Plant genetic diversity and population differentiation in the fragmented alpine landscape

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Chapter 1

General introduction

THE AIM OF THIS THESIS

The aim of this thesis is to study alpine plant life with a focus on genetic variation, population differentiation and adaptation due to two major environmental gradients: altitude and succession (Chapter 2, 3 and 4). Additionally, genetic diversity, plant growth and competition ability was measured in plant populations of different sizes in recently fragmented habitats (Chapter 5). The main objectives and research questions of this thesis were:

- How is genetic variation and population differentiation shaped by the naturally fragmented alpine environment? May gene flow counteract genetic drift or does the alpine conditions interrupt gene exchange? (Chapter 2)
- Does the amount of clonal *vs*. sexual reproduction in alpine plants differ among contrasting habitats? How is growth and reproduction affected by environmental conditions? (Chapter 3)
- Does selection favour larger seeds at high altitude, as more reserves provided for seedlings are beneficial in harsh alpine environments? (Chapter 4)
- Is molecular variability and fitness of a common grassland plant species reduced in recently fragmented habitats? Do small populations suffer from genetic erosion? (Chapter 5)

PLANTS IN FRAGMENTED HABITATS

Anthropogenic destruction and fragmentation of the formerly continuous natural landscape is increasing and has become now a significant threat to the maintenance of biodiversity in many terrestrial ecosystems (Young, Boyle & Brown 1996). The threat lies not only in the local extinction of species, but also in the potential loss of genetic diversity and fitness of remnant populations. Habitat fragmentation is especially pronounced in tropical rainforests and temperate grasslands (Soulé & Orians 2001). Management changes reduce habitats in the cultural landscape in Central Europe, e.g. nutrient-poor grasslands, which were formed by long-lasting human activities. Populations of formerly common plant species are now small in numbers and isolated from each other by large distances (see this thesis, Chapter 5). Smaller populations are more vulnerable to demographic as well as environmental

stochasticity and to genetic erosion (Lande 1988; Menges 2000; Schemske *et al.* 1994; Young *et al.* 1996).

Natural habitats may also be fragmented to a smaller or larger extend resulting in suitable habitat 'islands' for a species surrounded by non-suitable habitats (Begon, Harper & Townsend 1995; Bossuyt, Honnay & Hermy 2003; Pither, Shore & Kellman 2003; Vucetich *et al.* 2001). This thesis focuses on species from the naturally fragmented alpine landscape with a clonally repdroducing herb as the main study species (Chapter 2, 3, and 4). In alpine habitats, spatially isolated populations are probably more the rule than the exception (Klimes *et al.* 1997). Additionally to the consequences of fragmented habitats, alpine plants have to cope with harsh environmental conditions, e.g. short and cold vegetation periods, unstable and low fertility soils, desiccating winds, and high solar radiation. Climatic conditions change with altitude, exposition, and degree of slope resulting in steep environmental gradients. Thus, the island nature of alpine landscape provides unbeaten opportunities for comparative ecological research, for study plant adaptation along gradients, and for study the effect of natural habitat fragmentation and isolation on genetic variation and population differentiation.

POPULATION DIFFERENTIATION

***Genetic differentiation* –** Fragmented landscapes particularly affect the partitioning of genetic diversity among populations; variation within population may decrease and differentiation among populations may increase (see this thesis, Chapter 2 and 5). In small, isolated populations, species persistence is seriously affected by enhanced random genetic drift, increased inbreeding, limited gene flow, and reduced mate availability (Young *et al.* 1996 and references therein). As genetic drift is selectively neutral, both deleterious and advantageous alleles can get lost, while the level of heterozygosity decreases (Lacy 1987). In the short term, a loss of genetic diversity can reduce plant performance and lower population viability (Barrett & Kohn 1991; Ellstrand & Elam 1993). In the longer term, reduced genetic diversity may limit the potential for further adaptive evolution (Falconer & Mackay 1996). Selection may reduce the frequency of deleterious alleles responsible for inbreeding depression and thereby increase mean individual fitness. Hence, plants of naturally fragmented populations (see this thesis, Chapter 2) are hypothesised to be less affected by small population size and isolation than plants of recently fragmented populations (see this thesis,

Chapter 5). Moreover, natural fragmentation is in general a long-lasting process with species-coevolution.

Beside population size and fragmentation, genetic variation and differentiation depends on breeding system or reproductive mode. In clonal plants with rare repeated seedling recruitment, differences in the success of particular genets beside random processes are expected to decrease population genetic diversity over time, while genetic differences among populations increase (Soane & Watkinson 1979; Watkinson & Powell 1993; see this thesis, Chapter 2). Due to replicated genotypes within populations, effective size of local populations may be reduced and enhance genetic drift (Chung & Kang 1996; Jones & Gliddon 1999; Young *et al.* 2002). But given the possible long life of individual genets, very few new genets need to be added annually to maintain genetic diversity (Widen, Cronberg & Widen 1994). Indeed, similar levels of genetic diversity in clonal as in non-clonal plants is presented in many studies (Ellstrand & Roose 1987; Hamrick & Godt 1989). On the other hand, studies of genetic diversity in naturally fragmented alpine areas are small in numbers, and there are few indications for a decreased genetic diversity at high altitude (Holderegger, Stehlik & Abbott 2002; Till-Bottraud & Gaudeul 2002). If effects on molecular diversity of naturally isolated habitat are increased by clonal reproductive behaviour is one of the questions in this thesis (Chapter 2).

Fitness differentiation – The opposing abiotic and biotic conditions along steep environmental gradients in the Alps can lead to major modifications in selective forces on plant life history traits (Cody & Overton 1996; Stearns 1992), resulting in genetic adaptation to local conditions, even between populations of the same species (Mitchell-Olds & Shaw 1987; see this thesis, Chapter 3 and 4). Low gene flow due to spatial isolation of populations may even increase the degree of local differentiation. However, phenotypic plasticity rather than genetic differentiation may be an alternative way of matching genotypes to environment, with increasing environmental variability favouring greater levels of plasticity (Schlichting 1986; Sultan 1987).

In general, plant growth and reproduction is determined by the genotype and a variety of interactions with the abiotic and biotic environment. In this thesis plant performance is studied in greenhouse experiments, where plants grew in common environments (i.e. quantitative genetic experiments, Chapter 3 and 5). Differentiation in phenotypic variation

should then be largely genetic.

A FOCUS ON REPRODUCTION

Clonal growth evolved many times in different taxa and is one of the most noticeable adaptation to severe environmental conditions in cold environments, even in pioneer communities and particularly in late successional alpine grasslands (Callaghan 1988; Hartmann 1957; Klimes *et al.* 1997; Stöcklin 1992). Available resources are usually limited (Cody 1966) but environmental shortage may be overcome by clonal reproduction due to low cost for the mother plant, as clonal offspring are partly self-sustenance. Even though clonal reproduction is less costly than sexual reproduction (Harper 1977), a pronounced allocation to vegetative reproduction will lower the investment for seed production (see this thesis, Chapter 3) because meristems may be more limited than carbon or other resources (Watson 1984). The consequence is a trade-off between reproductive modes (Geber 1990; Harper 1977; Prati & Schmid 2000; Watson 1984).

Clonal propagation may result in rapid, but spatially limited spread of genotypes and may improve population persistence during phases lacking sexual reproduction. Long-distance dispersed seeds, however, connect fragmented populations in the patchy alpine landscape (migration) or found new populations in unoccupied habitats (colonisation). Metapopulation models predict opposing selection for traits favouring dispersal during colonisation and selection against genes promoting migration once a population has been established (Olivieri, Michalakis & Gouyon 1995). In early successional sites, sexual reproduction is expected to be favoured whereas in late successional sites clonal reproduction may be more frequent. Along an altitudinal gradient, the trade-off between reproductive behaviour may result in increased vegetative growth at higher elevations, because reproduction by seeds may be hampered by the harsh alpine conditions (e.g. Young *et al.* 2002). The effects of successional and altitudinal contrasting habitats (early *vs*. late and low *vs*. high, respectively) on plant growth and reproduction is studied in two greenhouse experiments in Chapter 3.

Seed size is a crucial character of a plant's life history. Especially, in the severe alpine environment selection for larger seeds is expected (see this thesis, Chapter 4), as larger seeds may have a higher establishment success, but smaller seeds are commonly better dispersed.

Due to limited resources a trade-off among seed size and number is expected (Harper 1977; Smith & Fretwell 1974). Numbers are directly related to fitness and a selection pressure for more but smaller seeds should always operate. Seed size is expected to vary mainly in response to differences in selection pressure towards larger seeds (Westoby, Jurado & Leishman 1992). Along an elevational gradient, Baker (1972) conducted the most detailed across-species study and reported decreasing seed weight with altitude. But Baker (1972) did not consider phylogeny, whereas phylogenetic constraints or niche conservatism explain the lack of major differences in seed size between taxonomically related species in a study of Lord, Westoby & Leishman (1995). In the Swiss Alps, Landolt (1967) compared pairs of related lowland and alpine species but made no quantitative measurements of seed weights. The question if seed weight increases with altitude among populations of the same species or among closely related species pairs, to account for phylogenetic consequences, is still not answered and is the topic of Chapter 4.

THIS DOCTORAL THESIS

The study species, *Geum reptans*

The clonal pioneer *Geum reptans* L. (Rosaceae) is very well suited to study plant population diversity, differentiation, and adaptation in the naturally fragmented alpine landscape. *Geum reptans* occur in contrasting habitats between 2000 and 3000 m a.sl., and in early and late successional sites. If changing selection pressure along successional and altitudinal trajectory influence the proportion of clonal *vs.* sexual reproduction can be well studied in *G. reptans*; both reproductive modes relay on meristems in leaf axils. These meristems are preformed to a flower head or stolon in the season prior to emerging. On aboveground stolons one terminal daughter rosette is built. Clonal integration lasts only for the establishment of new clonal rosettes.

The study species, *Scabiosa columbaria*

The perennial *Scabiosa columbaria* is L. (Dipsacaceae) very well suited to study genetic variability, population differentiation, plant growth, and competition ability in recently

fragmented habitats. This species is still common but restricted to nutrient poor grassland remnants in the Swiss Jura.

Experimental approach

Three different approaches were used in my thesis. Firstly, in a molecular study, genetic diversity, and differentiation was measured with neutral genetic markers, and the importance of gene flow and drift was assessed in *Geum reptans*. Secondly, in two quantitative genetic greenhouse experiments, genetic diversity and differentiation in respect to growth and reproductive behaviour was measured in *G. reptans*. Thirdly, in a reciprocal transplant experiment, adaptation of *G. reptans* to low and high altitude was intended to be measured. Unfortunately, the last experiment failed, because too many transplanted individuals did not survive the first winter. To compensate for this experiment, variation of seed weights along altitudinal gradients was studied in the field and using samples from the seed collection of the Botanical Institute of the University of Basel, Switzerland.

Additionally, genetic diversity and differentiation of neutral genetic markers as well as plant growth and competition ability in a quantitative genetic greenhouse experiment was measured in *Scabiosa columbaria.*

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 A. R. Pluess & J. Stöcklin. **Population genetic diversity of the clonal plant *Geum reptans* in the Swiss Alps.** *American Journal of Botany*, accepted.

In this manuscript I ask how important gene flow and genetic drift are in an alpine clonal pioneer plant, to what extend populations are differentiated, and if genetic variation differs among successional age of the population or altitude. To answer this question, 20 populations of *Geum reptans* were sampled in a spatially hierarchical design with distances among populations ranging from 0.2 to 208 km. These populations origin from different altitudes, early, medium, and late successional habitats and were studied with randomly amplified polymorphic DNA (RAPD) profiles. Seed and pollen dispersability was estimated by direct

measurements.

Chapter 3 A. R. Pluess & J. Stöcklin. **The importance of population origin and environment on clonal and sexual reproduction in the alpine plant *Geum reptans*.** *Functional Ecology*, accepted.

In this manuscript I ask to what extend plant growth and reproduction in the clonal *Geum reptans* can be explained by differences in population origin and environment. To answer this question clonal offsprings collected at natural sites grew in two separate greenhouse experiments: a competition-experiment comparing plants from early *vs*. late successional habitats (N = 172) and a temperature-experiment comparing plants from low *vs*. high altitude (N = 206). The treatments within the specific experiment were chosen according to one of the most important environmental conditions at population origins. This approach allows to test if plants from late successional sites are favored in the competition treatment, and if plants from higher altitude are favored at low temperature, indicating adaptation. Furthermore, if clonal and sexual reproduction is affected differently by plant size and if there is a trade-off between the two reproductive modes is tested.

Chapter 4 A. R. Pluess, W. Schütz & J. Stöcklin. **Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species.** Submitted.

In this manuscript I ask if seed weight increases with altitude. To answer this question seed weight differences were measured (i) between 29 closely-related species-pairs from the Alps, with one species occurring at low altitude and a corresponding species from high elevations and (ii) among populations within four alpine species (*Carex flacca*, *Epilobium fleischeri*, *Saxifraga oppositifolia*, and *Scabiosa lucida*) occurring over large altitudinal gradients. Seed samples from natural sites were collected in the Swiss Alps or came from the seed collection of the Botanical Institute of the University of Basel, Switzerland.

Chapter 5 A. R. Pluess & J. Stöcklin (2004). **Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size.** *Conservation Genetics*, 5: 145-156.

In this manuscript I ask if genetic variability and fitness is reduced in recently fragmented

populations of the common species *Scabiosa columbaria*. To answer this question seeds of eleven populations containing c. 90 up to c. 2000 flowering individuals were collected in Mesobromion remnants of the Swiss Jura. Plants were raised from seeds in the greenhouse to test for plant fitness and competition ability with *Bromus erectus*. The same material was used to measure genetic variation within and among populations with RAPD profiles.

For this manuscript I used data from my diploma thesis. During my PhD I have redone part of the molecular data analysis and calculations of fitness measurements. While writing this publication and going through the revising process requested by the *Conservation Genetics* journal, I learned how to analyse and present molecular data. This positively influenced and shortened the publication process of the molecular study with *Geum reptans*.

Finally, the concluding general summary present the most important findings of this thesis.

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Chapter 2

Population genetic diversity of the clonal plant *Geum reptans* in the Swiss Alps

Andrea R. Pluess & Jürg Stöcklin

American Journal of Botany, accepted

ABSTRACT

In the alpine landscape most plant populations are spatially isolated due to extreme patchiness and strong natural fragmentation. We used RAPD-PCR (randomly amplified polymorphic DNA polymerase chain reaction) for a study of the genetic diversity within and among 20 populations of *Geum reptans*, an outcrossing clonal plant species in the Swiss Alps. Populations were sampled at different altitudes, in early-, medium- and late-successional habitats (population origin) using a spatially hierarchical design, with distances among populations ranging from 0.2 to 208 km. Seed and pollen dispersibility was estimated by direct measurements. Seed dispersibility by wind was low with only 0.015 % of the seeds flying over 100 m. Observed pollen flow was even more restricted. Molecular diversity within populations was irrespective of population origin (H_e = 0.22 ± 0.004) and similar to the average of other RAPD studies. Contrary to our expectation, populations were only moderately differentiated (G_{st} = 0.14). However, there was a clear spatial genetic structure and a positive relationship between pairwise genetic and geographic distances. Our results indicate considerable gene flow among populations within the same regional area, and we found no indication for genetic depletion during succession or in peripheral habitats. We conclude that, despite the high natural fragmentation and the importance of vegetative reproduction in this alpine plant, gene flow and repeated seedling recruitment during succession might be more frequent than commonly suggested.

Key words. *Geum reptans*; molecular diversity; pioneer plant species; RAPD; Rosaceae; successional habitats.

INTRODUCTION

Alpine plant life is characterized by habitats with steep environmental gradients, sharp boundaries, strong natural fragmentation, and high disturbance frequency. As growth conditions are impaired by hostile climatic conditions, establishment from seeds is restricted to safe sites (Urbanska and Schütz, 1986). A high proportion of alpine plants are characterized by clonal growth even in pioneer communities on glacier foreland, alpine screes, or moraines (Hartmann, 1957; Gray, 1993; Stöcklin and Bäumler, 1996; Klimes et al., 1997). Clonality and spatial isolation of populations may lower genetic diversity within and increase genetic separation among populations (Barrett and Kohn, 1991; McLellan et al., 1997; Gaudeul et al., 2000; Landergott et al., 2001; Cheon et al., 2002; Despres et al., 2002). So far, however, molecular studies in alpine plants mostly considered phylogeny or the migration of species from refuge sites after the ice ages, concentrating on the molecular patterns created by historical gene flow (e.g., Bauert et al., 1998; Holderegger et al., 2002; Stehlik, 2002; Tribsch et al., 2002). The consequence of the island nature of alpine vegetation for actual gene flow is fairly unknown. Direct measurements of pollen movement and seed dispersal tend to underestimate the importance of long-distance dispersal for the movement of genes (Ouborg et al., 1999; Cain et al., 2000). Thus, indirect measurements using molecular markers are needed for a better understanding of genetic diversity patterns in fragmented alpine environments. The genetic consequences of clonality in long-lived plants are also poorly known (McLellan et al., 1997).

Plant molecular studies have shown that fragmentation of habitats and small population size may negatively affect population genetic diversity (Ellstrand and Elam, 1993; Fenster and Dudash, 1994; Fischer and Matthies, 1998; Luijten et al., 2000; Paschke et al., 2002). The genetically less diverse populations have a reduced ability to buffer the effects of poor environmental conditions or competition (e.g., Fischer et al., 2000; Pluess and Stöcklin, 2004). The fragmented alpine landscape might particularly affect the partitioning of genetic diversity among populations and strong selection forces under the harsh environmental conditions might strengthen population differentiation. In general, populations of clonal plants exhibit considerable levels of genetic diversity (Ellstrand and Roose, 1987; Parker and Hamrick, 1992; Widen et al., 1994; Hamrick and Godt, 1997). It has been suggested that this is also true for long-lived clonal plants from alpine habitats (Steinger et al., 1996; Diggle et al., 1998; Holderegger et al., 2002). However, in a recent review by Till-Bottraud and Gaudeul (2002),

only two studies of alpine plants (Gugerli et al., 1999; Jones and Gliddon, 1999) were available with a suitable sampling design to study the pattern of genetic diversity within and among populations accurately, i.e., comprising at least 10 populations and 20 individuals per population.

We selected a clonal plant for our study because vegetative reproduction is one of the most noticeable adaptations to severe environments and nutrient poorness in cold environments (Callaghan, 1988; Klimes et al., 1997). Clonal growth has benefits, like the ability to forage for resources, to support the establishment of offspring, or to minimize the mortality risk of a genet. On the other hand, there are costs, like the easy transmission of diseases or a reduced availability of resources for sexual reproduction (Jackson et al., 1985; Callaghan et al., 1992; Klimes et al., 1997). It is generally assumed that reproduction from seeds is infrequent in clonal plants, and this might be particularly true for plants from cold environments (see review in Eriksson, 1989). If no repeated seedling recruitment takes place after colonization, differences in the success of particular genets and random processes are expected to decrease genetic diversity over time (Soane and Watkinson, 1979; Watkinson and Powell, 1993). Moreover, clonal growth may act as an enhancer of genetic drift by reducing the effective size of local populations (Chung and Kang, 1996; Jones and Gliddon, 1999). However, simulation models have shown that even rare establishment from seeds is sufficient to maintain genetic diversity in long-lived clonal plants (Watkinson and Powell, 1993).

The assumption that clonal reproduction is necessarily correlated with a reduced level of reproduction by seeds must not always be true (Eriksson, 1989; Stöcklin and Bäumler, 1996). Well-established populations of clonal species may be an important source of seeds for the colonization of nearby patches or unoccupied habitats. Eriksson (1992) even suggested that clonal growth may have been selected more frequently in lineages with seeds adapted for long-distance dispersal. However, extremely localized dispersal is common in many plants (Freckleton and Watkinson, 2002) and short-range dispersal might be expected to dominate the colonization processes. Less isolated habitats are more likely to be colonized than are more isolated ones (Harrison et al., 2000), which may result in a spatial genetic structure with nearby populations being more related than more distant ones. Even in alpine species with good dispersal mechanisms, populations may tend to be genetically aggregated because potential habitats are isolated from each other and colonizations from nearby sites are more likely than random dispersal events.

Here, we focus on the genetic diversity within and among populations of the clonal pioneer *Geum reptans* L.. This species occurs mainly on glacier forelands and is able to persist until later successional stages. Population increase of this species is rapid by vegetative offspring produced at the end of stolons. At the same time the plant produces plenty of seeds which are wind-dispersed by conspicuously elongated, feathery styles on the nutlets (Rusterholz et al., 1993). We studied the genetic structure of 20 populations in the Swiss Alps within a core area on two nearby glacier forelands, in a regional area surrounding this core area, and from three sites distant to the core area (Fig. 1). Furthermore, we measured gene flow via seeds and pollen directly in the field for a comparison with the molecular data. Populations occur on a gradient from early- to late-successional communities and at different altitudes. We hypothesize, that (1) the genetic diversity in populations of this clonal pioneer plant is high, but, because of the fragmentation of the alpine landscape, we expect populations to be genetically structured in space. (2) Genetic variation is higher in populations from early-successional communities than in populations of later successional stages due to high seedling recruitment in early succession and loss of genotypes due to competition pressure in later succession. (3) Populations from lowest and highest altitudes are genetically less diverse than populations from medium altitudes, because environmental constraints at the elevational distribution boundaries of a species are expected to reduce the number of successful genotypes (Lesica and Allendorf, 1995, and references therein). (4) Based on direct estimates in the field, the potential of gene flow via seeds is larger than via pollen.

Genetic variation and differentiation were measured using RAPD-PCR. This method potentially provides a much higher number of markers than do allozyme analysis and it is now well established as a sensitive method for detecting genetic diversity (Nybom and Bartish, 2000).

