Climatic oscillations and the fragmentation of plant populations – genetic diversity within and among populations of the glacial relict plants *Saxifraga paniculata* (Saxifragaceae) and *Sesleria albicans* (Poaceae)

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Contents

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I
Genetic diversity within and among populations of glacial relicts - a general introduction

1 Origin and distribution of glacial relict plant populations

The climatic oscillations of the Quaternary starting 700,000 years ago with a dominant series of cold and dry periods (each lasting approximately 100,000 years) and being interupted by shorter intervals (approximately 10-20,000 years) of warmer and moister interglacial climates (Webb & Bartlein 1992) had an enormous influence on the distribution of plants.

Population sizes and ranges of many temperate plant species were greatly reduced during the glacial times. Quaternary palaeoecology in Europe has revealed a series of southward range contractions of both plant and animal species during the last glacial period, followed by rapid northward range expansions in the wake of deglaciation (Hewitt 1996). During the glaciations, vast areas in northern Europe, North America, and Asia were covered by massive ice-sheets, although independent centres of glaciation occured at low latitude mountains such as the Alps and the Himalayas (Figure 1). The central European lowlands between the Scandinavian and Alpine ice sheets were unglaciated and covered by a tundra vegetation (Hantke 1978, Lang 1994). The plants, which grew in this tundra during the glaciations were adapted to a cold climate. In the course of the postglacial warming the range of these plants contracted to the higher altitudes of the Alps and/or the higher latitudes of northern Europe.

Only in a few habitats in central Europe, such as fens or rocky habitats, populations of arctic or alpine plant species have survived. There are numerous plant species (Table 1), such as *Saxifraga paniculata* or *Sesleria albicans*, which are widely distributed in the higher altitudes of the Alps and only occur with isolated populations in the lower mountains of central Europe. The populations of these rare plants are thought to be glacial relict populations.

2 Population genetics of rare glacial relict plants

Populations of glacial relict plants were fragmented at least since the end of the last glaciation. Furthermore, the relict populations colonize habitats, which differ from each other with regard to ecological conditions and selection pressures. The genetic structure of glacial relict populations, therefore, was tremendously influenced by the population genetic processes of long-term isolation and differentiation.

The fragmentation and differentiation of populations are evolutionary processes, which as well presently take place in the central European man-made landscapes.

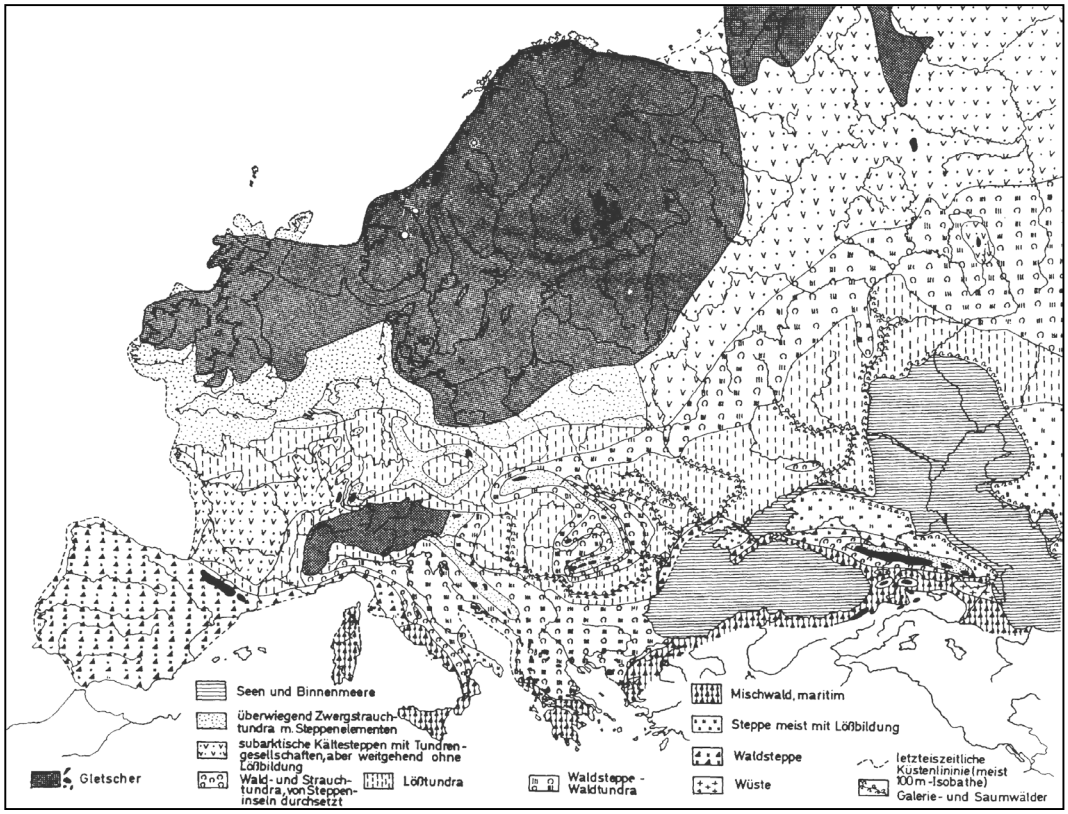

Figure 1: Vegetation in Europe during the Würm glaciation (20,000 years before today). Scandinavia and the Alps were occupied by massive ice sheets while the central European lowlands were covered by tundra vegetation (taken from Walter & Straka 1970).

On the one hand, many rare plant species are currently threatened by extinction. Species richness in central Europe declined tremendously due to the intensification of agricultural practices during the last decades (Sukopp & Trepl 1987). Even those species, which were still widespread, decline in their distribution (Korneck et al. 1996). The extinction of species, however, is not only due to the destruction, but also to the fragmentation of habitats and the isolation of predominantly small plant populations (Young & Clarke 2000).

On the other hand many new taxa have evolved by population differentiation in the central European man-made landscapes. The opening of the landscapes caused an increase of species richness in the central European flora (Sukopp & Trepl 1987). Furthermore, it resulted in a differentiation of ecotypes especially due to the development of different land use practices since the beginning of the Neolithic period, as they created new habitats with differing ecological conditions (den Nijs et al. 1999, van Raamsdonk & den Nijs 1999).

Table 1: Arctic-alpine (o) and alpine (x) glacial relict plant species in central European mountain regions (Vogesen, Black forest, Swabian Alb, Franconian Alb, Bohemian Forest, Erzgebirge, Harz) (according to Thorn 1960, slightly changed).

	Vogesen	Black Forest	Swabian Alb	Franconian Alb	Bohemian Forest	Erzgebirge	Harz
Poa alpina	o				o		
Poa laxa		o					
Agrostis rupestris					x		
Phleum alpinum					o		
Sesleria albicans			x	x			x
Carex bigelowii						o	o
Carex magellanica					o	o	
Carex vaginata							o
Carex sempervirens			x				
Juncus trifidus					x		
Luzula desvauxii	o	o					
Veratrum album	o		o				
Allium victorialis	o	o					
Streptopus amplexifolius	o	o			o	o	
Crocus albiflorus		x	x				
Nigritella nigra		o					
Traunsteinera globosa	x	x	x			x	
Salix bicolor	o						o
Salix hastata	o						o
Salix appendiculata		x				x	
Salix myrtilloides					o		
Betula nana					o	o	o
Alnus viridis		o					
Rumex alpinus	x	x				x	
Rumex alpestris	x	x			x	x	x
Polygionum viviparum			o	o			
Silene rupestris	o	o					
Gypsophila repens							x
Sagina saginoides		o			o	o	
Moehringia muscosa					x		
Anemone narcissiflora	o		o				
Anemone alpina	o						o
Ranunculus montanus			x	x			
Kernera saxatilis				x			
Draba aizoides			x	x			
Cardamine resedifolia					x		
Cardaminopsis petraea				o			o
Arabis alpina			o	o			o
Sedum roseum	o						
Sedum annuum	o	o					
Sedum alpestre	x						
Saxifraga paniculata	o	o	o	o			
Saxifraga decipiens	o		o	o		o	o
Saxifraga stellaris	o	o					
Ribes petraeum	x	x	x				

	Vogesen	Black Forest	Swabian Alb	Franconian Alb	Bohemian Forest	Erzgebirge	Harz
Potentilla aurea		o					
Sibbaldia procumbens	o						
Alchemilla hoppeana		x					
Alchemilla fissa	x						
Epilobium alpestre	x	x				x	
Epilobium nutans	x	x			x	x	
Epilobium anagallidifolium	o	o			o	o	
Epilobium alsinifolium		o			o		
Anthriscus nitidus	x	x	x				x
Athamanta cretensis			x				
Ligusticum mutellina		x			x		
Primula auricula		x		x			
Androsace carnea	x						
Androsace lactea			x				
Soldanella alpina		x					
Soldanella montana					x		
Gentiana lutea	x	x	x				
Gentiana pannonica					x		
Gentiana clusii		x					
Veronica fruticans	o	o					
Bartsia alpina	o	o					
Euphrasia minima	o						
Euphrasia picta						x	
Euphrasia salisburgensis			o				
Rhinanthus pulcher						x	
Pedicularis foliosa	x		x				
Galium anisophllon				x	x		
Valeriana tripteris	x	x	x				
Campanula cochlearifolia		x	x				
Campanula scheuchzeri		o			o		
Adenostyles alliariae	x	x					
Aster bellidiastrum		x	x	x			
Aster alpinus						x	x
Gnaphalium norvegicum	o	o			o	o	
Gnaphalium supinum		o					
Homogyne alpina		x			x	x	
Doronicum austriacum					x		
Leontodon helveticus	o	o					
Cicerbita plumieri	x	x					
Crepis pyrenaica	x	x					
Crepis alpestris			x	x			
Hieracium bupleuroides			x	x			
Hieracium humile	x	x	x				
Hieracium alpinum	o						o
Hieracium prenanthoides	o	o					

Reflecting these facts, relict populations are highly interesting objects for the investigation of population genetic processes. The fragmented distribution of relict populations allows, on the one hand, to study the consequences of longterm isolation for the genetic diversity of plant populations. The occurrence of relict populations in ecologically differing habitats permits, on the other hand, to analyze the genetic differentiation of populations and the development of ecotypes. An investigation of the genetic diversity within and among glacial relict populations, therefore, can shed light upon population genetic questions of general interest.

3 Population genetics and the fragmentation of populations

Apart from habitat destruction, which is generally considered to be the most inportant reason for the extinction of species, the fragmentation of habitats is a significant threat to the maintenance of biodiversity in many terrestrial ecosystems. Initial predictions about the genetic consequences of habitat fragmentation focus on the reduced size and increased spatial isolation of populations occupying habitat remnants. Such population changes lead to an increased interpopulational genetic divergence and to an erosion of genetic variation.

The loss of populations associated with habitat fragmentation generally reduces interpopulation geneflow (Young et al. 1993). Provided that a population is stable and assuming an Island Model of geneflow, interpopulation divergence will increase due to random genetic drift, especially when population sizes and gene flow are low. As demonstrated by Powell & Powell (1987), a 100 m wide clearing formed an effective barrier to the movement of pollinators. Conversely, only a very small amount of gene flow (approximately one individual per generation) is required to reduce the loss of alleles due to genetic drift (Young et al. 1996). Fragmented and isolated populations, therefore, very often show high levels of interpopulational differentiation (Bauert et al. 1998, Cardoso et al. 1998, Fischer & Matthies 1998).

Reductions in population size at the time of fragmentation create genetic bottlenecks because the remaining individuals contain only a small sample of the original genepool. Subsequent to this initial loss of variation, remnant populations that remain small and isolated for several generations continue to loose alleles due to random genetic drift. Many investigations confirmed that reductions in genetic variation accompany reductions in plant population size associated with habitat fragmentation (van Treuren et al. 1991, Prober & Brown 1994, Raijmann et al. 1994). Furthermore, many studies have demonstrated a positive logarithmic relationship between remnant population size and heterozygosity, suggesting that decreases in heterozygosity are correlated to reductions in population size (Prober & Brown 1994, Raijmann et al. 1994). Although the degree of inbreeding depression also depends on environmental factors (Barrett & Kohn 1991) and on the stage in the plant life cycle (Charlesworth & Charlesworth 1987, Husband & Schemske 1996, Byers & Waller 1999), small populations often suffer from a greater inbreeding depression, compared to large ones because of the reduced effectiveness of selection relative to genetic drift (Booy et al. 2000). A large number of studies

in natural populations showed a relationship between heterozygosity and various fitness components, such as viability, growth rate, developmental stability and physiological variables (Mitton & Grant 1984, Allendorf & Leary 1986, Mitton 1994). Especially in ecologically unstable environments, a higher level of heterozygosity corresponds to a higher individual fitness (Rankevich et al. 1996, Nevo et al. 1997). In the short term, a loss of heterozygosity can reduce individual fitness and lower remnant population viability. In the longer term, reduced allelic richness may even limit a species‘ ability to respond to changing selection pressures (Frankel et al. 1995).

Concerning central European glacial relict plants little is known, to date, about the consequences of fragmentation and isolation for the genetic diversity within and among relict populations. Initial investigations, however, suggest comparatively high levels of diversity within and a strong differentiation among glacial relict populations, as demonstrated for *Biscutella laevigata* and *Saxifraga aizoides* (Dannemann 2000, Lutz et al. 2000).

4 Population genetics and the differentiation of populations

Due to human activities, the central European man-made landscape consists of very different habitats, exhibiting a multitude of ecological conditions. Populations of many plant species can be found in habitats such as forests, meadows, pastures or farmland. The colonized ecosystems vary to a high degree from each other with regard to the selection pressures, which may have an effect on the plant populations growing in them. Plants can genetically adapt to the different environments, besides the phenotypic plasticity, which allows them to react on ecological changes.

The differentiation of new ecospecies (Grant 1981) in man-made landscapes has been reported for genera such as *Hieracium* (Asteraceae) (Gottschlich & Raabe 1991, Gottschlich 1996), *Taraxacum* (Asteraceae) (Doll 1982) and *Ranunculus* (Ranunculaceae) (Melzheimer & Lohwasser 1997, Melzheimer 1998), whereas the differentiation of new ecotypes (Grant 1981) has been observed for *Galium aparine* (Rubiaceae) (Groll & Mahn 1986, Mahn 1989), *Rhinanthus alectorolophus* (Scrophulariaceae) (Zopfi 1993a,b) or *Euphrasia rostkoviana* (Scrophulariaceae) (Zopfi 1998). Reflecting this fact, genetic differentiation of populations is of tremendous interest because it is the basic requirement for the development of new taxa.

Climatic and edaphic conditions are directly related to the microgeographic differentiation of plants (Briggs & Walters 1984, Nevo et al. 1988). Several studies demonstrated genetic differentiation among populations from ecosystems with high or low levels of radiation intensity (Nevo et al. 1988, Owuor et al. 1998). Furthermore, Huff et al. (1998) reported of genetic divergence of populations growing on soils of varying fertility. The geographic altitude is another factor, which has an influence on the differentiation of plant populations. Gunter et al. (1996), for example, observed genetic differences among upland and lowland

populations of *Panicum virgatum*. Since ecological conditions show extreme variation among ecosystems, genetic differentiation of populations in different habitats can be observed, as demonstrated by Hsao & Lee (1999). They found significant differences among plant populations from grassland and forest biomes. Ecological parameters, therefore, have a tremendous influence on the genetic differentiation of populations (Kölliker et al. 1998).

Man-made open habitats show a considerably higher environmental heterogeneity than forest biomes. This results in higher evolutionary rates and an increased genetic diversity (Antonovics 1971, Hoffmann & Parsons 1991, Parsons 1994). Different types and intensity of land use are generally thought to make a contribution to the differentiation within and among plant populations (Poschlod et al. 2000). Fertilization and defoliation, for example, are known to cause population differentiation (Snaydon 1987). As demonstrated by Kölliker et al. (1998) fertilization and higher cutting frequencies can also result in a reduction of genetic variability within populations. Different cutting regimes, furthermore, can cause genetic differentiaton among populations (Sweeney & Danneberger 1995). The development of „seasonal" ecotypes in *Rhinanthus alectorolophus* and *Euphrasia rostkoviana* shows the strong influence of grassland management on the differentiation of populations (Zopfi 1993, 1998). Different land use practices, therefore, may considerably contribute to the differentiation of populations in the man-made landscape.

Regarding central European glacial relict plants, to date, nothing is known about the genetic differentiation of populations in different habitats and the development of ecotypes. The investigation of non-relict plant populations, however, suggests that a differentiation of isolated populations in ecologically differing habitats is quite possible.

5 Methods for the measurement of genetic diversity

For several decades, numerous studies on population genetics used isozymes to detect genetic variation, the genetic basis of isozyme patterns, therefore, being well established in many species (Booy et al. 2000). Isozymes can provide informative genetic markers, using straightforward laboratory procedures that are relatively rapid and inexpensive (Peakall et al. 1995). There are, nevertheless, some widely recognized limitations of isozymes. The detection of genetic variation is limited to protein coding loci, which may lead to an underestimates of genetic diversity (Clegg 1989) and may not always be representative of the entire genome (Schaal et al. 1991). Isozymes are also tissue-specific in many cases, and protein expression can respond to varying environmental conditions (Peakall et al. 1995).

Due to these facts, many workers have shifted to use nuclear DNA markers such as RFLP's (Restriction Fragment Length Polymorphisms). RFLP's overcome some of the limitations of isozymes, but because they are more difficult, time consuming and costly to assay than isozymes, their use has been limited for extensive surveys of the genetic variation in plants. With the development of the PCR (po-

lymerase chain reaction) for the direct amplification of DNA fragments, the use of DNA markers in population studies has increased tremendously. One great advantage of PCR-based DNA markers is the fact that they detect a higher level of diversity than isozymes. DNA markers, such as microsatellites, AFLP's (Amplified Fragment Length Polymorphisms) or RAPD's (Random Amplified Polymorphic DNA) are, therefore, qualified for the investigation of rare plants, which presumably show low levels of genetic variability.

Microsatellites are tandem repeates of one to four base-pair units that are dispersed throughout the genome and were subject to genetic variation due to slippage during replication. This resulted in the variation of the number of repeat units at the site (Tautz et al. 1986). Specific repeats have been detected and used for genetic analysis in various plants (Morgante & Olivieri 1993, Thomas & Scott 1993). As demonstrated by Kijas et al. (1995) microsatellites are conserved across a range of citrus and related species. However, from present experience, suitably variable microsatellate loci may not be as easy to find in plants as in animals, and flanking primers developed at considerable expense for one species seem to have a limited use outside that species (Bachmann 1997).

AFLP and RAPD analysis are PCR-based assays employing random primers to generate banding patterns. By using single, short primers both techniques are capable of scanning a genome for the many inverted priming sites that are close enough to be amplified. They do not require any information about the target genome and only a small amount of DNA is necessary. Both methods are simple and quick techniques and can provide a very large number of polymorphic bands (Welsh & McClelland 1990, Williams et al. 1990, Vos et al 1995, Travis et al. 1996). However, AFLP analysis allows to determine heterozygosity which is not possible when using RAPD analysis since the amplified RAPD fragments form a banding pattern inherited in Mendelian fashion as dominant characters (Williams et al. 1990).

Furthermore, the squencing of nuclear DNA can also be used for the detection of genetic variability. At present, the technically most convenient and universally accessible nuclear DNA sequences with sufficient variability to distinguish species of a genus or even populations of a species are the internal transcribed spacers, ITS1 and ITS2, between the conserved rDNA sequences coding for ribosomal RNA. The ITS region is highly informative. ITS sequencing, therefore, has become widely used by systematists (Baldwin et al. 1995) and has also proven to be informative for biogeographic studies in some angiosperm genera (Baldwin 1993, Vargas et al. 1998).

6 Aim of the investigation

In the study presented here, RAPD and ITS analysis were used to investigate the genetic diversity within and among populations of the glacial relict plants *Saxifraga paniculata* Mill. and *Sesleria albicans* Kit. ex Schultes.

Since population fragmentation and differentiation are evolutionary processes, which were presently thrown into gear by human influences in the central European man-made landscape, the investigation of genetic diversity within and among glacial relict populations can shed light upon population genetic processes of general interest.

The aim of this study was to answer the following questions:

- What is the partitioning of genetic diversity within and among isolated populations of glacial relicts?
- Is there a geographic differentiation among glacial relict populations from different regions?
- What is the level of diversity within relict populations from different geographic regions?
- Is there a higher level of diversity within central populations than within peripheral relict populations from the boundary of the distribution area?
- Is there a lower level of genetic diversity within small populations than within large populations?
- What is the influence of mating system, longevity and clonality on the partitioning and the level of genetic diversity?
- Is there a genetic or even ecotypic differentiation among relict populations from different types of habitats?
- Are there different levels of genetic diversity within relict populations from habitats with differing ecological conditions?

7 References

Allendorf, F.W. & Leary, R.F. (1986): Heterozygosity and fitness in natural populations of animals. In: Soule, M.E. (ed.): Conservation biology. The science of scarcity and diversity. Sinauer, Sunderland, 57-76.

Antonovics, J. (1971): The effects of a heterogenous environment on the genetics of natural populations. American Scientist 59: 593-559.

Bachmann, K. (1997): Nuclear DNA markers in plant biosystematic research. Opera Botanica 132: 137-148.

Baldwin, B.G. (1993): Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. American Journal of Botany 80: 222-238.

Baldwin, B.G.; Sanderson, M.J.; Porter, J.M.; Wojciechowski, M.F.; Campbell, C.S. & Donoghue, M.J. (1995): The ITS region of the nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247-277.

Barrett, S.C.H. & Kohn, J.R. (1991): Genetic and evolutionary consequences of small population sizes in plants: implications for conservation. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 3-30.

Bauert, M.R.; Kälin, M.; Baltisberger, M. & Edwards, P.J. (1998): No genetic variation within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. Molecular Ecology 7: 1519-1527.

Booy, G.; Hendriks, R.J.J.; Smulders, M.J.M.; van Groenendal, J.M. & Vosman, B. (2000): Genetic diversity and the survival of species. Plant Biology 2: 379-395.

Briggs, D. & Walters, S.M. (1984): Plant variation and evolution. 2 ed., Cambridge University Press, Cambridge.

Byers, D.L. & Waller, D.M. (1999): Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annual Reviews in Ecology and Systematics 30: 479-513.

Cardoso, M.A.; Provan, J.; Powell, W.; Ferreiras, C.G. & de Oliveira, D.E. (1998): High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinoideae). Molecular Ecology 7: 601-608.

Charlesworth, D. & Charlesworth, B. (1987): Inbreeding depression and its evolutionary consequences. Annual Reviews in Ecology and Systematics 18: 237-268.

Clegg, M.T. (1989): Molecular diversity in plant populations. In: Brown, H.D.; Clegg, M.T.; Kahler, A.L. & Weir, B.S. (eds.): Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, 98-115.

Dannemann, A. (2000): Der Einfluss von Fragmentierung und Populationsgröße auf die genetische Variation und Fitness von seltenen Pflanzenarten am Beispiel von *Biscutella laevigata* (Brassicaceae). Dissertationes Botanicae 330. J.Cramer, Berlin, Stuttgart.

Den Nijs, H.C.M.; Marhold, K. & Hurka, H. (1999): Plant evolution in disturbed habitats: an introduction. Folia Geobotanica 34: 399-403.

Doll, R. (1982): Grundriß der Evolution der Gattung *Taraxacum* ZINN. Feddes Repertorium 93: 481-624.

Fischer, M. & Matthies, D. (1998): RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). American Journal of Botany 85: 811-819.

Frankel, O.H.; Brown, A.H.D. & Burdon, J.J. (1995): The conservation of plant biodiversity, Cambridge University Press, Cambridge.

Gottschlich, G. (1996): *Hieracium* L. In: Sebald, O.; Seybold, S.; Philippi, G. & Wörz, A. (eds.): Die Farn- und Blütenpflanzen Baden-Württembergs. Ulmer Verlag, Stuttgart.

Gottschlich, G. & Raabe, U. (1991): Zur Verbreitung, Ökologie und Taxonomie der Gattung *Hieracium* L. (Compositae) in Westfalen und angrenzenden Gebieten. Abhandlungen aus dem Westfälischen Museum für Naturkunde 53.

Grant, V. (1981): Plant speciation. Columbia University Press, New York.

Groll, U. & Mahn, E.G. (1986): Zur Entwicklung ausgewählter Populationen des Kletten-Labkrautes (*Galium aparine* L.). Flora 178: 93-110.

Gunter, L.E.; Tuskan, G.A. & Wullschleger, S.D. (1996): Diversity of populations of switchgrass based on RAPD markers. Crop Science 36: 1017-1022.

Hantke, R. (1978): Eiszeitalter, Band 1. Ott, Thun.

Hewitt, G.M. (1996): Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247-276.

Hoffmann, A.A. & Parsons, P.A. (1991): Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford.

Hsao, J.Y. & Lee, M. (1999): Genetic diversity and microgeographic differentiation of yushan cane (*Yushania niitakayamensis*; Poaceae) in Taiwan. Molecular Ecology 8: 263-270.

Huff, D.R.; Quinn, J.A.; Higgins, B. & Palazzo, A.J. (1998): Random amplified polymorphic DNA (RAPD) variation among native little bluestem [*Schizachyrium scoparium* (Michx.) Nash] populations from sites of high and low fertility in forest and grassland biomes. Molecular Ecology 7: 1591-1597.

Husband, B.C. & Schemske, D.W. (1996): Evolution of the magnitude of inbreeding depression in plants. Evolution 50: 54-70.

Kijas, J.M.H.; Fowler, J.C.S. & Thomas, M.R. (1995): An evaluation of sequence tagged microsatellite site markers for genetic analysis within citrus and related species. Genome 38: 349-355.

Kölliker, R.; Stadelmann, F.J.; Reidy, B. & Nösberger, J. (1998): Fertilization and defoliation frequency affect genetic diversity of *Festuca pratensis* Huds. in permanent grasslands. Molecular Ecology 7: 1557-1567.

Korneck, D.; Schnittler, M. & Vollmer, I. (1996): Rote Liste der Farn- und Blütenpflanzen (*Pteridophyta* et *Spermatophyta*) Deutschlands. Schriftenreihe für Vegetationskunde 28: 21-188.

Lang, G. (1994): Quartäre Vegetationsgeschichte Mitteleuropas, Jena.

Lutz, E.; Schneller, J. & Holderegger, R. (2000): Understanding population history for conservation purposes: Population genetics of *Saxifraga aizoides* (Saxifragaceae) in the lowlands and the lower mountains north of the Alps. American Journal of Botany 87: 583-590.

Mahn, E.G. (1989): Anpassungen annueller Pflanzenpopulationen an anthropogen veränderte Umweltvariablen. Verhandlungen der Gesellschaft für Ökologie 18: 655-663.

Melzheimer, V. & Lohwasser, U. (1997): *Ranunculus auricomus* agg. Kritische Anmerkungen zum gegenwärtigen Stand der Forschung. Floristische Rundbriefe 31: 89-98.

Melzheimer, V. (1998): *Ranunculus auricomus* agg. In: Wisskirchen, R. & Haeupler, H. (eds.): Standardliste der Farn- und Blütenpflanzen Deutschlands. Ulmer Verlag, Stuttgart, 400-409.

Mitton, J.B. (1994): Molecular approaches to population biology. Annual Reviews in Ecology and Systematics 25: 45-69.

Mitton, J.B. & Grant, M.C. (1984): Associations among protein heterozygosity, growth rate and developmental homoeostasis. Annual Reviews in Ecology and Systematics 15: 479-499.

Morgante, M. & Olivieri, A.M. (1993): PCR amplified microsatellites as markers in plant genetics. Plant Journal 3: 175-182.

Nevo, E.; Beiles, A. & Krugman, T. (1988): Natural selection of allozyme polymorphisms: a microgeographic differentiation in wild emmer wheat (*Triticum dicoccoides*). Theoretical and Applied Genetics 75: 529-538.

Nevo, E.; Appelbaum-Elkaher, I.; Garty, J. & Beiles, A. (1997): Natural selection causes microscale allozyme diversity in wild barley and a lichen at „Evolution canyon“, Mt. Carmel, Israel. Evolution 47: 1329-1341.

Owuor, E.D.; Fahima, T.; Beiles, A.; Korol, A. & Nevo, E. (1997): Population genetic response to microsite ecological stress in wild barley, *Hordeum spontaneum*. Molecular Ecology 6: 1177-1187.

Parsons, P.A. (1994): Habitats, stress and evolutionary rates. Journal for Evolutionary Biology: 387-397.

Peakall, R.; Smouse, P.E. & Huff, D.R. (1995): Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloe dactyloides*. Molecular Ecology 4: 135-147.

Poschlod, P.; Dannemann, A., Kahmen, S.; Melzheimer, V.; Biedermann, H.; Mengel, C.; Neugebauer, K.R. & Pantle, I. (2000): Genes in the landscape. Changes in central European land use and its impact on genetic diversity of plants. Schriftenreihe für Vegetationskunde 32: 111-127.

Powell, A.H. & Powell, G.V.N. (1987): Population dynamics of male euglossine bees in Amazonian forest fragments. Biotropica 19: 176-179.

Prober, S.M. & Brown, A.H.D. (1994): Conservation of the grassy white box woodlands: Population genetics and fragmentation of *Eucalyptus albens*. Conservation Biology 8: 1003-1013.

Raijman, L.E.L.; van Leeuwen, N.C.; Kersten, R.; Oostermeijer, J.G.B.; den Niijs, H.C.M. & Menken, S.B.J. (1994): Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonathe* L.. Conservation Biology 8: 1014-1026.

Rankevich, D.; Lavie, B.; Nevo, E.; Beiles, A.; Arad, Z. (1996): Genetic and physiological adaptations of the prosobranch landsnail *Pomatia olivieri* to microclimatic stresses on Mt. Carmel, Israel. Israelian Journal of Zoology 42: 425-441.

Schaal, B.A.; Leverich, W.J. & Rogstad, S.H. (1991): Comparison of methods for assessing genetic variation in plant biology. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 123-134.

Snaydon, R.W. (1987): Population responses to environmental disturbance. In: Van Andel, J.; Bakker, J.P.; Snaydon, R.W.: Disturbance in Grasslands. Dr. W. Junk Publishers, Dordrecht.

Sukopp, H. & Trepl, L. (1987): Extinction and naturalization of plant species as related to ecosystem structure and function. Ecological Studies 61: 245-276.

Sweeney, P.M. & Danneberger, T.K. (1995): RAPD characterization of *Poa annua* L. populations in golf course greens and fairways. Crop Science 35: 1676-1680.

Tautz, D.; Trick, M.; Dover, G.A. (1986): Cryptic simplicity in DNA is a major source of variation. Nature 322: 652-656.

Thomas, M.R. & Scott, N.S. (1993): Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequnece-tagged sites (STSs). Theoretical and Applied Genetics 86: 985-990.

Thorn, K. (1960): Bemerkungen zu einer Übersichtskarte vermutlicher Glazialreliktpflanzen Deutschlands. Mitteilungen der floristisch-soziologischen Arbeitsgemeinschaft 8: 81-85.

Travis, S.E.; Maschinski, J. & Keim, P. (1996): An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. Molecular Ecology 5: 735-745.

Van Raamsdonk, L.W.D. & den Nijs, H.C.M. (eds.) (1999): Plant evolution in man-made habitats. Proceedings of the VIIth International IOPB Symposium Amsterdam. Hugo de Vriess Laboratory, Amsterdam.

Van Treuren, R.; Bijlsma, R.; van Delden, W. & Ouborg, N.J. (1991): The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. Heredity 66: 181-189.

Vargas, P.; Baldwin, B. & Constance, L. (1998): Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). Proceedings of the National Academy of Sciences, USA 95: 235-240.

Vos, P.; Hogers, R.; Bleeker, M.; Reijnans, M.; van de Lee, T.; Hornes, M.; Frijtjers, A.; Pot, J.; Peleman, J.; Kuiper, M. & Zabeau, M. (1995): AFLP: a new technique for DNA fingerprinting. Nucleic Acid Research 23: 4407-4414.

Walter, H. & Straka, H. (1970): Arealkunde. Floristisch-historische Geobotanik. Ulmer, Stuttgart.

Webb, T. & Bartlein, P.J. (1992): Global changes during the last 3 million years: climatic controls and biotic response. Annual Reviews in Ecology and Sytematics 23: 141-173.

Welsh, J. & Mc Clelland, M. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research 18: 7213-7218.

Williams, J.G.K.; Kubelik, A.R.; Livak, K.J. Rafalski, J.A. & Tingey, S.V. (1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research 18: 6531-6535.

Young, A.G.; Merriam, H.G. & Warwick, S.I. (1993): The effects of forest fragmentation on genetic variation in *Acer saccarum* Marsh. (sugar maple) populations. Heredity 71:277-289.

Young, A.; Boyle T. & Brown, T. (1996): The population genetic consequences of habitat fragmentation for plants. Tree 11/10: 413-418.

Young, A. & Clarke, G. (eds.) (2000): Genetics, Demography and Viability of Fragmented Populations. Cambridge University Press, Cambridge.

Zopfi, H.-J. (1993a): Ecotypic variation in *Rhinanthus alectorolophus* (Scopoli) Pollich (Scrophulariaceae) in relation to grassland management I. Morphological delimitations and habitats of seasonal ecotypes. Flora 188: 15-39.

Zopfi, H.-J. (1993b): Ecotypic variation in *Rhinanthus alectorolophus* (Scopoli) Pollich (Scrophulariaceae) in relation to grassland management II. The genotypic basis of seasonal ecotypes. Flora 188: 153-173.

Zopfi, H.-J. (1998): The genetic basis of ecotypic variants of *Euphrasia rostkoviana* Hayne (Scrophulariaceae) in relation to grassland management. Flora 193: 41-58.

II
Genetic diversity within and among populations of the rare glacial relict plant Saxifraga paniculata Mill. (Saxifragaceae) in central Europe

1 Introduction

Many plant species are currently threatened by extinction due to the destruction of their habitats resulting either directly or indirectly from human activities (Falk & Holsinger 1991). Habitat protection is, therefore, generally considered the most important step for the conservation of endangered species. However, the maintenance of genetic diversity is also regarded to be essential for the survival of endangered plants. High levels of genetic diversity allow the species to respond to the selection pressures imposed by pests and disease and to facilitate adaption to future environmental change (Barrett & Kohn 1991, Holsinger & Gottlieb 1991). The maintenance of existing levels of genetic diversity is, therefore, of considerable significance for the long-term survival of endangered plants.

Data on genetic diversity within and among populations of rare and endangered species play a significant role in the formulation of appropriate management strategies directed towards their conservation and development, besides being advantageous in the understanding of their structure, evolutionary relationships, taxonomy and demography (Milligan et al. 1994). Consequently, the study of population genetics has been identified as one of the main priorities for conservation (Holsinger & Gottlieb 1991).

Saxifraga paniculata Mill. (Saxifragaceae) is an arctic-alpine plant species, which is distributed widely in the Alps, but isolated outlying populations can also be found in the lower European highlands. These peripheral populations in the mountains of central Europe are considered to be rare and endangered glacial relict populations (Wilmanns & Rupp 1966, Walter & Straka 1970, Oberdorfer 1990). The present distribution of *Saxifraga paniculata* is a result of climatic changes after the last glaciation. It is supposed, that *S. paniculata* was widely distributed in the tundra, which covered once the unglaciated central European lowlands during the last glaciation (Wilmanns & Rupp 1966, Hantke 1978 and 1980, Lang 1994). Due to the warmer climatic conditions, which were not so suitable to an arctic-alpine plant species, population size and range of *Saxifraga paniculata* were greatly reduced since the end of the pleistocene (Bresinsky 1965). *Saxifraga paniculata*, therefore, is an "old rare" species in central Europe (Huenneke 1991), which includes small populations that were exposed to isolation for a very long time.

Small populations generally exhibit lower levels of genetic diversity relative to congeneric species with large populations sizes (Hamrick & Godt 1989). Causes for the reduction of genetic diversity can be attributed to the effects of inbreeding, leading to an increase in the number of homozygotes in the population, and genetic drift producing random changes in allele frequencies (Ellstrand & Elam

1993). Numerous studies have demonstrated that small, isolated populations of so called "new rare" species tend to have lower levels of genetic diversity than large, extensive populations (van Treuren et al. 1991, Raijmann et al. 1994, Prober & Brown 1994, Frankham 1996, Godt et al. 1996, Fischer & Matthies 1998, Menges & Dolan 1998). Habitat destruction and fragmentation of plant populations break up large, genetically variable populations into isolated, small, inbreeding subpopulations that are then subject to the consequences of drift (Ellstrand & Elam 1993, Oostermeijer et al. 1996, Young et al. 1996).

Although little is known about the genetic diversity within and among populations of glacial relicts in central Europe (Dannemann 2000, Lutz et al. 2000), it is supposed that „old rare" species are better adapted to processes connected to small population size and isolation (Schmidt & Jensen 2000). Numerous studies from North America provide substantial evidence that putative relict plant populations harbour high levels of genetic diversity (Lewis & Crawford 1995, Soltis et al. 1997, Allphin et al. 1998). It seems to be a typical phenomenon, that plants from glacial refugia show high levels of diversity. Therefore, this study focuses on the genetic diversity within and among rare glacial relict populations of *Saxifraga paniculata*.

Despite its rarity, *Saxifraga paniculata* shows an extensive clonal reproduction. Although clonal growing species are thought to exhibit low levels of genetic diversity, only a few studies exist, which have revealed low levels of genetic variation in clonal plants (Sharitz et al. 1980, Gray et al. 1991). For large angiosperm clones with little clonal intermingling intraclonal pollination may occur and lead to high levels of inbreeding and subsequent inbreeding deppression (Luijten et al. 1996). However, most investigations of clonal plants report of at least moderate levels of genetic variation within populations (Ellstrand & Roose 1987, Hamrick & Godt 1989). For this reason, the study also focuses on the effects of clonal reproduction on the genetic variability within populations of rare and endangered species.

Random Amplified Polymorphic DNA (RAPD) analysis is a PCR-based assay capable of analysing genetic relatedness among and within populations (Welsh & McClelland 1990, Williams et al. 1990). RAPD analysis has been successfully used for plant germplasm characterization in a large number of studies (Kump & Javornik 1995, Chan & Sun 1997, Whitkus et al. 1998, Ahmad 1999, Fahima et al. 1999, Mengistu et al. 2000). Its utilization in investigations of the genetic diversity of rare or threatened plant species is also proven (Rossetto et al. 1995, Smith & Pham 1996, Martin et al. 1997, Palacios & Gonzalez-Candelas 1997, Cardoso et al. 1998, Ayres & Ryan 1999, Maki & Horie 1999, Dannemann 2000, Tansley & Brown 2000). Furthermore, RAPD analysis has been successfully used to detect genetic diversity in different species of Saxifraga (Bauert et al. 1998, Tollefsrud et al. 1998, Gugerli et al. 1999). In addition, the use of the analysis of molecular variance technique (AMOVA) (Excoffier et al. 1992) to analyse RAPD marker variation has been shown to be effective for population analysis of highly heterozygous, outcrossing plant species (Huff et al. 1993, Huff 1997).

The sequencing of the internal transcribed spacer (ITS) region of nuclear 18S-26SrDNA can also be used for detecting genetic variation at the population level. The ITS region has become widely used by systematists (Baldwin et al. 1995) and has also proven to be informative for biogeographic studies in some angiosperm genera (Baldwin 1993, Vargas et al. 1998). Sequencing of the ITS region seems to be promising for detecting variation in *Saxifraga* even at the populational level (Brochmann et al. 1998), and it was used successfully for an investigation of *S. tridactylites* and *S. osloensis*. (Brochmann et al. 1996).

