study species were pollinated by bumble bees, smaller hymenoptera, or flies which usually show flight activity within a range of < 1 km (see Tab. 1, Osborne et al., 1999; Darvill et al., 2004). A life-form effect, e.g. annuals vs. long-lived perennials (Nybom, 2004), is also unlikely because the species all are perennial taxa. Although ramet age varied among species, population persistence as well as genet persistence for clonal species can exceed more than several 100 yrs.

A possible explanation for our different Φ_{st}-values may be found in the breeding systems. Higher levels of among-population diversity are reported in selfing species as opposed to obligatory outbreeders (Hamrick and Godt, 1989; Nybom and Bartish, 2000; Nybom, 2004). *E.fleischeri* is known to be a mixed-mating species, i.e. generally outcrossing but allowing for selfing, and should therefore tend to be more differentiated than populations of *G. reptans* or *C. thyrsoides*. Our data indicates such a relationship but a general conclusion is difficult given only three species that differ also in a number of other life-history traits. In comparison with the few studies on alpine plants that employ dominant nuclear markers, our results for the outcrossing *G. reptans* (Φ_{st} = 14.8) and *C. thyrsoides* (Φ_{st} = 16.8) are within the broad range of Φ_{st}-values calculated for the outcrossing *Saxifraga oppositifolia* (5 %, Gugerli et al., 1999), *Phyteuma globularifolia* (13 %, Schönswetter et al., 2002), *Trollius europeus* (16 %, Despres et al., 2002), *Erithrichum nanum* (17 %, Stehlik et al., 2001) and *Bupleurum stellatum* (22

%, Schönswetter and Tribsch, 2005). *Epilobium fleischeri* partitioned 22.7% of its genetic diversity among populations, which is considerably lower than the mixed-mating *Eryngium alpestris* (42 %, Gaudeul et al., 2000) or the selfing *Saxifraga cespitosa* (42 %, Tollefsrud et al., 1998) so that *Epilobium fleischeri* seems to behave like an outcrossing species. However, *Eryngium alpestris* is adapted to exozoochory which is assumed to lead to higher Φ_{st}-values than wind-dispersal (Nybom, 2004). We could therefore argue that a high LDD potential in *Epilobium fleischeri* may reduce the differentiating effects of its mating system.

Unfortunately, up to date there is no study that attempted to elucidate the complex interaction of life-history traits on genetic diversity partitioning in a standardized geographic setting. In this context, it is important to note that for the above mentioned alpine species we did not use the 'global Φ_{st}-values' of each literature source but those values associated with 'genetic differentiation among populations within regions' in which case the size of a region was similar to our study. This reduced the bias of geographic scale which may have been the reason behind a high 'mean RAPD Φ_{st}-value' of 27% for outcrossing species as listed in a review by (Nybom, 2004). To conclude at this point, our three species have Φ_{st}-values between 14.8% and 22.7% which demonstrate a relatively restricted differentiation of populations and thus, we cannot confirm our initial hypothesis that natural fragmentation in the alpine environment has led to a particularly high population differentiation. The biology of a species appears to have a major influence on genetic diversity partitioning and largely masks an effect of spatial isolation.

Conclusion

Our results indicate that natural fragmentation has led to a significant decline of relatedness between population pairs with increasing geographic distance. However, this pattern of isolation by distance also shows a considerable amount of variation with high levels of differentiation even at small spatial scales (< 5 km). This suggests that genetic connectivity of alpine plant populations has a strong stochastic component at all spatial scales and further, that population similarity is not directly associated with the long-distance dispersal potential of a species. Other life-history traits (e.g. breeding system) or a combination thereof may considerably influence genetic diversity partitioning in alpine plants and in this respect, alpine plants do not differ from lowland plants of fragmented or continuous populations. Also, natural fragmentation does not necessarily result in particularly high levels of mean genetic population differentiation or in a loss of genetic diversity within populations of alpine plants. Even small populations of less than 50 reproductive individuals can maintain comparably high levels of genetic diversity.

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Appendix 1 a. *Epilobium fleischeri*. Sampling location, population abbreviation, co-ordinates, elevation, number of sampled plants per population (N), population size, H_e: Nei's expected heterozygosity, SI: Shannon diversity index, P_p: percentage of polymorphic loci. NA: not available

	Location	Population	Co-ordinates[a]	Elevation[b]	N	Pop. size	H_e	SI	Pp (%)
1	Zermatt, Zmutt, VS	ZM1	617' 580/095' 125	2260	20	NA	0.20	0.33	82.69
2	Zermatt, Zmutt, VS	ZM2	618' 600/094' 555	2183	20	NA	0.18	0.29	71.15
3	Zermatt, Zmutt, VS	ZM3	618' 980/094' 760	2189	20	NA	0.21	0.33	86.54
4	Teifbach, VS	TEI	621' 620/094' 780	1930	20	NA	0.18	0.28	73.08
5	Furgg, VS	FUG	620' 000/092' 925	2435	20	NA	0.22	0.35	80.77
6	Furggbach, VS	FUB	621' 145/093' 675	2000	20	NA	0.20	0.31	69.23
7	Gornergletscher, VS	GO1	623' 180/092' 510	2100	20	NA	0.17	0.27	71.15
8	Gornergletscher, VS	GO2	623' 020/092' 870	2050	20	NA	0.22	0.34	78.85
9	Gornergletscher, VS	GO3	622' 130/093' 710	2020	20	NA	0.18	0.29	75.00
10	Findelgeltscher, VS	FIN	628' 880/095' 025	2470	20	NA	0.20	0.32	78.85
11	Leukerbad, VS	LEU	613' 600/137' 510	1700	20	NA	0.14	0.23	73.08
12	Lämmerenboden, VS	LAE	610' 650/138' 500	2310	20	NA	0.20	0.32	76.92
13	Loetschental, VS	LOE	635' 910/144' 375	2090	20	NA	0.20	0.30	67.31
14	Unteralptal, UR	UNT	691' 100/164' 650	1560	20	NA	0.15	0.24	65.38
15	Muttgletscher, VS	MUT	673' 700/158' 120	2060	20	NA	0.19	0.30	75.00
16	Rhonegletscher, VS	RHO	672' 050/158' 100	1790	20	NA	0.22	0.34	78.85
17	Steingletscher, BE	STE	675' 850/175' 350	1940	20	NA	0.21	0.32	76.92
18	Scaletta, GR	SC1	790' 200/176' 525	2100	20	NA	0.19	0.31	75.00
19	Scaletta, GR	SC2	790' 925/175' 900	2250	20	NA	0.21	0.33	80.77
20	Morteratsch, GR	MOR	791' 800/145' 500	2010	20	NA	0.13	0.22	59.62

[a] Co-ordinates according to the Swiss topographical maps (Bundsamt für Landestopographie, Wabern, Switzerland).
[b] Elevation in meters above sea level

Appendix 1 b. *Geum reptans*.

	Location	Population	Co-ordinates[a]	Elevation[b]	N	Pop. size	H_e	SI	Pp (%)
1	Fluhseeli, BE	FLS	604' 700/139' 700	2070	17	1500	0.16	0.24	48.98
2	Muttgletscher, VS	MUT	674' 500/156' 600	2520	18	5000	0.22	0.33	63.27
3	Blauberg, UR	BLA	675' 030/157' 920	2580	17	7000	0.22	0.33	65.31
4	Val Fex, GR	FEX	781' 325/137' 730	2140	20	3500	0.24	0.36	81.63
5	Diavolezza, GR	DIA	794' 025/143' 500	2980	19	1000	0.24	0.36	71.43
6	Val da Cambrena, GR	CAM	797' 100/142' 300	2340	20	8000	0.22	0.33	65.31
7	Piz Languard, GR	LAN	793' 075/151' 450	3080	20	1500	0.22	0.33	73.47
8	Vadret da Porchab., GR	KEE	787' 100/168' 020	2680	20	5000	0.22	0.33	69.39
9	Vadret da Porchab., GR	KEL	785' 165/168' 460	2340	20	5000	0.22	0.34	79.59
10	Sertig, Gletschtälli, GR	SER	787' 450/173' 800	2460	20	5000	0.21	0.32	69.39
11	Scalettapass, GR	SPA	789' 935/174' 380	2600	20	1500	0.19	0.29	67.35
12	Scaletta, GR	SCE	791' 600/175' 430	2500	20	2000	0.22	0.34	73.47
13	Scaletta, GR	SCL	791' 750/175' 500	2330	20	8000	0.22	0.33	73.47
14	Vadret da Grialet., GR	GR4	792' 785/175' 850	2630	20	3000	0.23	0.35	81.63
15	Vadret da Grialet., GR	GR2	793' 220/175' 380	2660	20	9000	0.25	0.37	73.47
16	Vadret da Grialet., GR	GR1	793' 800/175' 300	2600	20	2000	0.22	0.34	79.59
17	Vadret da Radönt, GR	RAD	792' 585/178' 485	2640	20	4000	0.23	0.36	79.59
18	Flüela Schwarzh., GR	SWH	791' 400/178' 750	2900	16	500	0.22	0.34	69.39
19	Flüelapass, GR	FLU	791' 700/180' 300	2420	19	4000	0.19	0.29	71.43
20	Vadret Tuoi, GR	TUO	806' 275/191' 300	2610	20	5000	0.21	0.31	69.39

Appendix 1 c. *Campanula thyrsoides*.

	Location	Population	Co-ordinates[a]	Elevation[b]	N	Pop. size	H_e	SI	Pp (%)
1	Col du Marchairux, VD	JUM	508' 900/156' 400	1440	23	1000	0.17	0.25	53.19
2	Les Amburnez, VD	JUA	507' 480/155' 100	1340	23	1000	0.22	0.32	57.45
3	Pre du Rolle, VD	JUR	508' 983/155' 652	1377	23	150	0.16	0.25	57.45
4	Pres de Four, VD	JUF	498' 400/148' 450	1430	23	10000	0.21	0.32	65.96
5	Col du Jamon, VD	JAA	564' 830/145' 050	1630	23	100	0.18	0.27	59.57
6	Col du Jamon, VD	JAC	564' 589/144' 944	1670	23	80	0.20	0.31	63.83
7	Lac du Fully, VS	FUL	574' 000/113' 200	2100	23	500	0.21	0.31	57.45
8	Trient, Les Tseppes, VS	TRI	564' 350/099' 500	2020	23	50	0.24	0.35	65.96
9	Lac du Moiry, VS	MOI	609' 932/109' 638	2266	23	50000	0.21	0.31	57.45
10	Stockhorn, BE	STO	607' 737/171' 103	1980	23	100	0.22	0.34	68.09
11	Schynige Platte, BE	SPO	636' 225/167' 625	1990	23	600	0.22	0.34	68.09
12	Schynige Platte, BE	SPU	636' 600/167' 150	1890	23	500	0.20	0.30	57.45
13	Furka, UR/VS	FUR	674' 850/158' 825	2430	23	30000	0.22	0.33	68.09
14	Unterschächen, UR	UNB	702' 500/193' 200	1900	23	500	0.20	0.29	55.32
15	Langwies, Listbod., GR	LAL	776' 750/191' 510	2000	23	300	0.18	0.28	59.57
16	Langwies, Strassb., GR	LAS	775' 875/190' 550	1870	23	7000	0.21	0.33	76.6
17	Langwies, Holzbüel, GR	LAH	775' 010/188' 875	1700	23	50	0.19	0.29	53.19
18	Vals, Peil, GR	VAL	735' 375/160' 425	1850	23	100	0.20	0.30	70.21
19	Safiental, GR	SAF	742' 851/174' 289	1857	23	50	0.22	0.33	61.7
20	Medels, Parjurs, GR	MED	742' 800/157' 700	1870	23	50	0.22	0.33	61.7
21	Monstein, Mäschen. GR	MOM	779' 668/173' 708	1961	23	45	0.18	0.29	68.09
22	Monstein, Fanex., GR	MOF	780' 750/174' 910	2220	23	250	0.19	0.29	59.57
23	Parsennmeder, GR	PMA	784' 030/191' 473	1995	23	5000	0.20	0.30	57.45
24	Parsennmeder, GR	PMB	784' 478/191' 548	1910	23	100	0.19	0.29	57.45
25	Churwalden, Joch, GR	CHJ	762' 300/185' 100	1890	23	150	0.21	0.32	59.57
26	St. Antönien, GR	STA	782' 203/201' 989	1943	23	250	0.22	0.32	61.7
27	Alp Laret, GR	LAR	784' 234/153' 944	2180	23	300	0.19	0.28	55.32
28	Albula Pass, Naz, GR	NAZ	778' 193/162' 751	1755	23	150	0.21	0.32	63.83
29	Schuol, La Motta, GR	SCM	816' 400/188' 400	2142	23	2000	0.19	0.28	57.45
30	Ftan, Prui, GR	FTA	812' 505/187' 750	2100	23	150	0.18	0.27	55.32
31	Tschlin, Alp Tea, GR	TEA	828' 250/198' 250	2200	23	150	0.20	0.30	61.7
32	Tschlin, Alp Tea, GR	TEB	827' 800/198' 000	2150	23	200	0.19	0.30	63.83

[a] Co-ordinates according to the Swiss topographical maps (Bundsamt für Landestopographie, Wabern, Switzerland).

[b] Elevation in meters above sea level

Chapter 3

Evolutionary demography of the long-lived monocarpic perennial *Campanula thyrsoides* in the Swiss Alps

Patrick Kuss, Mark Rees, Hafdís Hanna Ægisdóttir, Jürg Stöcklin

Summary

The optimal timing of reproduction is a key factor for population persistence in a variable environment. This is especially crucial for monocarpic species in which reproduction is fatal. Here we study the demography of the long-lived monocarpic perennial *Campanula thyrsoides* in order to understand the mechanisms behind delayed flowering in a temperate alpine mountain system usually devoid of monocarpic plants. We used a novel approach combining permanent plot and herb chronology data from two populations in the Swiss Alps to parameterize site-specific integral projection models (IPMs). The existing IPMs were extended to incorporate the biological constraint that rosette size cannot readily be measured in the year of flowering. We found all main demographic functions, i.e. growth, probability of flowering, probability of survival, and fecundity, to be strongly size-dependent with a pronounced threshold size of flowering. Mostly, there was good agreement between model predictions and the quantitative field study regarding population growth rate, λ, net reproductive rate, R_0, and generation time, T, with survival and growth being the critical determinants of λ, contributing to 88-90% to overall elasticities. We found only weak selection pressure operating on the observed flowering strategy which was close to the predicted environmental stable strategy (ESS). Additional simulations showed that stable $\lambda \approx 1$ can be maintained at extremely low establishment rates and likewise, with increased microsite availability, populations increase rapidly. We conclude (1) that *C. thyrsoides* follows a simple flowering strategy where the gain from delaying flowering comes from increased fecundity as a result of growth and (2) that the combination of methods applied have proven adequate to investigate the demography of long-lived monocarpic species even in a comparably short census period of three years.

Keywords: Alpine plant demography, elasticity analysis, evolutionary stable strategy, flowering threshold, integral projection model, semelparity, size structured populations, stable size distribution

Introduction

Monocarpic (= semelparous or hapaxant) perennials are plants that delay flowering for more than one year and die after first reproduction. In contrast to strictly annual or biennial species which are numerous in taxa and found in many habitats throughout the world, monocarpic perennial species are far less abundant and predominately found in moderately disturbed lowland sites (sand dunes, ruderal and/or arid and semi-arid habitats) or in the alpine zone of subtropical and tropical mountains (bogs, paramo; e.g. Bruinenberg 1980; Körner, 2003; Young and Augspurger, 1991). In some extreme cases plants delay flowering for many decades, forming giant rosettes and reaching sizes of several meters in height (e.g. *Puya spp*., *Espeletia spp*., *Lobelia spp*., *Echium spp*.; Körner, 2003). On the other hand, the flora of high-latitudes or temperate mountain ranges is almost devoid of monocarpic perennials and within the European Alps *Campanula thyrsoides* is one of the few representative of this life-form occurring from tree-line ecotone into the alpine belt (Aeschimann *et al.*, 2005).

The flowering strategy of monocarpic plants can be understood in terms of the benefit which accrue through growth and consequent increases in fitness and cost which are the result of death before flowering (Metcalf et al., 2003). Although in a variable environment other factors such as non-linear averaging and non-equilibrium dynamics can be important (Rees et al., 2004). The basic demographic functions follow common relationships and are largely size-related: with increasing plant size the relative growth rate decreases, and the probabilities of survival and flowering as well as the fecundity increases. Also, in some species the absence of a persistent seed bank means that the only effective buffer to fluctuations in the environment is a considerable variability in generation time (Young and Augspurger, 1991). Most likely this generation overlap can be attributed to different growth conditions in adjacent microhabitats and to a smaller extent seems to be genetically determined (de Jong and Klinkhamer, 1988; Wesselingh et al., 1996; Young and Augspurger, 1991). The simple demography of monocarpic species allows testing of evolutionary ideas because a species' cost of reproduction is easily quantified and the time of flowering is a key determinant of Darwinian fitness. In this context Klinkhamer and de Jong (1983) have shown that delayed flowering in monocarpic species will be favored in situations with large environmental fluctuation and further that the monocarpic life-history may be an alternative to polycarpy (= iteroparity) because generation overlap also leads to temporal averaging of reproductive success. In all cases, populations follow an evolutionary stable strategy (ESS) when a resident phenotype cannot be invaded and replaced by a mutant phenotype with a different flowering strategy (Maynard Smith, 1989) be it e.g. early- vs. late-flowering or monocarpy vs. polycarpy. However, due to the longevity of many alpine monocarpic species and the associated difficulty in following their whole life-cycle, the majority of demographic and evolutionary investigations have focused on temperate lowland species with generation times of less than 5 years (see review by Metcalf et al., 2003). Recent developments in age determination of non-woody plants applying 'herb chronology' techniques (Dietz and Ullmann, 1998; Schweingruber and Poschlod, 2005; von Arx and Dietz, 2006) as well as in modelling population demographic behaviour using Integral Projection Models (Easterling et al., 2000; Ellner and Rees, 2006) have greatly facilitated the investigation of alpine monocarps with field campaigns restricted to a short census period.

The Integral Projection Model (IPM) is a powerful and flexible modelling framework that describes how a continuously size-structured population changes in discrete time (Easterling et al., 2000), and the approach has recently been generalised by Ellner and Rees (2006) to cover complex life cycles where individuals are classified by multiple traits (i.e. size, age and condition). IPMs allow a more natural description of a continuously size structured population than traditional matrix models (Caswell, 2001) because division of populations into distinct classes or 'stages' is avoided. This is of particular importance because in many continuously size-structured populations no natural stage boundaries exist and an arbitrary selection of 'stages' will bias sensitivities and elasticities (Enright et al., 1995). However, IPMs have many properties in common with matrix models such that predictions of the population growth rate, λ, generation time T, net reproductive rate, R_0 (= rate of population increase over a generation), as well as stable age- and size-distributions, sensitivities and elasticities can readily be made (Easterling, 1998; Ellner and Rees, 2006). Furthermore, the framework of an IPM facilitates the assessment of the evolutionary stable strategy (ESS) and thus can avoid time-consuming individual-based simulations of the evolutionary process (Ellner and Rees, 2006).

In this study we use a novel approach that combines permanent plot data from a 3-year census period and a single season herb-chronology survey of two populations of *C. thyrsoides* in the Swiss Alps to parameterize site-specific IPMs. We extended existing IPM framework to incorporate the biological constraint that rosette size cannot readily be measured in the year of flowering which results in an additional time lag in the model. Our objectives are to investigate this rare species' strategy to persist in and cope with the unpredictability of the alpine environment by assessing demographic functions and life-stage transitions that determine λ, R_0, and T. We further evaluate whether the observed flowering strategy of *C. thyrsoides* is an evolutionary stable strategy and to which extent this long-lived alpine monocarpic species behaves similar to short-lived monocarpic species previously studied in the lowlands.