MATERIALS AND METHODS

The plant species – The alpine pioneer Geum reptans L. (Rosaceae) is a hemicryptophytic outcrossing perennial plant. The distribution of the species extends from the Alps to the Carpathians, the Illyric mountains, and Macedonia at altitudes between 1950 and 3800 m above sea level (a.s.l.) (Hegi, 1995). Geum reptans preferentially occurs on virgin soils of early-successional habitats on glacier forelands, moraines, mountain ridges, and river

beds with a low chalk content and persists in such sites until later successional herbaceous communities (Hegi, 1995), but is absent from closed grasslands (Rusterholz et al., 1993).

Individuals consist of 1-7 rosettes (rarely more). Age at first reproduction, measured by counting growth rings in the main root of small reproducing individuals, was ~ 5 yr in late- and ~ 10 yr in early-successional communities and maximum age, observed in very large individuals, was ~ 30 yr (A. Pluess and J. Stöcklin, personal observation). Adults reproduce vegetatively by forming new rosettes at the end of aboveground stolons or sexually by seeds borne on a single-flowered stem. Buds are preformed in autumn and emerge in the following spring. Flowers are proterogynous, pollinated by inseccts and produce approximately 100 seeds (T. Weppler and J. Stöcklin, unpublished data). Viable seeds are only produced by outcrossing, indicating self-incompatibility (Rusterholz et al., 1993). After pollination, the style develops into an elongated feathery structure of up to 3 cm length, which facilitates dispersal by wind. Stolons grow to a length of up to 100 cm with a terminal rosette with adventive roots. At the end of the growing season, stolons wither and unrooted rosettes die.

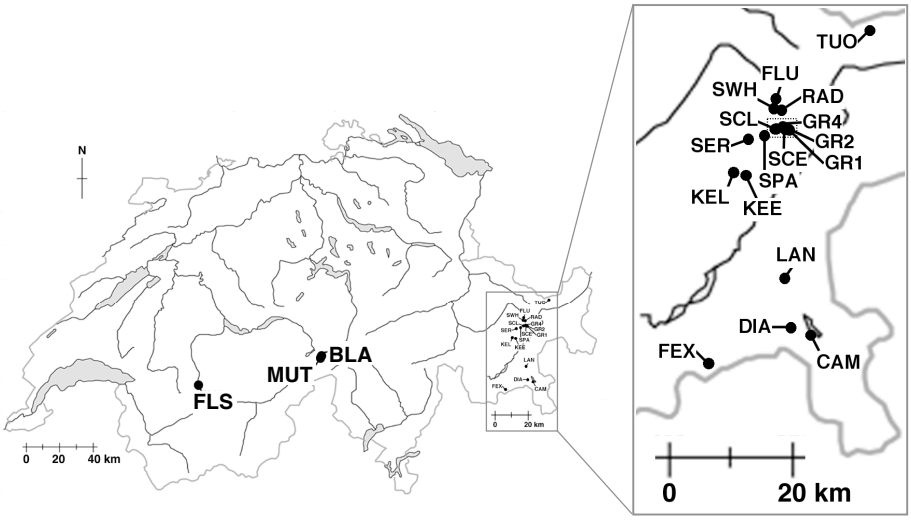

Fig. 1: Geographic distribution of the studied populations of the alpine plant *Geum reptans* in Switzerland. Populations are grouped into a core area (dashed line quadrate in the enlarged map section, including SCL, SCE, GR4, GR2 and GR1), in a regional area (enlarged map section, including 17 populations) and three populations at great distance to the core area.

Sampling design – We sampled all available populations ($N = 5$) in a core area of 0.55 × 2.8 km, which included the foreland of two glaciers (Scaletta glacier and Grialetsch glacier near Davos in the eastern part of the Swiss Alps), and all available populations in a regional

area of 25 × 54 km (+ 12 populations; Fig. 1; Tables 1, 3). To include populations more distant to the core area, we sampled three additional populations: two populations from the Central Alps (Muttgletscher and Blauberg; 120 km distant from the core area) and one population in the Bernese Alps (Fluhseeli; 200 km distant from the core area). In all but one population, we collected tissue of young leaves from 20 randomly chosen individuals along a transect of 100 m in autumn 2001. To keep the risk of resampling the same clone low, spacing between individuals was at least 4 m. In the population at Flüela Schwarzhorn, only 16 individuals with a minimum spacing of 4 m were available. Leaf material was dried with silica gel and stored at room temperature until analysis.

Estimates of population sizes were obtained by measurements of the area of each population multiplied with an average density estimate (Table 1).

RAPD-PCR – Of each individual, 20 mg dry leaf material was grounded (Retsch MM2, Retsch GmbH & Co KG, Haan, Germany) for the extraction of total DNA with a DNeasy plant mini kit (Qiagen GmbH, Hilden, Germany). DNA concentrations were determined by fluometry (Turner Design, Sunnyvale, California, USA) with PicoGreen dsDNA quantitation reagent (Molecular Probes Inc., Eugene, Oregon, USA). From 21 decamer primers (Kit P Operon Technologies Inc., Alameda, California, USA and M-6 Microsynth, Balgach, Switzerland), five were selected for the complete survey (OPP-8 [ACA TCG CCC A]; OPP-9 [GTG GTC CGC A]; OPP-17 [TGA CCC GCC T]; OPP-19 [GGG AAG GAC A]; M-6 [GTG GGC TGA C]) after a detailed preliminary screening with three individuals each from four populations. $MgCl_2$ concentration was optimized for each primer. Reproducibility of RAPD banding pattern was tested with timely repeated amplifications of the 12 individuals and confirmed with consistent amplifications. Furthermore one individual was used as a standard marker, beside the 1 kb ladder, for scoring bands and to confirm consistent amplifications during the whole study. 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 (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA), additional 0.5 mmol/L MgCl for the primers OPP-17, OPP-19, and M-6, and 1 U Taq DNA Polymerase (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA). To endorse 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 per-

Tab. 1: Location, population abbreviation, co-ordinates elevation, number of sampled plants per population (*N*), habitat type and estimated population size of 20 study populations of *Geum reptans* in the Swiss Alps.

	Location	Popu-lation	Co-ordinates*	Elevation m a.s.l.	*N*	Habitat type°	Population size
1	Fluhseeli, BE	FLS	604‘700 / 139‘700	2070	17	low	1500
2	Muttgletscher, VS	MUT	674‘500 / 156‘600	2520	18	early sc	5000
3	Blauberg, UR	BLA	675‘030 / 157‘920	2580	17	late sc	7000
4	Val Fex, GR	FEX	781‘325 / 137‘730	2140	20	low	3500
5	Diavolezza, GR	DIA	794‘025 / 143‘500	2980	19	high	1000
6	Val da Cambrena, GR	CAM	797‘100 / 142‘300	2340	20	late sc	8000
7	Piz Languard, GR	LAN	793‘075 / 151‘450	3080	20	high	1500
8	Vadret da Porchabella GR	KEE	787‘100 / 168‘020	2680	20	early sc	5000
9	Vadret da Porchabella GR	KEL	785‘165 / 168‘460	2340	20	late sc	5000
10	Sertig, Gletschtälli, GR	SER	787‘450 / 173‘800	2460	20	late sc	5000
11	Scalettapass, GR	SPA	789‘935 / 174‘380	2600	20	medium sc	1500
12	Scaletta, GR	SCE	791‘600 / 175‘430	2500	20	early sc	2000
13	Scaletta, GR	SCL	791‘750 / 175‘500	2330	20	late sc	8000
14	Vadret da Grialetsch, GR	GR4	792‘785 / 175‘850	2630	20	medium sc	3000
15	Vadret da Grialetsch, GR	GR2	793‘220 / 175‘380	2660	20	medium sc	9000
16	Vadret da Grialetsch, GR	GR1	793‘800 / 175‘300	2600	20	early sc	2000
17	Vadret da Radönt, GR	RAD	792‘585 / 178‘485	2640	20	early sc	4000
18	Flüela Schwarzhorn, GR	SWH	791‘400 / 178‘750	2900	16	high	500
19	Flüelapass, GR	FLU	791‘700 / 180‘300	2420	19	late sc	4000
20	Vadret Tuoi, GR	TUO	806‘275 / 191‘300	2610	20	early sc	5000

*Co-ordinates according to the Swiss topographical maps (Bundesamt für Landestopographie, Wabern, Switzerland).
°Habitat type includes low and high sites, early-, medium- and late-successional (sc) sites.

formed 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. 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, ~ 2.5 h). The banding patterns were made visible with ethidium bromide under UV light. The presence or absence of bands was scored for clear and reproducible bands with estimated fragment lengths of between 500 and 2000 base pairs. The final data set contained 386 individuals instead of 396 individuals due to the failure of the amplification in 10 individuals (Table 1).

RAPD analysis – Statistical analyses of RAPD banding patterns was based on the following assumptions: (1) RAPD fragments behave as diploid, dominant markers with alleles being either present (amplified) or absent (nonamplified); (2) comigrating fragments represent homologous loci; (3) polymorphic loci are inherited in a nuclear (Mendelian) fashion (Arafeh et al., 2002); and (4) populations are in Hardy-Weinberg equilibrium (HWE; $F_{is} = 0$). Even though the information on the heterozygosity of populations was lacking, HWE should not be violated, because a pollination experiment in *G. reptans* resulted in a very low seed set after self-pollination with no germinating seeds (Rusterholz et al., 1993). Assuming that the populations are in HWE, allele frequencies were estimated based on the square root of the frequency of the null (recessive) allele. Only polymorphic bands were taken into account. To avoid biased results, data analyses were restricted 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).

The molecular diversity within populations was quantified as (1) Nei's expected heterozygosity (H_e), (2) the Shannon index (SI; Lewontin, 1972) and (3) 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, SI, and P_p. H_e and SI were calculated with POPGENE (version 1.21; Yeh et al., 1997). The relations of the three molecular indices and the population sizes assessed were calculated as a nonparametric Spearman's Rho (r_s) correlation. The relation of altitude and the molecular indices was calculated as two-polynomial regression. We used JMP (version 3.1; 1995, SAS Institute, Cary, North Carolina, USA) for calculations of correlations.

The molecular differentiation between population pairs was quantified with the pairwise F_{st}, calculated with Arlequin (Schneider et al., 2000). The *F*-statistic was calculated across all bands. To test for isolation by distance (Slatkin, 1993), the genetic distance matrix (pairwise F_{st} values) and the geographic distance matrix was correlated (Mantel test, implemented in Arlequin). Significance levels were obtained after performing 10 100 and 10 000 random permutations for the pairwise genetic distances (F_{st}) and the Mantel test, respectively. Moreover, a UPGMA cluster analysis of pairwise Nei's unbiased genetic distances (Nei, 1978; TFPGA [tools for population genetic analysis]: Miller, 1997), and bootstrapping of 10 000 replicates, was calculated and displayed as a dendrogram to test for spatial separation.

Among-population differentiation was calculated using Nei's (1973) fixation index G_{st} with POPGENE and the fixation index F_{st} by calculating the molecular analysis of variance (AMOVA, implemented in Arlequin). G_{st} values are identical to F_{st} values if a locus consists of two alleles as applicable in RAPD marker analysis (Nybom and Bartish, 2000). Besides calculating G_{st} for the whole data set, it was calculated for the regional and the core areas separately. Furthermore, as genetic diversity correlates with the spatial scale of the distribution of populations (Nybom and Bartish, 2000), we calculated the fixation index for only those populations with 20, 10, or 2 km distance to each other. All fixation indices were calculated with polymorphic bands less frequent than 1-(3/*N*) (Lynch and Milligan, 1994). To test for differences in the level of genetic diversity between populations from early-, medium-, and late-successional stages as well as low (2070 – 2140 m a.s.l.), medium (2330 – 2680 m a.s.l.), and high (2900 – 3080 m a.s.l.) elevation, we used AMOVA, which enables the extraction of variance components, beside the calculation of the fixation index. Euclidean squared distances among individuals were computed prior to the AMOVA and significance level for AMOVA was evaluated after 16 000 random permutations.

Directly observed gene flow – Gene flow occurs via pollen and seeds. (1) We directly measured pollen dispersal distances on the foreland of the Muttgletscher on two midsummer days with good weather conditions (24 July 2001 and 19 July 2002). The frequency of pollinator groups (flies, syrphids, and bumble bees) was estimated by counting flower visitations by insects during 6 h of observation. Dispersal distances of pollen were measured using fluorescent dye (Stockhouse, 1976; fluorescent dye from Radiant colour, Brussels, Belgium). Early in the morning, the anthers of four flowers were marked in an area of 10 × 25 m with different colored fluorescent dyes. Insects visited the flowers and dispersed the colorpowder during the day. With a UV torch, we searched the fluorescent powder after sunset and measured the distance to the initially marked flower. (2) Seed dispersal distances by wind were estimated with simulations using "PAPPUS" (Tackenberg, 2003). With this model, seed dispersal distances are calculated based on the terminal velocity of seeds, spatially explicit landscape data (from the core area), and the assessment of thermally induced turbulence and convection currents. Wind measurements took place on the Scaletta glacier foreland during the period of seed release in 2001. The wind measurements during the week with the best dispersal conditions were taken for a calculation of the dispersal spectra and estimates of the

maximal dispersal distances. To consider the numbers of seeds dispersed, the absolute number of seeds produced by the population of *G. reptans* within the investigated area was estimated by measuring the density of flowering ramets in the area (for more details see O. Tackenberg and J. Stöcklin, unpublished data).

RESULTS

Using five primers, we detected 51 polymorphic RAPD bands, of which two were rejected due to their high frequency. Out of the 386 investigated individuals 384 RAPD phenotypes were found. Two of the populations from the highest altitude contained the same RAPD phenotype twice. None of the 49 scored bands was fixed at the level of populations.

Molecular diversity within populations – The molecular diversities of individual populations calculated from polymorphic RAPD bands are listed in Table 2: Nei's expected heterozygosity, H_e, ranged from 0.16 to 0.25 (CV = 8.7 %) with a mean of 0.22 (SE = 0.004); Shannon indices (SI) of RAPD phenotypic diversity ranged from 0.24 to 0.37 (CV = 8.7 %), with a mean of 0.33 (SE = 0.01) and percentage of polymorphic bands (P_p) within populations ranged from 49.0 to 81.6 % (CV = 10.7 %), with a mean of 71.3 % (SE = 1.7).
Population size had no influence on molecular variation (H_e: r_s = 0.04, P = 0.88; SI: r_s = -0.05, P = 0.84; P_p: r_s = -0.08, P = 0.72). Molecular variation within populations was also not correlated with the altitude of the populations (two-polynomial correlation: H_e: r^2 = 0.17, P = 0.21; SI: r^2 = 0.18, P = 0.18; P_p: r^2 = 0.11, P = 0.38).

Pairwise differentiation and spatial structure of the populations – Genetic differentiation (F_{st}) between population pairs ranged from 0.02 to 0.45 (mean = 0.15, SE = 0.01, CV = 62.3 %). All but one of the 190 pairwise F_{st} values were significant (tested against 10 100 random permutations). The two early-successional populations of recently deglaciated sites from neighboring glacier forelands in the core area were genetically not differentiated (SCE and GR1, P = 0.06), even though the distance among them was 2.2 km. The genetic differentiation between the most distant population (FLS) and populations from the regional area ranged from 0.23 to 0.45 (mean = 0.37, SE = 0.01, CV = 10.5 %).

Tab. 2: Molecular variation measured as 'expected heterozygosity' (H_e), the Shannon Index (SI), and the percentage of polymorphic bands (P_p) per population as well as their means (±SE) and the coefficient of variation for all populations (CV).

Population	**FLS**	**MUT**	**BLA**	**FEX**	**DIA**	**CAM**	**LAN**	**KEE**	**KEL**	**SER**	**SPA**	**SCE**
H_e	0.16	0.22	0.22	0.24	0.24	0.22	0.22	0.22	0.22	0.21	0.19	0.22
SI	0.24	0.33	0.33	0.36	0.36	0.33	0.33	0.33	0.34	0.32	0.29	0.34
P_p (%)	48.98	63.27	65.31	81.63	71.43	65.31	73.47	69.39	79.59	69.39	67.35	73.47

									Summary	
Population	**SCL**	**GR4**	**GR2**	**GR1**	**RAD**	**SWH**	**FLU**	**TUO**	**mean (SE)**	**CV**
H_e	0.22	0.23	0.25	0.22	0.23	0.22	0.19	0.21	0.22 (0.004)	0.09
SI	0.33	0.35	0.37	0.34	0.36	0.34	0.29	0.31	0.33 (0.01)	0.09
P_p (%)	73.47	81.63	73.47	79.59	79.59	69.39	71.43	69.39	71.33 (1.7)	0.11

The pairwise values of genetic differentiation (F_{st}) among the 20 populations correlated significantly with the spatial distances (Mantel test: $R = 0.81$, $P < 0.001$; Fig. 2).

Geographic regions were separated in a UPGMA dendrogram based on Nei's (1978) unbiased measure of genetic distances (Fig. 3): the most western (FLS) and the two populations from central Switzerland (MUT, BLA; Fig. 1) were well separated from the populations of the regional area in the east. As above, the two early-successional populations from two neighboring glacier forelands were grouped together. Within the regional area and

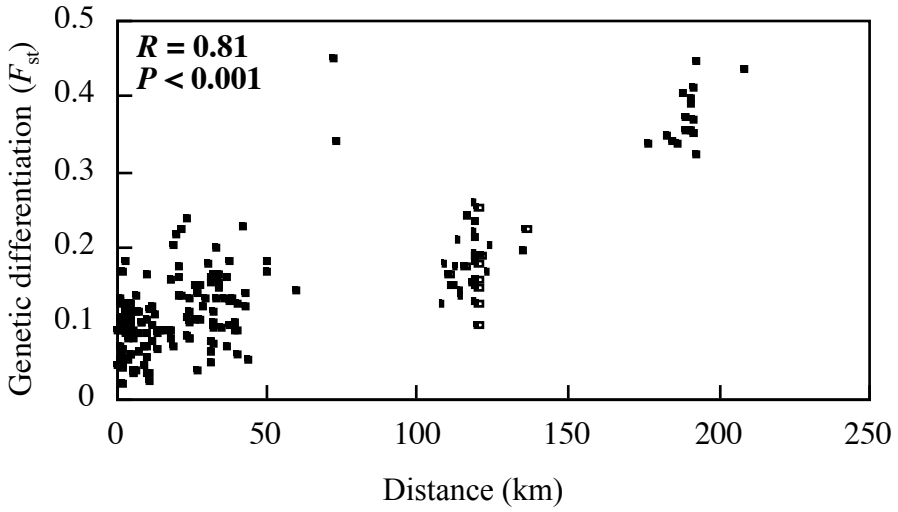

Fig. 2: Matrix correlation of genetic (pairwise F_{st}-values) and geographic distances among 20 populations of *Geum reptans*.

within the core area no clear genetic structure of the populations was found, populations from the same glacier foreland were not grouped tightly (the branches of the UPGMA analysis collapsed under the 50 % majority rule consensus).

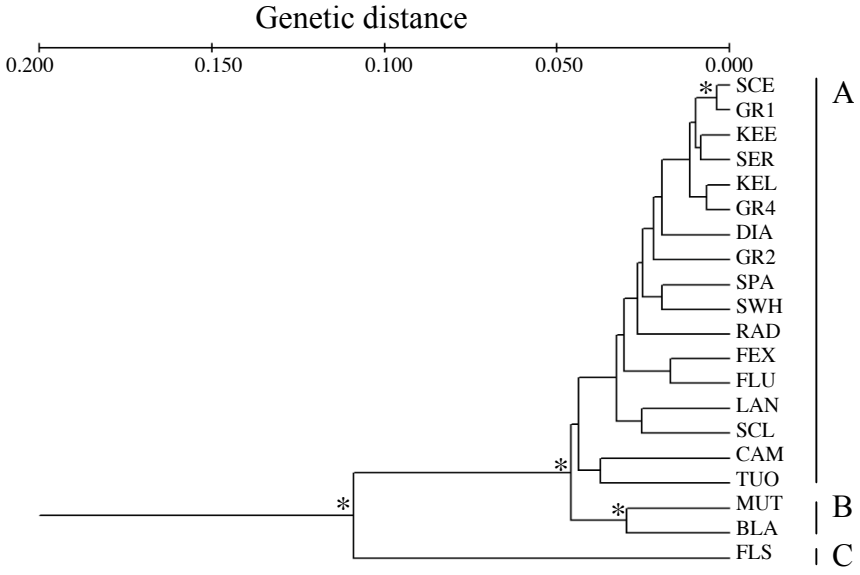

Fig. 3: Dendrogram of the UPGMA cluster analysis based on Nei's (1978) unbiased measure of genetic distance. Populations of the core and the regional area (A), the two central populations (B), and the most western population (C) were separated (* indicate bootstrap values larger than 50 %, based on 10 000 permutations).

Spatial differentiation – RAPD band variation among populations explained 14 % of the total molecular variation: G_{st} = 0.14 (SD = 0.08); H_t = 0.25 (SD = 0.03); and H_s = 0.22 (SD = 0.02). The AMOVA revealed a significant difference among populations (F_{st} = 15 %, $P < 0.001$). The proportion of molecular variation (G_{st}) among the populations of the regional and the core area was 11 % (48 polymorphic bands; one band was excluded, see Materials and Methods) and 6 % (43 polymorphic bands), respectively (Table 3). If only populations that were at least 20, 10, or 2 km distant from each other were analyzed, molecular differentiation was: G_{st} = 0.18 (SD = 0.13; five populations, 44 polymorphic bands), G_{st} = 0.16 (SD = 0.10; eight populations, 47 polymorphic bands), and G_{st} = 0.14 (SD = 0.08; 15 populations, 49 polymorphic bands), respectively.

Tab. 3: Proportion of molecular variation explained among populations (G_{st}) in the core area, the regional area and the whole study area. The table includes population number, the number of populations, the spatial area, and the distances among the groups of populations considered.