Previous studies of *Saxifraga paniculata* centered on systematics (Conti et al. 1999), morphology (Ovstedal 1998) and ecophysiology (Neuner et al. 1999). Data on the genetic diversity of the rare *Saxifraga paniculata*, however, have not been reported to date. In this investigation RAPD analysis, AMOVA and ITS analysis were used to detect genetic diversity within and among populations of the arctic-alpine plant species *Saxifraga paniculata* Mill. (Saxifragaceae).

The following questions were addressed:
(1) What is the effect of long-term isolation since the end of the last glaciation on partitioning of molecular variance and on levels of genetic diversity?
(2) Is there a geographic differentiation among the populations?
(3) What is the effect of population size, mating system, longevity and clonality on the genetic diversity within and among populations?
(4) Are there different levels of genetic diversity in populations from different regions and of different size?

2 Material and methods

2.1 Species description

Saxifraga paniculata Mill. (Saxifragaceae) is a perennial, prostrate and iteroparous arctic-alpine plant species that forms multiramet cushions and mats which consist out of hemispherical rosettes of one to six centimeters in diameter. The leaves are obovate to oblong-lingulate, semisucculent, evergreen and usually lime-encrusted (Webb & Gornall 1989). The flowering stem is branched only in its upper third to form a small panicle with white or pale cream flowers of common *Saxifraga* type. The cushions and mats consist of numerous non-flowering and flowering rosettes with flowering ramets dying off after seed set. The insect-pollinated, diploid *S. paniculata* (2n = 28) is mainly outbred, selfing has only been observed in the high arctic up to now (Kaplan 1995).
Dispersal occurs by seeds and vegetatively via detachment of rosettes, which easily root again (Wilmanns & Rupp 1966). *S. paniculata* lives in rocky habitats: in crevices, on ledges and in sparsely growing plant communities on rock debris (Kaplan 1995). The cushion plant prefers limestone, but also occurs on slate or porphyry.

S. paniculata is distributed throughout the mountains of central and southern Europe, from the Vosges and central Poland to northern Spain, southern Italy and central Greece (Webb & Gornall 1989). It also occurs in the arctic from Norway, Iceland and Greenland to the northeastern North America (Labrador, Baffinsland, Quebec, Ontario, Newfoundland, New Brunswick and Vermont).
In the Alps *S. paniculata* is widely distributed, but there also exist isolated outlying populations in the other European highlands. These rare peripheral populations in the lower mountains of central Europe are thought to be glacial relict populations (Wilmanns & Rupp 1966, Walter & Straka 1970). *S. paniculata* in Germany is endangered because of its rareness (Korneck et al. 1996) and, therefore, registered in the red list of threatened species. *Saxifraga paniculata* is legally protected in Germany.

S. paniculata is morphologically very variable (Ovstedal 1998), especially in the Alps. Engler & Irmscher (1919) describe a lot of different varieties, but most of them are not accepted (Kaplan 1995). Webb & Gornall (1989) subdivide the species into a typical subspecies (*ssp. paniculata*) and a subspecies from Caucasia (*ssp. cartilaginea*). However, a satisfying intraspecific classification does not exist up to now.

2.2 Study sites

To investigate the genetic diversity within and among populations of *Saxifraga paniculata*, 30 populations at 17 locations in west and southwest Germany, in the northern and southern Alps and in Romania were selected (Table 1). The three populations from west Germany were located near Kirn in the valley of the Nahe, whereas three of the nineteen southwest German populations were sampled on the western Swabian Alb near Balingen and Fridingen. Five populations were located

on the middle Swabian Alb near Münsingen and Metzingen, while ten populations grew on the eastern Swabian Alb near Geislingen and Ulm. Only one population originated from the Black Forest. The north alpine populations grew on the Nebelhorn near Oberstdorf, while the south alpine populations were located near Bozen in South Tyrol. The plants from Romania grew in the valley of Thorenburg near Turda (Figure 2).

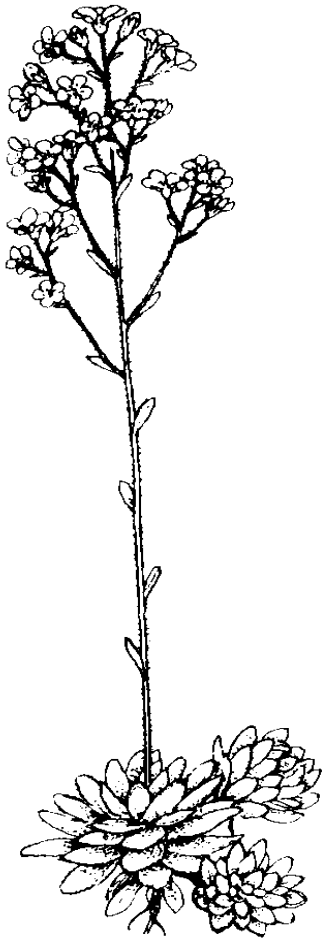

Figure 1: Habit of *Saxifraga paniculata* (taken from Rothmaler 1991). The plant forms multiramet cushions and mats. The flowering stem is branched only in its upper third to form a small panicle with white or pale cream flowers of common *Saxifraga* type.

2.3 Plant material and sampling conditions

In spring and summer of 1999 plant material was collected in situ from all selected populations. Only few young leaves per plant were sampled to minimize the impact on the rare plants. Two populations were considered as separated when they were at least 70 m apart from each other. If possible, leaf material was sampled from four plants per population. Only at Hausen and Kahlenstein, where the populations consisted of only one to three plants, less material was collected. The populations Brunkenstein 1, Lochenstein and Wutachschlucht were sampled by collecting leaves of eight to twelve plants on the right side, in the middle and on the left side of the population. At Lochenstein and Hausen 2 leaf material was picked from ten and five rosettes within growing mats of two and one square me-

Table 1: Sampled populations of *Saxifraga paniculata* with abbreviation (Abb.) description of location, region, population size (P. size = number of counted individuals), geographical latitude (Lat.), longitude (Long.), altitude (Alt), sample size (S. size = number of collected individuals) and geological underground.

Nr	Population	Abb.	Location	Region	P.size	Lat. (N)	Long. (E)	Alt.	S. size	Geology
1	**Brunkenstein 1**	Bru 1	Brunkenstein	West Germany	400	49°49´	7°31´	220	12	Green slate
2	**Brunkenstein 2**	Bru 2	Brunkenstein	West Germany	22	49°50´	7°29´	220	4	Green slate
3	**Brunkenstein 3**	Bru 3	Brunkenstein	West Germany	12	49°49´	7°29´	220	4	Green slate
4	**Eichbühl**	Eic	Eichbühl	Southwest Germany	83	48°18´	9°30´	600	4	Limestone
5	**Gerberhöhle**	Ger	Gerberhöhle	Southwest Germany	9	48°18´	9°30´	600	4	Limestone
6	**Finstertal**	Fin	Finstertal	Southwest Germany	12	48°04´	9°00´	750	4	Limestone
7	**Altstadtfels**	Alt	Altstadtfels	Southwest Germany	18	48°03´	8°58´	800	4	Limestone
8	**Hausen 1**	Hau 1	Hausen	Southwest Germany	4	48°37´	9°47´	710	3	Limestone
9	**Hausen 2**	Hau 2	Hausen	Southwest Germany	3	48°37´	9°47´	710	2	Limestone
10	**Kahlenstein 1**	Kah 1	Kahlenstein	Southwest Germany	2	48°36´	9°48	650	2	Limestone
11	**Kahlenstein 2**	Kah 2	Kahlenstein	Southwest Germany	1	48°36´	9°48	650	1	Limestone
12	**Roßberg 1**	Roß 1	Roßberg	Southwest Germany	22	48°30´	9°20´	700	4	Limestone
13	**Roßberg 2**	Roß 2	Roßberg	Southwest Germany	14	48°30´	9°20´	700	4	Limestone
14	**Roßberg 3**	Roß 3	Roßberg	Southwest Germany	60	48°30´	9°20´	700	4	Limestone
15	**Lautertal 1**	KLt 1	Lautertal	Southwest Germany	8	48°26´	9°52´	600	4	Limestone
16	**Lautertal 2**	KLt 2	Lautertal	Southwest Germany	10	48°26´	9°52´	600	4	Limestone
17	**Lautertal 3**	KLt 3	Lautertal	Southwest Germany	7	48°26´	9°52´	600	4	Limestone
18	**Lautertal 4**	KLt 4	Lautertal	Southwest Germany	15	48°26´	9°52´	600	4	Limestone
19	**Weilerhalde 1**	Wei 1	Weilerhalde	Southwest Germany	9	48°24´	9°47´	650	4	Limestone
20	**Weilerhalde 2**	Wei 2	Weilerhalde	Southwest Germany	17	48°24´	9°47´	650	4	Limestone
21	**Lochenstein**	Loc	Lochenstein	Southwest Germany	62	48°11´	8°50´	950	12	Limestone
22	**Wutachschlucht**	Wut	Wutachschlucht	Southwest Germany	60	47°52´	8°16´	700	8	Porphry
23	**Zeigergrat**	Zei	Zeigergrat	Northern Alps	4	47°24´	10°21´	1750	4	Marl
24	**Seealpe**	See	Seealpe	Northern Alps	93	47°25´	10°21´	1650	4	Marl
25	**Karneid 1**	Kar 1	Karneid	Southern Alps	121	46°29´	11°25´	900	4	Porphyry
26	**Karneid 2**	Kar 2	Karneid	Southern Alps	39	46°29´	11°25´	900	4	Porphyry
27	**Karneid 3**	Kar 3	Karneid	Southern Alps	180	46°29´	11°25´	900	4	Porphyry
28	**Steinegg 1**	Ste 1	Steinegg	Southern Alps	147	46°30´	11°26´	1000	4	Porphyry
29	**Steinegg 2**	Ste 2	Steinegg	Southern Alps	27	46°30´	11°26´	1000	4	Porphyry
30	**Turda**	Tur	Turda	Romania	-	46°33´	23°41´	440	3	Limestone

meters, respectively. Plant material was placed into plastic bags and stored on ice in the field for transportation and in the laboratory at -35°C. After sampling the plant material the population size was determined by counting individuals (every continous cushion was considered as one plant) and the associated plant species were reported to describe the plant communities in which *Saxifraga paniculata* grew.

2.4 Population size and plant communities

In 1999 the investigated population sizes ranged from one to 400 individuals per population, most of them including population sizes between 10 and 100 individuals. Nine of 30 populations consisted of less than 10 and only four populations comprised more than 100 individuals (Table 1).

The investigated populations of *Saxifraga paniculata* were located on rocks and debris, in crevices, on ledges of limestone, slate, marl and porphyry as well as in plant communities, which were typical for rocky habitats (Table 2). The plants from west Germany grew in the *Saxifraga paniculata* association whereas the populations from southwest Germany were located in the *Drabo-Hieracietum humilis*, the *Diantho-Festucetum pallentis*, the *Alysso-Sedetum alyssoides* or the *Valeriano-Seslerietum albicantis*. In contrast the north alpine populations were located in the *Seslerio-Caricetum sempervirentis* and the south alpine populations in the *Sclerantho-Sempervivetum arachnoidei*.

Table 2: Plant communities in which the investigated populations of *Saxifraga paniculata* were located, with description of characteristic species and the geographic region.

Plant community	Characteristic species	Region
Saxifraga paniculata association	*Asplenium trichomanes, Polypodium vulgare, Sedum rupestre*	West Germany
Drabo-Hieracietum humilis	*Draba aizoides, Hieracium humile, Asplenium trichomanes*	Southwest Germany
Diantho-Festucetum pallentis	*Dianthus gratianopolitanus, Alyssum montanum, Minuartia setacea*	Southwest Germany
Alysso-Sedetum alyssoides	*Sedum album, Sedum acre, Thlaspi perfoliatum*	Southwest Germany
Valeriano-Seslerietum albicantis	*Valeriana tripteris, Sesleria albicans, Carduus defloratus*	Southwest Germany
Seslerio-Caricetum sempervirentis	*Gentiana clusii, Nigritella nigra, Carex sempervirens*	Northern Alps
Sclerantho-Sempervivetum arachnoidei	*Sempervivum arachnoideum, Asplenium septentrionale, Sedum dasyphyllum*	Southern Alps

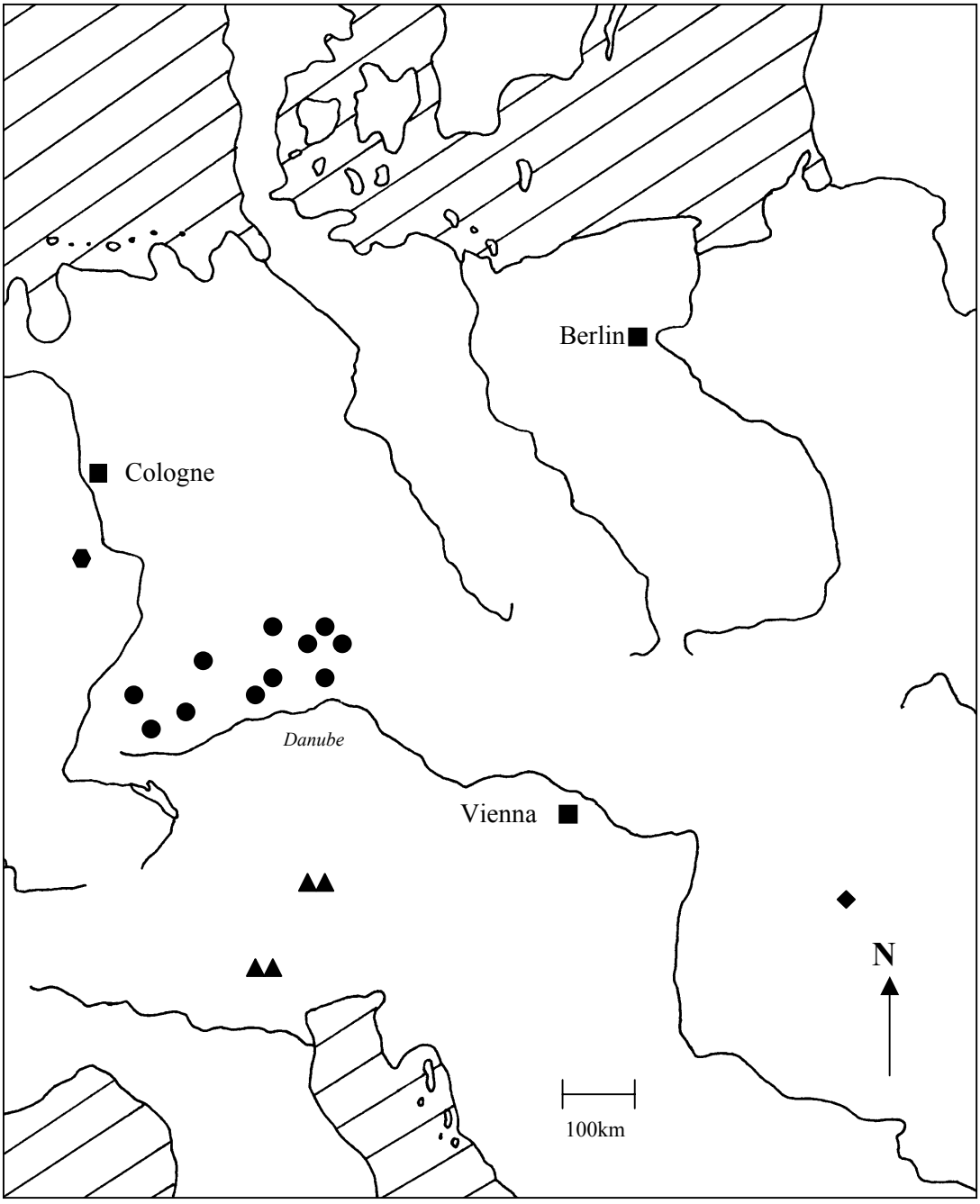

Figure 2: Geographic position of the study sites. 30 populations of *Saxifraga paniculata* were investigated at 17 locations in west Germany (hexagon) and southwest Germany (circles), in the northern and southern Alps (triangles) and in Romania (rhombus).

2.5 DNA isolation

The DNA was isolated from frozen plant material of individual plants using the CTAB (cetyltriammonium bromide) method (Rogers & Bendich 1988) adapted as follows: Approximately 40-60 mg leaf material was ground in liquid nitrogen in a 1.5 mL Eppendorf tube followed by addition of 700 µL extraction buffer (100 mM Tris HCl, pH 9.5; 20 mM EDTA, pH 8.0; 1.4 M NaCl; 1% PEG; 2% CTAB; 2.5 µL/mL β-mercapto ethanol). Incubation was at 74°C for 30 min, shaking every 5-10 min. Subsequently the mixture was extracted twice with an equal volume of chloroform/isoamylalcohol (24:1), and centrifuged at 15,000g at 4°C for 10 min. Adding an equal volume of isopropanol (5°C) the DNA was precipitated and pelleted by centrifugation at 20,000g at 4°C for 15 min. The DNA was washed with 70 % cold ethanol (4°C) for 5 min and air-dried for 15-30 min. The DNA was resuspended in 200 µL TE buffer (10 mM Tris-HCl, pH 8.0; 1mM EDTA, pH 8.0). The concentration was estimated spectrophotometrically (Uvikon 930, Kontron Instruments, Germany) at 260 nm, and the purity measured by the ratio of the absorbances at 260 nm and 280 nm. For PCR only template DNA was used with a purity of 1.8-2.1 in a dilution of 15 ng/µL.

2.6 RAPD analysis

2.6.1 DNA amplification

For DNA amplification the polymerase chain reaction (PCR) was used with arbitrary 10-mer oligonucleotide primers (Roth, Karlsruhe, Germany) for RAPD amplification (Williams et al. 1990). All primers employed in this study were random sequence primers (Table 3) with G+C contents of up to 70 %. A total of 75 primers were initially screened for amplification on *S. paniculata*.

In a final volume of 15 µL amplification mixtures contained 10 mM Tris-HCl (pH 9.5), 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM dNTP, 0.3 µM primer, 1 U Taq polymerase (Pharmacia, Freiburg, Germany) and 30 ng of genomic DNA.

The mixture was overlaid with two drops of mineral oil. The polymerase chain reaction (PCR) was run in a thermal cycler (Autogene, Grant Instruments, Cambridge, UK). The thermal cycling program started with denaturation for 120 s at 94°C, followed by 35 cycles of 12 s denaturation at 94°C, 48 s annealing at 36°C and 90 s extension at 72°C. A final extension at 72°C for 10 min concluded the DNA amplification.

PCR products were kept at 4°C until they were loaded. The amplificated products were separated on 1.5 % agarose gels in 1×TBE (Tris-borate-EDTA) buffer (Sambrook et al. 1989) at 180 V for 1.5 h, using a 100 base-pair-ladder as a fragment size marker (Roth, Karlsruhe, Germany) and visualized by ethidium bromide staining. Each sample was repeated at least once in a separate amplification reaction.

2.6.2 Data scoring

For data scoring, the banding patterns were recorded using a trans-illuminating gel documentation system (Gel Print 2000i, BioPhotonics Corporation, Ann Arbor, USA). The image profiles and molecular weight of each band were determined by the program RFLPSCAN (Scanalytics CSPI Inc., Billerica, USA). Pictures were examined for strong, clearly defined bands, reproducible in duplicate amplifications. Each band was scored across all individuals as either present or absent. When individuals did not give clear, easily scored signals, all bands of this fragment size were excluded from the analysis.

In the data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0. The basic data structure finally consisted of a binomial (0,1) matrix of 131 rows, representing the investigated individuals and 319 columns, representing the scored RAPD markers. Since RAPD markers are dominant, it was assumed, that each band represented the phenotype at a single bi-allelic locus (Williams et al. 1990).

2.6.3 Statistical analysis

The binomial matrix was used to calculate the level of polymorphism (percentage of polymorphic bands) for each population and to compute similarities between individuals of *S. paniculata* using the Jaccard's similarity coefficient, calculated as $J = a/(n-d)$, where a is the number of positive matches (i.e. the presence of a band in both samples), d is the number of negative matches (i.e. the absence of a band in both samples), and n is the total sample size including both the numbers of matches and „unmatches". The genetic distances were calculated as $GD = 1-J$ using the data from the Jaccard's similarity coefficient matrix. The minimum, maximum and mean genetic distance between the individuals for each population were compared in order to describe the molecular variance within the populations.

The genetic relatedness among species was obtained by clustering. Dendrograms were generated from the similarity coefficient matrix using the unweighted pair group method (UPGMA) as described by Sneath & Sokal (1973). The calculation of the Jaccard's similarity coefficient matrix and the generation of the bootstrapped dendrograms was done with the program TREECON 1.3b (van de Peer & de Wachter 1994).

The binomial matrix was also applied to an analysis of molecular variance in RAPD patterns by the program WINAMOVA 1.55 (Excoffier et al. 1992, Stewart & Excoffier 1996). AMOVA was originally developed for RFLP haplotypes, but it is also appropriate for RAPD phenotypes (Huff et al. 1993, Palacios et al. 1997). AMOVA analyses were based on the pairwise squared euclidian distances among molecular phenotypes, which are equal to the number of different band states, because band states can only take the values 0 or 1. Since a sum of squares in a conventional analysis of variance (ANOVA) can be written as a sum of all squared pairwise differences, AMOVA is closely related to ANOVA. It allowed the calculation of variance components and their significance levels for variation

among groups of populations, among populations within groups and within populations. Additionally the AMOVA sums of squares divided by n-1 were calculated for each population to describe the molecular variance within the populations. Because significance tests in AMOVA are based on permutation procedures, they are essentially assumption free (Excoffieret al. 1992). The program also extracts analogs of F statistics (so-called Φ statistics).

Homogeneity of molecular variance in pairs of populations was tested using Bartlett tests (Bartlett 1937), which are also implemented in the WINAMOVA 1.55 program. Pairwise genetic distances (Φ_{ST}) among the 30 populations and their levels of significance were also obtained from the AMOVA. These values allow the estimation of gene flow as the number of individuals migrating between populations per generation, using the equation of Wright (1951) (N_em = ¼[1/Φ_{ST}-1]), where N_em is the product of effective population size and migration rate, and Φ_{ST} is obtained from the AMOVA. For each analysis, 1,000 permutations were performed to obtain significance levels. To investigate possible differences bet-ween the main geographical regions, two different AMOVAs were carried out, in which the populations were grouped together not only according to their locations but also according to their regions of origin.

A Mantel test was used to determine whether the matrix of mean genetic distances (mean Φ_{ST}) between the locations was correlated with the matrix of geographic distances between the locations or not (Mantel 1967). The Mantel test was conducted with the program TFPGA 1.3 (Miller 1997).

To analyze the influence of population size on the genetic diversity of populations, it was calculated whether populations size correlated with the level of polymorphism, the maximum genetic distance taken from the Jaccard similarity coefficient matrix, or the AMOVA sums of squares divided by n-1.

2.7 ITS analysis

For ITS analysis individuals of thirteen populations (Eichbühl, Wutachschlucht, Brunkenstein 1, Weilerhalde 1, Lochenstein, Lautertal 1, Roßberg 1, Kahlenstein 1, Finstertal, Karneid 1, Steinegg 1, Zeigergrat, Turda) were selected from all five geographic regions (Table 1). The nuclear ITS region, comprising ITS 1, the 5.8SrDNA, and ITS 2, was amplified by PCR (polymerase chain reaction) using the primer pair ITS5-307R (for sequences see Conti et al. 1999). Amplifications were performed with the following temperature cycle repeated 35 times: 1 min at 94°C, 2 min at 45°C and 3 min at 72°C. Amplified products were cleaned using the NucleoSpin Extract (Macherey-Nagel, Düren, Germany), following the protocols provided by the manufacturer.

Purified products were then sequenced directly using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Weiterstadt, Germany). For cycle sequencing the primer pair ITS5-307F was used. Unincorpo-

rated dye terminators were removed using a 3 mol/L sodium acetate and absolute alcohol precipitation, as recommended by the manufacturer. Samples were then loaded in a 5% polyacrylamide gel on an ABI 373A DNA Sequencer (Applied Biosystems, Weiterstadt, Germany). Sequencing data were edited and assembled using the program Sequencher 3.1.1 (GeneCodes Corporation, Ann Arbor, USA). Thirteen sequences of *Saxifraga paniculata* were compared and aligned by visual inspection using the same program. To find the optimal model of sequence evolution, the program Modeltest 3.04 (Posada 2000) was used. The sequence matrix was finally used to construct an UPGMA dendrogram with the program PAUP 4.0b5 (Swofford 2001).

3 Results

3.1 RAPD banding

Five samples were randomly selected from the thirty populations of *Saxifraga paniculata* and subjected to RAPD analysis with 75 decamer random primers. Amplification products were obtained with all individuals and primers from which fifteen were selected giving rise to clear reproducible and distinct banding patterns.

In total 319 reproducible fragments were amplified with a varying number per primer i.e. B06 produced 30 scorable bands, while primer I16 only amplified 12 fragments (Table 3). The size of the amplified fragments ranged from 336 to 3,687 bp. The fifteen primers produced between 61.5 % and 100 % polymorphic bands. In total 91.2 % of the bands were polymorphic, only 8.8 % were scored in all individuals from all populations. Reflecting this high level of polymorphism, the investigated populations represented 126 different RAPD phenotypes, while only two plants showed the same banding pattern.

Table 3: Primers employed with the number of RAPD markers obtained, their sequence, the size of the fragments, and the percentage of polymorphic markers (P) for each primer.

Primer	Sequence (5´→3´)	[CG] %	Size (bp) Min-max	Polymorphic Bands	Monomorphic Bands	Total	P (%)
B01	- GTT TCG CTC C -	60	563 – 2109	22	1	23	95,7
B06	- TGC TCT GCC C -	70	762 – 3240	26	4	30	86,7
B07	- GGT GAC GCA G -	70	507 – 2866	26	0	26	100
B11	- GTA GAC CCG T -	60	460 – 2125	24	0	24	100
B12	- CCT TGA CGC A -	60	336 – 1954	24	1	25	96
B18	- CCA CAG CAG T -	60	564 – 3810	16	3	19	84,2
D03	- GTC GCC GTC A -	70	486 – 2161	21	2	23	91,3
D08	- GTG TGC CCC A -	70	404 – 1893	18	2	20	90
D11	- AGC GCC ATT G -	60	567 – 2772	27	1	28	96,4
D18	- GAG AGC CAA C -	60	381 – 1486	22	1	23	95,7
I02	- GGA GGA GAG G -	70	561 – 3189	17	2	19	89,5
I03	- CAG AAG CCC A -	60	459 – 1843	8	5	13	61,5
I04	- CCG CCT AGT C -	70	967 – 3731	12	2	14	85,7
I11	- ACA TGC CGT G -	60	357 – 3687	18	2	20	90
I16	- TCT CCG CCC T -	70	463 - 2137	10	2	12	83,3
Total			336 – 3687	291	28	319	91,2

3.2 AMOVA analysis and variance partitioning

When the 30 populations were arranged in 17 groups according to their locations (Table 1 and 4), 43.60 % molecular variance was found within the populations, followed by 40.95 % variation among the locations and only 15.45 % among the populations within the different locations.

The variation in RAPD banding patterns among populations, among populations within locations and within populations was highly significant (AMOVA $P<0.001$). The correlation of RAPD phenotypes within locations relative to the correlation of random pairs drawn from the whole sample **(Φ_{ST})** was 0.56, the correlation among random phenotypes within populations relative to the correlation of random pairs drawn from the whole sample **(Φ_{CT})** was 0.40, and the correlation of random phenotypes within locations, relative to that of random pairs drawn from the population **(Φ_{SC})**, was 0.26.
Different results were obtained when the populations were arranged in five groups corresponding to their region of origin. In this case, only 28.3 % variation was found among regions, while 39.1 % variation was observed within populations and 32.6 % variance was found among the different populations within the regions (Table 4). In this analysis the variation in RAPD banding pattern was also highly significant (AMOVA $P<0.001$). Φ_{ST} was 0.60, while Φ_{CT} and Φ_{SC} were 0.28 and 0.45, respectively.
The AMOVA calculation of molecular variance was carried out for only 28 of the 30 populations of *S. paniculata*, because the populations number 10 and 11 were monomorphic. Molecular variance was significantly different among the 28 populations ($P<0.001$). Of the 378 pairwise Bartlett tests of homogeneity of population variation 370 tests showed highly significant differences between the populations (Table A1, appendix). Only eight tests were not significant.

Table 4: Summary of analysis of molecular variance (AMOVA). Plants represented 30 populations of *Saxifraga paniculata* that were growing at 17 locations in five regions (see Table 1). The analysis was based on RAPD phenotypes consisting of 319 band states. Levels of significance were based on 1,000 iteration steps. (SS: sum of squares, MS: mean squares, %: apportionment of genetic variability, *P*: level of significance).

Level of variation	SS	MS	%	*P*
Populations grouped together in 17 locations:				
Among locations	1967.730	131.182	40.95	<0.001
Among populations within locations	386.379	32.198	15.45	<0.001
Within populations	1306.250	13.063	43.60	<0.001
Populations grouped together in 5 regions:				
Among regions	971.832	242.958	28.30	<0.001
Among populations within regions	1465.520	58.621	32.60	<0.001
Within populations	1306.250	12.933	39.10	<0.001

3.3 Genetic distances (Φ_{ST}) and geneflow among populations

411 of the 435 pairwise genetic distances (Φ_{ST}) between populations were highly significant (Table A2, appendix). Only two had levels of marginal significance ($P<0.05$). Maximum Φ_{ST} (varying between 1 and 0) was 0.96, found between the southwest German population Kahlenstein 1 and the Romanian population Turda.

Minimum Φ_{ST} was only 0.01, which was observed between the populations Karneid 1 and Karneid 3 in the southern Alps.
From the genetic distances (Φ_{ST}) the geneflow was calculated as the number of individuals migrating between populations per generation (Table A3, appendix), using the equation of Wright (1951). Mean geneflow between populations was 0.36, which means that less than one individual per generation is migrating between the populations. The geneflow was also calculated among and within the different localities and found to give very low levels of migration among the localities (0.26) but a considerably higher geneflow between the populations within the same localities (2.86).
The mean genetic distance (Φ_{ST}) and the geographic distance between the different growing locations were determined to investigate the connection between geographic distance and genetic distance. The result clearly shows: geographic distance did explain the genetic distance among locations. The matrix of 136 pairwise genetic distances (Φ_{ST}) among the 17 locations of *S. paniculata* was highly significantly correlated with the corresponding matrix of geographic distances. The calculated Mantel test shows a correlation coefficient of $r = 0.63$ and a significance of $p<0.001$ (Figure 3). Maximum values of mean genetic distance (Φ_{ST}) can be found between the central European locations and the Romanian location Turda, which is geographically most distant to all other locations.

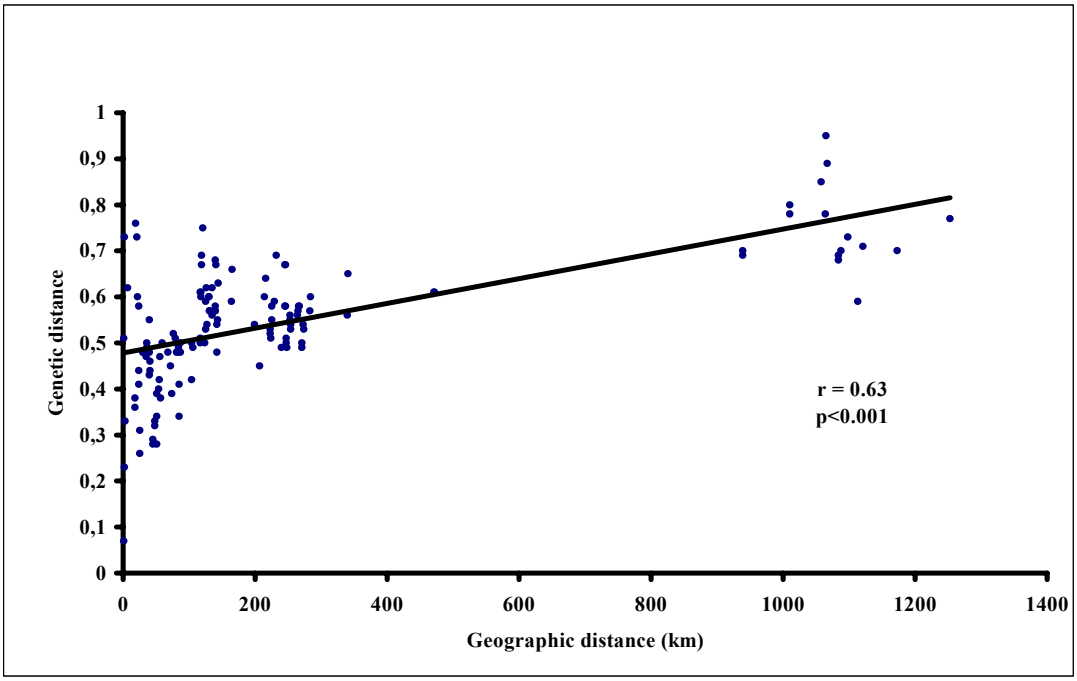

Figure 3: The calculated Mantel test showed the correlation between the mean genetic distances (Φ_{ST}) and the geographic distances among the 17 locations of *Saxifraga paniculata* in central Europe, the Alps and Romania.

3.4 Genetic diversity within relict populations

The 30 populations of *S. paniculata* showed varying levels of polymorphism. The southwest German populations Kahlenstein 1 and 2 were populations of a very small size (one and two individuals) and no polymorphisms. Levels of polymorphism and genetic distances among individuals therefore could not be calculated for these populations. In the other populations the levels of polymorphism ranged from 4.2 % in the Turda population to 53.7 % in the Lochenstein population.
The genetic distances between all investigated individuals within the populations were taken from the Jaccard's similarity coefficient matrix, also showing very different values (Table 5). Minimum and maximum genetic distance within populations ranged from 0 % and 4.1 %, respectively, in the Turda population, to 23.2 % and 30.8 %, respectively in the Steinegg 1 population. The mean genetic distance varied between 2.8 % in the Turda population and 25.0 % in the Steinegg 1 population. The sums of euclidian squares/n-1, taken from the AMOVA analysis, can also be used as a measurement of genetic diversity and ranged from 6.2 in the Lautertal 1 population to 18.5 in the Gerberhöhle population. The lowest levels of polymorphism and genetic distances were, therefore, determined for the Romanian Turda population and the highest for the alpine Steinegg 1 population.

Table 5: Percentage of polymorphic bands and genetic distances within all investigated populations of *Saxifraga paniculata*.

Nr	Population	Polymorphic bands (%)	Min Genetic Distance Jaccard (%)	Max Genetic Distance Jaccard (%)	Mean Genetic Distance Jaccard (%)	Sums of euclidian squares/n-1
1	**Brunkenstein 1**	37.7	06.6	21.0	15.2	17.1
2	**Brunkenstein 2**	23.9	10.5	18.8	14.7	10.0
3	**Brunkenstein 3**	18.1	08.3	14.9	11.1	07.4
4	**Eichbühl**	37.5	19.6	26.7	22.2	17.3
5	**Gerberhöhle**	38.2	20.5	26.6	23.7	18.5
6	**Finstertal**	36.1	19.3	26.4	22.2	17.9
7	**Altstadtfels**	37.2	13.7	24.8	21.5	16.8
8	**Hausen 1**	11.9	00.0	11.9	07.9	10.3
9	**Hausen 2**	13.0	12.9	12.9	06.5	08.5
12	**Roßberg 1**	28.5	12.2	18.1	15.7	11.3
13	**Roßberg 2**	37.7	17.5	27.7	22.4	17.3
14	**Roßberg 3**	37.7	19.0	25.7	22.5	17.2
15	**Lautertal 1**	15.9	01.5	12.7	08.7	06.2
16	**Lautertal 2**	22.5	09.9	15.8	12.0	08.6
17	**Lautertal 3**	22.5	05.8	16.4	12.9	09.3
18	**Lautertal 4**	18.3	01.4	14.9	10.1	07.7
19	**Weilerhalde 1**	25.5	09.7	20.9	15.0	11.0
20	**Weilerhalde 2**	27.2	14.0	19.4	16.9	12.2
21	**Lochenstein**	53.7	10.8	30.6	23.4	17.8
22	**Wutachschlucht**	40.0	12.2	21.9	17.0	12.8
23	**Zeigergrat**	27.7	12.8	21.2	15.9	11.8
24	**Seealpe**	29.9	14.1	22.8	17.4	13.0
25	**Karneid 1**	40.6	22.6	25.3	24.4	17.8
26	**Karneid 2**	26.0	02.3	21.5	15.9	11.4
27	**Karneid 3**	39.8	20.7	27.9	24.1	17.6
28	**Steinegg 1**	41.5	23.2	30.8	25.0	18.3
29	**Steinegg 2**	33.3	18.0	22.4	20.9	15.1
30	**Turda**	04.2	00.0	04.1	02.7	17.6

3.5 Genetic diversity within populations from different geographic regions

To compare the three main geographic regions (west Germany, southwest Germany and the Alps) of the investigated *S. paniculata* populations, the mean levels of polymorphism and mean genetic distances were calculated (Table 6). The mean percentage of polymorphic bands ranged from 26.6 % in west Germany to 29.6 % in southwest Germany and 34.1 % in the Alps. The minimum genetic distance varied from 8.5 % in west Germany to 11.8 % in southwest Germany and 16.2 % in the Alps. Maximum genetic distance showed values between 18.2 % in west Germany, 21.2 % in southwest Germany and 24.5 % in the Alps. The mean genetic distance ranged from 13.7 % in west Germany to 16.5 % in southwest Germany and 20.5 % in the Alps. The sums of euclidian squares/n-1 varied between 11.5 in west Germany, to 13 in southwest Germany and 15 in the Alps. The lowest levels of polymorphism and genetic distances were, therefore, determined for west Germany and the highest for the Alps.

Table 6: Genetic variation within the populations of *Saxifraga paniculata* in west Germany, southwest Germany and the Alps. For each geographic region mean percentage of polymorphic bands and mean genetic distances were calculated.

Region	West Germany	Southwest Germany	Alps
Polymorphic bands (%)	26.6	29.6	34.1
Jaccard-Distance Min (%)	08.5	11.8	16.2
Jaccard-Distance Max (%)	18.2	21.2	24.5
Jaccard-Distance Mean (%)	13.7	16.5	20.5
Sums of euclidian squares/n-1	11.5	13.0	15.0

3.6 Molecular variance and population size

The *S. paniculata* population sizes ranged from a very large number of plants (400) to just one individual (Table 1). The level of polymorphism correlated highly significantly with the population size (Spearman's rank-correlation coefficient r_s= 0.67, P<0.001, Figure 4a). A significant correlation was also observed between population size and maximum genetic distance, taken from the Jaccard's similarity index matrix (r_s=0.59, P<0.005, Figure 4b). The sums of euclidian squares/n-1, which also can be used for the measuremant of genetic diversity within populations, were only marginally significantly correlated with the population size (r_s=0.59, P<0.05, Figure 4c).

The results of the correlation analysis clearly show that smaller populations harbour lower levels of genetic diversity than larger populations.

