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ORIGINAL PAPER

# High genetic differentiation in populations of the rare alpine plant species *Campanula thyrsoides* on a small mountain

**Eva S. Frei · J. F. Scheepens · Jürg Stöcklin**

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**Abstract** Changes in climate and traditional land use have contributed to a loss and fragmentation of suitable habitats for many alpine plant species. Despite the importance of these changes, our knowledge of the consequences for gene flow and genetic diversity is still poor, especially in rare taxa and at fine spatial scales. Here, we investigated the genetic diversity in a rare alpine plant on a small and highly structured mountain in the Swiss Alps. Using microsatellite markers and Bayesian cluster analyses, we investigated genetic diversity within and among 24 populations of *Campanula thyrsoides* L. We also tested whether landscape structure has affected genetic structure by correlating genetic diversity with landscape and population features, which were assessed in a four-year monitoring period. The recorded genetic diversity ($H_e = 0.714$) and genetic differentiation ($G'_{ST} = 0.32$) at distances of 1–10 km were remarkably high. Clustering analyses revealed a split of populations into two genetically different spatial groups, but between-population genetic distances were neither correlated to geographic distance, elevation nor slope. The high differentiation and genetic bottlenecks may indicate strong founder effects, although the number of alleles was not decreased in bottlenecked populations. We conclude that stochastic colonisation by seeds is most important for shaping the genetic structure of *C. thyrsoides* on this small mountain. The high genetic diversity even in small populations may indicate that occasional gene flow is strong enough to overcome negative effects of bottlenecks. Nevertheless, further fragmentation and isolation of habitats may threaten this rare plant in the future.

**Keywords** *Campanula thyrsoides* · European Alps · Genetic bottlenecks · Isolation by distance · Landscape genetics · Microsatellite markers

## Introduction

In the topographically highly structured landscape of the European Alps, loss and fragmentation of suitable habitats, changes in traditional land use and global warming cause reduction in species diversity (Dirnböck et al. 2003; Stöcklin et al. 2007). From the perspective of preserving intraspecific genetic diversity in the European Alps, an important question is whether genetic diversity in alpine taxa is currently threatened (Becker et al. 2007). Changes in human land use may affect genetic diversity of common alpine plants negatively (e.g. Rudmann-Maurer et al. 2007), but little is known about neutral genetic drift affecting genetic diversity and structure in alpine plants, especially in rare plants and at fine spatial scales. Gene flow by pollen or seed dispersal, neutral genetic drift and natural selection are among the most important evolutionary forces shaping the genetic diversity of plant populations at different spatial scales (Loveless and Hamrick 1984). Gene flow is strongly dependent on the structure of the landscape, breeding system, pollination vectors and adaptations of seeds for dispersal (Kalisz et al. 2001; Gaudeul et al. 2007; Yan et al. 2009). Since gene flow is more likely among geographically close populations, isolation by distance (IBD; Hutchison and Templeton 1999) can create spatial genetic structure. The study of genetic structure in a landscape context has the

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E. S. Frei (✉) · J. F. Scheepens · J. Stöcklin
Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland
e-mail: eva.frei@gmx.ch

potential to improve our understanding of how topography and geographical isolation influence gene flow and population differentiation in plant species (Sork et al. 1999; Manel et al. 2003).

In a fragmented landscape, founder effects are likely because only a few seeds may reach a suitable habitat for colonisation located far away from the source and, in addition, genetic exchange among established populations is restricted (Wade and McCauley 1988). Moreover, as a consequence of the small size of newly established populations, genetic bottlenecks may occur. Identifying recent bottlenecks is important since bottlenecks may reduce the adaptive potential of a population because of the loss of genetic variation and an increase of the individual inbreeding coefficients (i.e. founder effects, Nei et al. 1975; Ellstrand and Elam 1993). High genetic differentiation among spatially isolated populations might be expected as a result of random genetic drift, restricted gene flow or selection in a heterogeneous landscape (Till-Bottraud and Gaudeul 2002; Shimono et al. 2009). Landscape heterogeneity is especially high in mountainous environments, where, for instance, variability in slope, exposure, temperature and snow cover is large (Scherrer and Körner 2010). In contrast to expectations, some studies reported that populations in fragmented landscapes are genetically not much differentiated (Jacquemyn et al. 2004; He et al. 2010). Such low genetic differentiation among spatially separated populations is sometimes explained with extreme long-distance seed or pollen dispersal (Bacles et al. 2006; Yang et al. 2008).

The naturally heterogeneous landscape of the Alps offers an excellent opportunity to study the effect of landscape structure on genetic differentiation in plants. Numerous studies have investigated spatial genetic structure in alpine plant species at large spatial scales, i.e., populations sampled >10 km distant from each other (Schönswetter et al. 2005; Kuss et al. 2008a; Alvarez et al. 2009). However, genetic structure in alpine plants has rarely been investigated at an intermediate (1–10 km) or a fine spatial scale (<1 km, i.e. within populations; but see Gaudeul and Till-Bottraud 2008). Likewise, an IBD pattern has been found frequently in plants from the Alps (Pluess and Stöcklin 2004; Gaudeul 2006; Stöcklin et al. 2009), but only a few studies have compared IBD patterns at different spatial scales of the Alps (e.g. Stehlik et al. 2001). In the present study, we investigated the genetic diversity within and among 24 populations of the alpine plant species *Campanula thyrsoides* L. on a small mountain (Schynige Platte, area of c. 10 km$^2$) in the Swiss Alps (Fig. 1a). In Switzerland, *C. thyrsoides* is red-listed because the species is regionally scarce (Moser et al. 2002). On the Schynige Platte, the study species occurs in numerous populations usually of small size in a mosaic of highly fragmented (semi-)natural habitats, including pastures and screes. The populations are spatially separated by forest patches and avalanche corridors (Fig. 1b). Furthermore, the study populations on the Schynige Platte are far away from *C. thyrsoides* populations on other mountains, with the nearest population on the Faulhorn being 4 km away. Geographical distances between neighbouring populations range from less than a hundred to a few hundred metres (for details see Supplementary material, Table S1).

The following questions were addressed: (1) How large is the genetic diversity within populations of *C. thyrsoides* and is there evidence of recent genetic bottlenecks in small populations? (2) How large is the genetic differentiation among the 24 populations in an area of 10 km$^2$ and if present, is it spatially structured? (3) Is genetic differentiation distance-dependent (IBD) and related to variation in topography (elevation, slope) or population features? Finally, our results are interpreted in the light of previous studies of *C. thyrsoides* based on microsatellite data at the larger scales of the Central Swiss Alps (18,000 km$^2$) and the entire Alpine range (190,000 km$^2$).