Spatial scale	Population	No. of populations	Spatial area (km^2)	Distances among populations (km)		G_{st} (%)
				range	mean (SD)	
Core area	12 – 16	5	1.2	0.2 – 2.2	1.2 (0.6)	6
Regional area	4 – 19	17	1‘300	0.2 – 59.1	17.9 (14.3)	11
Whole area	1 – 20	20	10‘800	0.2 – 208.1	51.7 (59.3)	14

Differentiation due to successional stage and altitude – Genetic variation was not significantly different among early-, medium- and late-successional populations. Moreover, successional stage in the AMOVA model did not explain any variation among populations (AMOVA, 15 populations; $F_{\text{among groups}} = 0$, $P = 0.66$). If populations are grouped into elevational classes (low, medium, and high), the percentage of variation explained by this grouping factor was low (AMOVA: 2.4 %; $P = 0.06$). This corresponds to the lack of relationship of molecular variation and altitude reported above.

Direct estimates of pollen and seed dispersal distances – In total, we observed 435 flower visitations of pollinators on *G. reptans* within 6 h, i.e., 3.5 visitors per flower and per hour. *Geum reptan*s is pollinated mainly by flies of different sizes (94 %), followed by syrphids (4.6 %), and bumble bees (1.4 %). The traces of fluorescing dyes were found 50 times. The frequency of the observed dispersal distances decreased dramatically from 4 cm to 11.5 m with a single rare dispersal event over 30 m (Fig. 4). The seed dispersal spectra obtained from simulations with the model PAPPUS showed that most seeds (99.9 %) are dispersed < 10 m (Fig. 5). Long-distance seed dispersal with seeds dispersed over > 100 m and 1000 m occurred in only 0.015 % and 0.005 % of all cases, respectively. Based on estimates of the yearly seed production on the Scaletta glacier foreland of > 10 Mio seeds, on this particular glacier foreland ~ 1580 and ~ 520 seeds are dispersed over > 100 m and 1000 m, respectively.

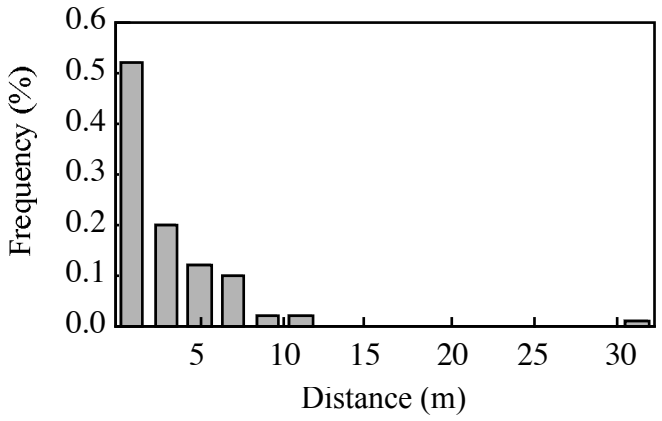

Fig. 4: Frequency of pollinator flight distances grouped in intervals of 2 m (N = 50).

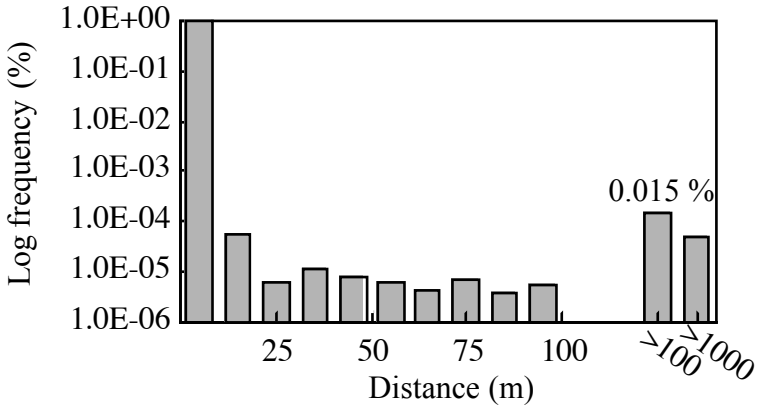

Fig. 5: Frequency of seeds dispersed by wind, grouped in intervals of 10 m. Assessment of seed dispersal distances were calculated with the model 'Pappus' (Tackenberg, 2003).

DISCUSSION

In spite of highly fragmented habitats, the observed genetic differentiation among populations of *Geum reptans* in the Swiss Alps was not particularly high compared with other RAPD studies. Nevertheless, we observed a clear spatial genetic structure with a relatively low differentiation among regional populations and a stronger differentiation between regions, indicating that the exchange of genes decreases with distance. Molecular variation within population was similar across all populations and did not depend on successional stage,

elevation, or population size. Direct observations of pollen and seed dispersal indicate that the potential of gene flow from seeds is larger than from pollen.

Genetic diversity within populations of a clonal alpine species – The amplification of randomly selected gene loci (RAPD-PCR) is usually a more sensitive method to detect genetic variation in plant species compared to gene product level methods (e.g., isozymes) (Nybom and Bartish, 2000). RAPDs are sometimes questioned with respect to reproducibility and the biallelic nature of DNA bands. Heterozygotes cannot be separated from homozygotes and Hardy-Weinberg equilibrium has to be assumed for analysis. Our analysis is based on well reproducible RAPD banding patterns and because selfed seeds in *G. reptans* are nonviable (Rusterholz et al., 1993), selfing can be neglected.

Isozyme studies suggest that within and among populations genetic variation does not depend on either sexual and/or clonal reproduction (Hamrick and Godt, 1989). Furthermore, from the now available studies of alpine plants, there is little indication that plant species from high altitude have lower levels of genetic diversity compared to lowland plants (Holderegger et al., 2002; Till-Bottraud and Gaudeul, 2002). We can confirm both statements with our RAPD data of *G. reptans*: mean molecular variation within populations ($H_e = 0.22 \pm 0.004$) was neither particularly high nor low and similar to the mean of 41 RAPD studies reviewed by Nybom and Bartish (2000) ($H_e = 0.21 \pm 0.12$). The mean molecular variation reported in this review is probably somehow underestimated because sexually as well as clonally reproducing species were included. To avoid a biased estimate of genetic diversity in studies of clonal plants, each genotype should be collected only once (McLellan et al., 1997). In *Cladium jamaicense*, for example, overall genetic variation was underestimated if only genotypic diversity was considered (Ivey and Richards, 2001). But in *Viola riviniana* genotypic diversity and overall genetic diversity were almost identical (Auge et al., 2001). We tried to avoid sampling the same genetic individual twice and indeed 99.5 % of all our samples were genetically different. This high resolution of RAPD phenotypes also indicates that single genets of *G. reptans* only exceptionally grow > 4 m in diameter, as supposed when we chose the sampling design. Our results with *G. reptans* support the statement of Hamrick and Godt (1989) that, in general, clonal plants are genetically as diverse as nonclonal plants (Ellstrand and Roose, 1987; Widen et al., 1994). Genotypic diversity in the studied populations was high, as only exceptionally the same genotype was found twice.

Effects of successional and elevational gradients on genetic variation within populations – We assumed high initial recruitment from seeds when a population is founded after the retreat of a glacier, as several other authors did (e.g., MacDonald and Lieffers, 1991; Jelinski and Cheliak, 1992). Later, sexual recruitment might be low in clonal plants (Eriksson, 1989). It was also suggested that diversity should decline due to increased selection pressure during succession (Till-Bottraud and Gaudeul, 2002). In *G. reptans* a low genetic diversity might be expected particularly in late-successional habitats or in peripheral altitudinal habitats, because selection might be particularly strong at the distributional limits of a species. We found no evidence that population genetic variability in *G. reptans* is affected by environmental gradients. Changes in population genetic variability due to ecological conditions are rarely observed (Shimizu et al., 2002; Young et al., 2002; but see Gugerli et al., 1999; Auge et al., 2001; Li and Ge, 2001; Stenström et al., 2001; Bonnin et al., 2002). For example, in *Parnassia palustris* habitat type affected neither within-population genetic diversity nor genetic and phenotypic differentiation among populations (Bonnin et al., 2002) and in the alpine *Saxifraga oppositifolia* no effect of altitude on genetic population variability was detected (Gugerli et al., 1999). Along environmental gradients, several parameters such as individual age, overlap of generations, or recruitment frequency in climatically favorable years may change and influence intrapopulation genetic variation (Molau, 1997). But these parameters appear to have either no major or opposing effects on genetic variation in most cases studied so far, and this also holds for *G. reptans*. Long ramet life-span and potentially immortal genets may enhance the maintenance of genetic diversity in *G. reptans*, as observed in other studies of clonal plants (Lee and Chung, 1999; Brzosko et al., 2002). A high disturbance frequency in alpine habitats may allow repeated seedling establishment at least in favorable years in spite of the general harsh environmental conditions. Nevertheless, sexual reproduction alone does not guarantee the preservation of genetic variation. Various factors, such as drift, inbreeding, and strong selection, may result in genetic depletion (McLellan et al., 1997) if there is no gene flow from immigrating seeds or pollen. Our results suggest that the loss of genotypes during succession might be negligible and repeated seedling recruitment takes place irrespective of environmental conditions.

Population size may be critical for the maintenance of genetic variation (review by Frankham, 1996). In large populations, genetic drift is insignificant, but it becomes important in small populations and may be particularly pronounced after dramatic reduction in range size

and fragmentation of habitats of a species (Srikwan and Woodruff, 2000). Alpine habitats are naturally fragmented, population sizes are expected to be stable, and, indeed, even though population sizes were very variable (CV = 61.7 %), we found no effect of population size on genetic diversity in *G. reptans*.

Genetic differentiation and gene flow in the fragmented alpine environment – Due to high natural fragmentation and limited gene flow, we expected a comparably high genetic differentiation among populations in the alpine *G. reptans*. However, the observed estimate of genetic variation among all populations (G_{st} = 0.14) is not particularly high if compared with the mean reported in the review of 41 RAPD studies by Nybom and Bartish (2000) (G_{st} = 0.29 ± 0.21). In addition to the amount of gene flow and drift, G_{st} values are dependent on life history traits, colonization events, and the extent of the area under study, with a positive correlation of genetic differentiation and maximum spatial distance between population pairs (Nybom and Bartish, 2000). In our study, genetic differentiation increased from the core area, to the regional, to the whole area. However, the average spatial distances in our study are much lower than the mean distances among populations in most of the studies reviewed by Nybom and Bartish (2000), which in part might explain the comparably low value of population differentiation in *G. reptans*. In *Trollius europaeus*, a similar estimate of genetic differentiation among populations in the Alps was observed, but in this case genetic and geographic distances were not correlated (Despres et al., 2002).

In our study, we observed considerable variation among pairwise population F_{st} values (ranging from 0.02 to 0.45), indicating random genetic drift. Even population pairs on the same glacier foreland were significantly differentiated, but to a lower degree than the more distant populations. This suggests that gene flow among populations of *G. reptans* is dependent on distance. The nonsignificant genetic difference between the two youngest populations in adjacent valleys may however indicate that younger populations are not necessarily founded by the nearest and elder populations from the same glacier forland, but by immigrants from more than one population in the same region. In *G. reptans*, we do not expect genetic bottlenecks in populations because our results indicate that gene flow is not restricted to single habitats (i.e., single glacier foreland) and because even early-successional populations were not genetically depleted. The observed gene flow via pollinators was very low, but the calculated seed dispersal distances may explain the results of the molecular study.

Even if the simulation model predicts that only a small proportion of *G. reptans* seeds is dispersed over long distances, this may be sufficient for colonization and gene flow over considerable distances because of the large numbers of seeds produced every year. To infer the relative contribution of seed and pollen to gene flow, genetic population structure due to maternally inherited and nuclear genes should be compared (McCauley, 1995). However, we have not yet been successful in finding variation in maternally inherited genes in *G. reptans* with RFLP (random fragment length polymorphism).

Conclusion – Our results indicate that an alpine plant species may exhibit a similar level of genetic variation as lowland species. We observed no severe consequences of the highly fragmented habitats of *Geum reptans* for molecular genetic diversity and genetic differentiation among populations was not particularly high. In addition, clonal reproduction in this species has no severe consequences for population genetic variability and neither did successional age or elevation of the populations. Clearly, our results indicate that, at least within the same region, considerable gene flow is occurring among populations of *G. reptans* over larger distances, probably mainly by seed. Nevertheless, we observed a clear genetic structure according to the geographic distribution of the studied populations. Even populations within the same glacier foreland were significantly differentiated, but to a small degree, while genetic differentiation increased among more distant populations. We conclude that, in spite of the highly fragmented alpine landscape, random genetic drift is not the main factor determining population genetic structure of a species like *G. reptans* and that gene flow might be more important than commonly suggested.

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Chapter 3

The importance of population origin and environment on clonal and sexual reproduction in the alpine plant *Geum reptans*

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Functional Ecology, accepted

ABSTRACT

1. Reproductive behaviour of plants may change in contrasting habitats. In two separate greenhouse experiments, we studied effects of population origin (early *vs.* late successional and low *vs*. high altitudinal habitats) and environmental effects (competition and temperature) on plant size and sexual *vs.* clonal reproduction in *Geum reptans*.
2. Plant size and reproduction differed significantly among populations, but only plant size differed between contrasting habitats.
3. If plants grew with competition or at warm temperature, plant size and reproduction was reduced and more plants reproduced only with stolons. Individuals with flowers were larger than plants which reproduced only with stolons, indicating a lower minimum plant size for clonal than for sexual reproduction.
4. Populations of different origin changed only little in their response to environmental treatments. Plants from early successional habitats tended to produce more flowers in the competition-free treatment, whereas in plants from late successional habitats it was the opposite.
5. Results indicate limited adaptation in reproductive behaviour to contrasting habitats. Nevertheless, great size-dependent plasticity in the proportion of sexual *vs*. clonal reproduction ensures population persistence and reproduction in a large range of habitat conditions.

Keywords: competition, plasticity, reproductive allocation, temperature, trade-off

INTRODUCTION

Steep environmental gradients are ubiquitous in alpine ecosystems. Climatic conditions change with altitude, exposition, and slope resulting in a patchy distribution of microhabitats. Changes in abiotic and biotic conditions can lead to major modifications in selection pressure on plant life history traits (Stearns 1992; Cody & Overton 1996). Clonal growth is among the most noticeable adaptations to severe climatic conditions and nutrient poorness in cold environments (Callaghan 1988; Klimes *et al.* 1997). Recruitment by seeds is commonly assumed to be restricted in the cold and by short seasons (Jelinski & Cheliak 1992; Eriksson 1997). Indeed, many alpine plants reproduce clonally even in pioneer communities and particularly in late successional grasslands (Gray 1993; Stöcklin & Bäumler 1996; Klimes *et al.* 1997).

Clonal plants tend to allocate more biomass to vegetative propagation than to sexual reproduction (Abrahamson 1980; Cook 1985; Eriksson 1997). As resources are usually limited (Cody 1966), a pronounced allocation to vegetative reproduction will lower the investment for seeds resulting in a trade-off between the two reproductive modes (Harper 1977; Watson 1984; Piquot *et al.* 1998; Prati & Schmid 2000; Ronsheim & Bever 2000 but see Cain & Damman 1997). In plants, meristem allocation may be even more important than resource partitioning, because meristems available for reproduction may be more limited than carbon or other resources (Watson 1984; Geber 1990; Bonser & Aarssen 1996). The future availability of reproductive meristems is increased by clonal reproduction (Eriksson 1989). Extensive vegetative propagation may result in rapid, but spatially limited spread of genotypes and may improve population persistence during phases of lacking sexual reproduction. Long-distance dispersed seeds, however, connect fragmented populations in the patchy alpine landscape or found new populations in unoccupied habitats. But seed production is more nutrient demanding than vegetative reproduction (Harper 1977; Watson 1984). The onset of clonal and sexual reproduction in general may be determined by different minimum plant size requirements (Schmid, Bazzaz & Weiner 1995). In most cases sexual reproduction increases with increasing plant size (Weiner 1988), whereas the relative allocation to clonal propagation appears to be constant over a large range of plant sizes (Schmid *et al.* 1995).

Selection pressure affecting trade-offs differ in response to environmental heterogeneity (Bazzaz *et al.* 1987; Sultan 1987; Hutchings 1988) and may lead to local adaptation (Bradshaw 1984; Galen, Shore & Deyoe 1991). We expect variation in the

partitioning of resources and meristems to reproductive strategies in contrasting habitats. The spatial isolation of alpine habitats may even increase the degree of local differentiation. However, phenotypic plasticity rather than genetic differentiation may be an alternative way of matching genotypes to environment, with increasing environmental heterogeneity favouring greater levels of plasticity (Schlichting 1986; Sultan 1987). How the highly structured alpine landscape affects trait differentiation among sites and to what an extend such effects are genetically based is fairly unknown.

Here, we ask if genetic population differentiation in growth and reproductive behaviour is affected by two prominent alpine environmental gradients: succession and elevation. The most important factor changing along successional gradients after the retreat of glaciers is the increasing competition pressure while soil development proceeds and vegetation cover increases. With increasing altitude temperature and vegetation period are decreasing resulting in more stressful abiotic conditions for plant life and particularly seedling establishment. Metapopulation models predict that individuals with high dispersal ability will be favoured in early successional habitats, because new populations are more likely founded by high-dispersal genotypes (Olivieri, Michalakis & Gouyon 1995; Olivieri & Gouyon 1997). During succession, genotypes with an affinity to maintenance functions, e.g. clonal propagation might be more successful. Opposing selection for sexual and clonal reproduction in early and late successional habitats was reported by Piquot *et al.* (1998), whereas Van Kleunen, Fischer & Schmid (2001) found an increase in sexual reproduction at higher density. What the altitudinal gradient is concerned, vegetative reproduction may increase at higher altitudes within species (e.g. Young *et al.* 2002), or among species (Klimes *et al.* 1997). Other studies report higher allocation to clonal than to sexual reproduction in harsh environments (Bostock 1980), a maximum relative investment in clonal reproduction at medium altitude (Douglas 1981), or no altitudinal effect of reproductive allocation at all (Williams, Mack & Black 1995). Large differences in reproductive behaviour among populations of *Geum reptans* L. have been observed (T. Weppler and J. Stöcklin; unpublished), but to which extend these differences have a genetic background is unknown.

In two separate experiments, we studied effects of population origin (genetic effects) and environment on growth and the relative importance of sexual and clonal reproduction in *Geum reptans*. In a competition-experiment plants from early and late successional populations grew with or without competition with *Poa alpina* L.. In the second experiment,

plants from low and high altitudinal populations were exposed to different temperatures (cold and warm). *G. reptans* is especially suited to study the trade-off between reproductive modes, because meristems in leaf axils are used for either sexual or clonal reproduction. We addressed the following hypothesis: (i) reproductive behaviour of plants from early and late successional habitats differs. (ii) The relative importance of clonal growth is increased in populations from high altitude compared to populations from lower elevations. (iii) Plants from late successional populations will be favoured in the competition treatment and plants from higher populations will be favoured at low temperature, indicating adaptation. Additionally, these two experiments allow to test, if sexual and clonal reproduction are affected by plant size and if there is a trade-off between the two reproductive modes.

MATERIALS AND METHODS

Study species – The clonal *Geum reptans* (Rosaceae) is a perennial outcrossing rosette plant, and occurs preferentially on glacier forelands and moraines on siliceous rocks between 1950 and 3800 m a.sl. (Hegi 1995). The plant is one of the first pioneers on virgin soils and persists long during succession. In the competition-experiment, early successional habitats refer to recently (several years to a few decades) deglaciated areas and late successional habitats are at least 100 years free of ice.

G. reptans is reproducing clonally by forming new rosettes (ramets) with adventitious roots at the end of aboveground stolons, and sexually by producing flowering stems with a single terminal flower head. Reproductive meristems are located in the axils of leaves and are preformed in the season prior to emerging. At the end of summer, stolons, not established daughter rosettes, and leaves of adult plants die back.

Experimental design – In the competition-experiment a total of 192 plants from four early and four late successional populations were grown either with or without *Poa alpina*, i.e. 12 individuals each per population and treatment (Table 1). Plants grew in two air-conditioned greenhouse compartments with an ecological relevant air-temperature regime of 10 °C at night and 20 °C during daytime. In the temperature-experiment a total of 208 plants from five low and four high altitudinal populations were exposed to two temperature treatments. Per

population 12 individuals grew at cold temperature (7.5 °C at night and 17.5 °C during daytime) and 12 individuals at warmer temperature (12 °C at night and 22 °C during daytime) in two air-conditioned greenhouse compartments per treatment (in one population only 8 individuals per treatment were available, Table 1). The temperature difference of 4.5 K equals a difference in altitude of 750 m under the estimate of a lapse rate of 0.60 K per 100 m (Körner 1999). Plants within greenhouse compartments were randomised every second month. Within each compartment a temperature sensor measured air-temperature continuously and regulated the connected air-conditioner (Airwell Type R-407C, ACE Klimatechnik GmbH, Frankfurt, Germany) to cool the compartment to the required temperature. During daytime, temperature rose due to incident solar radiation and the ambient air-temperature around the chamber. All plants were kept inside the greenhouse from April to October, but overwintered outside in the Garden of the Institute of Botany, University of Basel, Basel (270 m a.sl.), Switzerland, where plants experienced frost.

Tab.1: Location, population abbreviation, elevation, habitat type, and number of individuals per population and treatment of 17 populations of *Geum reptans* in the Swiss Alps used in two greenhouse experiments with a competition- and a temperature-treatment, respectively.

