Genetic differentiation among populations of *Sesleria albicans* Kit. ex Schultes (Poaceae) from ecologically different habitats in central Europe

C Reisch¹, P Poschlod¹ and R Wingender²

¹Lehrstuhl für Botanik, Universität Regensburg, D-93040 Regensburg, Germany; ²Institut für Landwirtschaftliche Botanik, Universität Bonn, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

As observed for many other plant species, the populations of *Sesleria albicans* in Central Europe are located in habitats, which differ to a high degree from each other with regard to ecological factors such as nutrients, light and water as well as in type of land use. The species colonizes limestone cliffs, pavements, screes, grazed and mown grasslands, heaths, fens and open woodlands. In this study, we used random amplified polymorphic DNA (RAPD) analysis to investigate the genetic differentiation among 25 populations of *S. albicans* from six different types of habitat (beech forests, alpine and lowland rocky ridges, lowland screes, fens, calcareous grasslands). With RAPD analysis, 344 fragments could be amplified, of which 95.9% were polymorphic. The level of polymorphism ranged from 29.7 to 56.7%

polymorphic bands per population and was correlated with population size. In an analysis of molecular variance (AMOVA), used to detect variation among individuals within populations, among populations from the same habitat and among different habitats, most of the genetic variation was found within populations (62.06%) and among populations from the same habitat (33.36%). In contrast, only a very low level of differentiation could be observed among different habitats (4.58%). The results of our study give only little evidence for an ecotypic differentiation of *Sesleria albicans*. This differentiation is principally conceivable, but obviously not related to the investigated RAPD loci.

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Introduction

Sesleria albicans Kit. ex Schultes (Poaceae) is an alpine, perennial grass, which is widely distributed in the Alps, but also occurs in the lower mountains of south and central Germany (Dixon, 1982). Populations of *S. albicans* are located in ecologically very different habitats, which means that the plant colonizes beech forests, as well as naturally and manmade open habitats such as alpine and lowland rocky ridges, lowland screes, fens or calcareous grasslands. Furthermore, the plant grows on different geological materials and under a multitude of climatic conditions. As a consequence, the habitats in which the populations of *S. albicans* are located differ to a high degree from each other with regard to ecological factors such as nutrients, light and water, just as in the type of land use by grazing or mowing (Table 1).

Genetic variation within and among populations is strongly affected by ecological factors (Huff *et al.*, 1998; Kölliker *et al.*, 1998; Hsao and Lee, 1999), and microgeographic differentiation, caused by these factors, is reported for many species (Nevo *et al.*, 1988; Nevo and Beiles, 1989; Owuor *et al.*, 1997; Hsao and Lee, 1999). Ecological differences among the habitats colonized by a

plant species can, therefore, result in the development of ecotypes (Gunter *et al.*, 1996).

Moreover, different land-use practices are thought to make a contribution to the genetic differentiation of populations (Poschlod and Jackel, 1993; Poschlod *et al.*, 2000). Fertilization and defoliation are generally known to cause population differentiation and different types of grassland management can result in the development of ecotypic variants (Sweeney and Danneberger, 1995; Zopfi, 1998).

S. albicans colonizes wet and dry, sunny and shady, manmade and natural habitats. Differences in the length and width of leaves and spikes among populations of *S. albicans* from different habitats have been observed under common garden conditions (Dixon, 1982) and the populations growing in fens are supposed to be separate varieties or ecotypes. Furthermore, a morphometric analysis (Reisch and Poschlod, 2003) indicates differentiation among populations of *S. albicans*. Genetic or ecotypic differentiation among the populations of *S. albicans*, which grow in different habitats and under variable ecological conditions, can therefore be supposed.

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

Correspondence: C Reisch, Lehrstuhl für Botanik, Universität Regensburg, D-93040 Regensburg, Germany

E-mail: christoph.reisch@biologie.uni-regensburg.de

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Table 1 Habitats in which the sampled populations of *S. albicans* were located

Habitats	Temperature fluctuations	Water fluctuations	Nutrient stress	Type of land use
Lowland scree	High	High	High	None
Alpine rocky ridge	High	High	High	None
Lowland rocky ridge	High	High	High	None
Calcareous grassland	Medium	Medium	Medium	Grazing
Fen	Medium	None	Medium	Mowing
Beech forest	Low	Low	Low	None

For each habitat, information about ecological conditions and land use is given.

of breeding systems and the analysis of ecographical variation (Weising *et al.*, 1995).