## Materials and methods

### Study species and region

*Campanula thyrsoides* is a bell flower native to the European Alps, the adjacent Dinaric Alps and Jura Mountains (Aeschimann et al. 2004). The species is one of the few monocarps in the Alps and individuals are long-lived with a mean flowering age of 10 years and a range of 3–16 years (Kuss et al. 2007, 2008b). Plants are mainly pollinated by bumblebees (Ægisdóttir et al. 2009). Most seeds are dispersed within a distance of 10 m from the mother plant and a persistent soil seed bank is likely absent (Kuss et al. 2007). There are several biological reasons for the rarity of *C. thyrsoides*, including its very specific habitat requirements (Wüest 2008), monocarpic life-cycle (Kuss et al. 2007) and strong gametophytic self-incompatibility system (Ægisdóttir et al. 2007a). Moreover, recent field experiments have indicated that *C. thyrsoides* is strongly dispersal and microsite limited, meaning that even though a lot of suitable habitats and microsites are available, the species spread and seedling recruitment is poor (Frei et al. 2012). Climate or land-use changes are possible threats to the dispersal-limited *C. thyrsoides*. Migration to higher elevation in response to climate change, as observed in other alpine plants (e.g. Frei et al. 2010), might be too slow or limited in *C. thyrsoides* because suitable grassland habitats in the Alps are shrinking as a consequence of abandonment or overgrazing (Stöcklin et al. 2007).

The study region is called "Schynige Platte" and is a small mountain (centered at 46° 39′ 12″N; 7° 54′ 42″E) in

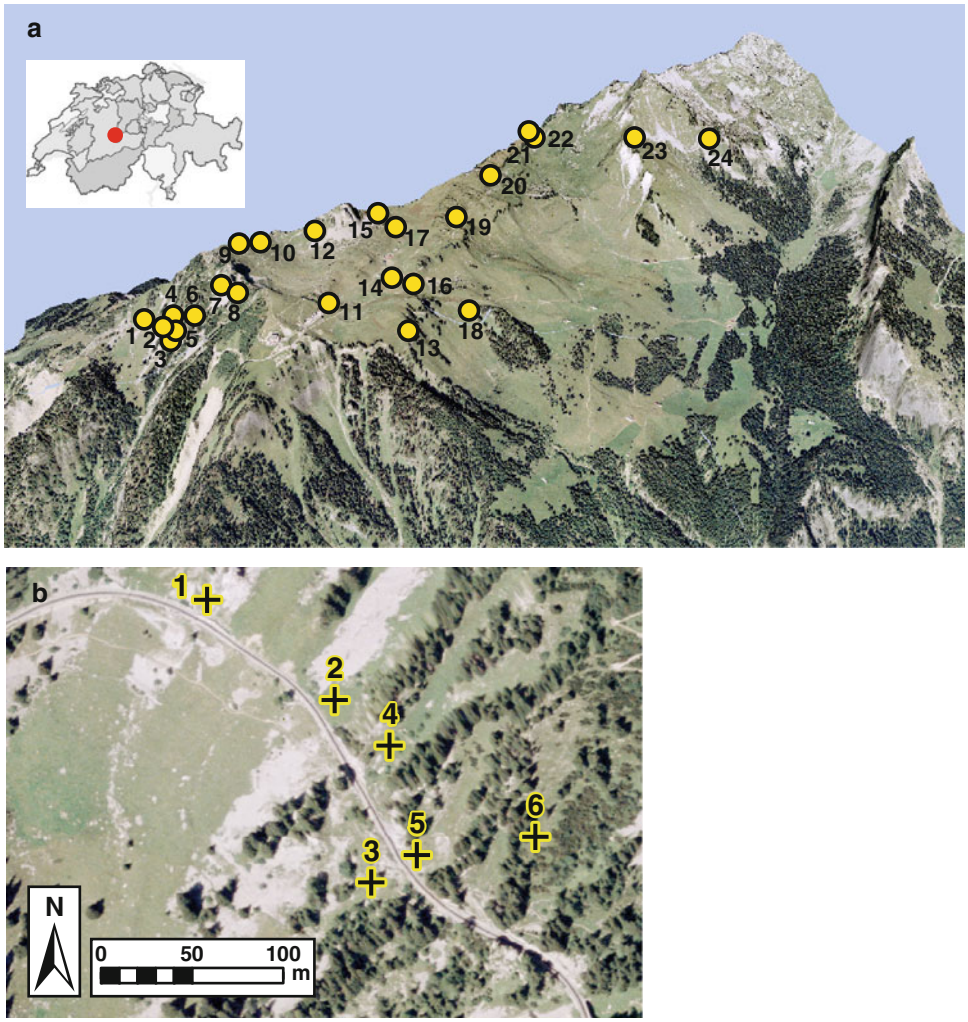


**Fig. 1** Study region on the mountain "Schynige Platte" in the Swiss Alps showing **a** the locations of all investigated populations (no. 1–24) of *Campanula thyrsoides* and **b** the locations of populations no. 1–6 in a more detailed view. For geographical distances between populations see Supplementary material, Table S1. The map is reproduced with the permission of Swisstopo, Berne, Switzerland (BA100596)

the northern Swiss Alps, consisting of calcareous bedrock. The region covers an area of about 10 km$^2$ and includes an altitudinal range of 1,800–2,100 m a.s.l. Part of the region has been used as summer pasture for cattle for at least 60 years (Lüdi 1948), but probably for centuries.

## Field monitoring

In August 2005, we mapped all 24 populations of *C. thyrsoides* occuring within the study region, with one population (no. 11) located in a Botanical Garden (Table 1; Fig. 1). Each population (i.e. centre of its area) was georeferenced with a GPS (GARMIN eTrex Summit, Berne, Switzerland) with a precision of ±5 m. The distances between all 276 population pairs range from 11 to 2,710 m, with three pairs separated by <50 m and 13 pairs by <100 m (see Table S1). In June 2006, we estimated the total size of each population either by counting all non-flowering individuals (rosettes) and flowering individuals, or, in large populations by extrapolating it from the average number of individuals counted in five sub-plots. Mean population size was 209 individuals, with a range of 12–780 individuals (Table 1). In addition to population size, we measured elevation, exposure, slope, occupied area, and vegetation cover (Table 1). Plant density was calculated by dividing population size by occupied area. The number of flowering individuals was counted during peak flowering in summer of each year from 2005 to 2009. Vegetation cover was estimated as a proxy for the strength of competition.

