Annals of Botany **99**: 647–651, 2007
doi:10.1093/aob/mcl290, available online at www.aob.oxfordjournals.org

OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Spatial Genetic Structure and Clonal Diversity in an Alpine Population of *Salix herbacea* (Salicaceae)

CHRISTOPH REISCH*, SOPHIA SCHURM and PETER POSCHLOD

University of Regensburg, Institute of Botany, D-93040 Regensburg, Germany

Received: 28 September 2006 Revision requested: 31 October 2006 Accepted: 6 December 2006 Published electronically: 22 January 2007

• *Background and Aims* Many alpine plant species combine clonal and sexual reproduction to minimize the risks of flowering and seed production in high mountain regions. The spatial genetic structure and diversity of these alpine species is strongly affected by different clonal strategies (phalanx or guerrilla) and the proportion of generative and vegetative reproduction.
• *Methods* The clonal structure of the alpine plant species *Salix herbacea* was investigated in a 3 × 3 m plot of an alpine meadow using microsatellite (simple sequence repeat; SSR) analysis. The data obtained were compared with the results of a random amplified polymorphic DNA (RAPD) analysis.
• *Key Results* SSR analysis, based on three loci and 16 alleles, revealed 24 different genotypes and a proportion of distinguishable genotypes of 0·18. Six SSR clones were found consisting of at least five samples, 17 clones consisting of more than two samples and seven single genotypes. Mean clone size comprising at least five samples was 0·96 m^2, and spatial autocorrelation analysis showed strong similarity of samples up to 130 cm. RAPD analysis revealed a higher level of clonal diversity but a comparable number of larger clones and a similar spatial structure.
• *Conclusions* The spatial genetic structure as well as the occurrence of single genotypes revealed in this study suggests both clonal and sexual propagation and repeated seedling recruitment in established populations of *S. herbacea* and is thus suggestive of a relaxed phalanx strategy.

Key words: *Salix herbacea*, genotypic diversity, RAPD, SSR, molecular marker, clonality, spatial genetic structure.

INTRODUCTION

Alpine habitats are characterized by challenging environmental conditions. Short vegetation periods, limited nutrient resources, strong winds or long periods of snow cover are typical environmental factors at higher elevations (Barry, 1981; Körner, 2003). These factors have a large impact on the distribution of alpine plant species and the composition of plant communities (Kikvidze *et al.*, 2005; Moser *et al.*, 2005). Severe climatic conditions can hamper sexual reproduction in alpine regions, which means that flowering and seed production can be a risky mode of reproduction at higher altitudes (Körner, 2003; Urbanska, Schütz, 1986). One way to cope with this constraint is clonal propagation, which is generally supposed to increase in importance with increasing elevation (Bliss, 1971). A high proportion of alpine plant species are characterized by clonal growth (Stöcklin and Bäumler, 1996; Klimes *et al.*, 1997). The advantages of clonal reproduction are to avoid the risks of sexual reproduction, to share resources through clonal integration and reduce the mortality of genets. On the other hand, diseases can be transmitted more easily among ramets, and resources for sexual reproduction are limited (Klimes *et al.*, 1997).

Clonal reproduction of plant species can follow different strategies. The two most extreme types of clonal reproduction are the 'phalanx' and the 'guerrilla' strategy (Harper, 1977; Stöcklin, 1992). Plants of the guerrilla type exhibit a dispersive growth form, distributing their ramets at larger distances. In contrast, plants of the phalanx type show a dense growth form, clustering their ramets closely together and forming cushions. Many plant species show, however, intermediate growth forms combining aspects of both strategies (Körner, 2003). For clonal alpine plant species, the extreme types of this categorization are following either a conservative strategy (phalanx species with low levels of plasticity and strong integration) or an explorative strategy (guerrilla species with high levels of plasticity and only low levels of integration) (Stöcklin, 1992). At the spatial scale, the different types of clonal reproduction result in a different distribution of ramets in the habitat. Ramets of phalanx species are theoretically found close to each other, while ramets of guerrilla species are supposed to be more distant.

