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Spatial genotypical diversity of *Sesleria albicans* (Poaceae) in a dry grassland community

Monika Janišová[1] & Dušan Gömöry[2]

[1]*Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK-84523 Bratislava, Slovakia; e-mail: monika.janisova@savba.sk*
[2]*Department of Phytology, Faculty of Forestry, Technical University of Zvolen, Masarykova 24, SK-96053 Zvolen, Slovakia; e-mail: gomory@vsld.tuzvo.sk*

Abstract: Spatial genotypical diversity of *Sesleria albicans* Kit. ex Schultes was studied in a dry grassland community by isozyme analysis. The aim was to identify the genetical individuals within the studied population and to assess the species' clonal growth parameters. Vegetative mobility and branching intensity were measured in field for the sake of the correct interpretation of the results. Five isozyme systems were analyzed and interpreted (MDH, MNR, 6-PGDH, SkDH, LAP). Altogether, 98 distinct isozyme profiles were identified within an area of 2 m^2. Average genotype identity rapidly decreased with distance. Several very remote ramets (more than 1 m) belonging to the same genet were identified. The longest distance between ramets of the same genotype was 153 cm. With average annual rhizome increment of 13.59 mm assessed for the studied population the age of genet with the most remote ramets exceeded 56 years by a bi-directional growth and 112 years by a growth in single direction. Number of daughter tillers produced by a tiller per year was 0.67 (branching intensity, median) and the median of tiller life span was 2.5 years. The high genotypical diversity of *S. albicans* in the studied population could be a result of both, regular and continuous seed production with subsequent seedling recruitment and long life span of genets.

Key words: clonal growth; dry grasslands; identification of genets; life span; reproductive strategy; vegetative mobility

Abbreviations: MDH, malate dehydrogenase; MNR, menadione reductase; 6-PGDH, 6-phosphogluconate dehydrogenase; SkDH, shikimate dehydrogenase; LAP, leucine aminopeptidase; ADH, alcohol dehydrogenase, PVP, polyvinylpyrrolidon; DTT, dithiothreitol

Introduction

Sesleria albicans is a caespitose grass forming both intra- and extravaginal tillers. It has a submediterranean-subatlantic distribution in Europe (Conert 1998). The species occurs in ecologically very different habitats, it colonizes beech forests, as well as naturally and manmade open habitats such as alpine and lowland rocky ridges, lowland screes, fens or calcareous grasslands (Reisch et al. 2003). Its occurrence in the Western Carpathians is concentrated on the montane and subalpine belt over the calcareous bedrock, where it dominates several community types. Occasionally, it enters dry grasslands at lower altitudes and here it was considered to be a relic dealpine species (Ellenberg 1986; Conert 1998). This assumption is indirect and based upon logical inference from the actual distribution and the ecological requirements of the species. Recent studies of *S. albicans* genetic pattern gave no evidence for its glacial relic endemism (Reisch et al. 2002). Some authors (Pignatti & Pignatti 1975) support the hypothesis that *S. albicans* has reached high altitudes in the Alps post-glacially, having survived the last glaciation in mountain gorges in the southern Alps.

S. albicans is characterized by a large phenotypic plasticity (Urbas & Zobel 2000) and it can change the growth form according to the given environmental conditions. In the studied region, it is frequent in two types of dry grassland communities obtaining two growth forms. In the upper parts of N-facing ridges, it forms contiguous closed stands composed of loose tussocks. On S-facing slopes, the species grows in dense tussocks or clumps occurring exclusively in open communities on gravel in the earliest successional stages.

Both, morphological and physiological differences between populations of *S. albicans* were revealed and regarded as genetically fixed (Lloyd & Woolhouse 1976; 1978). A high genetic diversity within populations (Reisch et al. 2002) could indicate that sexual reproduction and successful seedling growth does take place at a high enough frequency to reduce the effects of inbreeding and to maintain variability. The longevity of genets could be one of the reasons (along with frequent recruitment from seedlings) for the maintenance of genetic diversity within populations (Lysák et al. 2000; Urbas & Zobel 2000; Reisch & Poschlod 2003).