Random amplified polymorphic DNA (RAPD) analysis has been successfully used to detect genetic variation of plant populations in a large number of studies (Fahima *et al.*, 1999; Mengistu *et al.*, 2000; Persson and Gustavsson, 2001; Arafeh *et al.*, 2002) and to analyse genetic differentiation among populations from ecologically different habitats (Owuor *et al.*, 1997; Huff *et al.*, 1998; Kölliker *et al.*, 1998; Hsao and Lee, 1999). Moreover, the analysis of molecular variance technique (AMOVA) (Excoffier *et al.*, 1992) is a powerful tool to analyse RAPD marker variation within and among plant populations (Huff *et al.*, 1993; Huff, 1997). In the study presented here, we therefore used RAPDs and AMOVA to find out whether there exists a genetic differentiation among populations from habitats, which differ from each other with regard to ecological conditions and land use.

Materials and methods

Species description

S. albicans Kit. ex Schultes (Poaceae) is a perennial grass with deeply rooting rhizomes which form small, persistent cushions of numerous tillers. The diploid ($2n=28$) and wind-pollinated plant usually flowers from March to June. The flowers are slightly proteandrous, and self-pollination, therefore, is probably of rare occurrence. 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 means of vegetative propagation.

S. albicans 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 in the lower mountains of south and central Germany (Dixon, 1982). *S. 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 former Yugoslavia and Albania and in two localities in Iceland.

Study sites

To investigate the genetic diversity within and among populations of *S. albicans* from different types of habitats, 25 populations in southwest, west and central Germany

Figure 1 Geographic position of the study sites. In all, 25 populations of *S. albicans* in central Europe and the Alps were investigated. Populations grew in six different types of habitats: lowland screes (hexagons), lowland rocky ridges (inverted triangles), limestone grasslands (circles), beech forests (squares), fens (rhombuses) and alpine rocky ridges (triangles).

and in the northern and southern Alps were selected (Figure 1). Plants from six different types of habitat like lowland screes and lowland rocky ridges, calcareous grasslands, beech forests, fens and alpine rocky ridges (Table 2) were compared.

Plant material and sampling conditions

Seed material was collected *in situ* all across the population from at least 20 different plants, and was used to germinate plants from each population in a common garden. After germination, 10 individuals per population were planted in small pots with common garden soil and cultivated for 1 year. Since we focused on the differentiation among populations from different habitats in this study, we decided to analyse a relatively small number of plants from a large number of populations rather than a large number of plants from a small number of populations. After the first flowering of the individuals, therefore, leaves from four plants per population were sampled and stored at -35°C in the laboratory.

DNA isolation

The DNA was isolated from frozen plant material of individual plants using the cetyltrimmonium bromide (CTAB) method (Rogers and Bendich, 1994) 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 $\mu\text{l}/\text{ml}$ β -mercaptoethanol).

Table 2 Sampled populations of *S. albicans* with abbreviation (Abb.) description of habitat, region, geographical latitude (La.), longitude (Lo.), altitude (Alt.), number of sampled individuals (S.) and population size (P. size, in m² covered by *Sesleria*)