Plant species often combine clonal and sexual reproduction. As a result, clonal species generally exhibit a level of genetic variability comparable with that of non-clonal species (Ellstrand and Roose, 1987; Widén *et al.*, 1994; Hamrick and Godt, 1996). It is expected that this observation is also true for alpine clonal species (Steinger *et al.*, 1996; Holderegger *et al.*, 2002), although there are only a few studies which explicitly tested this hypothesis (Pluess and Stöcklin, 2004). Considering the genetic variability of clonal species, it has been shown that the genetic diversity depends on the establishment of seedlings (Eriksson, 1989, 1992). In the case of initial seedling recruitment (ISR), where the establishment of seedlings occurs only during colonization, different levels of success of genets

* For correspondence. E-mail christoph.reisch@biologie.uni-regensburg.de

and competition reduce the genetic diversity during a population's history (Watkinson and Powell, 1993). Repeated seedling recruitment (RSR) contributes, in contrast, to the formation and preservation of genetic diversity in populations of clonal plants. Simulation experiments have shown that even the rare establishment from seeds is sufficient to maintain genetic diversity within populations of clonal plants (Watkinson and Powell, 1993).

Different strategies of clonal growth and combination with generative and vegetative reproduction lead to complex spatial patterns of genets at the local scale. The spatial genetic patterns of clonal species have been analysed in many studies (Luijten *et al.*, 1996; Gabrielsen and Brochmann, 1998; Reusch *et al.*, 1998; Persson and Gustavsson, 2001; Garnier *et al.*, 2002; Kleijn and Steinger, 2002; Ziegenhagen *et al.*, 2003; Richards *et al.*, 2004). There are, however, only a few studies dealing with the clonal diversity of alpine plant species at the local scale (Steinger *et al.*, 1996; Escaravage *et al.*, 1998; Pornon *et al.*, 2000).

In the study presented here, clonal spread of the alpine willow *Salix herbacea* was analysed. The species exhibits a clonal system of underground rhizomes with aboveground leaves forming extensive mats often covering several square metres in snowbeds and alpine meadows. The clonal strategy of. *S. herbacea* was analysed within a single 3×3 m study plot and the following questions were asked. (*a*) How large is the clonal diversity of alpine *S. herbacea* at the local scale? (*b*) Which is the clonal strategy of *S. herbacea* — phalanx or guerrilla? (*c*) Does the species reproduce mainly clonally or is there also indication for sexual reproduction?

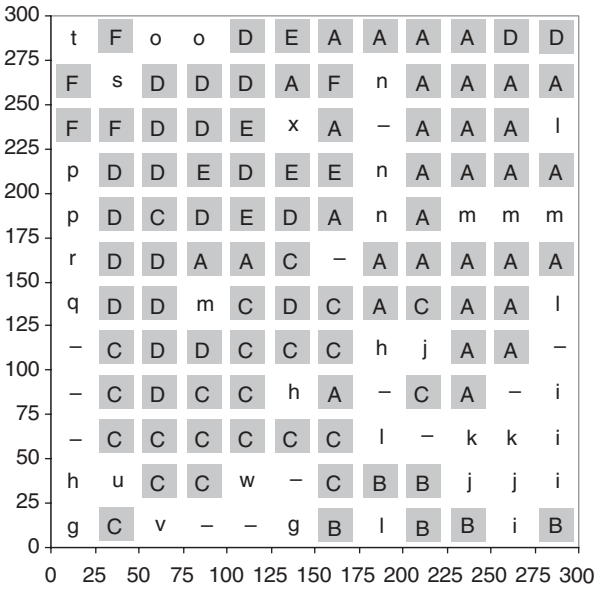

FIG. 1. Sample design and clonal structure of alpine *S. herbacea* estimated with microsatellites. Within the study plot consisting of 144 sub-plots, 138 samples were collected. In six sub-plots no plant material was available (–). Another six samples were omitted from the analysis due to PCR problems (–). The analysis of the remaining 132 samples revealed six clones consisting of more than five samples (A–F), 17 clones consisting of more than two samples (g–p) and seven single genotypes (q–x).