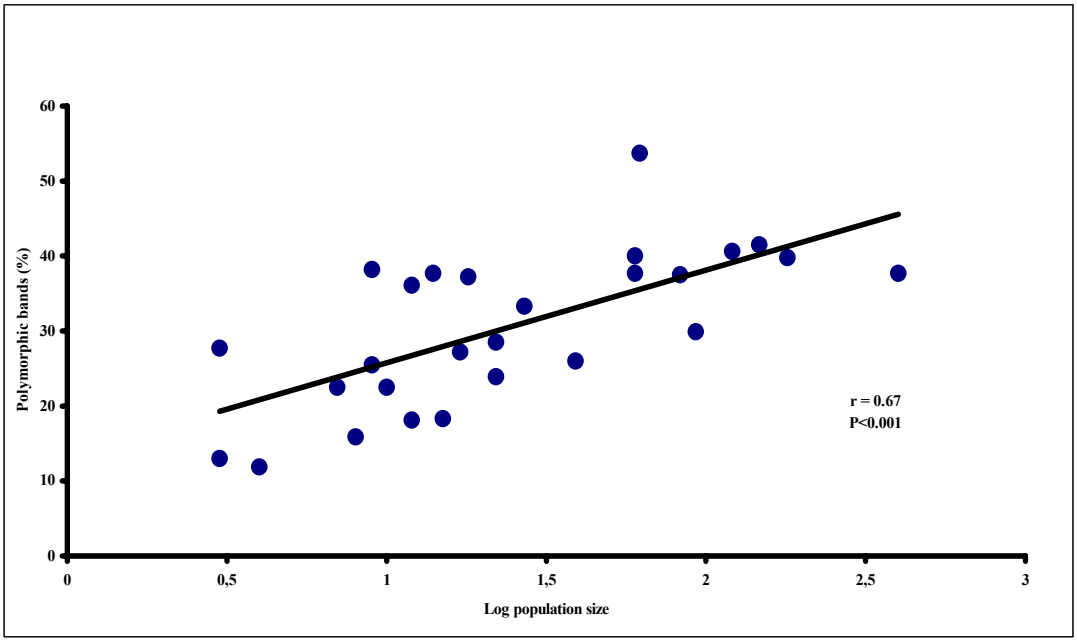

Figure 4a: Correlation between population size and the percentage of polymorphic bands per population, calculated for *Saxifraga paniculata*.

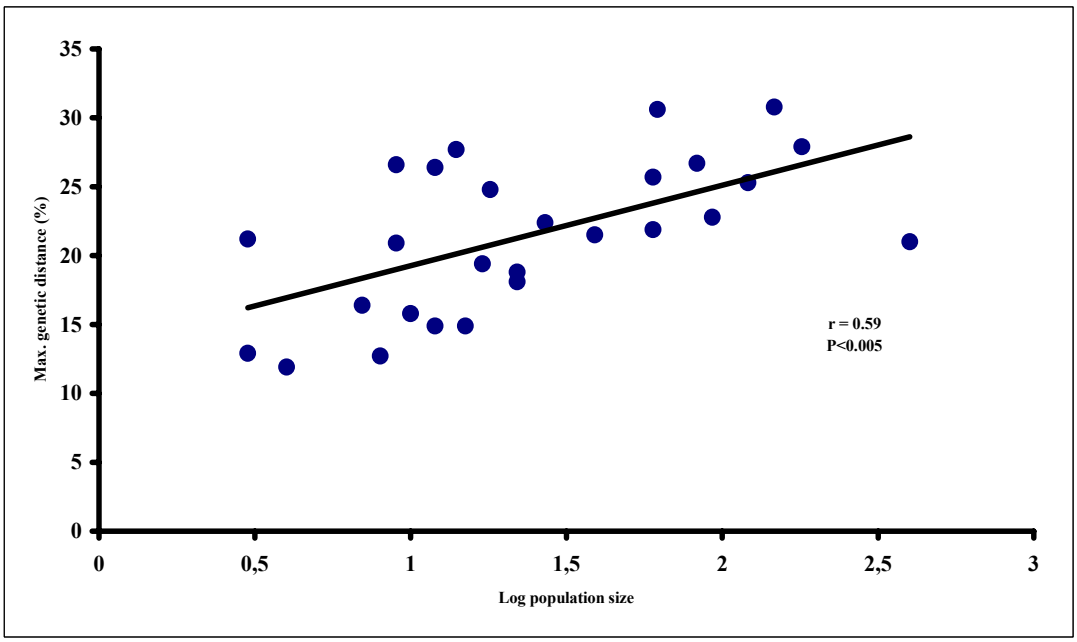

Figure 4b: Correlation between population size and the maximum genetic distance within every population, calculated for *Saxifraga paniculata*.

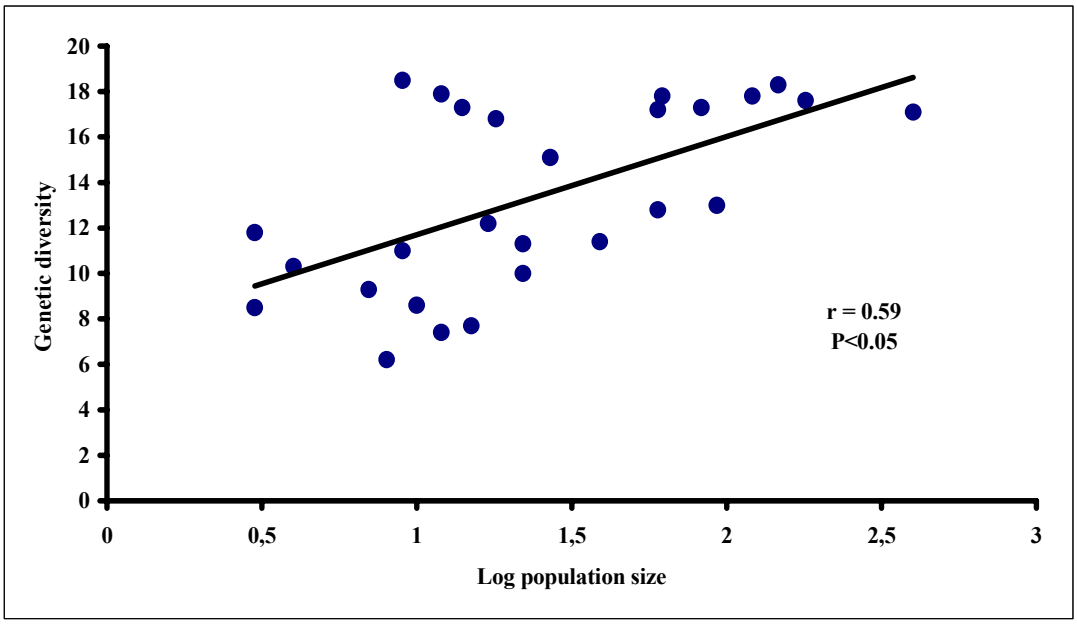

Figure 4c: Correlation between population size and the genetic diversity (sums of euclidian squares/n-1) per population, calculated for *Saxifraga paniculata*.

3.7 Population clustering

An unrooted and bootstrapped UPGMA dendrogram based on 319 RAPD markers clustered the 131 individuals within distinct groups according to their origin from different geographical regions (Figure 5). The dendrogram showed four major geographical groups with high reliability (bootstrap values higher than 50 %).

One group comprised the single population from Romania. Another two groups consisted of five populations from the southern Alps and two populations from the northern Alps. The most extensive cluster is formed by all glacial relict populations from southwest and west Germany. The bootstrap values of the four major groups ranged from 100 % (Romania) and 99 % (southern Alps) to 74 % (northern Alps) and 59 % (west and southwest Germany).

Except from two locations in the southern Alps (Steinegg and Karneid) and two locations in southwest Germany (Eichbühl and Gerberhöhle), in all four clusters the locations formed very well defined groups. They could be distinguished from each other and had high bootstrap values, which means that the clusters have high levels of reliability.

All individuals of a population were grouped together in 21 of the 30 investigated populations. In the south alpine cluster the populations from the locations Steinegg and Karneid could not be separated clearly from each other. The populations 2 and 3 of Lautertal and the populations 2 and 3 of Roßberg could also not be clearly distinguished from each other in the cluster which consisted out of the individuals from west and southwest Germany.

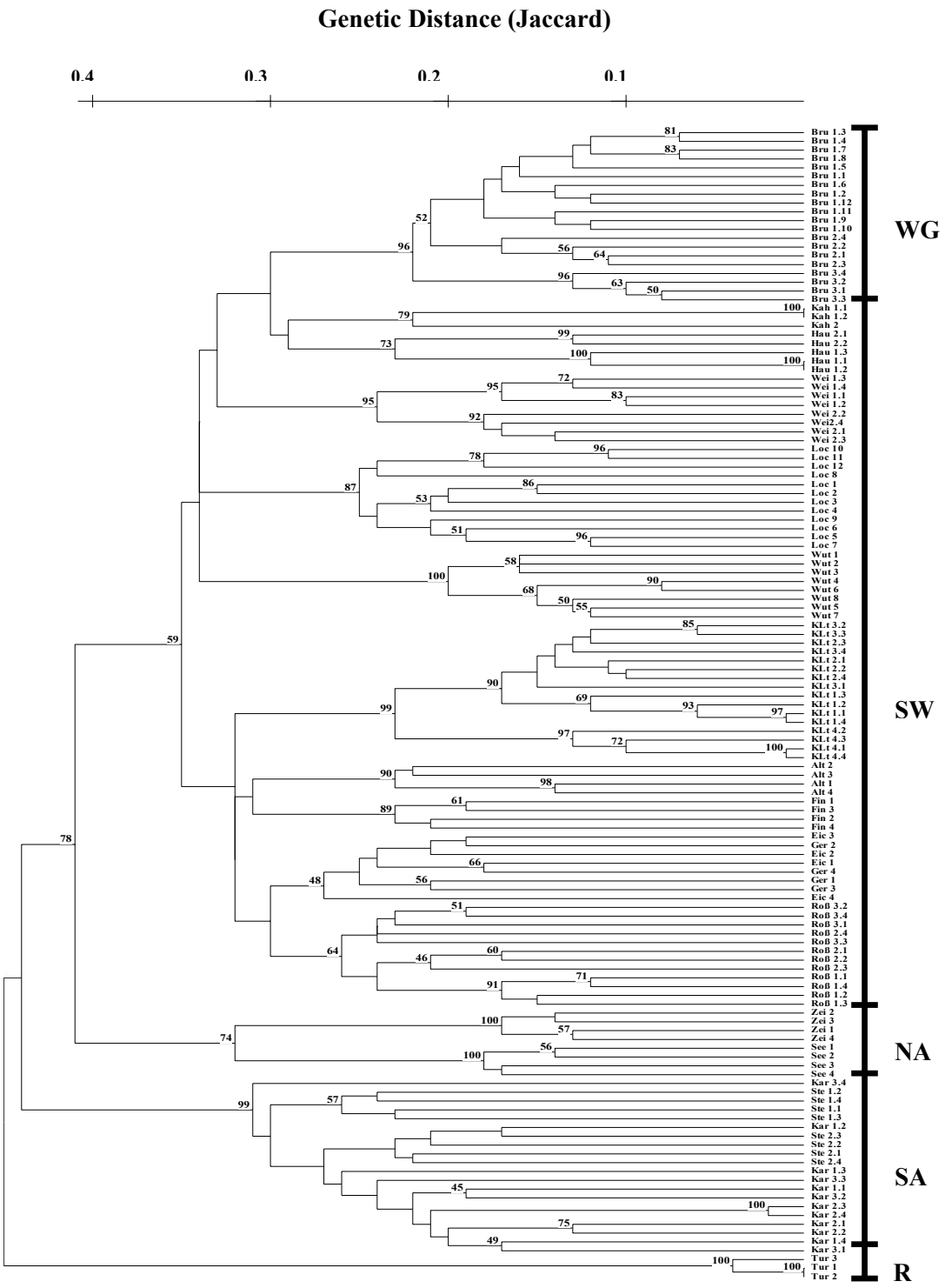

Figure 5: Bootstrapped cluster analysis (UPGMA) of 131 plants of *Saxifraga paniculata* using 319 RAPD markers, based on the Jaccard's similarity coefficient matrix. Numbers at the nodes indicate the probability of the branch when the bootstrap analysis was calculated with 1,000 iterations. Population abbreviations follow Table 1 (WG = west Germany, SW = southwest Germany, NA = northern Alps, SA = southern Alps, R = Romania).

Within the cluster which consists of the relict populations from west and southwest Germany the locations were not grouped together according to their geographical origin. Although the population Wutachschlucht grew in the Black Forest, the individuals were not seperated from the other southwest German populations, which were sampled on the Swabian Alb. The populations were located in different mountain regions, but these regions did not form separate clusters in this investigation. Nevertheless, the cluster analysis clearly illustrated the geographical differentiation of *Saxifraga paniculata*.

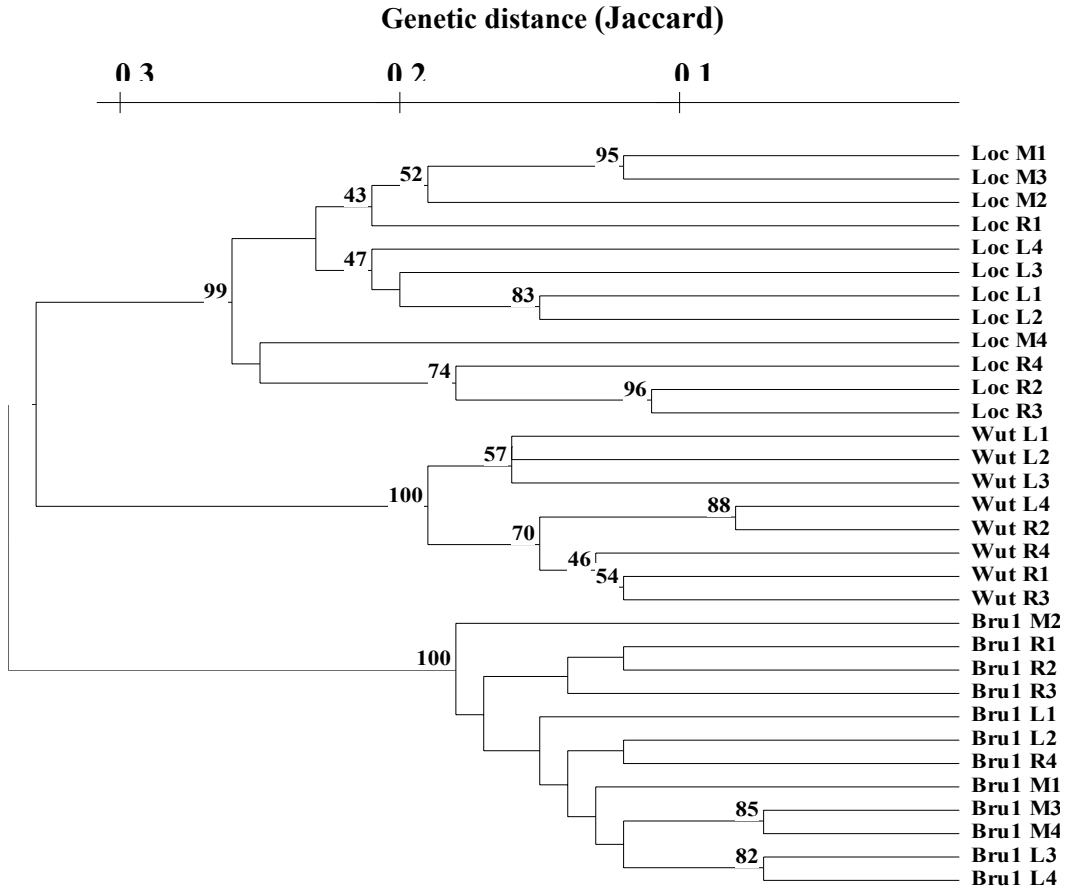

Figure 6: Bootstrapped cluster analysis (UPGMA) of 32 plants from three populations of *Saxifraga paniculata* using 319 RAPD markers, based on the Jaccard's similarity coefficient matrix. The plants were sampled on the left side (L), in the middle (M) or on the right side (R) of the population. Numbers at the nodes indicate the probability of the branch when the bootstrap analysis was calculated with 1,000 iterations. Population abbreviations follow Table 1.

3.8 Subpopulation clustering

To test if a subpopulation structure existed within populations individual plants of *Saxifraga paniculata* were sampled on the left side, in the middle and on the right side of the populations Brunkenstein 1, Wutachschlucht and Lochenstein. The unrooted and bootstrapped UPGMA dendrogram based on 319 RAPD markers showed the clear separation of the three populations. The individuals within the populations, however, did not form groups corresponding to their origin from the left, the middle, or the right side of the population (Figure 6).

3.9 Genetic diversity among nearest neighbour plants

Individuals of *S. paniculata* form cushions due to their clonal growth. In some of the investigated populations the plants formed large mats, which occasionally covered several square meters. To test whether mats represented a clone or different individuals, which became neighbours upon clonal growth, material was collected from ten rosettes in a two square meter mat at Lochenstein and from five rosettes in a one square meter mat at Hausen 2.

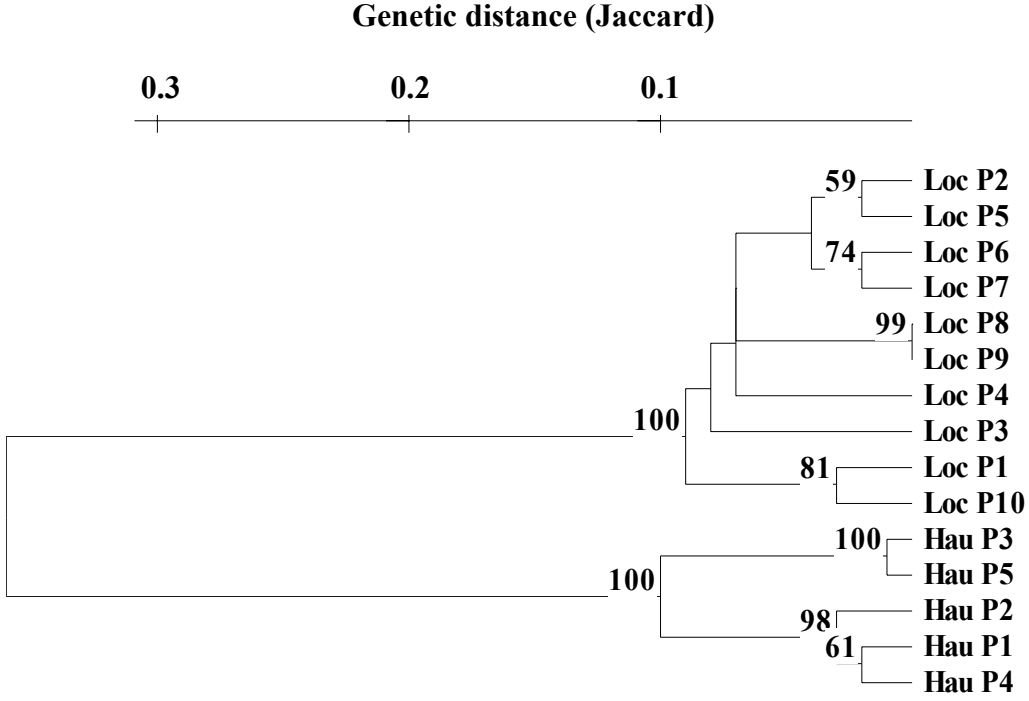

Figure 7: Bootstrapped cluster analysis (UPGMA) of 15 samples from two mats of the *Saxifraga paniculata* populations Lochenstein and Hausen 2, based on the Jaccard's similarity coefficient matrix. For cluster analysis 152 RAPD markers were used. Numbers at the nodes indicate the probability of the branch when the bootstrap analysis was calculated with 1,000 iterations. Population abbreviations follow Table 1.

A total of 131 amplified fragments were obtained using plants from Lochenstein and 108 fragments from Hausen plants. The levels of polymorphism ranged from 17.5 % in Lochenstein to 11.5 % in Hausen 2. The maximum and minimum genetic distance, taken from the Jaccard's similarity index matrix, varied from 12.6 % and 0 %, respectively, at Lochenstein to 11.7 % and 0.9 %, respectively, at Hausen 2. The mean genetic distance between individuals from the same mat was 7.1 %. The unrooted and bootstrapped UPGMA dendrogram based on 152 RAPD markers (Figure 7), which grouped the samples according to their origin, indicated that the populations were well defined. All individuals showed different banding patterns. Thus ten different genotypes could be identified at Lochenstein, while five were obtained at Hausen 2.

3.10 ITS analysis

The sequencing of the internal transcribed spacers obtained 585 base positions of which 14 were variable (2.4 %). Except for one population, all investigated plants from the southwest and west German populations had identical ITS sequences.

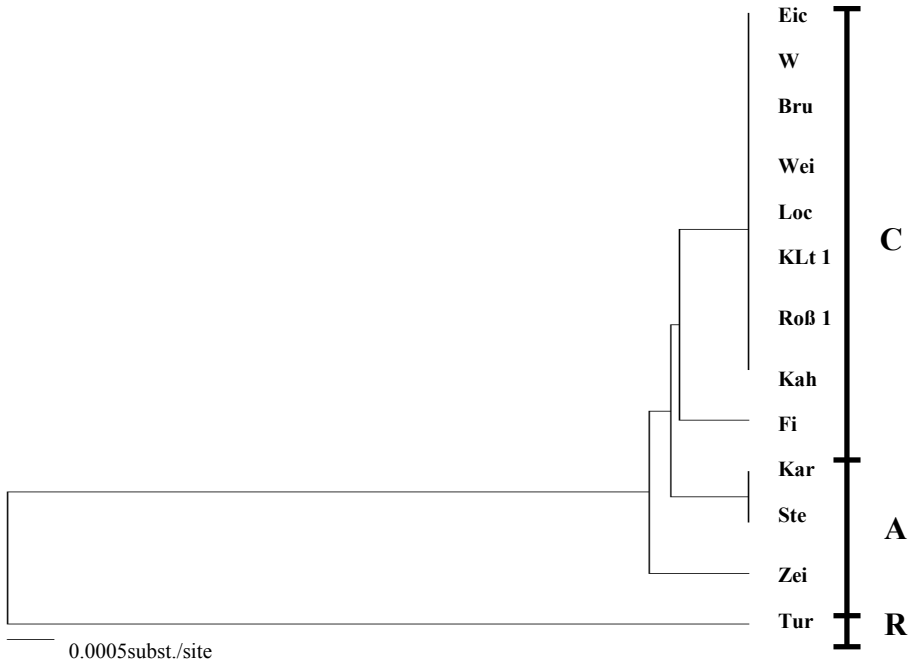

Figure 8: Cluster analysis (UPGMA) based on thirteen ITS sequences of *Saxifraga paniculata*. Individuals from thirteen populations were investigated in all five geographical regions (CE = central Europe, A = northern and southern Alps, R = Romania). Population abbreviations follow Table 1.

Only the Finstertal population showed one base substitution. One substitution was also observed in the alpine populations Karneid 1, Steinegg 1, and Zeigergrat. Most base substitutions were found in the Romanian population Turda. The individual from this population differed in 10 base substitutions from all other investigated plants.
The dendrogram (UPGMA), constructed with the ITS sequence data, corroborates the distinct geographical clustering, observed in the RAPD analysis (Figure 8). ITS clustering showed three main clusters. The first cluster contained all central European relict populations from west and southwest Germany, while the second cluster was formed by the alpine populations. The third cluster only contained the most divergent population from Romania.

4 Discussion

4.1 Partitioning of molecular variance within and among populations

The investigated populations of *Saxifraga paniculata* showed 41 % molecular variation among the locations and 44 % within populations, whereas only 15 % variance was found among populations within the same location. The results of the analysis, therefore, showed a distinct geographical partitioning of molecular variance, which also was impressively illustrated by the cluster analysis of the RAPD and ITS data. Both UPGMA dendrograms showed clear geographical clusters of *Saxifraga paniculata* populations from Romania, from the southern Alps, from the northern Alps and from central Germany. Moreover, the mean genetic distance correlated significantly the linear geographic distance between the locations, which suggests, that the locations are isolated and geneflow by insect-pollination is limited. For this reason, the greatest genetic distances were revealed between the geographically most distant populations. The results of this study, therefore, are in agreement with Ehrlich & Raven (1969), who observed that already distances of 15 metres to a few kilometres can effectively isolate two populations of insect-pollinated plant species. The estimates of N_em corroborated this observation. The present study revealed a low level of migration between the isolated locations, which resulted in considerable geographical differentiation. In contrast, the populations of the same location were comparatively similar to each other. The higher level of geneflow within locations and populations, furthermore, prevented the differentiation of subpopulations.

Glacial relicts often show high levels of interpopulational genetic differentiation (Demesure et al. 1996, Le Corre et al. 1997, Tremblay & Schoen 1999). Bauert et al. (1998) observed strong differentiation among rare relict populations of *Saxifraga cernua* (Saxifragaceae) in the Alps. Cardoso et al. (1998) investigated relict populations of the woody plant *Caesalpinia echinata* (Caesalpiniaceae) in Brazil, which had expanded in distribution during the cold periods of the Quaternary and contracted to few sites with the return of the warmer climate, as reported for *Saxifraga paniculata* in central Europe. *Caesalpinia echinata* also showed considerable differentiation among the rare and isolated populations. Reflecting these facts the strong geographical differentiation of *Saxifraga paniculata* clearly can be attributed to the long-term isolation since the end of the last glaciation.

Empirical data indicate, that the differentiation of isolated populations may require many thousands of generations. Loveless & Hamrick (1987) found no unique alleles between *Cirsium canescens* (Asteraceae) and its putatively derived species *C. pitcheri* (Asteraceae), although the two are thought to have diverged 11,000 years ago. Lesica et al. (1988) were unable to detect any genetic variation among four populations of the aquatic plant *Howellia aquatilis* (Campanulaceae) and postulated that mutation did not have sufficient time to establish differences among the populations in the last 10,000 years.
Moreover, little differentiation among populations is observed in plants, which occupied previously glaciated terrain. Gabrielsen et al. (1997) found 64 % of the RAPD variation within populations in an investigation of the arctic-alpine *Saxifraga oppositifolia* (Saxifragaceae). According to their results, the extant nordic

populations were established after glacial retreatment by massive, centripetal immigration from genetically variable, periglacial populations. Mosseler et al. (1993) observed no genetic differentiation in the conifer *Pinus resinosa* (Pinaceae), which passed through long-term expansion after the last glaciation in North America. Niebling & Conkle (1990) also found no interpopulational differentiation in the conifer *Pinus washoensis* (Pinaceae), although the populations were seperated 8,000 years ago. The strong geographical differentiation of *Saxifraga paniculata*, therefore, is an unequivocal indication for the long-term isolation of the populations.

The analysis of molecular variance, carried out in the present study revealed approximately as much variation within populations as among locations. Numerous studies provide substantial evidence that putative relict plant populations harbour high levels of genetic diversity (Comes & Kadereit 1998). Genetically variable populations were reported for many herbaceous, perennial and insect-pollinated relict plants. Lutz et al. (2000) determined considerable genetic diversity within relict populations of *Saxifraga aizoides* (Saxifragaceae). Abbott et al. (1995) found high levels of diversity in putative high arctic refugials of *Saxifraga oppositifolia*, whereas Soltis et al. (1997) observed the same in North American refugial populations of *Tolmiea menziesii* (Saxifragaceae) and *Tellima grandiflora* (Saxifragaceae). Considerable levels of diversity in rare glacial relict populations were, furthermore, reported for *Biscutella laevigata* (Brassicaceae) (Dannemann 2000), *Arctomecon humilis* (Papaveraceae) (Allphin et al. 1998) and the genus *Polygonella* (Polygonaceae) (Lewis & Crawford 1995). Glacial refugia, therefore, are sanctuaries for allelic richness, although the level of genetic diversity depends on the estimator which is used for the assessment of variability (Widmer & Lexer 2001). It seems to be a typical phenomenon, that plants from glacial refugia show high levels of intrapopulational diversity. Reflecting the fact, that historical events may have a profound influence on determining and partitioning of the genetic diversity in plant species (Parks et al. 1994, Huang et al. 1998) the considerable level of diversity within the relict populations strongly supports the assumption that *Saxifraga paniculata* was present in central Europe before the last glaciation and presumably even during the warmer interglacials.

The present investigation showed that the north alpine populations had as much interpopulational differentiation as the populations in central Europe, whereas more intermingling of individuals could be observed in the south alpine populations. When considering intrapopulational diversity slightly higher values were observed in the populations from the Alps. It is, therefore, difficult to decide whether the analysed alpine populations are glacial relicts or derived from non-alpine relict populations after glaciation. Investigations showed, that already during the last interglacial period an alpine flora was present in the Alps (Lang 1994). This flora was mostly wiped out by the Würm glaciation, but mountain peaks of the central Alps and ranges of the northern and southern lower Alps protruded through the ice sheet (Hantke 1978, 1980). According to Burga & Perret (1998), especially the south alpine mountain ranges played a critical role as nunataks. This is demonstrated by the present richness of local endemic plant species in this region. Hantke (1978) suggested that some alpine species survived the pleistocene

glaciations in the nunataks of the lower Alps and migrated from there to occupy their present locations. The alpine populations of *Saxifraga paniculata* showed an inter- and intraspecific diversity which is comparable to the central European populations. It is, therefore, carefully supposed that the alpine populations are relict populations as well. However, without further investigations it is virtually impossible to decide finally whether these populations were glacial relicts or migrated to their locations after glaciation.

4.2 Levels of genetic diversity within relict populations

In central Europe *Saxifraga paniculata* is a rare and endangered plant species. Rare or geographically restricted species are usually thought to have low levels of genetic variability. Rarity can thus be interpreted as either a cause or a consequence of limited diversity for many rare plant species (Hamrick & Godt 1989, Soltis 1992, Frankham 1996, Odasz & Savolainen 1996, Godt et al. 1997). However, several studies have found high levels of allozyme variability in rare or narrow endemic species (Karron 1987, Hamrick & Godt 1989, Lewis & Crawford 1995). Gitzendanner & Soltis (2000) compared patterns of genetic variation in rare and widespread plant congeners and showed, that rare species exhibit significantly lower genetic diversity. However, additionally they observed that the genetic diversity of rare species encompasses almost the same range as it is found in widespread congeners and that a high degree of correlation exists within a genus for all measures of diversity.

For this reason, it is not unexpected to find rare species with a high level of genetic diversity. Although most investigations use allozymes, both low and high levels of diversity in rare plant species were detected using RAPDs. Smith & Pham (1996) demonstrated high levels of diversity in the narrow endemic *Allium aaseae* (Alliaceae), a perennial and insect-pollinated geophyte, which is only found along the Boise Front in North America. Tansley & Brown (2000) found strong RAPD variation in the rare and endangered south African shrub *Leucadendron elimense* (Proteaceae). The small populations of the insect-pollinated and perennial woody plant showed higher levels of diversity than ubiquitous species of the same genus. Furthermore, Martin et al. (1997) observed strong genetic variability in the endemic species *Erodium paularense* (Geraniaceae), a perennial and insect-pollinated chamaephyte with woody stems which can be found in central Spain.

In contrast, low levels of diversity were reported for *Argyroxiphicum sandwicense* (Asteraceae), a perennial and insect-pollinated plant with rosettes from Hawaii (Friar et al. 1996). Glover & Abbott (1995) found nearly no genetic variation in the Scotish endemic *Primula scotica* (Primulaceae), which is only found in the north of Scotland and reproduces predominantly by selfing. Low levels of genetic variability were, furthermore, reported for the rare, swedish populations of the perennial and insect-pollinated *Vicia pisiformis* (Fabaceae) (Gustafsson & Gustafsson 1994).

In *Saxifraga paniculata*, the proportion of polymorphic bands per populations ranged from 12 to 54 %. These values were similar to those, found by Martin et al. (1997) in the narrow endemic *Erodium paularense* (44 to 51 %) and Smith & Pham (1996) in the rare endemic *Allium aaseae* (40-63 %). In genetically depauperated populations of *Argyroxiphicum sandwicense* the proportion of polymorphic bands only ranged from 12 to 15 %.
In this investigation, 91.2 % of the RAPD fragments were polymorphic. Bauert et al. (1998) were not able to detect any polymorphic band in rare populations of *Saxifraga cernua*, which is presumably due to the strong vegetative propagation of this species by bulbils. Brauner et al. (1992) found 24.5 % polymorphic fragments in *Lactoris fernandeza* (Lactoridaceae), while Morden & Loeffler (1999) observed 44 % in *Haplostachys haplostachya* (Lamiceae). Both species are young and rare island endemics, which possibly explains the limited variability. Higher levels of polymorphism were, however, observed in more widespread species. Chan & Sun (1997) found 69.5 % polymorphic bands in wild species of *Amaranthus* (Amaranthaceae), Gallois et al. (1998) 73.2 % in *Fagus sylvatica* (Fagaceae) and Nebauer et al. (1999) 90.6 % in *Digitalis obscura* (Scrophulariaceae). Gugerli et al. (1999) even found 97.7 % polymorphic bands in widespread populations of *Saxifraga oppositifolia*. Comparing these results, *Saxifraga paniculata* showed a large genetic diversity with a high percentage of polymorphic bands, despite of its rarity in central Europe. The analysis of further *Saxifraga* species would be impressive to test, if a high level of polymorphism is characteristic for this genus.

The level of genetic diversity depends on different historical events and life history traits to a high degree (Hamrick et al. 1979, Loveless & Hamrick 1984, Hamrick & Godt 1989). Apart from the distribution range, factors like population size, mating system, clonality and longevity exhibit an enormous influence on the genetic variability of plant species, which is discussed in the following.

4.2.1 Effects of population size

The population size of *S. paniculata* ranged from one to 400 individuals, with most populations containing 10 to 100 plants. These results are in agreement with the investigation of Ellstrand & Elam (1993), who observed, that most populations of rare plants contain fewer than 100 individuals. Small populations generally exhibit lower levels of genetic diversity compared to congeneric species with large population sizes (Hamrick & Godt 1989). Causes for this reduction can be attributed to the effects of inbreeding, leading to an increase in the number of homozygotes in the population and genetic drift, producing random changes in allele frequencies (Ellstrand & Elam 1993).

Due to habitat destruction by human influence the populations of many plant species are broken up into rare, small and isolated subpopulations. Such species are considered as „new rare“ species. Numerous studies have demonstrated that small, fragmented populations of these plant species tend to have lower levels of genetic diversity than large, extensive populations (van Treuren et al. 1991, Prober & Brown 1994, Raijmann et al. 1994, Frankham 1996, Godt et al. 1996, Fischer &

Matthies 1998, Menges & Dolan 1998). Only in some investigations genetic diversity was not correlated with population size (Dolan 1994, Ouborg & van Treuren 1994, Greimler & Dobes 2000).

Species with naturally limited distribution, such as *Saxifraga paniculata*, are considered as „old rare" species. The populations of these species were exposed to the influence of fragmentation and isolation at least since the end of the last glaciation. As reported for many „new rare" species, *Saxifraga paniculata* showed a significant correlation between genetic diversity (level of polymorphism, maximum genetic distance, sums of euclidian squares/n-1) and population size. *S. paniculata* maintained considerable genetic variation, although the population sizes were not very large. Nevertheless, larger populations obviously preserved higher genetic diversity than smaller populations, which is presumably due to lower levels of inbreeding in larger populations. A comparable pattern was described for the glacial relict plant *Biscutella laevigata*. The level of polymorphism within populations of this species was significantly correlated with population size. However, despite long-term isolation, even small relict populations maintained high levels of diversity (Dannemann 2000).

4.2.2 Effects of mating system

The genetic diversity maintained within a population is known to be affected by the mating system. Selfing species tend to have lower genetic variation within populations, although the differences in genetic diversity between selfing and outcrossing species at the species level are not statistically significant (Hamrick & Godt 1989). However, the genetic structure of populations is more differentiated in selfing species than in outcrossing species (Hamrick & Godt 1989). In a study of genetic diversity in populations of *Plantago*, the obligatory outcrossing *Plantago lanceolata* exhibited a high variation within and moderate differentiation between populations. In contrast, the highly selfing *P. major* showed relatively little variation within but pronounced differentiation between populations (Wolff et al. 1994). Gabrielsen & Brochmann (1998) observed higher levels of genetic diversity in Scandinavian populations of the mainly vegetative propagating *Saxifraga cernua* compared to the values found by Bauert et al. (1998) in alpine populations. They explain this finding with a higher degree of sexual reproduction in Scandinavia. Reflecting these facts the allogamous mating system of *Saxifraga paniculata*, contributes considerably to the maintenance of genetic diversity. Approximately as much RAPD variability was found within compared to those among populations. On the one hand this is due to the allogamous mating system of *S. paniculata* and on the other hand to the long-term isolation of the populations.

4.2.3 Effects of longevity and clonality

Long living perennials usually show greater variability than shorter-lived species (Hamrick & Godt 1989). A long life-span, therefore, seems to contribute to the

maintenance of genetic diversity in populations. Ayres & Ryan (1997, 1999) and Ranker (1994) emphasize the importance of longevity for the preservation of genetic diversity in populations of the perennial herb *Wyethia reticulata* (Asteraceae) and the fern *Adenophorus periens* (Grammitidaceae). Since very old ages were reported for clonal growing plants (Kemperman & Barnes 1976, Steinger et al. 1996), the multiramet, clonal structure of *S. paniculata* suggests that the plant can reach considerable ages. An age-determination by means of annual rings could shed light upon the real age of *Saxifraga paniculata* plants (Dietz & Ullmann 1997). However, the presumably long life-span of *S. paniculata* seems to contribute to maintain genetic diversity in the relict populations. This was also observed for the relict plant *Biscutella laevigata*, which has long-term persisting, subterranean rhizomes (Dannemann 2000).

Although clonality seems to have a strong influence on the genetic diversity, only few studies have revealed low levels of genetic variation in clonal plants (Sharitz et al. 1980, Gray et al. 1991, Sydes & Peakall 1998). Intraclonal pollination may occur in large angiosperm clones with little clonal intermingling and lead to high levels of inbreeding and subsequent inbreeding depression (Luijten et al. 1996). However, most investigations of clonal plants reported of high levels of genetic variation within populations. In a review on isozyme diversity, Hamrick & Godt (1989) found no significant differences between the levels of allelic variation within populations of 416 sexual species and 56 species with both sexual and asexual reproduction. Ellstrand & Roose (1987) focused on clonal genetic diversity within populations of clonal plants where sexual recruitment seems to be absent or extremely rare. They found that most vegetatively reproducing species had high clonal diversity within populations.

Saxifraga paniculata showed, despite strong clonal propagation, high levels of genetic diversity. Clonality, therefore, has no negative influence on the intrapopulational genetic diversity of *Saxifraga paniculata*. This is in correspondence with Dannemann (2000), who observed high levels of genetic diversity within populations of the clonal growing relict plant *Biscutella laevigata*.
A long life-span and mixed reproduction systems, consisting out of sexual and asexual reproduction, therefore, seem to be particularly suitable to create high levels of genetic diversity.

4.3 Genetic diversity in central and peripheral populations

Saxifraga paniculata showed different levels of intrapopulational diversity in the Alps compared to central Europe. For example, a mean of 34.1 % polymorphic bands was found in the alpine populations, while observing only 29.6 % in southwest German populations and 26.6 % in the west German populations. These results were all the more important, since only seven alpine populations showed a higher diversity than 19 southwest German populations. Reflecting the present geographic range, *S. paniculata* is mainly distributed in the Alps, while the central European populations were peripheral populations near the outer boundary of the geographic range. The peripheral populations from central Europe exhibited

slightly lower intrapopulational genetic diversity, than the central populations from the Alps. These results are in correspondence with Lesica & Allendorf (1995), who postulated reduced gene flow (isolation), small population size and founder effects in peripheral populations, which all promote genetic drift and result in reduced genetic variation and increased differentiation. Generally, disjunct populations and populations located at the extremes of a species range have frequently lower levels of genetic diversity than more centrally located populations (Schwaegerle & Schaal 1979, Yeh & Layton 1979, Shumaker & Babbel 1980, Guries & Ledig 1982, Furnier & Adams 1986), as also seen for *Saxifraga paniculata*.