In natural populations of *S. albicans,* a reliable distinguishing between genets and ramets is impossi-

ble and the demographical study is thus difficult. The determination of tiller membership to genets has shown to be inevitable for the study of the species' demographical characteristics. It is also important for estimating the reproductive strategy of the species and the role of both generative reproduction and clonal growth in the total reproduction. The presented study includes results of *S. albicans* isozyme analysis, which was aimed to assign the analyzed tillers to the individual genotypes.

The ability of species to spread horizontally depends on the combination of two parameters (Sammul 2003): first, the ability of a ramet to produce new offspring (branching intensity), and second, the distance from a mother ramet to a daughter ramet (ramet vegetative mobility). We measured these parameters in the studied population to enable a precise interpretation of the results of isozyme analysis.

Material and methods

S. albicans Kit. ex Schult. ($2n = 28$) is a perennial polycarpic grass with deeply rooting rhizomes, hemicryptophyte (Conert 1998) or chamaephyte (Dixon 1982), with vegetative survival and slow winter growth of leaves and developing inflorescences (Reisch et al. 2003). The plant is wind-pollinated and flowers usually from March to June. The flowers are slightly proteandrous, and self-pollinating, therefore, is probably of rare occurrence (Reisch et al. 2003). Established plants set seed every year, although isolated plants sometimes yield very few seeds (Dixon 1982). The species is very well adapted to drought but also tolerates moist conditions (Lloyd & Woolhouse 1978; Dixon 1986).

The distribution of *S. albicans* in Europe is submediterranean-subatlantic. It occurs in a wide range of altitudes from lowland (minimum 5 m a.s.l., coastal populations in Lancashire, UK) to the alpine belt (maximum 3 120 m a.s.l. in Wallis, Switzerland).

We analyzed the population of *S. albicans* in the vicinity of the village Lúka nad Váhom (48°39′25″ N, 17°54′20″ E) at the altitude 380–390 m a.s.l. The geological bedrock is built by trias dolomites, on which a shallow protorendzina soil has developed. The climate is warm with the mean annual temperature 9.2 °C and the mean annual precipitation 625 mm (data from the meteorological station in Piešťany). In the studied grasslands of Považský Inovec Mts (Western Carpathians), *S. albicans* belongs to dominant species. The samples of *S. albicans* for the isozyme analysis were obtained in April 1995 on a permanent plot of 2 m^2 in dry grassland community belonging to the association *Carici humilis-Seslerietum calcariae* Sillinger 1930 of the class *Festuco-Brometea* Br-Bl. et R. Tx. 1943. The studied plot was located on a N-facing slope approximately 2.5 m below the top of the mountain ridge. The slope was uniform with average inclination of 31°. The fine soil reached to the depth from 4 to 16 cm. The herb layer covered 95–100% of the plot area and was dominated by *S. albicans*. Other very frequent species were *Genista pilosa* L., *Carex humilis* Leyss. and *Anthericum ramosum* L. *Hypnum cupressiforme* Hedw. was dominant in the moss layer.

In the permanent plot, we analyzed 200 tillers, which were selected as follows: The plot was divided into 10 by 10 cm quadrats (200 quadrats altogether). In each quadrat two coordinates (x and y) were randomly generated and the tiller located at the position defined by the generated coordinates (respectively the closest one) was selected for the analyses. The position of the selected tillers was mapped (Fig. 1).

To measure the clonal growth paramerets, 15 clonal fragments (polycormons) were excavated in October 2006 in close vicinity of the above-mentioned permanent plot. The number of tillers collected this way was 150. Among them, 104 tillers were unbroken and undamaged, suitable for measurements. For each tiller on each clonal fragment, two parameters were estimated: vegetative mobility (the annual increase of rhizome in mm) and branching intensity (number of rhizome branches i.e. daughter tillers per tiller per year). Determining the annual rhizome increment is possible due to the differences in the formation of nodes and internodes in different season (short and thick spring internodes and longer thinner internodes formed later in the year). These morphological differences allowed us also to estimate the tiller age. The tiller life span was estimated for dead tillers. The branching intensity was calculated as the number of daughter tillers per tiller divided by tiller life span. For both, branching intensity and life span we used median values as their frequency distributions were skewed.