MATERIALS AND METHODS

Species description

Salix herbacea L. is a clonal, diploid ($2n = 38$), dioecious, prostrate dwarf shrub with an extensive ramifying system of tough, branched, underground rhizomes, forming loose, flattened mats (Beerling, 1998). Individual ramets can reach an age of >40 years (Schweingruber and Poschlod, 2006). The species is insect and wind pollinated (Beerling, 1998). The seeds are small (66–136 μg) and dispersed by wind. *Salix herbacea* is an amphi-atlantic species with a typical arctic–alpine distribution in Europe (Beerling, 1998). It occurs mainly in the arctic and sub-arctic, and extends southwards in the mountains of the Pyrenees, Appenines and Bulgaria (Tutin *et al.*, 1964). In the Alps, *S. herbacea* is widespread in snowbeds and alpine meadows (Ellenberg, 1988).

Study design and sampling procedure

To investigate the spatial genetic pattern of *S. herbacea* at the local scale, a study plot (300×300 cm) was established consisting of 144 sub-plots (25×25 cm) in an alpine meadow dominated by *S. herbacea* and *Carex curvula* near the Greitspitze mountain (2200 m above sea level) in the central Alps (Fig. 1). The species covered more or less continuously the whole plot, but was not found in all sub-plots. Where possible, one sample was collected per sub-plot (Fig. 1). Fresh leaf material was taken from 138 samples; they were placed in a cryogenic container and stored at −80 °C in the laboratory.

Molecular and statistical analyses

DNA was isolated from frozen plant material by a modified CTAB (cetyltriammonium bromide) method (Rogers and Bendich, 1994) using about 10–30 mg of leaf material as described before (Reisch *et al.*, 2005). Clonal diversity was analysed with nuclear microsatellites (simple sequence repeats; SSRs) using primers, which were already established for *S. herbacea* in previous studies (Stamati *et al.*, 2003). After an initial screening of seven loci for polymorphisms with eight random samples, the primer pairs gSIMTC011, gSIMTC024 and gSIMTC035 were selected (Table 1). Primers were M13tail-labelled (Oetting *et al.*, 1995) with the fluorescent dyes IRD 700 (MWG), Cy 5 (MWG) and D2 (Proligo). Amplification reactions (10 μL) contained 200 μM dNTP (PeqLab), 1·5 pM labelled M13 primer (10 pM), 0·1 pM gSIMCT forward primer (1 pM), 1·5 pM gSIMCT reverse primer (10 pM), 0·25 U of *Taq* polymerase (Qiagen) with buffer and 20 ng of genomic DNA. Polymerase chain reactions (PCRs) were run in a thermal cycler (Cyclone Gradient, PeqLab, Nürnberg, Germany) with an initial step (5 min at 94 °C), 40 cycles (1 min at 94 °C, 1 min at 50 °C, 1 min at

TABLE 1. *SSR primers used, their GenBank accession IDs and sequence, the size of the amplified products and the number of detected alleles in alpine* S. herbacea

Primer	GenBank ID	Sequence (5′ → 3′)	Size of the fragments	Alleles
gSIMCT011	BV006744	TTCATCTCCCCGTTCACTTC ACCGTTAGGATGGCATCTCG	306, 420	2
gSIMCT024	BV006745	TCATTTGCTCGATGAGGTTG GTGGTAGTTGCAAAAGGGGA	312, 314, 318, 320, 322, 324, 326, 328, 330	9
gSIMTC035	BV006748	ACACATGACTCCCCTTCGTC TCTTATGGTCGTGGTGGTGA	312, 316, 322, 328, 420	5

72 °C) and a final extension step of 8 min at 72 °C. The success of a PCR was confirmed on 1·5% agarose gels. The length of amplified fragments was determined on a CEQ 8000 automated sequencer (Beckman Coulter) using an internal size standard (CEQ 400, Beckman Coulter). Based on SSR variation, the genotype of each sample was determined. The data obtained about the spatial genetic structure and clonal diversity results were compared with the results of a random amplified polymorphic DNA (RAPD) analysis (Williams *et al.*, 1990). After an extensive screening, eight primers were selected and used to perform RAPD analysis as described before (Reisch *et al.*, 2005). Due to PCR problems, six samples were omitted from the SSR and one sample from the RAPD analysis.