5 References

Abbott, R.J.; Chapman, H.M.; Crawford, R.M.M. & Forbes, D.G. (1995): Molecular diversity and derivations of populations of *Silene acaulis* and *Saxifraga oppositifolia* from the high arctic and more southerly latitudes. Molecular Ecology 4: 199-207.

Ahmad, F. (1999): Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. Theoretical and Applied Genetics 98: 657-663.

Allphin, L.; Windham, M.D. & Harper, K.T. (1998): Genetic diversity and gene-flow in the endangered dwarf poppy, *Arctomecon humilis* (Papaveraceae). American Journal of Botany 85: 1251-1261.

Ayres, D.R. & Ryan, F.J. (1999): Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. American Journal of Botany 86: 344-353.

Ayres, D.R. & Ryan, F.J. (1997): The clonal and population structure of a rare endemic plant *Wyethia reticulata* (Asteraceae): allozyme and RAPD analysis. Molecular Ecology 6: 761-772.

Baldwin, B.G.; Sanderson, M.J.; Porter, J.M.; Wojciechowski, M.F.; Campbell, C.S. & Donoghue, M.J. (1995): The ITS region of the nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247-277.

Barrett, S.C.H. & Kohn, J.R. (1991): Genetic and evolutionary consequences of small population sizes in plants: implications for conservation. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 3-30.

Baldwin, B.G. (1993): Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. American Journal of Botany 80: 222-238.

Bartlett, M.S. (1937): Some examples of statistical methods of research on agriculture and applied biology. Journal of the Royal Statistical Society, Supplement 4: 137-170.

Bauert, M.R.; Kälin, M.; Baltisberger, M. & Edwards, P.J. (1998): No genetic variation within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. Molecular Ecology 7: 1519-1527.

Brauner, S.; Crawford, D.J. & Stuessy, T.F. (1992): Ribosomal and RAPD variation in the rare plant family *Lactoridaceae*. American Journal of Botany 79: 1436-1439.

Bresinsky, A. (1965): Zur Kenntnis des circumalpinen Florenelements im Vorland nördlich der Alpen. Berichte der Bayerischen Botanischen Gesellschaft 38: 5-67.

Brochmann, C.; Nilsson, T. & Gabrielsen, T.M. (1996): A classic example of postglacial allopolyploid speciation re-examined using RAPD markers and nucleotide sequences: *Saxifraga osloensis* (Saxifragaceae). Symbolae Botaniceae Upsaliensis 31: 75-89.

Brochmann, C.; Xiang, Q.-Y.; Brunsfeld, S.J.; Soltis, D.E. & Soltis, P.S. (1998): Molecular evidence for polyploid origins in *Saxifraga* (Saxifragaceae): The narrow endemic *S. svalbardensis* and its widespread allies. American Journal of Botany 85: 135-143.

Burga, C.A. & Perret, R. (1998): Vegetation und Klima der Schweiz seit dem jüngeren Eiszeitalter. Ott, Thun.

Cardoso, M.A.; Provan J.; Powell, W.; Ferreiras, C.G. & de Oliveira, D.E. (1998): High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinoideae). Molecular Ecology 7: 601-608.

Chan, K.F. & Sun, M. (1997): Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. Theoretical and Applied Genetics 95: 865-873.

Comes, H.P. & Kadereit, J.W. (1998): The effects of quaternary climatic changes on plant distribution and evolution. Trends in Plant Science 3: 432-438.

Conti, E.; Soltis, D.E.; Hardig, T.M. & Schneider, J. (1999): Phylogenetic Relationships of the Silver Saxifrages (*Saxifraga*, Sect. *Ligulatae* Haworth): Implications for the Evolution of Subtrate Specifity, Life Histories, and Biogeography. Molecular Phylogenetics and Evolution 13: 536-555.

Dannemann, A. (2000): Der Einfluss von Fragmentierung und Populationsgröße auf die genetische Variation und Fitness von seltenen Pflanzenarten am Beispiel von *Biscutella laevigata* (Brassicaceae). Dissertationes Botanicae 330. J.Cramer, Berlin, Stuttgart.

Demesure, B.; Comps, B. & Petit, R.J. (1996): Chloroplast DNA phylogeography of the common beech in Europe. Evolution 50: 2515-2520.

Dietz, H. & Ullmann, I. (1997): Age-determination of dicotyledonous herbaceous perennials by means of annual rings: exception or rule? Annals of Botany 80: 377-379.

Dolan, R.W. (1994): Patterns of isozyme variation in relation to population size, isolation, and phytogeographic history in Royal Catchfly (*Silene regia*; Caryphyllaceae). American Journal of Botany 81: 965-972.

Ehrlich, P.R. & Raven, P.H. (1969): Differentiation of populations. Science 165: 1228-1232.

Ellstrand, N.C. & Elam, D.R. (1993): Population genetic consequences of small population size: implications for plant conservation. Annual Reviews of Ecology and Systematics 24: 217 242.

Ellstrand, N.C. & Roose, M.L. (1987): Patterns of genotypic diversity in clonal plant species. American Journal of Botany 74: 123-131.

Engler, A. & Irmscher, E. (1919): Das Pflanzenreich IV. Engelmann, Leipzig.

Excoffier, L.; Smouse, P.E. & Quattro, J.M. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitrochondrical DNA restriction data. Genetics 131: 479-491.

Fahima, T.; Sun, G.L.; Beharav, A.; Krugman, T.; Beiles, A. & Nevo, E. (1999): RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theoretical and Applied Genetics 98: 434-447.

Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York.

Fischer, M. & Matthies, D. (1998): RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). American Journal of Botany 85: 811-819.

Frankham, R. (1996): Relationship of genetic variation to population size in wildlife. Conservation Biology 10: 1500-1508.

Friar, E.A.; Robichau, R.H. & Mount, D.W. (1996): Molecular genetic variation following a population crash in the endangerd Mauna Kea silversword, *Argyroxiphium sandwicense* (Asteraceae). Molecular Ecology 5: 687-691.

Furnier, G.R. & Adams, W.T. (1986): Geographic patterns of allozyme variation in Jeffrey pine. American Journal of Botany 73: 1009-1015.

Gabrielsen, T.M.; Bachmann, K.; Jakobsen, K.S. & Brochmann, C. (1997): Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. Molecular Ecology 6: 831-842.

Gabrielsen, T.M. & Brochmann, C. (1998): Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. Molecular Ecology 7: 1701-1708.

Gallois, A.; Audran, J.C. & Burrus, M. (1998): Assessment of genetic relationships and population discrimination among *Fagus sylvatica* L. by RAPD. Theoretical and Applied Genetics 97: 211-219.

Gitzendanner, M.A. & Soltis, P.S. (2000): Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87: 783-792.

Glover, B.J. & Abbott, R.J. (1995): Low genetic diversity in the Scotish endemic *Primula scotica* Hook. New Phytologist 129: 147-153.

Godt, M.J.W.; Johnson, B.R. & Hamrick, J.L. (1996): Genetic diversity and population size in four rare southern appalachian plant species. Conservation Biology 10: 796-805.

Godt, M.J.W.; Walker, J. & Hamrick, J.L. (1997): Genetic diversity in the endangered Lily *Harperocallis flava* and a close relative *Tofielda racemosa*. Conservation Biology 11: 361-366.

Gray, A.J.; Marshall, D.F. & Raybould, A.F. (1991): A century of evolution in *Spartina anglica*. Advances in Ecological Research 21: 1-60.

Greimler, J. & Dobes, C. (2000): High genetic differentiation in relict lowland populations of *Gentianella austriaca* (A. and J. Kern.) Holub (Gentianaceae). Plant Biology 2: 628-637.

Gugerli, F.; Eichenberger, K. & Schneller, J.J. (1999): Promiscuity in populations of the cushion plant *Saxifraga oppostifolia* in the Swiss Alps as inferred from random amplified polymorphic DNA (RAPD). Molecular Ecology 8: 453-461.

Guries, R.P. & Ledig, F.T. (1982): Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). Evolution 36: 387-402.

Gustafsson, L. & Gustafsson, P. (1994): Low genetic variation in swedish populations of the rare species *Vicia pisiformis* (Fabaceae) revealed with rflp (rDNA) and RAPD. Plant Systematics and Evolution 189: 133-148.

Hamrick, J.L. & Godt, W. (1989): Allozyme diversity in plant species. In: Brown, A.H.D.; Clegg, M.T.; Kahler, A.L. & Weir, B.S. (eds.): Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, M.A., 43-63.

Hamrick, J.L.; Linhart, Y.B. & Mitton, J.B. (1979): Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annual Reviews of Ecology and Systematics 10: 173-200.

Hantke, R. (1978): Eiszeitalter, Band 1. Ott, Thun.

Hantke, R. (1980): Eiszeitalter, Band 2. Ott, Thun.

Holsinger, K.E. & Gottlieb, L.D. (1991): Conservation of rare and endangered species: priciples and prospects. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 195-208.

Huang, H.; Dane, F. & Kubisak, T.L. (1998): Allozyme and RAPD analysis of the genetic diversity and geographic variation in wild populations of the American Chestnut. American Journal of Botany 85: 1013-1021.

Huenneke, L.F. (1991): Ecological implications of genetic variation in plant populations. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 31-44.

Huff, D.R.; Peakall, R. & Smouse, P.E. (1993): RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. Theoretical and Applied Genetics 86: 927-934.

Huff, D.R. (1997): RAPD characterization of heterogenous perennial ryegrass cultivars. Crop Science 37: 557-564.

Kaplan, K. (1995): Saxifragaceae. In: Weber, H.E. (ed.): Gustav Hegi, Illustrierte Flora von Mitteleuropa, IV (2A). Blackwell, Berlin, 130-229.

Karron, J.D. (1987): A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evolutionary Ecology 1:47-58.

Kemperman, J.A. & Barnes, B.V. (1976): Clone size in American Aspens. Canadian Journal of Botany 76: 755-761.

Korneck, D.; Schnittler, M. & Vollmer, I. (1996): Rote Liste der Farn- und Blütenpflanzen (*Pteridophyta* et *Spermatophyta*) Deutschlands. Schriftenreihe für Vegetationskunde 28: 21-188.

Kump, B. & Javornik, B. (1996): Evaluation of genetic variability among common buckwheat (*Fagopyrum esculentum* Moench) populations by RAPD markers. Plant Science 114: 149-158.

Lang, G. (1994): Quartäre Vegetationsgeschichte Mitteleuropas, Jena.

Le Corre, V.; Dumoulin-Lapegue, S. & Kremer, A. (1997): Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. Molecular Ecology 6: 519-529.

Lesica, P. & Allendorf, F.W. (1995): When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Lesica, P.; Leary, R.F.; Allendorf, F.W. & Bilderback, D.E. (1988): Lack of genetic diverstity within and among populations of an endangered plant, *Howellia aquatilis*. Conservation Biology 2: 275-282.

Lewis, P.O. & Crawford, D.J. (1995): Pleistocene refugium endemics exhibit greater allozymic diversity than widespread congeners in the genus *Polygonella* (Polygonaceae). American Journal of Botany 82: 141-149.

Loveless, M.D. & Hamrick, J.L. (1987): Genetic organization and evolutionary history in two north American species of *Cirsium*. Evolution 42: 254-265.

Loveless, M.D. & Hamrick, J.L. (1984): Ecological determinants of genetic structure in plant populations. Annual Reviews of Ecology and Systematics 15: 65-95.

Luijten, S.H.; Oostermeijer, J.G.B.; van Leeuwen, N.C. & den Nijs, H.C.M. (1996): Reproductive success and clonal genetic structure of the rare *Arnica montana* (Compositae) in the Netherlands. Plant Systematics and Evolution 201: 13-30.

Lutz, E.; Schneller, J. & Holderegger, R. (2000): Understanding population history for conservation purposes: Population genetics of *Saxifraga aizoides* (Saxifragaceae) in the lowlands and the lower mountains north of the Alps. American Journal of Botany 87: 583-590.

Maki, M. & Horie, S. (1999): Random amplified polymorphic DNA (RAPD) markers reveal less genetic variation in the endangered plant *Cerastium fischerianum* var. *molle* than in the widespread conspecific *C. fischerianum* var. *fischerianum* (Caryophyllaceae). Molecular Ecology 8: 145-150.

Mantel, N. (1967): The detection of disease clustering and a generalized regression approach. Cancer research 27: 209-220.

Martin, C., Gonzalez-Benito, M.E. & Iriondo, J.M. (1997): Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. & Izco. Molecular Ecology 6: 813-820.

Menges, E.S. & Dolan, R.W. (1998): Demographic variability of populations of *Silene regia* in midwestern prairies: relationships with fire management, genetic variation, geographic location, population size and isolation. Journal of Ecology 86: 63-78.

Mengistu, L.W.; Mueller-Warrant, G.W. & Barker, R.E. (2000): Genetic diversity of *Poa annua* in western Oregon grass seed crops. Theoretical and Applied Genetics 101: 70-79.

Miller, M.P. (1997): Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Milligan, B.G.; Leebens-Mack, J. & Strand, A.E. (1994): Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3: 423-435.

Morden, C.W. & Loeffler, W. (1999): Fragmentation and genetic differentiation among subpopulations of the endangered Hawaiian mint *Haplostachys haplostachya* (Lamiaceae). Molecular Ecology 8: 617-625.

Mosseler, A.J.; Egger, K.N. & Innes, D.J. (1993): Life history and the loss of genetic diversity in red pine. American Journal of Botany 80: Abstract 240.

Nebauer, S.G.; del Castillo-Agudo, L. & Segura, J. (1999): RAPD variation within and among natural populations of outcrossing willow-leafed foxglove (*Digitalis obscura* L.). Theoretical and Applied Genetics 98: 985-994.

Neuner, G.; Braun, V.; Buchner O. & Taschler, D. (1999): Leaf rosette closure in the alpine rock species *Saxifraga paniculata* Mill.: significance for survival of drought and heat under high irradication. Plant Cell and Environment 22: 1539-1548.

Niebling, C.R. & Conkle, M.T. (1990): Diversity of washoe pine and comparisons with allozymes of ponderosa pine races. Canadian Journal of Forest Research 20: 298-308.

Oberdorfer, E. (1990): Pflanzensoziologische Exkursionsflora. Ulmer, Stuttgart.

Odasz, A.M. & Savolainen, O. (1996): Genetic variation in populations of the arctic perennial *Pedicularis dasyantha* (Scrophulariaceae), on Svalbard, Norway. American Journal of Botany 83: 1379-1385.

Ostermeijer, J.G.B.; Berholz, A. & Poschlod, P. (1996): Genetical aspects of fragmented plant populations. In: Settele, J.; Margules, C.R.; Poschlod, P. & Henle, K. (eds.): Species survival in fragmented landscapes. Kluwer Academic publishers, Dordrecht, 93-101.

Ouborg, N.J. & van Treuren, R. (1994): The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. Evolution 48: 996-1008.

Ovstedal, D.O. (1998): Variation within some nordic *Saxifraga* species (Saxifragaceae). Nordic Journal of Botany 18: 171-181.

Palacios, C. & Gonzalez-Candelas, F. (1997): Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufourii* (Plumbaginaceae). Molecular Ecology 6: 1107-1121.

Parks, C.R.; Wendel, J.F.; Sewell, M.M. & Qiu, Y.-L. (1994): The significance of allozyme variation and introgression in the *Liriodendron tulipifera* complex (Magnoliaceae). American Journal of Botany 81: 878-889.

Posada, D. & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Prober, S.M. & Brown, A.H.D. (1994): Conservation of the grassy white box woodlands: Population genetics and fragmentation of *Eucalyptus albens*. Conservation Biology 8: 1003-1013.

Raijman, L.E.L.; van Leeuwen, N.C.; Kersten, R.; Oostermeijer, J.G.B.; den Nijs, H.C.M. & Menken, S.B.J. (1994): Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L.. Conservation Biology 8: 1014-1026.

Ranker, T.A. (1994): Evolution of high genetic variability in the rare hawaiian fern *Adenophorus periens* and implications for conservation management. Biological Conservation 70: 19-24.

Rogers, S.O. & Bendich, A.J. (1988): Extraction of DNA from plant tissues. In: Gelvin, S.B.; Schilperoort, R.A. & Verma, D.S. (eds): Plant molecular biology manual. Kluwer Academic Publishers, Dordrecht, 1-10.

Rothmaler, W. (1991): Atlas der Gefäßpflanzen, Verlag Volk und Wissen, Berlin.

Rossetto, M.; Weaver, P.K. & Dixon, K.W. (1995): Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Molecular Ecology 4: 321-329.

Sambrook, J.; Fritsch, E.F. & Maniatis, T. (1989): Molecular cloning: a laboratory manual, 2 ed. Cold Spring Harbour Laboratory Press, New York.

Schmidt, K. & Jensen, K. (2000): Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components. American Journal of Botany 87: 678-689.

Schwaegerle, K.E. & Schaal, B.A. (1979): Genetic variability and founder effect in the in the pitcher plant *Sarracenia purpurea* L. Evolution 33: 1210-1218.

Sharitz, R.R.; Winereiter, S.A.; Smith, M.H. & Edwin, H.L. (1980): Comparisons of isozymes among *Typha* species in the Eastern United States. American Journal of Botany 67: 1297-1303.

Shumaker, K.M. & Babbel, G.R. (1980): Patterns of allozyme similarity in ecologically central and marginal populations of *Hordeum jubatum* in Utah. Evolution 34:110-116.

Simberloff, D. (1988): The contribution of population and community biology to conservation science. Annual Reviews of Ecology and Systematics 19: 473-512.

Smith, J.F. & Pham, T.V. (1996): Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). American Journal of Botany 83(6): 717-726.

Sneath, P.H.A. & Sokal, R.R. (1973): Numerical Taxonomy. The principles and practice of numerical classification. W.H. Freeman, San Francisco.

Soltis, D.E.; Gitzendanner, M.A.; Strenge, D.D. & Soltis, P.S. (1997): Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Plant Systematics and Evolution 206: 353-373.

Soltis, P.S.; Soltis, D.E.; Tucker, T.L. & Lang, F.A. (1992): Allozyme variability is absent in the narrow endemic *Bensoniella oregona* (Saxifragaceae). Conservation Biology 6: 131-134.

Steinger, T.; Körner, C. & Schmid, B. (1996): Long-term persistence in a changing climate: DNA analysis suggests very old ages of clones of alpine *Carex curvula*. Oecologia 105: 94-99.

Stewart, C.N. & Excoffier, L. (1996): Assessing population genetic structure and variability with RAPD data: application to *Vaccinium macrocarpum* (American Cranberry). Journal of Evolutionary Biology: 153-171.

Stewart, C.N. & Porter, D.M. (1995): RAPD profiling in biological conservation: an application to estimating clonal variation in rare and endangered *Iliamna* in Virginia. Biological Conservation 74: 135-142.

Swofford, D. L. (2001). PAUP. Phylogenetic Analysis Using Parsimony. Sunderland.

Sydes, M.A. & Peakall, R. (1998): Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragoceae) revealed by allozymes and RAPDs. Molecular Ecology 7: 87-93.

Tansley, S.A. & Brown, C.R. (2000): RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. Biological Conservation 95: 39-48.

Tollefsrud, M.M.; Bachmann, K.; Jakobsen, K.S. & Brochmann, C. (1998): Glacial survival does not matter – II: RAPD phylogeography of Nordic *Saxifraga cespitosa*. Molecular Ecology 7: 1217-1232.

Tremblay, N.O. & Schoen, J. (1999): Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. Molecular Ecology 8: 1187-1198.

van de Peer, Y. & de Wachter, R. (1994): TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Applic. Biosci. 10, 569-570.

van Treuren, R.; Bijlsma, R.; van Delden, W. & Ouborg, N.J. (1991): The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. Heredity 66: 181-189.

Vargas, P.; Baldwin, B. & Constance, L. (1998): Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). Proceedings of the National Academy of Sciences, USA 95: 235-240.

Walter, H. & Straka, H. (1970): Arealkunde. Floristisch-historische Geobotanik. Ulmer, Stuttgart.

Webb, D.A. & Gornall, R.J. (1989): Saxifrages of Europe. Christopher Helm, London.

Welsh, J. & Mc Clelland, M. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research 18: 7213-7218.

Whitkus, R.; de la Cruz, M.; Mota-Brava, L. & Gomez-Pompa, A. (1998): Genetic diversity and relationships of cacao (*Theobroma cacao* L.) in southern Mexiko. Theoretical and Applied Genetics 96: 621-627.

Widmer, A. & Lexer, C. (2001): Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends in Ecology and Evolution 16: 267-269.

Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. & Tingey, S.V. (1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research 18: 6531-6535.

Wilmanns, O. & Rupp, S. (1966): Welche Faktoren bestimmen die Verbreitung alpiner Felsspaltenpflanzen auf der Schwäbischen Alb? Veröffentlichungen der Landesstelle für Naturschutz und Landschaftspflege Baden-Württemberg 34: 62-85.

Wolff, K.; Rogstad, S.H. & Schaal, B.A. (1994): Population and species variation of minisatellite DNA in *Plantago*. Theoretical and Applied Genetics 87: 733-740.

Wright, S. (1951): Variability within and among natural populations. Volume 4. The University of Chicago Press, Chicago.

Yeh, F.C. & Layton, C. (1979): The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* ssp. *latifolia*. Canadian Journal of Genetics and Cytology 21: 487-503.

Young, A.; Boyle, T. & Brown, T. (1996): The population genetic consequences of habitat fragmentation for plants. Tree 11/10: 413-418.

III
Genetic diversity within and among populations of the glacial relict plant Sesleria albicans Kit. ex Schultes (Poaceae) from different habitats in central Europe

1 Introduction

The climatic changes during the pleistocene had an enormous influence on the distribution of plants (Comes & Kadereit 1998). Quaternary palaeoecology in Europe has revealed range contractions and expansions for many species in the wake of deglaciation and glaciation (Hewitt 1996). *Sesleria albicans* Kit. ex Schultes (Poaceae) is an alpine, perennial grass which is widely distributed in the Alps but also occurs with isolated populations in the lower mountains of south and central Germany (Dixon 1982). The present distribution of *Sesleria albicans* is a result of the climatic warming after the last glaciation. During the glacial periods *Sesleria albicans* was distributed widely in the unglaciated lowlands of central Europe (Bresinsky 1965, Walter & Straka 1970) which were covered by tundra vegetation (Hantke 1979, 1980). Due to the postglacial return of the forest trees, the plant was displaced from most habitats, which were formerly covered by glacial tundra. The present outlying lowland populations, therefore, are considered to be glacial relicts (Bresinsky 1965, Walter & Straka 1970, Conert 1999).

These populations of *S. albicans* were subjected to fragmentation and long-term isolation since the end of the last glaciation. High levels of interpopulational genetic variation and a distinct geographical clustering among populations could, therefore, be expected for *Sesleria albicans*. Furthermore, numerous studies have demonstrated that small, isolated populations of "new rare" species tend to have lower levels of genetic diversity than large, extensive populations (van Treuren et al. 1991, Raijmann et al. 1994, Prober & Brown 1994, Frankham 1996, Godt et al. 1996, Fischer & Matthies 1998, Menges & Dolan 1998). However, little is known about the genetic diversity within and among populations of glacial relicts in central Europe, which are so called "old rare" species (Dannemann 2000, Lutz et al. 2000). The study presented here, therefore, focuses on the effects of long-term isolation on genetic diversity within and among the populations the glacial relict plant *Sesleria albicans*.

The relict populations of *S. albicans* are located in very different habitats and plant communities. *Sesleria albicans* colonizes beech forests, as well as naturally open habitats such as alpine and lowland rocky ridges, rocky river valley slopes and fens, or man-made open habitats like calcareous grasslands. Furthermore, the plants grow on different geological materials and under a multitude of climatic conditions. The habitats in which the populations of *Sesleria albicans* are located, therefore, differ to a high degree from each other with regard to ecological factors such as nutrients, light and water just as in type and intensity of land use.

Different land use practices are generally thought to make a contribution to the genetic differentiation of populations (Poschlod & Jackel 1993, Poschlod et al. 2000). As demonstrated by many authors, different types of grassland manage-

ment can result in the development of ecotypic variants (van Tienderen & van der Toorn 1991, Briggs & Block 1992, Sweeney & Danneberger 1995, Zopfi 1998). Fertilization and defoliation are generally known to cause population differentiation (Snaydon 1987).

Moreover, genetic variation within and among populations is affected by ecological factors (Kölliker et al. 1998). Ecotypic development strongly depends on climatic and edaphic conditions (Briggs & Walters 1984, Nevo et al. 1988) and microgeographic differentiation, caused by these factors, is reported for many species (Nevo et al. 1988, Nevo & Beiles 1989, Owuor et al. 1997, Hsao & Lee 1999). Therefore, differences in ecological conditions can also result in the development of ecotypes (Gunter et al. 1996) or biotypes (Colosi & Schaal 1994).

Populations of *Sesleria albicans* colonizing wet and dry habitats are supposed to be separate varieties or ecotypes (Braun-Blanquet 1932, Sebald et al. 1998) and Dixon (1982) reported of differences in the length and width of leaves and spikes in populations of *S. albicans* from different habitats. Considering these facts, a high degree of genetic variability can be presumed. This study therefore focuses on the ecotypic differentiation among the populations of *Sesleria albicans*, which grow in different habitats under variable ecological conditions.

Although habitat protection is normally considered to be of foremost importance in the conservation of species, the maintenance of high levels of genetic diversity within the species is essential to allow them to respond to the selection pressures imposed by pests and disease, and to facilitate adaption to future environmental change (Barrett & Kohn 1991, Holsinger & Gottlieb 1991). Data on the genetic diversity of plant populations can, therefore, play a significant role in the formulation of appropriate management strategies directed towards the conservation of species, besides being advantageous in the understanding of their structure, evolutionary relationships, taxonomy and demography (Milligan et al. 1994). Consequently, the study of population genetics has been identified as one of the main priorities for conservation (Holsinger & Gottlieb 1991).

In the past decade, the detection of genetic diversity has been improved by the advent of new molecular techniques. Different methods of DNA fingerprinting have proved to be useful tools with a wide range of applications in plant population genetic studies, such as the detection of genetic variation within and among populations, the characterization of clones, the analysis of breeding systems and the analysis of ecographical variation (Weising et al. 1995). Random Amplified Polymorphic DNA (RAPD) analysis is a PCR-based assay capable of analysing genetic relatedness among and within populations (Welsh & McClelland 1990, Williams et al. 1990). In addition, the use of the analysis of molecular variance technique (AMOVA) (Excoffier et al. 1992) to analyse RAPD marker variation has been shown to be effective for population analysis of highly heterozygous, outcrossing plant species (Huff et al. 1993, Huff 1997).
RAPD analysis has been successfully used for plant germplasm characterization in a large number of studies (Kump & Javornik 1995, Chan & Sun 1997, Whitkus et al. 1998, Ahmad 1999, Fahima et al. 1999, Mengistu et al. 2000). Its utilization

in investigations of the genetic diversity of rare or threatened plant species is also proven (Rossetto et al. 1995, Smith & Pham 1996, Martin et al. 1997, Palacios & Gonzalez-Candelas 1997, Cardoso et al. 1998, Ayres & Ryan 1999, Maki & Horie 1999, Dannemann 2000, Tansley & Brown 2000). Furthermore, RAPD analysis has been successfully used to detect genetic diversity in grass species (Hsao & Rieseberg 1994, Gunter et al. 1996, Huff 1997, Mengistu et al. 2000) and to analyze genetic differentiation among populations from ecologically different habitats (Owuor 1997, Huff et al. 1998, Kölliker et al. 1998, Hsao & Lee 1999).

Previous studies of *Sesleria albicans* mostly centered on systematics (Ujhelyi 1938, Deyl 1946, Strgar 1966), ecology (Lloyd & Woolhouse 1978, Dixon 1982, 1996) and phytosociology (Schmidt 2000). Data on the genetic diversity within and among populations of *Sesleria albicans*, however, have not been reported to date. In this investigation, therefore, RAPD analysis and AMOVA were used to detect the genetic diversity within and among relict populations of *Sesleria albicans* in different habitats.

The following questions were addressed:
(1) What is the influence of long-term isolation since the end of the last glaciation on partitioning of molecular variance and on levels of genetic diversity?
(2) Is there a geographic differentiation among the populations?
(3) Are there different levels of genetic diversity in populations of different size and from different regions or habitats?
(4) Is there a genetic or even ecotypic differentiation among populations from different habitats?

2 Material and methods

2.1 Species description

Sesleria albicans Kit. ex Schultes (Poaceae) is a perennial grass with deeply rooting rhizomes which form small, persistent cushions of numerous tillers. The culms are slender, erect and smooth to the base, arising terminally from certain tillers. The leaves are mostly basal, sharply keeled and hairless, with characteristic persistent sheaths surrounding the bases of the tillers. They are parallel-sided to the tip, which is contracted abruptly to a hooded point. Mature leaves are green to glaucus above, dark green beneath and up to six millimeters wide. The culm leaves, usually two, are very short and generally less than one centimeter long. The glistening panicles are oblong to spherical, spicate and dense, one to three centimeters long and three to ten millimeters wide. Usually they are yellowy-white on the upper part and steely-blue or purplish-green on the lower part (Dixon 1982, Conert 1999).

The diploid and wind-pollinated *Sesleria albicans* (2n = 28) usually flowers from March to June. The flowers are slightly proteandrous, and self-pollination, therefore, is probably of rare occurence. On established plants seed set occurs every year, although isolated plants sometimes yield very few seeds (Dixon 1982).
The species is a chamaephyte with vegetative survival and slow winter growth of leaves and developing inflorescences. No specialized propagules are produced for vegetative regeneration. Detached tillers, however, could provide an effective mean of vegetative propagation.

Sesleria albicans Kit. ex. Schultes and the most closely related species *Sesleria caerulea* (L.) Ard. were formerly regarded as two subspecies in the species *Sesleria caerulea* (L.) Ard. (as *S. caerulea* (L.) Ard *ssp. calcaria* Celak. and *S. caerulea* (L.) Ard *ssp. uliginosa* Celak.). However, most authors meanwhile consider both taxa as separate species (Deyl 1980, Conert 1999).

Both species are widely distributed. While *Sesleria caerulea* is found from northern Sweden through Eastern Europe to Bulgaria, *Sesleria albicans* has a more fragmented distribution. It is widely distributed in the Alps, Carpathians and Pyrenees, but also occurs in Great Britain and Ireland, in the foothills of the Alps in France, Germany and Italy, and with isolated populations in the lower mountains of south and central Germany (Dixon 1982). These outlying lowland populations are considered to be glacial relicts (Bresinsky 1965, Walter & Straka 1970, Conert 1999). *Sesleria albicans* also occurs in isolated sites in the plains of Poland, in the river valleys of northern France and Belgium, in the Sierra Nevada of Spain, in Jugoslavia and Albania, and in two localities in Iceland.

Sesleria albicans shows a broad ecological amplitude. It mainly grows on limestone (Conert 1999, Schmidt 2000), but is also reported from serpentine and phonolite (Deyl 1946), slates (Dixon 1982), sandstone (Tüxen 1937) and porphyry (Haffner 1962). The plant is located in very different types of habitats such as limestone cliffs, pavements, screes, grasslands, heaths and open woodlands (Dixon 1982, Oberdorfer 1990, Conert 1999).

S. albicans is adapted very well to drought (Lloyd & Woolhouse 1978, Dixon 1986) but also tolerates moist conditions (Dixon 1996). The grass prefers dry habitats, but is also found in fens or seasonally flooded grasslands (Bresinsky 1965, Dixon 1982). *Sesleria albicans* colonizes natural habitats such as beech forests, rocky ridges, rocky river valley slopes and fens as well as man-made open habitats such as calcareous grasslands. The plants which are growing in fens in the Alps and the Prealps are supposed to be an own variety (*Sesleria caerulea ssp. calcaria var. pseudouliginosa*) or ecotype (Braun-Blanquet 1932, Sebald et al. 1998).

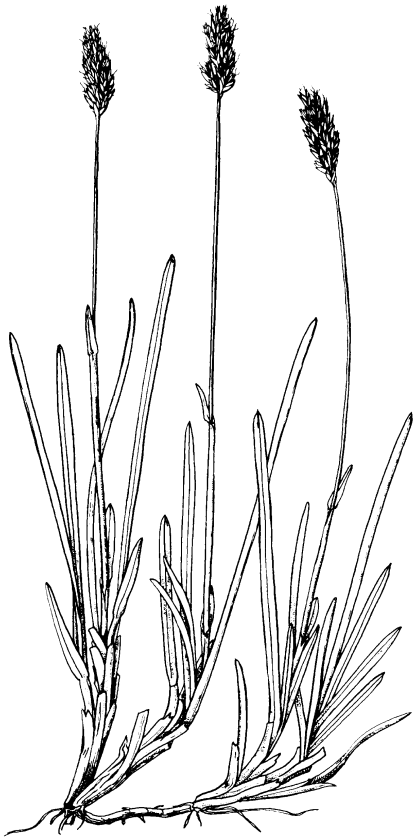

Figure 1: Habit of *Sesleria albicans* Kit. ex Schultes (taken from Hubbard 1985). The plant is a perennial grass which forms small, persistent cushions of numerous tillers.

2.2 Study sites

To investigate the genetic diversity within and among populations of *Sesleria albicans* from different types of habitats, 25 populations in southwest, west and central Germany and in the northern and southern Alps were selected. Plants from six different types of habitats like rocky river valley slopes, lowland rocky ridges, calcareous grasslands, beech forests, fens and alpine rocky ridges (Table 1) were compared.

From rocky river valley slopes five populations were selected which were located in the valleys of the Neckar, Tauber, Nahe, Ahr and Alme. The southwest German

populations from lowland rocky ridges, calcareous grasslands and beechforests were located on the Swabian Alb, whereas the west German populations from calcareous grasslands and beechforests grew in the Eifel. The populations of *Sesleria albicans* which grew in fens were located in the south German Prealps. The alpine rocky ridge populations were sampled on the Nebelhorn near Oberstdorf and in the valley of Eggen near Bozen in South Tyrol.
To investigate the relationship between *Sesleria albicans* and the closely related *Sesleria caerulea* additionally one population of *Sesleria caerulea* from Öland in Sweden was analysed.

2.3 Plant material and sampling conditions

In spring and summer of 1999 seed material was collected in situ from all selected populations. Seeds all across the population from at least twenty different plants were sampled. Two populations were considered as separate when they were at least 70 m apart from each other. After sampling the seeds population size was estimated (in square meters covered by *Sesleria albicans*) and associated plant species were reported to describe the plant communities in which *Sesleria albicans* grew. Seed material was used to cultivate plants from each population in a common garden. After germination, ten individuals per population were planted in small pots with common garden soil and cultivated for one year. In spring of 2000 ten young leaves from four plants per populations were sampled and stored at –35°C in the laboratory.

2.4 Population size and plant communities

In 1999 the size of the investigated *Sesleria albicans* populations ranged from four to 20000 square meters (Table 1). However, most of the investigated populations had 60 to 1000 square meters extent. Six of 22 populations were smaller than 60 square meters and the same number of populations was larger than 1000 square meters.
Sesleria albicans showed a broad ecological amplitude and the plants, therefore, grew in their habitats in very different plant communities (Table 2). Although most populations were located on limestone, plants which were growing on slate and porphyry also were observed.

The populations of *Sesleria albicans* which were located on rocky river valley slopes in the valley of the Nahe and the Ahr grew in a glacial relict association called *Genista pilosa-Sesleria varia* association, whereas the population from the valley of the Mühlenbach in central Germany was part of the *Asplenietum trichomano-ruta murariae* and the populations from the southwest German valley of the Neckar and the Tauber grew in the *Teucrio-Seslerietum*.

The lowland rocky ridge populations from southwest Germany were located in a plant community called *Bromo-Seslerietum*. In contrast the *Sesleria albicans* populations from the west German calcareous grasslands were living in the

Table 1: Sampled populations of *Sesleria albicans* with abbreviation (Abb.) description of habitat, region, population size (P. size) in square meters, geographical latitude (Lat.), longitude (Long.), altitude (Alt), number of sampled individuals (S. ind.) and geological underground (n.d.a.. = no description available)

Nr	Population	Abb.	Species	P. size (m²)	Habitat	Region	Lat. (N)	Long. (E)	Alt.	S. ind.	Geology
1	**Mühlenbachtal**	Müh	S. albicans	5	Rocky river valley slope	Central Germany	51°27′	8°37′	300	4	Limestone
2	**Engelslay**	Eng	S. albicans	6	Rocky river valley slope	West Germany	50°31′	6°58′	200	4	Porphyry
3	**Brunkenstein**	Bru	S. albicans	4	Rocky river valley slope	West Germany	49°49′	7°31′	220	4	Slate
4	**Felsengärten**	Fel	S. albicans	2000	Rocky river valley slope	Southwest Germany	48°59′	9°12′	280	4	Limestone
5	**Gissigheim**	Gis	S. albicans	80	Rocky river valley slope	Southwest Germany	49°45′	9°35′	250	4	Limestone
6	**Rauher Fels**	Rau	S. albicans	300	Lowland rocky ridge	Southwest Germany	48°03′	8°59′	780	4	Limestone
7	**Spielburg**	Spi	S. albicans	60	Lowland rocky ridge	Southwest Germany	48°43′	9°45′	650	4	Limestone
8	**Indelhausen**	Ind	S. albicans	100	Lowland rocky ridge	Southwest Germany	48°18′	9°30′	600	4	Limestone
9	**Bürgle**	Bür	S. albicans	15000	Calcarous Grassland	Southwest Germany	48°17′	8°59′	740	4	Limestone
10	**Zeller Horn**	Zel	S. albicans	8000	Calcarous Grassland	Southwest Germany	48°17′	9°00′	900	4	Limestone
11	**Irrenberg**	Irr	S. albicans	12000	Calcarous Grassland	Southwest Germany	48°15′	8°56′	920	4	Limestone
12	**Lampertstal**	Lam	S. albicans	1000	Calcarous Grassland	West Germany	50°22′	6°42′	440	4	Limestone
13	**Steinfeld**	Ste	S. albicans	15000	Calcarous Grassland	West Germany	50°22′	6°38	500	4	Limestone
14	**Hundsrücken**	Hun	S. albicans	20000	Calcarous Grassland	West Germany	50°29′	6°34	500	4	Limestone
15	**Nähberg**	Näh	S. albicans	300	Beech Forest	Southwest Germany	48°17′	9°00′	880	4	Limestone
16	**Eybachtal**	Eyb	S. albicans	300	Beech Forest	Southwest Germany	48°40′	9°54′	600	4	Limestone
17	**Blindloch**	Bli	S. albicans	100	Beech Forest	Southwest Germany	48°02′	8°58′	750	4	Limestone
18	**Pütz**	Püt	S. albicans	100	Beech Forest	West Germany	50°32′	6°33′	450	4	Limestone
19	**Urfttal**	Urf	S. albicans	1000	Beech Forest	West Germany	50°32′	6°35′	450	4	Limestone
20	**Evagarten**	Eva	S. albicans	30	Fen	Southwest Germany	47°52′	9°22′	600	4	Limestone
21	**Höll**	Höl	S. albicans	20	Fen	Southwest Germany	47°48′	9°48′	600	4	Limestone
22	**Federmähder**	Fed	S. albicans	20	Fen	Southwest Germany	48°28′	10°19′	450	4	Limestone
23	**Füssen**	Füs	S. albicans	n.d.a.	Fen	Southwest Germany	47°36′	10°45′		4	Limestone
24	**Nebelhorn**	Neb	S. albicans	n.d.a.	Alpine rocky ridge	Northern Alps	47°25′	10°21′	1600	4	Limestone
25	**Eggental**	Egg	S. albicans	n.d.a.	Alpine rocky ridge	Southern Alps	46°30′	11°25′	650	4	Porphyry
26	**Öland**	Öla	S. coerulea	n.d.a.	Rocky Grassland	Southern Scandinavia	56°15′	16°45′	50	4	Limestone

Gentiano-Koelerietum seslerietosum, while the southwest German populations colonized the *Koelerio-Seslerietum*.