Location	Population	Longitude (m)/ Latitude (m)[1]	Elevation m a.sl.	Habitat type[2]	No. of plants in the treatment:	
Competition-experiment					**without comp.**	**with comp.**
Scaletta early, GR	SCE	791600/175430	2500	early ss	12	11
Muttgletscher, VS	MUT	674500/156600	2520	early ss	10	8
Vadret da Radönt, GR	RAD	792585/178485	2640	early ss	12	12
Vadret da Grialetsch, GR	GRI	793500/175250	2660	early ss	12	12
Scaletta late, GR	SCL	791750/175500	2330	late ss	10	10
Val da Cambrena; GR	CAM	797100/142300	2340	late ss	12	12
Flüelapass, GR	FLU	791700/180300	2420	late ss	12	12
Blauberg, UR	BLA	675030/157920	2580	late ss	9	9
Temperature-experiment					**cold temp.**	**warm temp.**
Fluhseeli, BE	FLS	604700/139700	2070	low	13	14
Steinlimigletscher, BE	STE	675025/173850	2080	low	11	13
Val Roseg, GR	ROS	786125/142900	2120	low	6	5
Val Fex, GR	FEX	781325/137730	2140	low	13	14
Muttbach, VS	MUB	674250/157550	2150	low	12	10
Eggishorn, VS	EGH	650400/142290	2860	high	12	12
Flüela Schwarzhorn, GR	SWH	791400/178750	2900	high	12	12
Diavolezza, GR	DIA	794025/143500	2980	high	13	12
Piz Languard, GR	LAN	793075/151450	3080	high	10	12

[1] Longitude and latitude according to the Swiss topographical maps (Bundesamt für Landestopographie, Wabern, Switzerland)
[2] Early and late successional (ss) habitats; low and high habitats.

Plant material was collected in early autumn 2000 and 2001 for the competition-, and the temperature-experiment, respectively. Per population, we collected c. 30 daughter rosettes with their stolons each from a different mother plant. The randomly chosen mother plants were located along a transect and were at least 4 m distant from each other, except in the population at 'Eggishorn' and 'Flüela Schwarzhorn' where plants were aggregated along cracks. Because of few reproducing adults in the populations of 'Val Roseg', 'Muttbach', and 'Flüela Schwarzhorn' more than one clonal offspring from 3, 4, and 7 mother plants (respectively) were collected and allocated equally to treatments. Collected plants were kept in moist bags in the dark at 4 °C until planting. By the end of October, 24 plants per population with equal size were planted into single rectangular pots of 9 x 9 cm and 10 cm in height. Pots were filled with 2 cm of pumice-gravel as drainage, followed by a 1:1 mixture of sand and pot-soil. Plants were randomly allocated to the two treatments in each experiment. After three weeks, in one half of the pots of the competition-experiment four randomly chosen bulbills of *Poa alpina* were planted as competitors, one in each edge of the pot. *P. alpina* bulbills were collected at four sites at different altitudes and kept in a moist bag in the dark at 4 °C until planting. After the first winter the number of *P. alpina* individuals per pot were reduced to one and every second month individuals of *P. alpina* were reduced to two tillers. In each experiment, plants within populations were randomly and equally partitioned to greenhouse compartments. Statistically, greenhouse compartments were treated as blocks. In the temperature-experiment blocks and temperature treatments fall together.

The remaining ramets were potted the same way. During the first winter 14 and 21 individuals died in the competition- and the temperature-experiment, respectively. Whenever possible, dead plants were replaced by individuals of the same population or in the temperature-experiment of the same elevation. Plants which died later, were not replaced anymore. On July 15th 2002, the 161 remaining plants were brought to 2000 m a.sl. near Davos, Graubünden, Switzerland, for a comparison of their reproductive behaviour at a natural site with the plants in the greenhouse.

In the greenhouse, plants got additional light from 1 kW lamps 3 to 5 hours during daytime according to day-length. All plants were watered twice a week and received the same amount of full fertiliser (in 2002: 9 kg N ha^{-1}, and in 2003: 9 kg N ha^{-1} plus 23 kg N ha^{-1} with a higher K content; applied in equal portions during the vegetation period). Potassium facilitates growth of reproductive meristems.

Measurements – Number of leaves per plant were recorded at the beginning of the experiment and analysed to test for equal plant size between treatments and blocks. This measure was also used as a covariate in statistical analyses (see below).

Final harvest of both experiments was in July and August 2003, 3 and 2 years after the beginning of the competition- and the temperature-experiment, respectively. Numbers of rosettes, and green and dead leaves, produced in 2003, were counted. Above- and below-ground plant biomass was measured after drying at 80 °C for 48 h. Leaf axils of the leaves formed in the year of the final harvest were checked for reproductive meristems and if present, they were cut and stored in 50 % alcohol until determination whether they are future flowers or stolons. In the greenhouse, we observed only few individuals actually reproducing in 2003 (8.7 %), whereas 73.3 % of the remaining plants kept at 2000 m a.sl. were reproducing, despite a similar number of preformed reproductive meristems in both groups. Therefore, we judged preformed meristems as a more precise measurement of the reproductive potential of populations in the greenhouse than the actual number of flowers and stolons. In the common garden vernalisation was probably too short to initiate sufficiently preformed meristems to grow out because of the milder climate during winter compared with the conditions at 2000 m a.sl.. In the previous years, reproduction was constantly low and should not have influenced reproductive allocation in the year of the final harvest.

Statistics – Vegetative and reproductive measurements were used to test for effects of origin (habitat type and population) and environment (treatment) by hierarchical analysis of covariance (ANCOVAs). In the competition-experiment, habitat type (early and late succession) was tested against population, treatment was tested against the interaction of treatment and population, and population as well as the interaction of habitat type and treatment were tested against the remaining residual. In the temperature-experiment, treatment was tested against block, habitat type (low and high altitude) was tested against the population, and population, the interaction of treatment and habitat type as well as the interaction of treatment and population were tested against the remaining residual. Plant measurements were ln-transformed to test for relative differences and the proportion of stolons on all reproductive organs was arcsin-transformed. Because several individuals neither produced stolons nor flowers the requirement for the ANCOVA could not be fulfilled with individual data points. Instead, we used means of reproductive meristems per population within treatment and block

for the ANCOVA. Correlations were also calculated with population-means with a non-parametric Spearman's Rho (r_s) procedure instead of parametric Pearson's product-moment (R) tests.

Phenotypic measurements of experimental plants include a genetic and a maternal environmental component. The maternal environment may become manifest in stolons influencing the nutrition and growth of clonal offsprings. Therefore, we used the number of leaves at the beginning of the experiment as a measure of maternal effects. In both experiments this measure did not affect the number of reproductive meristems ($P > 0.1$), and did not influence plant size at the final harvest in the competition-experiment, but influenced plant size at the final harvest in the temperature-experiment ($R = 0.22$, $P < 0.01$ for leaves and above-ground biomass). Because of the possibility of maternal effects, we consequently used in all the analyses the number of leaves at the beginning of the experiments as a covariable. With this approach we feel confident that we were able to reduce maternal effects in the final data set to a large extent.

Plants were classified according to their reproductive meristems in individuals with stolons, with flowers, with stolons and flowers, or non-reproducing individuals. If preferences of reproductive behaviour differed between habitat types or treatments was tested with a Poisson log-linear Model.

All statistical analyses were done with R (Ihaka & Gentleman 1996), a shareware package with high similarity to S-PLUS.

RESULTS

Effects of population origin on plant size and reproduction – All measured traits of plant size and reproduction in the temperature-experiment and four of eight traits in the competition-experiment differed significantly among populations indicating population differentiation (Table 3). But there were only few effects on plant size and reproduction due to population origin from early and late successional habitats in the competition-experiment, and from low and high altitudes in the temperature-experiment: plants from early successional habitats produced 14 % more aboveground biomass and 15 % more below-ground biomass ($P < 0.05$ for both terms, Tables 2A and 3A), and plants from high altitude produced 18 % more

leaves (P = 0.06, Tables 2B and 3B). Furthermore, in two cases we found marginal significant interaction terms for origin x treatment, indicating that the response to environmental treatments was different among habitats or populations. In the competition-experiment plants from early successional populations produced more flowers in the treatment without competition, whereas plants from late successional populations produced more flowers in the treatment with competition (P = 0.07 for the interaction of habitat x treatment, Table 3A, Fig. 1). In the temperature-experiment, the difference in the number of reproductive meristems in the warm and cold temperature treatment was depending from populations (P = 0.07 for the interaction of population x treatment, Table 3B).

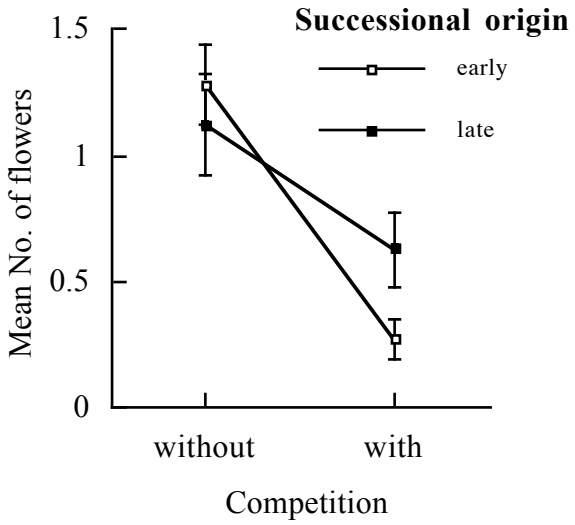

Fig. 1: Mean numbers of flowers (± SE) of *Geum reptans* populations from early and late successional habitats in a competition-experiment with or without *Poa alpina* as a competitor (P = 0.07 for competition x habitat origin in ANCOVA).

Effects of environment on plant size and reproduction – In the competition-experiment plant size of *G. reptans* was significantly reduced by competition with *Poa alpina*: above-ground biomass was reduced by 61%, below-ground biomass by 72 %, number of rosettes by 33 %, and number of leaves by 40 % (P = 0.00001 for all terms, Tables 2A and 3A). The frequency of plants reproducing either by flowers, stolons, or with both reproductive modes shifted between the competition treatments. More individuals were reproducing clonally or not at all in the treatment with competition at the expense of individuals with flowers or both reproductive modes (P < 0.001, Fig. 2A). Plants grown with competition

produced 50 % less reproductive meristems in total, 37.5 % less stolons, and 62.5 % less flowers ($P < 0.01$ for all terms, Tables 2A and 3A). In general, plants produced relatively more stolons than flowers (60 ± 2.4 %, Table 2A). The proportion of stolons, however, did not change significantly between treatments ($P = 0.293$, Tables 2A and 3A).

Tab. 2: Plant size and reproduction (mean ± SE) of *Geum reptans* from different origins in two experiments: (A) Competition-experiment with plants from early and late successional habitats with and without competition from *Poa alpina* and (B) temperature-experiment with plants from low and high altitude grown under cold or warm temperature-treatment. For differences among traits see Tab. 3.

A

Competition-treatment	**without competition**		**with competition**	
Successional habitat	**early**	**late**	**early**	**late**
Plant size				
Above-ground biomass (g)	1.93 ± 0.07	1.67 ± 0.07	0.83 ± 0.05	0.67 ± 0.05
Below-ground biomass (g)	3.00 ± 0.13	2.76 ± 0.13	0.97 ± 0.07	0.69 ± 0.06
No. of rosettes	1.80 ± 0.15	1.79 ± 0.14	1.15 ± 0.07	1.16 ± 0.07
No. of leafs	24.5 ± 1.31	21.3 ± 1.07	14.0 ± 0.72	13.3 ± 0.69
Reproduction				
No. of reproductive meristems	2.78 ± 0.18	2.93 ± 0.25	1.24 ± 0.16	1.60 ± 0.21
No. of flowers	1.28 ± 0.16	1.12 ± 0.20	0.27 ± 0.08	0.63 ± 0.15
No. of stolons	1.50 ± 0.15	1.81 ± 0.20	0.98 ± 0.12	0.98 ± 0.15
% Stolons	56.0 ± 4.95	59.7 ± 5.58	65.7 ± 6.79	55.4 ± 6.81

B

Temperature-treatment	**warm**		**cold**	
Altitude of habitat	**low**	**high**	**low**	**high**
Plant size				
Above-ground biomass (g)	1.55 ± 0.06	1.59 ± 0.06	1.61 ± 0.06	1.73 ± 0.07
Below-ground biomass (g)	1.61 ± 0.07	1.63 ± 0.09	1.61 ± 0.07	1.56 ± 0.08
No. of rosettes	2.21 ± 0.15	2.40 ± 0.16	2.25 ± 0.14	2.66 ± 0.17
No. of leafs	23.6 ± 1.07	28.0 ± 1.09	24.8± 1.11	31.3 ± 1.37
Reproduction				
No. of reproductive meristems	2.86 ± 0.31	2.96 ± 0.35	4.16 ± 0.25	4.94 ± 0.38
No. of flowers	1.39 ± 0.26	1.19 ± 0.33	1.69 ± 0.19	1.89 ± 0.35
No. of stolons	1.46 ± 0.13	1.77 ± 0.19	2.49 ± 0.20	3.04 ± 0.27
% Stolons	55.1 ± 5.15	64.3 ± 5.70	59.8 ± 3.45	63.6 ± 4.61

The temperature treatment had only weak influence on above-ground biomass and leaf number per plant, and no influence on below-ground biomass and the number of rosettes (Tables 2B and 3B). But temperature influenced the reproductive behaviour of plants. In warm temperature, more individuals produced only stolons, only flowers, or were non-reproducing at the expense of individuals with both reproductive modes ($P < 0.001$, Fig. 2B). In total, in

Tab. 3: ANCOVA summary of the effect of habitat type, population, treatment, and interaction of treatment with habitat type or population on plant size (above- and below-ground biomass, No. of rosettes, or No. of leaves) and reproductive meristems (total, flower, or stolon, and percentage of stolons on total reproductive meristems) in two experiments: (A) Competition-experiment, (B) temperature-experiment.

A

Competition-experiment	DF	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Plant size		**Above-ground biomass**		**Below-ground biomass**		**No. of rosettes**		**No. of leaves**	
Covariable°	1	0.021		0.090		0.024		0.028	
Block	1	0.192		0.007		0.047		0.597	
Habitat (early *vs*. late succession)	1	0.771	*	1.195	*	0.00001	n.s.	0.582	n.s.
Population	6	0.069	*	0.109	n.s.	0.093	n.s.	0.165	n.s.
Competition-treatment	1	10.226	***	27.328	***	5.691	***	11.484	***
Habitat x Treatment	1	0.0001	n.s.	0.084	n.s.	0.011	n.s.	0.106	n.s.
Population x Treatment	6	0.032	n.s.	0.049	n.s.	0.039	n.s.	0.086	n.s.
Residual	154	0.031		0.062		0.168		0.103	
Reproduction		**Reproductive meristems**		**Flowers**		**Stolons**		**% Stolon**	
Covariable°	1	0.077		0.068		0.001		23.8	
Block	1	0.080	n.s.	0.001	n.s.	0.222	n.s.	539.6	n.s.
Habitat (early *vs*. late succession)	1	0.029	n.s.	0.006	n.s.	0.055	n.s.	13.8	n.s.
Population	6	0.391	**	0.181	0.06	0.443	**	1003.4	*
Competition-treatment	1	4.344	***	1.456	***	1.912	*	156.1	n.s.
Habitat x Treatment	1	0.055	n.s.	0.265	0.07	0.256	n.s.	1029.8	0.09
Population x Treatment	6	0.106	n.s.	0.030	n.s.	0.191	n.s.	117.7	n.s.
Residual	14	0.077		0.066		0.095		327.3	

B

Temperature-experiment	DF	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Plant size		**Above-ground biomass**		**Below-ground biomass**		**No. of rosettes**		**No. of leaves**	
Covariable°	1	0.642		0.398		0.554		1.080	
Temperature-treatment	1	0.163	0.07	0.022	n.s.	0.233	n.s.	0.298	0.08
Block	2	0.012		0.036		0.200		0.027	
Habitat (low *vs*. high altitude)	1	0.009	n.s.	0.167	n.s.	0.831	n.s.	1.700	0.06
Population	7	0.300	***	0.342	*	0.836	**	0.338	***
Habitat x Treatment	1	0.023	n.s.	0.010	n.s.	0.098	n.s.	0.046	n.s.
Population x Treatment	7	0.095	n.s.	0.044	n.s.	0.167	n.s.	0.076	n.s.
Residual	185	0.074		0.136		0.235		0.086	
Reproduction		**Reproductive meristems**		**Flowers**		**Stolons**		**% Stolon**	
Covaribale°	1	0.266		0.088		0.067		54.1	
Temperature-treatment	1	2.691	**	0.554	n.s.	1.243	*	25.7	n.s.
Block	2	0.022		0.118		0.016		189.9	
Habitat (low *vs*. high altitude)	1	0.632	n.s.	0.058	n.s.	0.339	n.s.	542.3	n.s.
Population	7	0.605	***	0.577	***	0.187	**	933.5	*
Habitat x Treatment	1	0.0002	n.s.	0.003	n.s.	0.0003	n.s.	23.6	n.s.
Population x Treatment	7	0.132	0.07	0.061	n.s.	0.042	n.s.	409.6	n.s.
Residual	15	0.054		0.061		0.041		229.7	

°No. of leaves at the beginning of the experiment; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and n.s. $P > 0.1$

the cold-temperature treatment plants produced 55 % more reproductive meristems, and 68.8 % more stolons, whereas the amount of flowers was unaffected by temperature (Tables 2B and 3B). Again, plants produced relatively more stolons than flowers (59 ± 3 %) and the proportion of stolons did not differ between treatments (P = 0.791, Tables 2B and 3B).

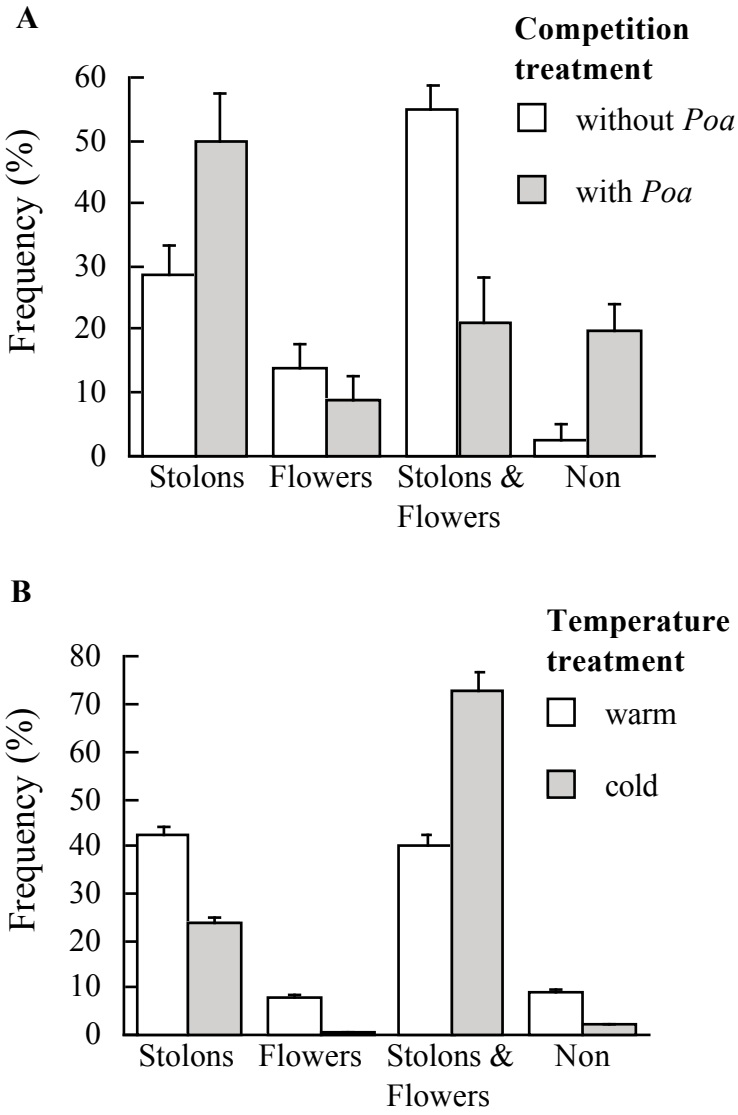

Fig. 2: Frequency of *Geum reptans* individuals reproducing either by flowers, stolons, or both reproductive modes in two experiments: (A) Competition-experiment (N = 172) and (B) temperature-experiment (N = 206). Frequencies change significantly among the treatments in both experiments (Poisson log-linear model: $P < 0.001$). Non-reproducing individuals are marked with 'Non'.

Size-dependence of reproduction – In general, larger plants reproduced more than smaller plants. In both experiments, mean above-ground biomass was largest in plants

reproducing simultaneously with flowers and stolons. Biomass of plants reproducing only clonally or not at all was significantly lower compared to biomass of sexual plants (paired t-Tests: $P < 0.001$ in both experiments, Table 4). Biomass did not differ between plants reproducing only sexually or with both reproductive modes ($P > 0.1$ for both experiments).

In the competition-experiment, the total number of reproductive meristems increased with increasing above-ground plant weight ($r_s = 0.66$, $P = 0.007$; $r_s = 0.51$, $P = 0.04$ and $r_s = 0.81$, $P = 0.02$ for total reproductive meristems, stolons, and flowers, respectively; N = 32) or increasing number of leaves. The percentage of stolons decreased with increasing above-ground biomass and increasing leaf numbers ($r_s = -0.49$, $P = 0.05$ and $r_s = -0.55$, $P = 0.03$, respectively; N = 32).

In the temperature-experiment, plants with more leaves produced more reproductive meristems and more stolons, whereas the number of flowers was not affected ($r_s = 0.60$, $P = 0.01$; $r_s = 0.54$, $P = 0.02$ and $r_s = 0.23$, $P = 0.35$, respectively; N = 36). The number of reproductive meristems did not correlate with above-ground biomass ($P > 0.05$ for all terms; N = 36) and the percentage of stolons did not change in relation to plant size ($r_s = -0.31$, $P = 0.20$ and $r_s = 0.07$, $P = 0.75$ for above-ground biomass and numbers of leaves, respectively; N = 36).

Tab. 4: Mean above-ground biomass (g; ± SE) of *Geum reptans* individuals with only stolons, only flowers, both reproductive modes, or no reproduction at all in a competition- and a temperature-experiment (different superscript indicate significant differences among means).

	Mean above-ground biomass (g) of individuals with:			
	Stolons	Flowers	Stolons and flowers	No reproduction
Competition-experiment	1.13 ± 0.07^b	1.53 ± 0.13^a	1.57 ± 0.07^a	0.63 ± 0.14^c
Temperature-experiment	1.47 ± 0.05^b	1.63 ± 0.14^a	1.72 ± 0.04^a	1.34 ± 0.13^b

Trade-off among stolons and flowers – In both experiments, the number of stolons decreased with increasing number of flowers, indicating the expected trade-off between reproductive modes (R = -0.18, $P = 0.02$ and R = -0.16, $P = 0.02$ with all reproductive individuals in the competition- (N = 153) and the temperature- (N = 194) experiment, respectively).