Fragment data were used to assess the number of different multilocus genotypes in the plot. The proportion of distinguishable multilocus genotypes (PD) was calculated as G/N (Ellstrand and Roose, 1987), where G is the number of distinct genotypes and N is the number of individuals sampled. The probability of identity (PI) was calculated for each of the three analysed SSR loci and all loci together, using the program GenalEx (Peakall and Smouse, 2001). Samples with the same genotype were considered as originating from the same clone (Table 2). The size of clones was estimated by the area of colonized sub-plots occupied by a distinct multilocus genotype (Table 3). The distribution of clones composed of at least five samples within the study plot was mapped (Fig. 1) and the spatial genetic structure was analysed using autocorrelation analysis in SGS (Degen *et al.*, 2001) as the mean genetic distances between all pairs of individuals belonging to a given spatial distance class (Fig. 2). The overall mean genetic distances between all samples serves as a reference of random spatial structure. Monte Carlo permutation (500 replications) was applied to test for significant deviation from random spatial distribution (Diniz-Filho and Pires de Campos Telles, 2002).

RESULTS

In the SSR analysis, 16 alleles at three loci were revealed. Primer gSIMTC011 produced two alleles, gSIMTC024 produced nine alleles and gSIMTC035 produced five alleles (Table 1). The PI was 0·57 for gSIMTC011, 0·16 for gSIMTC024, 0·33 for gSIMTC035 and 0·03 for all loci together. The 132 analysed samples produced 24 different multilocus genotypes. The PD was 0·18. Six clones consisting of at least five samples were observed, 17 clones were composed of at least two samples and there were seven single genotypes (Table 2). Clone size ranged from 0·31 to 2·06 m^2 with an average of 0·96 m^2 (Table 3). Clones consisting of at least five individuals were intermingled (Fig. 1) and spatial autocorrelation analysis revealed a strong similarity of samples at distances up to 130 cm (Fig. 2).

In the RAPD analysis, the 137 analysed samples produced 52 different multilocus RAPD genotypes. The PD was 0·36. Seven clones consisting of at least five samples were observed, 17 clones were composed of at least two samples, and there were 35 single genotypes. Clone size ranged from 0·31 to 1·37 m^2 with an average of 0·70 m^2. Clones consisting of at least five individuals were intermingled, and spatial autocorrelation analysis showed a strong similarity of samples at distances up to 130 cm.

DISCUSSION

The clonal diversity (PD) revealed for *S. herbacea* in this study was 0·18 in the SSR and 0·36 in the RAPD analysis. According to previous studies, the genotypic diversity (PD) of clonal species varies from 0·02 to 1·0 (Ellstrand and Roose, 1987). Based on an extensive literature survey,

TABLE 2. *Clonal diversity within the analysed plot of alpine* S. herbacea *measured as the number of detected genotypes, proportion of distinguishable genotypes, the number of detected single genotypes and number of detected clones, composed of either ≥2 or ≥5 samples*

Analysed samples	Detected genotypes	Percentage of distinguishable genotypes	Detected single genotypes	Detected clones ≥2	Detected clones ≥5
132	24	0·18	7	17	6

TABLE 3. *Size of the analysed clones of alpine* S. herbacea *consisting of at least five samples*