Isozyme analysis

The youngest growing leaves were used for the analysis (assimilating tissues together with the lower part without pigments). Plant tissues were homogenized in a 0.1M Tris-HCl extraction buffer pH 7.3 with the addition of PVP 40, PVP 360, EDTA II, Tween 80, PEG, 2–mercaptoethanol (1% each), 0.025% DTT, and 0.5% Na-ascorbate. Enzyme separation was performed electrophoretically in 12% (w/v) starch gels using two buffer systems (discontinuous lithium borate – Tris citrate pH 8.1, and continuous Tris-citrate buffer pH 7.0). Staining procedures essentially followed Cheliak & Pitel (1984).

Five isozyme systems were observed: MDH (malate dehydrogenase), MNR (menadione reductase), 6-PGDH (6-phosphogluconate dehydrogenase), SkDH (shikimate dehydrogenase) a LAP (leucine aminopeptidase). Moreover, ADH (alcohol dehydrogenase) was analyzed, but the zymograms were too complex to allow inference on the genetic control of this enzyme system. ADH phenotypes (banding patterns) were thus used as an additional criterion for the discrimination of clonal groups for those pairs of individuals, which exhibited identical multilocus records in the remaining enzyme loci or when multilocus records were incomplete and repeated isozyme analysis could not be conducted because of smaller size or dying shoots.

To assess the spatial pattern of clone distribution, two approaches were used. First, genotype identity (binary variable: *GI* equals 1 for a pair of individuals with identical multilocus genotypes, 0 for non-identical genotypes) was tested for relationship with spatial distance by a simple Mantel test with 100 000 random permutations using the *zt* program (Bonnet and Van de Peer, Ghent University, Belgium). Second, we averaged genotype identities over 10 cm distance classes using the SGS program (Degen et al. 2001). Under non-random distribution of genotypes (as expected in the case of clonal spread), average genotype identities in short distance classes are expected to be higher than over large distances. 95% confidence intervals for average genotype identities were derived from 1 000 random permutations (Fig. 2). Each permutation consisted of a random redistribution of genetic or phenotypic data over the spatial co-ordinates of the sampled individuals. For each of the spatial distance classes, observed values were compared with the distribution obtained after 1 000 permutations. Then