DISCUSSION

In both greenhouse experiments, we found significant differences in growth and reproduction among populations of *Geum reptans* and some evidence for adaptive population differentiation in contrasting habitats. If plants grew under environmental stress (competition, warmer temperature), plant size and reproduction was reduced and fewer plants reproduced sexually or simultaneously with both reproductive modes. In general, larger plants reproduced more, but the minimum plant size for clonal reproduction was definitively smaller than for sexual reproduction. This shift in reproduction with plant size was mostly responsible for the different reproductive behaviour of plants in different experimental environments. The observed genetic differences among populations was only in few cases explained by contrasting habitats and is probably mainly a result of drift, as populations of *G. reptans* are spatially isolated and gene flow is limited (Pluess & Stöcklin, in press).

Plant size and reproductive investment – Reproductive behaviour of *G. reptans* was highly dependent on plant size, with more offsprings in larger plants, similar to many other studies, e.g. *Viola sororia* (Solbrig 1981), *Scabiosa columbaria* (Van Treuren *et al.* 1993), *Epilobium dodonaei* and *E. fleischeri* (Stöcklin & Favre 1994), *Pennisetum setaceum* (Williams *et al.* 1995), and *Asarum canadense* (Cain & Damman 1997), however not in *Ranunculus acris* (Hemborg & Karlsson 1998). The threshold size in *G. reptans* for clonal reproduction was significantly lower than for sexual reproduction (Table 4), as predicted by life history theory and observed in several species (e.g. Schmid et al. 1995). The preference of smaller plants to reproduce clonally may have a physiological and an evolutionary reason. Clonal propagation in plants is to a high degree self-sustainable because of the photosynthesis of the stolon itself, the leaflets at stolon nodes, and the leaves of the new rosette at the end of a stolon. Seed production is usually more costly (Harper 1977; Watson 1984). Secondly, from an evolutionary point of view, it may be favourable to invest first in clonal persistence and to postpone the more uncertain production of seeds. Such a strategy is particularly suited in harsh alpine habitats where the establishment of seeds is more risky (Stöcklin & Bäumler 1996). As a consequence of a smaller threshold size for clonal reproduction, the relative allocation to sexual reproduction increases in larger plants (Weiner 1988; Schmid & Weiner 1993). In the competition-experiment, the percentage of flowers was positively related to plant size. In the temperature-experiment, size variation was lower and the percentage of flowers was

independent of plant size. Differences in size may largely explain treatment effects on reproduction in the competition-experiment but not in the temperature-experiment. As all plants within each experiment were of the same age, size variation was smaller than commonly observed in field populations. Nevertheless, large differences in reproductive behaviour, similar size effects, and an increase of flower production in larger plants were found in 20 field populations of *G. reptans* (T. Weppler & J. Stöcklin, unpublished data). Thus, effects of age and environmental conditions on plant size may explain in part the different reproductive behaviour of field populations.

Effects of environment – Competition reduced plant size and reproduction drastically, indicating low competition ability of *G. reptans*. In the competition treatment, more plants reproduced by stolons only, probably as a consequence of the general lower plant size rather than as a direct consequence of competition (see above), whereas in the treatment without competition more plants reproduced with both reproductive modes. Interestingly, this shift in reproductive behaviour of individuals did not change the population mean of percent stolon production, because in the treatment without competition many larger plants increased not only flower but also stolon production. We conclude, that large individual size variability in stolon and flower production may largely buffer the effects of contrasting habitats. Similarly, in a greenhouse experiment with *Uvularia perfoliata* the effect of ramet size on reproduction was stronger than the light-treatment (Wijesinghe & Whigham 1997).

The temperature treatment had marginally significant effects on plant size, but particularly the number of reproductive meristems, notably of stolons was reduced with warmer temperature. Alpine plants may not be well adapted to warmer temperature. Plants from high altitudes grown at lowland-temperature frequently have a higher respiration than lowland plants due to insufficient acclimation and may therefore have a reduced growth and reproduction, or they even die (Körner 1999). Metabolic limitations may explain the reduced plant performance at warmer temperature in our experiment. Stolon production was more negatively affected than flower production (Tables 2B and 3B), indicating that the fate of reproductive meristems may be influenced directly by environmental conditions. A shift towards a higher stolon production with lower temperature is an advantage for a plant like *G. reptans*, because at higher altitude and lower temperature population persistence from clonal reproduction is more important (Abrahamson 1980; Bostock 1980). Our results also suggest,

that unfavourable temperature regimes at low altitude may be in part responsible for the lower distributional limits of *G. reptans*.

Effects of origin – Significant differences in size and reproduction among populations in both experiments indicate genetic differentiation among populations, as experimental conditions were common for all plants. There were some effects from contrasting habitats, but they were not very strong (see below). Population differentiation is therefore mainly random in nature and based on drift. That the differences result from adaptations to unknown habitat conditions cannot be fully excluded, but is unlikely, since unknown differences among habitats are supposed to be much smaller than the tested differences between contrasting successional and altitudinal habitats.

In addition to unspecific population differentiation, we found indications for a differentiation at least in the competition-experiment (Table 3) giving some support to the hypothesis of adaptations in *G. reptans* to contrasting environments. Particularly, early successional plants produced more flowers in the absence of competition compared to plants from late succession (Fig. 1) and at the same time, plants (irrespective of their smaller size) from late successional populations had twice as much flowers than plants from early succession when they competed with *Poa* (P = 0.07, Tables 2A and 3A), suggesting a specialisation to contrasting successional habitats. With respect to stolons, plants from late succession invested more in stolons without, and plants from early succession more in stolons with competition ($P < 0.1$, Table 2A and 3A). These interactions were only marginally significant, but since we had to use, for statistical reasons, population means instead of individual data points, statistical power was low and we consider these results as an indication for the described effects. Results suggest, that plants of *G. reptans* from late successional populations are better adapted to produce flowers in a competitive environment than plants from early successional populations, probably because they better compete for scarce nutrients. In the temperature-experiment, the tendential increase in the number of leaves in populations from high altitude (P = 0.06, Table 3B) and similar above-ground biomass in low and high altitudinal habitats may indicate smaller leaf size at high altitude. Smaller leaves may be an adaptation to the colder and harsher climatic conditions at high altitude.

As we found in both experiments a trade-off between clonal and sexual reproduction, opposing selection in contrasting habitats on reproductive behaviour in *G. reptans* is at least

possible, as predicted from general metapopulation models that included evolutionary constraints via a trade-off between life-history traits (Olivieri et al. 1995). A trade-off between the two reproductive modes in contrasting habitats was found in *Rumex acetosella* (Houssard & Escarre 1995) and in *Sparganium erectum* (Piquot *et al.* 1998). These species allocated more resources to sexual reproduction in young populations, while in *Ranunculus repens* sexual reproduction increased with density (Van Kleunen *et al*. 2001). In *G. reptans*, however, the percentage of stolons only changed among populations but not between contrasting habitats, indicating no particular selection pressure on reproductive modes constraint by a trade-off.

Conclusion – Despite large differences among alpine habitats, our results indicate that the selective forces shaping growth and reproduction of *Geum reptans* in contrasting habitats are probably not strong enough for pronounced adaptive population differentiation. There are indications that plants from late successional populations are better adapted to competition, but they were weak. Results suggest that population genetic differences in *G. reptans* are mostly an effect of drift as already suggested from results of a molecular study (Pluess and Stöcklin, in press). Size dependent plasticity in reproductive behaviour may explain why differences in the relative contribution of sexual *vs*. clonal reproduction in contrasting habitats were not stronger. Environmental effects on reproductive behaviour were pronounced, related to plant size, and may shift reproduction of plants towards stolon production under stressful conditions (competition, warmer temperature).

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Chapter 4

Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species

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Submitted

ABSTRACT

Seed weight is a crucial plant life history trait, determining establishment success and dispersal ability. Especially in stressful environments larger seeds may be selected at the expense of seed number, because larger seeds have a better chance of giving rise to an established offspring. We tested the hypotheses that between related species-pairs and among populations of a single species, a similar trend for increasing seed weight with altitude should be present. Firstly, we measured seed weights from 29 closely-related species-pairs, with one species occurring in lowland areas and a corresponding species from high altitudes. Seeds of the alpine species were 28 ± 8 % larger than seeds from lowland species ($P < 0.01$). Compared to the related lowland species 55 % of the alpine species had heavier seeds, 3 % (one species) had lighter, and 41 % had seeds of approximately equal weight. Secondly, we compared seed weights among populations of four species from different habitats and with different life histories. Seeds from between 11 and 34 populations per species were sampled along altitudinal gradients of 800 – 1500 m (c 800 m in *Scabiosa lucida*, c 1000 m in *Saxifraga oppositifolia*, c 1000 m in *Epilobium fleischeri*, and c 1500 m in *Carex flacca*). We found no indication for heavier seeds at higher altitude within the four species, but considerable variation in seed weight within and among populations. From the significant heavier seeds in alpine species compared to lowland species we conclude that selection tends to favour larger seeds at high altitudes, but it is not unanimously operating. Results suggest that seed weight only rarely changes as a single trait, but rather evolves as an element of a correlated set of characters involving taxonomic differentiation at the species or at least infra-specific level.

Keywords: Seed size, *Carex flacca*, *Epilobium fleischeri*, *Saxifraga oppositifolia*, *Scabiosa lucida*

INTRODUCTION

Seed weight is a critical character of a plant's life history. Particularly in adverse conditions or high competition pressure, larger seeds may have a higher establishment success, as they provide more reserves for seedlings. But smaller seeds are commonly better dispersed over large distances. Resources are limited and a plant may allocate them into fewer, larger seeds or into many, smaller ones (Harper et al. 1970; Smith and Fretwell 1974). Under stressful environmental conditions, selection for larger seeds is expected. Seed numbers are related directly to fitness and a selection pressure for more but smaller seeds should operate similarly for all species. Thus, seed size is expected to vary mainly in response to differences in selection pressure towards larger seeds (Westoby et al. 1992).

Here we ask if seed weight is changing along altitudinal gradients in the Alps, as favourable conditions for seed recruitment decrease continuously from low altitude to the top of mountains (Jolls and Bock 1983) where short and cold summers, long snow cover, and soil instability constrain seedling establishment (Bliss 1971; Urbanska and Schütz 1986; Rusterholz et al. 1993; Chambers 1995a, 1995b; Stöcklin and Bäumler 1996). The compromise between seed size and number may be resolved differently at different elevation. Indeed, variation in seed size among populations living in different environmental conditions appear to be common (Winn 1988).

However, seed weight within species was considered for a long time to be relatively constant (Harper 1977), and selection was predicted to produce one single optimal seed weight within mother plants (Smith and Fretwell 1974). More recently, many studies have emphasised that seed size does vary within species and heritability of seed size in populations of wild plants was shown to be low (e.g. Schaal 1980; Wolfe 1995). Nevertheless, differences in seed sizes within genera or even families are frequently small (e.g. Hodgson and Mackey 1986; Mazer 1989, 1990; Lord et al. 1995). This is explained by phylogenetic constraints or niche conservatism (Lord et al. 1995). Adaptive changes may be restricted by a species' evolutionary history, i.e., complex pattern of covariation among functionally related traits (Pigliucci 2003).

In the most detailed across-species study of variation in seed weight with environmental conditions, seed weight of herbaceous species of the Californian flora decreased with altitude as well as with decreasing soil moisture (Baker 1972). As differences in altitude and moisture availability are confounded in the Mediterranean climate of California

the conclusions were questioned (Körner 2003). Phylogenetic relationships among species are important but were not considered by Baker (1972), whereas Landolt (1967) compared seed weight in pairs of congeneric lowland and alpine species. Quantitatively measured seed size tended to increase rather than decrease with altitude (Landolt 1967). A number of studies, dealing with single species and a focus on several aspects of plant life history, reported on variation in seed weight with altitude, but a consistent picture is not apparent. Most of these studies observed an increase (Mariko et al. 1993; Oyama 1993; Holm 1994; Lord 1994; Piano et al. 1996; Ayana and Bekele 2000; Boulli et al. 2001; Blionis and Vokou 2002), some others a decrease (Totland and Birks 1996) or no alteration (Holm 1994; Kaya and Temerit 1994; Gera et al. 2000) in seed weight with altitude.

We combined two approaches to assess if there are differences in seed weight with altitude. We hypothesized that between related species and among populations within species a similar trend for increasing seed weight with altitude should be observed. Firstly, we selected 29 closely-related species-pairs from the Alps, with one species occurring at low altitude and a corresponding species from high elevations. We quantified mean individual seed weight using seed samples from the seed collection of the Botanical Institute at the University of Basel, Switzerland. We considered each species-pair in this across-species comparison as a phylogenetically independent replicate of seed weight and altitude. Secondly, we selected four species occurring over a large altitudinal gradient, with large differences in seed weight and seed dispersal capacities (*Carex flacca* Schreber, *Epilobium fleischeri* Hochstetter, *Saxifraga oppositifolia* L., *Scabiosa lucida* Vill.). Seeds were collected in populations along an altitudinal gradient. The relationship between altitude and seed weight was tested for each of the four species separately using regression analysis.

MATERIALS AND METHODS

Comparison of seed weight between related species from low and high altitude – We selected randomly 29 species-pairs (Table 1) from a list of 53 lowland and 38 closely-related alpine plants published by Landolt (1967). The list was initially compiled for a comparison of morphological characteristics between lowland and alpine species-pairs of open habitats in Switzerland. Species of each pair grow on similar soils with similar moisture conditions according to the moisture indicator values (Lauber and Wagner 2001). Therefore, differences

of elevation are primarily due to climatic changes, e.g. decreasing temperature and vegetation period with increasing altitude. The lowland species occur mainly in the colline region, and the related alpine species occur mainly in the subalpine/alpine region. The two species within a pair are not only from the same genus, but are sister species and may frequently hybridise (Landolt 1967). The list of pairs in our study includes species from 14 families and 27 genera: 25 genera were represented by one species-pair, and only two genera are represented by two species-pairs (*Helianthemum* and *Plantago*). The nomenclature for plant species follows Hess et al. (1976).

For all 58 species we measured mean individual seed weight using seed samples from the seed collection of the Botanical Institute, University of Basel, Switzerland. A seed sample in this collection includes seeds from plants of a single local population. For lowland species between 2 and 10 seed samples from different locations were available. For most alpine species seeds from between one and six populations were available, and for two species more population samples were available (*Epilobium fleischeri* and *Scabiosa lucida*). From each seed sample batches of at least 20 or, if available, 50 air-dried seeds were weighed, and mean weight per seed of a species was calculated using all available seed samples of this species. Values from the literature (Salisbury 1942; Lhotska and Chrtkova 1978; Grime et al. 1981; Müller-Schneider 1983; Bakker et al. 1997; Cerletti 1997; Akinola et al.1998; Milberg et al. 2000; VanAssche et al. 2002) complemented our measurements of 14 species (see Table 1). The age of the seed samples from the collection of the Botanical Institute (Basel, Switzerland) had no significant influence on seed weight (regression analysis with 47 seed samples from 11 randomly chosen species).

To answer the main question about difference in seed weights between lowland and alpine species, differences of the logarithmic seed weights were calculated of each species-pairs. With a t-test among all species-pairs it was tested, if these relative differences were significantly different from zero and thereby if alpine seeds were significantly heavier or lighter than lowland seeds. Tests for seed weight differences within each species-pairs were not possible, because in 21 of the 58 species seeds of only 1 or 2 populations were available. We used following approach to describe how many alpine species have heavier, equal, or lighter seed weights compared to the congeneric lowland species. We designated the seed weight of the lowland species as 100 %. The seed weight of the alpine species was calculated as a percentage of the seed weight of the corresponding lowland species. Seed weights of the

alpine species were considered to be different from the corresponding lowland species, when they exceeded a threshold value. A threshold value was used because of the large among-population variation in seed weight within many species. Based on the mean coefficient of variation (CV) of 19.9 ± 2.0 %, which was calculated from 24 randomly chosen species with seed samples from at least three populations and which was corrected for different sample numbers using the procedure described in Sokal and Rohlf (1995), we set the threshold value to ± 25 %.

Table 1: Mean weight per seed of 29 lowland and 29 closely-related alpine species, as well as the %-increase or decrease of seed weight of the alpine compared to the lowland species. Main data sources are seed samples from the seed collection of the Botanical Institute, University of Basel, Switzerland.

Lowland species		**Alpine species**		
	mean weight per seed (mg)[a]		mean weight per seed (mg)	% difference of alpine vs lowland species[b]
Achillea collina Becker	0.16	*A. stricta* Schleicher	0.22	+ 38 ‡
Anthoxanthum odoratum L.	0.60 [3]	*A. alpinum* Löve et Löve	0.51	- 15
Anthyllis macrocephala Wenderoth	2.71	*A. alpestris* (Kit.) Rchb./Hegetschw.	5.33	+ 97 ‡
Arenaria leptoclados(Rchb.) Guss.	0.04	*A. marschlinsii* Koch	0.08	+ 90 ‡
Artemisia campestris L.	0.12	*A. borealis* Pall.	0.22	+ 76 ‡
Campanula rotundifolia L.	0.06 [3,4,5]	*C. scheuchzeri* Vill.	0.09	+ 43 ‡
Centaurea scabiosa L.	7.49 [3,4]	*C. alpestris* Hegetschw.	8.82	+ 18
Cerastium caespitosum Gilib.	0.09	*C. fontanum* Baumg.	0.13	+ 48 ‡
Chrysanthemum leucanthemum L.	0.42	*C. montanum* All.	0.62	+ 49 ‡
*Epilobium dodonae*i Vill.	0.30 [3]	*E. fleischeri* Hochst.	0.17	- 42 ‡
Erigeron acer L.	0.13 [3]	*E. angulosus* Gaud.	0.11	- 12
Galium pumilum Murray	0.50	*G. anisophyllum* Vill.	0.48	- 4
Helianthemum canum (L.) Baumg.	0.39 [3]	*H. alpestre* (Jacq.) DC.	0.60	+ 54 ‡
Helianthemum nummularium (L.) Miller	0.94 [5]	*H. grandiflorum* (Scop.) Lam.	1.59	+ 70 ‡
Herniaria incana Lam.	0.14	*H. alpina* Vill.	0.21	+ 47 ‡
Juniperus communis L.	12.29	*J. nana* Willd.	10.54	- 14
Lotus corniculatus L.	1.42 [1,2,3,4,5,7]	*L. alpinus* (DC.) Schleicher	1.98	+ 39 ‡
Onobrychis arenaria (Kit.) DC.	7.53	*O. montana* DC.	11.72	+ 56 ‡
Phleum boehmeri Wib.	0.12	*P. hirsutum* Honck.	0.30	+ 150 ‡
Plantago lanceolata L.	1.96 [3,4,5,6,7]	*P. atrata* Hoppe	1.52	- 22
Plantago serpentina All.	1.00	*P. alpina* L.	0.84	- 16
Rumex acetosa L.	0.97 [7,8,9]	*R. arifolius* All.	1.42	+ 46 ‡
Sagina subulata (Schwarz) Presl	0.02	*S. linnaei* Presl	0.02	+ 40 ‡
Satureja acinos L. Scheele	0.28	*S. alpina* (L.) Scheele	0.26	- 7
Scabiosa columbaria L.	1.73 [5]	*S. lucida* Vill.	1.53	- 12
Scrophularia canina L.	0.34	*S. juratensis* Schleicher	0.43	+ 28 ‡
Silene vulagris (Moench) Garcke	0.85 [3,4]	*S. willdenowii* Sweet	0.98	+ 16
Trifoium pratense L.	1.54 [6]	*T. nivale* Sieber	1.33	- 13
Veronica serpyllifolia L.	0.08 [4]	*V. tenella* All.	0.06	- 23

[a]Numbers refer to included data from the literature: [1]Salisbury 1942; [2]Lhotska and Chrtkova 1978; [3]Grime et al. 1981; [4]Müller-Schneider 1983; [5]Bakker et al. 1997; [6]Cerletti 1997; [7]Akinola et al. 1998; [8]Milberg et al. 2000; [9]VanAssche 2002

[b]Mean weight per seed among species-pair is considered different (indicated by ‡) based on a threshold value of ± 25 % (see Materials and methods for explanation).

Altitudinal variation in seed weight among populations of four species – To assess the variation in seed weight among populations, we selected four species with different seed sizes, different dispersal capacities, and occurrence in different alpine habitats, but with a similar distribution over a large altitudinal gradient. *Carex flacca* and *Saxifraga oppositifolia*

have seeds without particular structures to assist seed dispersal, while seeds of *Epilobium fleischeri* and *Scabiosa lucida* are adapted for wind dispersal. *E. fleischeri* and *S. oppositifolia* are species from alpine screes and moraines, while *C. flacca* and *S. lucida* occur preferentially in grasslands. The altitudinal gradient covered c. 800 m in *S. lucida*, 1000 m in *S. oppositifolia*, 1000 m in *E. fleischeri*, and 1500 m in *C. flacca* (Table 2). In late summer 2000 and 2001, as well as in 1996 for *S. oppositifolia,* we collected seeds in 34 populations of *E. fleischeri*, in 14 populations of *S. oppositifolia*, and in 11 populations of *S. lucida*. In each population we sampled seeds of 15 to 25 plants separately and weighed at least 10 or, if available, 20 air-dried seeds per plant. Mean seed sizes of single plants were averaged per population. In *C. flacca* we used bulk samples with seeds from different individuals or ramets from 32 populations collected either in late summer 2000 in the Alps or taken from samples in the seed collection of the Botanical Institute at the University of Basel, Switzerland. Samples from the seed collection included mostly populations from low altitudes. In *C. flacca*, population means were calculated from 50 air-dried seeds per population. For all four species we calculated a coefficient of variation (CV) for individual seed weights based on population means, and for each population (except in the case of *C. flacca*, where we have no means for individual plants due to the marked clonal growth of this species) a coefficient of variation was calculated based on means for individual plants. All CVs were corrected for different sample numbers using the procedure described in Sokal and Rohlf (1995).

