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## **Genetic structure of fragmented plant populations: a comparison of two arctic – alpine species with contrasting breeding systems (*a new project*)**

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### **Summary**

**1** Due to human activities, populations of many species have become small, fragmented and isolated in recent years. Theoretical models predict that this will affect patterns of genetic variation in two ways: a decrease of variation within populations and an increase of genetic differentiation among populations. However, these models are based mainly upon diploid, outbreeding organisms with separate sexes, and we have very little information about the consequences of fragmentation for the genetic structure of populations of long-lived plant species with different breeding systems. Such studies are particularly necessary to formulate realistic plans for management of fragmented populations and to assess conservation priorities.

**2** The main objective of this project is to investigate patterns of genetic variation within and among populations of two plant species whose populations in the Alps have been fragmented since the end of the last glaciation. The two species, *Saxifraga cernua* and *Ranunculus pygmaeus*, have been chosen because of their similar arctic-alpine disjunctions and their contrasting breeding system and ploidy level. The main objective of the project is to test whether the theoretically predicted changes in genetic structure within and among populations have indeed occurred in these long-fragmented populations.

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### **Introduction**

Populations of many species have become small, fragmented and isolated in recent years due to human activities. Populations that decline in size become increasingly affected by stochastic events, causing fluctuations in population size, demographic parameters and changes in genetic structure, and, as a consequence, the risk of population extinction increases significantly (Bijlsma *et al.*

1994). For over a decade, much attention has focused on the potential genetic risk associated with small population size, particularly from inbreeding and genetic drift. Genetic drift is thought to change the distribution of genetic variation in two ways: to decrease variation within populations and to increase differentiation among populations (Ellstrand & Elam 1993).

The theoretical risks are often straightforward extensions of population genetic theory; but relevant data have been slow to appear and are sometimes conflicting (Ellstrand & Elam 1993). Most theoretical work on the stochastic loss of variation through bottlenecks and founder events has considered diploid, outbreeding organisms with separate sexes. This genetic system is common among many animal groups, but is unrepresentative for most plant species in which polyploidy, hermaphroditism, and varying degrees of self-fertilisation commonly occur. Generalisations on the harmful effects of inbreeding may need to be modified from those available for most animals because many plant species regularly inbreed (Barrett & Kohn 1991). The breeding system significantly influences genetic diversity within and among populations. Widespread, long-lived, wind-pollinated, outbreeding species maintain more genetic variation within populations than do species with other combinations of traits (Hamrick *et al.* 1991). However, there are several exceptions to this generalisation (Karron 1991). Empirical data on the significance of genetic erosion are still very scarce, but are clearly needed to evaluate the role of genetic stochasticity in relation to the other stochastic factors (Bijlsma *et al.* 1994). Such studies are particularly necessary to formulate realistic plans for management of fragmented populations and to assess conservation priorities.

Most comparative studies on rare or isolated plant populations have examined congeneric species (reviewed by Jain 1994); an exception are the studies of Ouborg *et al.* (1991), van Treuren (1993) and Bijlsma *et al.* (1994) on *Salvia pratensis* and *Scabiosa columbaria*. These studies compared the response to inbreeding and demographic stochasticity of small and of large populations. However, little attention has been directed to

the population structure of isolated or fragmented arctic and alpine plant populations (e.g. Odasz *et al.* 1991).

## Methods

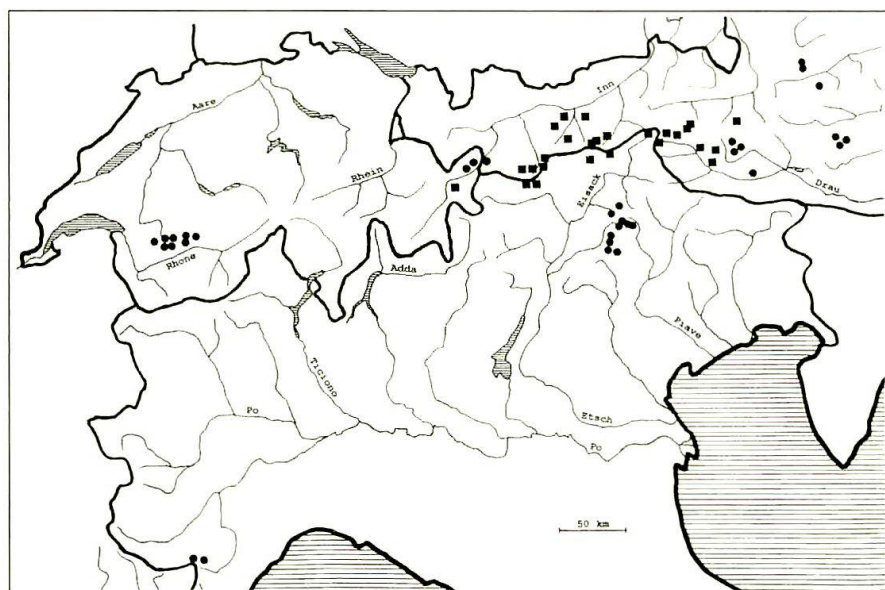
### EXPERIMENTAL SYSTEM

The arctic and alpine tundra is one of the few relatively undisturbed biomes of the earth. Therefore arctic and alpine plant species offer excellent material to investigate the natural structure of populations. Several species which are common throughout the arctic are found in the Alps in only a few relict populations on mountain peaks or in snowbed communities. These populations have a long history of isolation and fragmentation, having managed to survive since the Pleistocene glaciation.