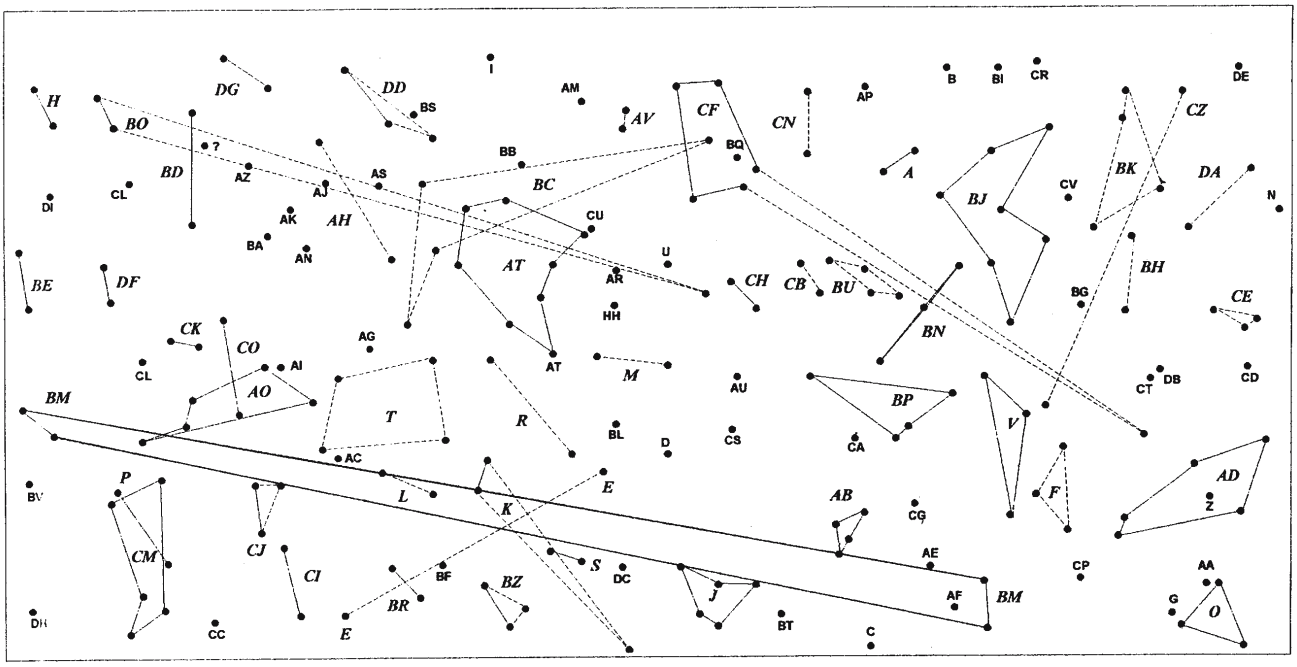

Fig. 1. Distribution map of the analyzed tillers in the permanent plot (2 × 1 m) and their membership to the distinguished genotypes. Genotypes are depicted by letter or couple of letters (larger font in italics is used for genotypes with numerous analysed tillers). Tillers with genotype membership confirmed by all studied isozyme systems are connected by a full line. The tillers indicated to belong to the same genotype, but with this membership not verified by at least four enzymes and ADH are joined by a dashed line.

a 95% confidence interval for the average genetic identity was constructed as the interval from the 25th to the 975th ordered permutation estimates (Manly 1997).

Results

In the studied locality, *S. albicans* formed exclusively intravaginal tillers. The number of daughter tillers produced by a tiller per year (branching intensity) was 0.67 (median), SD = 0.61, quartile range = 1, with maximum value 3. Tiller life span was 2.5 years (median), SD = 1.32, quartile range = 1, with maximum value 7 years. The vegetative mobility of tillers determined by average annual rhizome increment was 13.59 mm, median = 13 mm, SD = 5.38, quartile range = 7.05 with maximum value 31 mm.

According to our observation, the length of rhizomes depends on the length of annual rhizome increments as well as on the longevity of old rhizome tissues. The subsequent tiller generations keep physically attached to each other for many years or even decades (usually 6–12 years in the measured tiller sample). The overall length of rhizomes with interconnected tiller generations reached usually from 5 to 20 cm in the studied population.

Complete isozyme profiles were obtained for 139 tillers. Based on the zymogram variation, 98 different isozyme profiles were identified within the five analyzed isozyme systems on the permanent plot. The position of individual genotypes is shown Fig. 1.

The analysis has identified several very remote tillers of the same genotype. The longest distance between ramets of the same genotype (among the tillers

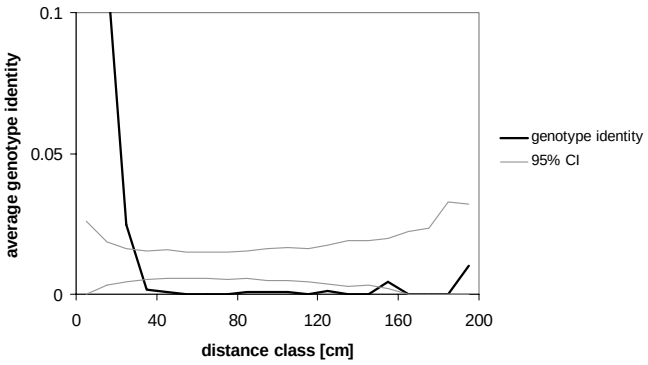

Fig. 2. Diagram of average genotype identities over 10 cm distance classes. 95% confidence intervals for *GI* derived from 1 000 random permutations are shown.

with complete isozyme profiles) was 153 cm (genotype BM, Fig. 1). For the remaining genotypes with complete isozyme profiles this distance did not exceed 35 cm. Accounting also tillers with incomplete isozyme profiles, large distances between tillers of the same genotype were observed also in the following cases: genotype BO (maximal distance between tillers 100 cm), CF (90 cm), BC (55 cm), CZ (53 cm), E (46 cm).