By regression analyses the relationship between altitude and population means of seed weight were tested for each of the four species. All analyses were calculated with JMP (Version 3.1; SAS Institute, Cary, N.C., USA, 1995).

RESULTS

Comparison of seed weight between related species from low and high altitude – Seeds of alpine species were 28 ± 8 % larger than seeds of the related lowland species ($df = 28$, $t = 2.76$, $P < 0.01$). The 29 species-pairs included 16 pairs (55 %) with heavier seeds, 1 pair (3 %) with lighter, and 12 pairs (41 %) with equal seed weights for alpine species (Table 1). The significant difference among lowland and alpine species remained ($df = 27$, $t = 2.48$, $P < 0.05$), when the Phleum species-pairs, where the seeds of the alpine species were 2.5 x heavier than the seeds of the lowland species, was excluded. If only those species-pairs with

the same moisture indicator values (Lauber and Wagner 2001) were tested, the relationship of seed weight remained the same (df = 19, t = 2.31, $P < 0.05$).

Altitudinal variation in seed weight among populations of four species – There was no general pattern of seed weight variation with altitude among the populations of the four species. In *Carex flacca* and *Epilobium fleischeri* we found no correlation of seed weight with altitude (r = 0.13, $P = 0.6$, Fig. 1; r = 0.004, $P = 0.98$; Fig. 2; respectively), whereas seed weight decreased significantly with altitude in *Saxifraga oppositifolia* and *Scabiosa lucida* (r = -0.77, $P < 0.001$, Fig. 3; r = -0.61, $P < 0.05$, Fig. 4; respectively).

Table 2: Number and altitudinal range of sampled populations, mean individual seed weight (x ± SD) and coefficient of variation (CV) among and within populations in four species from the Swiss Alps.

Species	No. of populations	altitudinal range (m a.s.l.)	weight per seed (mg) (x ± SD)	CV among populations	CV within populations
Carex flacca Schreber	32	430 – 2100	0.77 ± 0.13	17%	–
Epilobium fleischeri Hochstette	34	1500 – 2500	0.17 ± 0.02	12%	11 – 31 %
Saxifraga oppositifolia L.	14	2040 – 3020	0.07 ± 0.02	29%	20 – 36 %
Scabiosa lucida Vill.	11	1650 – 2400	1.53 ± 0.28	19%	24 – 51 %

Seed weight variation among population means was relatively high with coefficients of variation of 17 % in *C. flacca*, 12 % in *E. fleischeri*, 29 % in *S. oppositifolia*, and 19 % in *S. lucida* (Table 2). Variation among seed families within populations was between moderate and high with coefficients of seed weight variation between 11 and 51 % (Table 2). The coefficients of seed weight variation within populations did not correlate with the altitude of the populations in *E. fleischeri*, *S. oppositifolia* and *S. lucida* (data not shown).

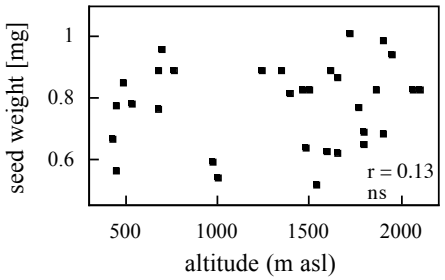

Fig. 1: Means for weight per seed of *Carex flacca* within 32 populations from different altitudes.

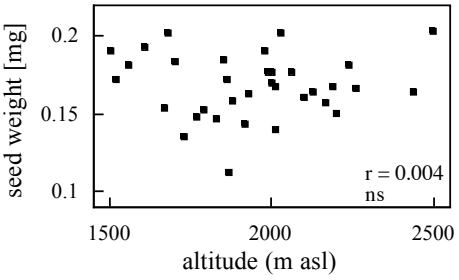

Fig. 2: Means for weight per seed of *Epilobium fleischeri* within 34 populations from different altitudes.

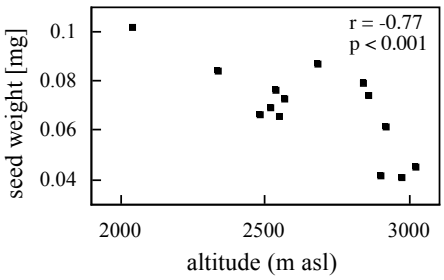

Fig. 3: Means for weight per seed of *Saxifraga oppositifolia* within 14 populations from different altitudes.

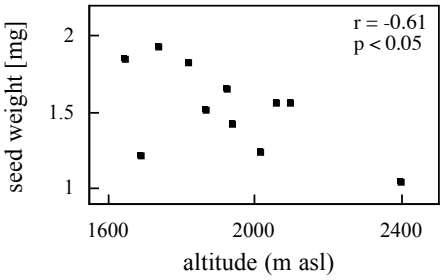

Fig. 4: Means for weight per seed of *Scabiosa lucida* within 11 populations from different altitudes.

DISCUSSION

Seed weights of related species – In the overall comparison of seed weight differences of lowland vs. alpine species, we observed significantly heavier seeds in alpine species. This result suggests that selection tends to favour the formation of larger seeds in species from higher altitudes, because environmental conditions for seedling establishment are more severe and larger seedlings are at an advantage. At any given stage during development, seedlings of larger-seeded species should have more reserves available to compensate for various environmental stresses (Leishman et al. 2000).

However, the formation of larger seeds in alpine plants is not general. A considerable number of species-pairs (41%) did not differ much in seed weight, and in one species-pair seed weight was lower at high altitude. The reason for this may be explained by functional, developmental, or genetic co-variation among traits imposing constraints that keep changes in size and shape of seeds within narrow limits inside a taxonomic lineage (Hodgson and Mackey 1986; Pigliucci 2003). Furthermore, the ancestors of a particular species-pair may have acquired a combination of traits enabling them to succeed in a particular habitat type (Lord et al. 1995). The descendants within the same lineage could have migrated to higher altitudes, following climatic warming, where they still occupy similar habitats and, thereby, may have preserved the characteristic trait combination.

From the 29 species-pairs, only the alpine *Epilobium fleischeri* produced markedly smaller seeds than the related lowland species (*E. dodonaei*). Stöcklin and Favre (1994)

studied the variation in reproductive components of these two species in detail and reported a similar number of seeds per stem. However, they found a lower fruit number per stem of the alpine plant, due to an increase in number of seeds per fruit and a reduction in seed weight. The combination of a lower seed weight with a higher seed number per fruit in *E. fleischeri* probably results from strong selection for better dispersal ability in this early successional plant from glacier foreland (Stöcklin and Favre 1994). In the island nature of the alpine landscape, the small and efficiently dispersed seeds of *E. fleischeri* have a better chance of colonising new sites, while a reduced probability of seed establishment is compensated for by clonal growth, an ability absent from the lowland *E. dodonaei*. The case of *Epilobium*, though an exception among the 29 species-pairs, illustrates that selection for high dispersal ability of seeds may counteract the suggested selection pressure for larger seeds in adverse conditions.

Intraspecific differences in seed weights – In the comparison of seed weights among populations of the four species studied, we found no indication of larger seed size at higher altitude, even though we selected species with different life history traits and from different habitats. There are several possible reasons for this result. Firstly, a change of the trait seed weight can affect several other traits and may thereby lead to speciation. Indeed, seed weight has been characterised as one element of a coevolving complex of characters (Venable and Brown 1988; Rees 1997) including dispersal, seed dormancy, plant mass, niche specialisation, and competition ability. Therefore, an increase in seed size at higher altitude is probably frequently related to changes of the taxonomic status of the plants involved. For instance Mariko et al. (1993) reported larger seeds at higher altitude in *Reynoutria japonica* on Mt. Fuji. They classified the involved populations into two distinct ecotypes, with the larger seeded ecotype growing at higher altitude. A similar observation was reported by Blionis and Vokou (2002) for *Campanula spatulata*. Elevation played a primary role in subspecies formation in this species; in the subspecies from higher altitude a marked divergence in morphological and phenological characters was observed in addition to larger seeds. Here, for the assessment of intraspecific differences in seed weight, we carefully avoided choosing species with a taxonomic differentiation at the subspecies level. This may not only explain partially the absence of an intraspecific increase in seed weight but also why the altitudinal gradient covered by the four species selected for intraspecific differences in seed weight does not exceed 1000 m, or in the case of *Carex flacca* 1500 m.

Secondly, plants stand still but populations are connected by gene flow via pollen and seeds. Gene flow by pollen is restricted by pollinator movement. Gene flow by seeds is also considered to be low, particularly in alpine habitats, where natural "barriers" like mountain ridges and valleys separate populations. However, because of high turbulence and convective updrafts, seeds at high altitudes are far better dispersed by wind than in lowland habitats. As an example, in *E. fleischeri*, 3.6 % and 1.2 % of all seeds are dispersed over more than 100 and 1000 m respectively (O. Tackenberg and J. Stöcklin, unpublished). When gene flow between populations reaches a substantial degree, potential differences due to differences in selection pressure will be homogenised, and no pattern of seed size variation with altitude will evolve.

Thirdly, the lack of evidence for an increase of seed weight with altitude within species may be due to large phenotypic variation in seed weight. Randomness in the abiotic environment within a population (Wolfe 1995), in the course of a vegetation period (Winn 1991), and developmental constraints within individuals (Silvertown 1989) may create large variation in seed weights among and within populations. Winn (1991) discussed the possible reasons why individual plants frequently fail to produce a uniform seed size in detail for the case of *Prunella vulgaris*. Similar observations were reported for a number of other plants (Stöcklin and Favre 1994; Wolfe 1995; Eriksson 1999). In our study, phenotypic variation in seed weight of the four study-species is mostly much higher within populations than between populations (Table 2). The high within-individual variation in seed weight in particular is a weak basis for the operation of selection towards larger seeds at higher altitude.

Instead of an increase we found a decrease of seed weight with altitude in *S. oppositifolia* and *S. lucida*, which can most likely be explained by the climatic conditions at higher sites. Weather conditions, like temperature and season length as well as resource availability are suggested to be important in determining the reproductive output of single plants (Totland and Birks 1996). It is expected that plants at high altitudes may face limiting conditions for producing and filling up seeds. Therefore, in the absence of a selection towards higher seeds, a negative elevational trend in the weight of seeds collected at field sites is not surprising. Seeds produced under controlled conditions in the greenhouse would be necessary to test if environmental conditions had such an overriding impact that a positive elevational trend in seed size was masked.

Conclusion – Our result of the comparison of seed weight between closely-related species-pairs supports the hypotheses that selection tends to favour larger seeds in stressful environments at high altitudes. The trend for larger seeds with elevation is however not unanimously operating and was not observed in all species-pairs studied. We were not able to detect a similar trend among populations of the four species assessed for intra-specific variation in seed weight along altitudinal gradients. Our results suggest that seed weight probably only rarely changes as a single trait, but instead usually evolves as an element of a more complex and correlated set of characters involving differentiation at the species or at least at the infra-specific level and depending on the specific life history of a plant.

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Chapter 5

Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size

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ABSTRACT

Due to changes in land use, remnants of unfertilised, nutrient-poor calcareous grassland in the Swiss Jura are small in area and are highly fragmented. We selected 89 seed families from eleven populations of various sizes of *Scabiosa columbaria* for a study of molecular diversity, and used the same material in a greenhouse experiment to measure variation in fitness-related traits and the ability of populations to cope with competition. Using RAPD-PCR we detected 71 RAPD-phenotypes among 87 genotypes. Molecular diversity within populations was variable and relatively high, with an expected heterozygosity H_e ranging from 0.09 to 0.24. H_e, the Shannon index (SI) and the percentage of polymorphic bands were not correlated with population size, but the smallest populations had the lowest molecular diversity (H_e, SI). Population differentiation was moderate with 12 % of the molecular diversity among populations. Measures of fitness in the greenhouse differed among seed families ($p < 0.001$), but not among populations. Mean above-ground biomass was largely reduced when plants had to compete with *Bromus*. Mean fitness of populations decreased when molecular diversity (H_e) was low, but only when plants had to compete with *Bromus* ($p = 0.02$). Accordingly, the relative competition ability of *Scabiosa* plants decreased when molecular diversity (H_e) was low ($p = 0.01$). Our results suggest an increased risk of local extinction of *Scabiosa columbaria* in the Swiss Jura caused by a decreased viability and reduced phenotypic plasticity due to genetic erosion in small populations.

Key words: Competition ability, molecular variation, population differentiation, genetic erosion, conservation

INTRODUCTION

Over the last decades nutrient-poor calcareous grassland has declined drastically in the Swiss Jura due to changes in agricultural land use (see Zoller et al. 1986). Only 20 % of the area of nutrient-poor grassland (Mesobromion) mapped around 1950 by Zoller et al. (1986) was still intact 35 years later (Stöcklin et al. 2000). Today, many populations of species characteristic of nutrient-poor grasslands are small in numbers and are isolated from each other by large distances. Conservation efforts stopped the ongoing destruction of Mesobromion remnants, but small populations may still suffer from a decrease in genetic diversity due to inbreeding and reduced gene flow between populations (Lacy 1987; Frankham 1996). Their fitness and competition ability may be reduced (Fenster and Dudash 1994; Young et al. 1996), and their risk of local extinction may be increased. Fischer and Stöcklin (1997) and Stöcklin and Fischer (1999) found that local extinctions of small plant populations occurred frequently even in intact grassland remnants in the Swiss Jura.

While many studies of endangered plants have examined variation in molecular markers, a few have also considered the evidence for a decrease in fitness with population size. Fischer and Matthies (1998) observed a decreased fitness with smaller population size in the short-lived *Gentianella germanica*, and Kery et al. (2000) observed the same in *Primula veris* and *Gentiana lutea*. Several other studies have confirmed that small populations are frequently not only genetically less diverse but also less viable (Oostermeijer et al. 1994; Lammi et al. 1999; Buza et al. 2000; Luijten et al. 2000; Schmidt and Jensen 2000; Mavraganis and Eckert 2001). However, levels of genetic diversity and fitness are not always related (Podolsky 2001; Reed and Frankham 2001), and molecular data by itself is not sufficient to judge if populations are endangered through genetic erosion. Data on both genetic diversity and population viability in fitness-related traits are necessary to determine whether populations are threatened. Furthermore, it is important to know whether populations of an endangered species are still capable of responding to changing environmental conditions. Plastic adaptations allow individual organisms to maintain function and fitness across a range of diverse environments (Schlichting 1986; Sultan 2000). Small or genetically less diverse populations in particular may have a reduced ability to buffer the effects of poor environmental conditions or competition. For instance Kery et al. (2000) observed that plants from small populations of *Primula veris* were less able to respond plastically to an increase in nutrient availability than plants from larger populations. A similar reduction in the phenotypic

response ability to an increase of competition was observed in populations with low molecular diversity in *Ranunculus reptans* (Fischer et al. 2000). The ability to cope with competition may be particularly important for endangered species. In this study, we combined the evaluation of molecular diversity in populations of different sizes from grassland remnants with a greenhouse experiment using the same populations to measure fitness and the phenotypic response to competition as a measure of plasticity.

We selected *Scabiosa columbaria* for this study for two reasons. Firstly, this geographically widespread species is restricted, in the Swiss Jura, to nutrient-poor Mesobromion remnants, and populations vary in size from less than a hundred to several thousand individuals. Secondly, variation in molecular diversity and fitness-related traits in populations at the margins of the geographical range of this species have been studied in the Netherlands and in Sweden. In the Netherlands, genotypic and phenotypic variation correlated with population size, indicating that genetic erosion occurs in small populations (Bijlsma et al. 1991; Ouborg et al. 1991; Van Treuren et al. 1991; Van Treuren et al. 1993; Bijlsma et al. 1994; Van Treuren et al. 1994). In Sweden, a high phenotypic differentiation among populations was observed, but there was no correlation between genetic diversity and population size (Waldmann and Andersson 1998). Peripheral populations might be more prone to fitness losses due to genetic erosion than populations from central regions, because peripheral populations are more likely to occur in ecologically marginal habitats (Lesica and Allendorf 1995). In the Swiss Jura, *Scabiosa columbaria* is near the centre of its distribution and the results of the present study can be compared to the effects observed in the more marginal populations in the Netherlands.

We selected plants from eleven populations from grassland remnants, containing c. 90 up to c. 2000 flowering individuals, for a molecular study using RAPD-PCR. We used the same material for a greenhouse experiment, investigating differences in fitness-related traits and plasticity among these populations in response to competition.

We used the molecular approach to address two questions: (1) How large is the molecular diversity in *Scabiosa columbaria* from grassland remnants in the Swiss Jura, and how is this diversity partitioned within and among populations? (2) Is there a difference in molecular diversity due to population size? The greenhouse study was designed to address two additional questions: (3) Do populations differ in fitness-related traits, and if so can these differences be related to population size and to molecular diversity measured with RAPD-

PCR? (4) Are such effects dependent on environmental conditions, such as the presence or absence of *Bromus erectus* as a competitor?

MATERIALS AND METHODS

Study species, sites and seed material – *Scabiosa columbaria* L. (Dipsacaceae) is a perennial species with a maximum life span of ten years (Grime et al. 1988). Flowering rosettes have stems 20 to 80 cm in height, with one to a few flower heads (Hegi 1918). Each flower head produces up to 100 achaena (Grime et al. 1988). The flowers are mainly pollinated by Aphidae (Hymenoptera), Syrphidae (Diptera) and Rhopalocerae (Lepidoptera) (Knuth 1898). *S. columbaria* is predominantly an outcrossing species (Van Treuren et al. 1994).

The distribution of *S. columbaria* extends throughout Eurasia and NW Africa (Hegi 1918) on nutrient-poor soils (Lauber and Wagner 1996). In the Swiss Jura *S. columbaria* occurs in nutrient-poor grassland (Mesobromion) remnants. In Central Europe such extensively used grassland probably reached its greatest extension in the 19th century when arable fields of low productivity were replaced by meadows (Zoller 1954; Behre and Jacomet 1991; Pott 1995). After the Second World War the area of such grassland declined drastically due to changes in land use (e.g. abandonment, fertilisation), restricting species which had previously been widely distributed for several centuries to small and isolated habitats.

For this study seed material of a total of 89 individuals of *S. columbaria* from eleven populations of different sizes were collected in autumn 1997. In general, seeds of nine mother plants (seed families; Tab. 1) per population were randomly chosen with a spacing of several meters along a transect through the population. The mean number of flowering individuals during the years 1996 to 2000 was used as a measure of population size, with the exception of two populations for which data from only one year were available (Tab. 1). The selected populations were distributed over an area of 37 x 11 km.

RAPD-PCR – individual from each of the 89 seed families was chosen for the molecular analysis. Leaf material was sampled from plants raised in the greenhouse (see below) and was shock frozen i liquid nitrogen (- 98 °C). After freeze drying (Unicryo MC

Tab. 1: Population size, location, habitat area, land use, the number of studied seed families and the molecular diversity (H_e) in eleven populations of *Scabiosa columbaria* from the Swiss Jura from an area of 400 km^2. Population size was measured as the mean number of flowering individuals during the years 1996-2000.

Site	Population size	Longitude [m][1]	Latitude [m][1]	Altitude [m]	Habitat area [ha]	Land use in 1996	No. of studied seed families	Molecular diversity (H_e)
SLB	93	591375	251520	805	3.39	pasture	4	0.089
OLT	102	598800	251250	590	0.94	fallow	7	0.137
HEL	131	623240	244785	860	3.46	pasture	9	0.175
HAS	158*	596500	249100	405	0.8	pasture	9	0.158
LIW	248	599500	251200	670	2.99	pasture	9	0.237
CDM	500	586500	250550	755	2.93	pasture	9	0.239
NEW	544	608975	255825	520	7.9	pasture	8	0.195
RIT	603	604750	254075	440	8.4	pasture	9	0.183
LCO	901*	599180	246175	640	8.09	pasture	7	0.202
RDS	920	595800	250850	520	0.08	hay meadow	9	0.197
BEU	2003	605150	246125	625	5.18	pasture	9	0.165

[1] Longitude and latitude according to the Swiss topographical maps (Bundesamt für Landestopographie, Wabern, Switzerland).

* Number of flowering plants was only available for the year 1996.

4L, Uniequip GmbH, Martinsried, Germany) and grinding of the leaf material (Retsch MM2, Retsch GmbH and Co KG, Haan, Germany), DNA was extracted with a DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany). From sixty primers ten base pairs long, the ones with the highest C/G nucleotide contents (i.e. those with higher binding stability due to three hydrogen bonds compared to two in A/T nucleotides) were screened and the band patterns were checked for repeatability. The first six primers with reproducible, polymorphic banding patterns were selected for the screening of all 89 sampled plants (see Appendix 1 for primer sequences).

Amplifications were carried out in 25 µL volumes, containing 5 µl of template DNA (5 ng DNA/µl); 11.3 µl ddH_2O; 0.5 µl MgCl (25 mM); 0.5 µl dNTP's (5 mM); 2.5 µl TaqPolymerase Buffer (10x; amersham pharmacia biotech); 5 µl Primer (5 µM); and 0.2 µl Taq DNA Polymerase (5000 units/ml; amersham pharmacia biotech). Polymerase chain reactions (PCR) were performed in a thermal cycler (PTC-100, MJ Research, Inc., Watertown, Mass., USA) with denaturing of the DNA at 93 °C for 1 min followed by 34 cycles of: 30 s at 92 °C; 30 s at 37 °C; and 1.5 min at 72 °C. Following the 34 cycles, a final step of 5 min at

72 °C was carried out. Samples were kept at 4 °C until analysis. PCR products were separated on 1.6 % agarose gels (Sea Kem LE agarose) in 1x TAE (Tris/Acetat/EDTA) buffer in an electrical field (170 mV, c. 1.5 h). The banding pattern was made visible with ethidium bromide in an UV-light source. The presence or absence of bands was scored for 16 good visible bands with a length of between 500 and 2000 base pairs. Two samples were not analysed with all six primers due to low quantity of the DNA extraction. Therefore the final presence-absence matrix contained 87 individuals scored for 16 bands.