The west German beech forest populations grew in the *Hordelymo-Fagetum seslerietosum*, whereas the southwest German populations colonized the *Carici-Fagetum seslerietosum*.

The populations of *S. albicans* which were located in the prealpine fens colonized the *Caricetum davallianae* and the *Primulo-Schoenetum*, while the populations from the northern and southern Alps were growing in the *Seslerio-Caricetum sempervirentis* and the *Valeriano-Seslerietum albicantis*.

Table 2: Plant communities in which the investigated populations of *Sesleria albicans* were located, with description of characteristic species, habitat, and geographic region.

Plant community	Characteristic species	Habitat	Region
Asplenietum trichomano-ruta murariae	*Asplenium trichomanes, Asplenium ruta-muraria, Mycelis muralis*	Rocky river valley slope	Central Germany
Genista pilosa-Sesleria varia association	*Genista pilosa, Teucrium scorodonia, Deschampsia flexuosa*	Rocky river valley slope	West Germany
Teucrio-Seslerietum	*Anthericum ramosum, Teucrium chamaedrys, Euphorbia cyparissias*	Rocky river valley slope	Southwest Germany
Bromo-Seslerietum	*Globularia punctata, Teucrium monta-num, Thymus froelichianus*	Lowland rocky ridge	Southwest Germany
Koelerio-Seslerietum	*Gentianella ciliata, Cirsium acaule, Bromus erectus, Koeleria pyramidata*	Limestone Grassland	Southwest Germany
Gentiano-Koelerietum seslerietosum	*Gentianella germanica, Cirsium acaule, Bromus erectus, Koeleria pyramidata*	Limestone Grassland	West Germany
Carici-Fagetum seslerietosum	*Carex digitata, Carex montana, Vincetoxicum hirundinaria*	Beech Forest	Southwest Germany
Hordelymo-Fagetum seslerietosum	*Sorbus torminalis, Viburnum lantana, Euonymus europaeus*	Beech Forest	West Germany
Caricetum davallianae	*Carex davalliana, Carex panicea, Crepis paludosa, Pinguicula vulgaris*	Fen	Southwest Germany
Primulo-Schoenetum	*Schoenus ferrugineus, Primula farinosa, Tofieldia calyculata*	Fen	Southwest Germany
Seslerio-Caricetum sempervirentis	*Gentiana clusii, Carex sempervirens, Festuca pulchella*	Alpine rocky ridge	Northern Alps
Valeriano-Seslerietum albicantis	*Valeriana tripteris, Calamagrostis varia*	Alpine rocky ridge	Southern Alps

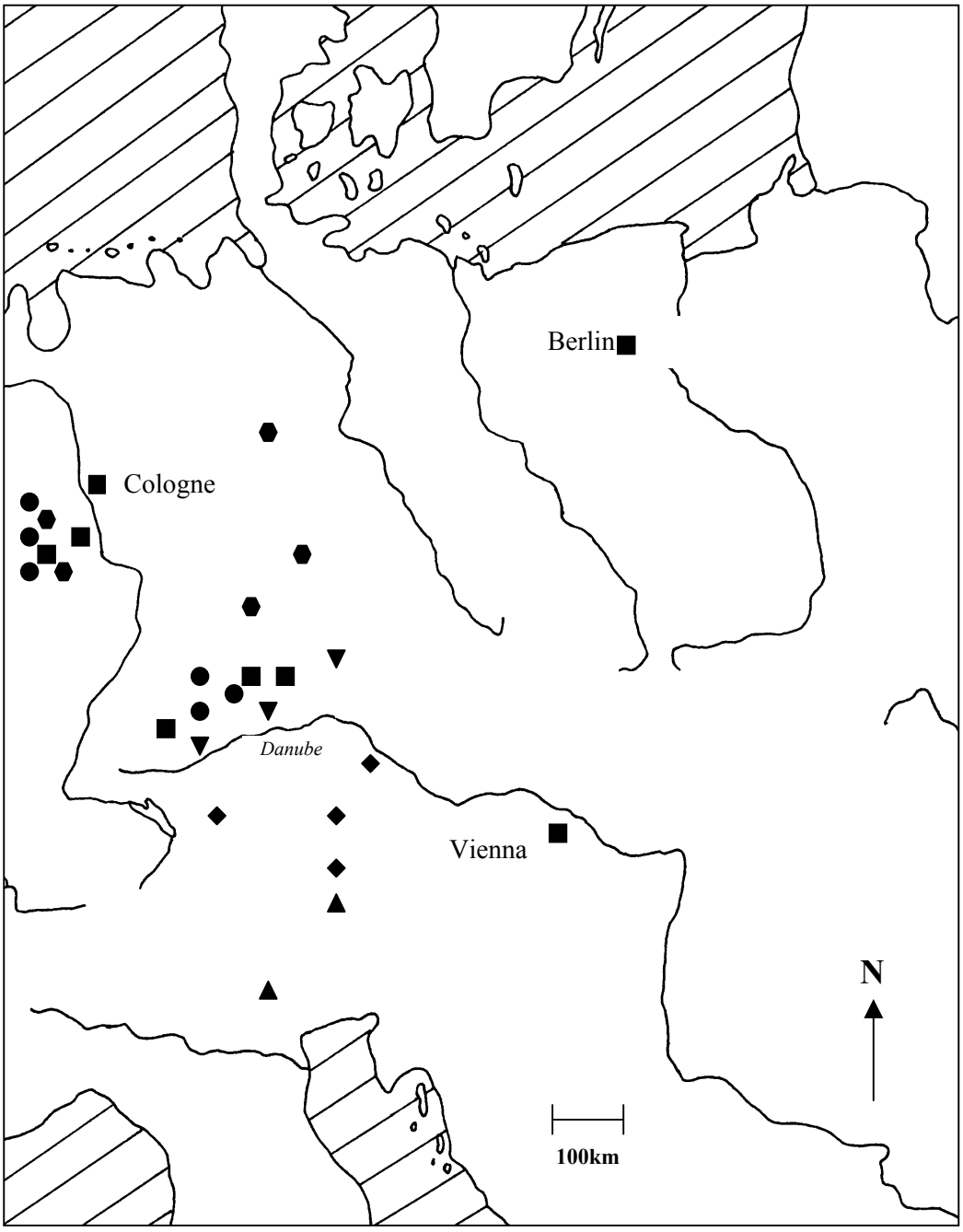

Figure 2: Geographic position of the study sites. 25 populations of *Sesleria albicans* in central Europe and the Alps were investigated. Populations grew in six different types of habitats: rocky river valley slopes (hexagons), lowland rocky ridges (inverted triangles), limestone grasslands (circles), beech forests (squares), fens (rhombuses) and alpine rocky ridges (triangles).

2.5 DNA isolation

The DNA was isolated from frozen plant material of individual plants using the CTAB (cetyltriammonium bromide) method (Rogers & Bendich 1988) adapted as follows: Approximately 40-60 mg leaf material was ground in liquid nitrogen in a 1.5 mL Eppendorf tube followed by addition of 700 µL extraction buffer (100 mM Tris HCl, pH 9.5; 20 mM EDTA, pH 8.0; 1.4 M NaCl; 1% PEG; 2% CTAB; 2.5 µL/mL β-mercapto ethanol). Incubation was at 74°C for 30 min shaking every 5-10 min. Subsequently the mixture was extracted twice with an equal volume of chloroform/isoamylalcohol (24:1), and centrifuged at 15,000g at 4°C for 10 min. Adding an equal volume of isopropanol (5°C), the DNA was precipitated and pelleted by centrifugation at 20,000g at 4°C for 15 min. The DNA was washed with 70 % cold ethanol (4°C) for 5 min and air-dried for 15-30 min. The DNA was resuspended in 200 µL TE buffer (10 mM Tris-HCl, pH 8.0; 1mM EDTA, pH 8.0). The concentration was estimated spectrophotometrically (Uvikon 930, Kontron Instruments, Germany) at 260 nm, and the purity measured by the ratio of the absorbances at 260 nm and 280 nm. For PCR only template DNA was used with a purity of 1.8-2.1 in a dilution of 15 ng/µL.

2.6 RAPD analysis

2.6.1 DNA amplification

For DNA amplification the polymerase chain reaction (PCR) was used with arbitrary 10-mer oligonucleotide primers (Roth, Karlsruhe, Germany) for RAPD amplification (Williams et al. 1990). All primers employed in this study were random sequence primers (Table 3) with G+C contents of up to 70 %. A total of 75 primers were initially screened for amplification on *S. albicans*.

In a final volume of 15 µL amplification mixtures contained 10 mM Tris-HCl (pH 9.5), 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM dNTP, 0.3 µM primer, 1 U Taq polymerase (Pharmacia, Freiburg, Germany) and 30 ng of genomic DNA. The mixture was overlaid with two drops of mineral oil. The polymerase chain reaction (PCR) was run in a thermal cycler (Autogene, Grant Instruments, Cambridge, UK). The thermal cycling program started with denaturation for 120 s at 94°C, followed by 35 cycles of 12 s denaturation at 94°C, 48 s annealing at 36°C and 90 s extension at 72°C. A final extension at 72°C for 10 min concluded the DNA amplification. PCR products were kept at 4°C until they were loaded.

The amplificated products were separated on 1.5 % agarose gels in 1×TBE (Tris-borate-EDTA) buffer (Sambrook et al. 1989) at 180 V for 1.5 h, using a 100 base-pair-ladder as a fragment size marker (Roth, Karlsruhe, Germany) and visualized by ethidium bromide staining. Each sample was repeated at least once in a separate amplification reaction.

2.6.2 Data scoring

For data scoring, the banding patterns were recorded using a trans-illuminating gel documentation system (Gel Print 2000i, BioPhotonics Corporation, Ann Arbor, USA). The image profiles and molecular weight of each band were determined by the program RFLPSCAN (Scanalytics CSPI Inc., Billerica, USA). Pictures were examined for strong, clearly defined bands, reproducible in duplicate amplifications. Each band was scored across all individuals as either present or absent. When individuals did not give clear, easily scored signals, all bands of this fragment size were excluded from the analysis.

In the data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0. The basic data structure finally consisted of a binomial (0,1) matrix of 104 rows, representing the investigated individuals and 344 columns, representing the scored RAPD markers. Since RAPD markers are dominant, it was assumed that each band represented the phenotype at a single bi-allelic locus (Williams et al. 1990).

2.6.3 Statistical analysis

The binomial matrix was used to calculate the level of polymorphism (percentage of polymorphic bands) for each population and to compute similarities between individuals of *S. albicans* using the Jaccard's similarity coefficient, calculated as $J = a/(n-d)$, where a is the number of positive matches (i.e. the presence of a band in both samples), d is the number of negative matches (i.e. the absence of a band in both samples), and n is the total sample size including both the numbers of matches and „unmatches". The genetic distances were calculated as $GD = 1-J$ using the data from the Jaccard's similarity coefficient matrix. The minimum, maximum and mean genetic distance between the individuals for each population were compared in order to describe the molecular variance within the populations.

The genetic relatedness among species was obtained by clustering. Dendrograms were generated from the similarity coefficient matrix using the unweighted pair group method (UPGMA) as described by Sneath & Sokal (1973). The calculation of the Jaccard's similarity coefficient matrix and the generation of the bootstrapped dendrograms was done with the program TREECON 1.3b (van de Peer & de Wachter 1994).

The binomial matrix was also applied to an analysis of molecular variance in RAPD patterns by the program WINAMOVA 1.55 (Excoffier et al. 1992, Stewart & Excoffier 1996). AMOVA was originally developed for RFLP haplotypes, but it is also appropriate for RAPD phenotypes (Huff et al., 1993, Palacios & Gonzalez-Candelas 1997). AMOVA analyses were based on the pairwise squared euclidian distances among molecular phenotypes, which are equal to the number of different band states, because band states can only take the values 0 or 1. Since a sum of squares in a conventional analysis of variance (ANOVA) can be written as a sum of all squared pairwise differences, AMOVA is closely related to ANOVA. It allowed the calculation of variance components and their significance

levels for variation among groups of populations, among populations within groups and within populations.

Additionally the AMOVA sums of squares divided by n-1 were calculated for each population to describe the molecular variance within the populations. Because significance tests in AMOVA are based on permutation procedures, they are essentially assumption free (Excoffieret al. 1992). The program also extracts analogs of F statistics (so-called Φ statistics).

Homogeneity of molecular variance in pairs of populations was tested using Bartlett tests (Bartlett 1937), which are also implemented in the WINAMOVA 1.55 program. Pairwise genetic distances (Φ_{ST}) among the 30 populations and their levels of significance were also obtained from the AMOVA. These values allow the estimation of gene flow as the number of individuals migrating between populations per generation, using the equation of Wright (1951) ($N_em = ¼[1/\Phi_{ST}-1]$), where N_em is the product of effective population size and migration rate, and Φ_{ST} is obtained from the AMOVA. For each analysis, 1,000 permutations were performed to obtain significance levels. To investigate possible differences between the habitats and geographical regions, several AMOVAs were carried out in which the populations were grouped together according to their habitats and regions of origin.

A Mantel test was used to determine whether the matrix of mean genetic distances (mean Φ_{ST}) between the locations was correlated with the matrix of geographic distances between the locations or not (Mantel 1967). The Mantel test was conducted with the program TFPGA 1.3 (Miller 1997).

To analyze the influence of the population size on the genetic diversity of populations, it was calculated whether population size correlated with the level of polymorphism, the minimum, maximum and mean genetic distance taken from the Jaccard similarity coefficient matrix or the AMOVA sums of squares divided by n-1.

3 Results

3.1 RAPD banding

Five samples were randomly selected from the 25 populations of *Sesleria albicans* and subjected to RAPD analysis with 75 decamer random primers. Amplification products were obtained with all individuals and primers from which fifteen were selected, giving rise to clear reproducible and distinct banding patterns. In total 344 reproducible fragments were amplified with a varying number per primer. For example, primer I01 produced 35 scorable bands, while primer I15 only amplified 11 fragments (Table 3).

The size of the amplified fragments ranged from 210 to 4,123 bp. The fifteen primers produced between 90.1 % and 100 % polymorphic bands. In total 95.9 % of the bands were polymorphic, only 4.1 % were scored in all individuals from all populations. Reflecting this high level of polymorphism, all investigated individuals showed their own RAPD phenotypes.

Table 3: Primers employed with the number of RAPD markers obtained, their sequence, the size of the fragments, and the percentage of polymorphic markers (P) for each primer.

Primer	Sequence (5´→3´)	[CG] %	Size (bp) min-max	Polymorphic Bands	Monomorphic Bands	Total	P (%)
P1	- CGG TCA CTG T -	60	617 – 2130	20	2	22	90.9
P7	- CAG TCC GAG C -	70	467 – 2000	19	0	19	100.0
B01	- GTT TCG CTC C -	60	487 – 2596	23	1	24	95.8
B07	- GGT GAC GCA G -	70	587 – 2312	22	2	24	91.7
B11	- GTA GAC CCG T -	60	650 – 2483	29	0	29	100.0
B18	- CCA CAG CAG T -	60	210 – 4123	26	0	26	100.0
D11	- AGC GCC ATT G -	60	624 – 2572	22	2	24	91.7
D12	- CAC CGT ATC C -	60	512 – 2500	21	0	21	100.0
I01	- ACC TGG ACA C -	60	441 – 2170	34	1	35	97.1
I04	- CCG CCT AGT C -	70	510 – 2075	23	1	24	95.8
I15	- TCA TCC GAG G -	60	776 – 2349	10	1	11	90.1
I16	- TCT CCG CCC T -	70	450 – 2383	20	1	21	95.2
160/3	- CTA CAC AGG C -	60	499 – 2777	22	2	24	91.7
160/4	- GTC CTT AGC G -	60	330 – 2262	22	1	23	95.7
170/1	- CAT CCC GAA C -	60	644 – 2141	17	0	17	100.0
Total			210 – 4123	330	14	344	95.9

3.2 AMOVA analysis and variance partitioning

The highest level of molecular variance was found within populations when arranging the 25 populations of *Sesleria albicans* in three groups according to their origin from different geographical regions (Table 1). 61.22 % variation was observed within populations, whereas 34.25 % was found among populations within regions and only 4.53 % variance was observed among the different regions (Table 4).

Table 4: Summary of analysis of molecular variance (Three-level-AMOVA). 25 populations of *Sesleria albicans* were investigated from three geographic regions that were growing in six different types of habitats (Table 1). The analysis was based on RAPD phenotypes consisting of 344 band states. Levels of significance were based on 1,000 iteration steps. (SS: sums of squares, MS: mean squares, %: apportionment of genetic variability, *P*: level of significance).

Level of variation	SS	MS	%	*P*
25 populations grouped together in 3 regions:				
Among regions	263.996	131.998	**4.53**	<0.001
Among populations within regions	1806.883	82.131	**34.25**	<0.001
Within populations	1902.500	25.367	**61.22**	<0.001
25 populations from all geographic regions grouped together in 6 types of habitats:				
Among habitats	552.488	110.498	**4.58**	<0.001
Among populations within habitats	1518.391	79.915	**33.36**	<0.001
Within populations	1902.500	25.367	**62.06**	<0.001
Populations from southwest Germany grouped together in 5 types of habitats:				
Among habitats	387.675	96.919	**2.69**	<0.001
Among populations within habitats	838.953	83.896	**36.06**	<0.001
Within populations	1125.500	25.011	**61.26**	<0.001
Populations from west and central Germany grouped together in 5 types of habitats:				
Among habitats	159.875	79.937	**3.38**	0.075
Among populations within habitats	334.375	66.875	**28.38**	<0.001
Within populations	602.500	25.104	**68.23**	<0.001
Populations from the Swabian Alb grouped together in 3 types of habitats:				
Among habitats	172.944	86.472	**2.33**	<0.001
Among populations within habitats	452.333	75.389	**30.82**	<0.001
Within populations	715.750	26.509	**66.85**	<0.001
Populations from the Eifel grouped together in 2 types of habitats:				
Among habitats	63.191	63.192	**0.90**	<0.001
Among populations within habitats	180.208	60.069	**22.52**	<0.001
Within populations	414.000	27.600	**76.58**	<0.001

The results were similar, when the 25 populations of *Sesleria albicans* were arranged in six groups according to their origin from different types of habitats. In this case the highest level of molecular variance was also observed within populations. 62.06 % variation was within populations, whereas 33.36 % was found among populations within habiats and only 4.58 % variance was observed among the different habitats (Table 4).

The variation in RAPD banding patterns among populations, among populations within habitats and within populations was highly significant (AMOVA $P<0.001$). The correlation of RAPD phenotypes within habitats relative to the correlation of random pairs drawn from the whole sample **(Φ_{ST})** was 0.38. The correlation among random phenotypes within populations relative to the correlation of random pairs drawn from the whole sample **(Φ_{CT})** was 0.35, and the correlation of random phenotypes within habitats, relative to that of random pairs drawn from the population **(Φ_{SC})** was 0.045.

The partitioning of molecular variance was corroborated by separate three-level AMOVA's, which were conducted for the populations from southwest and west Germany only (Table 4). In both cases 61.26 % and 68.23 % molecular variance, respectively, were observed within the populations. Only 2.69 % and 3.38 % molecular variation, respectively, were found among the different habitats.

This partitioning of molecular variance was also observed, when analyzing populations from different habitats, which were located close to each other in the same mountain region. Comparing only populations from the Swabian Alb 2.33 % variation was found among populations from different habitats. In an AMOVA conducted for the populations from the Eifel only 0.90 % variation was observed among populations from beech forests and calcareous grasslands.

Further two-level-AMOVA's were carried out to analyze the partitioning of molecular variance within and among populations from one habitat only (Table 5). In this analysis 52.61 % to 74.76 % molecular variance were observed within and 25.24 % to 47.39 % variation among populations from the same habitat. The populations from the calcareous grasslands showed the highest level of variation within populations (74.76 %), followed by the lowland and alpine rocky ridges (68.00 % and 67.15 %), beech forests (64.94 %) and rocky river valley slopes (60.64 %). The populations from the prealpine fens exhibited the lowest level of intrapopulational variation (52.61 %).

The AMOVA calculation of molecular variance (Bartlett test) was carried out for all investigated populations. Molecular variance was significantly different among the 25 populations ($P<0.001$). Of the 300 pairwise Bartlett tests of homogeneity of population variation all tests showed highly significant differences between the populations (Table A4, appendix).

Table 5: Summary of analysis of molecular variance (Two-level-AMOVA). 25 populations of *Sesleria albicans* were investigated that were growing in six different types of habitats (Table 1). The analysis was based on RAPD phenotypes consisting of 344 band states. Levels of significance were based on 1000 iteration steps. (SS: sums of squares, MS: mean squares, %: apportionment of genetic variability, *P*: level of significance).

Level of variation	SS	MS	%	*P*
5 populations from rocky river valley slopes grouped together:				
Among populations within habitat	321.000	80.250	**39.36**	<0.001
Within populations	334.750	22.317	**60.64**	<0.001
3 populations from lowland rocky ridges grouped together:				
Among populations within habitat	151.500	75.750	**32.00**	<0.001
Within populations	236.500	26.278	**68.00**	<0.001
6 populations from limestone grassland grouped together:				
Among populations within habitat	330.541	66.108	**25.24**	<0.001
Within populations	506.250	28.125	**74.76**	<0.001
5 populations from beech forests grouped together:				
Among populations within habitat	326.100	81.525	**35.06**	<0.001
Within populations	387.000	25.800	**64.94**	<0.001
4 populations from fens grouped together:				
Among populations within habitat	303.250	101.083	**47.39**	<0.001
Within populations	263.500	21.958	**52.61**	<0.001
2 populations from alpine rocky ridges grouped together:				
Among populations within habitat	86.000	86.000	**32.85**	<0.001
Within populations	174.500	29.083	**67.15**	<0.001

3.3 Genetic distances (Φ_{ST}) and geneflow among populations

273 of the 300 pairwise genetic distances (Φ_{ST}) between populations were highly significant (Table A5, appendix). Only two had levels of marginal significance ($P<0.05$). Maximum Φ_{ST} (varying from 0 to 1) was 0.63, found between the southwest German population Federmähder and the west German population Engelslay. Minimum Φ_{ST} was only 0.13 which was observed between population Lampertstal and Urfttal, both in west Germany.

From the genetic distances (Φ_{ST}) geneflow was calculated (Table A6, appendix) as the number of individuals migrating between populations per generation, using the equation of Wright (1951). Mean geneflow between populations was 0.47, which means that less than one individual per generation is migrating between populations.

To analyze the connection between geographic distance and genetic distance the correlation of these two parameters between all investigated populations were calculated. The matrix of 300 pairwise genetic distances (Φ_{ST}) among the 25 populations of *S. albicans* (Table A5, appendix) was not significantly correlated with the corresponding matrix of geographic distances. The calculated Mantel test shows a correlation coefficient of r=0.17 and a significance of p=0.06 (Figure 3).

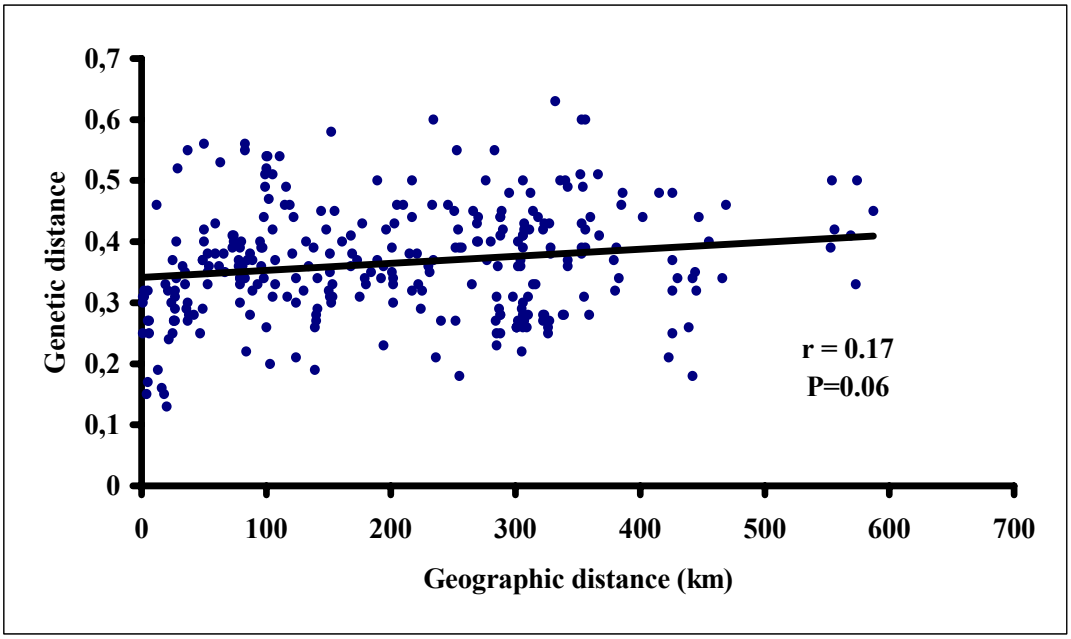

Figure 3: The calculated Mantel test showed no correlation between the genetic distances (Φ_{ST}) and the geographic distances among the 25 populations of *Sesleria albicans* in central Europe and the Alps.

3.4 Genetic diversity within populations

The 25 populations of *S. albicans* showed varying levels of intrapopulational polymorphism. The percentage of polymorphic bands ranged from 29.7 % in the Federmähder population to 56.7 % in the Eggental population.
The genetic distances within the populations, taken from the Jaccard's similarity coefficient matrix between all investigated individuals also were very different (Table 6). Minimum genetic distance within population ranged from 2.8 % in the Mühlenbachtal population to 31.8 % in the Eggental population, while maximum

genetic distance showed values between 18.8 % in the Federmähder population and 39.9 % in the populations Rauher Fels and Eggental. Mean genetic distance varied between 17.0 % in the Federmähder population and 35.9 % in Eggental. The sums of euclidian squares/n-1, which were taken from the AMOVA analysis and can also be used for the measurement of genetic diversity within populations, ranged from 14.0 in the Federmähder population to 31.4 in the Urfttal population.

Table 6: Percentage of polymorphic bands, minimum, maximum and mean genetic Jaccard-Distances just as the sums of euclidian squares/n-1 within populations of Sesleria albicans:

Nr.	Population	Polymorphic Bands (%)	Min Genetic Distance Jaccard (%)	Max Genetic Distance Jaccard (%)	Mean Genetic Distance Jaccard (%)	Sums of euclidian squares/n-1
1	**Mühlenbachtal**	42.3	02.8	32.9	24.7	20.8
2	**Engelslay**	39.0	18.6	27.3	22.9	19.0
3	**Brunkenstein**	43.6	21.8	32.6	26.3	23.0
4	**Felsengärten**	49.2	27.5	33.7	30.7	26.3
5	**Gissigheim**	44.0	23.5	29.6	26.7	22.4
6	**Rauher Fels**	52.8	26.0	39.9	34.2	29.2
7	**Spielburg**	41.7	19.4	29.5	24.9	21.6
8	**Indelhausen**	50.0	27.8	37.0	31.2	28.0
9	**Bürgle**	55.6	25.6	38.3	34.5	28.4
10	**Zeller Horn**	51.9	28.7	36.8	32.8	27.4
11	**Irrenberg**	51.1	26.2	36.6	32.2	26.8
12	**Lampertstal**	53.7	32.5	35.9	34.8	30.2
13	**Steinfeld**	53.4	29.8	37.0	33.1	28.5
14	**Hundsrücken**	48.5	29.0	34.8	31.3	27.3
15	**Nähberg**	54.2	28.8	38.1	33.5	28.0
16	**Eybachtal**	47.0	23.6	33.9	29.2	24.6
17	**Blindloch**	44.0	21.5	33.3	28.1	24.4
18	**Pütz**	40.4	21.3	30.1	25.4	20.5
19	**Urfttal**	55.7	31.2	37.0	35.2	31.4
20	**Evagarten**	48.7	25.6	32.6	29.8	25.9
21	**Höll**	40.1	03.3	32.8	25.1	21.4
22	**Federmähder**	29.7	14.3	18.8	17.0	14.0
23	**Füssen**	50.8	29.7	34.4	33.0	26.5
24	**Nebelhorn**	55.0	29.7	38.2	35.3	30.1
25	**Eggental**	56.7	31.8	39.9	35.9	28.1

3.5 Genetic diversity within populations from different habitats and regions

To compare the levels of genetic variation within populations from different types of habitats, mean levels of polymorphism and mean genetic distances for each habitat were calculated (Table 7). When all populations from all geographic regions and habitats were compared, the highest levels of intrapopulational diversity were observed within the alpine rocky ridge populations, followed by calcareous grasslands, beechforests and lowland rocky ridges. The lowest level of intrapopulational variation was observed on the rocky river valley slopes and in the fens.

Table 7: Genetic variation within the populations of *Sesleria albicans* in different habitats (Rocky river valley slopes, lowland rocky ridges, calcareous grasslands, beechforests, fens and alpine rocky ridges) from different geographic regions. For each habitat mean percentage of polymorphic bands and mean genetic distances were calculated.

Habitat	Fen	Rocky river valley slope	Lowland rocky ridge	Beech-forest	Calcareous grassland	Alpine rocky ridge
Populations from all geographic regions:						
Polymorphic bands (%)	42,3	43,6	48,2	48,3	52,4	55,9
Jaccard-Dist. Min (%)	18,2	18,8	24,4	25,3	28,6	30,8
Jaccard-Dist. Max (%)	29,7	31,2	35,5	34,5	36,6	39,1
Jaccard-Dist. Mean (%)	26,2	26,3	30,1	30,3	33,1	35,6
S. of euc. Squares/n-1	22,0	22,3	26,3	25,8	28,1	29,1
Populations from southwest Germany only:						
Polymorphic bands (%)	42,3	46.6	48.2	48.4	52.8	-
Jaccard-Dist. Min (%)	18,2	25.5	24.4	24.6	26.8	-
Jaccard-Dist. Max (%)	29,7	31.7	35.5	35.1	37.2	-
Jaccard-Dist. Mean (%)	26,2	28.7	30.1	30.3	33.2	-
S. of euc. Squares/n-1	22,0	24,4	26,3	25,7	27,5	-
Populations from west Germany only:						
Polymorphic bands (%)	-	41.6	-	48.1	51.9	-
Jaccard-Dist. Min (%)	-	14.4	-	26.3	30.4	-
Jaccard-Dist. Max (%)	-	30.9	-	33.6	35.9	-
Jaccard-Dist. Mean (%)	-	24.6	-	30.3	33.1	-
S. of euc. Squares/n-1	-	21,0	-	26,0	28,7	-

The same pattern was observed, when comparing populations from different habitats in southwest or west Germany only. In southwest Germany the lowest level of intrapopulational diversity was found in the fens and on the rocky river valley slopes, whereas the highest diversity was observed in the calcareous grasslands. The west German populations also showed lower levels of variation on the rocky river valley slopes and higher levels of diversity in the calcareous grasslands.

The results were different, when comparing intrapopulational diversity of all populations from the Alps with all populations from southwest Germany and west Germany (Table 8). In this case approximately as much variation was found within the southwest German as within the west German populations. However, the alpine populations showed higher variation than the lowland populations.

Table 8: Genetic variation within the populations of *Sesleria albicans* from West and Central Germany, from Southwest Germany and the Alps. For each geographic region mean percentage of polymorphic bands and mean genetic distances were calculated.

Region	West and Central Germany	Southwest Germany	Alps
Polymorphic bands (%)	47.1	47.3	55.9
Jaccard-Distance Min (%)	23.4	23.4	30.8
Jaccard-Distance Max (%)	33.5	33.7	39.1
Jaccard-Distance Mean (%)	29.2	29.5	35.6
Sums of euclidian squares/n-1	25.1	25.0	29.0

3.6 Molecular variance and population size

The *S. albicans* population sizes ranged from 20,000 to just a few square meters (Table 1). The level of polymorphism correlated highly significant with the population size (Spearman's rank-correlation coefficient r_s=0.7, *P*<0.001, Figure 4a). A highly significant correlation was also observed between population size and the minimum and mean genetic distance, taken from the Jaccard's similarity index matrix (r_s=0.7, *P*<0.001, Figure 4b).

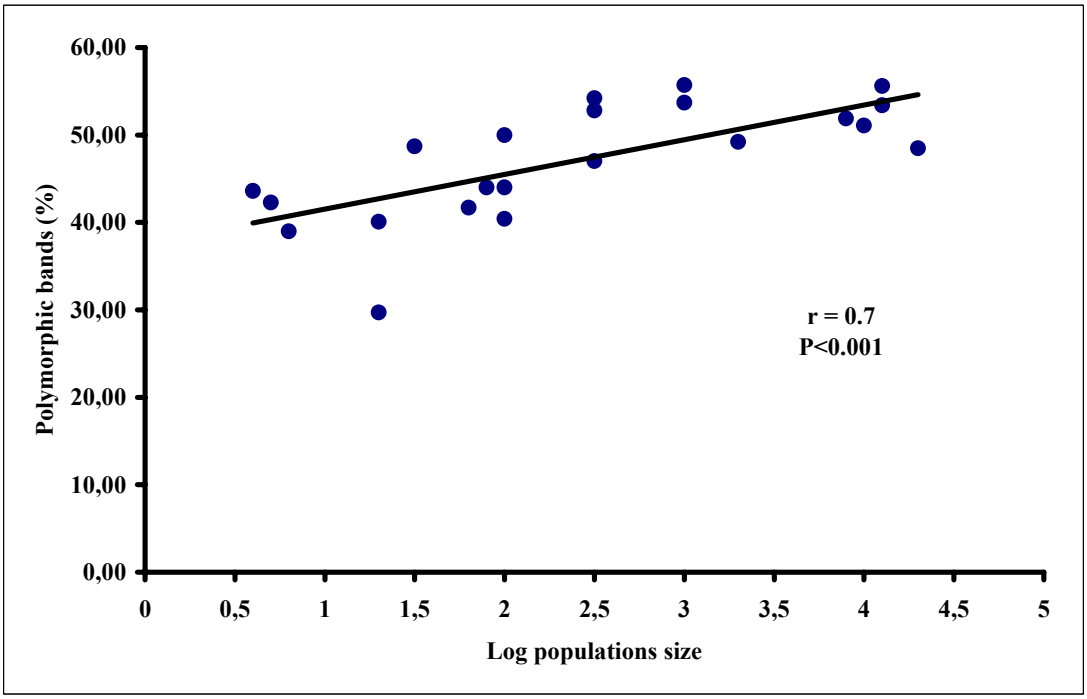

Figure 4a: Correlation between population size and the percentage of polymorphic bands per population, calculated for *Sesleria albicans*.

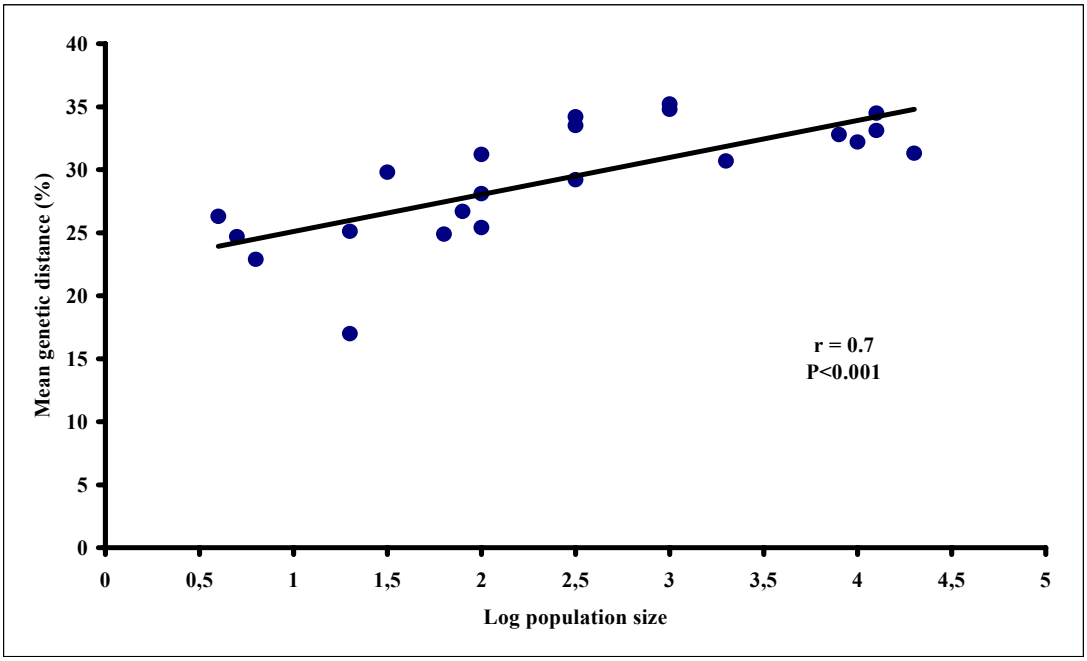

Figure 4b: Correlation between population size and mean genetic distance per population, calculated for *Sesleria albicans*.

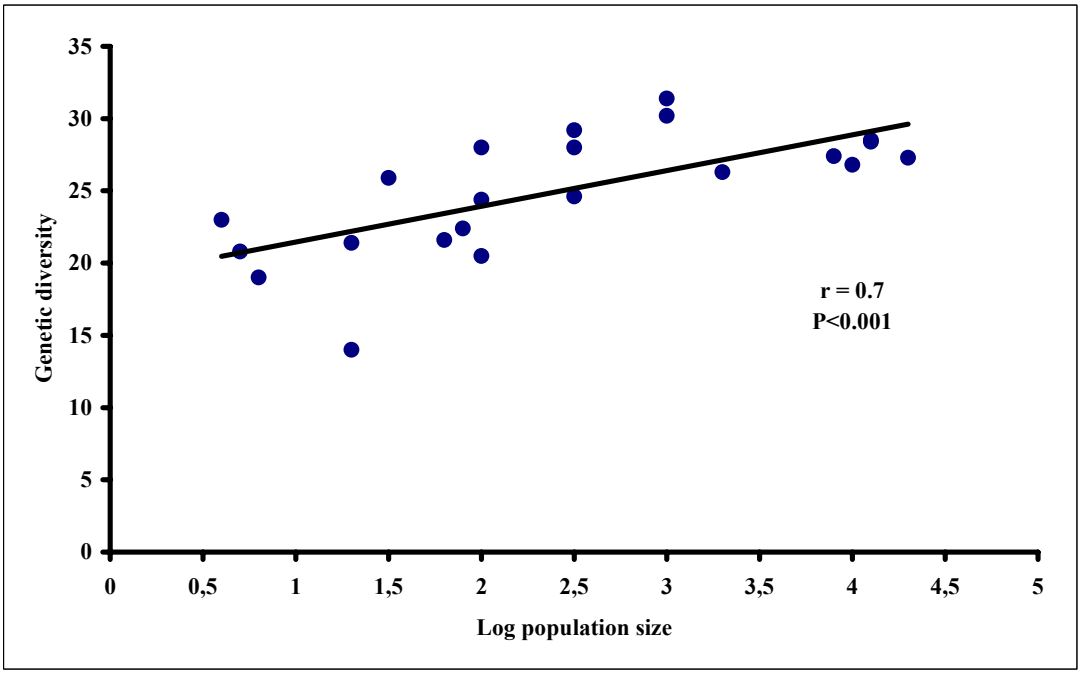

Figure 4c: Correlation between population size and genetic diversity (sums of euclidian squares/n-1) per population, calculated for *Sesleria albicans*.