*Saxifraga cernua* is circumpolar distributed. In the Alps there are only few relict populations (Fig. 1; Melchior 1934). *Saxifraga cernua* is polyploid ( $2n = 48-70$ , Zhukova & Petrovsky 1971) and forms in the inflorescence a terminal flower and lateral bulbils (Fig. 2). Although viable seeds have been observed in Scandinavia, *S. cernua* reproduces almost exclusively asexually by bulbils. It perennates by basal bulbs and sometimes by very short rhizomes (Molau 1992).

*Ranunculus pygmaeus* is also circumpolar distributed with relict populations in the Alps (Fig. 1; Rikli 1899). It is diploid throughout its distribution (Jalas & Suominen 1989) and reproduces only by seeds (Fig. 3). *Ranunculus pygmaeus* appears to be a typical outbreeder. The investigation of the breeding system of different *Ranunculus* taxa revealed that diploid species normally produce viable pollen and have a marked self-incompatibility (e.g. *R. montanus* s.l., Landolt 1954; *R. polyanthemus* s.l., Hess 1955, Baltisberger 1980; *R. alpestris* s.l., *R. segueri*, Baltis-





**Fig. 1.** Relict populations of *Saxifraga cernua* (●) and *Ranunculus pygmaeus* (■) in the Alps. Distribution of *S. cernua* after Melchior (1934), Sutter (1976) and Welten & Sutter (1982).



**Fig. 2.** *Saxifraga cernua* produces normally one white, terminal flower and red, lateral bulbils partly with young leaves. It is polyploid and reproduces almost exclusively asexually by bulbils. Flowers setting seeds have been observed in Scandinavia. *Saxifraga cernua* is circumpolar distributed with several relict populations in the Alps.



**Fig. 3.** *Ranunculus pygmaeus* is about 5 cm tall, leaves are three to five lobed, flowers yellow. It is diploid, produces no runners and reproduces only by seeds. *Ranunculus pygmaeus* is circumpolar distributed with one relict population in the Swiss Alps and several populations in the Austrian and Italian Alps.

berger & Müller 1981, Müller & Baltisberger 1984, Baltisberger 1994; *R. aconitifolius*, *R. platanifolius*, *R. kuepferi*, Huber 1988). Polyploid species have a reduced pollen fertility and reproduce apomictically (Vuille & Küpfer 1985; Huber 1988).

*Saxifraga cernua* and *Ranunculus pygmaeus* were selected as experimental system because they have the same arctic-alpine disjunction but differ in ploidy level and reproductive system. In Scandinavia both species are common and occur throughout the whole range of the mountains (Jalas & Suominen 1989).

The alpine relict populations of *S. cernua* and *R. pygmaeus* grow on mountain peaks, in shadowy rock niches and in snowbed communities; they might be vulnerable to the predicted climatic changes and consequent upward migration of vegetation belts, as documented by Grabherr *et al.* (1994).

#### MOLECULAR TECHNIQUES IN ECOLOGICAL GENETICS

The new techniques which have recently become available from molecular biology, offer powerful tools for those interested in ecological genetics. Questions about the importance of genetic variation, micro-evolution and the history of plant distribution can now be investigated much more directly than before (Bachmann 1994). Several techniques are available for determining the levels of genetic variation within and between natural populations. These include allozymes, restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), micro- and minisatellites.

In this study we will use starch gel electrophoresis to detect allozyme polymorphism and the RAPD technique, which might be a key to finding residual genetic variation (Bachmann 1994). The combination of allozyme and RAPD techniques offers a very

convenient approach to investigate genetic structure in fragmented populations. It does not produce the same sensitivity or resolution as some other methods (e.g. DNA sequencing) but has the advantage of being fast and it can be used on a large number of samples.

#### Objectives and significance of the project

The main objective is to study patterns of genetic variation within and among populations of *S. cernua* and *R. pygmaeus* in the Alps and in Scandinavia in relation to their breeding system, population size and degree of fragmentation. The relict alpine populations offer the opportunity of investigating the effect of natural long fragmentation on the genetic structure of populations. We will also analyse the demographic structure of the genetically investigated populations. Concerning the breeding systems we try to answer following questions: Does *S. cernua* produce viable pollen and seeds in the Alps and is *R. pygmaeus* selfincompatible like other diploid *Ranunculus* spp.?

The core of this project is to test whether the hypothesis that genetic drift in small, fragmented populations leads to a decrease of variation within populations and an increase of differentiation among populations is true for long-time fragmented populations of two species with contrasting breeding systems and ploidy level. The practical importance of this work is that it attempts to understand the significance of genetic variation for survival of small populations. Can we draw conclusions about the genetic structure of small, fragmented plant populations, and what lessons can we learn for the conservation of these species, whether *in situ* or by re-establishment of new populations?

The genetic structure of arctic-alpine species is poorly known but evidence is



growing that even under these adverse environment sexual reproduction is of high importance for the maintenance of viable populations (Bauert 1996).

Due to human activities many plant and animal populations became or will become fragmented. The insight in the genetic structure of long-term fragmented populations will help to improve conservation strategies for organism in relation to their reproductive system.

Plants provide useful monitoring systems of environmental change. Slow climatic changes as well as extreme events will affect populations. Rapid changes in size, viability or disappearance of relict ice-age populations which have persisted in the Alps for several thousand years, would be important indicators of climatic change. The assessment of the population size of the relict populations of *S. cernua* and *R. pygmaeus* in the Alps will provide the base for future investigations and long-term monitoring.

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