## Molecular analysis

In 2006, we sampled leaf material of 12 individuals within each population, totally 288 individuals. Individuals were sampled randomly within a population and, if possible, separated by at least 3 m. Genomic DNA was extracted from 2 mg of silica-gel dried leaf tissue using a NucleoSpin 96 Plant II extraction kit according to the standard protocol of the manufacturer (Macherey–Nagel GmbH, Düren, Germany). The DNA concentration was quantified with a

**Table 1** Location of 24 populations of *Campanula thyrsoides* from the mountain "Schynige Platte" in the Swiss Alps with population and landscape features

| Pop | Lat. °N (Swiss Grid) | Long. °E (Swiss Grid) | Elevation (m a.s.l.) | Exp | Slope (°) | Area ($m^2$) | Cover (%) | Density | Size | No. flowering |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 167,230 | 635,610 | 1,885 | SW | 45 | 60 | 60 | 0.50 | 30 | 7 (3–12) |
| 2 | 167,175 | 635,680 | 1,885 | SW | 55 | 120 | 95 | 0.83 | 100 | 10 (3–15) |
| **3** | **167,075** | **635,700** | **1,900** | **SW** | **45** | **300** | **95** | **1.00** | **300** | **9 (5–10)** |
| 4 | 167,150 | 635,710 | 1,900 | SW | 50 | 150 | 95 | 1.60 | 240 | 32 (23–51) |
| 5 | 167,090 | 635,725 | 1,900 | SW | 50 | 200 | 95 | 1.00 | 200 | 16 (2–20) |
| 6 | 167,100 | 635,790 | 1,950 | SW | 40 | 300 | 90 | 0.14 | 42 | 8 (5–9) |
| 7 | 167,150 | 635,900 | 2,010 | W | 45 | 150 | 95 | 0.13 | 20 | 6 (1–8) |
| 8 | 167,090 | 635,940 | 2,000 | W | 45 | 25 | 85 | 5.60 | 140 | 15 (10–19) |
| 9 | 167,450 | 635,975 | 2,040 | S | 35 | 25 | 70 | 0.48 | 12 | 2 (2–4) |
| 10 | 167,475 | 636,050 | 2,020 | S | 35 | 75 | 90 | 0.33 | 25 | 7 (6–13) |
| **11** | **167,000** | **636,240** | **1,980** | **E** | **40** | **2,500** | **95** | **0.16** | **400** | **71 (50–100)** |
| 12 | 167,600 | 636,275 | 1,980 | SW | 30 | 3,000 | 90 | 0.15 | 460 | 40 (30–50) |
| 13 | 166,850 | 636,490 | 1,900 | SE | 30 | 50 | 85 | 3.00 | 150 | 9 (5–15) |
| 14 | 167,200 | 636,500 | 1,925 | SE | 40 | 700 | 87 | 1.00 | 700 | 105 (69–167) |
| 15 | 167,750 | 636,540 | 1,960 | E | 50 | 300 | 90 | 0.10 | 30 | 5 (4–10) |
| 16 | 167,175 | 636,575 | 1,910 | SE | 25 | 400 | 92 | 0.63 | 250 | 34 (24–48) |
| 17 | 167,630 | 636,590 | 1,930 | SE | 50 | 500 | 90 | 0.12 | 60 | 16 (4–30) |
| 18 | 167,040 | 636,750 | 1,840 | S | 65 | 400 | 85 | 0.08 | 30 | 16 (4–40) |
| 19 | 167,650 | 636,850 | 1,950 | SE | 30 | 1,000 | 95 | 0.30 | 300 | 58 (28–105) |
| 20 | 168,030 | 637,090 | 1,940 | SE | 15 | 800 | 97 | 0.98 | 780 | 57 (9–100) |
| **21** | **168,430** | **637,400** | **2,010** | **SE** | **25** | **300** | **90** | **0.57** | **170** | **32 (24–40)** |
| **22** | **168,425** | **637,410** | **1,970** | **SE** | **40** | **3,000** | **90** | **0.13** | **380** | **75 (35–146)** |
| 23 | 168,125 | 637,800 | 2,030 | SE | 40 | 200 | 85 | 0.75 | 150 | 30 (26–35) |
| 24 | 168,125 | 638,175 | 1,980 | S | 45 | 4,000 | 90 | 0.01 | 40 | 6 (0–8) |
| Mean | | | 1,950 | | 40 | 773 | 89 | 0.82 | 209 | 28 |
| SD | | | 53 | | 11 | 1,140 | 6 | 1.24 | 212 | 27 |

All population and landscape features were assessed in 2006 with the exception of number of flowering individuals, showing the mean and range from the years 2005 to 2009. Populations are ordered from west to east. The site of population no. 11 lies within a Botanical Garden. Bold values indicate bottlenecked populations (see Table 3)

*Pop* population, *Exp* exposure (*S* South, *W* West, *E* East), *Area* area occupied by a population, *Cover* coverage of herb layer, *Density* individuals per $m^2$, *Size* population size (number of flowering and non-flowering individuals), *No. flowering* number of flowering individuals

NanoDrop ND-1000 spectrophotometer (Witec AG, Littau, Switzerland). For genotyping plants, we used microsatellites (SSRs) as selectively neutral molecular markers to reach high enough resolution for detection of smallest genetic differences among individuals (Selkoe and Toonen 2006). In a pre-analysis, we tested eight microsatellites developed for *C. thyrsoides* (Ægisdóttir et al. 2007b). For the final analysis, we selected the five microsatellites that reached the highest reproducibility (see Table S2).

PCR amplification was performed in a 10-µL volume containing 1× PCR buffer (with 1.5 mM $MgCl_2$ included in the buffer; Qiagen, Hombrechtikon, Switzerland), 0.15 mM of each dNTP (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), 0.3 µM of the forward and reverse primers (Ecogenics, Zürich-Schlieren, Switzerland), 0.5 U Hotstar Taq polymerase (Qiagen, Hombrechtikon, Switzerland) and 0.5 µL DNA. Amplifications were always run in the same thermal cycler (Techne TC-412, Witec AG, Littau, Switzerland). After initial denaturation for 15 min at 95 °C, PCR was performed for 38 cycles: annealing at primer-specific temperature (see Table S2) for 30 s, 30 s at 72 °C, and 30 s at 95 °C. The PCR finished with 1 min at the primer-specific annealing temperature, followed by a final extension at 72 °C for 30 min. After amplification, the PCR products were separated at an electrical field of 120 V, using Spreadex®-gels in a submerged gel apparatus SEA-2000 (Elchrom Scientific AG, Cham, Switzerland). Depending on the pair of primers, gels were run 110–130 min at a temperature of 55 °C and stained for 30 min with ethidium bromide.