The sums of euclidian squares/n-1 which were taken from the AMOVA calculation and can be used for the measurement of genetic diversity within populations, also were correlated highly significant with the population size (r_s=0.7, P<0.001, Figure 4c). In contrast, the correlation between population size and maximum genetic distance was not so strong (r_s=0.6, P<0.005).

3.7 Population clustering

In an unrooted and bootstrapped UPGMA dendrogram, based on 344 RAPD markers the 104 individuals of the two species *Sesleria albicans* and *Sesleria caerulea* were clearly seperated from each other (Figure 5).

The dendrogram therefore consisted of two major groups. One small group comprised the individuals of *Sesleria caerulea*. The other large group consisted of all individuals of *Sesleria albicans*. The genetic distances between the individuals of the two species were much higher than the genetic distances between the individuals of *Sesleria albicans*. In this analysis the two closely related *Sesleria* species, therefore, showed distinct genetic differences.

Without any exception, all populations of *Sesleria albicans* and *Sesleria caerulea* could be discriminated from each other. 16 of 26 populations had bootstrap values which were higher than 80 %. The clustering of these populations, therefore, had considerable reliability. *Sesleria albicans* showed, however, only a weak clustering of populations according to their origin from different geographical regions. The south alpine Eggental population was separated with a high bootstrap value from all populations, which were located in the northern Alps and in central Germany. The other populations of *Sesleria albicans*, however, did not form groups according to their common geographical origin.

Furthermore, the populations from the same habitats did not form independent clusters. Except for the west German grassland populations and the southwest German rocky ridge populations most investigated populations were mixed thoroughly, without respect to their origin from different habitats.

To investigate whether there is habitat specific clustering within different geographic regions, further cluster analyses were conducted for the populations from southwest (Figure 6) and west Germany only and also no habitat specific population groups were observed. Even when only the populations from the beechforests, rocky ridges, and grasslands on the Swabian Alb were analysed no clustering of the populations from the same habiat could be found.

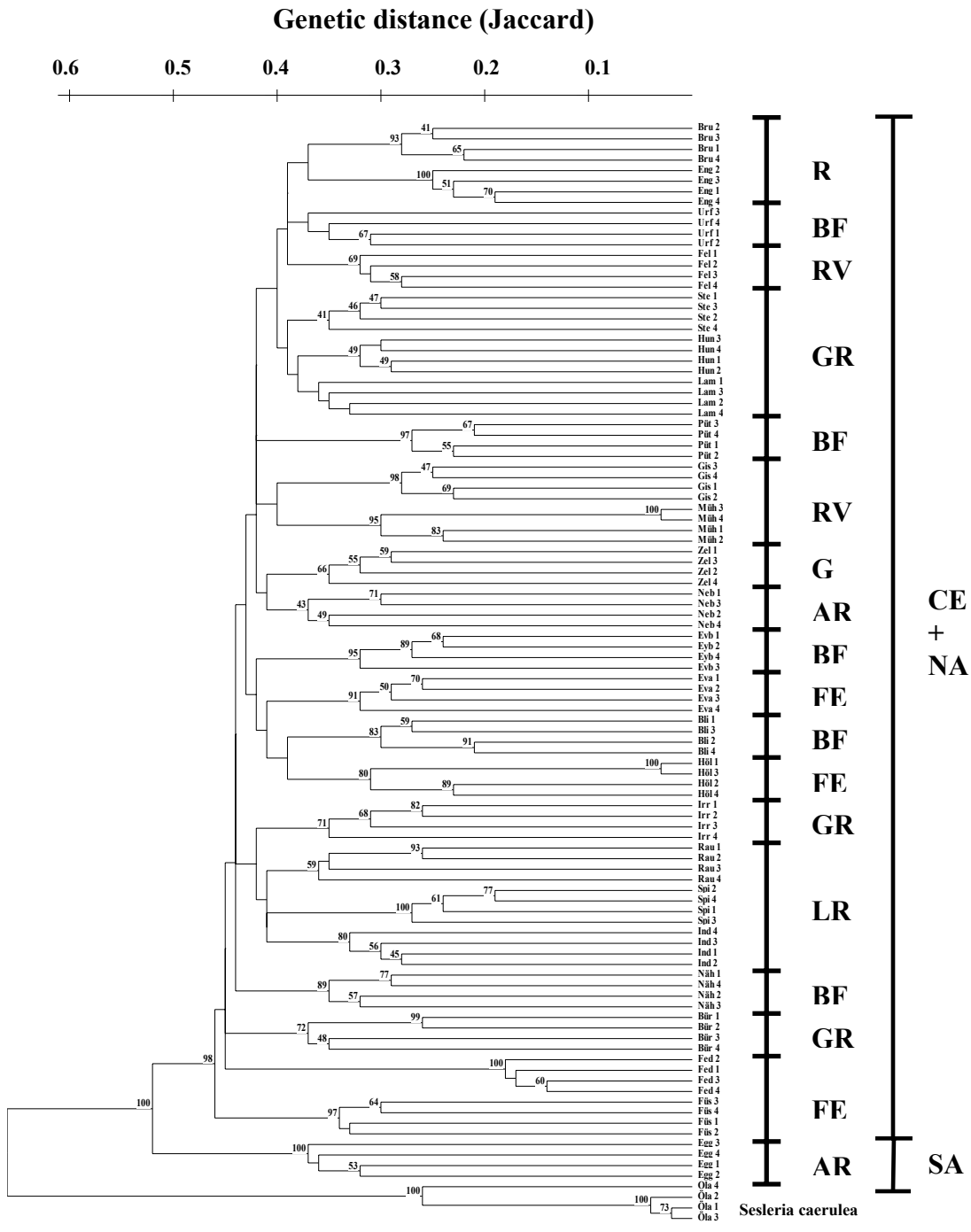

Figure 5: Bootstrapped cluster analysis (UPGMA) of 104 plants of *Sesleria albicans* from the Alps (SA, NA) respectively central Europe (CE) and *S. caerulea* from south Scandinavia (Öla) using 344 RAPD markers, based on the Jaccard's similarity coefficient matrix. Plant material of *S. albicans* was sampled in six different habitats (GR = grassland, RV = rocky river valley slope, BF = beech forest, LR = lowland rocky ridge, FE = fen, AR = alpine rocky ridge). The cluster analysis showed that the populations were clearly separated but not grouped together according to their origin from the same habitat. Numbers at the nodes indicate the probability of the branch when the bootstrap analysis was calculated with 1,000 iterations. Population abbreviations follow Table 1.

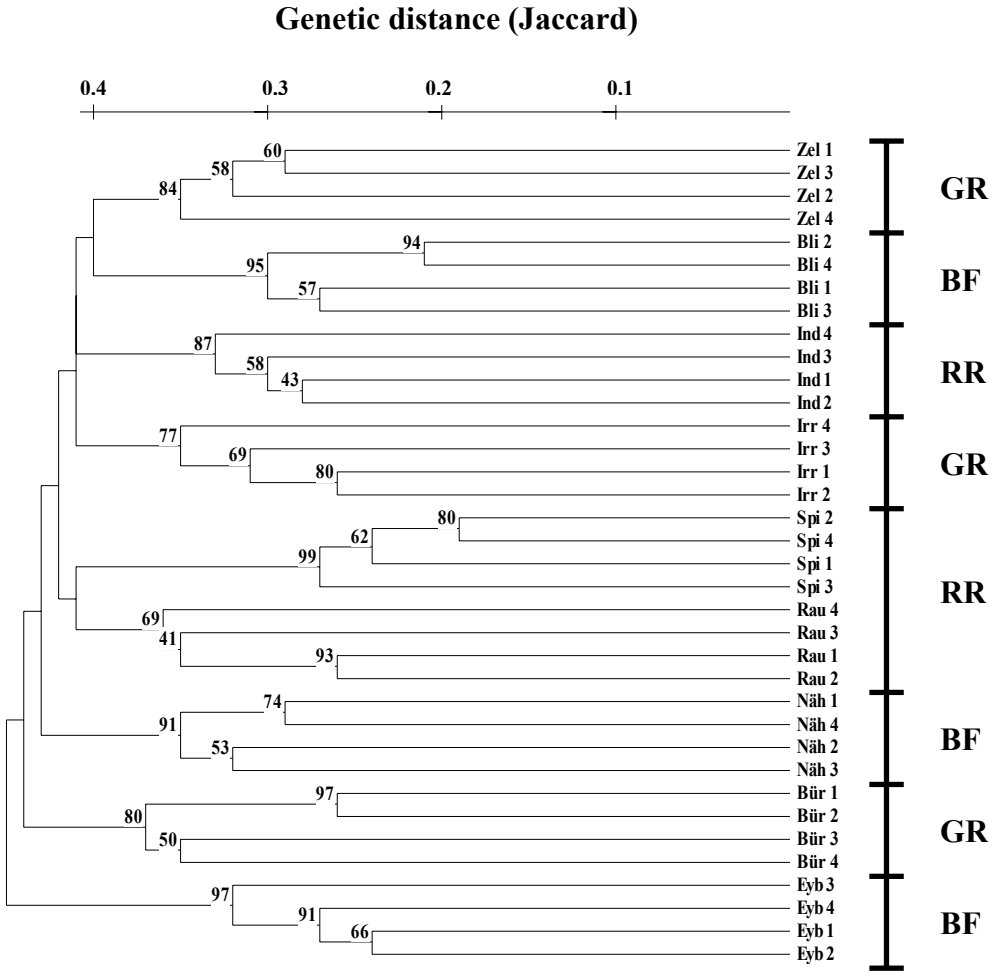

Figure 6: Bootstrapped cluster analysis (UPGMA) of 56 plants of *Sesleria albicans* from the Swabian Alb using 344 RAPD markers, based on the Jaccard's similarity coefficient matrix. Plant material was sampled in three different habitats (GR = grassland, RR = rocky ridge, BF = beech forest). The cluster analysis showed that the populations were clearly separated but not grouped together according to their origin from the same habitat. Numbers at the nodes indicate the probability of the branch when the bootstrap analysis was calculated with 1,000 iterations. Population abbreviations follow Table 1.

4 Discussion

4.1 Partitioning of molecular variance within and among relict populations

The isolation of the central European relict populations of *Sesleria albicans* is a result of population fragmentation since the end of the last glaciation. Isolated glacial relicts often show strong geographical differentiation, as demonstrated by Cardoso et al. (1998), who found a distinct geographical pattern among relict populations of the woody and insect-pollinated plant *Caesalpinia echinata* (Caesalpiniaceae) in Brazil. Moreover, Bauert et al. (1998) reported of distinct geographical clustering of relict populations of the perennial and mainly vegetative propagating *Saxifraga cernua* (Saxifragaceae) in the Alps. However, in an analysis of molecular variance of *Sesleria albicans* 61 % variation were found within populations, 34 % variation were observed among populations and only 5 % molecular variance were found among different regions. *Sesleria albicans*, therefore, only showed weak geographical differentiation. In the UPGMA clustering populations were clearly separated, but the dendrograms also showed that no regional geographic partitioning among the investigated relict populations in central Europe exists. The populations were mixed thoroughly, independent from the fact whether there were small or large geographic distances between them. Additionally, genetic distance and linear geographic distance between populations of *Sesleria albicans* did not significantly correlate. Moreover, geneflow among populations was only 0.47. This represents a very low level of migration. Fischer & Matthies (1998) and Schmidt & Jensen (2000) observed similar values among isolated populations of the insect-pollinated plants *Gentianella germanica* (Gentianaceae) and *Pedicularis palustris* (Scrophulariaceae) which indicated that the populations were subjected to effects of genetic drift.

Sesleria albicans showed no geographical clustering in central Europe as expected for plant populations, that were subjected to long-term isolation. This lack of regional differentiation is presumably due to the fact that long periods of time are required for population differentiation. Loveless & Hamrick (1987) found no unique alleles between *Cirsium canescens* (Asteraceae) and its putatively derived species *C. pitcheri* (Asteraceae), although the two are thought to have diverged 11,000 years ago. Furthermore, Lesica et al. (1988) were unable to detect any genetic variation among four populations of the aquatic plant *Howellia aquatilis* (Campanulaceae) and postulated that mutation did not have sufficient time to establish differences among the populations in the last 10,000 years. The isolation of *Sesleria albicans* since the end of the last glaciation resulted in a genetic differentiation among the populations. However, it seems possible that there was not enough time to create a regional differentiation since the beginning of the fragmentation.

Another reason for lower interpopulational differentiation is the breeding system of *Sesleria albicans*, which is a wind-pollinated and mainly outcrossing species (Dixon 1982). Such plants tend to have lower genetic variation among populations than selfing and insect-pollinated plants, although the differences in genetic diversity between selfing and outcrossing species at the species level are not statistically significant (Hamrick & Godt 1989). Population genetic structure, therefore,

is geographically more differentiated in insect-pollinated species than in wind-pollinated species (Loveless & Hamrick 1984, Hamrick & Godt 1989). Wind-pollinated plants can exchange pollen across large distances, and reach higher levels of geneflow and lower levels of differentiation than insect-pollinated plants. In *Sesleria albicans*, however, we observed a low level of geneflow among the investigated populations. Since Ellstrand & Elam (1993) argue that one immigrant per every second generation or one interpopulational mating per generation (N_em=0.5) will be sufficient to prevent strong differentiation, even this low level of geneflow could be a reason for the lack of a distinct geographical pattern.

Plants which did not have enough time for differentiation because they colonized new habitats after glaciation, very often show low levels of differentiation as demonstrated for the arctic-alpine plant species *Saxifraga oppositifolia* (Saxifragaceae) (Gabrielsen et al. 1997) or the conifer *Pinus resinosa* (Pinaceae) (Mosseler et al. 1993).

Sesleria albicans has a partitioning of molecular variation, which was not expected for a glacial relict plant but has also been observed in many other grass species. Kölliker et al. (1998) for example observed 71 % variation within populations of *Festuca pratensis*, 20 % variance among populations from the same site, and only 9 % variation among different sites. Mengistu et al. (2000) even observed 87 % variability within populations of *Poa annua*, while they found only 2 % variation among populations from the same site and 8 % variance among different sites. Furthermore, Baum et al. (1997) found 74.5 % variation within populations of *Hordeum spontaneum*, whereas they found 25 % among populations from the same site and only 0.5 % RAPD variation among different sites.
Lack of a regional differentiation was also observed in *Hordeum vulgare* (Demissie et al. 1998) and *Triticum urartu* (Castagna et al. 1997). In an investigation of the discontinously and patchy distributed *Triticum dicoccoides*, Nevo & Beiles (1989) observed a sharp local differentiation over short geographic distances, but only a weak differentiation among populations from different countries. They described a genetic mosaic, which was also reported for *Avena sterilis* (Beer et al. 1993) and is presumably due to a fragmented population structure as observed for *Sesleria albicans* in this investigation.

In conclusion it can be stated that the partitioning of molecular variance showed no geographical differentiation among populations of *Sesleria albicans* from central Europe, despite of the long-term-isolation since the end of the last glaciation. This is presumably due to the long periods, which are required for population differentiation and to the wind-pollinated breeding system of *Sesleria albicans*.

4.2 Levels of genetic diversity within relict populations

Compared to more widespread plant species, the populations of *Sesleria albicans* were comparatively rare, especially the isolated outlying populations in west and central Germany. Limited genetic diversity within populations has been reported

for many rare species (Hamrick & Godt 1989, Soltis et al. 1992, Odasz and Savolainen 1996). Nevertheless, Gitzendanner & Soltis (2000) observed that levels of diversity for rare species encompass almost the same range as found in widespread congeners and that a high degree of correlation within a genus for all measures of diversity exists. It is, therefore, not astonishing, that both high and low levels of RAPD variation were reported for rare species (Gustafsson & Gustafsson 1994, Glover & Abbott 1995, Friar et al. 1996, Martin et al. 1997, Smith & Pham 1996, Tansley & Brown 2000).

In *Sesleria albicans*, the percentage of polymorphic bands per population ranged from 29.7 % to 56.7 %. Martin et al. (1997) found 44 to 51 % in the narrow endemic *Erodium paularense* (Geraniaceae), a perennial and insect-pollinated chamaephyte with woody stems which can be found in central Spain. Smith & Pham (1996) observed 40-63 % in the rare endemic *Allium aaseae* (Alliaceae), a perennial and insect-pollinated geophyte which is only located along the Boise Front in North America. In genetically depauperated populations of *Argyroxiphicum sandwicense* (Asteraceae), a perennial and insect-pollinated plant with rosettes from Hawaii, the proportion of polymorphic bands only ranged from 12 to 15 % (Friar et al. 1996), whereas the widespread *Poa annua* (Poaceae) showed between 44 % and 89 % polymorphic bands per population.

In this investigation, 95.9 % of the RAPD fragments were polymorphic. Huff et al. 1993 found 85.6 % of polymorphic bands in the grass *Buchloe dactyloides*, Fahima et al. (1999) 81.4 % in *Triticum dicoccoides* and Kölliker et al. (1998) 68 % in *Festuca pratensis*. Compared with these results, *Sesleria albicans* showed with 95.9 % polymorphic bands a large genetic diversity, despite of its relictual and isolated status in central Europe.

The level of genetic diversity depends to a high degree on different historical events and life history traits (Hamrick et al. 1979, Loveless & Hamrick 1984, Hamrick & Godt 1989). Apart from the distribution range, factors like population size, mating system, longevity and clonality exhibit an enormous influence on the genetic variability of plant species. Moreover, ecological amplitude (Babbel & Selander 1974) and morphological variability (Ge et al. 1999) can be positively correlated with genetic diversity which is dicussed in the following.

4.2.1 Effects of population size

In *S. albicans* population size ranged from few to 20,000 square meters, the differences in size being tremendous. Small populations generally exhibit lower levels of genetic diversity compared to congeneric species with large population sizes (Hamrick & Godt 1989). Reasons for this reduction of genetic variation can be attributed to the effects of inbreeding, which increases the number of homozygotes in the population and genetic drift that leads to random changes in allele frequencies (Ellstrand & Elam 1993). Habitat destruction and fragmentation of large populations will have the same effect by breaking up large, genetically variable populations into isolated, small inbreeding subpopulations which are then

subject to the consequences of drift (Ellstrand & Elam 1993, Oostermeijer et al. 1996, Young et al. 1996).

Many studies reported that small, fragmented populations of so called „new rare" species tend to have lower levels of genetic diversity than large, extensive populations (van Treuren et al. 1991, Raijmann et al. 1994, Prober & Brown 1994, Frankham 1996, Godt et al. 1996, Fischer & Matthies 1998, Menges & Dolan 1998). In only some investigations, genetic diversity was not correlated with population size (Dolan 1994, Ouborg & Van Treuren 1994, Greimler & Dobes 2000).

The populations of the „old rare" species *Sesleria albicans* were exposed to the influence of fragmentation and isolation at least since the end of the last glaciation. As reported for many „new rare" species, a significant correlation between genetic diversity (level of polymorphism, minimum, maximum and mean genetic distance, sums of euclidian squares/n-1) and population size was observed. Extensive populations of *Sesleria albicans*, therefore, maintained higher genetic variation, than small populations which is presumably due to a lower level of inbreeding in large populations.

4.2.2 Effects of longevity and clonality

Sesleria albicans is a perennial grass with deeply rooting rhizomes which form small persistent cushions of numerous tillers. Very old ages were reported for clonal growing species, such as *Carex curvula* (Cyperacae) (Steinger et al. 1996). Long living perennials generally show greater variability than shorter-lived species (Hamrick & Godt 1989). Ayres & Ryan (1997, 1999) and Ranker (1994) emphasize the importance of longevity for the preservation of genetic diversity in populations of *Wyethia reticulata* (Asteraceae) and *Adenophorus periens* (Grammitidaceae). Hsao & Rieseberg (1994) found considerable diversity in the long living populations of *Yushania niitakayamensis* (Poaceae) and ascribe this diversity to higher frequencies of sexual reproduction in the evolutionary history of the species.

The clonal structure of *S. albicans* suggests that the plant can reach considerable ages. An age-determination by means of annual rings could shed light upon the real age of *Sesleria albicans* plants (Dietz & Ullmann 1997). However, the presumably long life-span of *S. albicans* seems to contribute to maintain genetic diversity in the relict populations. This was also observed for the relict plant *Biscutella laevigata* which has long-term persisting, subterranean rhizomes (Dannemann 2000).

4.2.3 Effects of the ecological amplitude

Genetic diversity and ecological amplitude are often positively correlated (Babbel & Selander 1974). Moreover, the presence of genetic variation has been shown to enable a population to adapt to different environments (Bradshaw 1984, Zangerl & Bazzaz 1984) and may allow better adaptation to small-scale patchy and heterogenous environments (Schmidt & Antonovics 1986). *Sesleria albicans* shows a broad ecologal amplitude (Dixon 1982, Schmidt 2000). The investigated populations grew in very different plant communities and colonized natural or seminatural habitats, as well as wet and dry or sunny and shady places. As observed by Deyl (1946), the grass is morphologically very variable. Since high levels of genetic diversity can go along with a high degree of morphological variability (Ge et al. 1999), it is not astonishing that *S. albicans* showed high levels of genetic diversity.

4.3 Genetic diversity in central and peripheral populations

Sesleria albicans showed different levels of intrapopulational diversity in the Alps compared to central Europe. For example a mean of 55.9 % polymorphic bands was observed in the alpine populations, while 47.3 % were found in southwest German populations and 47.1 % in the west and central German populations. Reflecting the present geographic range, *S. albicans* is mainly distributed in the Alps, while the central European populations are peripheral populations near the outer boundary of the geographic range. The peripheral populations from central Europe exhibited lower intrapopulational genetic diversity than the central populations from the Alps. Nevo & Beiles (1989) also observed significant differences in central and marginal populations of *Triticum dicoccoides* in Israel. These results are in correspondence with Lesica & Allendorf (1995), who postulated reduced gene flow (isolation), small population size and founder effects in peripheral populations which all promote genetic drift and result in reduced genetic variation and increased differentiation. Generally disjunct populations and populations located at the extremes of a species range frequently have lower levels of genetic diversity than more centrally located populations (Schwaegerle & Schaal 1979, Yeh & Layton 1979, Shumaker & Babbel 1980, Guries & Ledig 1982, Furnier & Adams 1986) as also seen for *Sesleria albicans*.

4.4 Genetic differentiation among populations from different habitats

The habitats colonized by *Sesleria albicans* differ to a high degree from each other in intensity of land use and with regard to the heterogeneity of the environment and to the availability of nutrients, light and water.

Different types and the intensity of land use are generally thought to make a contribution to the differentiation among and within populations (Poschlod & Jackel 1993, Poschlod et al. 2000). As demonstrated by Zopfi (1993a,b; 1998) different types of grassland management resulted in the development of several ecotypic variants of *Rhinanthus alectorolophus* (Scrophulariaceae) and *Euphrasia rostko-*

rostkoviana (Scrophulariaceae). Van Tienderen & van der Toorn (1991) showed local adaptions of the perennial species *Plantago lanceolata* (Plantaginaceae) to meadows or pastures and Briggs & Block (1992) found adaptions in the length of developing time to weeding pressure in *Senecio vulgaris* (Asteraceae). Moreover, microenvironmental differentiation of *Poa annua* (Poaceae) as a result of different cutting management in populations was reported by Sweeney & Danneberger (1995). Fertilization and defoliation are generally known to cause population differentiation (Snaydon 1987).

Genetic variation within and among populations is affected by ecological factors (Kölliker et al. 1998). Ecotypic development strongly depends on climatic and edaphic conditions (Briggs & Walters 1984, Nevo et al. 1988). These factors can cause microgeographic differentiation, as reported for many grasses such as *Hordeum spontaneum* (Owuor et al. 1997), *Triticum dicoccoides* (Nevo et al. 1988, Nevo & Beiles 1989), *Yushania niitakayamensis* (Hsao & Lee 1999), *Avena barbata* (Hamrick & Allard 1972) or *Aegilops peregrina* (Nevo et al. 1994). Gunter et al. (1996) found genetic differentiation among upland and lowland ecotypes of *Panicum virgatum*, while Colosi & Schaal (1994) observed genetic differences among different biotypes of *Panicum miliaceum*. Because populations of *Sesleria albicans* from fens colonize wet habitats, they were thought to be an own variety or ecotype (Braun-Blanquet 1932, Sebald et al. 1998). Considering these facts, an ecotypical differentiation among the populations of *Sesleria albicans*, which grew in different habitats under various ecological conditions, could have been assumed.

The results of this investigation, however, indicate only a low level of ecotypic differentiation. In an analysis of molecular variance 4.58 % variation was observed among the different habitats. The rest of molecular variability was found among populations from the same habitat and within populations. This pattern of molecular partitioning did only marginally change, when populations from the same geographic regions were analysed. Hsao & Lee (1999) found comparable results in an investigation of *Yushania niitakayamensis* (Poaceae) from grassland and forest undergrowth. They reported significant microgeographic differentiation among populations and observed 2.91 % to 7.99 % molecular variance among populations from different habitats. Huff et al. (1998) reported different results from an analysis of the grass *Schizachyrium scoparium* (Poaceae). They found no significant differentiation among populations from forest and grassland biomes but observed 3.18 % variation among populations from different soil fertility levels. Kölliker et al. (1998) also observed no significant differentiation among populations of *Festuca pratensis* (Poaceae) from sites with differing levels of fertilization and defoliation.

In an UPGMA cluster analysis the populations were not grouped together according to their origin from the same habitat. When comparing only populations that were located close to each other on the same mountain region, there was also no clustering of populations from the same habitat. Not even the populations from the fens, although they were supposed to be an own variety, were arranged close

to each other. The results of this study do, therefore, contradict the assumption, that these populations form an own taxon.

Sesleria albicans generally seems to have a high level of pheno- and genotypic plasticity. This attribute obviously allows the species to colonize very different habitats without being genetically adapted in a specific way to the same habitats.

4.5 Levels of genetic diversity within populations from different habitats

In the analyses of molecular variance within populations the highest levels of intrapopulational diversity were observed within populations from the alpine rocky ridges, while the lowest levels of intrapopulational diversity were found in the populations from the rocky river valley slopes and the fens.

Former investigations have shown that fertilization and higher cutting frequencies reduce the variability within populations (Kölliker et al. 1998). Additionally, increased genetic variability and evolutionary rates were described for heterogenous environments and under environmental stress in open habitats (Antonovics 1971, Hoffmann & Parsons 1991, Parsons 1994). Owuor et al. (1997) observed higher levels of genetic diversity in *Hordeum spontaneum* growing in sunny, open habitats than in shady forestlike habitats. Nevo et al. (1988) observed the highest genetic diversity of *Triticum dicoccoides* in a sunny and rocky habitat which is the ecologically most variable of all investigated habitats. It is supposed that selecting factors of e.g. habitat quality have a stronger effect in open habitats than under the leaf canopy of forests (Poschlod & Schumacher 1998). Genetic diversity generally rises with increasing spatiotemporal ecological heterogeneity in accordance with the prediction of the niche width-variation hypothesis (van Valen 1965), which predicts a positive correlation between ecological and genetic diversity.

In this investigation the highest diversity was observed within the alpine rocky ridges which are the most heterogenous habitats. However, the lowest levels of intrapopulational diversity were found within the rocky river valley slopes and fens, which also are heterogenous habitats, and not in the less heterogenous calcareous grasslands or beechforests. The level of diversity, therefore, must be influenced by additional factors.

As established in other investigations (Nevo et al. 1988), possible ecotypic differentiations can be superimposed by population genetic processes such as founder-effects or genetic drift. The genetic diversity within the relictual populations of *Sesleria albicans* seems to be strongly influenced by population size. The populations from the fens and the rocky river valley slopes were very much smaller than the populations from the calcareous grasslands and the beech forests. The higher intrapopulational diversity within populations from grasslands and the lower diversity within poulations from fens and rocky river valley slopes is, therefore, most likely due to the effects of population size than to ecotypic differentiation.

4.6 Genetic relationship between *Sesleria albicans* and *Sesleria caerulea*

S. albicans and the most closely related species *S. caerulea*, were formerly regarded as two subspecies of the species *S. caerulea* (as *S. caerulea* ssp. *calcaria* and *S. caerulea* ssp. *uliginosa*). Because of differences in distribution and morphology (Ujhelyi 1938) most authors meanwhile consider both taxa as separate species (Deyl 1980, Conert 1999).

As this investigation shows, there also exist significant genetic differences between *Sesleria albicans* and *Sesleria caerulea*. Much higher genetic distances were observed among the populations of the two *Sesleria* species than among all investigated populations of *S. albicans*. Our results, therefore, clearly support the separation of the two species.

5 References

Ahmad, F. (1999): Random Amplified Polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. Theoretical and Applied Genetics 98: 657-663.

Antonovics, J. (1971): The effects of a heterogenous environment on the genetics of natural populations. American Scientist 59: 593-559.

Ayres, D.R. & Ryan, F.J. (1999): Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. American Journal of Botany 86: 344-353.

Ayres, D.R. & Ryan, F.J. (1997): The clonal and population structure of a rare endemic plant *Wyethia reticulata* (Asteraceae): allozyme and RAPD analysis. Molecular Ecology 6: 761-772.

Babbel, G.R. & Selander, R.K. (1974): Genetic variability in edaphically restricted and widespread plant species. Evolution 28: 619-630.

Barrett, S.C.H. & Kohn, J.R. (1991): Genetic and evolutionary consequences of small population sizes in plants: implications for conservation. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 3-30.

Bartlett, M.S. (1937): Some examples of statistical methods of research on agriculture and applied biology. Journal of the Royal Statistical Society, Supplement 4: 137-170.

Bauert, M.R.; Kälin, M.; Baltisberger, M. & Edwards, P.J. (1998): No genetic variation within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. Molecular Ecology 7: 1519-1527.

Baum, B.R.; Nevo, E.; Johnson, D.A. & Beiles, A. (1997): Genetic diversity in wild barley (*Hordeum spontaneum* C. Koch) in the Near East: a molecular analysis using Random Amplified Polymorphic DNA (RAPD) markers. Genetic Ressources and Crop Evolution 44: 147-157.

Beer, S.C.; Goffreda, J.; Phillips, T.D.; Murphy, J.P. & Sorrells, M.E. (1993): Assessment of genetic variation in *Avena sterilis* using morphological traits, isozymes and RFLP's. Crop Science 33: 1386-1393.

Bradshaw, A.D. (1984): Ecological significance of genetic variation between populations. In: Dirzo, R. & Sarukhan, J. (eds): Perspectives in Plant Population Ecology. Sinauer, Sunderland, 213-228.

Braun-Blanquet, J. (1932): Flora von Graubünden 1: 137.

Bresinsky, A. (1965): Zur Kenntnis des circumalpinen Florenelements im Vorland nördlich der Alpen. Berichte der Bayerischen Botanischen Gesellschaft 38: 5-67.

Briggs, D. & Block, M. (1992): Genecological studies of groundsel (*Senecio vulgaris* L.). I. Maintenance of population variation in the Cambridge Botanic Garden. New Phytologist 121: 257-266.

Briggs, D. & Walters, S.M. (1984): Plant variation and evolution. 2 ed., Cambridge University Press, Cambridge.

Cardoso, M.A.; Provan, J.; Powell, W.; Ferreiras, C.G. & de Oliveira, D.E. (1998): High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinoideae). Molecular Ecology 7: 601-608.

Castagna, R.; Gnocchi, S.; Perenzin, M. & Heun, M. (1997): Genetic variability of the wild diploid wheat *Triticum urartu* revealed by RFLP and RAPD markers. Theoretical and Applied Genetics 94: 424-430.

Chan, K.F. & Sun, M. (1997): Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. Theoretical and Applied Genetics 95: 865-873.

Colosi, J.C. & Schaal, B.A. (1994): RAPD's distinguish among biotypes of proso millet (*Panicum miliaceum* L.) and detect significant genetic variation among populations of the recently introduced black-grained weed biotype. American Journal of Botany 81: 69.

Comes, H.P. & Kadereit, J.W. (1998): The effects of quaternary climatic changes on plant distribution and evolution. Trends in Plant Science 3: 432-438.

Conert, H.J. (1998): *Sesleria albicans*. In: Hegi, G.: Illustrierte Flora von Mitteleuropa. I (3), Blackwell, Berlin: 479-482.

Dannemann, A. (2000): Der Einfluss von Fragmentierung und Populationsgröße auf die genetische Variation und Fitness von seltenen Pflanzenarten am Beispiel von *Biscutella laevigata* (Brassicaceae). Dissertationes Botanicae 330, J.Cramer, Berlin, Stuttgart.

Demissie, A.; Bjornstad, A. & Kleinhofs, A. (1998): Restriction fragment length polymorphisms in landrace barleys from Ethopia in relation to geographic, altitude and agro-ecological factors. Crop Science 38: 237-243.

Deyl, M. (1980): *Sesleria albicans*. In: Tutin, T.G.; Heywood, V.H.; Burgess, N.A.; Moore, D.M.; Valentine, D.H.; Walters, S.M. & Webb, D.A. (eds.): Flora europaea 5: 176.

Deyl, M. (1946): Study of the genus *Sesleria*. Opera Botanica Cechica 3.

Dietz, H. & Ullmann, I. (1997): Age-determination of dicotyledonous herbaceous perennials by means of annual rings: exception or rule? Annals of Botany 80: 377-379.

Dixon, J.M. (1996): Waterlogging and survival in *Sesleria albicans*. New Phytologist 133: 415-422.

Dixon, J.M. (1986): Drought resistance in *Sesleria albicans* Kit. ex Schultes, compared with *Agrostis capillaris* L. and *Cynosurus cristatus* L. New Phytologist 103: 559-572.

Dixon, J.M. (1982): Biological Flora of the British Isles: 151. *Sesleria albicans* Kit. ex Schultes. Journal of Ecology 70: 667-684.

Dolan, R.W. (1994): Patterns of isozyme variation in relation to population size, isolation, and phytogeographic history in Royal Catchfly (*Silene regia*; Caryphyllaceae). American Journal of Botany 81: 965-972.

Ellstrand, N.C. & Elam, D.R. (1993): Population genetic consequences of small population size: implications for plant conservation. Annual Reviews of Ecology and Systematics 24: 217-242.

Excoffier, L.; Smouse, P.E. & Quattro, J.M. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitrochondrical DNA restriction data. Genetics 131: 479-491.

Fahima, T.; Sun, G.L.; Beharav, A.; Krugman, T.; Beiles, A. & Nevo, E. (1999): RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theoretical and Applied Genetics 98: 434-447.

Fischer, M. & Matthies, D. (1998): RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). American Journal of Botany 85: 811-819.

Frankham, R. (1996): Relationship of genetic variation to population size in wildlife. Conservation Biology 10: 1500-1508.

Friar, E.A.; Robichau, R.H. & Mount, D.W. (1996): Molecular genetic variation following a population crash in the endangerd Mauna Kea silversword, *Argyroxiphium sandwicense* (Asteraceae). Molecular Ecology 5: 687-691.

Furnier, G.R. & Adams, W.T. (1986): Geographic patterns of allozyme variation in Jeffrey pine. American Journal of Botany 73: 1009-1015.

Gabrielsen, T.M.; Bachmann, K.; Jakobsen, K.S. & Brochmann, C. (1997): Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. Molecular Ecology 6: 831-842.

Ge, S.; Wang, K.-Q.; Hong, D.-Y.; Zhang, W.-H. & Zu, Y.-G. (1999): Comparison of genetic diversity in the endangered *Adenophora lobophylla* and its widespread congener, *A. potaninii*. Conservation Biology 13: 509-513.

Gitzendanner, M.A. & Soltis, P.S. (2000): Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87: 783-792.

Glover, B.J. & Abbott, R.J. (1995): Low genetic diversity in the Scotish endemic *Primula scotica* Hook. New Phytologist 129: 147-153.

Godt, M.J.W.; Johnson, B.R. & Hamrick, J.L. (1996): Genetic diversity and population size in four rare southern appalachian plant species. Conservation Biology 10: 796-805.

Greimler, J. & Dobes, C. (2000): High genetic differentiation in relict lowland populations of *Gentianella austriaca* (A. and J. Kern.) Holub (Gentianaceae). Plant Biology 2: 628-637.

Gunter, L.E.; Tuskan, G.A. & Wullschleger, S.D. (1996): Diversity of populations of switchgrass based on RAPD markers. Crop Science 36: 1017-1022.

Guries, R.P. & Ledig, F.T. (1982): Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). Evolution 36: 387-402.

Gustafsson, L. & Gustafsson, P. (1994): Low genetic variation in swedish populations of the rare species *Vicia pisiformis* (Fabaceae) revealed with RFLP (rDNA) and RAPD. Plant Systematics and Evolution 189: 133-148.

Haffner, W. (1969): Das Pflanzenkleid des Naheberglandes und des südlichen Hunsrück in ökologisch-geographischer Sicht. Decheniana Beihefte 15: 1-145.

Hamrick, J.L. & Allard, R.W. (1972): Microgeographical variation in allozyme frequencies in *Avena barbata*. Proceedings of the National Academy of Sciences of the USA 69: 2100-2104.

Hamrick, J.L. & Godt, W. (1989): Allozyme diversity in plant species. In: Brown, A.H.D.; Clegg, M.T.; Kahler, A.L. & Weir, B.S. (eds.): Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, 229-253.

Hamrick, J.L.; Linhart, Y.B. & Mitton, J.B. (1979): Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annual Reviews of Ecology and Systematics 10: 173-200.

Hantke, R. (1978): Eiszeitalter, Band 1. Ott, Thun.

Hantke, R. (1980): Eiszeitalter, Band 2. Ott, Thun.

Hewitt, G.M. (1996): Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247-276.

Hoffmann, A.A. & Parsons, P.A. (1991): Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford.

Holsinger, K.E. & Gottlieb, L.D. (1991): Conservation of rare and endangered species: principles and prospects. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 195-208.

Hsao, J.Y. & Lee, M. (1999): Genetic diversity and microgeographic differentiation of yushan cane (*Yushania niitakayamensis*; Poaceae) in Taiwan. Molecular Ecology 8: 263-270.

Hsao, J.Y. & Rieseberg, L.H. (1994): Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, Poaceae) in Taiwan. Molecular Ecology 3: 201-208.

Hubbard, C.E. (1985): Gräser. Ulmer Verlag, Stuttgart.

Huff, D.R. (1997): RAPD characterization of heterogenous perennial ryegrass cultivars. Crop Science 37: 557-564.

Huff, D.R.; Peakall, R. & Smouse, P.E. (1993): RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. Theoretical and Applied Genetics 86: 927-934.

Huff, D.R.; Quinn, J.A.; Higgins, B. & Palazzo, A.J. (1998): Random amplified polymorphic DNA (RAPD) variation among native little bluestem [*Schizachyrium scoparium* (Michx.) Nash] populations from sites of high and low fertility in forest and grassland biomes. Molecular Ecology 7: 1591-1597.

Kölliker, R.; Stadelmann, F.J.; Reidy, B. & Nösberger, J. (1998): Fertilization and defoliation frequency affect genetic diversity of *Festuca pratensis* Huds. in permanent grasslands. Molecular Ecology 7: 1557-1567.

Kump, B. & Javornik, B. (1996): Evaluation of genetic variability among common buckwheat (*Fagopyrum esculentum* Moench) populations by RAPD markers. Plant Science 114: 149-158.

Lesica, P.; Leary, R.F.; Allendorf, F.W. & Bilderback, D.E. (1988): Lack of genetic diverstity within and among populations of an endangered plant, *Howellia aquatilis*. Conservation Biology 2: 275-282.