Mantel test revealed a highly significant correlation between genotype identity and distance ($r = -0.148$; $P < 0.0001$). This is an expected outcome, but it proves that clonal groups arising through lateral growth are spatially continuous. Average genotype identity rapidly decreased with distance (Fig. 2). In the shortest distance class 0–10 cm, the observed average genetic identity (0.345) was considerably higher than the upper 95%

confidence limit (0.026) derived from 1000 random permutations of genotypes over spatial positions. The same applies to further two distance classes up to 30 cm. This means that the probability that two randomly chosen plants separated by less than 30 cm belong to the same clone was significantly higher than expected under random distribution of genotypes. In contrast, for distance classes between 30 and 150 cm, the observed average genotype identities were significantly lower than expected by chance. Thus, plants separated by more than 30 cm likely belong to different clonal groups.

Discussion

The estimated clonal growth parameters for the studied population were very similar to those estimated by Kull (1995), Tamm et al. (2002) and Sammul et al. (2003) for a coastal population of *S. albicans* in Estonia. The annual increment of rhizome was identical for the compared populations (median = 13 mm), while the maximum value was higher for the Estonian population (86.6 mm in comparison to 31 mm in our population).

Compared to other plant species, *S. albicans* showed a high level of genetic variability (Reisch et al. 2003). It is a perennial, allogamous species with very broad ecological amplitude and these biological characteristics all contribute to creation and maintenance of a high level of genetic variability. High genetic diversity is known for a lot of clonal herb populations (cf. Widén et al. 1994). The number of identified genotypes in the studied population (49 genets per m^2) is considerably higher than the number of genotypes observed for other grasses, e.g. 1.67 genets per m^2 for *Brachypodium pinnatum* (Schläpfer & Fischer 1998), 27–33 genets per m^2 for *Festuca rubra* (Suzuki et al. 1999), 7.67 genets per m^2 for *Festuca novae-zelandiae* (Lord 1993). This fact could imply that in *S. albicans* generative reproduction plays an important role. Nevertheless, according to theoretical models, even a low seeds availability is sufficient for maintaining the genetic diversity (Eriksson 1993), under the condition that the replacement from seeds is continuous. For *S. albicans* the annual seed production is not high, compared to other grass species (about 400 seeds per tussock according to Dixon 1982; up to 1 000 seeds according to the BIDS database), however, the seed production is regular even at the most extreme locations and the seed persistence is high (Söyrinki 1954–56; personal observation). The existence of a seed bank in the soil has not been proved (Dixon 1982). Germination was high (80–95%, personal observation) and even after 4 years the germination potential was not significantly reduced (Dixon 1982).

The predominance of the generative reproduction was observed for the majority of grasses with dense tussocks, like *Festuca valesiaca* and *F. rupicola* (Hroudová-Pučelíková 1972), *Festuca pallens* (personal observation), *Festuca novae-zealandiae* (Lord 1993). However, a large distance between tiller aggregations of the same genotype supports the idea of frequent vegetative reproduction in the studied species. High age of genets and spatial spreading by clonal growth with the subsequent fragmentation (Lord 1993; Wilhalm 1995) could explain both the high number of genotypes recorded and the presence of numerous overlapping tussocks.