Methods

Biology of the species

Campanula thyrsoides L. (Campanulaceae) is native to the European Alps and adjacent mountain ranges to the East (Dinarids and Balcans) and North-West (Jura) usually found from treeline ecotone into the alpine belt (1600 to 2500 m a.s.l.; Chapter 5). The plant is rare but locally abundant with average populations consisting of a few hundred to several thousand individuals. Typical habitats are species-rich grasslands and screes on limestone or carbonate-bearing schists, and is characteristic species of the Caricion ferrugineae syntaxa (Grabherr and Mucina 1993). Traditionally, *C. thyrsoides* has been considered to be a biennial species (Hegi, 1975) but plants can live up to 16 years growing significantly older with increasing altitude (Hänger, unpublished). The species shows a two-phased rosette growth each year: a spring rosette which reaches maximum size at the end of the growing season, and a distinct summer rosette with smaller leaves that is initiated within the spring rosette few weeks prior to peak season conditions. Rosette leaves generally wither over winter. In less than 10% of all plants two or rarely more sister rosettes are formed either due to herbivory or other damage to the meristem. The sister rosettes are autonomous regarding size-related flowering thresholds but will die once flowering has been initiated in any sister rosette. Comparable to many other monocarps, *C. thyrsoides* forms a large tap root which can grow 1 m long. Flowering plants display a dense spike of around 100 yellow flowers that are pollinated by bumble bees and other hymenoptera. Individuals produce between 15000 and 50000 viable seeds as the only mean of reproduction. Seed dispersal is restricted to the close vicinity of the mother plant with little potential for long-distance wind dispersal (Chapter 2). Seeds germinate after snowmelt in the following spring and no evidence exists of a persistent seed bank (Hegi, 1975; Jäger, 2000). *C. thyrsoides* is protected by law in most of the European Alpine countries but the species is not endangered in its native range (Korneck et al., 1996; Landolt, 1991; Niklfeld and Schratt-Ehrendorfer, 1999).

Data collection

Population data on *Campanula thyrsoides* were used from two sites in the Swiss Alps collected from July 2003 until August 2005. One site was at Schynige Platte (SP), Canton Berne (N 46°40’ O 7°54’, 1990 m, SW-exp., 30°), and the other at Furka Pass (FU), Canton Valais (N 46°34’ O 8°25’, 2430 m, S-exp., 40°). At both sites, individuals of *C. thyrsoides* were growing at low densities within alpine meadows. Twenty-one (SP) and thirty-five (FU) 1 × 1-m quadrates were distributed at random within each population, with the prerequisite to cover a minimum of 300 plants per site. Within each plot, the position, number of leaves, length and width of longest leaf (in mm) of each non-flowering plant was recorded on visits at the peak of the growing season (SP: end July; FU: mid August). For flowering individuals, we counted the number of capsules and extrapolated seed set per individual by averaging counts of viable seeds per 5 capsules of 10 individuals per site. To test viability, a common garden was established at FU and seeds from both sites were sown into cleaned soil collected in the field. Germination success was recorded after snowmelt in the following year. The total data set includes 515 individuals with 1371 observations from SP and 416 individuals with 998 observations from FU. For each year and population the annual population growth rates could then be calculated from the life-table entries: $\lambda = n(t+1)/n(t)$.

In 2005, plants from two plots at each site were harvested to measure above-ground biomass in addition to morphometric data. Stained cross sections of roots were used to age each individual (SP: 122 inds.; FU: 132 inds.) applying standard herb chronology techniques (von Arx and Dietz, 2006) Thin sections were photographed through the phototube of a dissecting microscope (Wild M3Z, Heerbrugg, Switzerland) using a digital camera (Nikon CoolPix 990 [Nikon, Tokyo, Japan]). In 90% of all plants, minimum and maximum age estimates differed by no more than one year. When different counts were obtained from the same individual, the mean of minimum and maximum age was taken (rounded up if the mean was not an integer) and used as an estimate of true plant age. Mean generation time for each population could then be calculated as the mean flowering age + one year. With λ and T known, it is straightforward to estimate the net reproductive rate, $R_0 = \lambda^T$ (Silvertown and Charlesworth, 2005).

As our measure of plant size we used the log-transformed product of number of leaves and length of longest leaf per vegetative rosette. This size parameter was highly correlated with the log-transformed above-ground dry weight (*cor* = 0.91, $P < 0.001$) and thus, seems to be the best non-destructive census measure of plant size in this species.

Data Analysis

Statistical models were fitted to the combined data from both sites, including site effects when they were significant. The fitted models are given in Table 1. Annual changes in plant size were size (P<0.0001) and site (P<0.0002) dependent, and are fitted by a linear model with size and site dependent variance (P<0.0001; see Figure 1a). These models were fitted using generalized least squares and the significance levels are derived from likelihood ratios. The probabilities of flowering, $p_f(x)$, and of survival, $s(x)$, were each modelled using logistic regressions and in both cases the interaction between size and site were significant ($p_f(x): \chi_1^2 = 8.46$, P<0.004, Figure 1b; $s(x): \chi_1^2 = 12.36$, P<0.0004, Figure 1c) suggesting models with separate intercept and size slopes for each site. Seed production was modelled using linear regression and was size ($F_{1,50} = 62.0$, P<0.0001) and site-dependent ($F_{1,50} = 7.15$, P<0.01), suggesting a model with site-specific intercept and a common slope (Figure 1d). We have no information on the distribution of seedling sizes from plants of different sizes and so we assume seedling size is independent of adult size. The distribution of seedling sizes was site-dependent ($F_{1,669} = 65.92$, P<0.0001) and modelled as a Gaussian distribution with site specific mean and common variance. The probability of seed establishment was estimated by dividing the total number of seedlings recorded by the total seed production the previous year at each site.

Despite seed production per quadrate varying from 0 to 68000 seeds the maximum number of seedlings recorded in a quadrate the following year was 25. At both sites there was no relationship between per quadrate seed production and subsequent recruitment (FU: $r_s = 0.09$, $P > 0.4$, $n = 70$; SP: $r_s = 0.17$, $P > 0.2$, $n = 42$). At Furka Pass, plant densities per 1 × 1 m plot ranged from 2 to 70 individuals with a mean of 18.1 and at Schynige Platte from 1 to 56 individuals with a mean of 13.3. We found no indication that seedling establishment was negatively density dependent. At Furka Pass, recruitment was independent of plant densities ($r_s = 0.00$, $P = 2.9$, $n = 105$), while at Schynige Platte

Table 1. Statistical models and parameter estimates describing demographic processes of *Campanula thyrsoides*. The models are functions of log rosette size, x, which is the product of the length of the longest leaf and the number of leaves (for details see text). The predicted values are the conditional mean, $\hat{y}$, and variance, σ_g^2, of log size next year given the current size; s: survival probability; p_f: flowering probability; f_n: fecundity; p_e: establishment rate. Figures in brackets are standard errors.

Demographic process	**Model**
Growth	FU: $\hat{y} = 1.18(0.08) + 0.88(0.01)x$ SP: $\hat{y} = 1.07(0.09) + 0.88(0.01)x$ Variance about the growth curve: FU: $\sigma_g^2 = 4.60\exp(-0.49\hat{y})$ SP: $\sigma_g^2 = 4.60\exp(-0.42\hat{y})$ $n = 1092$, $P<0.0001$
Survival probability	FU: $\text{Logit}(s) = -0.48(0.57) + 0.63(0.12)x$ SP: $\text{Logit}(s) = 3.6780(1.12) - 0.11(0.18)x$ $n = 1226$, $P<0.0001$
Flowering probability	FU: $\text{Logit}(p_f) = -47.19(9.35) + 5.91(1.20)x$ SP: $\text{Logit}(p_f) = -22.04(3.37) + 2.74(0.44)x$
Fecundity (seeds per flowering plant)	FU: $f_n = \exp(1.61(0.92) + 1.01(0.12)x)$ SP: $f_n = \exp(1.21(0.97) + 1.01(0.12)x)$ $n = 53$, $r_s = 0.49$, $P<0.0001$
Probability of seedling establishment	FU: $p_e = 0.00016$ SP: $p_e = 0.00078$
Distribution of seedling size	FU: Gaussian $\mu = 3.33(0.03)$ SP: Gaussian with $\mu = 4.00(0.08)$ $\sigma^2 = 0.58$, $n = 671$, $P<0.0001$

seedling establishment increased significantly with increasing plant densities ($r_s = 0.23$, $P < 0.001$, $n = 63$) suggesting facilitation of establishment. Our density-dependent model therefore assumes that population growth is limited by microsite availability.

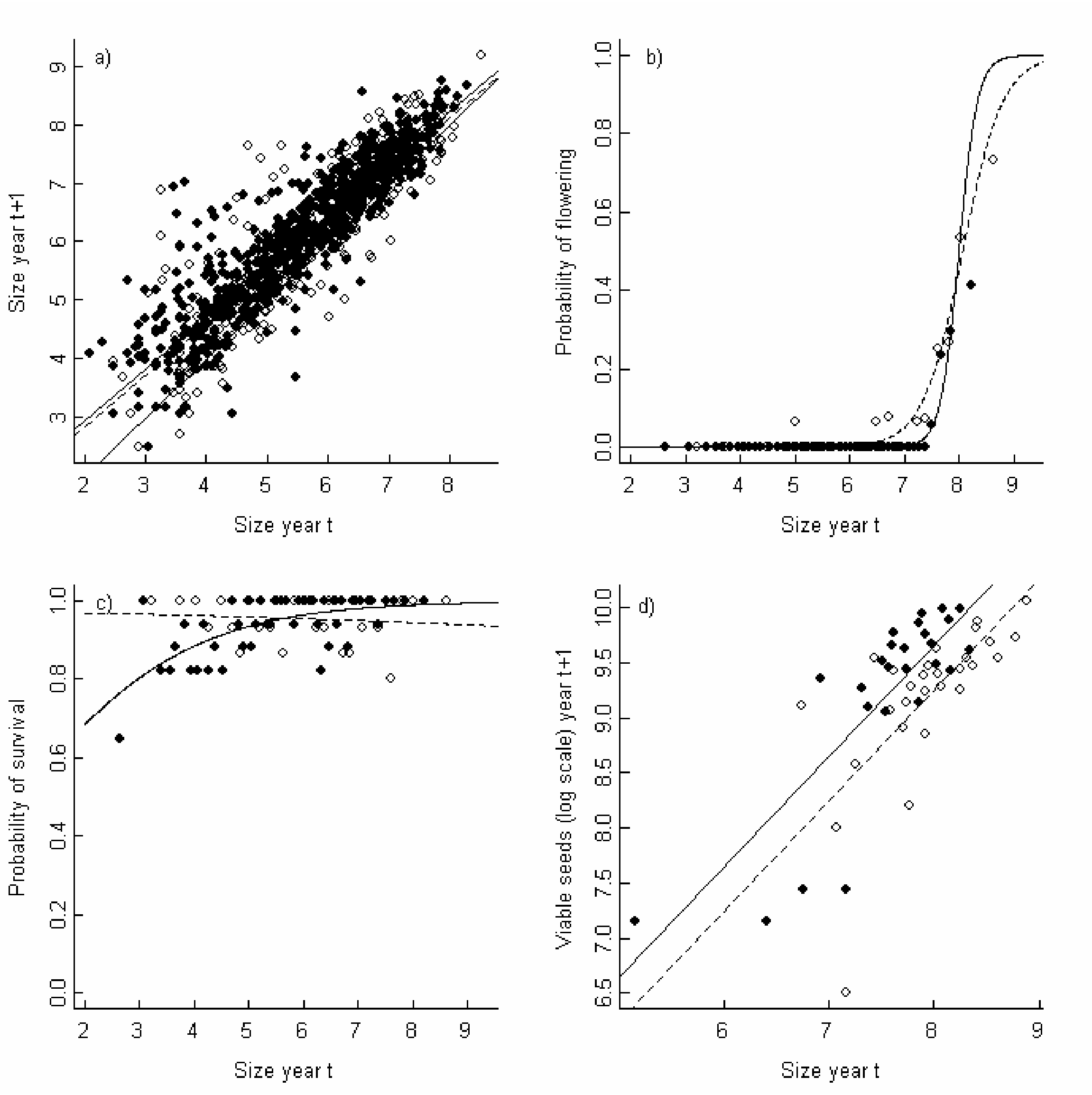

Figure 1. Demographic functions for *Campanula thyrsoides* a) growth relationship for plants size in successive years: note the lower line represents no growth, b) probability of flowering relationship: note for plotting the relationship the data have been binned however all statistical analysis was performed on the binary data, c) probability of survival relationship: again for plotting the data have been binned, and d) fecundity relationship. In all panels open symbols indicate FU and closed symbols SP. Size represents rosette size: log (number of leaves * length of longest leaf).

Integral projection model

The integral projection model describes how a continuously size-structured population changes in discrete time (Easterling *et al.*, 2000; Ellner & Rees, 2006). The state of the population is described by a distribution function $n(x,t)$, where $n(x,t)dx$ is the number of individuals with size in the range $[x, x+dx]$. The dynamics are then

$$\begin{aligned} n(y,t+1) &= \int_L^U [P(y,x)+F(y,x)]n(x,t)dx \\ &= \int_L^U K(y,x)n(x,t)dx. \end{aligned} \qquad 1$$

Where $K(y,x)$, known as the kernel, describes all possible transitions from size x to size y, including births, and $[L,U]$ is the set of all possible sizes. The kernel is composed of two parts describing the production of size *y* offspring by size *x* parents, $f(y,x)$, and the movement of individuals from size *x* to size *y*, $p(y,x)$. In *Campanula thyrsoides* a complication arises because the rosette size cannot be measured unambiguously in the year the plants flower. Therefore we predict the probability of flowering and seed production in year *t* as a function of size in year *t*-1, and any seedlings produced enter the population in year *t*+1. Therefore the integral projection models becomes

$$n(y,t+1) = \int_L^U P(y,x)n(x,t)dx + \int_L^U F(y,x)n(x,t-1)dx \qquad 2$$

In order to apply the model we must specify the dependence of survival, growth and fecundity on size. For *Campanula* we will write the fecundity function as

$$F(y,x) = p_e s(x) p_f(x) f_n(x) f_d(y) \qquad 3$$

where p_e is the probability of seedling establishment, $p_f(x)$ is the probability that an individual of size x flowers, $f_n(x)$ is the number of seeds produced, and $f_d(y)$ is the probability of producing a size *y* offspring. The survival-growth function is given by

$$P(y,x) = s(x)[1-p_f(x)]g(y,x) \qquad 4$$

where $s(x)$ is the probability of survival of an individual of size x, $g(y,x)$ is the probability of an individual of size x growing to size y. The probability of flowering, $p_f(x)$, enters the survival function, as reproduction is fatal in monocarpic species.

We solved the model numerically using the midpoint rule (Ellner & Rees, 2006). To do this we define *mesh points* x_i by dividing the interval [*L, U*] evenly into m size classes and set x_i at the midpoint of the *i*th class:

$$x_i = L + (i - 0.5)h, \quad i = 1, 2, \cdots, m \qquad 5$$

where $h = (U - L)/m$. The midpoint rule approximation to 2 is then

$$n(x_j, t+1) = h\sum_{i=1}^{m}[P(x_j, x_i)n(x_i, t) + F(x_j, x_i)n(x_i, t-1)], \qquad 6$$

which can be represented as a matrix multiplication

$$n(t+1) = \mathbf{K}n(t) \qquad 7$$

where **K** is the matrix of the form

$$\mathbf{K} = \begin{pmatrix} \mathbf{P} & \mathbf{F} \\ \mathbf{I} & \mathbf{0} \end{pmatrix}$$

where the $(i,j)^{\text{th}}$ entry of **F** is $hF(x_i, x_j)$, for **P** it's $hP(x_i, x_j)$, **I** is the $m \times m$ identity matrix, and $n(t) = (n(x_1, t), \cdots, n(x_m, t), n(x_1, t-1), \cdots, n(x_m, t-1))^T$. For the numerical calculations we used 100 mesh points for size and in the size × age model 50 age-classes.

Having constructed **K** it is then straightforward to calculate various statistics summarising population growth (λ, R_0; Ellner and Rees, 2006) and generation time ($T = \ln(R_0)/\ln(\lambda)$). The net reproductive rate R_0 is the long-term generation to generation population growth rate; so if g_0 is the total current population (generation 0), g_1 their total number of offspring counted at birth (generation 1), g_2 the total number of offspring they produce and so on, then

$$\lim_{k\to\infty} g_k / R_0^k = G \qquad 8$$

with the value of G depending on the initial population distribution (Ellner & Rees, 2006). As in matrix models, R_0 and λ are related: $\lambda - 1$ and $R_0 - 1$ have the same sign. To obtain estimates of the uncertainty associated with the various measures of population growth we used a bootstrap procedure, see Appendix A for details.

We further evaluated the influence of the establishment rate, p_e, on λ, R_0 and T by varying p_e from 0.00001 to 0.001 while keeping all other IPM parameters constant. For the calculation of the stable age distributions we constructed a size × age classified model using the recipe given in Ellner and Rees (2006) Appendix A and iterated this model until it converged on a stable state distribution. Note this is slightly inefficient as the distribution of offspring sizes is independent of parental size and so we can collapse the size and age structured model to a Leslie matrix; see Childs et al 2003 Appendix A for details.

The matrix **K** is useful for calculating various measures of population growth; however, it is inappropriate for sensitivity and elasticity analyses because the identity matrix, **I**, in the lower left hand corner is simply a computational device and not a biological transition. To get around this problem we used direct perturbation of the **P** and **F** to calculate the sensitivities and elasticities, see Appendix A of Ellner and Rees (2006) for details.

In order to predict how the probability of flowering varies with plant size (Figure 1b), the flowering strategy, we use the evolutionarily stable strategy (ESS) approach testing the invasiveness of an alternative phenotype into the resident population (Maynard Smith, 1989). The flowering strategy is characterised by a logistic regression with intercept β_0 and size slope β_s. When both of these parameters are allowed to evolve the predicted strategy is a step function; all plants should flower with probability 0 or 1 depending on whether their size is below or above a threshold (Childs *et al.*, 2003; Rees & Rose, 2002). However, *C. thyrsoides,* in common with many other monocarps, has a more gradual increase in the probability of flowering with size, suggesting a constraint or that the decision to flower is made sometime between censuses or there is genetic variation in the flowering threshold (Childs *et al.*, 2003; Metcalf *et al.*, 2003). We therefore constrain the ESS to match the observed gradual change in the probability of flowering with plant size by fixing the size slope, β_s, at its estimated value and allowing the intercept, β_0, to evolve. For systems such as *C. thyrsoides* where density dependence acts primarily on the probability of establishment, see above, the ESS can be characterised by maximising R_0 (Ellner & Rees, 2006; Mylius & Diekmann, 1995).

Results

Population growth rate

Measures of population growth differ significantly between sites (Table 2). The population from Furka Pass appears to be at approximate demographic equilibrium with $\lambda \approx 1$, and the 95% confidence interval includes $\lambda = 1$, whereas the population at Schynige Platte is predicted to increase rapidly, $\lambda = 1.19$, with a lower 95 % confidence interval of 1.07. Estimates of λ calculated from the site and year-specific life-table entries average to $\lambda = 1.08$ at each site such that both populations are predicted to moderately increase over time (Table 3). The life-table data also demonstrates a considerable interannual variation in population growth rates with a year of rapid increase followed, or preceded, by a year of almost demographic equilibrium. This observed variability is generally smaller than the

Table 2. Population growth rate, λ, net reproductive rate, R_0, and generation time, T, for *Campanula thyrsoides* extracted from the site specific integral projection models. The site differences are the means of the differences between site 1 and 2 parameters from the bootstrapped sample. The figures in brackets are 95% confidence intervals based on 5000 bootstrapped samples, see Methods and Appendix for details.

Site \ Parameter	λ	R_0	T
Furka	1.05 (0.96, 1.12)	1.67 (0.64, 3.19)	10.82 (10.18, 11.77)
Schynige Platte	1.19 (1.07, 1.30)	4.97 (2.19, 13.14)	10.37 (9.19, 11.70)
Site differences	-0.15 (-0.29, -0.02)	-4.44 (-11.79, -0.35)	0.58 (-0.90, 1.94)

95% confidence interval of the IPMs and also largely overlaps with it so that we have good agreement between the two methods. The simulated values for λ based on a range of establishment rates, p_e, show that the statistical significance in demographic processes observed between sites (Table 1) translates into different patterns of population growth (Figure 2). At equal p_e, the Furka population has considerably higher growth rates than the Schynige Platte population. The simulations also indicate that the potential for fast population growth is given at higher establishment rates as probable consequence of increased microsite-availability in disturbed habitats.

Generation time and *Net reproductive rate*

The mean generation time, T, for *Campanula* estimated with IPMs is approximately 10-11 years (Table 2), while T calculated with the herb chronology data approximates to 9-10 years (Table 3). The 95 % confidence intervals of the IPMs overlap with the standard errors of the herb chronology data so that we find good agreement between the two methods. We further do not find any significant differences between sites using either method. The simulated generation times with varying p_e are always higher at the Schynige Platte than for the Furka population (Figure 2). The net reproductive rates, R_0, is higher at

Table 3. Population growth rate, λ, net reproductive rate, R_0, and generation time, T, for *Campanula thyrsoides* extracted from the site specific life-tables entries in combination with the herb chronology survey. Values in square brackets are one year transitions, i.e. [2003/2004, 2004/2005]; see Methods for details.