Clone	No. of colonized sub-plots	Covered area (m^2)
A	33	2·06
B	6	0·37
C	22	1·37
D	20	1·25
E	7	0·43
F	5	0·31
Mean	15·5	0·96

a mean PD of 0·27 was found in clonal plants (Widén *et al.*, 1994). In previous SSR studies, PD values were reported of, for example, 0·43 for *Salix reinii* (Lian *et al.*, 2003) or 0·60 in *Quercus geminata* (Ainsworth *et al.*, 2003). In previous RAPD studies, clonal diversity at the local scale was, for example, 0·12 in *Saxifraga cernua* (Gabrielsen and Brochmann, 1998) or 0·23 in *Vaccinium idis-idea* (Persson and Gustavsson, 2001). The clonal diversity of *S. herbacea* was in the range observed for predominantly clonally reproducing plant species, although comparisons of clonal diversity are biased by different sampling scales and methods (Gabrielsen and Brochmann, 1998; Persson and Gustavsson, 2001; Reisch and Poschlod, 2004). However, as compared with the clonal diversity reported for the co-existing species *Carex curvula* (PD = 0·13) in the same habitat (Steinger *et al.*, 1996) using RAPDs, *S. herbacea* exhibited a rather high level of clonal diversity.

The spatial genetic analysis revealed a strong genetic similarity of samples up to 130 cm for both methods, which is obviously due to the proximity of samples with the same genotype. Similar results were also obtained in other small-scale studies of clonal species (Luijten *et al.*, 1996; Reusch *et al.*, 1999; Garnier *et al.*, 2002; Ziegenhagen *et al.*, 2003). Ramets of the same clone formed close patches, which built large mats. However, at the edge of the patches there was clonal intermingling. The species therefore does not seem to be a strict phalanx species, which is typical for alpine species (Körner, 2003). In previous studies, comparable patterns were observed for alpine *C. curvula* (Steinger *et al.*, 1996) and *Rhododendron ferrugineum* (Escaravage *et al.*, 1998; Pornon *et al.*, 2000).

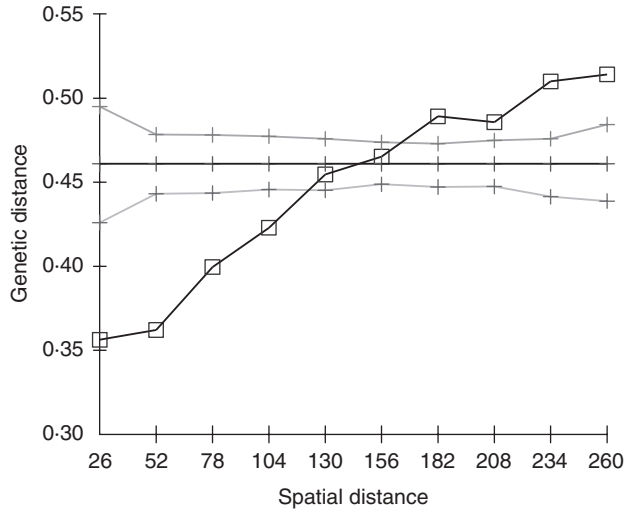

FIG. 2. Spatial autocorrelation of alpine *S. herbacea* based on microsatellites. The confidence interval is indicated by dashed lines.

In this study, the mean size of clones was 0·70 m^2 using RAPDs and 0·96 m^2 using SSRs. Clonal individuals are known to reach large sizes, as reported for *Pteridium aquilinium* (Oinonen, 1967). Since alpine species are generally small, the observed size of the *S. herbacea* clones is considerable. Nevertheless, some clones most probably extended farther outside of the studied plot. The different sizes of *S. herbacea* clones could also result from repeated seedling recruitment, which is suggested by the occurrence of numerous single genotypes within the study plot. Seedling recruitment of *S. herbacea* therefore does not seem to be a rare event in alpine meadows.

Comparing the results of both methods, clonal diversity was higher and clone size smaller with RAPDs than with SSRs. This is mainly due to the higher number of single genotypes detected with RAPDs. The number of larger clones and the spatial genetic structure within the study plot were, however, similar for both methods. The general spatial pattern of clonal structure in the present study was thus solid.