Lesica, P. & Allendorf, F.W. (1995): When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Lloyd, N.D.H. & Woolhouse, H.W. (1978): Leaf resistances in different populations of *Sesleria caerulea* (L.) Ard. New Phytologist 80: 79-85.

Loveless, M.D. & Hamrick, J.L. (1987): Genetic organization and evolutionary history in two north american species of *Cirsium*. Evolution 42: 254-265.

Loveless, M.D. & Hamrick, J.L. (1984): Ecological determinants of genetic structure in plant populations. Annual Reviews of Ecology and Systematics 15: 65-95.

Lutz, E.; Schneller, J. & Holderegger, R. (2000): Understanding population history for conservation purposes: Population genetics of *Saxifraga aizoides* (Saxifragaceae) in the lowlands and the lower mountains north of the Alps. American Journal of Botany 87: 583-590.

Maki, M. & Horie, S. (1999): Random amplified polymorphic DNA (RAPD) markers reveal less genetic variation in the endangered plant *Cerastium fischerianum* var. *molle* than in the widespread conspecific *C. fischerianum* var. *fischerianum* (Caryophyllaceae). Molecular Ecology 8: 145-150.

Mantel, N. (1967): The detection of disease clustering and a generalized regression approach. Cancer research 27: 209-220.

Martin, C.; Gonzalez-Benito, M.E. & Iriondo, J.M. (1997): Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. & Izco. Molecular Ecology 6: 813-820.

Menges, E.S. & Dolan, R.W. (1998): Demographic variability of populations of *Silene regia* in midwestern prairies: relationships with fire management, genetic variation, geographic location, population size and isolation. Journal of Ecology 86: 63-78.

Mengistu, L.W.; Mueller-Warrant, G.W. & Barker, R.E. (2000): Genetic diversity of *Poa annua* in western Oregon grass seed crops. Theoretical and Applied Genetics 101: 70-79.

Miller, M.P. (1997): Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Milligan, B.G.; Leebens-Mack, J. & Strand, A.E. (1994): Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3: 423-435.

Mosseler, A.J.; Egger, K.N. & Innes, D.J. (1993): Life history and the loss of genetic diversity in red pine. American Journal of Botany 80: Abstract 240.

Nevo, E. & Beiles, A. (1989): Genetic diversity of wild emmer wheat in Israel and Turkey. Structure, evolution and application in breeding. Theoretical and Applied Genetics 77: 421-455.

Nevo, E.; Beiles, A. & Krugman, T. (1988): Natural selection of allozyme polymorphisms: a microgeographic differentiation in wild emmer wheat (*Triticum dicoccoides*). Theoretical and Applied Genetics 75: 529-538.

Nevo, E.; Krugman, T. & Beiles, A. (1994): Edaphic natural selection of allozyme polymorphisms in *Aegilops peregrina* at a Galilee microsite in Israel. Heredity 72: 109-112.

Oberdorfer, E. (1990). Pflanzensoziologische Exkursionsflora. Ulmer Verlag, Stuttgart.

Odasz, A.M. & Savolainen, O. (1996): Genetic variation in populations of the arctic perennial *Pedicularis dasyantha* (Scrophulariaceae), on Svalbard, Norway. American Journal of Botany 83: 1379-1385.

Ostermeijer, J.G.B.; Berholz, A. & Poschlod, P. (1996): Genetical aspects of fragmented plant populations. In: Settele, J.; Margules, C.R.; Poschlod, P. & Henle, K. (eds.): Species survival in fragmented landscapes. Kluwer Academic publishers, Dordrecht, 93-101.

Ouborg, N.J. & van Treuren, R. (1994): The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. Evolution 48: 996-1008.

Owuor, E.D.; Fahima, T.; Beiles, A.; Korol, A. & Nevo, E. (1997): Population genetic response to microsite ecological stress in wild barley, *Hordeum spontaneum*. Molecular Ecology 6: 1177-1187.

Palacios, C. & Gonzalez-Candelas, F. (1997): Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufourii* (Plumbaginaceae). Molecular Ecology 6: 1107-1121.

Parsons, P.A. (1994): Habitats, stress and evolutionary rates. Journal for Evolutionary Biology: 387-397.

Poschlod, P.; Dannemann, A., Kahmen, S.; Melzheimer, V.; Biedermann, H.; Mengel, C.; Neugebauer, K.R. & Pantle, I. (2000): Genes in the landscape. Changes in central European land use and its impact on genetic diversity of plants. Schriftenreihe für Vegetationskunde 32: 111-127.

Poschlod, P. & Schumacher, W. (1998): Rückgang von Pflanzen und Pflanzengesellschaften des Grünlandes – Gefährdungsursachen und Handlungsbedarf. Schriftenreihe für Vegetationskunde 29: 83-99.

Poschlod, P. & Jackel, A.-K. (1993): Untersuchungen des Diasporenregens und der Diasporenbank auf zwei Kalkmagerrasenstandorten der Schwäbischen Alb. Flora 188: 49-71.

Prober, S.M. & Brown, A.H.D. (1994): Conservation of the grassy white box woodlands: Population genetics and fragmentation of *Eucalyptus albens*. Conservation Biology 8: 1003-1013.

Raijman, L.E.L.; van Leeuwen, N.C.; Kersten, R.; Oostermeijer, J.G.B.; den Nijs, H.C.M. & Menken, S.B.J. (1994): Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L.. Conservation Biology 8: 1014-1026.

Ranker, T.A. (1994): Evolution of high genetic variability in the rare hawaiian fern *Adenophorus periens* and implications for conservation management. Biological Conservation 70: 19-24.

Rogers, S.O. & Bendich, A.J. (1988): Extraction of DNA from plant tissues. In: Gelvin, S.B.; Schilperoort, R.A. & Verma, D.S. (eds): Plant molecular biology manual. Kluwer Academic Publishers, Dordrecht, 1-10.

Rossetto, M.; Weaver, P.K. & Dixon, K.W. (1995): Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Molecular Ecology 4: 321-329.

Sambrook, J.; Fritsch, E.F. & Maniatis, T. (1989): Molecular cloning: a laboratory manual, 2 ed. Cold Spring Harbour Laboratory Press, New York.

Schmidt, J. & Antonovics, J. (1986): Experimental studies of the evolutionary significance of sexual reproduction. III. Maternal and paternal effects during seedling establishment. Evolution 40: 817-829.

Schmidt, K. & Jensen, K. (2000): Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components. American Journal of Botany 87: 678-689.

Schmidt, M. (2000): Die Blaugrasrasen des nördlichen deutschen Mittelgebirgsraumes und ihre Kontaktgesellschaften. Dissertationes Botanicae 328. J. Cramer, Berlin, Stuttgart.

Schwaegerle, K.E. & Schaal, B.A. (1979): Genetic variability and founder effect in the in the pitcher plant *Sarracenia purpurea* L. Evolution 33: 1210-1218.

Shumaker, K.M. & Babbel, G.R. (1980): Patterns of allozyme similarity in ecologically central and marginal populations of *Hordeum jubatum* in Utah. Evolution 34:110-116.

Sebald, O.; Seybold, S.; Philippi, G. & Wörz, A. (1998): Die Farn- und Blütenpflanzen Baden-Württembergs, Vol. 7. Ulmer Verlag, Stuttgart.

Smith, J.F. & Pham, T.V. (1996): Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). American Journal of Botany 83(6): 717-726.

Snaydon, R.W. (1987): Population responses to environmental disturbance. In: Van Andel, J.; Bakker, J.P.; Snaydon, R.W.: Disturbance in Grasslands. Dr. W. Junk Publishers, Dordrecht.

Sneath, P.H.A. & Sokal, R.R. (1973): Numerical Taxonomy. The principles and practice of numerical classification. W.H. Freeman, San Francisco.

Soltis, P.S.; Soltis, D.E.; Tucker, T.L. & Lang, F.A. (1992): Allozyme variability is absent in the narrow endemic *Bensoniella oregona* (Saxifragaceae). Conservation Biology 6: 131-134.

Steinger, T.; Körner, C. & Schmid, B. (1996): Long-term persistence in a changing climate: DNA analysis suggests very old ages of clones of alpine *Carex curvula*. Oecologia 105: 94-99.

Stewart, C.N. & Excoffier, L. (1996): Assessing population genetic structure and variability with RAPD data: application to *Vaccinium macrocarpum* (American Cranberry). Journal of Evolutionary Biology: 153-171.

Strgar, V. (1966): Zur Unterscheidung der Komplexe *Sesleria calcaria* und *S. tenuifolia* in Slowenien und Kroatien. Biol. Vestn. 27: 71-74.

Sweeney, P.M. & Danneberger, T.K. (1995): RAPD Characterization of *Poa annua* L. populations in golf course greens and fairways. Crop Science 35: 1676-1680.

Tansley, S.A. & Brown, C.R. (2000): RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. Biological Conservation 95: 39-48.

Tüxen, R. (1937): Die Pflanzengesellschaften Nordwestdeutschlands. Mitteilungen der floristisch-soziologischen Arbeitsgemeinschaft in Niedersachsen 3: 1-170.

Ujhelyi, J. (1938): Sesleria-Studien. Index Horti Bot. Univ. Budapest. 3: 109-142.

van de Peer, Y. & de Wachter, R. (1994) TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Applic. Biosci. 10, 569-570.

van Tienderen, P.H. & van der Toorn, J. (1991): Genetic differentiation between populations of *Plantago lanceolata*. I. Local adaption in three contrasting habitats. Journal of Ecology 79: 27-42.

van Treuren, R.; Bijlsma, R.; van Delden, W. & Ouborg, N.J. (1991): The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. Heredity 66: 181-189.

van Valen, L. (1965): Morphological variation and width of ecological niche. American Naturalists 99: 377-390.

Walter, H. & Straka, H. (1970): Arealkunde. Floristisch-historische Geobotanik. Ulmer, Stuttgart.

Weising, K.; Nybom, H.; Wolff, K. & Meyer, W. (1995): DNA Fingerprinting in Plants and Fungi. CRC Press, Boca Raton.

Welsh, J. & Mc Clelland, M. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research 18: 7213-7218.

Whitkus, R.; de la Cruz, M.; Mota-Brava, L. & Gomez-Pompa, A. (1998): Genetic diversity and relationships of cacao (*Theobroma cacao* L.) in southern Mexiko. Theoretical and Applied Genetics 96: 621-627.

Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. & Tingey, S.V. (1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research 18: 6531-6535.

Wright, S. (1951): Variability within and among natural populations. Volume 4. The University of Chicago Press, Chicago.

Yeh, F.C. & Layton, C. (1979): The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* ssp. *latifolia*. Canadian Journal of Genetics and Cytology 21: 487-503.

Young, A.; Boyle, T. & Brown, T. (1996): The population genetic consequences of habitat fragmentation for plants. Tree 11/10: 413-418.

Zangerl, A.R. & Bazzaz, F.A. (1984): Niche partitioning between two phosphoglucoisomerase genotypes in *Amaranthus retroflexus*. Ecology 65: 218-222.

Zopfi, H.-J. (1993a): Ecotypic variation in *Rhinanthus alectorolophus* (Scopoli) Pollich (Scrophulariaceae) in relation to grassland management I. Morphological delimitations and habitats of seasonal ecotypes. Flora 188: 15-39.

Zopfi, H.-J. (1993b): Ecotypic variation in *Rhinanthus alectorolophus* (Scopoli) Pollich (Scrophulariaceae) in relation to grassland management II. The genotypic basis of seasonal ecotypes. Flora 188: 153-173.

Zopfi, H.-J. (1998): The genetic basis of ecotypic variants of *Euphrasia rostkoviana* Hayne (Scrophulariaceae) in relation to grassland management. Flora 193: 41-58.

IV
Genetic diversity within and among populations of *Saxifraga paniculata* Mill. and *Sesleria albicans* Kit. ex Schultes – a comparison of two glacial relict plants

1 Historical events and biological traits

The genetic diversity within and among plant populations is tremendously affected by historical events and the biological traits of the plant species. The genetic variability of the two glacial relict plants *Saxifraga paniculata* and *Sesleria albicans*, therefore, is strongly influenced by factors like population history, range of distribution, population size, vegetative propagation, longevity of the individuals and pollination system (Hamrick et al. 1979, Loveless & Hamrick 1984, Hamrick & Godt 1989). For this reason, the partitioning of genetic diversity and the level of variabiliy within populations of *S. paniculata* and *S. albicans* have to be considered and compared in the light of these factors.

Saxifraga paniculata and *Sesleria albicans* are both glacial relict plants and the species show, therefore, clear mutualities with respect to their population history and their range of distribution: They were widely distributed in the central European tundra during the last glaciation (Bresinsky 1965, Wilmanns & Rupp 1966). However, as a consequence of the post-glacial climatic warming the range of *S. paniculata* and *S. albicans* contracted to the higher altitudes of the Alps (Walter & Straka 1970). In central Europe both species were displaced from most habitats which formerly were covered by tundra, due to the return of forest tree cover. Today, *Saxifraga paniculata* and *Sesleria albicans*, therefore, are widely distributed in the Alps, whereas the central European lowland populations are considered to be glacial relicts (Walter & Straka 1970, Oberdorfer 1990).
Saxifraga and *Sesleria* also coincide with a view to population size. As a result of postglacial fragmentation the population sizes of *Saxifraga paniculata* and *Sesleria albicans* extend over a wide range and many populations of both species only consist of few individuals.
Furthermore, both species show strong vegetative reproduction by rosettes and tillers (Wilmanns & Rupp 1966, Dixon 1982, Kaplan 1995), respectively. Since very old ages were reported for clonal growing plants (Kemperman & Barnes 1976, Steinger et al. 1996), the multiramet, clonal structure of *S. paniculata* and *S. albicans* suggests that the plants can reach considerable ages.
Finally, *S. paniculata* and *S. albicans* show strong correspondence with respect to their pollination system. Both relict species are not self- but cross-pollinated (Kaplan 1995, Conert 1999) which is of enormous importance for the partitioning of genetic diversity within and among populations.

However, despite these correspondences, there are strong differences between the two species concerning the degree of rarity, the ecological amplitude of the plants and the medium which is used for pollination.

Relict populations of *Saxifraga paniculata* are much rarer than relict populations of *Sesleria albicans* (Oberdorfer 1990, Korneck et al. 1996). Since present and historical geneflow depend on the geographic distance between populations this difference can play an important role for the partitioning of genetic diversity.
Furthermore, *S. albicans* shows a substantially broader ecological amplitude than *S. paniculata* (Dixon 1982). *Saxifraga* is restricted to rocky natural habitats, whereas *Sesleria* can be found in rocky habitats, beech forests, fens and man-made calcareous grasslands as well.
Finally, *Saxifraga paniculata* is insect-pollinated whereas *Sesleria albicans* is wind-pollinated (Kaplan 1995, Conert 1999). Since geneflow is more restricted in insect- than in wind-pollinated plants, this factor is of special importance for the genetic diversity within and among populations.

Refering to the population history and the biological traits of the two relict plants, considerable mutualities and differences concerning the genetic diversity within and among populations of *Saxifraga paniculata* and *Sesleria albicans* had to be expected.

2 Partitioning of molecular variance and geographical clustering

The analysis of molecular variance, which was carried out for both species, revealed a very different partitioning of molecular variability within and among populations of the two glacial relicts (Table 1). *Saxifraga paniculata* exhibited approximately as much variance among the different regions (28 %), as within the populations (39 %). In contrast *Sesleria albicans* showed the very most variance within populations (61 %), and only a very low level of molecular variance among the regions (5 %) (see chapter II,3.2 and III,3.2).

Although both glacial relicts exhibited low levels of migration, mean geneflow was slightly higher in *S. albicans* (0.47) than in *S. paniculata* (0.36).
Furthermore, maximum and minimum genetic distance (Φ_{ST}) varied over a wider range in *Saxifraga paniculata* (0.01 – 0.96) than in *Sesleria albicans* (0.13 – 0.63) which means that the populations of *Sesleria* were more similar to each other than the populations of *Saxifraga* (see chapter II,3.3 and III,3.3).

The cluster analyses, carried out for both species with the data from the Jaccard's similarity coefficient matrix, revealed some mutualities but also significant differences. Both in the cluster analysis of *Saxifraga paniculata* and *Sesleria albicans*, the individuals were grouped together according to their origin from the same population. The different populations could, therefore, be clearly seperated from each other in both glacial relict plants (Table 1).

However, the populations of *Saxifraga*, which were located near to each other at the same location, clustered together while this could not be observed for the populations of *Sesleria*. Coroborating these results, the Mantel test, which was calculated for the populations of both species, showed a significant correlation of

the genetic distance (Φ_{ST}) and the geographic distance for *Saxifraga paniculata*, but not for *Sesleria albicans* (see chapter II,3.3 and III,3.3).

The results of this investigation, therefore, clearly showed a high level of geographic differentiation among relictual populations of *Saxifraga paniculata* and a low level of differentiation among relictual populations of *Sesleria albicans*.
The populations of *Saxifraga* were genetically more distant to each other, the geneflow was lower and considerable molecular variance could be observed among regions and within populations. In contrast, the populations of *Sesleria albicans* were genetically more related, the geneflow was higher, and molecular variance could be observed mainly within populations and hardly among regions.

These differences between *Saxifraga paniculata* and *Sesleria albicans* are directly connected with life history traits such as the pollination system, the glacial and post-glacial history of the populations and the distribution range of the relict plants during the glaciations and after the last glaciation.

Table 1: Geographical differentiation among populations of *Saxifraga paniculata* and *Sesleria albicans*. For each species information about the partitioning of molecular variance, the clustering of individuals and populations, the correlation of geographic and genetic distance, the minimum and maximum genetic distances, and the geneflow were given.

Geographical differentiation	***Saxifraga paniculata***	***Sesleria albicans***
Analysis of molecular variance (AMOVA):		
Level of variation among regions	28.30	04.53
Level of variation among populations within regions	32.60	34.25
Level of variation within populations	39.10	61.22
Cluster analysis:		
Clustering of individuals from the same population:	Yes	Yes
Clustering of populations located near to each other:	Yes	No
Correlation of geographic and genetic distance:		
Correlation coefficient (r):	0.63	0.17
Level of significance:	$p<0.01$	$p=0.06$
Genetic distances:		
Maximum genetic distance (Φ_{ST})	0.96	0.63
Minimum genetic distance (Φ_{ST})	0.01	0.13
Mean geneflow:		
N_em	0.36	0.47

The geographical differentiation of populations is generally known to be influenced by the pollination system of the plants. Geneflow among populations of insect-pollinated species is geographically more restricted than geneflow among populations of wind-pollinated plants (Govindaraju 1988, Hamrick & Godt 1989, Wingender 2000). A geographical differentiation is, therefore, more likely for insect- than wind-pollinated plants. Since *Saxifraga* is insect- and *Seslera* wind-pollinated, this difference presumably is the main reason for the higher level of geographical differentiation in *Saxifraga paniculata*. However, not all insect-pollinated relict species, show a clear geographical differentiation, as demonstrated by Dannemann (2000) for *Biscutella laevigata*. The populations of this glacial relict species exhibited no geographical clustering which is explained by a high level of historical geneflow that prevented regional differentiation.

Today *Saxifraga paniculata* is rarer than *Sesleria albicans* in central Europe (Oberdorfer 1990). However, nothing is known about the rarity of the two species during the interglacials and the last glaciation. Reflecting the present distribution in the Alps, it can be supposed that *Saxifraga paniculata* was always rarer than *Sesleria albicans*, even during the last glaciation. The more restricted distribution and the stronger geographical isolation of the populations probably could have made a contribution to the higher level of geographical differentiation observed in *Saxifraga paniculata*.

Finally, the geographical differentiation of populations is well known as a result of long-term isolation, due to restricted geneflow among populations, combined with increasing genetic drift and inbreeding (Ellstrand & Elam 1993, Fischer & Matthies 1998). Although many investigations demonstrated, that long periods of time are required for the differentiation of populations (Loveless & Hamrick 1987, Lesica et al. 1988, Niebling & Conkle 1990), little is known about the speed of differentiation. As demonstrated by studies of long-lived conifers, the generation period seems to play an important role in this question. This is also mentioned by Dannemann (2000), who explains the lack of a regional differentiation among populations of *Biscutella laevigata* with the long generation period of the relict plant. To date, nothing is known about the fact whether population differentiation proceeds faster in *Saxifraga paniculata* or in *Sesleria albicans*. However, a presumably faster development of *Saxifraga* could have intensified geographical differentiation among populations of *Saxifraga paniculata*.

3 Genetic diversity within populations of glacial relicts

Despite of their relictual status both *Saxifraga paniculata* and *Sesleria albicans* exhibited high levels of genetic diversity (Table 2). However, the percentage of polymorphic bands was slightly higher in *Sesleria* (95.9 %) than in *Saxifraga* (91.2 %). Furthermore *Sesleria* produced more bands (344) than *Saxifraga* (319) and the size of the fragments varied over a wider range in *Sesleria albicans* (210-4123) than in *Saxifraga paniculata* (336-3687) (see chapter II,3.1 and III,3.1).

Table 2: Total RAPD banding of *Saxifraga paniculata* and *Sesleria albicans*. For each species the size of the fragments, the number of polymorphic and monomorphic bands, and the percentage of polymorphic bands were given.

RAPD banding	***Saxifraga paniculata***	***Sesleria albicans***
Size of the fragments:	336 – 3687	210 – 4123
Number of polymorphic bands:	291	330
Number of monomorphic bands:	28	14
Number of bands total:	319	344
Percentage of polymorphic bands:	91.2	95.9

The genetic diversity within the populations of the relict plants ranged from 12 to 54 % in populations of *Saxifraga paniculata* and 30 to 57 % in populations of *Sesleria albicans* with a mean of 30 % and 48 %, respectively (see chapter II,3.4 and III,3.4). Mean genetic diversity, therefore, was higher in *Sesleria* than in *Saxifraga*. However, the level of genetic diversity within the populations varied over a wider range in populations of *Saxifraga* than of *Sesleria*. The genetic distances, taken from the Jaccard's similarity coefficient matrix and the matrix of squared euclidian distances also revealed higher levels of diversity within populations of *Sesleria* than in populations of *Saxifraga*. This, for example, was demonstrated by the mean Jaccard-Distance which was 16.7 % in *Saxifraga* and 29.9 % in *Sesleria* populations (Table 3). However, despite of their different levels of diversity within populations, genetic variability highly significant correlated with population size in *Saxifraga paniculata* and *Sesleria albicans*. Larger populations, therefore, showed higher genetic diversity than smaller ones in both species (see chapter II,3.3 and III3,3).

Table 3: Genetic variation within the populations of *Saxifraga paniculata* and *Sesleria albicans*. For each species mean percentage of polymorphic bands and mean genetic distances were calculated.

Genetic variation	***Saxifraga paniculata***	***Sesleria albicans***
Polymorphic bands (%):	29.5	47.9
Jaccard-Distance Min (%):	12.1	24.0
Jaccard-Distance Max (%):	20.8	34.0
Jaccard-Distance Mean (%):	16.7	29.9
Sums of euclidian squares/n-1:	13.4	25.3
Correlation with population size:	Yes	Yes

The genetic diversity within populations is directly related to the history of the populations, the rarity of the species, the pollination system, the longevity of the individuals and the ecological amplitude of the plant species.

Both high (Smith & Pham 1996, Tansley and Brown 2000) and low levels (Glover & Abbott 1995, Friar et al. 1996) of genetic diversity have been observed within rare populations. Although the level of genetic diversity depends on the estimator used to assess the variability (Widmer & Lexer 2001), relictual populations often show high levels of genetic diversity compared to widespread plant species, as demonstrated by many authors (Abbott et al. 1995, Lewis & Crawford 1995, Comes & Kadereit 1998, Dannemann 2000, Lutz et al. 2000). Since the populations of *Saxifraga paniculata* and *Sesleria albicans* were both relict plant populations, the results of this study were coroborated by the results reported for other relict populations.

Comparing *Saxifraga* and *Sesleria* the investigation of Gitzendanner & Soltis (2000) should be considered, who showed significantly lower genetic diversity in rare compared to wide-spread species. *Sesleria* is more widespread than *Saxifraga* and the higher level of diversity within *Sesleria* can at least partly be due to this fact.

Saxifraga paniculata and *Sesleria albicans* were both cross-pollinated. Cross-pollinated plants generally seem to have higher levels of genetic diversity within populations than self-pollinated species (Wolff et al. 1994, Wingender et al. 2000). The pollination system, therefore, makes a strong contribution to the high levels of genetic diversity found in populations of glacial relict plants in this investigation. According to Dannemann (2000), the high levels of genetic diversity observed in relict populations of *Biscutella laevigata* are mainly due to the allogamic pollination system. However, wind-pollinated plants generally exhibit higher levels of diversity than insect-pollinated species (Govindaraju 1988, Hamrick & Godt 1989, Wingender 2000). The higher level of genetic variability observed in the populations of *Sesleria* is, therefore, mainly due to the wind-pollination.

The longevity of plants also contributes to the higher level of genetic diversity within the investigated populations. Long-living perennials usually show greater variability than shorter-lived species (Hamrick & Godt 1989). Both *Saxifraga paniculata* and *Sesleria albicans* show strong vegetative propagation by rosettes and tillers, respectively. The multiramet, clonal structure of *S. paniculata* and *S. albicans* suggests that the plants can reach considerable ages. The longevity of *Saxifraga* and *Sesleria*, therefore, presumably plays an important role in maintaining the observed high levels of genetic diversity, as reported for the glacial relict *Biscutella laevigata* (Dannemann 2000) and other plant species before (Ayres & Ryan 1997, 1999).

Finally, *Sesleria albicans* exhibits a considerably broader ecological amplitude than *Saxifraga paniculata*. Since genetic diversity and ecological amplitude are often positively correlated (Babbel & Selander 1974), this may also have an influence on the higher levels of genetic diversity, observed in populations of *Sesleria albicans*.

4 Genetic diversity in central populations and peripheral relict populations

Both *Saxifraga paniculata* and *Sesleria albicans* showed different levels of intrapopulational diversity in the Alps compared to central Europe (Table 4). As demonstrated above, *Saxifraga* exhibited 29.6 % polymorphic bands in southwest Germany and 34.1 % in the Alps, while *Sesleria* showed 47.3 % in southwest Germany and 55.9 % in the Alps (see chapter II,3.5 and III,3.5). Despite the fact that a higher quantity of central European populations was investigated, the peripheral relict populations of both species were genetically less variable than the populations from the present main distribution area.

These results are in correspondence with Lesica & Allendorf (1995), who postulated reduced gene flow (isolation), small population size and founder effects in peripheral populations, which all promote genetic drift and result in reduced genetic variation and increased differentiation. Generally disjunct populations and populations located at the extremes of a species range have frequently lower levels of genetic diversity than more centrally located populations (Furnier & Adams 1986, Guries & Ledig 1982, Schwaegerle & Schaal 1979, Shumaker & Babbel 1980, Yeh & Layton 1979), which is in correspondence with the results presented in this study. However, not all glacial relicts show this genetic pattern, as demonstrated in an investigation of *Biscutella laevigata*. In this study, Dannemann (2000) found no higher levels of genetic diversity in populations which were located near to the centre of the distribution area.

Table 4: Genetic variation within the populations of *Saxifraga paniculata* and *Sesleria albicans* from west Germany, southwest Germany and the Alps. For each geographic region mean percentage of polymorphic bands and mean genetic distances were calculated.

Region	West Germany	Southwest Germany	Alps
Polymorphic bands (%):			
Saxifraga paniculata	26.6	29.6	34.1
Sesleria albicans	47.1	47.3	55.9
Jaccard-Distance Min (%):			
Saxifraga paniculata	08.5	11.8	16.2
Sesleria albicans	23.4	23.4	30.8
Jaccard-Distance Max (%):			
Saxifraga paniculata	18.2	21.2	24.5
Sesleria albicans	33.5	33.7	39.1
Jaccard-Distance Mean (%):			
Saxifraga paniculata	13.7	16.5	20.5
Sesleria albicans	29.2	29.5	35.6

5 Conclusions with respect to the conservation of glacial relict plants

A major goal of conservation biology is to preserve genetic variation and evolutionary processes in viable populations of ecologically important species in order to prevent potential extinction (Soule & Simberloff 1986). Loss of genetic diversity is thought, potentially, to lead to a decrease in a species' ability to survive environmental changes and demographic fluctuations, both in the short- and long-term (Ellstrand & Elam 1993, Milligan et al. 1994), although many authors caution that no causal link between low genetic variation and a population's long-term viability has yet been proven (Milligan et al. 1994, Schemske et al. 1994). The maintenance of at least a constant level of genetic variation is, therefore, generally considered essential for the long-term protection of a taxon (Frankel & Soule 1981, Simberloff 1988). Consequently the study of population genetics has been identified as one of the main priorities for conservation (Holsinger & Gottlieb 1991). Many of these studies resulted in strategies for conservation management (Rossetto et al. 1995, Stewart & Porter 1995, Ayres & Ryan 1997, Martin et al. 1997, Cardoso et al. 1998, Sydes & Peakall 1998).

The present investigation revealed, that *Saxifraga paniculata* maintained considerable genetic diversity both within and among populations despite and because of the long-term isolation at least since the end of the last glaciation. To ensure the long-term protection, conservation strategies should aim at the maintenance of the entire genetic diversity. Although larger populations showed higher genetic diversity than smaller ones, this study demonstrated that every population of *Saxifraga* shows uniqually genetic characteristics. For this reason all populations from all geographical regions are worth to be protected. However, special attention should be aimed in any case to the largest populations of every location and region.

This study also demonstrated, that *Sesleria albicans* shows only a low level of geographical and ecotypic differentiation. Most of the genetic variability of *Sesleria albicans* is located within the populations. From this point of view, it would not be necessary to preserve populations from different regions or habitats. However, even a low differentiation can be the starting point of a further evolutionary development. It would, therefore, be advisable in any case to prevent populations from all geographical regions and habitats.

Furthermore, the isolated central European populations of *Saxifraga paniculata* and *Sesleria albicans* are located near the outer boundary of the geographic range. Such populations are considered to have a high priority for the conservation of biodiversity in Europe (Korneck et al. 1996). This is in agreement with Lesica & Allendorf (1992), who emphasize the fact, that the occurrence of stress-induced heterozygous advantage in plants and the cocomitant increased retention of variation suggests that small populations that occasionally undergo moderate levels of stress may retain higher levels of heterozygosity than similar sized populations in benign environments. Geographical outliers, such as the investigated relict populations, are likely to occur in ecologically marginal or stressful conditions.

From that point of view the peripheral populations are in general of disproportionately importance for preserving the genetic diversity of the whole species (Lesica & Allendorf 1995). The protection of the peripheral populations of *Saxifraga paniculata* and *Sesleria albicans* in central Europe, therefore, requires special attention.

Since the establishment of new populations was not observed to date, both *Saxifraga paniculata* and *Sesleria albicans* seem to have low dispersal ability (Philippi 1984). The species, therefore, show no metapopulation dynamics. For this reason, conservation efforts have to focus on the preservation of persisting populations.

6 Biological traits and genetic diversity of further glacial relict plants

The genetic diversity within and among populations of glacial relicts is, as mentioned above, tremendously influenced by the biological traits and the distribution of the plants. The high level of genetic diversity observed within the investigated relict populations of *Saxifraga paniculata* and *Sesleria albicans* is, on the one hand, due to the allogamic pollination system of the plants and the longevity of the individuals. Different levels of diversity within populations of the two relict species can, on the other hand, be attributed to the media used for pollination (insects or wind), the degree of rarity, the generation period and the varying ecological amplitude.

An analysis concerning the biological traits of further central European glacial relicts (Table 5), showed that most relict plant species were perennial plants with an allogamic pollination system. Only few relict species were apomictic or exclusively self-pollinating plants. Considering the results of the present investigation discussed above, high levels of diversity can carefully be presumed for further glacial relict plant species. However, the central European glacial relict plants show strong differences concerning the medium used for pollination, the longevity of the species and the ecological amplitude. Variable high levels of diversity can, therefore, be presumed for different relict species. Since genetic diversity strongly depends on the longevity of plant species and long generation periods mainly can be observed in clonal growing plant species, genetic depauperation is more likely for plant species with weak than with strong vegetative propagation. Lower levels of genetic diversity, therefore, especially can be assumed for species such as *Pedicularis sceptrum-carolinum*, *Kernera saxatilis*, *Athamanta cretensis* or *Anemone narcissiflora*.

The analysis of the most important glacial relicts in central Europe also showed considerable differences with respect to the distribution of the plants (Table 5). Some glacial relicts are very rare while others are more widely distributed. Since the geographic differentiation of relict populations strongly depends on the level of geneflow among the more or less isolated plant populations, different levels of geographic differentiation can be assumed for different glacial relict species.

Table 5: Biological traits of central European glacial relict plant species (LS = life span, PO = pollination, VP = vegetative propagation, LF = life form, HA = habitat, RL = red list, PR = protection, DB = number of german topographic maps for which the species is reported, Per = perennial, W = windpollinated, I = insectpollinated, S = selfpollinated, A = apomict, Nph = nanophanerophyte, Hcr = hemicryptophyte, Cph = chamaephyte, Gph = geophyte, FO = forest, D = dwarf shrub vegetation, F = fen, R = rock, G = grassland).

Glacial relict species:	**LS**	**PO**	**VP**	**LF**	**HA**	**RL**	**PR**	**DB**
Prealps:								
Betula nana	Per	W	Weak	Nph	F/D	2	§	14
Betula humilis	Per	W	Weak	Nph	F/FO	2	§	39
Salix myrtlloides	Per	W	Middle	Nph	F	1	-	14
Carex heleonastes	Per	W	Strong	Hcr	F	1	-	20
Juncus stygius	Per	W	Strong	Hcr	F	1	§	3
Saxifraga hirculus	Per	I	Middle	Hcr	F	1	§	3
Pedicularis sceptrum-carolinum	Per	I	Weak	Hcr	F	2	§	16
Swabian Alb:								
Kernera saxatilis	Per	I/S	Weak	Cph	R	-	-	8
Draba aizoides	Per	I/S	Strong	Cph	R	-	§	20
Arabis alpina	Per	S	Middle	Cph	R	-	-	3
Athamanta cretensis	Per	I	Weak	Hcr	R	-	-	3
Androsace lactea	Per	I/S	Middle	Hcr	R	3	§	1
Gentiana lutea	Per	I	Middle	Hcr	G/FO	3	§	42
Campanula cochlearifolia	Per	I	Strong	Hcr	R	-	-	9
Hieracium humile	Per	A	Weak	Hcr	R	3	-	26
Traunsteinera globosa	Per	I/S	Weak	Gph	G	-	§	5
Carex sempervirens	Per	W	Strong	Hcr	G	-	-	7
Polygonum viviparum	Per	A	Strong	Hcr	G	-	-	4
Anemone narcissiflora	Per	I	Weak	Gph	G/FO	3	§	12
Ranunculus oreophilus	Per	I	Weak	Hcr	R	3	-	12
Pedicularis foliosa	Per	I	Weak	Hcr	G	-	§	1
Saxifraga paniculata	Per	I/S	Strong	Cph	R	-	§	41
Sesleria albicans	Per	W	Middle	Hcr	G/F/R/FO	-	-	57
Black Forest:								
Luzula desvauxii	Per	W	Strong	Hcr	R	R	-	2
Silene rupestris	Per	I/S	Middle	Hcr	R	-	-	11
Alchemilla hoppeana	Per	A	Weak	Cph	R	-	-	2
Primula auricula	Per	I	Weak	Hcr	R	3	§	3
Veronica fruticans	Per	I/S	Weak	Hcr	R	-	-	3
Potentilla aurea	Per	I	Middle	Hcr	G	-	-	3
Ligusticum mutellina	Per	I	Weak	Hcr	R	-	-	1
Soldanella alpina	Per	I	Weak	Hcr	G	-	§	2
Homogyne alpina	Per	I/S	Strong	Hcr	G/FO	-	-	2
Gnaphalium norvegicum	Per	I	Weak	Hcr	G	-	-	3
Gnaphalium supinum	Per	I	Strong	Hcr	G	-	-	1
Crepis pyrenaica	Per	I	Weak	Hcr	G	-	-	1
Saxifraga stellaris	Per	I/S	Strong	Cph	R	-	§	3
Bartsia alpina	Per	I	Weak	Gph	G	-	-	3
Harz:								
Gypsophila repens	Per	I	Strong	Cph	R	-	-	1
Cardaminopsis petraea	Per	I	Weak	Hcr	R	-	-	2
Anthriscus nitidus	Per	I	Weak	Hcr	FO	-	-	1
Aster alpinus	Per	I	Weak	Hcr	G	-	§	4
Carex vaginata	Per	W	Strong	Gph	G	R	-	2
Hieracium alpinum	Per	A	Weak	Hcr	G/D	-	-	2

In conclusion it has to be stated that is very difficult to make reliable predictions concerning the genetic diversity within and among populations of glacial relicts in general. Further investigations would, therefore, be of tremendous interest.

7 Prospects

The study presented here, impressively demonstrated the great advantages of DNA markers for the analysis of plant population genetic diversity. However, for several decades, numerous studies on population genetics used isozymes to detect variation. Although there exist many studies, data on RAPD variation in plant populations are comparatively rare. Further investigations using RAPD analysis would, therefore, be extremely desirable. Moreover, the use of codominant DNA markers, such as microsatellites or AFLP's, would give elucidating information about the levels of heterozygosity in the populations of the investigated plants. Additional analyses using further DNA markers would, therefore, be of tremendous interest.

In this study a very large number of plants was analysed. However, due to the aim of the investigation, most of the individuals were sampled in central European relict populations. To get an extensive overview on the genetic diversity within and among the populations of the two species, it would be elucidating to analyse more individuals of *Saxifraga paniculata* and *Sesleria albicans* from the Alps and from other regions in europe. Since *Saxifraga paniculata* is also distriduted in North America, an investigation of plants from this geographic region would be, furthermore, of strong systematic interest.

Finally, the present investigation revealed highly interesting and elucidating facts, concerning the genetic diversity of glacial relict plant populations. It shed light upon the genetic consequences of population isolation and differentiation. However, there are many other alpine relict species (Table 5), such as *Kernera saxatilis*, *Draba aizoides*, *Carex sempervirens* or *Traunsteinera globosa* and arctic-alpine relict species, such as *Gnaphalium norvegicum*, *Silene rupestris*, *Anemone narcissiflora* or *Saxifraga stellaris* which occur with rare and isolated populations in the lower mountains of central Europe. Since these central European relict species show common and different biological traits, an investigation of further relict plant species could shed light upon the general principles, determining the genetic diversity within and among populations of glacial relict plants.

8 References

Abbott, R.J.; Chapman, H.M.; Crawford, R.M.M. & Forbes, D.G. (1995): Molecular diversity and derivations of populations of *Silene acaulis* and *Saxifraga oppositifolia* from the high arctic and more southerly latitudes. Molecular Ecology 4: 199-207.

Ayres, D.R. & Ryan, F.J. (1999): Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. American Journal of Botany 86: 344-353.

Ayres, D.R. & Ryan, F.J. (1997): The clonal and population structure of a rare endemic plant *Wyethia reticulata* (Asteraceae): allozyme and RAPD analysis. Molecular Ecology 6: 761-772.

Babbel, G.R. & Selander, R.K. (1974): Genetic variability in edaphically restricted and widespread plant species. Evolution 28: 619-630.

Bresinsky, A. (1965): Zur Kenntnis des circumalpinen Florenelements im Vorland nördlich der Alpen. Berichte der Bayerischen Botanischen Gesellschaft 38: 5-67.