We visualized the banding pattern under ultraviolet light using an AlphaDigiDoc photo system (Alpha Innotech Corporation, San Leandro, USA). The alleles were scored automatically and the fragment length was estimated using the program IMAGE QUANT TL (GE Healthcare, Buckinghamshire, UK). Scoring was checked manually and all ambiguous PCR results (i.e. smear and stutter bands) were repeated to minimize scoring errors. For repeatability of the banding pattern (Bonin et al. 2004), we performed negative controls in DNA amplification and estimated the error rate by repeating complete PCR analysis for 60 blind samples. The resulting error rate was 2.2 %. To test for linkage disequilibrium of all pairs of loci, Fisher's method and GENEPOP version 4.0 (Rousset 2008) were used.

### Genetic diversity and bottlenecks

To estimate genetic diversity within all populations, the mean number of alleles ($N_A$) as well as the observed heterozygosity ($H_o$) and the expected heterozygosity ($H_e$) according to Nei (1978) were calculated for each population and averaged over all loci with the Excel Add-in GENALEX version 6.0 (Peakall and Smouse 2006). Population substructuring was evaluated by computing the inbreeding coefficient $F_{IS}$ (Weir and Cockerham 1984) as multilocus estimate for each population with the software GENEPOP version 4.0 (Rousset 2008). To assess the significance of the $F_{IS}$ values, departure from Hardy–Weinberg equilibrium (HWE) was evaluated with a multisampling score test at the population level for heterozygote deficit across all loci (Rousset and Raymond 1995). This test was performed with GENEPOP, using the Markov Chain algorithm (Guo and Thompson 1992) with 10,000 permutations, 20 batches and 5,000 iterations per batch.

The equilibrium between genetic drift and mutations determines the number and frequency distribution of alleles at neutral loci in natural populations. In case of a genetic bottleneck, the effective population size $N_e$ is decreased, reducing the genetic diversity through a decrease in heterozygosity and a loss of rare alleles (Cornuet and Luikart 1996). In populations which recently experienced a genetic bottleneck, an excess of heterozygosity may occur because the alleles are lost faster than the heterozygosity. However, it has been claimed that only if population size is reduced for a long time, a reduction in heterozygosity can be observed (Nei et al. 1975). If historic data of population size are not available, recent genetic bottlenecks (i.e. approximately within $2N_e$–$4N_e$ generations) can be inferred from tests for heterozygosity excess (Cornuet and Luikart 1996). In our study, we used the one-tailed Wilcoxon signed-rank test implemented in the software BOTTLENECK version 1.2 (Piry et al. 1999), because it is the most powerful and robust method to test for heterozygosity excess when applied to only a few polymorphic loci. This test compares the expected heterozygosity ($H_e$; Nei 1978) to the heterozygosity expected at mutation-drift equilibrium ($H_{eq}$) estimated from the observed number of alleles. However, tests for excess of heterozygosity ($H_e > H_{eq}$) should not be confused with tests for an excess of heterozygotes ($H_o > H_e$). Levels of heterozygosity excess were tested for significance using 1,000 permutations. Tests were performed using both the strict Stepwise Mutation Model (SMM) and the Two-Phase Model (TPM) with 95 % single-step mutations, 5 % multiple-step mutations and 12 % variance among multiple steps (G. Luikart, University of Montana, personal communication). Both mutation models are assumed for microsatellite evolution (Luikart and England 1999; Balloux and Lugon-Moulin 2002), but the TPM might be more appropriate for most microsatellites (Williamson-Natesan 2005).

### Genetic differentiation and structure

As a proxy for genetic differentiation among all populations, we calculated the standardized $G'_{ST}$ according to the formula 4b used in Hedrick (2005). For comparison with other molecular studies, we also calculated the two more commonly used differentiation measures, Wright's $F_{ST}$ (Weir and Cockerham 1984) and $G_{ST}$ (Nei 1973), although these indices are less suited than $G'_{ST}$ to cope with the problem of high values of heterozygosity in the highly polymorphic microsatellites (Heller and Siegismund 2009). $F_{ST}$ and 95 % confidence intervals were calculated with 1,000 bootstraps using the program GENETIX version 4.05 (Belkhir et al. 2004). $G_{ST}$ and 95 % confidence intervals were computed with 1,000 bootstrap resamplings using the statistical package DEMEtics (Jueterbock et al. 2011) in R version 2.13.0 (R Development Core Team 2011).

To investigate the genetic structure of populations, we performed two different Bayesian cluster analyses. In the first analysis, we used the program STRUCTURE version 2.3 (Hubisz et al. 2009) for the assignment of individuals to genetic clusters and selected the "admixture" model with independent allele frequencies (Pritchard et al. 2000). The alternative model options ("no admixture" model and correlated allele frequencies) yielded highly similar results (not shown). After a burn-in period of 10,000 cycles, 10,000 Markov Chain Monte Carlo iterations were performed for $K$ (number of clusters) ranging from 1 to 10. We estimated the most likely value of $K$ by performing 100 simulations for each $K$ (i.e. using the ad hoc statistics $\Delta K$; Evanno et al. 2005). Another Bayesian cluster analysis was performed with the software BAPS version 5.3 (Corander et al. 2008). The analysis with BAPS allowed us to include the information about the location of populations prior to the assignment of populations to clusters. The model was fitted

with a maximum of 20 genetic clusters and the optimal partition was assessed with 50 independent simulations. During the structuring process, the landscape occupied by a discrete population is divided into a Voronoi tessellation (Deussen et al. 2000). For visual presentation of the tessellation, cells with different shades of grey represent genetically differentiated spatial groups.