S. albicans has long-living rhizomes with short internodia which contain the nutrient supplies (Serebrjakova 1971). The rhizome length depends on habitat conditions (Wilhalm 1995) – the short ones are common in poor habitats exposed to stress or disturbance (drought, high radiation or grazing), the long ones (the overall rhizome length up to 60 cm) occur in plants growing in forest understorey or over nutrient richer soils (Dixon 1982). The process of physiological integration of tillers within tussocks still remains poorly known. The clonal fragmentation is common mostly in stoloniferous and rhizomatous species (Vorontzova & Zaugolnova 1985). Extensive areas of grasslands may be covered only by several clones of considerable age as shown by e.g. *Festuca rubra*, *Holcus mollis* or *Carex curvula* (Harberd 1961, 1967; Steinger et al. 1996). An intensive centrifugal growth was recorded in caespitose grasses such as *Nardus stricta* (Chadwick 1960) and *Bromus erectus* (Austin 1968). Similar process of spatial spreading can be assumed here as in rhizomatous grasses, but at much lower rates (Grubb 1990). If we know the annual spreading rate in *S. albicans* and suppose, that the most remote tillers with identical isozyme profiles are parts of the same clone, the clone age can be estimated. With the annual increment of 13.59, the greatest recorded distance of 153 cm could be achieved in 56 years assuming that the growth started in the middle distance and proceeded in both directions. By a growth in a single direction, the genet age could reach 112 years. A long life span of *S. albicans* (100 years or more) was reported also by Reisch et al. (2002).

Although Dixon (1982) mentions difficulties with growing *S. albicans* from isolated tillers, in our planting experiment the separated tillers survived almost up to 100% (Janišová, unpublished). Detached tillers can thus provide an effective means of vegetative propagation. Therefore, we consider the tussock rejuvenation from the isolated tillers after tussock fragmentation to be a possible way of vegetative reproduction.

Recently, increasing amounts of data on population structure of *S. albicans* have been published (see the cited literature). The future research could be focused on the following questions: a) What are the basic demographic characteristics of *S. albicans* at the level of ramets (tillers) and genets (tussocks)? b) Which of these characteristics are genetically predetermined and which are affected by the changing environmental conditions? c) What is the level of physiological integration of tillers within tussocks? d) Which traits enable *S. albicans* to survive in extreme environmental conditions in terms of drought and disturbance (erosion pressure)? The future studies could preferably include a larger spectrum of *Sesleria* species as well as the hybrid zones between *S. albicans*, *S. tatrae* and *S. uliginosa*.

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References

Austin M. P. 1968. Pattern in a *Zerna erecta* dominated community. J. Ecol. **56:** 197–218.

Chadwick M.J. 1960. *Nardus stricta*, a weed of hill grazing, pp. 246–256. In: Harper J.L. (ed.), The Biology of Weeds, Blackwell Scientific Publications, Oxford.

Cheliak W.M. & Pitel J.A. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Information Report PI-X-42, Petawawa National Forest Institute, Chalk River.

Conert H.J. 1998. *Sesleria*, pp. 473–487. In: Hegi G. (ed.), Illustrierte Flora von Mitteleuropa. Berlin, Parey.

Degen B., Petit R. & Kremer A. 2001. SGS – Spatial Genetic Software: A computer program for analysis of spatial genetic and phenotypic structures of individuals and populations. J. Heredity **92:** 447–448.

Dixon J.M. 1982. Biological Flora of the British Isles 151: *Sesleria albicans* Kit ex Schultes. J. Ecol. **70:** 667–684.

Dixon J.M. 1986. Drought resistance in *Sesleria albicans* Kit. ex Schultes compared with *Agrostis capillaris* L. and *Cynosurus cristatus* L. New Phytol. **103:** 559–572.

Ellenberg H. 1986. Vegetation Mitteleuropas mit den Alpen in ökologischer Sicht. Verlag Eugen Ulmer, pp. 615–661.

Eriksson O. 1993. Dynamics of genets in clonal plants. Trends Ecol. Evol. **8(9):** 313–316.

Grubb P.J. 1990. Demographic studies on the perennials of chalk grassland, pp. 93–99. In: Hillier S. H., Walton D.W.H. & Wells D.A. (eds), Calcareous grasslands – ecology and management, Bluntisham Books, Huntingdon.

Harberd D.J. 1961. Observations on population structure and longevity of *Festuca rubra* L. New Phytol. **60:** 184–206.

Harberd D. J. 1967. Observations on natural clones of *Holcus mollis*. New Phytol. **66:** 401–408.