Statistical analysis of RAPD banding patterns were based on four assumptions: (i) RAPD fragments behave as diploid, dominant markers with alleles being either present (amplified) or absent (nonamplified); (ii) comigrating fragments represent homologous loci; (iii) polymorphic loci are inherited in a nuclear (Mendelian) fashion (Arafeh et al 2002); and (iv) populations are in Hardy-Weinberg equilibrium (HWE; $F_{is} = 0$). This last assumption is supported by results from Van Treuren et al. (1993, 1994). *S. columbaria* is highly susceptible to inbreeding and in their study with allozymes the inbreeding coefficient (F_{is}) in natural populations did not differ from zero. Assuming that the populations were in HWE, allele frequencies were estimated based on the square root of the frequency of the null (recessive) allele.

The molecular diversity within populations was quantified in three different ways: (i) Nei's expected heterozygosity (H_e), (ii) the percentage of polymorphic bands out of all polymorphic bands (%P) and (iii) the Shannon index (SI). The percentages of poylmorphic bands were corrected for unequal sample sizes by rarefaction (Heck et al. 1975). By rarefaction the mean percentage of polymporphic bands in a population was calculated using the percentage of polymorphic bands of all possible combinations of four individuals (the lowest n) out of all sampled individuals per population. For all calculations only the polymorphic markers were used. Moreover, we checked the whole data set for private polymorphism (unique to a given population). To quantify the variation of molecular diversity among populations we calculated the coefficient of variation (CV) for H_e, SI and %P. H_e and SI were calculated with POPGENE (Version 1.21; Yeh et al. 1997).

The molecular differentiation among populations was calculated by Nei's (1973) estimator G_{st}, the fixation index. G_{st} values are based on Wright's F- statistics and are identical to F_{st} values if a locus consists of two alleles as applicable in RAPD marker analysis (Nybom and Bartish 2000). The F-statistics was calculated across all bands by using POPGENE.

Genetic distances between each population pair were quantified with Nei's original pair-wise genetic distance (TFPGA Program, Version 1.3; Miller 1997) and the pair-wise genetic distance F_{st} (Arlequin, Version 1.1; Schneider et al. 1997). A Mantel test was used to test whether the matrix of Nei's original pair-wise genetic distance correlates with the matrix of pair-wise geographic distances (10.000 permuations; TFPGA Program).

Experimental design of the greenhouse study – In the greenhouse a total of 720 plants from 89 seed families (8 replicates per seed family) taken from eleven populations were exposed to two competition treatments (with and without competition with *Bromus erectus*). To eliminate the possible effects on the experiment of small pot size, we planted eight *S. columbaria* plants of different populations into rectangular pots of 16.7 x 26.6 x 21.5 cm (in the statistical analysis these pots were considered as subplots). In the treatment which included interspecific competition seeds of *Bromus erectus* were added. Thereby we were able to measure the response to interspecific competition while holding intraspecific competition constant in both treatments (Reynolds 1999).

Around 30 seeds per *S. columbaria* seed family were germinated separately in pot soil. After four weeks, eight seedlings per seed family (four in each competition treatment) were chosen randomly and planted into pots. The pots had a hole at the bottom of each wall and a drainage mat at the base, and were filled with 14.5 cm of marl, followed by a 5.5 cm layer of a 1:1 mixture of marl and natural soil from calcareous grassland and finally covered with 1.5 cm of the same natural soil. In the pots including interspecific competition c. 200 seeds of *Bromus erectus* were added. After germination the *Bromus* seedlings were reduced to 20 individuals per pot. The resulting 90 pots (45 per treatment) were randomly placed on 5 mobile greenhouse tables. Pots on the tables as well as the tables within the greenhouse were randomly repositioned every 4 weeks.

The experiment started on the 1st of June 1999, and the final harvest was seven months later, at the beginning of January 2000. Greenhouse conditions were similar during the whole experiment (14 hr day length, 1 kw-lamps were activated if outdoor light was lower than 180 μmol $m^{-2}s^{-1}$; day temperature was maintained at 25 °C, night temperature at 10 °C (using additional heating from November to January)). All plants were watered every two days and received the same amount of fertiliser (a full fertiliser, equivalent to 4.7 kg N ha^{-1} was applied in the first six weeks of the experiment in six equal portions).

Fitness measurements, competition ability and statistical analysis – After 210 days (Jan. 2001) the plants were harvested, subdivided into above- and below-ground material, and dried at 80 °C for 48 hours. Above-ground biomass was used as a fitness measure. In *S. columbaria*, as in other species, above-ground biomass correlates well with reproductive output (Van Treuren et al. 1993). To test for treatment effects and biomass differences among populations, and seed families within populations, a nested multi-factorial mixed model ANOVA type III was used.

We used the phenotypic response to competition as a measure of plasticity. The relative ability of a population to cope with competition was calculated as the proportional reduction in plant size in the treatment with *Bromus* compared with the plant size in the pure stand (Snaydon 1991). Following Snaydon (1991), the relative severity of competition was calculated using the population mean of within seed family reduction of biomass due to competition as the logarithm of the above-ground biomass per plant grown in a pure stand minus the logarithm of the above-ground biomass per plant grown in competition with *Bromus*. The measure for the relative severity of competition was subtracted from 1 to provide a measure of the ability to cope with competition: the lower the relative competition ability, the more reduced the plasticity of a population.

As a measure of fitness variation, means per population of within seed family coefficients of variation (CV) for above-ground biomass were calculated. The CV was corrected for different sampling size after Sokal and Rohlf (1995): $CV^* = [1+(1/4n)] \times [SDx100/mean]$.

Because of the low sample size we calculated non parametric Spearman's Rho (r_s) correlations. JMP (Version 3.1; 1995, SAS Institute, Cary, N.C., USA) was used for all analyses. Data sets were transformed if necessary to meet the requirements of ANOVA.

RESULTS

RAPD-phenotypes and polymorphism – The eleven populations of *Scabiosa columbaria* differed greatly in the composition of their RAPD-phenotypes. The 87 plants comprised 71 different RAPD-phenotypes. Seven RAPD-phenotypes were found in more than one population; only one phenotype was found twice in the same population. In each individual population, between 5 and 12 of the 16 reproducible RAPD-bands were

polymorphic, with a mean of 8.9 (SE = 0.61). No private bands (unique to one population) were found.

Molecular diversity within populations and population size – The molecular diversity of individual populations calculated from polymorphic RAPD-bands was relatively high: Nei's expected heterozygosity H_e ranged from 0.089 to 0.239 (CV = 23.4 %) with a mean of 0.18 (SE = 0.01). The Shannon index ranged from 0.14 to 0.36 (CV = 23.6 %) with a mean of 0.27 (SE = 0.02) and the percentage of polymorphic bands within populations after rarefaction ranged from 31.3 % to 59.0 % (CV = 17.6 %) with a mean of 44.0 % (SE = 2.3).

There was no significant relationship between the molecular diversity within population (H_e) and logarithmic population size (r_s = 0.49, p = 0.13), neither when the Shannon index (r_s = 0.46, p = 0.15) nor when the percentage of polymorphic bands (r_s = 0.41, p = 0.21) was used as a measure of molecular diversity. However, the two smallest populations showed the lowest molecular diversity (H_e = 0.14 and 0.09 in OLT and SLB, respectively; SI = 0.21 and 0.14 in OLT and SLB, respectively). Furthermore, OLT was the only population in which a RAPD-phenotype was repeated, and SLB had the lowest percentage of polymorphic bands.

Molecular differentiation among populations – The G_{st} value indicated a moderate genetic differentiation among populations. 12 % of the RAPD-band variation was among and 88 % within populations (G_{st} = 0.12 (SD = 0.07); H_t = 0.21 (SD = 0.03); H_s = 0.18 (SD = 0.02)).

Genetic and geographic distances – The 55 pair-wise genetic distances among the eleven populations were generally small. Nei's original pair-wise genetic distances calculated from RAPD-band frequencies varied between 0.01 and 0.09. Genetic distances from pair-wise combinations of populations were significant in nine cases (F_{st} statistics calculated with the program Arlequin, see methods). The pair-wise genetic distances among populations did not correlate with the corresponding geographic distances (r = -0.1, p = 0.65; Mantel-test).

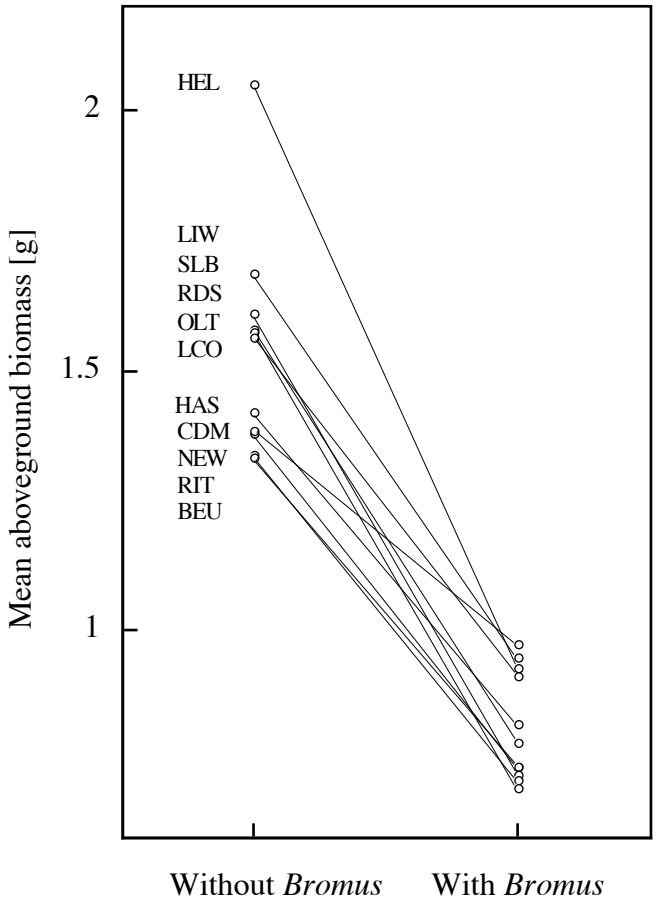

Fig. 1: Mean above-ground biomass of eleven populations of *Scabiosa columbaria* from a common garden experiment in two treatments (including *Bromus erectus* as a competitor or not). See Tab. 1 for the acronyms of populations.

Fitness measurements in the greenhouse – The competition treatment had a significant effect on the growth of *S. columbaria*. Mean above-ground biomass of individuals was reduced by 57 % (0.59 g ± 0.03 instead of 1.03 g ± 0.03 without *Bromus,* $p < 0.001$; Fig. 1). Root biomass was even more reduced (by 77 %, $p < 0.001$) when plants had to compete with *Bromus erectus*. Populations and seed families within populations did not differ in their response to competition ($p = 0.17$, $p = 0.18$ for the interaction of treatment with populations or seed families, respectively; Tab. 2). But mean above-ground biomass differed significantly among seed families within populations ($p < 0.001$, Tab. 2). Differences among seed families within populations explained 12.2 %, and random variation among individuals explained 85.3 % of the total variation in biomass.

Tab. 2: Nested, mixed-model ANOVA type III for above-ground biomass of seed families from eleven *Scabiosa columbaria* populations grown in the greenhouse for 210 days. Plants were grown with or without competition from *Bromus*.

	df	SS	MS	Variance component		p
				absolute	in %	
Competition	1	17.04	17.04			***
Competition * Population	10	1.11	0.11			0.17
Competition * Seed family	78	7.05	0.09			0.18
Plot	4	0.93	0.23	0.00148	1.62	*
Subplots within plot	80	5.72	0.07	0	0	0.67
among Populations	10	2.09	0.21	0.00080	**0.86**	0.25
Seed families within Populations	78	12.11	0.16	0.01114	**12.21**	***
Residuals	457	35.58	0.08	0.07787	**85.31**	
Total	718				100.00	

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Fitness, relative competition ability and population size – Between fitness within populations (i.e. above-ground biomass means for seed families within populations) and logarithmic population size we found no significant relationship ($r_s = -0.25$, $p = 0.47$); neither for the treatment without competition ($r_s = -0.56$, $p = 0.07$), nor when *Bromus* was added as a competitor ($r_s = 0.10$, $p = 0.77$).

The relative competition ability was also not correlated with logarithmic population size ($r_s = 0.38$, $p = 0.25$; Fig. 2). However, the three smallest populations showed the highest reduction in biomass indicating a larger loss of fitness due to competition with *Bromus*.

Fitness, relative competition ability and molecular diversity – Fitness within populations (i.e. above-ground biomass means for seed families within populations) increased with molecular diversity when *S. columbaria* had to compete with *Bromus* (H_e: $r_s = 0.69$, $p = 0.02$, Fig. 3 A; SI: $r_s = 0.67$, $p = 0.02$; %P: $r_s = 0.71$, $p = 0.01$). However, when plants were grown without competition with *Bromus*, fitness was not correlated with molecular diversity (H_e: $r_s = -0.05$, $p = 0.89$, Fig. 3 B; SI: $r_s = -0.08$, $p = 0.81$; %P: $r_s = -0.03$, $p = 0.94$).

The relative competition ability also increased with molecular diversity within populations (H_e: $r_s = 0.65$, $p = 0.03$, Fig. 4; SI: $r_s = 0.68$, $p = 0.02$; %P: $r_s = 0.61$, $p = 0.047$). Thus, competition had a larger negative effect on populations with a low molecular diversity, i.e. populations with a low molecular diversity were less able to resist to competition.

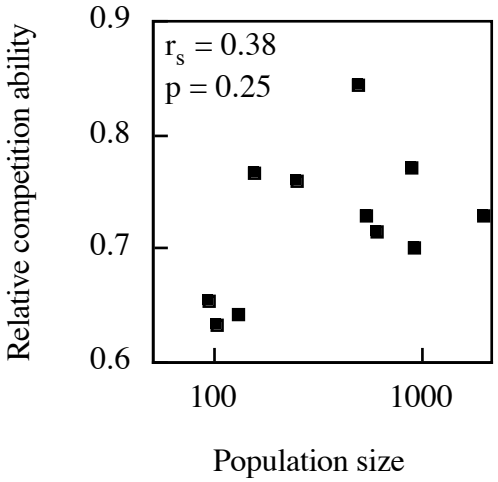

Fig. 2: Spearman's rank correlation of the relative competition ability (a measure of phenotypic plasticity) with log population size for eleven *Scabiosa columbaria* populations in a common environment (the relative competition ability is calculated as the proportional reduction in plant size of *S. columbaria* in a mixture with *Bromus* compared with the plant size in the pure stand, see methods for details).

Biomass variation and molecular diversity – Populations with low molecular diversity had higher mean coefficient of biomass variation (CV*) among seed families within populations (H_e: r_s = -0.86, p = 0.0006; SI: r_s = -0.84, p = 0.001; %P: r_s = -0.60, p = 0.05). The effect was also present, when the treatment including competition with *Bromus* was considered separately (H_e: r_s = -0.68, p = 0.02; SI: r_s = -0.67, p = 0.02; %P: r_s = -0.65,

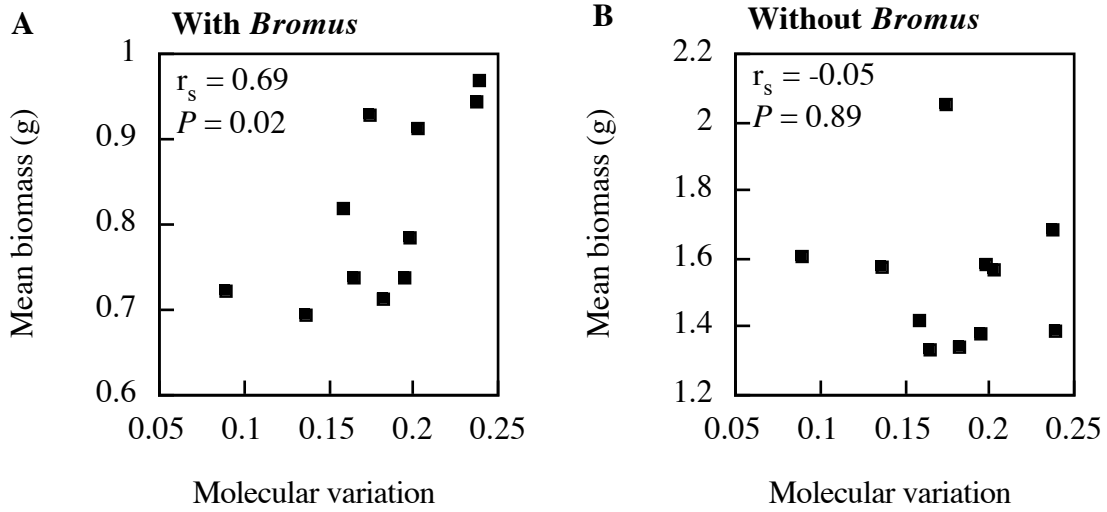

Fig. 3: Spearman's rank correlation of mean above-ground biomass of seed families in eleven populations of *Scabiosa columbaria* with the corresponding molecular diversity (H_e) from RAPD-PCR. Plants were grown in a common environment: (A) plants had to compete or (B) had not to compete with *Bromus*.

p = 0.03), but was not apparent in the treatment without competition with *Bromus* (H_e: r_s = -0.31, p = 0.34; SI: r_s = -0.23, p = 0.48; %P: r_s = -0.02, p = 0.96). This result indicates higher fitness variation in populations with lower molecular diversity, particularly in the treatment including interspecific competition.

DISCUSSION

With RAPD-PCR we found a relatively high molecular diversity in the eleven populations of *Scabiosa columbaria* from grassland remnants in the Swiss Jura, weak indications of a lower molecular diversity in small populations, and evidence for reduced competition ability in populations with decreased molecular diversity in the greenhouse experiment. In particular, we observed lower population viability with decreasing molecular diversity when plants had to compete with *Bromus*. The large differences in levels of molecular diversity observed among populations may explain why we found this correlation in spite of the small sample size per population in our study. Our results suggest that populations of *Scabiosa* with a low molecular diversity in the Swiss Jura are at risk of local extinction. However, there was only weak indirect evidence for a poorer plant performance in small populations, indicating that population size is not always the best indicator for population viability.

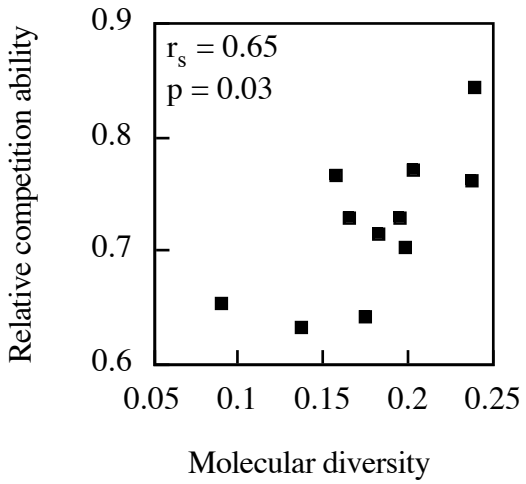

Fig. 4: Spearman's rank correlation of the relative competition ability (a measure of phenotypic plasticity) in a common environment of eleven *Scabiosa columbaria* populations with the corresponding molecular diversity (H_e) from RAPD-PCR (the relative competition ability is calculated as the proportional

reduction in plant size of *S. columbaria* in a mixture with *Bromus* compared with the plant size in the pure stand, see methods for details).

Genetic diversity and population differentiation – The amplification of randomly selected gene loci (RAPD-PCR-technique) is usually a more sensitive method to detect genetic variation in plant species compared to gene product level methods (e.g. isozymes), and is now well established as a sensitive method for detecting genetic structure among populations (Nybom and Bartish 2000). We found 71 different RAPD-phenotypes among the 87 *Scabiosa* plants. None of the sampled populations was monomorphic, and measures of molecular diversity (Nei's expected heterozygosity) revealed a variable and relatively high level of genetic diversity across the eleven populations. The within-population gene diversity ranged from 0.089 to 0.239 (0.18 (SD = 0.04)), comparable to the mean of 0.214 (SD = 0.117) reported recently for 41 RAPD-marker studies reviewed by Nybom and Bartish (2000). However, the proportion of among-population genetic diversity (G_{st}) was relatively low in our study (only 12 %). Similar values based on RAPD-markers have been reported by Vazquez et al. (1999) for *Sideritis pusilla* (11.3 %) and Papa et al. (1998) for *Hordeum vulgare* (11 %). In the endangered *Gentianella germanica* from the Swiss Jura, Fischer and Matthies (1998) observed a much higher coefficient for RAPD (37 %), and the mean value reported for 31 RAPD studies by Nybom and Bartish (2000) was 29 %. In their review, these authors demonstrated that with RAPD-data among-population diversity increases with the geographic distribution of sampled populations. The comparably low among-population diversity in *Scabiosa* may in part reflect our restricted study area. Our results indicate some genetic differentiation among the populations of *Scabiosa,* but this is probably not very pronounced.

A lack of a relationship between genetic and geographic distances was found in other studies as well, such as in Grünbauer et al. (1999). Gene flow among populations of characteristic species from grassland remnants is probably low because changes in agricultural land use strongly enhanced their isolation (Stöcklin et al. 2000). Seed dispersal over several kilometres is unlikely in *Scabiosa* because of the generally short dispersal distances of seeds (Cain et al. 2000). Gene flow due to pollen transfer might be more important. *Scabiosa columbaria* is visited by honeybees (Müller 1873) which forage in a diameter of up to 6 km around their beehive. Migrating butterfly species might occasionally visit inflorescences of *Scabiosa* (Müller 1873), but since butterflies are not effective pollinators (A.

Erhardt, personal communication) this probably does not matter for gene flow. The lack of any significant relationship between genetic and geographic distances, and the lack of a spatial pattern in our molecular data in general, suggests that the observed population differentiation might result from random genetic processes, and that migration between remnants is possibly too low to compensate for this.