To check for IBD (Rousset 1997), we tested for correlation between genetic distances, measured as $F_{ST}/(1-F_{ST})$, and the $\log_{10}$ of spatial distances using a Mantel test (Mantel 1967) with 1,000 permutations in the program ARLEQUIN version 3.5 (Excoffier and Lischer 2010). The pairwise genetic differences ($F_{ST}$) were calculated using the same program and their significance was tested with 1,000 permutations. Additional Mantel tests were performed to test for IBD within the two spatial groups separately, previously inferred from Bayesian cluster analyses (Figs. 2, 3). To test whether landscape features have affected the genetic structure, we correlated the pairwise genetic distances with environmental distances (i.e. differences in elevation and slope) using simple Mantel tests (Manel et al. 2003). Partial Mantel tests were used to control the response variable for a possible correlation of the two other explanatory variables, as recommended by Smouse et al. (1986). Finally, we investigated whether genetic diversity is correlated with population and landscape features. Thus, $H_e$ and $F_{IS}$ indices were related to environmental parameters measured in the field (Table 1) with linear regressions using Pearson's correlation analysis and R version 2.13.0 (R Development Core Team 2011).

## Results

A total of 42 unambiguously scorable and reproducible alleles ranging from 92 to 188 bp at five microsatellite loci were detected across all 288 individuals. We found no evidence for linkage disequilibrium between any of the pairs of loci (Table S3).

### Genetic diversity and bottlenecks

The number of alleles per locus averaged over all populations was $5.0 \pm 0.6$ ($\pm$SD) with a range of 3.8–6.2 (Table 2). The observed and expected heterozygosity averaged over all populations and loci were $H_o = 0.746$ and $H_e = 0.714$, respectively (Table 2). In one population (no. 17), a significant positive $F_{IS}$ value (0.195) was found (Table 2). A significant excess of heterozygosity in four out of 24 populations, based on the Wilcoxon's test under both mutation models, indicated recent genetic bottlenecks in these populations (Table 3).

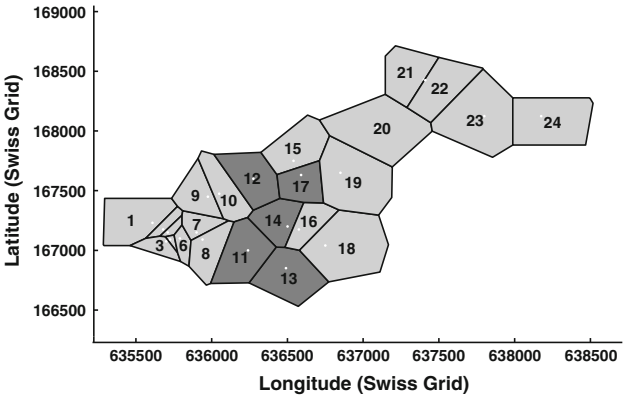


**Fig. 3** Results of a Bayesian cluster analysis using the program BAPS (Corander et al. 2008), a priori location information and microsatellite data of 24 populations ($n = 288$) of *Campanula thyrsoides* from the Schynige Platte. Shown is the most likely partition of 50 independent runs. The two clusters are represented by different shades in *grey*. For a better visual presentation, population no. 2, 4 and 5 are not numbered (but see Fig. 1 for locations)

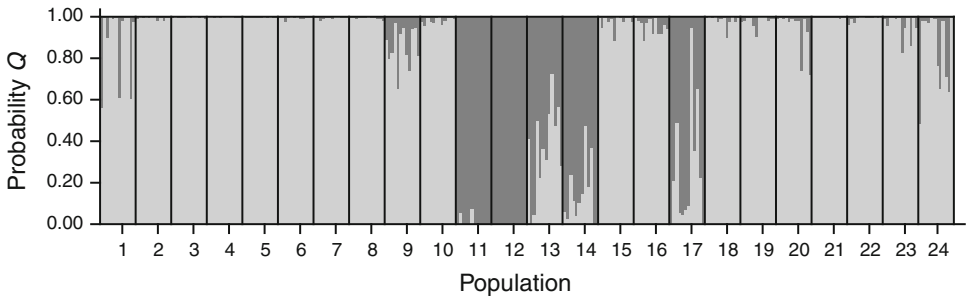


**Fig. 2** Results of a Bayesian cluster analysis using the program STRUCTURE (Hubisz et al. 2009) and microsatellite data of 24 populations of *Campanula thyrsoides* from the Schynige Platte for the most likely number of clusters $K = 2$. The two clusters are represented by different shades in *grey*. Individuals ($n = 288$) are grouped to populations aligned from west (*left*) to east (*right*). *Bars* indicate the assignment probability $Q$ of individuals to each of the clusters. Shown is the simulation run with the highest likelihood for posterior distribution of data out of 100 independent runs

**Table 2** Mean number of alleles per locus and genetic diversity indices in 24 populations of *Campanula thyrsoides* from the Schynige Platte

| Pop | $N_A$ | $H_o$ | $H_e$ | $F_{IS}$ | $P$ value | Pop | $N_A$ | $H_o$ | $H_e$ | $F_{IS}$ | $P$ value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.4 | 0.839 | 0.741 | −0.140 | 0.9815 | 13 | 6.2 | 0.799 | 0.778 | −0.034 | 0.6500 |
| 2 | 6.0 | 0.833 | 0.757 | −0.106 | 0.6286 | 14 | 5.6 | 0.751 | 0.747 | −0.006 | 0.3665 |
| **3** | **4.4** | **0.824** | **0.710** | **−0.161** | **0.9591** | 15 | 4.0 | 0.743 | 0.664 | −0.131 | 0.7433 |
| 4 | 4.8 | 0.708 | 0.662 | −0.071 | 0.6776 | 16 | 5.0 | 0.739 | 0.712 | −0.043 | 0.5440 |
| 5 | 5.6 | 0.700 | 0.686 | −0.001 | 0.1490 | 17 | 5.0 | 0.579 | 0.704 | 0.195 | 0.0001 |
| 6 | 4.4 | 0.822 | 0.724 | −0.147 | 0.8599 | 18 | 5.4 | 0.677 | 0.733 | 0.082 | 0.2865 |
| 7 | 5.0 | 0.677 | 0.672 | −0.010 | 0.5587 | 19 | 5.2 | 0.717 | 0.735 | 0.023 | 0.1848 |
| 8 | 4.6 | 0.608 | 0.576 | −0.056 | 0.8652 | 20 | 4.6 | 0.830 | 0.678 | −0.246 | 0.9976 |
| 9 | 5.0 | 0.762 | 0.689 | −0.112 | 0.8702 | **21** | **3.8** | **0.697** | **0.681** | **−0.037** | **0.4495** |
| 10 | 5.6 | 0.793 | 0.772 | −0.029 | 0.4100 | **22** | **4.2** | **0.783** | **0.717** | **−0.096** | **0.8919** |
| **11** | **5.4** | **0.854** | **0.780** | **−0.107** | **0.9573** | 23 | 4.4 | 0.707 | 0.732 | 0.031 | 0.4547 |
| 12 | 5.8 | 0.777 | 0.761 | −0.024 | 0.6408 | 24 | 5.2 | 0.684 | 0.723 | 0.059 | 0.2231 |
| | | | | | | Mean | 5.0 | 0.746 | 0.714 | | |
| | | | | | | SD | 0.6 | 0.073 | 0.046 | | |