Cardoso, M.A.; Provan J.; Powell, W.; Ferreiras, C.G. & de Oliveira, D.E. (1998): High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinoideae). Molecular Ecology 7: 601-608.

Comes, H.P. & Kadereit, J.W. (1998): The effects of quaternary climatic changes on plant distribution and evolution. Trends in Plant Science 3: 432-438.

Conert, H.J. (1998): *Sesleria albicans*. In: Hegi, G.: Illustrierte Flora von Mitteleuropa. I (3), Blackwell, Berlin: 479-482.

Dannemann, A. (2000): Der Einfluss von Fragmentierung und Populationsgröße auf die genetische Variation und Fitness von seltenen Pflanzenarten am Beispiel von *Biscutella laevigata* (Brassicaceae). Dissertationes Botanicae 330. J.Cramer, Berlin, Stuttgart.

Dixon, J.M. (1982): Biological Flora of the British Isles: 151. *Sesleria albicans* Kit. ex Schultes. Journal of Ecology 70: 667-684.

Ellstrand, N.C. & Elam, D.R. (1993): Population genetic consequences of small population size: implications for plant conservation. Annual Reviews of Ecology and Systematics 24: 217 242.

Fischer, M. & Matthies, D. (1998): RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). American Journal of Botany 85: 811-819.

Frankel, R. & Soule, M.E. (1981): Conservation and Evolution. Cambridge University Press, Cambridge.

Friar, E.A.; Robichau, R.H. & Mount, D.W. (1996): Molecular genetic variation following a population crash in the endangerd Mauna Kea silversword, *Argyroxiphium sandwicense* (Asteraceae). Molecular Ecology 5: 687-691.

Furnier, G.R. & Adams, W.T. (1986): Geographic patterns of allozyme variation in Jeffrey pine. American Journal of Botany 73: 1009-1015.

Gitzendanner, M.A. & Soltis, P.S. (2000): Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87: 783-792.

Glover, B.J. & Abbott, R.J. (1995): Low genetic diversity in the Scotish endemic *Primula scotica* Hook. New Phytologist 129: 147-153.

Govindaraju, D.R. (1988): Relationship between dispersal ability and levels of geneflow in plants. Oikos 52: 31-35.

Guries, R.P. & Ledig, F.T. (1982): Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). Evolution 36: 387-402.

Hamrick, J.L. & Godt, M.J.W. (1989): Allozyme diversity in plant species. In: Brown, A.H.D.; Clegg, M.T.; Kahler, A.L. & Weir, B.S. (eds.): Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, M.A., 43-63.

Hamrick, J.L.; Linhart, Y.B. & Mitton, J.B. (1979): Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annual Reviews of Ecology and Systematics 10: 173-200.

Holsinger, K.E. & Gottlieb, L.D. (1991): Conservation of rare and endangered species: priciples and prospects. In: Falk, D.A. & Holsinger, K.A. (1991): Genetics and conservation of rare plants. Oxford University Press, New York, 195-208.

Kaplan, K. (1995): Saxifragaceae. In: Weber, H.E. (ed.): Gustav Hegi, Illustrierte Flora von Mitteleuropa, IV (2A). Blackwell, Berlin, 130-229.

Kemperman, J.A. & Barnes, B.V. (1976): Clone size in American Aspens. Canadian Journal of Botany 76: 755-761.

Korneck, D.; Schnittler, M. & Vollmer, I. (1996): Rote Liste der Farn- und Blütenpflanzen (*Pteridophyta* et *Spermatophyta*) Deutschlands. Schriftenreihe für Vegetationskunde 28: 21-188.

Lesica, P.; Leary, R.F.; Allendorf, F.W. & Bilderback, D.E. (1988): Lack of genetic diverstity within and among populations of an endangered plant, *Howellia aquatilis*. Conservation Biology 2: 275-282.

Lesica, P. & Allendorf, F.W. (1992): Are small populations of plants worth preserving? Conservation Biology 6: 135-139.

Lesica, P. & Allendorf, F.W. (1995): When are peripheral populations valuable for conservation? Conservation Biology 9: 753-760.

Lewis, P.O. & Crawford, D.J. (1995): Pleistocene refugium endemics exhibit greater allozymic diversity than widespread congeners in the genus *Polygonella* (Polygonaceae). American Journal of Botany 82: 141-149.

Loveless, M.D. & Hamrick, J.L. (1984): Ecological determinants of genetic structure in plant populations. Annual Reviews of Ecology and Systematics 15: 65-95.

Loveless, M.D. & Hamrick, J.L. (1987): Genetic organization and evolutionary history in two north american species of *Cirsium*. Evolution 42: 254-265.

Lutz, E.; Schneller, J. & Holderegger, R. (2000): Understanding population history for conservation purposes: Population genetics of *Saxifraga aizoides* (Saxifragaceae) in the lowlands and the lower mountains north of the Alps. American Journal of Botany 87: 583-590.

Martin, C.; Gonzalez-Benito, M.E. & Iriondo, J.M. (1997): Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. & Izco. Molecular Ecology 6: 813-820.

Milligan, B.G.; Leebens-Mack, J. & Strand, A.E. (1994): Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3: 423-435.

Niebling, C.R. & Conkle, M.T. (1990): Diversity of washoe pine and comparisons with allozymes of ponderosa pine races. Canadian Journal of Forest Research 20: 298-308.

Oberdorfer, E. (1990): Pflanzensoziologische Exkursionsflora. Ulmer, Stuttgart.

Philippi, G. (1984): Trockenrasen, Sandfluren und thermophile Saumgesellschaften des Tauber-Main-Gebietes. Veröffentlichungen aus Naturschutz und Landschaftspflege 57/58: 533-618.

Rossetto, M.; Weaver, P.K. & Dixon, K.W. (1995): Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Molecular Ecology 4: 321-329.

Schemske, D.W.; Husband, B.C.; Ruckelshaus, M.H.; Goddwillie, C.; Parker, I.M. & Bishop, J.G. (1994): Evaluating approaches to the conservation of rare and endangered plants. Ecology 74: 584-606.

Schwaegerle, K.E. & Schaal, B.A. (1979): Genetic variability and founder effect in the in the pitcher plant *Sarracenia purpurea* L. Evolution 33: 1210-1218.

Shumaker, K.M. & Babbel, G.R. (1980): Patterns of allozyme similarity in ecologically central and marginal populations of *Hordeum jubatum* in Utah. Evolution 34:110-116.

Simberloff, D. (1988): The contribution of population and community biology to conservation science. Annual Reviews of Ecology and Systematics 19: 473-512.

Smith, J.F. & Pham, T.V. (1996): Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). American Journal of Botany 83(6): 717-726.

Soule, M.E. & Simberloff, D. (1986): What do genetics and ecology tell us about the design of nature reserves? Biological Conservation 35: 19-40.

Steinger, T.; Körner, C. & Schmid, B. (1996): Long-term persistence in a changing climate: DNA analysis suggests very old ages of clones of alpine *Carex curvula*. Oecologia 105: 94-99.

Stewart, C.N. & Porter, D.M. (1995): RAPD profiling in biological conservation: an application to estimating clonal variation in rare and endangered *Iliamna* in Virginia. Biological Conservation 74: 135-142.

Sydes, M.A. & Peakall, R. (1998): Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragoceae) revealed by allozymes and RAPDs. Molecular Ecology 7: 87-93.

Tansley, S.A. & Brown, C.R. (2000): RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. Biological Conservation 95: 39-48.

Walter, H. & Straka, H. (1970): Arealkunde. Floristisch-historische Geobotanik. Ulmer, Stuttgart.

Widmer, A. & Lexer, C. (2001): Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends in Ecology and Evolution 16: 267-269.

Wilmanns, O. & Rupp, S. (1966): Welche Faktoren bestimmen die Verbreitung alpiner Felsspaltenpflanzen auf der Schwäbischen Alb? Veröffentlichungen der Landesstelle für Naturschutz und Landschaftspflege Baden-Württemberg 34: 62-85.

Wingender, R. (2000): Impact of breeding systems on the genetic diversity of natural plant populations. Schriftenreihe für Vegetationskunde 32: 31-35.

Wolff, K.; Rogstad, S.H. & Schaal, B.A. (1994): Population and species variation of minisatellite DNA in *Plantago*. Theoretical and Applied Genetics 87: 733-740.

Yeh, F.C. & Layton, C. (1979): The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* ssp. *latifolia*. Canadian Journal of Genetics and Cytology 21: 487-503.

V
Summary

Saxifraga paniculata and *Sesleria albicans* are widely distributed in the Alps, but also occur with isolated populations in the lower European highlands. The present distribution of the two species is a result of the climatic changes after the last glaciation. Due to the warmer conditions, which were not so suited for alpine and arctic-alpine plants, population size and range of *Saxifraga paniculata* and *Sesleria albicans* were greatly reduced since the end of the pleistocene. The peripheral and isolated populations in the mountains of central Europe are, therefore, considered to be glacial relict populations.

Populations of *Saxifraga paniculata* and *Sesleria albicans* were fragmented at least since the end of the last glaciation. Furthermore, the relict populations colonize habitats, which differ from each other with regard to ecological conditions and selection pressures. In the present investigation this was used to study the consequences of longterm isolation for the genetic diversity of plant populations and to analyze the genetic differentiation of populations and the development of ecotypes.

To investigate the genetic diversity within and among populations in this study 30 populations (131 individuals) of *Saxifraga paniculata* and 25 populations (100 individuals) of *Sesleria albicans* from central Europe and the Alps were subjected to RAPD and ITS analysis.

The results clearly showed a high level of geographic differentiation among relictual populations of *Saxifraga paniculata*. In an analysis of molecular variance approximately as much variability could be observed among geographic regions as within populations and in a cluster analysis the populations from the same region or location were grouped together. These results were coroborated by a significant correlation of the genetic distance (Φ_{ST}) and the geographic distance between the populations of *Saxifraga paniculata*.

In contrast, *Sesleria albicans* exhibited only a low level of geographic differentiation. In an analysis of molecular variance most of the variability was observed within populations and in a cluster analysis the populations were mixed up thoroughly independent from the fact whether they were located near to each other or not. A significant correlation of the genetic distance (Φ_{ST}) and the geographic distance could, furthermore, not be observed for *Sesleria albicans*.

These findings can be explained with the pollination system and the geographic distribution of the two species. *Saxifraga paniculata* is an insect-pollinated plant species with a limited distribution which promotes geographic differentiation. In contrast, *Sesleria albicans* is a wind-pollinated plant species with a wider geographic distribution which prevents a regional differentiation.

Both *Saxifraga paniculata* and *Sesleria albicans* exhibited considerable levels of genetic diversity within populations. However, genetic diversity was slightly higher within populations of *Sesleria*. Furthermore, genetic variability highly significant correlated with population size in both species. Larger populations, therefore, showed higher genetic diversity than smaller ones in both species. Moreover, peripheral relict populations of both species were genetically less variable than the populations from the present main distribution area in the Alps.

The considerable genetic diversity observed within relict populations of *Saxifraga paniculata* and *Sesleria albicans* is characteristic for relict plant populations. It is mainly due to the allogamic pollination system of the two plant species and the longevity of the individuals, which both contributes to the maintenance of genetic variability. The sightly higher level of molecular diversity within populations of *Sesleria* can be attributed to the fact that the species is wind-pollinated, shows a broader ecological amplitude and is more widely distributed than *Saxifraga*.

The lower level of genetic diversity within small and/or peripheral relict populations can be explained by the effects of inbreeding, leading to an increase in the number of homozygotes in the population and genetic drift, producing random changes in allele frequencies which results in reduced genetic variation.

Although the habitats colonized by *Sesleria albicans* differ to a high degree from each other in intensity of land use and with regard to the heterogeneity of the environment and to the availability of nutrients, light and water, in this investigation only a low level of ecotypic differentiation could be observed. *Sesleria albicans* generally seems to have a high level of pheno- and genotypic plasticity. This attribute obviously allows the species to colonize very different habitats without being genetically adapted in a specific way to the same habitats.

Since the establishment of new populations was not observed to date, *Saxifraga paniculata* and *Sesleria albicans* seem to show no metapopulation dynamics. For this reason, conservation efforts have to focus on the preservation of persisting populations.

Loss of genetic diversity is thought, potentially, to lead to a decrease in a species' ability to survive environmental changes and demographic fluctuations. Conservation strategies, therefore, should aim at the maintenance of the entire genetic diversity of a species to ensure long-term protection.

This study demonstrated that every population of *Saxifraga paniculata* and *Sesleria albicans* shows uniqually genetic characteristics. Furthermore, the isolated central European populations of the two species are located near the outer boundary of the geographic range. The relict populations are, for this reason, considered to have a high priority for the conservation of biodiversity in Europe. The protection of the peripheral populations of *Saxifraga paniculata* and *Sesleria albicans* in central Europe requires, therefore, special attention.

VI
Appendix

Table A1: Pairwise test of heteroscedascity of molecular variance among 30 populations of *Saxifraga paniculata*. Bartlett's B is given for each pair of populations. Levels of significance above diagonal: * P<0.05; ** P<0.01; *** P<0.001. P values indicate the probability that a random B is larger than the observed B and are based on 1,000 iterations. Populations are numerated as in Table 1 (p. 19).

	1	2	3	4	5	6	7	8	9	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
2	2.20		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
3	3.89	2.62		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
4	5.94	3.21	3.91			***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
5	6.18	3.40	4.05	1.03		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
6	5.68	3.20	3.91	1.83	1.90		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
7	6.44	3.36	4.05	2.01	2.12	2.12		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
8	5.58	3.68	4.21	2.96	3.18	322	3.40		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
9	3.07	2.11	2.66	1.80	1.84	1.95	1.98	2.05		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	
12	6.31	3.74	4.32	2.25	2.42	3.07	2.93	3.60	2.18		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
13	6.06	3.28	3.73	1.56	1.75	2.26	1.90	3.17	1.70	1.67		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
14	6.12	3.25	3.96	1.64	2.02	2.42	2.56	3.21	1.75	2.14	1.19		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
15	9.05	5.53	6.21	3.83	3.87	4.05	4.01	5.31	3.82	4.76	3.98	4.62		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
16	7.69	4.64	5.56	3.25	3.37	3.48	3.46	4.80	3.04	3.85	3.34	3.87	2.17		***	***	***	***	***	***	***	***	***	***	***	***	***	***
17	7.99	4.71	5.35	3.27	3.31	354	3.41	4.63	2.90	3.76	3.22	3.67	2.53	1.01		***	***	***	***	***	***	***	***	***	***	***	***	***
18	8.56	5.30	5.81	3.61	3.57	3.93	3.77	4.96	3.36	4.24	3.70	3.99	3.93	3.05	2.90		***	***	***	***	***	***	***	***	***	***	***	***
19	6.80	3.88	4.67	2.86	3.01	3.36	3.27	3.68	2.38	3.83	2.92	3.03	4.95	4.29	4.14	4.59		***	***	***	***	***	***	***	***	***	***	***
20	6.21	3.58	4.40	2.44	2.71	2.90	2.63	3.56	2.07	3.38	2.57	2.80	4.69	3.89	3.83	4.51	2.33		***	***	***	***	***	***	***	***	***	***
21	9.85	4.24	5.49	2.72	3.23	3.61	3.43	3.48	2.02	4.21	3.51	3.87	5.90	5.25	5.30	5.62	4.41	3.64		***	***	***	***	***	***	***	***	***
22	9.52	5.23	6.15	3.64	4.53	4.53	4.17	5.08	2.89	4.93	3.71	4.51	6.70	5.89	6.17	6.58	5.33	4.62	6.44		***	***	***	***	***	***	***	***
23	7.92	4.33	4.96	4.17	4.10	4.02	4.25	4.69	2.63	4.96	4.12	4.20	6.63	6.01	5.78	6.31	5.05	4.62	6.03	6.89		***	***	***	***	***	***	***
24	6.06	3.39	4.12	3.28	3.23	3.23	3.44	3.92	2.07	4.09	3.29	3.35	5.77	5.08	4.94	5.37	4.36	3.98	5.00	5.56	3.26		***		***			***
25	6.89	3.60	4.67	2.67	2.76	3.13	3.22	3.83	1.86	3.71	2.77	2.85	5.22	4.56	4.51	4.98	3.72	3.59	4.60	4.96	3.86	3.49				***	***	***
26	7.92	4.41	5.53	3.72	3.87	3.95	4.21	4.61	2.52	4.75	3.72	3.84	6.23	5.58	5.51	6.08	4.69	4.54	5.89	5.99	4.80	4.24	1.17		***	***	***	***
27	7.11	3.62	4.75	3.07	3.03	3.31	3.44	4.15	2.15	3.98	3.07	3.25	5.33	4.73	4.63	5.30	3.89	3.70	4.94	5.40	3.96	3.76	0.80	1.62		***	***	***
28	7.09	3.63	4.74	3.02	3.17	3.46	3.55	4.01	2.13	4.04	3.10	3.29	5.53	4.77	4.63	5.46	392	3.82	5.03	5.68	4.05	3.76	1.48	2.11	1.61		***	***
29	7.72	4.03	5.14	3.34	3.31	3.71	3.75	4.25	2.31	4.38	3.33	3.51	5.83	5.25	5.21	5.74	4.18	4.01	5.39	5.92	4.29	3.84	1.16	2.12	1.52	1.69		***
30	9.37	6.60	7.47	6.17	6.18	6.41	6.42	6.89	4.83	6.99	6.20	6.53	8.40	7.61	7.62	8.23	7.11	6.72	7.68	8.17	7.29	6.94	5.97	6.60	6.12	5.95	63.9	

Table A2: Pairwise genetic distances (Φ_{ST}) among 30 populations of *Saxifraga paniculata*. Levels of significance above diagonal: * P<0.05; ** P<0.01; *** P<0.001. P values indicate the probability that a random Φ_{ST} is larger than the observed Φ_{ST} and are based on 1,000 iterations. Populations are numerated as in Table 1 (p. 19).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
2	0.22		***	***	***	***	***	***	***		***	***	***	***	***	***	***		***	***	***	***	***	***	***	***	***	***	***	***
3	0.39	0.41		***		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
4	0.53	0.48	0.54		***	***	***	***	***	***	***		***	***	***	***		***	***	***	***	***	***	***	***	***	***	***	***	***
5	0.54	0.50	0.55	0.07		***	***	***	***	***	*	***	*		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
6	0.52	0.48	0.53	0.28	0.29		***	***	***	***	***	***		***	***		***	***	***	***	***	***	***	***	***	***	***	***	***	***
7	0.57	0.51	0.56	0.32	0.33	0.33		***	***	***		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
8	0.59	0.62	0.69	0.45	0.48	0.49	0.53		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
9	0.53	0.54	0.64	0.43	0.44	0.47	0.48	0.59		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*
10	0.60	0.70	0.78	0.57	0.54	0.55	0.57	0.86	0.82		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
11	0.53	0.56	0.68	0.39	0.33	0.44	0.39	0.75	0.50	0.47		***	***	***	***	***	***	***	***	***	***	***	***	***		***	***	***		***
12	0.57	0.57	0.62	0.34	0.36	0.47	0.46	0.60	0.55	0.71	0.56		***	***	***	***	***	***	***		***	***	***	***	***	***	***	***	***	***
13	0.54	0.49	0.52	0.22	0.26	0.36	0.29	0.49	0.40	0.54	0.31	0.21		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	
14	0.55	0.49	0.55	0.23	0.32	0.39	0.41	0.49	0.42	0.56	0.36	0.32	0.12		***		***	***	***		***	***	***	***	***	***	***	***	***	***
15	0.68	0.71	0.76	0.50	0.50	0.53	0.53	0.77	0.78	0.85	0.80	0.65	0.52	0.60		***	***	***	*		***	***	***	***	***	***	***	***	***	***
16	0.64	0.65	0.72	0.47	0.48	0.50	0.50	0.73	0.70	0.78	0.70	0.58	0.48	0.55	0.33		***	***	***	***	***	***	***	***	***	***	***	***	***	***
17	0.65	0.66	0.71	0.48	0.48	0.52	0.51	0.72	0.68	0.77	0.68	0.57	0.48	0.54	0.39	0.06		***	***	***	***	***		***	***	***	***	***	***	***
18	0.67	0.70	0.74	0.50	0.49	0.54	0.53	0.75	0.73	0.81	0.74	0.61	0.51	0.55	0.59	0.48	0.46		***		***	***	***	***	***	***	***	***	***	***
19	0.60	0.59	0.65	0.44	0.46	0.51	0.50	0.61	0.59	0.68	0.56	0.58	0.45	0.47	0.66	0.62	0.61	0.64			***	***	***	***	***	***	***	***	***	***
20	0.57	0.55	0.62	0.38	0.42	0.45	0.41	0.59	0.53	0.65	0.53	0.53	0.40	0.44	0.64	0.58	0.58	0.63	0.37		***	***	***	***	***	***	***	***	***	***
21	0.47	0.40	0.48	0.28	0.34	0.38	0.36	0.34	0.34	0.49	0.34	0.41	0.37	0.40	0.49	0.47	0.48	0.49	0.43	0.36		***	***	***	***	***	***	***	***	***
22	0.56	0.56	0.61	0.42	0.50	0.50	0.47	0.60	0.54	0.61	0.56	0.54	0.43	0.50	0.63	0.60	0.62	0.63	0.57	0.52	0.42		***	***	***	***	***	***	***	***
23	0.65	0.63	0.67	0.61	0.60	0.59	0.62	0.71	0.63	0.75	0.61	0.68	0.60	0.61	0.77	0.75	0.73	0.76	0.69	0.65	0.55	0.66		***	***	***	***	***	***	***
24	0.56	0.53	0.59	0.51	0.50	0.50	0.53	0.63	0.52	0.67	0.46	0.61	0.51	0.52	0.71	0.68	0.67	0.70	0.63	0.60	0.48	0.59	0.51		***	***	***	***	***	***
25	0.59	0.53	0.62	0.43	0.44	0.50	0.51	0.58	0.45	0.63	0.38	0.55	0.45	0.46	0.65	0.62	0.62	0.65	0.55	0.54	0.46	0.54	0.57	0.54			***	***	***	***
26	0.65	0.64	0.71	0.56	0.57	0.58	0.61	0.70	0.62	0.75	0.62	0.67	0.56	0.57	0.75	0.72	0.72	0.75	0.66	0.65	0.54	0.61	0.67	0.62	0.08		***	***	***	
27	0.60	0.53	0.63	0.49	0.48	0.52	0.54	0.62	0.52	0.65	0.41	0.58	0.49	0.51	0.66	0.64	0.63	0.67	0.57	0.56	0.49	0.57	0.58	0.57	0.01	0.20		***	***	***
28	0.60	0.53	0.62	0.48	0.50	0.54	0.55	0.60	0.51	0.64	0.44	0.59	0.49	0.52	0.67	0.64	0.63	0.68	0.57	0.57	0.49	0.59	0.59	0.56	0.20	0.31	0.23		***	***
29	0.64	0.59	0.67	0.52	0.52	0.57	0.57	0.65	0.57	0.69	0.50	0.63	0.52	0.54	0.71	0.69	0.69	0.71	0.61	0.60	0.51	0.61	0.62	0.58	0.11	0.33	0.20	0.24		***
30	0.71	0.77	0.84	0.69	0.68	0.70	0.71	0.89	0.87	0.96	0.95	0.79	0.69	0.72	0.88	0.84	0.83	0.87	0.80	0.77	0.59	0.70	0.80	0.78	0.66	0.76	0.68	0.66	0.72	

Table A3: Geneflow among the 30 investigated populations of *Saxifraga paniculata* (N_em = 0.25 X (1 / Φ_{ST} – 1) calculated from Φ_{ST} values (Wright 1951). Populations are numerated as in Table 1 (p. 19).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	0.88																												
3	0.38	0.34																											
4	0.21	0.26	0.21																										
5	0.20	0.24	0.20	2.94																									
6	0.23	0.26	0.21	0.63	0.59																								
7	0.18	0.24	0.19	0.53	0.48	0.48																							
8	0.17	0.15	0.11	0.30	0.26	0.25	0.21																						
9	0.22	0.20	0.13	0.32	0.31	0.27	0.26	0.16																					
10	0.16	0.10	0.06	0.18	0.21	0.19	0.18	0.04	0.05																				
11	0.22	0.18	0.11	0.38	0.50	0.31	0.38	0.08	0.24	0.28																			
12	0.18	0.18	0.15	0.47	0.43	0.27	0.29	0.16	0.19	0.10	0.19																		
13	0.20	0.25	0.22	0.88	0.69	0.43	0.59	0.26	0.36	0.21	0.54	0.88																	
14	0.20	0.25	0.20	0.79	0.53	0.38	0.34	0.25	0.33	0.19	0.43	0.52	1.76																
15	0.11	0.09	0.07	0.24	0.24	0.22	0.21	0.07	0.07	0.04	0.06	0.13	0.22	0.16															
16	0.13	0.12	0.09	0.27	0.26	0.24	0.24	0.08	0.10	0.06	0.10	0.17	0.26	0.20	0.49														
17	0.13	0.12	0.10	0.26	0.26	0.23	0.24	0.09	0.11	0.07	0.11	0.18	0.26	0.21	0.38	3.35													
18	0.12	0.10	0.08	0.24	0.25	0.21	0.21	0.08	0.08	0.05	0.08	0.15	0.23	0.20	0.17	0.26	0.28												
19	0.16	0.17	0.13	0.31	0.29	0.23	0.24	0.15	0.16	0.11	0.19	0.17	0.30	0.28	0.12	0.14	0.15	0.13											
20	0.18	0.20	0.14	0.39	0.33	0.29	0.34	0.17	0.22	0.13	0.22	0.21	0.36	0.31	0.14	0.17	0.18	0.14	0.41										
21	0.27	0.36	0.26	0.62	0.48	0.40	0.44	0.46	0.48	0.26	0.47	0.35	0.42	0.36	0.25	0.27	0.26	0.25	0.32	0.42									
22	0.19	0.19	0.15	0.34	0.24	0.24	0.27	0.16	0.20	0.15	0.19	0.20	0.33	0.24	0.14	0.16	0.15	0.14	0.18	0.22	0.34								
23	0.13	0.14	0.12	0.16	0.16	0.17	0.15	0.10	0.14	0.07	0.15	0.11	0.16	0.15	0.07	0.08	0.08	0.07	0.11	0.12	0.20	0.12							
24	0.19	0.22	0.17	0.23	0.24	0.24	0.21	0.14	0.22	0.11	0.28	0.10	0.23	0.22	0.09	0.11	0.11	0.10	0.14	0.16	0.26	0.17	0.23						
25	0.17	0.21	0.15	0.32	0.30	0.24	0.23	0.17	0.30	0.14	0.39	0.19	0.30	0.29	0.13	0.15	0.14	0.13	0.19	0.20	0.28	0.21	0.18	0.21					
26	0.13	0.14	0.09	0.19	0.18	0.17	0.15	0.10	0.15	0.08	0.14	0.12	0.19	0.18	0.08	0.09	0.09	0.08	0.12	0.13	0.21	0.15	0.12	0.15	2.82				
27	0.16	0.21	0.14	0.25	0.26	0.22	0.21	0.14	0.22	0.13	0.35	0.17	0.25	0.23	0.12	0.14	0.14	0.11	0.18	0.19	0.26	0.18	0.17	0.18	52.9	0.96			
28	0.16	0.21	0.15	0.26	0.24	0.21	0.20	0.16	0.23	0.13	0.31	0.17	0.25	0.22	0.12	0.14	0.14	0.11	0.18	0.18	0.25	0.17	0.17	0.18	0.99	0.55	0.82		
29	0.14	0.17	0.12	0.22	0.22	0.18	0.18	0.13	0.18	0.10	0.24	0.14	0.22	0.20	0.10	0.11	0.11	0.09	0.15	0.16	0.23	0.15	0.14	0.17	1.97	0.50	0.95	0.77	
30	0.09	0.07	0.04	0.11	0.11	0.10	0.09	0.03	0.03	0.01	0.01	0.06	0.11	0.09	0.03	0.04	0.04	0.03	0.06	0.07	0.17	0.10	0.06	0.07	0.12	0.07	0.11	0.12	0.09

Table A4: Pairwise test of heteroscedascity of molecular variance among 25 Populations of *Sesleria albicans*. Bartlett's B is given for each pair of populations. Levels of significance above diagonal: * P< 0.05; ** P< 0.01; *** P< 0.001. P values indicate the probability that a random B is larger than the observed B and are based on 1,000 iterations. Populations are numerated as in Table 1 (p. 59).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
2	2.77		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
3	2.28	2.36		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
4	2.32	2.36	2.01		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
5	2.43	2.91	2.58	2.33		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
6	2.40	2.74	1.95	1.99	2.29		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
7	2.82	3.58	2.64	2.64	2.89	2.14		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
8	2.63	2.72	2.10	2.16	2.53	1.71	2.32		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
9	2.40	2.80	2.21	2.08	237	1.72	2.57	1.83		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
10	2.46	2.60	1.94	1.94	2.29	1.81	2.46	1.81	1.95		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
11	2.46	2.87	2.08	2.17	2.34	1.65	2.45	1.84	1.81	1.70		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
12	2.19	2.42	1.63	1.55	2.08	1.85	2.32	1.76	1.82	1.63	1.73		***	***	***	***	***	***	***	***	***	***	***	***	***	***
13	2.24	2.27	1.85	1.78	2.39	1.75	2.67	1.97	1.89	1.69	1.83	1.30		***	***	***	***	***	***	***	***	***	***	***	***	***
14	2.20	2.57	1.75	1.80	2.30	1.78	2.51	1.83	175	1.75	1.79	1.33	1.46		***	***	***	***	***	***	***	***	***	***	***	***
15	2.73	2.80	2.18	2.14	2.58	1.79	2.55	1.93	1.99	1.69	1.78	2.00	1.85	1.76		***	***	***	***	***	***	***	***	***	***	***
16	2.70	3.04	2.34	2.24	2.76	2.28	2.87	2.47	2.25	2.25	2.31	2.02	2.24	2.08	2.33		***	***	***	***	***	***	***	***	***	***
17	2.44	3.02	2.02	2.08	2.57	1.99	2.50	1.93	1.90	1.82	1.93	1.83	1.86	1.87	2.01	2.12		***	***	***	***	***	***	***	***	***
18	2.67	3.30	2.59	2.45	2.86	2.47	3.19	2.73	2.62	2.50	2.70	2.12	2.18	2.11	2.57	2.76	2.37		***	***	***	***	***	***	***	***
19	2.07	2.18	1.55	1.43	2.27	1.70	2.39	1.77	1.95	1.60	1.80	1.22	1.32	1.40	1.90	2.12	1.85	2.14		***	***	***	***	***	***	***
20	2.77	3.13	2.42	2.16	2.85	2.21	2.73	2.28	2.26	2.10	2.21	1.88	2.24	2.01	2.36	2.39	2.11	2.78	1.85		***	***	***	***	***	***
21	3.01	3.24	2.46	2.43	3.13	2.42	2.90	2.68	2.32	2.31	2.28	2.35	2.58	2.06	2.53	2.42	2.22	2.89	2.25	2.29		***	***	***	***	***
22	4.11	4.39	3.73	3.76	3.93	3.22	3.77	3.72	3.66	3.39	3.47	3.54	3.50	3.50	3.54	3.79	3.63	4.09	3.50	3.80	3.78		***	***	***
23	2.50	3.07	2.29	2.13	2.59	1.90	2.79	2.37	1.96	1.98	2.07	2.05	2.16	2.16	2.37	2.26	2.15	2.79	2.07	2.32	2.53	3.73		***	***
24	2.21	2.43	1.91	1.61	2.15	1.55	2.28	1.99	1.84	1.45	1.83	1.52	1.72	1.75	1.75	2.09	1.98	2.29	1.43	2.04	2.41	3.46	1.88		***
25	2.87	3.26	2.86	2.59	3.07	2.42	3.22	2.85	2.45	2.50	2.74	2.43	2.60	2.55	2.63	2.78	2.82	3.19	2.07	2.76	3.19	4.34	2.50	2.06	

Table A5: Pairwise genetic distances (Φ_{ST}) among 25 populations of *Sesleria albicans*. Levels of significance above diagonal: * P< 0.05; ** P< 0.01; *** P< 0.001. P values indicate the probability that a random genetic distance (Φ_{ST}) is larger than the observed distance and are based on 1,000 iterations. Populations are numerated as in Table 1 (p. 59).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1		***			***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***		***	***
2	0.45		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
3	0.37	0.38		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
4	0.37	0.37	0.31		***	***	***	***	***	***	***	***	***	***	***	***	***			***	***	***	***	***	***
5	0.39	0.46	0.42	0.37			***	***		***		***	***		***	***	*	***	***	***	***	***	***	***	***
6	0.37	0.42	0.29	0.31	0.36		***	***	***	***	***	***	***	***	***	***		***	***	***	***	***	***	***	***
7	0.45	0.55	0.43	0.42	0.46	0.33		***		***	***	***	***	***	***	***		***	*	***	***		***	***	***
8	0.42	0.42	0.33	0.34	0.40	0.25	0.37		***	***	***		***	***	***		***	***		***	***	***	***	***	***
9	0.38	0.44	0.35	0.33	0.38	0.25	0.41	0.28		***		***	***	***	***	***	***	***	***	***	***	***	***	***	***
10	0.39	0.41	0.30	0.30	0.36	0.27	0.39	0.27	0.30			***	***	***	***	***	***	***	***	***	***	***	***	***	***
11	0.39	0.45	0.33	0.34	0.37	0.24	0.39	0.28	0.27	0.25		***	***	***		***	***	***	***	***	***	***	***		***
12	0.33	0.37	0.22	0.21	0.32	0.28	0.36	0.26	0.27	0.23	0.25		***			***	***	***	***	***	***	***	***	***	
13	0.35	0.34	0.28	0.27	0.38	0.26	0.42	0.31	0.29	0.25	0.28	0.15		***		***	***	***	***	***	***	***	***	***	***
14	0.34	0.40	0.26	0.27	0.36	0.27	0.40	0.28	0.26	0.26	0.27	0.16	0.19		***			***	***	***		***	***	***	***
15	0.43	0.44	0.34	0.34	0.41	0.27	0.41	0.30	0.31	0.25	0.27	0.31	0.28	0.26		***	***	***	***	***	***	***	***	***	***
16	0.43	0.48	0.38	0.36	0.44	0.36	0.46	0.40	0.36	0.36	0.37	0.31	0.36	0.33	0.37		***	***	***	***	***	***	***	***	***
17	0.39	0.48	0.32	0.33	0.42	0.31	0.40	0.29	0.29	0.27	0.30	0.27	0.28	0.28	0.31	0.34		***	***	***	***	***	***	***	***
18	0.43	0.52	0.42	0.39	0.46	0.39	0.50	0.43	0.41	0.39	0.43	0.32	0.33	0.32	0.41	0.44	0.38		***	***	***	***	***		***
19	0.31	0.32	0.20	0.18	0.35	0.25	0.37	0.26	0.30	0.22	0.27	0.13	0.15	0.17	0.29	0.33	0.27	0.32		***	***	***	***		***
20	0.44	0.49	0.39	0.34	0.46	0.35	0.44	0.37	0.36	0.33	0.35	0.28	0.36	0.31	0.38	0.39	0.33	0.44	0.28		***	***	***	***	***
21	0.48	0.51	0.40	0.39	0.50	0.38	0.47	0.43	0.36	0.36	0.36	0.36	0.41	0.32	0.40	0.39	0.35	0.46	0.34	0.36		***	***	***	***
22	0.60	0.63	0.55	0.54	0.58	0.46	0.56	0.53	0.52	0.49	0.51	0.50	0.50	0.51	0.51	0.55	0.54	0.60	0.49	0.55	0.56		***	***	***
23	0.40	0.48	0.37	0.34	0.42	0.29	0.45	0.38	0.30	0.31	0.33	0.32	0.34	0.34	0.38	0.36	0.34	0.44	0.32	0.37	0.40	0.54		***	***
24	0.34	0.37	0.28	0.23	0.33	0.21	0.35	0.31	0.28	0.19	0.27	0.21	0.25	0.26	0.26	0.32	0.30	0.35	0.18	0.32	0.38	0.49	0.29		***
25	0.45	0.50	0.46	0.42	0.48	0.39	0.50	0.46	0.40	0.40	0.44	0.39	0.42	0.41	0.43	0.45	0.45	0.50	0.33	0.44	0.50	0.60	0.40	0.32	

Table A6: Geneflow among 25 investigated populations of *Sesleria albicans* ($N_em = 0.25$ X $(1 / \Phi_{ST} - 1)$ calculated from Φ_{ST} values (Wright 1951). Populations are numerated as in Table 1 (p. 59).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1																									
2	0.30																								
3	0.42	0.41																							
4	0.42	0.42	0.54																						
5	0.38	0.28	0.34	0.41																					
6	0.41	0.33	0.58	0.55	0.43																				
7	0.29	0.20	0.33	0.34	0.28	0.50																			
8	0.34	0.33	0.50	0.47	0.36	0.73	0.42																		
9	0.41	0.32	0.46	0.50	0.41	0.72	0.36	0.63																	
10	0.38	0.36	0.58	0.57	0.43	0.65	0.38	0.65	0.57																
11	0.38	0.30	0.51	0.47	0.41	0.78	0.38	0.63	0.65	0.73															
12	0.49	0.42	0.86	0.91	0.52	0.63	0.43	0.69	0.65	0.81	0.72														
13	0.46	0.47	0.64	0.68	0.40	0.70	0.33	0.55	0.60	0.74	0.63	1.37													
14	0.47	0.36	0.71	0.66	0.43	0.67	0.37	0.63	0.70	0.69	0.66	1.29	1.02												
15	0.32	0.32	0.47	0.48	0.35	0.66	0.36	0.58	0.54	0.74	0.67	0.54	0.62	0.69											
16	0.32	0.27	0.41	0.44	0.31	0.43	0.29	0.37	0.44	0.44	0.42	0.54	0.44	0.50	0.41										
17	0.38	0.27	0.53	0.50	0.35	0.55	0.36	0.58	0.60	0.65	0.58	0.66	0.63	0.62	0.54	0.48									
18	0.32	0.23	0.34	0.39	0.29	0.39	0.24	0.32	0.35	0.38	0.33	0.53	0.49	0.51	0.36	0.31	0.40								
19	0.56	0.53	0.98	1.11	0.45	0.74	0.42	0.69	0.57	0.85	0.67	1.65	1.32	1.16	0.60	0.50	0.65	0.53							
20	0.31	0.26	0.38	0.47	0.29	0.45	0.32	0.43	0.43	0.49	0.45	0.62	0.44	0.53	0.40	0.39	0.49	0.31	0.64						
21	0.26	0.24	0.37	0.39	0.25	0.40	0.28	0.33	0.43	0.43	0.44	0.43	0.36	0.53	0.37	0.39	0.45	0.28	0.47	0.43					
22	0.16	0.14	0.20	0.21	0.18	0.29	0.19	0.21	0.22	0.25	0.24	0.24	0.24	0.24	0.23	0.20	0.21	0.16	0.25	0.20	0.19				
23	0.37	0.27	0.42	0.48	0.34	0.6	0.30	0.40	0.56	0.55	0.50	0.52	0.47	0.47	0.40	0.43	0.47	0.31	0.51	0.41	0.36	0.21			
24	0.48	0.42	0.62	0.83	0.49	0.89	0.45	0.54	0.63	1.05	0.64	0.94	0.72	0.70	0.69	0.51	0.56	0.45	1.08	0.52	0.41	0.25	0.61		
25	0.30	0.24	0.29	0.34	0.26	0.38	0.24	0.29	0.37	0.36	0.31	0.38	0.34	0.35	0.33	0.30	0.30	0.25	0.51	0.31	0.25	0.16	0.36	0.51	