Population size, molecular diversity and population viability – Genetic erosion is a likely outcome of small population size especially in species which were formerly more common. Genetic drift is insignificant in large populations but becomes important when population size crashes to a small number following dramatic range size reduction and fragmentation (Srikwan and Woodruff 2000). In small populations drift as well as inbreeding can lead to a loss of less frequent alleles and thereby the level of homozygosity may increase. As a consequence, genetic diversity tends to be reduced and the chance that harmful alleles are expressed increases (Ellstrand and Elam 1993; Young et al. 1996). We expected to find genetic effects of small population size in *Scabiosa columbaria*, because strong inbreeding depression in experimental progenies from small and large populations of *S. columbaria* in the Netherlands suggests that this species is highly susceptible to inbreeding (Van Treuren et al. 1993). Nevertheless, no clear relationship between population size and the level of inbreeding was observed in the Netherlands indicating that the plants studied had not yet suffered severely from inbreeding because otherwise genetic load, causing fitness reductions, would have substantially reduced inbreeding depression in smaller populations.

In our molecular study we found only weak indications for a lower genetic diversity in small populations of *Scabiosa*. The smallest populations showed the lowest molecular diversity (H_e and SI). The greenhouse experiment provided additional evidence for a genetic deterioration in the studied populations. Offspring from populations with low molecular diversity were phenotypically less plastic, making them less able to cope with interspecific competition with *Bromus erectus* (Fig. 3); this is because they had a lower viability in the treatment including *Bromus* (Fig. 4). We cannot completely exclude the possibility that the negative effects on offspring fitness could be caused by maternal carry-over effects (Oostermeijer et al. 1994). However, a reduced performance of offspring is cautiously considered as an indication of genetic effects (Fischer and Matthies 1998; Kery et al. 2000). In our study, plant performance correlated with genetic diversity and thus genetic erosion is at

least in part responsible for the effect. Reduced performance is often linked with smaller population size (Fischer and Matthies 1998; Fischer et al. 2000; Kery et al. 2000), but in our study there was only weak indirect evidence that decreasing plant performance was due to decreasing population size. This may either be due to the difficulty of accurately measuring population size in perennial species, or because population size is only one of several possible reasons for a low genetic diversity (Vergeer et al. 2003). When evidence of reduced genetic diversity in small populations had been demonstrated, differences in the phenotypic performance of plants were not always correlated with population size. For instance, in the Netherlands variations in fitness traits were not related to population size in populations of *Salvia pratensis* or of *Scabiosa columbaria* (Ouborg et al. 1991; Van Treuren et al. 1993; Ouborg and Van Treuren 1995). Interestingly, in our study, plants from populations with a low molecular diversity were not only more affected by competition but they were as well phenotypically more variable, i.e. biomass variation of individuals around the seed family-mean was larger. This indicates that seed families in populations with low molecular diversity were also developmentally less stable, what might be an explanation for their reduced competition ability.

It is noteworthy that in our study fitness of *S. columbaria* only correlated with molecular diversity when plants suffered from additional competition with *Bromus*. Firstly, this indicates that the effect of harmful alleles is more severe under stressful conditions, and illustrates how important it is to include environmental variation in any study of plant fitness. Experiments have so far only been done to test the effect of stress on the magnitude of inbreeding depression (Wright 1977; Charlesworth and Charlesworth 1987; Reed et al. 2002); nevertheless Cheptou et al. (2000) concluded from results of a recent study that the effect of inbreeding depression in natural populations is more severe when competition occurs. Secondly, the reduced ability to resist competition suggests that a lower genetic diversity is likely to reduce the tolerance to changes in environmental conditions (Huenneke 1991). Similarly, Kery et al. (2000) observed that plants from smaller populations of the grassland species *Primula veris* were less able to respond to an increase in nutrient availability. Since phenotypic plasticity itself has a genetic basis, such a decrease in plasticity can result from inbreeding (Schlichting 1986). In isolated grassland remnants a reduced plasticity will strongly affect fitness in the long term, since such remnants are frequently subjected to changes in land use or gradual changes in nutrient availability from eutrophication.

Conclusions for conservation – *Scabiosa columbaria* is still relatively common in the Swiss Jura, and populations of this species were found in 36 out of 58 investigated remnants of calcareous grasslands (Ryf 1997). Nevertheless, our study shows that populations of this species may be endangered due to decreased plant viability caused by a reduced genetic diversity. Most notably, the decrease in phenotypic plasticity observed in the greenhouse study is likely to have negative consequences in the field at least in the long term, because this effect is an indication that populations with lower molecular diversity have a reduced potential for adaptation to future changes in habitat conditions. It has been argued that demographic factors are more important than genetics for the short term fate of local populations (Lande 1988), but it is now accepted that in small populations genetic effects may interact with demographic variability to produce an "extinction vortex", if population size is substantially reduced by stochastic events (Gilpin and Soulé 1986). Such effects are not unlikely in *Scabiosa columbaria,* because this plant is a short-lived perennial with a maximum life span of only a few years. In grassland remnants, considerable fluctuations in the number of flowering individuals have been observed from year to year, with several populations constantly below a hundred individuals (J. Stöcklin, unpublished). If a population in an isolated habitat becomes locally extinct, re-colonisation is uncertain because *Scabiosa* is considered to be a bad coloniser (Grime et al. 1988).

The results of the present study are in line with what has been found in the Netherlands and do not indicate that populations from the centre of a species distribution area are less prone to genetic erosion than peripheral populations. In their study with allozymes Van Treuren et al. (1991) observed a significant correlation between the size of populations and the level of polymorphism in *S. columbaria*, and concluded that small populations were genetically less variable and more differentiated. Together with the observation that *S. columbaria* is highly susceptible to inbreeding (Van Treuren et al. 1993), the decreased level of genetic and phenotypic variation in smaller populations from the Netherlands was considered as a clear indication of a significant increase in the extinction probability of small populations (Bijlsma et al. 1994). Since different types of molecular markers have been used, direct comparisons of the observed levels of diversity between the study in the Netherlands and our results are difficult. Population differentiation is probably stronger in the Netherlands compared to what we found in the Swiss Jura, but this may be an effect of our small study area. However, both the study from the Netherlands and our own results suggest that in this

species populations may genetically suffer from small size and isolation and that this may have consequences for their long-term survival. Restoration efforts should therefore not only tend towards increasing local population sizes, but should also consider genetic diversity taking into account the possible interactions between genetic and non-genetic effects on population viability.

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APPENDIX

Appendix 1: Primer sequences used for RAPD-PCR and the number of well reproducible polymorphic bands per primer.

	Primer sequence	**No. of polymorphic bands**
FMI, Basel, Switzerland	CACCTTTCCC	1
FMI, Basel, Switzerland	CACAGGCGGA	4
FMI, Basel, Switzerland	GGGCCACTCA	2
FMI, Basel, Switzerland	CCCTACCGAC	3
Microsynth, Balgach, Switzerland	GTGACGTAGG	4
Microsynth, Balgach, Switzerland	ACCCATGCGG	2
Total		16

Chapter 6

General summary

In this thesis the main focus is on genetic variation, population differentiation and adaptation of a clonal plant species (*Geum reptans*) in the naturally fragmented alpine landscape (Chapter 2 and 3). In addition, I studied the hypotheses that mean seed weight among and within species is increasing with altitude (Chapter 4). Finally, this theses includes a molecular and greenhouse study with *Scabiosa columbaria*, a plant from recently fragmented remnants of calcareous grassland (Chapter 5). The data for the last study is from my Diploma thesis, but data analysis and writing was mainly done during my PhD.

Alpine plant life is characterised by habitats with steep environmental gradients, sharp boundaries, strong natural fragmentation and high disturbance frequencies. Therefore, most plant populations are spatially isolated. Alpine areas are naturally fragmented in contrast to recently fragmented habitats, where habitat destruction increased as a consequence of human-impacts over the last decades. It is now well established, that populations in recently fragmented habitats may suffer from erosion of genetic variation and show increased interpopulation divergence as a consequence of isolation and reduced gene flow (Young, Boyle & Brown 1996). If similar effects are present in naturally fragmented landscapes is not well known. Partitioning of genetic variation among populations depends strongly on the breeding systems and on reproductive mode. Pronounced clonal propagation may decrease genetic variation within and increase genetic differentiation among populations. In general, the proportion of plants reproducing clonally increases with altitude (Klimes *et al.* 1997). Selection pressure on life history traits may differ dramatically among contrasting alpine habitats. Local adaptation of plants to abiotic and biotic environmental conditions may thereby further increase genetic separation among populations. I studied the effect of the naturally fragmented landscape on genetic population differentiation in the clonal plant *Geum reptans* using RAPD-PCR and greenhouse experiments (Chapter 2 and 3). The effects of recent fragmentation on molecular diversity, plant growth, and competition ability of populations of *Scabiosa columbaria* from remnants of calcareous grasslands in the Swiss Jura was also studied with RAPD-PCR in parallel to an experiment in the greenhouse (Chapter 5).

A main question of this thesis was, if populations from contrasting habitats (low *vs.* high elevation and early *vs.* late succession) differ in growth and reproductive traits and show adaptations to these conditions. A trade-off among seed size and number and among clonal and sexual reproduction may be expected, as available resources are always limited (Harper

1977). Opposing selection pressure on reproductive mode in contrasting habitats is predicted by metapopulation models: individuals with traits favouring sexual reproduction and seed dispersal should colonise open space preferentially, whereas selection acts against genes promoting migration once a population has been established (Olivieri, Michalakis & Gouyon 1995). In this thesis population differentiation in growth and reproductive behaviour, and adaptation to contrasting habitats was tested in two greenhouse experiments with the clonal alpine plant *Geum reptans* (Chapter 3). In Chapter 4, seed size variation was measured to test the hypothesis that mean seed weight is increasing with altitude, indicating adaptation to the more severe germination conditions at high altitudes, because more reserves provided for a seedling have many benefits (Grime & Jeffrey 1965; Leishman & Westoby 1994).

Geum reptans was chosen as a main study species. This alpine plant is particularly suited to study variation, differentiation, and adaptation in naturally fragmented habitats because it occurs in contrasting alpine habitats (low *vs*. high altitude, early *vs*. late succession) and is reproducing sexually as well as clonally with flower heads and stolons both relaying on the same apical meristems in rosette-leaf axils.

In recently fragmented remnants of calcareous grassland, *Scabiosa columbaria* was chosen as a study species. This species is common but restricted to grassland remnants in the Swiss Jura. Up to now, most studies on genetic consequences of habitat fragmentation concentrated on rare species, while more common species were neglected in this context.

MOLECULAR DIVERSITY AND DIFFERENTIATION IN FRAGMENTED LANDSCAPES

Mean molecular variation within 20 populations of the outcrossing *Geum reptans* (H_e = 0.22 ± 0.004) was neither particularly high nor low and similar to the mean of other RAPD-studies (Nybom & Bartish 2000; Chapter 2). The observed molecular variation suggests that clonal reproductive behaviour and the fragmented nature of alpine habitats both have no severe consequences on genetic diversity. Mean molecular variation within eleven populations of *Scabiosa columbaria* in recently fragmented habitats was lower than in *G. reptans* and lower than the mean of other RAPD-studies (H_e = 0.18 ± 0.04; Chapter 5), indicating genetic erosion. In both studies, molecular variation did not correlate with population size. But

measurements of molecular variation within populations were more variable in *S. columbaria* compared to *G. reptans*, with low levels of diversity in some populations. Again, this indicates genetic erosion in plants of recently fragmented grassland remnants, what may negatively influence fitness traits. Indeed, population viability in *S. columbaria* decreased with decreasing molecular diversity when plants had to compete with *Bromus* and competition ability decreased as well. On the other hand, competition ability of *G. reptans* was independent of molecular variation (R = 0.53, P = 0.18 for expected heterozygosity). Moreover competition ability did not change among early and late successional habitats (t-Test: P = 0.41), indicating no selection for competition during succession. Additionally, neutral molecular diversity was similar between contrasting habitats and thus, loss of genotypes during succession or with increasing or decreasing altitude may be neglected.

Plant populations in isolated habitats are expected to be highly differentiated. But in *G. reptans* populations were only moderately differentiated (G_{st} = 0.14), indicating considerable gene flow. *Scabiosa columbaria* populations were slightly less differentiated (G_{st} = 0.12). In general, genetic differentiation increases with increasing geographic scale (Nybom & Bartish 2000). The study area of *S. columbaria* is smaller than the study area of *G. reptans* and within the latter, genetic differentiation increases from the core to the regional, to the whole study area. In both cases, differences among population-pairs were considerable variable which indicates random genetic drift. Even *G. reptans* populations of the same glacier forefield were significantly differentiated, but to a small degree, while genetic differentiation increased among more distant populations. This suggests that gene flow among populations is dependent on distance. Moreover, a clear spatial genetic structure was observed in *G. reptans* according to the geographic distribution of the studied populations. The non-significant genetic difference between the two youngest populations in adjacent valleys suggest that they are not necessarily founded from propagules of the nearest or elder populations from the same glacier forefield, but by immigrants from several populations in the same region. Clearly, this reduces the possibility of a genetic bottleneck during colonisation of new sites. On the other hand, genetic differentiation among *S. columbaria* populations from grassland remnants in the Swiss Jura did not increase with distance and there was no spatial pattern in the molecular data. These findings suggest that migration of seeds or pollen between populations from recently fragmented grassland remnants is too low to compensate for random genetic processes.

PLANT DIFFERENTIATION IN CONTRASTING ALPINE HABITATS

In two greenhouse experiments I studied the effects of population origin and environment on population differentiation in *Geum reptans* (Chapter 3). In the competition- (8 populations, 172 individuals) and in the temperature-experiment (9 populations, 206 individuals), between early *vs*. late successional and low vs. high altitudinal *G. reptans* populations, respectively, I found differentiation in plant size and some indications for differences in reproductive behaviour between contrasting habitats. Plant performance differed significantly among all populations, indicating genetic drift. Accordingly, the molecular study of *G. reptans* with neutral genetic markers (Chapter 2) gave similar evidence for genetic drift. Growth under greenhouse conditions was superior in plants from early compared to late successional populations and in plants from high altitude populations compared to plants from low elevation. Numbers of reproductive organs depended on plant size, but reproducing individuals in plants of late succession were smaller than in plants from early succession. Plants of late successional populations may better exploit nutrients because of adaptation to competition in late successional habitats. I found some evidence for an influence of successional origin on flower production in the competition treatment: plants from early succession tended to produce more flowers in the absent of competition, whereas the opposite was the case in plants from late succession. Moreover, in early succession, plants tended to invest proportionally less in stolons if grown without competition than with competition and again, the opposite was found in plants from late succession. Therefore, results suggest opposing selection pressure in contrasting habitats of *G. reptans*, supporting the prediction of the metapopulation model of Olivieri *et al.* (1995) which included evolutionary constraints via a trade-off between life-history traits.

Selection pressure on the allocation of reproductive meristems among flowers and stolons is only possible, if there is a trade-off among numbers of flowers and stolons. In both experiments such a trade-off could be confirmed. However, the percentage of stolons only changed among populations but not between contrasting habitats, indicating no particular selection perssure on reproductive modes constraint by a trade-off.

Seed weight differed among alpine species and their lowland relatives (Chapter 4) with an increase in weight of 28 ± 8 % at high altitude, indicating that selection favours the formation of larger seeds in stressful environments at high altitude. Only in one of 29 closely-

related species-pairs the alpine species had markedly smaller seeds whereas in other species-pairs seed weights were similar. The reason for the not general trend to larger seeds in species at higher altitude may be explained by functional, developmental, or genetic co-variation among traits imposing constraints that keep changes in size and shape of seeds within a taxonomic lineage within narrow limits (Hodgson & Mackey 1986; Pigliucci 2003). Seed weight was characterised as one element of a co-evolving complex of characters (Rees 1997; Venable & Brown 1988) and an increase in seed size at higher altitude is probably frequently related to changes of the taxonomic status of the plants involved. This may explain why I found no indication of larger seed size at higher altitude among populations of four species (*Carex flacca*, *Epilobium fleischeri*, *Saxifraga oppositifolia*, and *Scabiosa lucida*), even though the species represents different life history traits and different habitats. Moreover, if gene flow between populations reaches a substantial degree, potential differences due to differences in selection pressure will be homogenised, and no pattern of seed size variation with altitude will evolve. Genetic drift may explain the large phenotypic variation in seed weight with mostly higher variation within populations than between populations. Correspondingly, the molecular study with *G. reptans* (Chapter 2) gives evidence, that in the naturally fragmented alpine environments gene flow and drift among and within plant populations may be similar in strength and may explain the spatial genetic variation.

THE IMPORTANCE OF ENVIRONMENT ON PLANT GROWTH AND REPRODUCTION

In both greenhouse experiments with *Geum reptans*, environmental conditions influenced plant size and reproduction (Chapter 3). If plants grow under higher environmental stress (e.g. competition, warmer temperature), fewer plants reproduced simultaneously sexually as well as clonally. Competition with *Poa alpina* reduced all measured plant traits drastically, indicating a low competitive ability of *G. reptans*. Due to the strong correlation of size and reproduction, I conclude that the allocation to sexual and clonal reproduction is mainly a response to plant size rather than a direct consequence of competition. In the temperature-experiment, plant size was slightly decreased and the amount of reproductive organs was smaller at warmer temperature. Alpine plants may not be well adapted to warmer

temperature. In addition to competitive exclusion in closed vegetation, unfavourable temperature conditions may be responsible for the lower distributional limit of *G. reptans*.

In the experiment with *Scabiosa columbaria* (Chapter 5), biomass was largely reduced when plants had to compete with *Bromus erectus*. Competition ability and plant biomass did not depend on population size but was positively related with molecular diversity. Populations with low molecular diversity were also developmentally less stable. This indicates reduced phenotypic plasticity when populations are genetically impoverished, making these populations less able to cope with competition. The study with *S. columbaria* of remnant populations gave only weak indirect evidence that decreasing plant performance was caused by decreasing population size.

REPRODUCTION AT HIGH ALTITUDE

Reproduction increased with increasing plant size in both greenhouse experiments with *Geum reptans* (Chapter 3). Smaller plants preferentially reproduced clonally whereas plant size was larger, but similar in plants with flowers or both reproductive modes. These results suggest that minimum plant size for the onset of clonal reproduction is smaller than for sexual reproduction.

Flowers of *G. reptans* are mainly pollinated by flies (Chapter 2): 94 % of all observed pollinators (N = 435) were flies of different sizes, 4.6 % were syrphids, and 1.4 % were bumble bees. Pollinators are suggested not to be limited at high altitude: I observed 3.5 visitors per flower and per hour on a sunny day. The frequency of the observed pollen dispersal distances (N = 50) decreased dramatically from 4 cm to 11.5 m with a single rare dispersal event over 30 m. The wind dispersed seeds (Chapter 2) are mostly not dispersed more than 10 m. However, 0.15 ‰ and 0.05 ‰ of all seeds are dispersed more than 100 and 1000 m, respectively. Given the large seed production per year, e.g. 10 Mio. seeds by *G. reptans* on the Scaletta glacier foreland, on this particular glacier foreland c. 1580 and c. 520 seeds are dispersed over more than 100 m and 1000 m, respectively. Due to the large amount of seeds produced, the low proportion of long distance dispersed seeds may be sufficient for colonization and gene flow. Recruitment in the field from seeds is suggested to be frequent, as I found mainly single *G. reptans*- genotypes in the molecular study (Chapter 2).

Seed size (Chapter 4) was increased in alpine plants compared to related lowland plant species, whereas seed size remained similar with increasing altitudes in *Carex flacca* and *Epilobium fleischeri* and even decreased with altitude in *Saxifraga oppositifolia* and *Scabiosa lucida*. Shorter season length, lower temperature, and reduced resource availability at higher altitudes most likely explain the decrease of seed weight with altitude. In the absence of selection towards higher seed weights, a negative elevational trend in the weight of seeds collected in the field is not surprising. Seeds produced under controlled conditions in the greenhouse would be necessary to test if environmental conditions had such an overriding impact that a positive elevational trend in seed size was masked in the field study.

CONCLUSION

Even though the alpine landscape is naturally fragmented and vegetative reproduction is important in *Geum reptans*, I conclude that gene flow and repeated seedling recruitment during succession might be more frequent than commonly suggested and is similar at different altitudes. Random genetic drift plays an important role in population differentiation and is suggested to account for the high variation in growth and reproduction among populations. Gene flow may additionally counteract selective forces in alpine plants from contrasting habitats: plants performed similar in most traits within the same environmental treatment irrespective of origin. This indicates limited adaptation to different habitats and a high phenotypic plasticity in *G. reptans*. Despite of the overall low competition strength and susceptibility to warm temperature, great size-dependent plasticity in the proportion of sexual *vs*. clonal reproduction ensures population peristence and reproduction in a lrage range of habitat conditions.

Selection for larger seeds along the altitudinal trajectory can be confirmed in a majority of alpine species, but is not generally operating. My results suggest that seed weight only rarely changes as a single trait, but rather evolves as an element of a correlated set of characters involving taxonomic differentiation at the species or at least infra-specific level.

In contrary to the findings with *G. reptans* in the naturally fragmented alpine landscape, *Scabiosa columbaria* from recently created habitat remnants is affected by habitat fragmentation more severely. Local extinction risk of *S. columbaria* is suggested to be increased as a consequence of a decreased viability and of reduced phenotypic plasticity due to

genetic erosion. However, there is only weak indirect evidence for a poorer plant performance of small populations, indicating that population size is not always the best indicator for population viability and genetic diversity. Restoration efforts should therefore not only tend towards increasing local population sizes, but should also consider genetic diversity by itself accounting for possible interactions between genetic and non-genetic effects on population viability.

My results suggest, that plants from naturally fragmented habitats are clearly less affected by isolation than plants in recently fragmented habitats. From the results with *Geum reptans* it can be concluded that phenotypic plasticity may be a successful strategy to cope with contrasting habitat conditions in the alpine landscape and probably also buffers against possible effects of natural fragmentation.

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