Site \ Parameter	λ	R_0	T
Furka	1.08	2.09	9.60
	[1.03, 1.14]	[1.33, 3.52]	SE = 0.66, n = 22
Schynige Platte	1.08	2.01	9.10
	[1.15, 1.01]	[3.57, 1.09]	SE = 0.41, n = 27

the Schynige Platte than at Furka Pass but the two populations do not differ significantly from each other (Table 2+3). At equal establishment rates, the Furka population generally has higher R_0-values as a result of a higher λ at the Furka site (Figure 2).

Stable age-structure

Using herb chronology data we can look at the age-structure of all plants and flowering individuals at each of the sites (Figure 3). Super-imposed on these distributions are the predicted stable age-distributions derived from the site-specific IPMs. At Furka Pass the IPMs provide remarkable good description of the age structure of all plants and flowering ones. In contrast at Schynige Platte where the population is predicted to increase rapidly there are substantial difference with too few young individuals in the total population, for flowering plants however the IPMs provides a reasonable description of the age distribution.

Elasticity analysis

We used elasticity analysis to partition the contributions of different sizes, ages and types of life-history transitions to λ. Partitioning the elasticities into survival-growth and reproduction components of the kernel, we find that the survival-growth transitions are the critical determinant of λ, (the summed survival-growth elasticities being 0.90 and 0.88 at the FU and SP sites, respectively). The survival-growth elasticities can be partitioned into

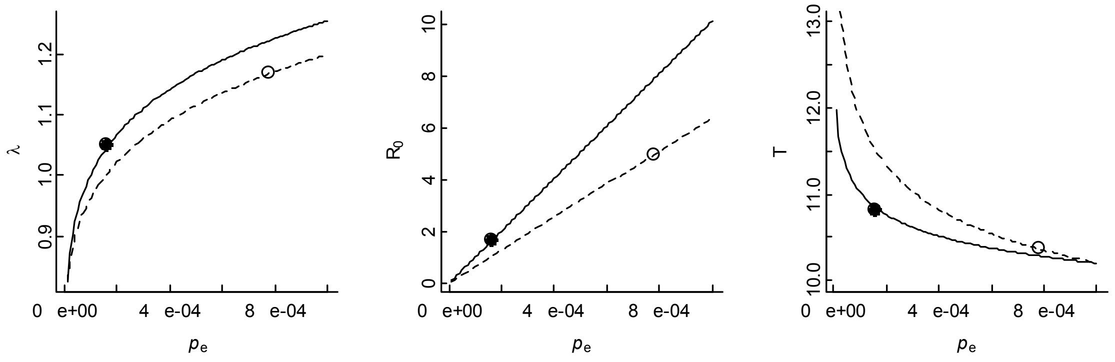

Figure 2. Predicted effect of changing the establishment rate p_e on population growth rate, λ, net reproductive rate, R_0, and generation time, T, for *Campanula thyrsoides*. Dots show observed values (solid: FU, open: SP). Lines represent simulated results from the p_e interval 0.00001 to 0.001 (solid: FU, dashed: SP).

contributions from plants of different sizes and ages (Figure 4). The contribution of growth to λ is dominated by transitions into the larger size range where reproduction occurs (Figure 4a,c). In contrast partitioning the elasticities by age indicates that young plant have the largest contribution to λ (Figure 4b,d), this is a consequence of the stable age distribution being dominated by young plants (Figure 3a,c). For the reproduction component of the kernel (Figure 5) the contributions of different size transitions are dominated by movement of individuals from large sizes to recruits; which is a consequence of larger individuals having higher probabilities of flowering and producing more seeds (Figure 5a,c). At the FU and SP site individuals of ages 8 and 6 years respectively make the largest contributions to λ (Figure 5b,d). This is because plants are on average larger as they get older and fecundity increases with size. However this effect is counteracted by older plants making up a smaller proportion of the stable age distribution (Figure 3b,d). For the Furka Pass population these values correspond well with the mean age at flowering of 8.6 years calculated from the herb chronology data. In contrast, the values for the Schynige Platte population are lower than the mean age at flowering of 8.1 years. This discrepancy is also seen between the stable age-distribution and the herb chronology data (Figure 3d).

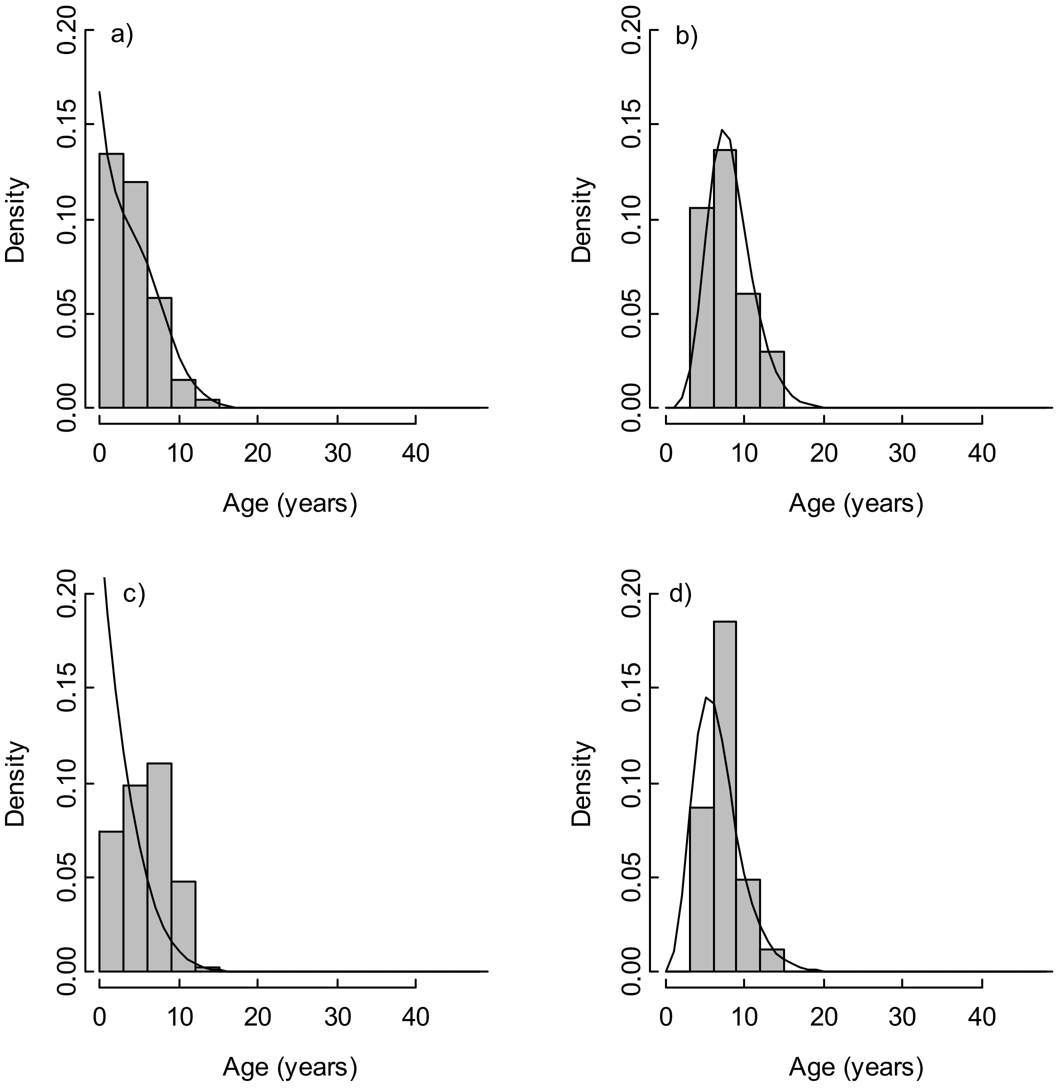

Figure 3. Age distributions of *Campanula thyrsoides* for the FU (top row) and SP (bottom row d) sites, for all plants (a and c) and for flowering plants only (b and d). The solid bars are the data, the lines the predicted stable age distributions from the site specific IPMs.

Evolutionary Analysis

The site-specific results for the ESS with respect to R_0 and λ are shown in Figure 6. At both sites, the ESS was found at smaller intercept values than the observed β_0 indicating that plants following the ESS would flower at larger sizes than real plants. This difference was most pronounced at the Furka population both for R_0 and λ (Figure 6a,b). At Schynige Platte the observed λ was almost identical with the estimated ESS (Figure 6d) and only weak selection for larger sizes at flowering can be found using R_0 as fitness measure. In all

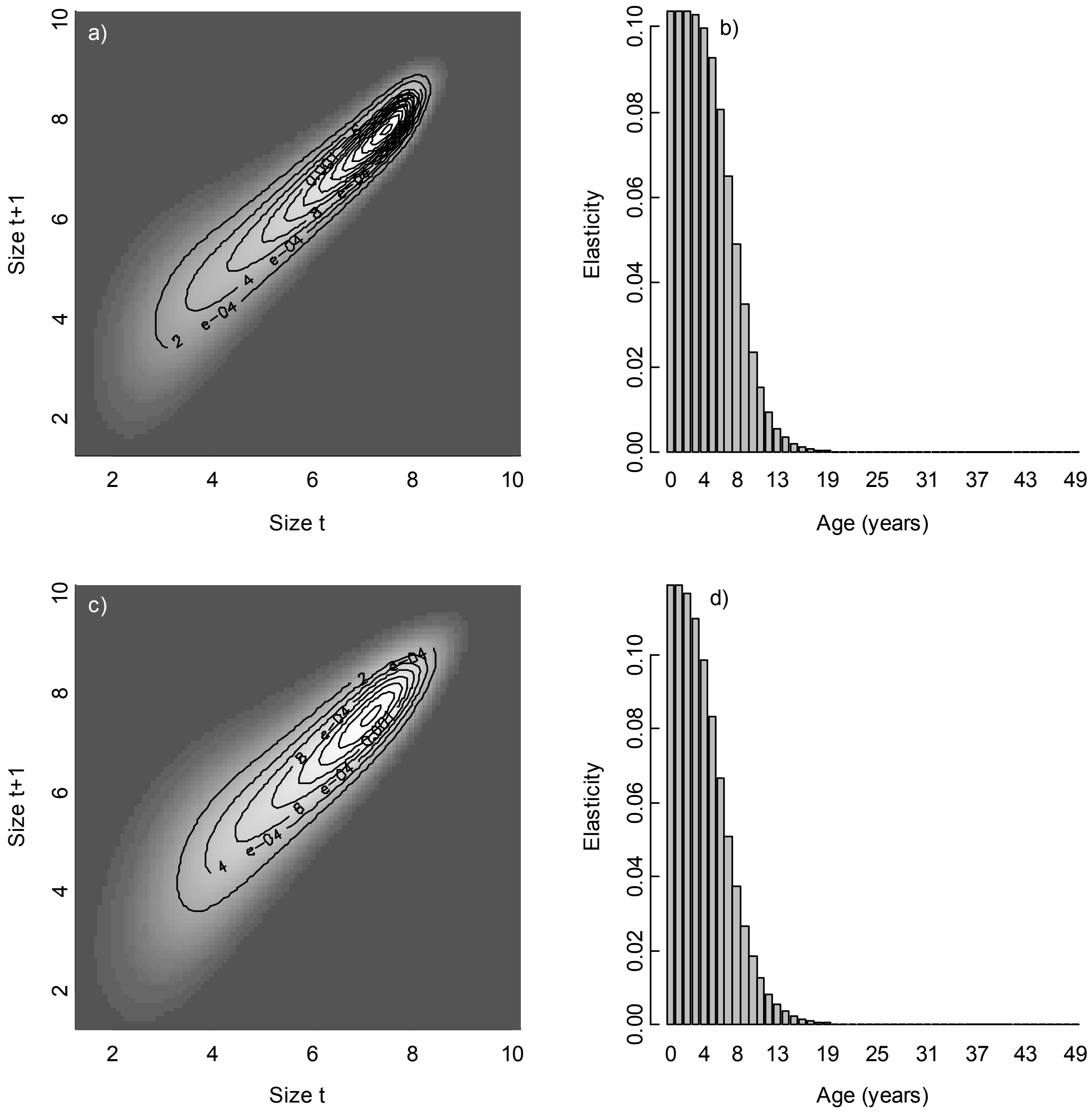

Figure 4. Survival-growth elasticities for *Campanula thyrsoides* for the Furka Pass (top row) and Schynige Platte (bottom row) sites partitioned according to size transitions from one year to the next (a and c) and age (b and d).

four cases, however, the uncertainty about the observed intercept, β_0, was always larger than the differences between estimated and observed values for R_0 and λ such that the observed flowering strategy seems to be very close to the evolutionary stable strategy.

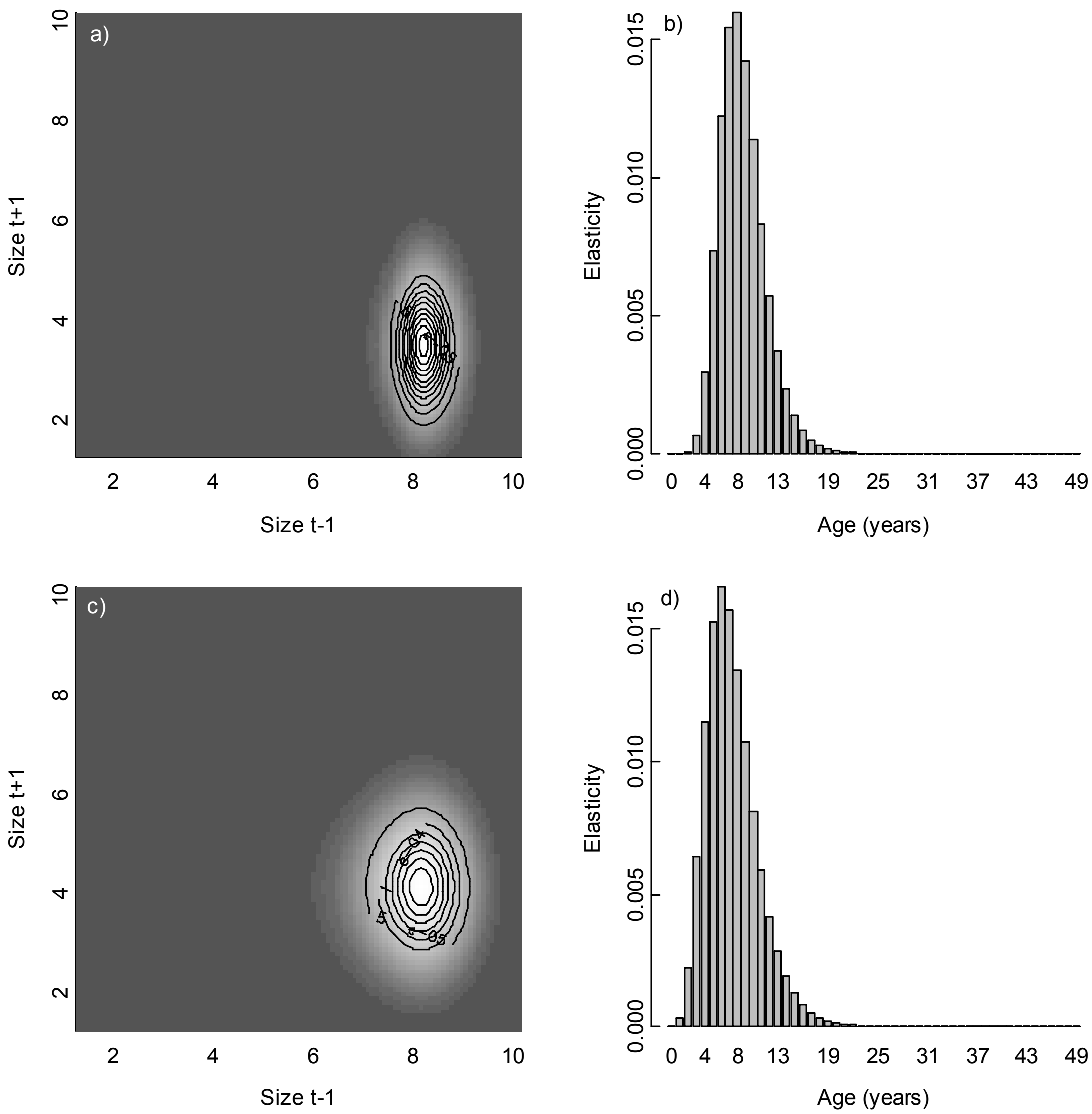

Figure 5. Fecundity elasticities for *Campanula thyrsoides* for the Furka Pass (top row) and Schynige Platte (bottom row) sites partitioned according to size transitions from one year to the next (a and c) and age (b and d).

Discussion

In this article, we have shown that IPMs can accurately describe the demography of the long-lived monocarpic perennial *Campanula thyrsoides* with data from a relatively short census period of three years. Demographic parameters and stable age-distributions calculated with the IPMs are largely in good agreement with the results obtained from life-table entries in combination with the herb chronology survey. Thus, IPMs are powerful tools for *C. thyrsoides* to further assess stable size- and age-structure, life-cycle elasticities and the evolutionary stable strategy.

Demographic functions

The demography of *C. thyrsoides* is strongly size-dependent and the four basic demographic functions (i.e. growth, probability of survival, probability of flowering, and reproductive output; Figure 1) generally follow the same pattern as other monocarpic species (Metcalf et al. 2003). The relative growth rate (RGR) for *C. thyrsoides* is a decreasing function of size and only little growth is observed close to the mean threshold value of flowering at size 8 on the log scale (Figure 1a, b). The substantial variance about the growth curves is most probably the result of spatial heterogeneity in microsite-related growth conditions and/or the length of the vegetation period since both factors are characteristic for the natural habitats of *C. thyrsoides* (Young and Augspurger 1991, Körner 2003). In plants with size-dependent flowering thresholds, this variance about the growth curve also has important genetic consequences because flowering within a cohort is spread over several years such that the probability of mating among close relatives and inbreeding-depression is largely reduced.