*P* value for the global test for heterozygote deficit. Data from 288 individuals were calculated across all five microsatellite markers. Bold values indicate recently bottlenecked populations (see Table 3)

*Pop* population, $N_A$ mean number of alleles per locus, $H_o$ observed heterozygosity (Nei 1978), $H_e$ expected heterozygosity (Nei 1978), $F_{IS}$ inbreeding coefficient (Weir and Cockerham 1984)

**Table 3** Tests for recent genetic bottlenecks in 24 populations of *Campanula thyrsoides* from the Schynige Platte

| Pop | SMM | TPM | Pop | SMM | TPM |
|---|---|---|---|---|---|
| 1 | 0.31 | 0.31 | 13 | 0.92 | 0.89 |
| 2 | 0.50 | 0.50 | 14 | 0.89 | 0.59 |
| 3 | 0.02* | 0.02* | 15 | 0.11 | 0.08 |
| 4 | 0.69 | 0.69 | 16 | 0.41 | 0.41 |
| 5 | 0.98 | 0.98 | 17 | 0.59 | 0.31 |
| 6 | 0.41 | 0.31 | 18 | 0.41 | 0.41 |
| 7 | 0.96 | 0.92 | 19 | 0.69 | 0.50 |
| 8 | 0.89 | 0.69 | 20 | 0.89 | 0.89 |
| 9 | 0.92 | 0.89 | 21 | 0.02* | 0.02* |
| 10 | 0.31 | 0.31 | 22 | 0.02* | 0.03* |
| 11 | 0.03* | 0.02* | 23 | 0.08 | 0.08 |
| 12 | 0.59 | 0.59 | 24 | 0.41 | 0.41 |

Shown are *P* values from the Wilcoxon's test that was performed under the Stepwise Mutation Model and the Two-Phase Model, both assumed for microsatellite evolution (Piry et al. 1999). Significant excess of heterozygosity indicates a genetic bottleneck and was tested with 1,000 permutations: * $P < 0.05$

*Pop* population, *SMM* Stepwise Mutation Model, *TPM* Two-Phase Model

### Genetic differentiation and structure

Genetic differentiation among all populations was $F_{ST} = 0.063$ (95 % CI 0.048–0.082), $G_{ST} = 0.099$ (95 % CI 0.088–0.112) and $G'_{ST} = 0.32$. The ad hoc statistics for results of Bayesian cluster analysis with STRUCTURE revealed two clusters to capture the genetic structure best (Fig. 2; Supplementary material, Fig. S4). For the most likely partition, the Bayesian simulations with BAPS also revealed two spatial groups, which include the same populations (Fig. 3). Genetic differentiation between pairs of populations ranged from $F_{ST} = 0.001$ to $F_{ST} = 0.191$, whereby 232 out of all 276 comparisons were significant at the 5 % level (see Table S1).

### Effects of geographic distance, topography and population features

There was no evidence for an IBD pattern, neither when including all 24 populations in the Mantel test nor when testing the larger or smaller spatial group separately (Table 4; Fig. 4a). Genetic distances were also not related to environmental distances, measured as changes in elevation or slope between populations, even when corrected for the significant ($P < 0.05$) positive correlations between geography and elevation or between elevation and slope (Table 4; Fig. 4b). Genetic diversity measured as $H_e$ correlated negatively with plant density (Fig. 5), but not with other population or landscape features (Table 5).

## Discussion

### High genetic diversity despite indications of genetic bottlenecks

Genetic diversity within populations of *C. thyrsoides* on the Schynige Platte was higher ($H_e = 0.714$) than the reported

average ($H_e = 0.61$) of other microsatellite studies (reviewed in Nybom 2004), but is well in line with diversity of this species in other populations from the Swiss Alps and Jura mountains (Ægisdóttir et al. 2009). The strong self-incompatibility system of this monocarpic and predominantly outcrossing species could be responsible for high genetic diversity in populations of *C. thyrsoides* (Nybom 2004; Ægisdóttir et al. 2007a). On the Schynige Platte, even small populations including 30 or less individuals harboured high genetic diversity (Tables 1, 2). In addition, genetic diversity measured as $H_e$ was negatively correlated with plant density (Fig. 5). This negative relationship could result from increased bi-parental inbreeding in denser populations as found in *Primula elatior* (Van Rossum et al. 2002) because of a density-dependent pollinator behaviour resulting in shorter pollination distances in denser populations (Schmitt 1983; Van Rossum et al. 2004).

The significant positive $F_{IS}$ value of population no. 17 (Table 2) may either indicate the presence of null alleles, bi-parental inbreeding or a sub-structure within this population (i.e. Wahlund inequality; Rosenberg and Calabrese 2004). Since no locus showed constant departures in HWE across all populations, undetected null alleles in our dataset are unlikely (Chapuis and Estoup 2007). Bi-parental inbreeding is a possible explanation because sister-crosses of *C. thyrsoides* may produce viable offspring that do not suffer from inbreeding depression (Ægisdóttir et al. 2007a). A population sub-structure caused by restricted gene flow is the most plausible explanation because of the low density of only 0.12 individuals per $m^2$ in population no. 17 (Table 1).