No.	Population	Abb.	Habitat	Region	La. (N)	Lo. (E)	Alt.	S.	P. size
1	Mühlenbachtal	Müh	Lowland scree	CG	51°27'	8°37'	300	4	5
2	Engelslay	Eng	Lowland scree	WG	50°31'	6°58'	200	4	6
3	Brunkenstein	Bru	Lowland scree	WG	49°49'	7°31'	220	4	4
4	Felsengärten	Fel	Lowland scree	SWG	48°59'	9°12'	280	4	2000
5	Gissigheim	Gis	Lowland scree	SWG	49°45'	9°35'	250	4	80
6	Rauher Fels	Rau	Lowland rocky ridge	SWG	48°03'	8°59'	780	4	300
7	Spielburg	Spi	Lowland rocky ridge	SWG	48°43'	9°45'	650	4	60
8	Indelhausen	Ind	Lowland rocky ridge	SWG	48°18'	9°30'	600	4	100
9	Bürgle	Bür	Calcarous grassland	SWG	48°17'	8°59'	740	4	15 000
10	Zeller Horn	Zel	Calcarous grassland	SWG	48°17'	9°00'	900	4	8000
11	Irrenberg	Irr	Calcarous grassland	SWG	48°15'	8°56'	920	4	12 000
12	Lampertstal	Lam	Calcarous grassland	WG	50°22'	6°42'	440	4	1000
13	Steinfeld	Ste	Calcarous grassland	WG	50°22'	6°38'	500	4	15 000
14	Hundsrucken	Hun	Calcarous grassland	WG	50°29'	6°34'	500	4	20 000
15	Nähberg	Näh	Beech forest	SWG	48°17'	9°00'	880	4	300
16	Eybachtal	Eyb	Beech forest	SWG	48°40'	9°54'	600	4	300
17	Blindloch	Bli	Beech forest	SWG	48°02'	8°58'	750	4	100
18	Pütz	Püt	Beech forest	WG	50°32'	6°33'	450	4	100
19	Urfthal	Urf	Beech forest	WG	50°32'	6°35'	450	4	1000
20	Evagarten	Eva	Fen	SWG	47°52'	9°22'	600	4	30
21	Höll	Höl	Fen	SWG	47°48'	9°48'	600	4	20
22	Federmähder	Fed	Fen	SWG	48°28'	10°19'	450	4	20
23	Füssen	Füs	Fen	SWG	47°36'	10°45'	500	4	50
24	Nebelhorn	Neb	Alpine rocky ridge	NA	47°25'	10°21'	1600	4	50 000
25	Eggental	Egg	Alpine rocky ridge	SA	46°30'	11°25'	650	4	40 000

CG = Central Germany, WG = west Germany, SWG = southwest Germany, NA = northern Alps, SA = southern Alps

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 15 000 g at 4°C for 10 min. Adding an equal volume of isopropanol (5°C), the DNA was precipitated and pelleted by centrifugation at 20 000 g 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; 1 mM 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 absorbance at 260 and 280 nm. For, polymerase chain reaction (PCR) only template DNA was used with a purity of 1.8–2.1 in a dilution of 15 ng/µl.

RAPD analysis

For DNA amplification, the 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* and 15 primers were selected, giving rise to clear reproducible and distinct banding patterns.

In a final volume of 15 µl, amplification mixtures contained 10 mM Tris-HCl (pH 9.5), 1.5 mM MgCl₂, 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 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 amplified products were separated on 1.5% agarose gels in 1 × TBE (Tris-borate-EDTA) buffer 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.

For data scoring, the banding patterns were recorded using a *trans*-illuminating gel documentation system (Gel Print 2000i, BioPhotronics 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 which were reproducible in duplicate amplifications. Each band was scored across all individuals as either present or absent. When individuals did not give clear or not reproducible and 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 100 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 biallelic locus (Williams *et al.*, 1990).

Statistical analysis

The binomial matrix was used to calculate the level of polymorphism (percentage of polymorphic bands) for

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
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
B18	–CCA CAG CAG T–	60	210–4123	26	0	26	100
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
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
Total			210–4123	330	14	344	95.9

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 (ie the presence of a band in both samples), d is the number of negative matches (ie 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 genetic relatedness among individuals was obtained by clustering. Dendrograms were generated from the similarity coefficient matrix using the UPGMA method (Sneath and 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 and de Wachter, 1994).

The binomial matrix was also applied to an AMOVA in RAPD patterns by the program WINAMOVA 1.55 (Excoffier *et al*, 1992; Stewart and Excoffier, 1996). AMOVA was originally developed for RFLP haplotypes, but it is also appropriate for RAPD phenotypes (Huff *et al*, 1993). 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. The program extracts analogies of F-statistics (so-called Φ -statistics). The 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 25 populations and their levels of significance were also obtained from the AMOVA. For each analysis, 1000

permutations were performed to obtain significance levels. As significance tests in AMOVA are based on permutation procedures, they are essentially assumption free (Excoffier *et al*, 1992). Additionally, the AMOVA sums of squares divided by $n-1$ were calculated for each population to describe the molecular variance within the populations.

Results

RAPD banding and levels of polymorphism

In the RAPD analysis of 100 individuals from 25 populations of *S. albicans* with 15 decamer random primers, 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 4123 bp. The 15 primers produced between 90.1 and 100% polymorphic bands. In total, 95.9% of the bands were polymorphic, and 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.

The percentage of polymorphic bands per population ranged from 29.7% in the Federmähdler population to 56.7% in the Eggental population (Table 4) and correlated highly significantly with population size (Spearman's rank-correlation coefficient $r_s = 0.7$, $P < 0.001$).