For our IPMs we used a non-destructive and continuous size measure that is highly correlated with above-ground biomass and thus is a biologically significant fitness measure. However, values on a log scale are not easy to interpret and for further field surveys, the easiest accessible parameter to predict flowering of a vegetative rosette is to count the number of leaves. Once a rosette consists of 20-30 leaves it will flower the following season with steadily increasing flowering probability, p_f, as more than 20 leaves are encountered. Further, we observed a site-dependent variability of p_f with a broader range of flowering sizes at SP but, at both sites, identical sizes at which 50% of the rosettes will flower the next season (Figure 1b). Therefore, the two populations seem to behave biologically very similar with respect to p_f and we have no reliable indication that higher altitude selects for smaller or larger sizes at flowering. The probability of survival, $s(x)$, generally increases with size in monocarpic plants (Metcalf et al., 2003), often with a considerable seasonal as well as density-dependent variability (e.g. Rees et al., 1999; de Jong et al., 2000; Rose, et al. 2002). Our short census period does not allow us to assess the annual variability of survival in *C. thyrsoides*, however, survival rates of 70-95% at seedling size, and > 90% during vegetative rosette stage are very high compared to other monocarpic species (e.g. *Oenothera glazioviana*: Kachi and Hirose, 1985; *Digitalis purpurea*: Sletvold, 2005; *Onopordum illyricum*: Rees et al., 1999) and only reached in

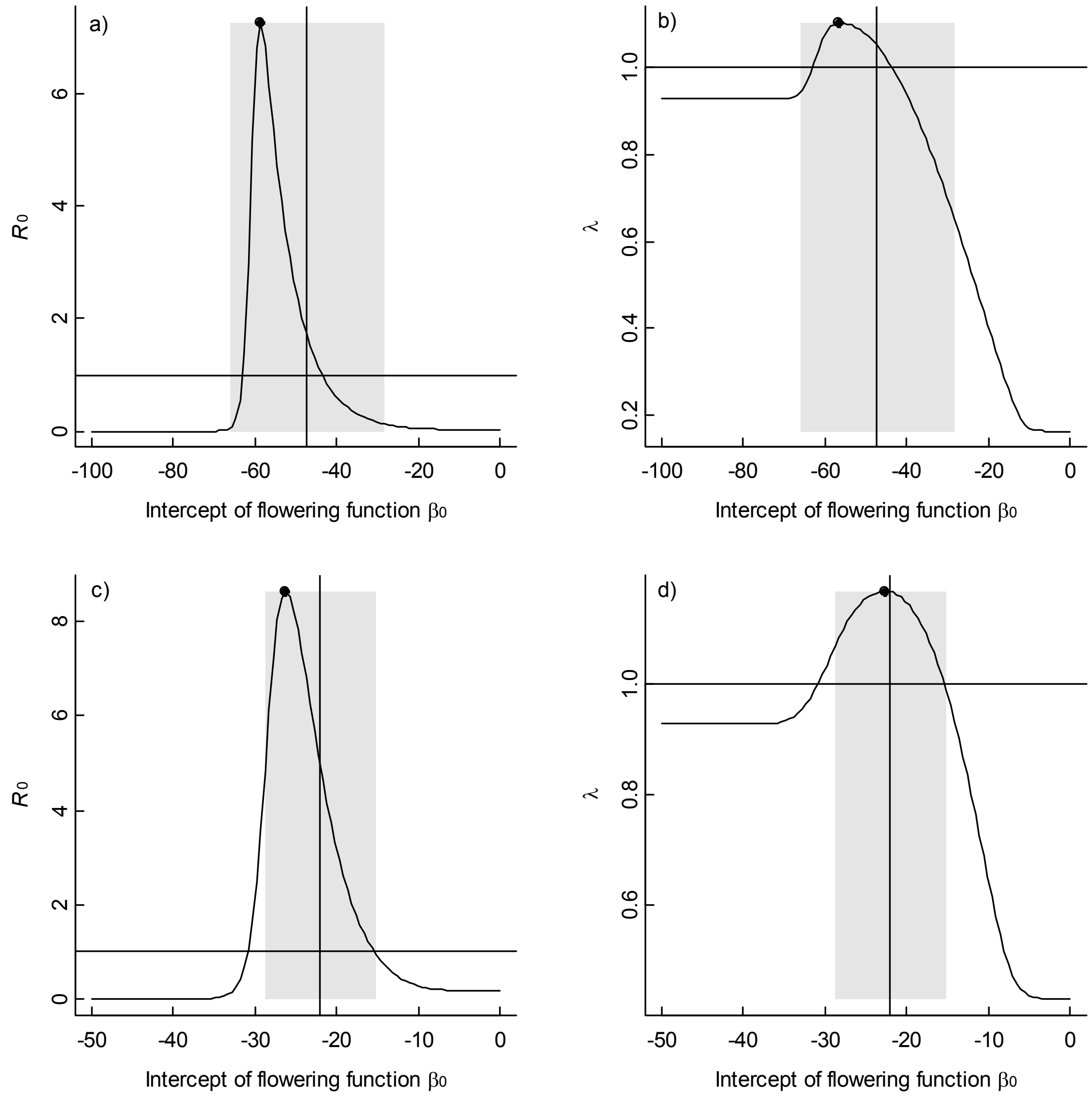

Figure 6. Predicted effects of changing the intercept of the flowering function β_0 on population growth rate, λ, and net reproductive rate, R_0, for *Campanula thyrsoides*: a) and b) Furka Pass, c) and d) Schynige Platte. Vertical lines represents observed β_0, horizontal lines the population equilibrium. The lines show the estimated values of λ and R_0 with varying β_0. Solid dots mark the ESS. The grey polygon describes the 2 SE uncertainty around the observed β_0.

favorable years in *Carlina vulgaris* (Rose et al., 2002). The probability of survival in *Campanula thyrsoides* increased with plant size at Furka Pass whereas in the Schynige Platte population $s(x)$ slowly decreases, but maintaining a high $s(x) > 90\%$ (Figure 1c).

These high survival rates at both sites stress the overall importance of survival for the demography of *C. thyrsoides* (see below).

As in many other monocarpic species (Metcalf et al., 2003), the reproductive output in *C. thyrsoides* increased with plant size. However, recruitment of seedlings was independent of seed production which varied between 0-68000 seeds per m^2. This represents an extreme and highly stabilizing form of density dependence, also observed in *Onopordum illyricum* (Rees et al. 1999). It is noteworthy that our *C. thyrsoides* plots are situated in stable alpine grassland communities in which higher recruitment seems unlikely. Along road shoulders and abandoned construction sites, we otherwise observe fast expanding populations, thus corroborating the importance of microsite-availability for population dynamics (Chapter 5).

Population dynamics, elasticities and ESS

The estimated demographic parameters, i.e. λ, R_0, and T, from the IPMs were largely in good agreement with the results calculated from life-table entries and herb chronology data. At both sites populations are predicted to increase over time which certainly reflects the situation encountered in the field. Given the short census period we are however limited in quantifying the interannual variability of λ, especially we cannot explore the possible increase in absolute seedling numbers after favorable years for flowering. As a long-lived species with considerable generation overlap *C. thyrsoides* can easily buffer single or recurrent years with no recruitment and assure population persistence. In the case of absent recruitment the age-distribution within a population will show a reduced number of young individuals and thus, lower numbers than predicted from the stable age-distribution. This simple relationship most likely explains the difference between observed age-distribution, i.e. single-season herb chronology data, and the stable age-distribution at Schynige Platte (Figure 3c). At the same site the observed age-distribution for flowering plants (Figure 3d) is very close to the stable age-distribution. Naturally, this reflects the insensitivity of flowering ages to years with no recruitment and is the result of a size-dependent flowering threshold. It is worth considering that in years where recruitment is absent while flowering continues, the probability of establishment may increase because reproductive plants will die and free favorable microsites for more seedlings than large plants. Then, high absolute recruitment can occur leading to an increase in λ. This short-term dynamics therefore does not accurately describe the long-term population growth. We found such a discrepancy at Schynige Platte where the IPM predicts a rapid population increase ($\lambda = 1.19$; Table 2) while observed values indicate only moderate population growth ($\lambda = 1.08$; Table 3) which matches the situation in the field.

The IPMs slightly overestimated generation time T (Table 2+3) and we found weak selection for flowering at larger sizes (Figure 6). The uncertainty about age and size at flowering was however larger than the differences between observed and estimated values. Given the high survival probabilities for vegetative rosettes we cannot argue for a conservative flowering strategy, i.e. flowering at suboptimal sizes to avoid the probability of dying before reproduction, as it has been proposed for *Lobelia inflata* (Simons and Johnston, 2003). *C. thyrsoides* can only gain from delaying flowering as long as it will grow larger until the next season. This interpretation is corroborated by the elasticity analyses which demonstrate the dominant importance of survival and growth for the population dynamics. In both *C. thyrsoides* populations the relative contribution of survival-growth (FU:88%; SP: 90%) and fecundity (FU:12%; SP: 10%) elasticities to λ were remarkably similar. Corresponding to our findings, survival-growth transitions are also most influential in short-lived monocarpic species. For example, in *Onopordum illyricum*, Ellner and Rees (2006) found survival-growth vs. fecundity transitions to contribute to 75% vs. 25% to λ, and in *Carlina vulgaris*, Childs et al. (2003) calculated a contribution of 66% vs. 34%. From all three species studied, *C. thyrsoides* demonstrates the largest contributions of survival and growth to λ and it seems likely that this increased importance is related to the species' higher longevity. The three species further show a comparable pattern of age-specificity of the survival-growth and fecundity elasticities (Figures 4b,d + 5b,d). The survival-growth elasticities commonly decrease with age and the fecundity elasticities have maximum values at intermediate ages (6 and 8 years in *Campanula thyrsoides*, 3 in *Onopordum illyricum* and 2 in *Carlina vulgaris*).

Complicating factors

Some aspects of the biology of *C. thyrsoides* were not included in our demographic model but may explain some of the variance encountered in demographic functions and parameters. These are most notably the genetic regulation of size-dependent flowering, the formation of sister rosettes as a result of damage to the apical meristem, and the role of herbivory. Several studies have shown that substantial genetic variance exists in natural populations for the threshold size of flowering. For example, in *Cynoglossum officinale* and *Senecio jacobaea* experimental selection for early flowering thresholds in the parent generation led to a substantial decrease of flowering size ranges in the offspring generation (Wesselingh and de Jong, 1995; Wesselingh and Klinkhamer, 1996). In the case of *C. thyrsoides* we have weak indications from a common garden experiment that half-sibs synchronize flowering even if grown at two different altitudes (Chapter 5). However, reliable estimates on the genetic basis of flowering time could not be obtained. A further complicating factor is the formation of sister-rosettes in about 10% of all plants. Sister-rosettes are autonomous with respect to flowering, but will die together in case one sister-rosette has completed seed set. Plants can be encountered with two or more flowering stalks and hereby considerably increase an individual's fecundity, but sister-rosettes can also die and be replace some years later. In the current IPMs we excluded individuals with more than one rosette due to the insufficient representation in our permanent plot data and the numerous peculiarities in their behaviour. Thirdly, herbivory can influence every aspect of plant performance and select for an optimal flowering strategy that minimizes size-related herbivory or, as proposed by Klinkhamer et al. (1997), may play a role in the evolution of the monocarpic perennial life-history when herbivory acts upon survival. Substantial herbivory in *C. thyrsoides* was only observed by leafroller moth larvae, *Cochylis cf. pallidana* and two weevil species, *Miarus cf. graminis* and *M. cf. abeillei*, that fed on immature seeds and could cause complete reproductive failure (Chapter 5). Seed-predation however was highly variable in space and years of occurrence and never observed in the permanent plots so that we did not incorporate pre-dispersal seed-predation in our IPMs.

Conclusion

Campanula thyrsoides seems to follow simple and well known rules to determine when to flower. We clearly observe that the gain from delaying flowering comes from increased fecundity as a result of growth. As growth slows down or stops the plant will flower because it cannot further maximize size-related reproductive output. The high survival rates facilitate such a flowering strategy and allow *C. thyrsoides* to efficiently buffer the temporal variability of the alpine environment. Our data further indicates that *C. thyrsoides* populations can slowly increase in stable alpine grasslands even with little recruitment success. Moderate disturbance that increases microsite-availability will most probably lead to rapid population growth and thus could explain the occurrences of recent and large *C. thyrsoides* populations on ruderal sites in the Alps.

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Appendix A1. Bootstrap details.

To obtain estimates of the uncertainty associated with the various measures of population growth, generation time and ESS flowering strategy we bootstrapped the data. Where site effects were significant we randomly sampled with replacement individual data pairs from within each site and fitted the site specific models to the combined data from both sites. This was done for the growth, survival and flowering probabilities, fecundity and the distribution of seedling sizes. For the probability of seedling establishment we resampled the data from each quadrate within each site then calculated p_e by dividing the total number of seedlings by the total seed production the previous year. Thus each complete bootstrapped sample allows an IPM to be constructed for both sites, and so site specific demographic parameters (i.e. λ, R_0 etc) can be estimated. The bootstrapped distributions for λ and the generation time, T, are symmetrical and approximately follow a normal distribution. In these cases the error involved in using the normal approximation to the 95% confidence interval is small (<1%).

To test if the various measures of population growth (λ and R_0) and generation time varied between sites we calculated the difference between the calculated statistics for each site, using each bootstrapped dataset.

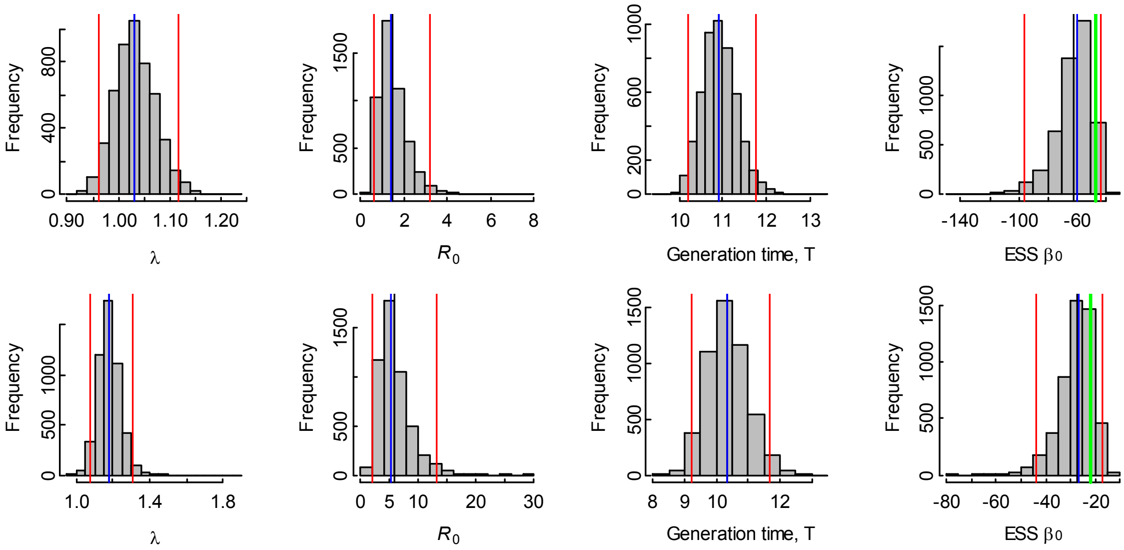

Figure A1. Bootstrapped distributions of population growth rate, λ, net reproductive rate, R_0, generation time, T, and intercept β_0 of the evolutionary stable flowering strategy (ESS) for *Campanula thyrsoides*. Flanking lines are the 2.5 and 97.5 percentiles (95% confidence intervals), central lines the means, off center the medians and the estimated values of β_0 are shown in bold black. Top row refers to population at Furka Pass, bottom to Schynige Platte.

Chapter 4

No inbreeding depression in an outcrossing Alpine species: the breeding system of *Campanula thyrsoides*

Hafdís Hanna Ægisdóttir, Daniela Jespersen, Patrick Kuss, Jürg Stöcklin

Summary

In plants living in fragmented landscapes, where populations are isolated from each other and in which long-distance dispersal is essential for colonization of empty sites, reproduction should be favoured by self-compatibility (Baker's law). Nevertheless, outcrossing mechanisms, such as self-incompatibility and dichogamy, are common in many species and often maintained by inbreeding depression in the fitness of selfed progeny. Here, we studied the breeding system and the consequences of selfing and sister mating in *Campanula thyrsoides*, a long-lived perennial monocarp, which is found in the naturally fragmented landscape of the Alps. An experiment with controlled pollinations was set up in the common garden with plants grown from seeds originating from 14 seed families, collected in the siliceous Central Alps, where this plant is found on isolated carbonate bearing outcrops.

Our results indicate that *C. thyrsoides* has a strong self-incompatibility system (SI) with no or low seed set in selfed flowers compared to outcrossed and sister-crossed flowers. Moreover, the SI system in *C. thyrsoides* did not break down with flower age as in some other *Campanula* species. Surprisingly, there was no significant difference in seed set, seed weight, germination percentage, seedling survival and size between outcrossed and sister-crossed offspring, which indicates no inbreeding depression.

We suggest that the absence of inbreeding depression in this outcrossing species might be a result of frequent bottlenecks during colonization of the isolated habitats in the alpine landscape.

Key words: Alpine plants, bottlenecks, fragmented landscape, self-incompatibility, Swiss Alps

Introduction

Self-compatibility is frequent among plants, despite the fact that many monoecious or hermaphroditic plant species have mechanisms which promote outcrossing and prevent self-fertilization, i.e. dichogamy, heterostyly or self-incompatibility system (Lande and Schemske 1985; Richards 1997; Byers and Waller 1999). The outcrossing habit of these species is often maintained by inbreeding depression, defined as a reduced fitness of selfed progeny due to the expression of mostly recessive deleterious mutations in homozygotes (partial dominance) or a decrease in heterozygotes that exhibit a fitness advantage over homozygotes (overdominance) (Lande and Schemske 1985; Frankham et al. 2002). Consequently, historically large and outcrossing populations are expected to have substantial inbreeding depression while predominantly selfing populations or populations that have experienced repeated bottlenecks are expected to have lower inbreeding depression due to selection, i.e. the populations have been purged of their genetic load (Charlesworth and Charlesworth 1987; Karron 1989; Barrett and Charlesworth 1991; Ouborg and van Treuren 1994).

Alpine habitats are characterized by great natural fragmentation and patchiness created by heterogeneous topography and related abiotic factors (Körner 1999, 2001). As a result, Alpine plant populations are often spatially isolated from each other, frequently by long distances. Establishment of new populations in the fragmented and heterogeneous Alpine landscape therefore depends on rare but critical long-distance dispersal (Harper 1977; Cain et al. 2000). Consequently, in isolated populations of species living in a fragmented landscape, self-compatibility should be favoured to ensure sexual reproduction (Baker's law, Baker 1955, 1967; Jain 1976).

Most *Campanula* species are self-incompatible (SI) and allogamous (Shetler 1979; Nyman 1993), but complete self-compatibility has also been recorded, e.g. in the arctic *C. uniflora* (Ægisdóttir and Thórhallsdóttir, in press). Self-sterility in plants is controlled by a self-incompatibility locus, named the S-locus that could be comprised of one or several loci. The S-locus has many different alleles and pollen is rejected when it carries the same alleles as the plant being pollinated. This self-incompatibility system can either be gametophytic (GSI) or sporophytic (SSI). In GSI systems, the pollen grain must match either of the alleles present in the diploid maternal tissue to be accepted, making semi-compatibility (sister mating) possible. In contrast, the pollen grains in the less common SSI must match both of the alleles present in the given female parent to be accepted, making semi-compatibility impossible (Richards 1997; Silvertown and Charlesworth 2001). It has been stated that some plants show variation and plasticity in SI as is the case in *Campanula rapunuculoides,* where self-fertility increases with flower age, i.e. delayed selfing (Richardson et al. 1990; Vogler et al. 1998).

In the genus *Campanula,* an interesting floral mechanism of protandrous flowers has evolved. In the male phase the pollen form a sheath around the hairy style before the stigma becomes receptive. In some *Campanula* species, late in the female phase, the stigmatic lobes bend backwards towards the style picking up pollen that had not been removed by insects. This process facilitates the late self-fertilization of ovules that have not been fertilized by outcrossing (Fægri and van der Pijl 1979; Ægisdóttir and Thórhallsdóttir, in press).

Campanula thyrsoides is a long-lived monocarpic perennial, living at high altitudes in the Alps, frequently in isolated habitats of calcareous soils. Although most *Campanula* species are self-incompatible and allogamous, self-compatible species should be favoured in plants living in patchy alpine habitats (Baker's law), as described above. Moreover, inbreeding depression is an important force for maintaining outcrossing in plants (Lande and Schemske 1985; Frankham et al. 2002). We therefore expect that inbred *C. thyrsoides* offspring suffer from inbreeding depression. For these reasons, we were interested in the following questions: (1) Is *C. thyrsoides* self-compatible? (2) If it isn't, does the SI (self-incompatibility) system break down with flower age? (3) Do inbred *C. thyrsoides* offspring suffer from inbreeding depression in seed set, seed weight, seed germination and seedling fitness?

Methods

Study species

Campanula thyrsoides is an alpine to subalpine long-lived monocarpic perennial, found on calcareous soils at approx. 1300-2800 m asl throughout the European Alps (Lauber and Wagner 2001). The species is rare but locally abundant with population sizes ranging from less than a hundred to more than 50,000 individuals. Prior to flowering, *C. thyrsoides* forms a basal rosette which overwinters and grows without producing flowers for about 2-16 years (Johanna Hänger, pers. comm.). In contrast, plants grown in greenhouses occasionally flower in their second year (pers. obs.). In the year of flowering, a 10-50 cm tall inflorences is formed that carries about 30-200 flowers in a compact spike (Jäger 2000 and pers. obs.). The flowers are protandrous and mainly pollinated by Hymenoptera, e.g. bumblebees and wasps. The plant dies after setting many small seeds in a multiseeded capsule (average seeds/capsule = 200) (Jäger, 2000; Ægisdóttir, pers. obs.).

C. thyrsoides is predominantly found in pastures, extensively used hay-meadows and disturbed areas, such as road sides. In Switzerland, it is common in the northern calcareous Alps, while in the central siliceous Alps, it is only found in isolated carbonate-bearing outcrops (Lauber and Wagner 2001).

Breeding system experiment

In the summer of 2002, we sampled seeds from 30 *C. thyrsoides* plants on the Furkapass (SUI 674850/158825, 2430 m asl) in the central Alps of Switzerland. The population is about 25 km away from the nearest *C. thyrsoides* population and the area's main soil type is of siliceous origin, but small stripes of calcareous soil also occur, on which *C. thyrsoides* can be found. We germinated seeds from each of the 30 motherplants and grew up 15 seedlings per plant (seed family). Of the total 450 seedlings, only 73 descendants flowered in 2004, of which 41 plants were used in the pollination experiment (3-5 plants per seed family stemming from 12 mother plants). Prior to flowering, we bagged the plants within 50 cm long insect excluders made of green 0.8

mm mosquito nets and stabilized the bags over two about 110 cm long crosswise attached iron wires in order to avoid direct contact between the bags and flowers.