**Table 4** Summary of Mantel test statistics for correlation analyses between genetic differentiation of 24 populations of *Campanula thyrsoides* from the Schynige Platte with elevation and slope

| | $r_M$ | $P$ value |
|---|---|---|
| GEO × ELE | 0.14 | 0.047 |
| GEO × SLO | 0.14 | 0.061 |
| ELE × SLO | 0.16 | 0.048 |
| GEN × GEO | −0.14 | 0.93 |
| GEN × GEO, only group 1 | −0.16 | 0.93 |
| GEN × GEO, only group 2 | 0.06 | 0.36 |
| (GEN × GEO) ELE | −0.13 | 0.93 |
| (GEN × GEO) SLO | −0.14 | 0.94 |
| (GEN × ELE) SLO | −0.06 | 0.73 |

Partial Mantel tests, with (Y1 × X1) X2 testing the partial correlation between Y1 and X1 by controlling for the effect of X2. Y1 is the response variable, and X1 and X2 are the explanatory variables. Group 1 and 2 represent the two spatial groups inferred from Bayesian cluster analyses

$r_M$ (partial) Mantel correlation coefficient (Smouse et al. 1986), *GEO* $\log_{10}$ of geographical distances, *ELE* differences in elevation, *SLO* differences in slope, *GEN* genetic distances measured as $F_{ST}/(1-F_{ST})$

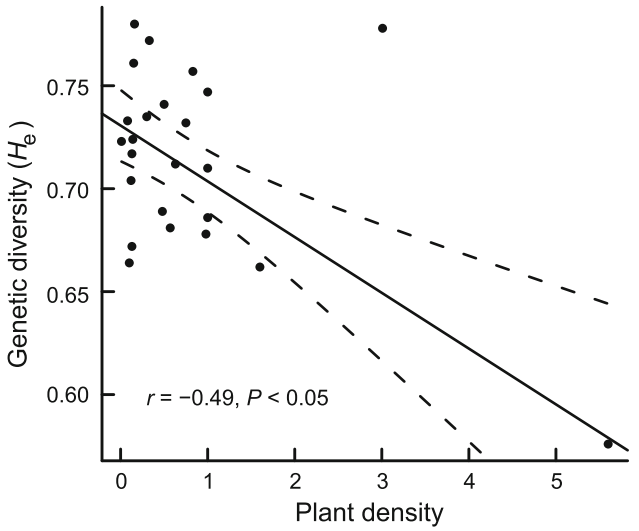


**Fig. 5** Correlation of mean genetic diversity, measured as $H_e$ (expected heterozygosity; Nei 1978), with plant density (total number of plants per $m^2$) of 24 populations of *Campanula thyrsoides* from the Schynige Platte. The *solid line* represents the linear regression and the *dotted lines* show the 95 % CI. For test statistics of linear regression, see Table 5

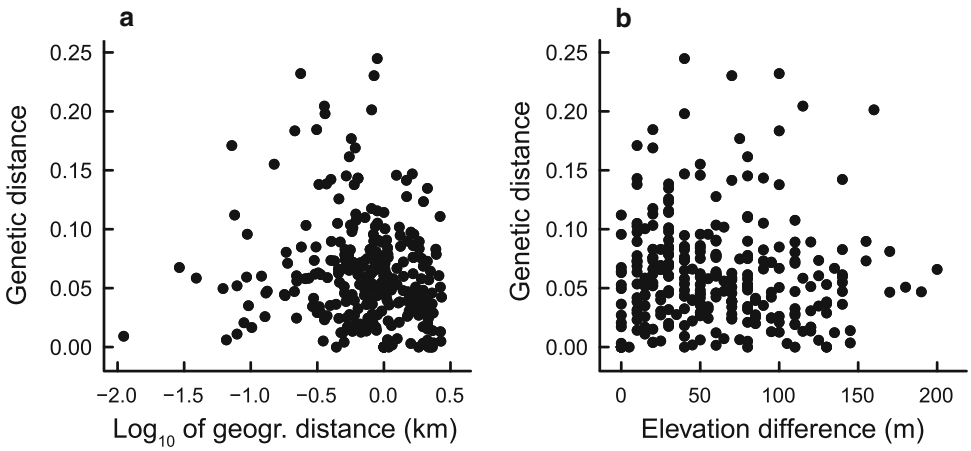


**Fig. 4** Correlations of **a** pairwise genetic distances measured as $F_{ST}/(1-F_{ST})$ with $\log_{10}$ of geographic distances and of **b** genetic distances with elevation differences of 24 populations of *Campanula thyrsoides* from the Schynige Platte. For Mantel test statistics, see Table 4

**Table 5** Linear regression analysis between genetic diversity measures of 24 populations of *Campanula thyrsoides* from the Schynige Platte with population and landscape features

| | $r_P$ | $P$ value |
|---|---|---|
| HET × ELE | −0.19 | 0.374 |
| HET × SLO | −0.09 | 0.668 |
| HET × ARE | 0.29 | 0.176 |
| HET × VCO | −0.05 | 0.809 |
| HET × DEN | −0.49 | 0.015 |
| HET × SIZE | 0.09 | 0.686 |
| FIS × ELE | −0.001 | 0.996 |
| FIS × SLO | 0.32 | 0.115 |
| FIS × ARE | 0.11 | 0.603 |
| FIS × VCO | 0.05 | 0.802 |
| FIS × DEN | −0.10 | 0.635 |
| FIS × SIZE | −0.18 | 0.411 |

$r_P$ Pearson's correlation coefficient, *HET* expected heterozygosity (Nei 1978), *ELE* elevation, *SLO* slope, *ARE* area occupied by a population; *VCO* vegetation cover, *DEN* plant density, *SIZE* $\log_{10}$ of population size; *FIS* inbreeding coefficient (Weir and Cockerham 1984)

**Table 6** Genetic differentiation among 24 populations of *Campanula thyrsoides* from the Schynige Platte compared with differentation among populations of this species from larger regions in the Alps

| | Area (km$^2$) | $n$ | $F_{ST}$ | $G_{ST}$ | $G'_{ST}$ |
|---|---|---|---|---|---|
| Schynige Platte | 10 | 24 | 0.06 | 0.10 | 0.32 |
| Central Swiss Alps[a] | 18,000 | 17 | 0.08 | 0.10 | 0.43 |
| European Alps[a] | 190,000 | 51 | 0.16 | 0.18 | 0.68 |

$n$ number of populations, $F_{ST}$ differentiation index (Weir and Cockerham 1984), $G_{ST}$ differentiation index (Nei 1973), $G'_{ST}$ standardized $G_{ST}$ (Hedrick 2005)

[a] Data from analyses using the same species and marker type (J. Stöcklin, University of Basel, unpublished data)

The number of alleles in recently bottlenecked populations was not reduced with exception of population no. 21 which harbours the smallest number of alleles (Table 2). These findings contradict expectations from theory that bottlenecked populations should have decreased numbers of alleles (Nei et al. 1975). Therefore, we assume that the detected bottlenecks may mirror founder effects during the establishment of new populations on the mountain several generations ago, since the bottlenecked populations are currently large and three out of four have high numbers of reproducing individuals (Table 1). Our results suggest either that the number of alleles in populations that have experienced genetic bottlenecks only decreased very little or that a decrease in the number of alleles has been overcome, e.g. by recent admixture. An ongoing admixture of populations may violate some of the assumptions of bottleneck tests (Cornuet and Luikart 1996). However, it has been shown in another microsatellite study (Shama et al. 2011) that Wilcoxon's test for bottlenecks is conservative.