In conclusion, the species exhibits a mixed reproduction system (Körner, 2003), which allows it to benefit from different strategies: clonality enhances individual longevity and maintains well adapted genotypes, while sexual reproduction allows evolution and adaptation to changing environmental conditions as well as the colonization of new habitat patches.

LITERATURE CITED

Ainsworth EA, Tranel PJ, Drake BG, Long SP. 2003. The clonal structure of *Quercus geminata* revealed by conserved microsatellite loci. *Molecular Ecology* **12**: 527–532.

Barry NC. 1981. *Mountain weather and climate.* London: Methuen.

Beerling DJ. 1998. Biological flora of the British isles: *Salix herbacea* L. *Journal of Ecology* **86**: 872–895.

Bliss LC. 1971. Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics* **2**: 405–438.

Degen B, Petit RJ, Kremer A. 2001. SGS – spatial genetic software: a computer program for analysis of spatial genetic structures of individuals and populations. *Journal of Heredity* **92**: 447–448.

Diniz-Filho JAF, Pires de Campos Telles M. 2002. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conservation Biology* **16**: 924–935.

Ellenberg H. 1988. *Vegetation ecology of Central Europe*, 4th edn. Cambridge: Cambridge University Press.

Ellstrand NC, Roose ML. 1987. Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* **74**: 123–131.

Eriksson O. 1989. Seedling dynamics and life histories in clonal plants. *Oikos* **55**: 231–238.

Eriksson O. 1992. Evolution of seed dispersal and recruitment in clonal plants. *Oikos* **63**: 439–448.

Escaravage N, Questiau S, Pornon A, Doche B, Taberlet P. 1998. Clonal diversity in a *Rhododendron ferrugineum* L. (Ericaceae) population inferred from AFLP markers. *Molecular Ecology* **7**: 975–982.

Gabrielsen TM, 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.

Garnier LKM, Durand J, Dajoz I. 2002. Limited seed dispersal and microspatial population structure of an agamospermous grass of West African savannahs, *Hyparrhenia diplandra* (Poaceae). *American Journal of Botany* **89**: 1785–1791.

Hamrick JL, Godt MJW. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**: 1291–1298.

Harper JL. 1977. *Population biology of plants.* London: Academic Press.

Holderegger R, Stehlik I, Abbott RJ. 2002. Molecular analysis of the Pleistocene history of *Saxifraga oppositifolia* in the Alps. *Molecular Ecology* **11**: 1409–1418.

Kikvidze Z, Pugnaire FI, Brooker RW, Choler P, Lortie CJ, Michalet R, Callaway RM. 2005. Linking patterns and processes in alpine plant communities: a global study. *Ecology* **86**: 1395–1400.

Kleijn D, Steinger T. 2002. Contrasting effects of grazing and hay cutting on the spatial and genetic structure of *Veratrum album*, an unpalatable, long-lived, clonal plant species. *Journal of Ecology* **90**: 360–370.

Klimes L, Klimesova J, Hendriks R, van Groenendal JM. 1997. Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H, van Groenendal JM eds. *The ecology and evolution of clonal plants*. Leiden: Backhuys, 1–29.

Körner C. 2003. *Alpine plant life*, 2nd edn. Berlin: Springer.

Lian C, Oishi R, Miyashita N, Nara K, Nakaya H, Wu B, Zhou Z, Hogetsu T. 2003. Genetic structure and reproduction dynamics of *Salix reinii* during primary succession on Mount Fuji, as revealed by nuclear and chloroplast microsatellite analysis. *Molecular Ecology* **12**: 609–618.

Luijten SH, Oostermeijer JGB, van Leeuwen NC, den Nijs HCM. 1996. Reproductive success and clonal genetic structure of the rare *Arnica montana* (Compositae) in the Netherlands. *Plant Systematics and Evolution* **201**: 13–30.

Moser D, Dullinger S, Englisch T, Niklfeld H, Plutzar C, Sauberer N, Zechmeister HG, Grabherr G. 2005. Environmental determinants of vascular plant species richness in the Austrian Alps. *Journal of Biogeography* **32**: 1117–1127.

Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers TA, King RA. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics* **30**: 450–458.

Oinonen E. 1967. The correlation between the size of Finnish bracken (*Pteridium aquilinium* (L.) Kuhn) clones and certain periods of site history. *Acta Forestalia Fennica* **83**: 1–51.

Peakall R, Smouse PE. 2001. GenalEx: *Ge*netic *An*alysis in *Ex*cel. Canberra: Australian National University. http://www.anu.edu.au/BoZo/GenAlEx.

Persson HA, Gustavsson BA. 2001. The extent of clonality and genetic diversity in ligonberry (*Vaccinium vitis-idea* L.) revealed by RAPDs and leaf-shape analysis. *Molecular Ecology* **10**: 1385–1397.

Pluess AR, Stöcklin J. 2004. Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. *American Journal of Botany* **91**: 2013–2021.

Pornon A, Escaravage N, Thomas P, Taberlet P. 2000. Dynamics of genotypic structure in clonal *Rhododendron ferrugineum* (Ericaceae) populations. *Molecular Ecology* **9**: 1099–1111.

Reisch C, Poschlod P. 2004. Clonal diversity and subpopulation structure in central European relict populations of *Saxifraga paniculata* Mill. (Saxifragaceae). *Feddes Repertorium* **115**: 239–247.

Reisch C, Anke A, Röhl M. 2005. Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. *Basic and Applied Ecology* **6**: 35–45.

Reusch TBH, Stam WT, Olsen JL. 1998. Size and estimated age of genets in eelgrass, *Zostera marina*, assessed with microsatellite markers. *Marine Biology* **133**: 519–525.

Reusch TBH, Hukriede W, Stam WT, Olsen JL. 1999. Differentiating between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity* **83**: 120–126.

Richards CL, Hamrick JL, Donovan LA, Mauricio R. 2004. Unexpectedly high clonal diversity of two salt marsh perennials across a severe environmental gradient. *Ecology Letters* **7**: 1155–1162.

Rogers SO, Bendich AJ. 1994. Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin SB, Schilperoort RA eds. *Plant molecular biology manual*. Dordrecht: Kluwer Academic Press, 1–8.

Schweingruber FH, Poschlod P. 2006. Growth rings in herbs and shrubs: life span, age determination and stem anatomy. *Forest, Snow and Landscape Research* **79**: 195–415.

Stamati K, Blackie S, Brown JWS, Russell SJ. 2003. A set of polymorphic SSR loci for subarctic willow (*Salix lanata*, *S. lapponum* and *S. herbacea*). *Molecular Ecology Notes* **3**: 280–282.

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.

Stöcklin J. 1992. Environment, morphology and growth of clonal plants – an overview. *Botanica Helvetica* **102**: 3–21.

Stöcklin J, Bäumler E. 1996. Seed rain, seedling establishment and clonal growth strategies on a glacier foreland. *Journal of Vegetation Science* **9**: 45–56.

Tutin TG, Heywood VH, Burgess NA, Moore DM, Valentine DH, Walters SM, Webb DA. 1964. *Flora Europaea*. Cambridge: Cambridge University Press.

Urbanska KM, Schütz M. 1986. Reproduction by seed in alpine plants and revegetation research above timber line. *Botanica Helvetica* **96**: 43–60.

Watkinson AR, Powell JC. 1993. Seedling recruitment and the maintenance of clonal diversity in plant populations – a computer simulation of *Ranunculus repens*. *Journal of Ecology* **81**: 707–717.

Widén B, Cronberg C, Widén M. 1994. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. *Folia Geobotanica et Phytotaxonomica* **29**: 245–263.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531–6535.

Ziegenhagen B, Bialozyt R, Kuhlenkamp V, Schulze I, Ulrich A, Wulf M. 2003. Spatial patterns of maternal lineages and clones of *Galium odoratum* in a large ancient woodland: inferences about seedling recruitment. *Journal of Ecology* **91**: 578–586.