To explore the breeding system and the consequences of selfing and sister mating in *C. thyrsoides*, we performed controlled pollinations with 4 treatments and 3 replicates by randomly choosing flowers of a single plant, i.e. we applied different treatment on the same flowering individual. The treatments were: a) spontaneous selfing: flowers from within the insect excluders left untreated to test whether seeds are produced in the absence of pollinators, b) hand-selfing: flowers pollinated with their own pollen on the second day of flowering to assess whether insect visitation was required for self-pollination, c) sister mating: flowers handpollinated on the second day of flowering with pollen from plants belonging to the same seed family, and d) outcrossing: flowers handpollinated on the second day of flowering with pollen from plants belonging to another seed family.

Additionally, we left 15 control plants from 14 seed families untreated and unbagged to establish the natural level of seed set. As in the experimental plants, we sampled 3 randomly chosen flowers from each plant. In order to test whether the self-incompatibility system would break down with flower age, we included 30 plants from 10 seed families, bagged them within insect excluders, and hand pollinated them with their own pollen just before the flower withered. We removed the insect excluders as soon as all the flowers, used for the experiment, had withered. In late summer, we collected the seeds, measured plant height, and counted the number of all capsules per plant. We categorized the seeds into a) well developed seeds (round, well filled), b) less developed seeds (flat, not well filled), and c) aborted seeds (very small). After sorting and counting the seeds, we determined their mass by weighing all seeds per capsule, instead of weighing every single seed.

Germination test

We ran germination tests for 25 days on 60 well developed seeds for each individual and treatment. We put eight control plants (from 6 seed families) and 27 plants from the outcrossed and sister-crossed treatments (11 seed families; 1-4 plants per family) to trial and placed twenty seeds on each filter paper in petri dishes and moistened with water.

We kept the seeds wet in an incubator with a 12-h photoperiod and 20°/10°C (day/night) temperature and estimated their germination (%) once a week.

Greenhouse experiment with F1 offspring

To find out if the offspring from the outcrossing (assumed inbreeding coefficient $F = 0$) and the sister-crossing treatment ($F = 0.125 – 0.25$; Wright, 1922) showed indication of inbreeding depression, we set up an experiment to compare the seedling survival and size of outcrossed and sister-crossed offspring.

We germinated seeds in petri dishes and transferred the seedlings into seedling trays with soil in the greenhouse (in both cases 288 seedlings from the same 6 seed families comprising 3 plants per seed family). Fifteen weeks after the transfer, we re-potted the seedlings and randomly arranged in the Botanical Garden of the University of Basel. We recorded survival rate and plant size (rosette diameter and number of leaves) at 15 weeks and at 25 weeks after re-potting.

Additionally, we germinated seeds from the control and the selfing treatments and handled the offspring (control: 58 offspring, 5 families, 10-13 per family, and selfing: 30 offspring, 6 families, 1-16 per family) as described above.

Data analysis

We performed statistical analyses using R 1.9.0. Prior to analysis, we log (log+1) transformed all data that did not meet the assumptions of an ANCOVA (Zar 1999). We tested the treatment effect on mean seed number, seed weight, and seed germination with an ANCOVA model categorizing families and treatments as factors and plant size and number of flowers per plant as covariables.

For the analysis, we included only individuals that met the minimum requirement of 20 seeds within at least one of the three capsules. This was done to exclude individuals where likely mistakes in handpollination had occurred. We also excluded outliers from the analysis (about 1-3 values per test) that alone changed the tests' result.

We seperately performed the statistical analysis for control vs. outcrossed and control vs. sister-crossed treatments. Only plants from seed families that had both control and treatment plants were included in the analysis, i.e. 26 treatment plants (from

11 families, 1-4 plants per family) and 7 control plants (6 seed families, 1-2 plants per family). Two control plants died. We excluded selfed flowers from the analysis since they did not produce any seeds in most cases (Table 1). To avoid pseudoreplication, we used mean number of seeds from the 3 capsules (replicated in each individual/treatment) in the model.

We tested the treatment effect on seedling survival and size of F1 offspring, in the outcrossed vs. sister-crossed plants with an ANOVA model with families and treatments as factors. When the size of the sister-crossed and the outcrossed F1 offspring was compared to the size of the selfed offspring and the controls, we ran the ANOVA with only treatment as a factor since the number and partition of seed families differed greatly between the treatments.

We calculated the self-compatibility index (SCI) as the number of selfed seeds divided by the sum of outcrossed and sister-crossed seeds, following controlled pollinations.

Results

Seed set

Most of the selfed flowers (spontaneous-, hand- or late selfed) set very few or no seeds with an average of five seed per capsule (sum of all seed categories). This led to a great difference in seed set between the treatments (Figure 1).

Seed set was prominent in outcrossed/sister-crossed flowers as well as in the control plants. Here, we found no significant difference in the mean number of seeds between control vs. outcrossed and control vs. sister-crossed flowers respectively (well developed seeds per capsule: $P = 0.33$, $P = 0.07$; less developed seeds per capsule: $P = 0.32$, $P = 0.86$; early aborted seeds per capsule: $P = 0.67$, $P = 0.25$; total no. of seeds: $P = 0.07$, $P = 0.13$).

Additional analyses of the outcrossed vs. sister-crossed treatments revealed no significant difference in the mean values of total no. of seeds per capsule ($P = 0.07$), well developed seeds per capsule ($P = 0.44$) and early aborted seeds per capsule ($P = 0.15$).

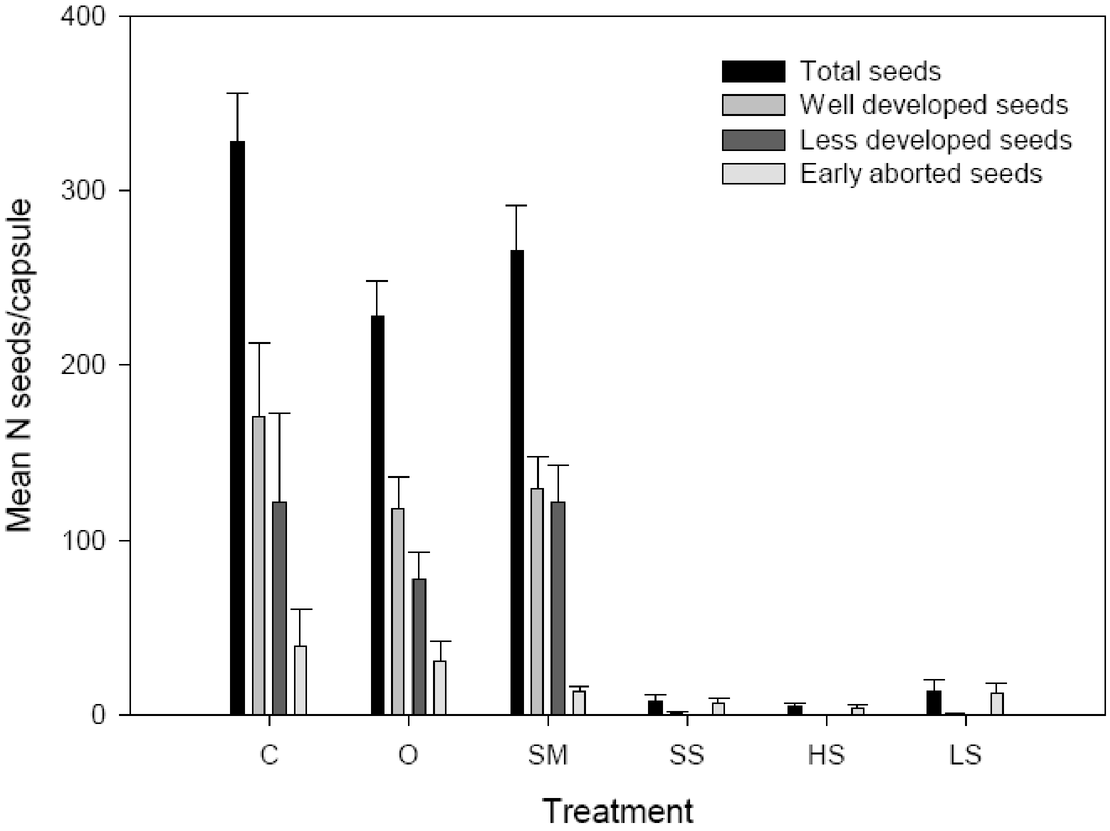

Figure 1. Number of a) total, b) well developed, c) less developed, and d) early aborted seeds per capsule in control (C), outcrossed (between seed families) (O), sister mated (inbred within seed families) (SM), spontaneously selfed (SS), hand selfed (HS), and late selfed (LS) *Campanula thyrsoides* plants from a pollination study in the common garden. Value represents mean ± standard error (SE). For *n* see Table 1.

However, the mean number of less developed seeds per capsule was significantly higher in sister-crossed- compared to outcrossed offspring ($P < 0.05$).

We detected no significant difference in number of seeds per capsule among the different seed families for total seeds, well developed and early aborted seeds ($P = 0.15$, $P = 0.19$, and $P = 0.74$, respectively). However, the number of less developed seeds per capsule differed significantly between seed families ($P < 0.05$).

Table 1: Mean weight (mg) of seeds per capsule (± SE) and mean germination percentage (± SE) in *Campanula thyrsoides* after different pollination treatments (*n*: number of plants per treatment)

Treatment	**Weight (mg)**	**Seed germination (%)**	***n***
Control	16.17 (± 2.42)	78.80 (± 3.93)	7
Outcrossing	13.71 (± 1.59)	88.65 (± 2.85)	26
Sister crossing	15.90 (± 1.84)	82.73 (± 3.85)	26
Hand-selfing	0.09 (± 0.04)	/	26
Spontaneous selfing	0.14 (± 0.07)	/	26
Late selfing	0.17 (± 0.08)	/	26

Self-compatibility system

C. thyrsoides is semi-compatible since it set as many seeds in outcrossed and sister-crossed flowers. However, when the number of outcrossed and sister-crossed seeds were compared with selfed seeds, the self-compatibility index (SCI) was very low for both young flowers (spontaneous and hand-selfing: 0.021) and old flowers (late selfing: 0.052). This indicates almost complete self-incompatibility among plants carrying the same alleles.

Seed weight

There was no significant difference in seed weight (mg) per capsule between controls vs. outcrossed and controls vs. sister-crossed seeds (P = 0.36, P = 0.86, respectively). Neither could we detect a significant difference between seed families (control vs. outcrossing, P = 0.36; control vs. sister crossing, P = 0.72). The only difference that we detected was a higher seed weight in sister-crossed seeds compared to outcrossed seeds (P <0.05).

Table 2. Mean rosette diameter (mm) (± SE) and number of leaves per rosette (± SE), 15 and 25 weeks after 2nd generation *Campanula thyrsoides* seedlings from four different pollination treatments had been re-potted (*n:* number of seedlings per treatment).

	Rosette diameter 15 weeks	**Rosette diameter 25 weeks**	**N leaves / rosette 25 weeks**	***n***
Control	63 (± 3.17)	176 (± 4,62)	55 (± 1.82)	58
Outcrossing	58 (± 6.61)	191 (± 4.22)	52 (± 2.06)	68
Sister crossing	55 (± 5.42)	181 (± 4.60)	50 (± 1.64)	79
Selfing	65 (± 4.4)	186 (± 7.55)	53 (± 2.37)	30

Seed germination

The seed germination was very high for both control, outcrossing and sister-crossing treatments. We recorded a 78.8 % germination success in seeds from the control treatment, 88.7% from outcrossing, and 82.7% from sister-crossing (Table 1). Germination percentage for seeds from control plants did not differ significantly from germination percentage observed in seeds from outcrossed and sister-crossed treatments (P = 0.60, P = 0.97, respectively). Slightly fewer seeds from the sister-crossing treatment germinated compared to seeds from the outcrossing treatment but the difference was only marginally significant (P = 0.07). There was no difference in the percentage of seed germination among seed families (P = 0.91).

Survival and size of seedlings

Fifteen weeks after the seedlings were transferred, many had died (mortality: 69 %), but the mortality percentage did not differ significantly between the outcrossing and the sister crossing treatments (P = 0.17). Additionally, there was no significant difference in the diameter of rosette (after 15 weeks: P = 0.46, after 25 weeks: P = 0.29) and number of leaves per rosette (after 25 weeks: P = 0.66) between the outcrossed and the sister-crossed plants (Table 2).

Moreover, we detected no significant difference in the rosette diameter (P = 0.14), and number of leaves per rosette (P = 0.35) between the offspring of control, selfed, sister-crossed and outcrossed plants 25 weeks after the seedlings transfer, although the rosette diameter was significantly larger in selfed offspring after 15 weeks ($P < 0.05$).

Discussion

Breeding system and seed set

Campanula thyrsoides had the same floral development as most other *Campanula* species being strongly protandrous with pollen deposited by the anthers directly onto the style before bud opening. Later the anthers withered and the stigma became receptive. This kind of temporal separation of male and female maturation, dichogamy, is usually regarded as an outbreeding mechanism (Bhardwaj and Eckert 2001; Silvertown and Charlesworth 2001). In our case this was truly so, since the results show us that *C. thyrsoides* is allogamous and self–incompatible like has been recorded to be the most common reproductive mode of the genus (Shetler 1979; Nyman 1993). Moreover, the SI system of *C. thyrsoides* proved to be of gametophytic origin since the flowers pollinated with pollen from plants from the same seed family (sister mating) produced as many seeds as outcrossed flowers (called semi-compatibility), but the same has been documented for other species in the Campanulaceae (Stephenson et al. 1992; Richards 1997; Steinbachs and Holsinger 2002).

The very low self compatibility index (spontaneous and hand-selfing: 0.021) indicates that *C. thyrsoides* has a very strong SI system. Plants with a self-compatibility index (SCI) of less than 0.15 are defined as strong SI plants while plants with a SCI of more than 0.40 are regarded as weak SI plants (Stephenson et al. 2000). Besides, there was no indication of a break down in the SI system with flower age as has been the case e.g. in *Campanula rapunculoides* (Vogler et al. 1998) since the SCI of the late selfing plants was only slightly higher (0.052) than in the spontaneous and hand-selfing (early selfing) plants.

The strong SI system and the allogamous habit of *C. thyrsoides* should not be very surprising since most *Campanula* species are self-incompatible and allogamous

(Shetler 1979; Nyman 1993). Since strict self-incompatibility is rare among alpine and arctic plants (Molau 1993; Brochmann and Steen 1999), a weaker SI system in *C. thyrsoides* is logically expected. For example, *C. uniflora* populations in Greenland and Iceland, occurring under very similar arctic conditions, showed self-compatibility and even preanthesis cleistogamy (Ægisdóttir and Thórhallsdóttir, in press).

As was previously mentioned, *C. thyrsoides* often lives in small and isolated populations in the fragmented Alpine landscape where self-compatibility should be favoured (Baker, 1955, 1967). Moreover, the species is monocarpic, which also might enhance self-compatibility (Barrett and Charlesworth, 1991). Nevertheless, this does not seem to favour self-compatibility in *C. thyrsoides*.

Inbreeding depression

Surprisingly, we found no indication of inbreeding depression in *C. thyrsoides* in this study as there was no significant difference in seed set, seed weight, and germination percentage between outcrossed and sister-crossed flowers. Equally, no difference was detected in the survival and size of outcrossed (assumed inbreeding coefficient: $F = 0$; sister-crossed offspring: $F = 0.125 – 0.25$). Why did we not detect any inbreeding depression in this outbreeding species? Living in the fragmented landscape of the Alps and consequently being spatially isolated from other populations could have caused frequent bottlenecks during colonization of isolated habitats. Since plant populations that have experienced repeated bottlenecks or pollinator failures are likely to exhibit reduced levels of inbreeding depression due to a reduction in genetic load, this could explain the low inbreeding depression in this outbreeding species. However, repeated bottlenecks might also select for reproductive assurance and thus lead to a break-down of the SI systems (Lande and Schemske 1985; Karron 1989; Glémin at al. 2001), but this was not observed in the studied population.

Moreover, Frankham et al. (2002 and references therein) argue that the degree of inbreeding depression also depends upon the amount of inbreeding. In this context, the ideal situtation to compare fitness values between plants is given for individuals with highly contrasting inbreeding coefficients, i.e. $F = 0.5$ for complete selfing and $F = 0$ for complete outcrossing. To recall, *C. thyrsoides* displayed maximum F values in a range of 0.125 – 0.25 and a decrease in fitness measures might have remained undetected due to the small contrast with completely outbred individuals. Inbreeding depression is also sometimes first detected in later stages of the life cycle, such as seedling biomass of the reproduction of second generation progeny (Karron, 1989), which we were not able to follow in this study. It is also possible that the control plants suffered from inbreeding depression because of fixed deleterious alleles. Moreover, since relatively few plants flowered in summer 2003, we can not completely exclude the possibility that those plants were more vigorous and less inbred than the plants which flowered later.

Conclusion

Like most other *Campanula* species, *C. thyrsoides* appeared to be both allogamous and self-incompatible. Since *C. thyrsoides* is a successful outcrosser, we expected to detect some negative inbreeding effects in inbred offspring (sister mating), which was not the case in our study. We conclude that the absence of inbreeding depression in this outcrossing Alpine species might be a result of frequent bottlenecks during colonization of isolated habitats in the fragmented Alpine landscape.

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Chapter 5

The biological flora of Central Europe: *Campanula thyrsoides* L.

Patrick Kuss, Hafdís Hanna Ægisdóttir, Jürg Stöcklin

Summary

Campanula thyrsoides is one of the few monocarpic perennials of temperate alpine mountain ecosystems and native to the European Alps and adjacent mountains ranges. Individuals are rare but locally abundant and the species is protected in most of the Alpine countries. The exceptionally yellow-flowering member of the Campanulaceae grows preferably in alpine meadows with a moderate disturbance regime on limestone or carbonate bearing schists. Traditionally regarded as a rosette-forming "biennial", individuals usually flower after 8 years and grow considerably older at higher altitudes. This article reviews the taxonomic status and morphology, the distribution, ecology, life cycle, population biology, genetics and legal protection status of this species.

Key words: Central Europe, ecology, monocarpic perennial, semelparity, species biology

Taxonomy

Campanula thyrsoides L.(Campanulaceae) – Yellow bellflower
Synonyms: *C. thyrsoidea* L.
C. thyrsoïdea L.

Two subspecies have been recognised that differ in morphological and palynological characteristics as well as in ecology and distribution (Podlech, 1964; Caramiello et al., 2002-2003). Nevertheless, transitional forms exist within and outside of their geographic contact zones (Podlech, 1964; Kuss, pers. obs.) and the two subspecies are sometimes regarded as altitudinal vicariants ((Tomažic, 1941) correct: Tomažič, 1941).

C. thyrsoides L. ssp. *thyrsoides*

Common names: Gewöhnliche Strauß-Glockenblume – Campanule en thyrse – Campanula gialla – Šopasta zvončica – Brunzina püschlada

C. thyrsoides L. ssp. *carniolica* (Sünd.) Podlech
Basio: *C. thyrsoidea* L. var. *carniolica* (Sündermann, 1925)

Common names: Krainer Strauß-Glockenblume – Campanule de Carniole – Campanula carniolica – Kransjka zvončica – Žućkastobijela zvončika

In the following text we will refer to *C. thyrsoides* ssp. *thyrsoides* as *C.* thyrsoides* and likewise to *C. thyrsoides* ssp. *carniolica* as *C.* carniolica*. Where information applies to both subspecies we use *C. thyrsoides*. Please note that *C.* thyrsoides* is by far the more common subspecies and therefore much more is known.

The genus *Campanula* includes approximately 350 species and occurs almost exclusively in the temperate zone of the Northern Hemisphere. Highest species diversity in the genus is found in the mountain ranges of the Eastern Mediterranean and the Caucasus (Fedorov and Kovanda, 1978). Within the European Alps 42 *Campanula* species are recognized of which 20 are endemic or subendemic (Aeschimann et al., 2005). *Campanula thyrsoides* is positioned into the large section *Campanula* s. str. which is characterized by dehiscent capsules with basal pores, and thus separated from the second section Rapunculus with lateral or sub-apical pores (Fedorov and Kovanda, 1978). This morphological classification and *C. thyrsoides*' position therein has been confirmed by molecular investigations (Eddie et al., 2003). For earlier classifications see de Candolle (1830), Schönland (1889-1894), and Fiori (1927). The genus *Campanula* hosts a range of life-forms, from annuals to long-lived polycarpic perennials, and the monocarpic (= semelparous or hapaxant) strategy of *C. thyrsoides* is also found in other species from various subsections (Shulkina, 1980) so that a monophyletic origin of semelparity can not been assumed. Monocarpic *Campanula* species are e.g. *C. alpina* Jacq, C. cervicaria L., C. macrochlamys Boiss. et Huet, *C. macrostachya* Waldst. et Kit. ex Willd., *C. medium* L., *C. mirabilis* Albov, *C. moesiaca* Velen., *C. patula* ssp. *patula* L., *C. patula* ssp. *costae* (Willk.) Nyman, *C. patula* ssp. *jahorinae* (K. Maly) Greuter & Burdet, *C. petraeae* L., *C. pyramidalis* L., *C. sibirica* ssp. *sibirica* L., *C. spicata* L. and *C. transsilvanica* Schur ex Andrae; see (Jäger, 2000; Bernini et al., 2002).