## High genetic differentiation and a spatial structure

Although the area of our study region was small (10 km$^2$), we observed a remarkably high genetic differentiation ($G'_{ST} = 0.32$) among the 24 populations of *C. thyrsoides* on the Schynige Platte. The high differentiation is astonishing, considering that population density is high and that the average minimal distance (based on the minimal distances for each population to another population) is only 214 m. Differentiation among the 24 populations on this mountain is also considerably high when compared with the values observed in this species in larger areas, with $G'_{ST}$ values of 0.43 and 0.68 in populations from the Central Swiss Alps (18,000 km$^2$) and the entire Alpine range (190,000 km$^2$), respectively (J. Stöcklin, University of Basel, unpublished data; Table 6). The results are a clear support for the assumption of a restricted seed dispersal ability of *C. thyrsoides* as has been observed in field experiments (Frei et al. 2012). The substantial genetic differentiation suggests that pollen flow is likewise low.

The high differentiation of *C. thyrsoides* found on the mountain is in concordance with the distinct spatial genetic structure inferred from both Bayesian cluster analyses (Figs. 2, 3). A smaller group of five populations (including population no. 11 located in the Botanical Garden) was bordered on both sides by a much larger group including the 19 other populations. Seed material of *C. thyrsoides* from outside the Schynige Platte was introduced into population no. 11 in the 1950s (O. Hegg, University of Berne, personal communication). Therefore, a plausible explanation for the spatial arrangement could be that foreign genetic material from population no. 11 in the Botanical Garden spread to several nearby populations over the last 50 years. This spread indicates that gene flow may occur over short distances among neighbouring populations, as evidenced by the existence of the two spatial groups (Fig. 2), while over larger distances of several hundred metres gene flow is more restricted, as discussed above.

In a small region, such as an isolated mountain area or an old crater of a volcano (López et al. 2010), founder effects may shape the spatial genetic structure of plant populations. Likewise, on the mountain "Schynige Platte", populations may become differentiated as a consequence of neutral genetic drift and founder effects, as indicated by genetic bottlenecks in several populations of *C. thyrsoides* (Table 3).

## No isolation by distance at the scale of a small mountain

The independence of genetic differentiation from geographic distance between populations of *C. thyrsoides* on

the Schynige Platte (Table 4) suggests that the presence of an IBD pattern depends on the spatial scale, since IBD was found at larger spatial scales (>10 km) when populations of *C. thyrsoides* from the entire Swiss Alps were investigated (Ægisdóttir et al. 2009). Such scale-dependent patterns of IBD were also found in the alpine plant species *Eryngium alpinum* and *Eritrichium nanum* (Gaudeul et al. 2000; Stehlik et al. 2001). Furthermore, the scale at which IBD can be observed in plants may be species-specific and thus may depending on flowering and dispersal features of the species under study (Hirao and Kudo 2004). At intermediate distances (1–10 km), addressed in the present study or by Vandepitte et al. (2007), no IBD could be observed. Some occasional long-distance seed dispersal may occur, and the frequency of such events is probably more likely than previously assumed (He et al. 2010). Unfortunately, detailed knowledge on the occurrence and frequency of long-distance seed dispersal in *C. thyrsoides* is missing and should be addressed in future studies. It is likely that secondary dispersal over snow or ice is a more efficient and important mechanism for long-distance dispersal of seeds than primary wind dispersal (e.g. Morton and Hogg 1989). Genetic diversity of *C. thyrsoides* on the Schynige Platte results probably from a combination of colonisation events by seeds not related to topographical features or distance and from a restricted pollen dispersal. In our study we did not directly measure gene flow, but Ægisdóttir et al. (2009) studied the pollination distances of *C. thyrsoides* within populations and found small distances of on average only 4.85 m. Thus, at short distances (<1 km) or within a single population, gene flow by seed dispersal may strongly affect the fine-scale genetic structure in *C. thyrsoides*. This is supported from a previous modelling study which showed that seed dispersal by wind in *C. thyrsoides* resulted in 99.9 % of the seeds being dispersed within 1 m of the mother plant (Kuss et al. 2007). We assume that at this fine-spatial scale, dispersal and hence gene flow is restricted, and therefore within a single population of *C. thyrsoides* a kin-structure and IBD could be detected as well (Gaudeul and Till-Bottraud 2008; Jacquemyn et al. 2009).

Similarly to geographic distance, we could not detect effects of topography on genetic distances among populations, supporting that the genetic relationship among populations of *C. thyrsoides* on the Schynige Platte is mostly a result of dispersal events occurring stochastically in time and space (i.e. not affected by distance or the particular topography). However, we cannot exclude that other elements of the highly structured landscape, not included here, have effects on the structuring of genetic diversity in *C. thyrsoides*.

## Conclusions

In summary, we found a high genetic diversity and differentiation in populations of the rare alpine plant *C. thyrsoides* on a small mountain. At the scale of only a few kilometres, IBD or effects of topography on genetic differentiation were absent and thus our results suggest that occasional long-distance seed dispersal and founder effects shaped the genetic structure of *C. thyrsoides* on the Schynige Platte. In the context of a topographically highly structured landscape, it is noticeable that stochastic dispersal events are relevant for the spatial genetic structure of populations at distances of a few kilometres, and that the connectivity among populations is restricted to short distances of a few hundred metres. The presence of two distinct groups of populations in this small area is probably a result of the introduction of plants into the Botanical Garden on the Schynige Platte. The great impact of this genotype introduction could be of interest from a conservation perspective, i.e. in the context of genetic introgression from populations of rare plants through reintroductions. Finally, the studied populations of *C. thyrsoides* are not immediately threatened by genetic erosion despite the small sizes of most of them. Although the seed dispersal of this species is restricted, occasional gene flow, possibly via pollen, seems to be sufficient to maintain genetic diversity and to overcome negative effects of bottlenecks in this monocarpic plant.

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