Hroudová-Pučelíková Z. 1972. A comparative study of the ecology of *Festuca valesiaca* Gaudin and *Festuca rupicola* Heuff. Folia Geobot. Phytotax. **7:** 53–79.

Kull K. 1995. Growth form parameters of clonal herbs, pp. 106–115. In: Aaviksoo K., Kull K., Paal J. & Trass H. (eds), Consortium Masingii: A Festschrift for Viktor Masing, Scripta Botanica 9, Tartu University.

Lord J.M. 1993. Does clonal fragmentation contribute to recruitment in *Festuca novae-zelandiae*? New Zealand J. Bot. **31:** 133–138.

Lloyd N.D. & Woolhouse H.W. 1976. The effect of temperature on photosynthesis and transpiration in populations of *Sesleria caerulea* (L.) Ard. New Phytol. **77:** 553–559.

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

Lysák M., Rostková A., Dixon J.M., Rossi G. & Doležel J. 2000. Limited genome size variation in *Sesleria albicans*. Ann. Bot. (Oxford) **86:** 399–403.

Manly B.F.J. 1997. Randomization, Bootstrap and Monte Carlo Methods in Biology. Chapman & Hall, London

Pignatti E. & Pignatti S. 1975. Syntaxonomy of the *Sesleria varia*-grasslands of the calcareous Alps. Vegetatio **30:** 5–14.

Reisch C. & Poschlod P. 2003: Intraspecific variation, land use and habitat quality – a phenologic and morphometric analysis of *Sesleria albicans* (Poaceae). Flora **198 (4):** 321–328.

Reisch C., Poschlod P. & Wingender R. 2002. Genetic variation of *Sesleria albicans* Kit. ex Schultes (Poaceae): Lack of evidence for glacial relict endemism in central Europe. Plant Biol. **4(6):** 711–719.

Reisch C., Poschlod P. & Wingender R. 2003. Genetic differentiation among populations of *Sesleria albicans* Kit. ex Schultes (Poaceae) from ecologically different habitats in central Europe. Heredity **91(5):** 519–527.

Sammul M., Kull K. & Tamm A. 2003. Clonal growth in a species-rich grassland: results of a 20–year fertilization experiment. Folia Geobot. **38:** 1–20.

Schläpfer F. & Fischer M. 1998. An isozyme study of clone diversity and relative importance of sexual and vegetative recruitment in the grass *Brachypodium pinnatum*. Ecography **21:** 351–360.

Serebrjakova T.I. 1971. Morfogenez pobegov i evolyutsiya zhiznennykh form zlakov. Nauka, Moskva, 358 pp.

Söyrinki N. 1954–1956. Vermehrungsökologische Studien in den Bayerischen Alpen. I. Ann. Bot. Soc. "Vanamo" **27/1:** 1–232.

Steinger T., Körner C. & Schmid, B. 1996. Long term perspective in a changing climate: DNA analysis suggest very old ages of clones of alpine *Carex curvula*. Oecologia **105:** 94–99.

Suzuki J., Herben T., Krahulec F. & Hara T. 1999. Size and spatial pattern of *Festuca rubra* genets in a mountain grassland: its relevance to genet establishment and dynamics. J. Ecol. **87:** 942–954.

Tamm A., Kull K. & Sammul M. 2002. Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. Evolutionary Ecology **15:** 383–401.

Urbas P. & Zobel K. 2000. Adaptive and inevitable morphological plasticity of three herbaceous species in a multi-species community: Field experiment with manipulated nutrients and light. Acta Oecol. **21(2):** 139–147.

Vorontzova L.I. & Zaugolnova L.B. 1985. Population biology of steppe plants, pp. 143–178. In: White J. (ed.), The Population Structure of Vegetation, Junk, Dordrecht.

Widén B., Cronberg N. & Widén M. 1994. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. Folia Geobot. Phytotax. **29:** 245–263.

Wilhalm T. 1995. A comparative study of clonal fragmentation in tussock-forming grasses. Abstracta Botanica **19:** 15–60.

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