Morphology

The two subspecies are differ mainly with respect to inflorescence height and spike density (*C.* thyrsoides*: 10-40 cm and dense, *C.* carniolica*: 40-100 cm and basal flowers rather lax), length of floral bracts (*C.* thyrsoides*: as long as corolla and not canaliculate, *C.* carniolica*: twice as long as corolla and canaliculate), canalicuate leaves in *C.* carniolica* (Sündermann, 1925; Podlech, 1964), as well as a number of (not further characterized) palynological features (Caramiello et al., 2002-2003). All further information applies to both subspecies. For color images of the two subspecies see Aeschimann et al. (2005).

Campanula thyrsoides has a thick, fleshy and whitish tap root, commonly 20-40 cm long, but occasionally protruding to a depth of 1 m (Figure 1). The roots as well as all other parts of the plant contain milky and sticky latex. The root architecture is fairly plastic with respect to branching of the main tab root and frequency of fine roots (< 1 mm) as a response to the diverse soil conditions. In water-logged or rocky soils the roots often grow almost horizontally spreading in several direction, while in mesic to

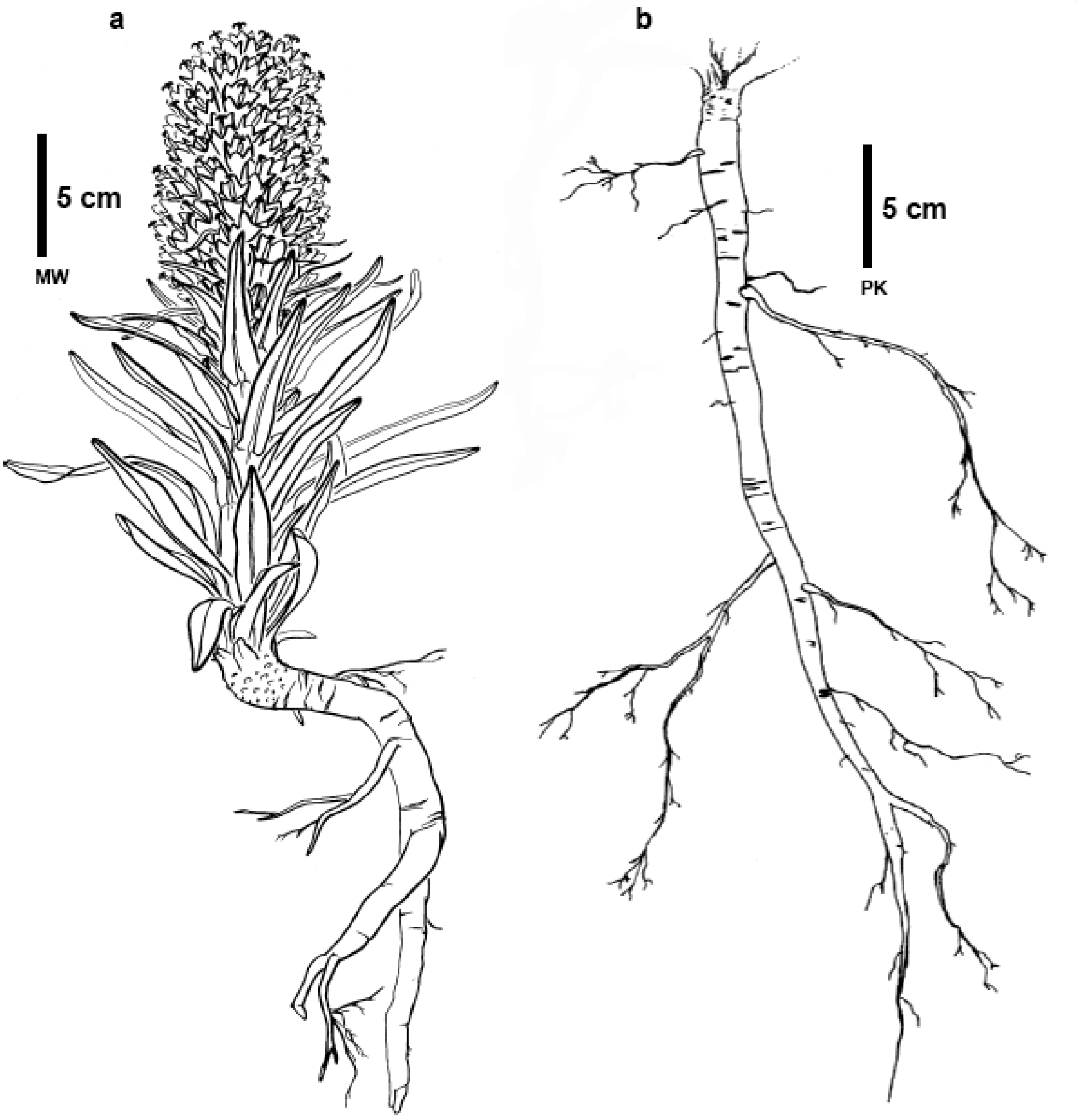

Figure 1. *Campanula thyrsoides*. (a) Habitus at flowering, garden plant, 2-years old; (b) root profile, vegetative plant, 8-years old, Furka Pass, Switzerland.

dry as well as in deep soils the roots are mostly unbranched and vertically expanding. The root collar of flowering plants measure around 11 mm (range: 4-20 mm).

Vegetative individuals form rosettes with a maximum diameter of 45 cm and 20-30 leaves (Figure 2 f-h; see Life-Cycle). Annual rosette growth is characterized by two phases of foliar development with two types of leaves. Spring leaves are oblong to ecliptical, entire margined, sometimes undulate, with an obtuse to acute apex, cuneate leaf base that slowly tapers into the petiole and are densely covered with stiff whitish-brownish hairs (Figure 2 a). The midrib is well developed. Depending on age, leaves

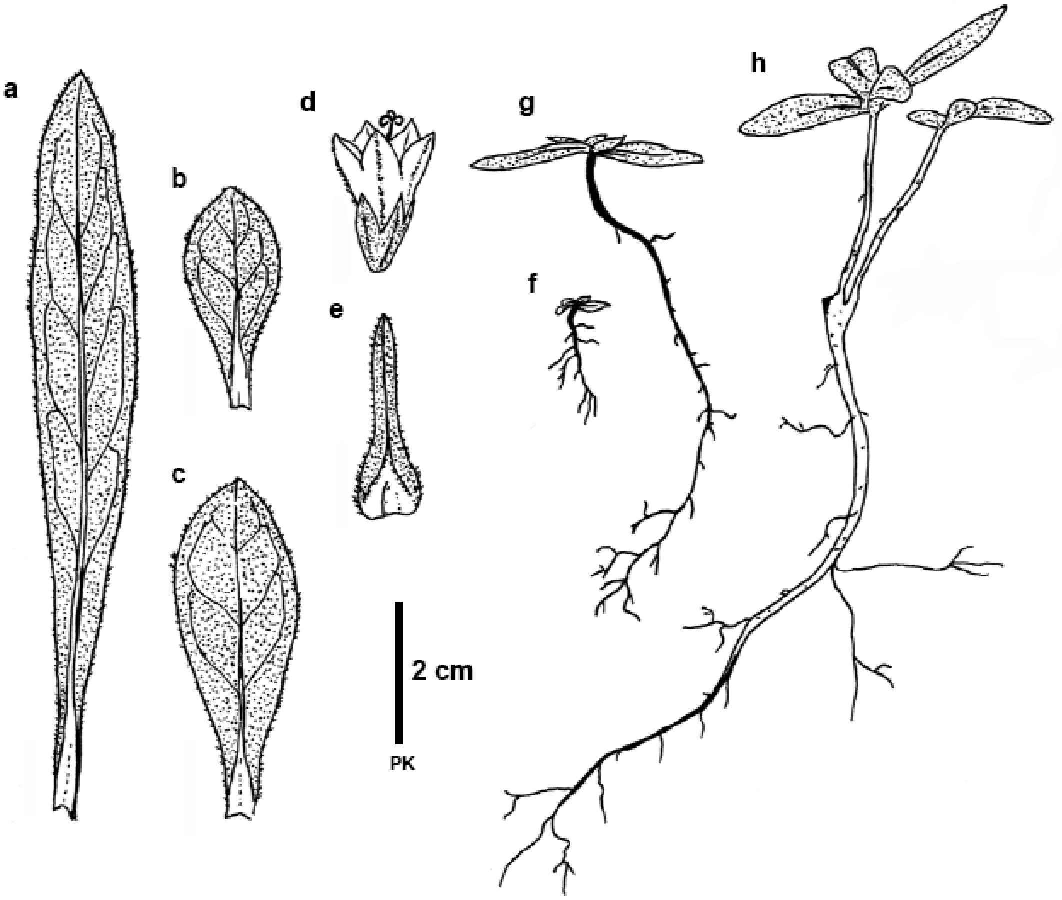

Figure 2. *Campanula thyrsoides*. (a) Spring leaf; (b,c) summer leaves; (d) flower; (e) bract; (f) seeling; (g) 1-year old vegetative plant; (h) 4-year old vegetative plant, note: two sister rosettes.

measure 6 × 4 mm to 225 × 35 mm. Summer leaves are 2-4 times smaller, obovate to oblanceolate and are formed at the center of the spring rosette (Figure 2 b,c). The majority of the rosette leaves wither over winter leaving a tunica of leave bases.

The flowering stalk is erect, 10-100cm tall (subspecies specific), angular, hirsute, densely foliose with sessile, alternate, 20 × 4 mm to 120 × 16 mm long, oblong to obovate, sometimes linear leaves. The inflorescence is composed of 50 to 200 flowers forming a dense or moderately lax spike (Figure 1 a). Flowers are erect, either single or arranged in cymules of 2-5 sessile flowers in the axils of short and pointed linear bracts (Figure 2 e). Calyx lobes are green, linear to lanceolate, acute, approximately half as long as the corolla and densely tomentose with stiff white hairs. The corolla is bell-shaped, 15-25 mm long, with villose nerves and a faint pleasant scent

(Figure 2 d). Unlike the majority of blue-flowered *Campanula* species, *C. thyrsoides* has yellow to pale-yellow flowers. In the European Alps, this color peculiarity is only shared by *C. albicans* Jacq. (white) and *C. petraea* L. (yellow; Aeschimann et al., 2005). Adamovic (1909) mentioned *C. thyrsoides* to have blue flowers throughout the Balcans. However, this has never been confirmed in the literature or from herbarium specimen. The flowers are hermaphroditic with five stamens, white filaments, yellow anthers, a single central style and three stigmatic lobes. The style is partly covered with retractable pollen-collecting hairs. These are more or less uniform in length, regularly arranged and have ascending tips that point toward the stigma (Nyman, 1993a).

The yellow pollen grains are monadic, 3- or 4-zonoporate and porous, radially symmetric, isopolar, oblate-sphaeroidal to subprolate in shape, appearing subcircular to circular in equatorial view (33.2 μ) and peritreme in polar view (32.7 μ). The surface is rugulate with spines which are uniformely distributed (base: 1.8 μ, heigth: 2.2 μ, distance: 2.0 μ). The aperture is situated in equatorial position, lolongate, circular or lalongate in shape and with indistinct annulus (P: 4.9 μ, E: 4.5 μ, mesoporium: 20.1 μ). The exine measures 2.2 μ, comprised of sexine: 1.2 μ and nexine: 1.0 μ (Caramiello et al., 2002-2003).

Capsules are erect, three-loculate, each locus containing approximately 40-60 seeds (see Reproduction). Well developed viable seeds are brownish, 1.2 mm long, compressed, sometimes with a rudimentarily developed winged margin. Less developed seeds are of similar size but lighter in color, thinner and rarely viable. Early aborted seeds are blackish to brownish round granules. Capsules open at the base with an outward retraction of the lower capsule tissue. Seeds are trapped by the withered concave bracts and the felted unicellular hairs of bracts, calyx and corolla. The withered infructescence remains upright as a winter stander for as long as a year. The seed arresting mechanism in combination with the lignified infructescence lead to a secondary diaspore presentation and an aboveground seed pool, essentially similar to the monocarpic *Campanula cervicaria* (Often, 1999) or the perennial *C. glomerata* (Emig and Leins, 1996). Seeds are only dispersed when strong winds, rain or animals shake the seeds out of the seed trap (see Reproduction).

In case the apical meristem of the flowering stalk is damaged due to herbivory or mowing, secondary shoots with pedicelate terminal flower cymules can be formed in the axils of the lower leaves. This leads to a peculiar, rather bushy habitus of the plant

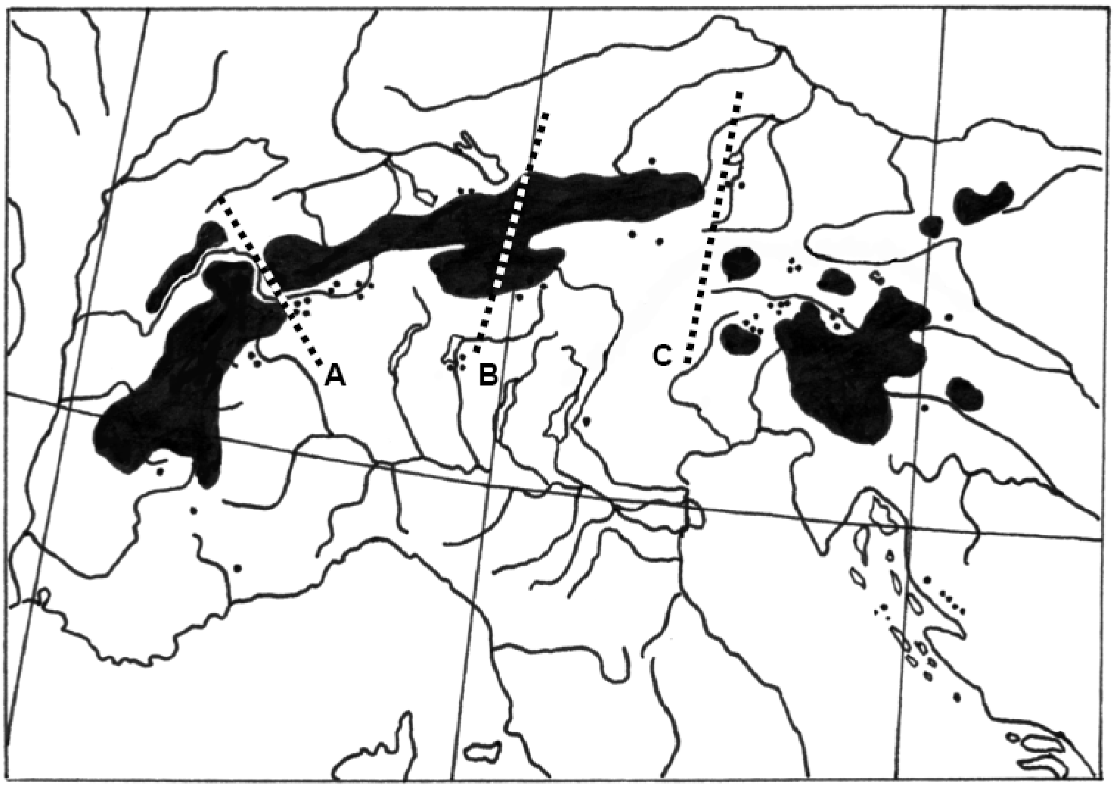

Figure 3. Geographic distribution of *Campanula thyrsoides*. Dashed lines represent prominent biogeographical borders separating glacial refugia for many species (Schönswetter et al., 2005). Line A has been confirmed for *C.* thyrsoides* (see Genetic Data). Line C separates the two subspecies: *C.* thyrsoides* – west of C, *C.* carniolica* – east of C.

sometimes referred to as *C. thyrsoides* L. var. *glomerata* Saut. (Schiebler, 1935) or *C. thyrsoides* f. *putata* (Hegi, 1975).

Geographical distribution

C. thyrsoides is native to the European Alps and adjacent mountain ranges to the East (Balcans and Dinarids) and North-West (Jura) and occurs in France, Italy, Switzerland, Lichtenstein, Austria, Germany, Slovenia, Croatia, Bosnia-Hercegovina and Bulgaria. An updated distribution map is presented in Figure 3, a generalized distribution map can be found in Aeschimann et al. (2005), and country specific information is available in print or electronically: Bosnia-Hervegovina (Šoljan, 2001), Croatia (Nikolić, 2006), France (Brisse et al., 1996-2006), Germany (Haeupler and Schönfelder, 2003), Switzerland (Welten and Sutter, 1982; Wohlgemuth, 1993). The two subspecies have separated distribution ranges with *C.* thyrsoides* occuring in the Western, Central and North-Eastern Alps as well as in the Jura mountains, thus having a cottian-helvetic-(north-noric) distribution pattern (Figure 3, west of C). *C.* carniolica* is only described from the South-Eastern Alps and the adjacent Dinarids and Balcans, hence a carnian distribution (Figure 3: east of C; Jäger, 2000). The morphological (and ecological) differentiation of the two subspecies has been hypothesizes to be a result of isolation and survival in different glacial refugia during the Quaternary (Jäger, 2000). Indeed, the review of Schönswetter et al. (2005) on glacial refugia in the Alps confirm the existence of an eastern alpine refugia which would correspond well with the present occurrence of *C.* carniolica*. However, *C.* thyrsoides* does not seem to be restricted to the Central and Western Alps but is also mentioned at single locations throughout the area of *C.* carniolica*. For example, *C.* thyrsoides* is reported from the Bosnian Klekovača mountain (Abadžić and Šilić, 1990), from Slovenian sites above 1600 m a.s.l. (Wraber, pers. comm.) and also characterizations from Bulgaria seem to refer to this subspecies (Stojanov and Kitanov, 1966). To further complicate things, large individuals (60 cm) with a lax inflorescence and long, caniculate bracts have been found in Switzerland at 1700 m a.s.l. and thus could belong to *C.* carniolica* (Kuss, pers. obs.). It remains therefore unclear to which extent glacial isolation has driven morphological and ecological differentiation and further, whether morphological differences are to be seen as a response to altitude. *C.* thyrsoides* is mostly found in subalpine to alpine habitats between 1600-2200 m a.s.l. with lowest occurences reported around 1010 m a.s.l. (Oytal, Bavarian Alps; Hegi, 1975) and 1040 m a.s.l. (Chapfensee, Switzerland; (Seitter, 1989)) and highest locations in Switzerland at 2800 m a.s.l. (Col de Sorebois; (Becherer, 1956)) and 2900 m a.s.l. (Val Mora; (Brunies, 1906)). In contrast, *C.* carniolica* grows preferentially in submontane to montane habitats between 400-1800 m a.s.l. with lowest reported occurrences around 300 m a.s.l. (Kozje, Slovenia; Hegi, 1975; Jäger, 2000).

Habitat

C. thyrsoides is a basiphilous species growing in shallow to deep soils derived from limestone, carbonate-bearing shists, dolomite or gypsum, and exceptionally granite with average pH-values between 5 to 8 (Béguin, 1972; Schubiger-Bossard, 1988; Vittoz, 1998) and little to moderate nitrogen availablity (Béguin, 1972). Typical soils are e.g. Lithic Leptosols [Syrosem] and Rendzic Leptosols [Eurendzina], Calcaric Regosols [Pararendzina] and Phaeozems [Mullgley], Gleyic Cambisols [Gley-Braunerde], Calcaric Cambisosls [Pararendzina] and Mollic Cambisols [Lockerbraunerde], seldom Folic Histosols [Tangelrednzina] or Haplic Histosols [Niedermoortorf] (e.g. Béguin, 1972; Vittoz, 1998); nomenclature follows World Reference Base for Soils and [German classification], Scheffer and Schachtschabel, 1998). *C. thyrsoides* can tolerate a wide range of water regimes from xeric/mesic to mesic/wet conditions and snow cover for 6-7 months. Plants are usually found in open and sunny habitats on moderately (0-10°) to steeply inclined (>60°) E-, S- or W-facing slopes. The species is adapted to and requires a moderate disturbance regime (meso- to euhemerobic) which creates microsites for successful germination of seeds. Disturbances can either be topography-induced processes, e.g. slope movement, or trampling by animals. Ellenberg’s indicator values represent only in part the ecological amplitude of the species: L8, T2, K4, F5, R7, N4, S0 (Ellenberg, 1996).

Communities

Most populations of *C.* thyrsoides* are found in natural and anthropogenic species-rich calcareous grasslands from treeline ecotone into the alpine belt but plants also occur in tall-herb communities of avalanche shoots and forest edges, alpine fens, forest clearings, screes, river banks and road shoulders. The wide ecological amplitude of the species, or maybe the existance of the particular niche in many habitats, poses some problems with respect to a phytosociological characterization such that occurrences are reported in a variety of associations, alliances, orders and classes (Table 1). *C.* thyrsoides* has been repeatedly regarded as a characteristic species of the alliance Caricion ferrugineae which comprises species-rich sub-alpine and alpine grasslands on calcareous substrates (Oberdorfer, 1977; Grabherr and Mucina, 1993; Ellenberg, 1996; Wilmanns, 1998) and this alliance certainly represents the “syntaxonomic optimum” of the subspecies (Aeschimann et al., 2005). Older literature refers to *C.* thyrsoides* as being characteristic for the association Carricetum ferrugineae (e.g. Braun-Blanquet, 1948) but this indicator status has been correctly transferred to the alliance level. Among the many syntaxa listed in Table 1, it is noteworthy that *C.* thyrsoides* is name-giving species for a plant community restricted to the Jura mountains, Campanulo thyrsoideae-Laserpitietum latifoliae, which occurs on steep, mesic, species-rich,

Table 1. Syntaxonomical units with occurrences of *Campanula thyrsoides* ssp. *thyrsoides*. Nomenclature follows Grabherr and Mucina (1993), Mucina et al. (1993) with additions from Julve (1988-2006) and Oberdorfer (1977). Bold: reported occurences; underlined: *C.* thyrsoides* is characteristic species; entries in [] denote geographic origin of relevés (A: Alps; CH: Switzerland, J: Jura), Syn.: Synonym.

- Seslerietea albicantis Oberd. 1978 corr. Oberd. 1990
  - Seslerietalia coeruleae Br.-Bl. in Br.-Bl. et Jenny 26
    - Seslerion coeruleae Br.-Bl. in Br.-Bl. et Jenny 26
      - **Seslerio-Caricetum sempervirentis** Br.-Bl. in Br.-Bl. et Jenny 26 (Reinalter, 2004) [**A**]
      - Alchemillo hoppeanae-Seslerietum caeruleae Luquet et Aubert 1930
      - Syn: **Alchemillo conjunctae-Seslerietum albicantis** (Vittoz, 1998) [**J**]
      - **Veronico fruticulosae-Agrostietum sideritetosum** Béguin 1972 (Béguin, 1972) [**J**]
    - **Caricion ferruginae** G. Br.-Bl. et J. Br.-Bl. 1931(Oberdorfer, 1977, Grabherr, 1993) [**A**]
      - **Caricetum ferrugineae** Lüdi 21(Oberdorfer, 1977, Reinalter, 2004) [**A**]
      - Trifolio thalii-Festucetum nigricantis Br.-Bl. in Br.-Bl. et Jenny 1926 corr. Grabherr [**A**]
      - Syn: Trifolio thalii-Festucetum violaceae Br.-Bl. in Br.-Bl. et Jenny 1926
      - Syn: **Festucetum violaceae** (Reinalter, 2004) [**CH**]
      - **Peucedano-Laserpitietum** J.-L. Richard 1977 (Richard, 1977; Theurillat, 1991) [**CH**]
      - **Senecioni-Caricetum sempervirentis** J.-L. Richard 1977 (Richard, 1977; Gillet et al., 1994) [**CH**]
    - Calamagrostion variae Sillinger 1929
      - Origano-Calamagrostietum variae Lippert ex Thiele 1978
      - Syn: **Laserpitio latifoliae-Calamagrostietum variae** (Kuhn 37, Moor 57) Th. Müll. 61(Béguin, 1972; Oberdorfer, 1978; Vittoz, 1998) [**J**]
      - Syn: **Calamagrostidetum variae** Gams 1927 (Gams, 1927) [**CH**]
      - **Campanulo thyrsoideae-Laserpitietum latifoliae** Béguin 1972 (Béguin, 1972, Theurillat, 1991)
- Mulgedio-Aconitetea Hadač et Klinka in Klinka et Hadač 1944
  - Adenostyletalia G. Br.-Bl. et J. Br.-Bl. 1931
    - Adenostylion alliariae Br.-Bl. 1926
      - **Cicerbitetum alpinae** Bolleter 1921 subassoc. **rhaponticetosum** (Kuss, unpublished) [**CH**]
- Koelerio-Corynephoretea Klika in Klika et Novák 1941
  - Alysso-Sedetalia Moravec 1967
    - Allyso allysoidis-Sedion albi Oberd. et T. Müller in T. Müller 1961
      - **Sedo acris-Poetum alpinae** Royer 1985 (Royer, 1985; Vittoz, 1998) [**J**]
- Festuco-Brometea Br.-Bl. et R. Tx. ex Klika et Hadač 1944
  - Brometalia erecti Br.-Bl. 1936
    - Bromion erecti Koch 1926
      - Onobrychido viciifoliae-Brometum T. Müller 1966
      - Syn: Carlino acaulis-Brometum Oberd. 1957
      - Syn: **Gentiano vernae-Brometum erecti** (Vittoz, 1998) [**J**]
      - **Ranunculo montani-Agrostietum cappilaris** (Vittoz, 1998) [**J**]
- Anemono nemorosae-Caricetea sylvaticae Gillet 1986 (see Gallandat et al., 1995)
  - Mercurialietalia perennis Gillet 1986
    - Actaeo spicatae-Mercurialion perennis Gillet 1986
      - **Valeriano montani-Polygonatetum verticillati** Gillet [**J**]
    - Seslerio caeruleae - Mercurialion perennis Gillet 1986
      - **Melampyro sylvatici-Calamagrostietum variae** Vittoz 1998 (Vittoz, 1998) [**J**]
- Thlaspietea rotundifolii Br.-Bl.1948
  - Epilobietalia fleischeri Moor 1958
    - Salicion incanae Aichinger 1933
      - **Epilobietum fleischeri** Frey 1922 (Schubiger-Bossard, 1988) [**CH**]

Table 1. continued

- Molinio-Arrhenatheretea R. tx. 1937 em. R. Tx. 1970
  - Poo alpinae-Trisetetalia Ellmauer et Mucina ordo nov. hoc loco
    - Polygono-Trisetion Br.-Bl. et R. Tx. ex Marschall 1947 nom. inv.
      - **Trisetum flavescentis** Rübel 1911(Reinalter, 2004) [**CH**]
- Scheuchzerio-Caricetea fuscae R. Tx. 1937
  - Caricetalia davallianae Br.-Bl. 1949
    - Caricion davallianae Klika
      - **Caricetum davallianae** Dutoit 1924 (Reinalter, 2004; Kuss, unpublished.) [**CH**]
- Caricetea curvulae Br.-Bl. 1948
  - Caricetalia curvulae Br.-Bl. in Br.-Bl. et Jenny 1926
    - Festucion variae Guinochet 1938
      - Hypochoerido uniflorae-Festucetum paniculatae Hartl in Theurillat 1989
      - Syn: **Laserpitio-Helictotrichetum pratensis** (Reinalter, 2004) [**CH**]
      - Syn: **Laserpitio-Avenetum pratensis** (Braun-Blanquet, 1969) [**CH**]
      - **Dracocephalo-Potentillietum** Br.-Bl. 1969 (Reinalter, 2004) [**CH**]
- Carici rupestris-Kobresietea bellardii Ohba 1974
  - Oxytropido-Kobresietalia Oberdorfer ex Albrecht 1969
    - Oxytropido-Elynion Br.-Bl. 1949
      - **Elynetum myosuroides** Rübel 1911 (Reinalter, 2004) [**CH**]

W-SW-exposed slopes between 1300 and 1500 m a.s.l. (Béguin, 1972). Also interesting to note is the occurence of *C.* thyrsoides* within the pioneer community Epilobietum fleischeri on the foreland of the Swiss Rhône-glacier on mostly silicious ground (Schubiger-Bossard, 1988). This very site, however, is in close vicinity to a ruderalized population of *C.* thyrsoides* along the Furka Pass road and thus, the sub-spontaneous appearance within the Epilobietum fleischeri is most probably related to disturbances during the road improvements works conducted in the 1960's.

Populations of *C.* carniolica* are found in similar habitats as *C.* thyrsoides* and additionally within meso-thermophilic pine forests and juniper meadows (Jäger, 2000; Aeschimann et al., 2005). However, little phytosociological literature was available that mentioned *C.* carniolica* or could be referred to this subspecies. Aeschimann et al. (2005) list *C.* carniolica*'s "syntaxonomic optimum" within the alliance Petasition paradoxi, which comprises communities of montane to sub-alpine screes of calcareous or marly substrate. Abadžić and Silić (1990) mention the subspecies' optimum in alpine grasslands within the order Seslerietalia juncifolia Ht 1930 (now integrated into Brometalia erecti; Horvat et al. 1974). *C.* carniolica* is further reported from a Slovenian endemic sub-mediterranean forest syntaxon, Cytisantho-Ostryetum Wraber 1960 (Ostryo carpinion; see Wraber, 1960; Horvat et al., 1974), as well as from dry to mesic grassland communities of the Scabioso hladnikianae-Caricetum humilis Tomažič 1941 (Bromion erecti Koch 1926; see Tomažič, 1941) and the Bromo-Plantaginetum mediae Horvat 1931 (Bromion erecti Koch 1926; see Šugar, 1972).

Responses to abiotic factors

Age at flowering significantly increases with altitude (see Reproduction).

Abundance

In most populations *C.* thyrsoides* is scarce and reaches a coverage of only 1–5 %. Within permanent plots of 1 × 1-m abundance of vegetative rosettes was generally between 0 and 20 individuals. On average, 5 % of a population flowers and sets seed in a given season. However, there is considerable interannual fluctuation of flowering individuals between 0 and 10 % which is largely explained by demographic stochasticity (see Life-Cycle) and seasonal climatic fluctuation. A special situation is encountered on ruderal sites, such as abandoned construction sites or road shoulders. In the latter case, *C.* thyrsoides* can dominate the plant community and more than 200 vegetative rosettes and seedlings per m2 have been found. Locally, populations follow mountain roads for several kilometers, being confined to the carbonate-bearing road construction material in an otherwise siliceous bedrock environment (e.g. roads to Lac de Moiry and Furka Pass, Switzerland).

Life cycle

C. thyrsoides is a rosette-forming monocarpic hemicryptophyte lacking means of vegetative propagation. Detailed investigations of the demography of *C.* thyrsoides* have been conducted at two sites in the Swiss Alps (Chapter 3). Seeds germinate directly after snow melt, depending on altitude and snow cover, between May and July, with low establishment rates (see Germination). Seedlings have a high survival probability between 75% and 95%. Once grown into a vegetative rosette, survival rates of individuals increase to 90%-95%. Spring rosette development (see Morphology) is initiated right after snowmelt, the summer rosette at the peak of the vegetation period, usually in August. Vegetative rosettes steadily grow in size and successively increase the number of leaves. Growth rate was not density-dependen ($R^2 = 0.001$, $P = 0.08$). A general trend was detected toward decreased growth with increasing plant size, which is characteristic for most monocarpic species (Metcalf et al., 2003). The probability of flowering was strongly size-dependent (measured as the rosette size the previous year) with an altitude-independent flowering threshold of 20-30 leaves. If sister-rosettes are present they are autonomous and must each reach this threshold in order to flower. Flowering buds are initiated in the year of flowering shortly after snow melt and weak evidence exists for an additional age-dependent genetic regulation of flowering exists. In a common garden experiment at two different altitudes (Basel 290 m a.s.l. and Furka Pass 2450 m a.s.l., Switzerland) individuals of the same seed family tended to flower synchronized at both sites. Mean age at flowering in natural sites is 8 years with considerable variability (see Reproduction). Flowering commonly occurs between June-September (*C.* thyrsoides*) or June-August (*C.* carniolica*). Seed maturation requires approximately 3-4 weeks and seed dispersal starts in late-August to September and can occur over the entire winter (see Reproduction). At high altitudes, cold years with a short vegetation period have been observed to lead to reproductive failure. Flowering plants and will die at the end of the vegetation period, their vegetative sister rosettes will die, too.

Demographic analysis of two *C.* thyrsoides* populations within stable alpine grassland communities revealed that populations can moderately increase even at very low establishment rates, pe ($\lambda \approx 1.08$, Furka: $p_e = 0.016\%$, Schynige Platte: $p_e = 0.078\%$; Chapter 3). Further elasticity analysis of the life-stage transitions using Integral Projection Models showed that survival and growth are the critical determinants of the population growth rate, λ, contributing 88-90% to the overall elasticities. Reproduction contributed only 10-12%. Additionally, the observed flowering strategy (size at flowering) was close to the predicted evolutionary stable strategy (ESS) indicating only weak selection for larger sizes at flowering.

Spatial distribution of plants within populations

See Abundance and Germination

Reproduction

Campanula thyrsoides is a monocarpic perennial, which flowers once and then dies. Traditionally, the species has been considered to be a „biennial“ plant (e.g. Murr, 1923-1926; Hayek, 1928-1931; Hegi, 1975) and only Jäger (2000) estimated flowering

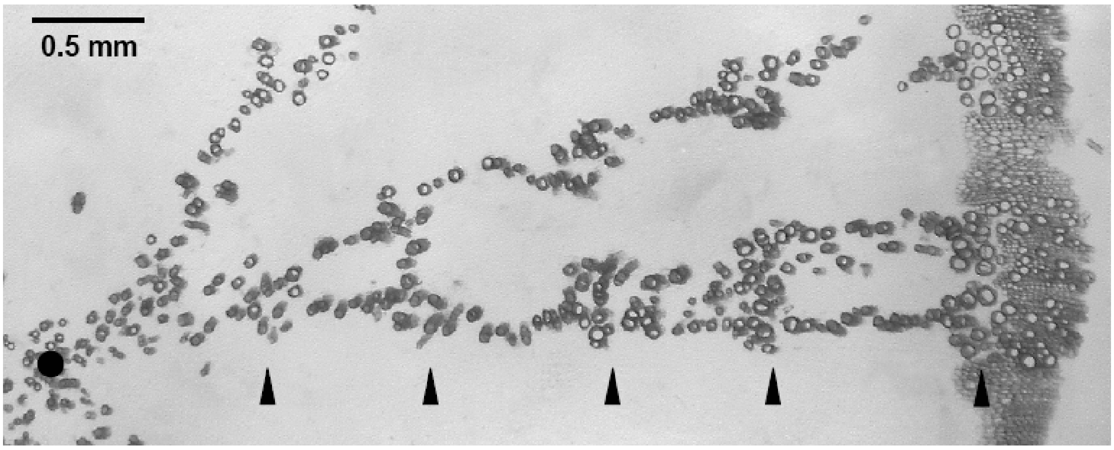

Figure 4. *Campanula thyrsoides*. Root cross-section of 5-year old vegetative plant. Dot: root center; arrows: wide-luminar spring cells.

plant ages to range between 5-10 years, deducing from leaf numbers of large rosettes and annual leaf number increase. A recent survey of plant ages for *C.* thyrsoides* along an altitudinal gradient from 1450 m to 2430 m a.s.l. in Switzerland using 'herb chronology' dating techniques (Figure 4) revealed a mean flowering age of 7.5 years and a high variability ranging from 3-16 yrs (*n* = 250; Hänger, unpublished). Flowering ages significantly increased with altitude (mean ages – Col du Marchairuz ,1450 m: 6.5 years, Schynige Platte, 1950 m: 7.8 years, Furka Pass, 2430 m: 8.3 years), such that the oldest flowering individuals were encountered at Furka Pass. Plants which flower in the second year were never encountered in the field and are also not very common (17%, *n* = 451) if grown under horticultural conditions in the lowlands.

C. thyrsoides has hermaphroditic strongly protandrous flowers. During bud stage, the anthers form a tube around the style, open introversely, such that pollen is deposited on the pollen-collecting hairs along the middle part of the style which then leads to secondary pollen presentation (Fægri and van der Pijl, 1979). During middle anthesis, late in the male phase, the three stigmatic lobes split open and the stigma becomes receptive. At that time, in a number of *Campanula* species, all pollen collecting hairs (PHCs) are retracted into the style starting at the uppermost part of the style and continuing downwards (Nyman, 1993a,b). The retraction of individual hairs starts at the base of the hair (comparable with the retraction of fingers of a glove) and is thought to be initiated by pollinator activity stimulating the PHCs. Late in anthesis, the stigmatic lobes bent backwards and can come in contact with the style. By then, all autologous pollen has usually either been collected by pollinators, fallen to the bottom of the flower or is hidden within the cavities of the retracted hairs such that self-pollination is avoided. However, in *C. thyrsoides*, PCH density is sparse and the retraction mechanism seems to be only imperfectly developed such that a sheath of pollen often remains around the style. Nevertheless, self-pollination is largely avoided because the uppermost part of the style is normally devoid of pollen so that the stigmatic lobes do not come in contact with autologous pollen.

C. thyrsoides is insect-pollinated, mainly by bumble bees and some smaller hymenopterans. Pollen transfer distances measured in at the Furka Pass using flourescent dye showed a maximum pollen flow distance of 39 m indicating pollen-mediated gene-flow to be restricted within single populations. Apomixis, vivipary or cleistogamy has not been observed. *C.* thyrsoides* is an obligatory outcrosser which barely sets viable seeds when experimentially selfed (Ægisdóttir et al., in press). *C.* thyrsoides* can be considered as semi-compatible, gametophytically self-incompatible, since it sets equally many seeds in outcrossed and sister-crossed flowers. In addition, the self-compatibility index (SCI) has been found to be very low for both young flowers (spontaneous and hand-selfing: 0.021) and old flowers (late selfing: 0.052) showing no breakdown of self-incompatibility with flower age (Ægisdóttir et al., in press). Breakdown of the SI-system is however known in other *Campanula* species (Richardson et al., 1990; Vogler et al., 1998). All mentioned pollination experiments revealed no pollen limitation of reproduction. Furthermore, hardly any indication of strong inbreeding depression was found for sister-crossed offsprings (fitness measures: seed set, seed weight, germination percentage, survival probability, size of offsprings; (Ægisdóttir et al., in press). Under natural conditions self-pollination is usually avoided due to pollen removal by insects and further because little pollen is present where the stigmatic lobes may touch the style. Additionally, the probability of sister-mating is low because only 10-20% of a cohort flower in the same season and individuals of a cohort most likely come from different mothers (Hänger, unpublished). Despite this, weak evidence of inbreeding depression exists when seed set from close neighbour crosses are compared to distant neighbour crosses (Ægisdóttir et al., in press).

In approximately 10 % of the plants, one or rarely up to six sister rosettes are formed, which are autonomous with respect to flowering initiation (Figure 2 h). However, vegetative reproduction is not possible because all sister rosettes are connected to the main tab root from which the mobile carbon reserves will be exclusively invested in any flowering rosette.

Seed set occurs in 90-100 % of all flowers under natural conditions. The number of flowers and thus, the number of seeds per plant is strongly dependent on the size of the rosette measured the previous year (Chapter 3). In *C.* thyrsoides*, an average plant had 60 flowers (± 34 SD, min: 28, max: 156, *n* = 122), with an average number of well-developed seeds per capsule of 147 (± 91 SD, min: 0, max: 633, *n* = 500). The number of seeds per capsule has been found to be size- and site-dependent with greatest seed production per capsule at the high altitude site at Furka Pass (Ægisdóttir et al., in press). Overall fecundity per plant is, again, strongly size-dependent (Chapter 3) and ranges between 15000 and 50000 viable seeds (*n* = 122). Seed/ovule ratio was 0.65 (± 0.13 SD, *n* = 500). Well-developed viable seeds weigh on average 0.11µg (± 0.03 SD, *n* = 100) and measure 1.05mm (± 0.15 SD, *n* = 100). The margin is only rudimentarily winged and thus, seeds lack special adaptations to dispersal. Primary dispersal is by wind, rain or animals shaking the seeds out of the withered infructescence (see Morphology). Long-distance dispersal has been modelled using a high-alpine wind data set implemented in the software PAPPUS (Tackenberg, 2003) with a fixed terminal falling velocity $V_{\mathrm{term}} = 1.36\ \mathrm{ms}^{-1}$ and a release height $H_{\mathrm{rel}} = 0.25$ m. About 99.99% of the seeds are dispersed within <10 m of the mother plant, and only 0.001% > 100 m and 0.001% >1000 m. With an average seed production of 1.5 million seeds per populations this implies that approximately 15 seeds are potentially dispersed over 1 km. Secondary anemochory over snow and ice is well probable after the release of seeds out of the lignified infructescence during winter time.

Germination

Seed germination is epigeal and occurs directly after snowmelt between May and July, depending on altitude and snow cover. Seedlings have a shortened epicotyl and condensed basal internodes (Figure 2 f). The cotyledons are oval, 4-6 mm long and 4-5 mm broad, with unicellular hairs mostly along the margin and infrequently on the surface of the cotyledon. In permanent plots we found an average germination rates of 0.016% (Furka Pass) and 0.078% (Schynige Platte). Despite seed production per 1 × 1 m quadrat varying from 0 to 68 000 seeds, the maximum number of seedlings recorded the following year was 25. At both sites there was no relationship between per quadrat seed production and subsequent recruitment. Further, we found no indication that seedling establishment was negatively density dependent. At Furka Pass, recruitment was independent of plant densities, while at Schynige Platte seedling establishment increased significantly with increasing plant densities (Chapter 3). These and the following results indicate that microsite limitation is most influential on the establishment rate of seedlings. In a common garden experiment at Furka Pass with seeds from 10 populations of different altitudes, germination rates varied greatly between 0 and 3% with no observed efect of altitudinal origin of the population. Seeds collected one year prior to the experiment and stored at 4°C still germinated at similar rates. For the common garden study, all seed were collected at the time of seed maturation in the fall and sown shortly afterward in cleaned autochthonous soil. Under laboratory conditions seed germination rates on wet filter paper reached 79% (Ægisdóttir et al., in press). In an additional experiment, application of varying levels of Giberrelic Acid (10-1000 mg/l) had no effect on germination success or timing of germination (Jespersen, 2005). A thorough soil seed bank experiment is still lacking but no literature or field indication exists for a persistent seed bank (Hegi, 1975).

Response to Competition and Management

C. thyrsoides is adapted to a moderate disturbance regime and populations persist within extensively used meadows and pastures most probably for hundreds of years. Unfortunately, no long-term permanent plot data including *C. thyrsoides* exists for Switzerland (Krüsi and Vittoz, pers. comm.) and only indirect evidence is available from old phytosociological relevés (e.g. Lüdi, 1948) that show almost no change in species' frequency/abundance compared to the current condition. There is also no indication that the traditional 2-year hay-making practice above the tree-line (German: *halbschürige Mahd*) of many Alpine cultures has diminished population numbers. However, recent intensification of grazing pressure by cows, and locally sheep, is reported to have reduced population numbers and sizes in the last decades (Seitter, 1989). In contrast, *C. thyrsoides* can been found on numerous road shoulders and construction sites where disturbance has stopped few decades ago. In such places, populations can rapidly expand and sometimes amount to several 100 000 individuals. *C. thyrsoides* usually occurs in naturally ahemerobe or oligohemerobe habitats such that little conservation measures have to be applied to assure long-term persistence (Hegg et al., 1993).

Herbivores and Pathogens

Alpine ungulates such as chamoix (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), ibex (*Capra ibex*) or marmot (*Marmota marmota*) are natural herbivores in habitats of *Campanula thyrsoides* and browsed flowering plants and rosettes have been observed in locations where no livestock grazing or human activity takes place. Nevertheless, herbivory under natural conditions seems very low. On the other hand, plants from cow and sheep pastures were often found decapitated.

Observed pre-dispersal seed predators in *C.* thyrsoides* were a small leafroller moth, *Cochylis* cf. *pallidana* Zeller (Tortricidae; Tortricinae), and two weevil species, *Miarus* cf. *graminis* (Gyll) and *M*. cf. *abeillei* (Desbrochers). Infection with *Cochylis* larvae and *Miarus* spp. were present in all 8 *C.* thyrsoides*' populations censused in Switzerland. About 40% of all plants were infected (range: 8-78%). In infected plants 14% of all capsules (range: 1-41%) were predated and predation caused complete seed loss. Seed predation by *Cochylis* larvae were usually higher than by *Miarus* spp. (67% vs. 33%, respectively). Interannual censuses however suggest an additional temporal variability of infection. In the literature *Cochylis pallidana* has been described to feed exclusively on *Jasione montana* (Razowski, 1970), a *Campanula* relative that is wide-spread throughout Europe. The moths feeding on *C.* thyrsoides* may therefore belong to a yet undescribed species. *Miarus graminis* has been recorded to feed on *Campanula glomerata* but little is known about *M. abeillie* (Stevenson et al., 1997).

Mycorrhiza

C. thyrsoides* is colonized by endomycorrhizal fungi with root colonization rates usually below 25 %. Only three fungal species could be isolated using spore trap cultures with soil collected in a typical habitat: *Glomus constrictum*, *G. invermaium* and *G. versiforma*. However, a larger spectrum of symbionts is most likely, given the range of natural habitats and the species' altitudinal distribution.

Physiological data

No data

Biochemical data

No specific data on *C. thyrsoides* available. A detailed study on mobile carbohydrates, its storage and allocation during different life-stages is currently performed (Ch. Schädel, Uni Basel, Switzerland). The white latex present in all plant parts is most likely inulin, or fructan, as these substances are characteristic for Campanulaceae serving as long-term winter storage compounds in tap roots and playing a functional role during floral development (for *C. rapunculoides* see Vergauwen et al., 2000).

Genetic data

C. thyrsoides is diploid, $2n = 34$ (Rosen, 1931; Larsen, 1954; Gadella, 1964), and this chromosome number is shared by about 42 % of the investigated members of the family Campanulaceae s.l. (Lammers, 1992). Additional chromosome counts by Sugiura (1942), with $2n = 48$, are considered incorrect and are commonly not taken into account for comparative studies (Gadella, 1964; Hess et al., 1972).

In a screening test with 18 allozyme systems on seedling tissue of *C.* thyrsoides*, no polymorphisms were detected (allozyme systems: AK, ADH, AO, ARK, AAT, G6PDH, GPI, HEX, IDH, LDH, MDH, NADP+(ME), PGM, 6PGDH, SOD, α-Trehalase, TPI, XDH; method: CAGE in TG buffer run for 15-30 min at 25mA; Ægisdóttir, unpublished).

A population genetic study on *C.* thyrsoides* of 32 populations in the Swiss Alps and Jura mountains using RAPD markers (Chapter 1) found moderately high within-population diversity values (Nei's genetic diversity, H_e: 0.20 (± 0.003 SE); Shannon Index, SI: 0.32 (± 0.006 SE); Percentage of polymorphic loci, P_p: 61.8 (± 1.3 SE); $n = 32$). Within-population diversity was not influenced by altitudinal location or population size. Even small populations of <100 individuals had comparably high levels of genetic diversity and none of the studied populations seems to be at immediate risk of extinction as a result of genetic depauperation. Spatial analysis of population relatedness detected a sharp genetic contrast between populations from the western part of Switzerland and the central and eastern populations. This delineation coincides with the proposed border of two distinct areas of post-glacial migration which are separated by the north-south running Aosta-Rhône-Valley (Schönswetter et al. 2005) and also visible in the geographic distribution map (Figure 3: line A). Morphological differences however could not be found between populations from the two distinct areas.

Among-population diversity within a single glacial refugia in Switzerland was relatively low, with a mean Φ_{st}-values of 16.8 % (± 0.003 SE) and G_{st}-value of 18.2 (± 0.03 SD). Pairwise Φ_{st}-values ranged from 2.3 % to 29.3 % and a significant isolation by distance behavior could be found ($R^2 = 0.32$, $P = 0.007$) indicating restricted gene flow. Despite the spatial isolation of populations, population differentiation was only moderately high and may be explained by the outcrossing breeding system of *C. thyrsoides*, which ensures successive gene pool mixing, and counteracts differentiation by drift.

A population genetic study with the same samples but applying microsatellites is currently in progress (H.H. Ægisdóttir, Uni Basel, Switzerland)

Hybrids

Hybrids of *C. thyrsoides* have not been observed in nature. In the only known experimental hybridization between the closely related *C. spicata* × *C. thyrsoides*, seedlings died in a very early stage (Gadella, 1964).

Status of the species

C. thyrsdoides is rare throughout its native range but locally abundant and therefore not considered endangered as a species. However, due to its regional rarity, *C. thyrsoides* is protected by law in a number of Alpine countries and listed according to the IUCN-criteria (e.g. Moser et al., 2002): Austria – '*near threatened*' (Niklfeld and Schratt-Ehrendorfer, 1999), Bulgaria – '*endangerd*' (Anchev, pers. comm.), Croatia – '*strictly protected*' (Nikolić, 2006), France – '*least concern*' (Ferrez 2004), Germany – '*vulnerable*' (Korneck et al., 1996), Switzerland – '*least concern*', but regionally '*vulnerable*' or '*near threatend*' (Moser et al., 2002). The species is not protected or mentioned in the 'Red Lists' of Italy (Pignatti et al., 2001) and Slovenia (Wraber, pers. comm.). Information from Bosnia-Herzegovina were not available. *C. thyrsoides* is not included in the Natura 2000 (Council of Europe, 1992), CITES (IUCN, 2004) or Bern Convention treaties (Council of Europe, 2006). The species has little commercial value, is rarely grown or sold as an ornamental plant and has no known medicinal use.

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Chapter 6

General discussion

General Discussion

This thesis studies important aspects of plant population survival in the rare monocarpic perennial *Campanula thyrsoides* in the Swiss Alps. It focuses on genetics (Chapter 2), demography (Chapter 3) and reproduction (Chapter 4) of this species and presents a concise description of its complex life-history (Chapter 5). In order to discuss the results of this thesis as an entity without copying the detailed discussions of each chapter, this chapter integrates our newly obtained information in a discussion about the nature of rarity in *C. thyrsoides*, including practical aspects for species conservation, and the rarity of the monocarpic life-history in the polycarpic Alpine environment. The chapter closes with an outlook on future research possibilities.

Do we need to apply specific conservation measures for this rare species?

There are a number of important findings from the various studies that have implications for applied conservation biology. The main aspect concerned is rarity. There are several reasons why a species is rare and usually rarity is a consequence of limited dispersal potential, low fecundity, short life-cycles, dependency on patchy or unpredictable resource availability, or vulnerability to human exploitation and land-use practices (cf. Meffe et al., 1997). *C. thyrsoides* seems to be naturally rare because of severe microsite limitation and poor dispersal ability.

Our modelling approach showed that populations will grow rapidly with increased probability of establishment and we have good indication that seedling recruitment is dependent on microsite availability (Chapter 3). In the field, *C. thyrsoides* is found in a variety of habitats some of which are clearly man-made, e.g. meadows, pastures and road shoulders, indicating that the species has actually profited from human intervention (Chapter 5). This is clearly observed in the form of plant densities which are extremely high on road shoulders. For the ruderalized population of *C. thyrsoides* along the Furka Pass road we have good indication that the population has expanded rapidly after the road improvement works in the 1960s. There is however also some information that the species has declined in recent years because of intensified grazing pressure by cows and especially sheep (Seitter, 1989) but certainly less sites are affected than have been created previously.

Locating populations of *C. thyrsoides* in the Swiss Alps was a laborious task and even apparently prime habitats, with respect to floristic composition and abiotic conditions, were mostly devoid of *C. thyrsoides*. Although, Brisse et al. (1996-2006) created a large database with phytosociological relevés from entire France which has been used to calculate the potential site-specific distribution of *C. thyrsoides* based on its probability to occur in combination with other species. The results show that there are many unoccupied sites that offer adequate conditions for population establishment. As we have seen, modelled seed dispersal potential of *C. thyrsoides* is extremely low (Chapter 5) such that reaching new and open sites is very improbable in a single season and appears only possible over the time-scale of several hundred or thousand years. The genetic data (Chapter 2) and the biogeographical pattern of the two subspecies (Chapter 5) indicate that *C. thyrsoides* has survived the last glaciation (> 12000 B.P.) in distinct refugia at the Southern rim of the alpine arc (cf. Schönswetter et al. 2005). With a maximum distance of ~300 km from a present location to a proposed glacial refugia the hypothetical velocity of post-glacial migration is about 25 m per year. From historic floras (Jaccard, 1895) and early phytosociological tables (Lüdi, 1948) we can assume that *C. thyrsoides* remains at a particular location for many decades or centuries. All these pieces of information confirm that colonization of new and open sites is possible over time through rare long-distance dispersal events. The high genetic dissimilarity of populations within confined geographic areas (Chapter 1) further demonstrates the stochasitic nature of dispersal and colonization.

Do we need to apply specific conservation measures for this rare species? *C. thyrsoides* is protected by law in the majority of the Alpine countries (Chapter 5) but the species is certainly not endangered. Rarity of *C. thyrsoides* is a consequence of biological and abiotic constraints, not of human intervention and in the light of global change the species rather shows affinities for further range expansion similar to ‘alpine ruderals’ as described by Körner (2003). The niche that *C. thyrsoides* occupies is found in a large variety of natural and semi-natural habitats which are characterized by different abiotic conditions. Further, populations of *C. thyrsoides* harbour a high amount of genetic variability and are only moderately dissimilar (Chapter 2) such that a population decline is not expected as a result of genetic depauperation. The high genetic diversity assures the species’ ‘adaptive capacity’ and ‘evolutionary potential’ to buffer change. In this context there appears to be no short- or long-term extinction risk for *C. thysroides* such that no specific conservation measures need to be taken.

Monocarpy in a polycarpic Alpine environment

It is a widespread belief that monocarpic plants are more commonly found in the lowlands and subtropical or tropical mountain systems than at high latitudes or in temperate alpine regions. At the same time the monocarpic life strategy requires a certain plant architectural pre-disposition (Silvertown, 1983) and is therefore found only in a small number of plant families (cf. Young and Augspurger, 1991; Körner, 2003; Metcalf et al., 2003) usually of high systematic rank. This raises the question whether *Campanla thyrsoides* is an exceptional representative of monocarpic plants in the polycarpic Alpine world because of ecological factors or because of phylogenetic and biogeographic constraints.

As we have seen there are simple rules by which monocarpic plants determine the optimal time at flowering (Chapter 2): the benefit from delaying flowering comes from increased fecundity as a result of growth and as soon as the probability of survival is smaller than the expected increase of fecundity a plant should flower. Size-dependent flowering thresholds are common in the majority of the hitherto analysed monocarpic species (Metcalf et al., 2003). Monocarpy has a number of advantages over polycarpy because the life-time reproductive output is often four times higher (Salisbury, 1943; Young and Augspurger, 1991), a single flowering event reduces the probability of herbivory, and resource storage in large tap roots increases resistence to drought and frost (Young and Augspurger, 1991; Körner, 2003). Moreover, habitats of monocarpic species are almost always characterized by a considerable amount of environmental variability, irrespective of geographic location (Young and Augspurger, 1991). There are however no reliable indications that the diurnal climate in subtropical and tropical alpine regions is more predictable for successful plant reproduction than the seasonal Swiss Alpine climate.

An additional important aspect about the evolution of monocarpy is related to plant architecture and thus also to plant systematics. Within the European flora a large part of the monocarpic plants are found within the Asteraceae and Apiaceae and even within these families monocarpy is a prominent life strategy (Silvertown, 1983). It is argued that plant architecture in these families facilitates the evolution of monocarpy because seed numbers increase allometrically with the size of an individual stem. Indeed, monocarpic species worldwide usually belong to a comparably small number of plant families of high systematic rank which are characterized by highly derived and aggregated inflorescences, e.g. Asteraceae, Campanulaceae, Boraginaceae, Onagraceae, Scophulariaceae, Apiaceae, Bromeliaceae (Silvertown, 1983; Young and Augspurger, 1991). Molecular analyses have however shown that there is no indication of a monophyletic origin of monocarpy within the genera *Saxifraga* (Saxifragaceae, Conti et al., 1999) and *Echium* (Boraginaceae, Böhle et al., 1996) or within the Campanulaceae (Eddie et al., 2003). Monocarpy may therefore have evolved 'stochastically' within plant groups that have a certain architectural pre-disposition. However, the vegetative rosette stage can be morphologically quite different even within the same families (e.g. Asteraceae, Campanulaceae): 'giant rosettes' in the tropics and 'regular' rosettes in other parts of the world (Young and Augspurger, 1991; Körner, 2003).

Combining all this information creates a considerable amount of complexity and it is obvious that there is no simple conclusive answer to our initial question especially because important numeric information is not available. It is unknown how many true monocarpic plants exist worldwide and how many plants have evolved facultative monocarpy; which refers to either life-span (biennial vs. perennial) or general reproductive strategy (monocarpy vs. polycarpy). It is further unknown at what frequency families with architectural pre-disposition occur in different biomes and at what frequency these families occur in the associated floras. Lastly, it is unknown whether the diurnal climate is more predictable for plant reproduction than the seasonal climate.

In this context *Campanula thyrsoides* certainly is able to grow oldest among all studied 'regular rosette' monocarpic species (cf. Körner, 2003; Metcalf et al., 2003). The case of *C. thyrsoides* demonstrates also that monocarpy can be a true alternative to polycarpy in the variable alpine environment. Nevertheless, given the absence of reliable data on ecological and phylogenetic constraints favoring monocarpy, it may as well be argued that the monocarpic strategy is as rarely realized in the Swiss Alps as in any other ecosystems.

Outlook

The results of this thesis answer many questions. At the same time new opportunities for research emerge that have the potential to advance our ecological understanding of the complex alpine environment. In the following I will briefly outline two ideas for future research and hope they will stimulate the next generation of doctoral candidates.

First, it appears most promising to explore in detail the reasons behind the ecological and morphological differentiation of the two subspecies of *C. thyrsoides*. The chorological map and molecular investigations indicate that a slow speciation process has started as a result of isolation in different glacial refugia. The information on the altitudinal range preferences however suggest a plastic response to different altitudinal conditions. This response can either be adaptive or genetically determined. Most probably the consequences of glacial isolation as well as the adaptive potential are involved in the ecological and morphological differentiation. I suggest to use three distinct approaches: 1) a thorough genetic survey using a complementary set of molecular markers with different resolutions in combination with 2) a field-based altitudinal and longitudinal transplant experiment and 3) three additional common garden experiments with plant material covering the entire distribution range of the species. This study would then have the potential to separate the relative effect of historic and present processes on the genetic and morphological characteristics of the species and provide general ecological information on plant responses to environmental variability.

Second, with the detailed analysis of the population demography of *C. thyrsoides* at hand it is now feasible to extend the modelling framework to incorporate explicit geographic information on the presence or absence of populations, the dynamics of microsite availability in response to vegetation succession and/or disturbance, and the potential for long-distance seed dispersal. I further suggest a complementary genetic study relying on maternal and biparental markers that focuses on the level of gene-flow within the same spatial context of the simulation model. The results from both studies combined can help to understand population dynamics at different time-scales and improve general meta-population theory for plants.

Conclusions

Campanula thyrsoides is one of the few monocarpic perennials in the Swiss Alps. Genetic connectivity among populations of this species is impeded due to the heterogeneity of the alpine environment and consequently, a significant pattern of decreasing population relatedness with increasing geographic distance can be found. The comparison with two other alpine species studied at the same spatial scale showed that the potential for long-distance seed dispersal does not necessarily translate into increased population similarity. The results further suggest that other life-history traits, especially the breeding system, may play an important role in genetic diversity partitioning. These findings corresponds well with the current knowledge about determinants of genetic diversity in plant populations. The molecular study also corroborates the theory on glacial refugia in the European Alps by delimiting two prominent areas of post-glacial plant migration. A very comforting result is the high amount of genetic diversity that can be found even in small populations such that the short-term adaptive potential of *Campanula thyrsoides* is assured.

Demographic analysis of two populations revealed that survival and growth are the most important determinants of population persistence. *C. thyrsoides* seems to follow an evolutionary stable flowering strategy which is characterized by an optimal size-dependent flowering threshold. Age at reproduction varied largely in this species and such a pronounced generation overlap efficiently buffers environmental variability. Simulation approaches further showed that *C. thyrsoides* is most likely microsite-limited and consequently has a large potential for population growth. In general, this study confirms the findings of similar investigations and the novel combination of methods applied have proven powerful to study in depth the important demographic parameters of a long-lived alpine plant, potentially facilitating future research.

The pollination experiment established firm proof for an obligatory outcrossing breeding system in *C. thyrsoides* which does not break down as flowers age. The study also demonstrated the minimal risk of population decline as a result of inbreeding depression.

From the three studies summarized above and the information accumulated in the general life-history analysis I conclude that *C. thyrsoides* is well adapted to the variability of the alpine environment, that the genetic and demographic requirements for short-term adaptive capacity and long-term evolutionary potential of this species is assured and that no specific conservation measures have to be taken for this naturally rare species.

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