Genetic distances (Φ_{ST}) among populations

In all, 273 of the 300 pairwise genetic distances (Φ_{ST}) between populations were highly significant. 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 Federmähdler and the west German population Engelslay. Minimum Φ_{ST} was only 0.13, which was observed between populations Lampertstal and Urfttal, both in west Germany. The matrix of 300 pairwise genetic distances (Φ_{ST}) among the

Table 4 Genetic diversity within populations of *S. albicans* measured as percentage of polymorphic bands per population

No.	Population	Polymorphic bands (%)
1	Mühlenbachtal	42.3
2	Engelslay	39.0
3	Brunkenstein	43.6
4	Felsengärten	49.2
5	Gissigheim	44.0
6	Rauher Fels	52.8
7	Spielburg	41.7
8	Indelhausen	50.0
9	Bürgle	55.6
10	Zeller Horn	51.9
11	Irrenberg	51.1
12	Lampertstal	53.7
13	Steinfeld	53.4
14	Hunds Rücken	48.5
15	Nähberg	54.2
16	Eybachtal	47.0
17	Blindloch	44.0
18	Pütz	40.4
19	Urfttal	55.7
20	Evagarten	48.7
21	Höll	40.1
22	Federmähder	29.7
23	Füssen	50.8
24	Nebelhorn	55.0
25	Eggental	56.7

25 populations of *S. albicans* was, however, not correlated significantly with the corresponding matrix of geographic distances, since the calculated Mantel test showed a correlation coefficient of $r=0.17$ and a significance of $P=0.06$.

Population clustering

In an unrooted and bootstrapped UPGMA dendrogram, based on 344 RAPD markers without any exception, all populations of *S. albicans* could be discriminated from each other (Figure 2). Except for the southwest German lowland rocky ridge populations, most investigated populations were mixed thoroughly, irrespective of their origin from different habitats. However, the south alpine population Eggental was separated from all other populations with a very high bootstrap value of 100%.

AMOVA analysis and variance partitioning

Arranging the 25 populations of *S. albicans* in six groups according to their origin from different types of habitat, a significant level of molecular differentiation among populations from different habitats was observed. A variance of 4.58% was found among different habitats, 33.36% among populations within habitats and 62.06% within populations (Table 5).

The partitioning of molecular variance was corroborated by separate three-level AMOVAs, which were conducted for the populations from southwest and west Germany only. In both cases, 61.26 and 68.23% molecular variance, respectively, were observed within the populations. Molecular variations of 2.69 and 3.38%, respectively, were found among the different habitats. This partitioning of molecular variance was also observed, when analysing 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, 0.90% variation was observed among populations from beech forests and calcareous grasslands (Table 5).

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. The variation in RAPD banding patterns among populations, among populations within habitats and within populations was also 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.

Discussion

In the investigation presented here, 95.9% of the RAPD fragments were polymorphic. Compared to other plant species, *S. albicans*, therefore, showed a high level of genetic variability. In a study of the widespread *Tanacetum vulgare*, 85% polymorphic bands were observed (Keskitalo et al, 1998), in the rare *Vicia pisiformis* 7.2% (Black-Samuelsson et al, 1997) and in apomictic species of *Rosa* section *Caninae* only 3% (Werlemark and Nybom, 2001). These differences are not astonishing, since the level of genetic variability strongly depends on the plant's life history traits (Hamrick and Godt, 1990; Nybom and Bartish, 2000). *S. albicans* is a perennial, allogamous plant species with a very broad ecological amplitude and these biological characteristics all contribute to create and maintain the observed high level of genetic variability (Babbal and Selander, 1974; Hamrick and Godt, 1990; Ge et al, 1999; Nybom and Bartish, 2000). Considering the intrapopulational genetic diversity of *S. albicans*, our study revealed a strong correlation of genetic variability and population size, as demonstrated for many other species (Frankham, 1996; Godt et al, 1996; Fischer and Matthies, 1998; Menges and Dolan, 1998). We observed low levels of variation in populations from fens and lowland screes, which were the smallest, and high levels of variation in populations from calcareous grasslands, which were the largest populations (Table 2). Since possible ecotypic differentiations are often superimposed by population genetic processes such as founder effects or genetic drift (Nevo et al, 1988), it is more likely that the different levels of intrapopulational diversity are due to the effects of population size rather than the influence of ecological conditions.

The habitats colonized by *S. albicans* differ to a high degree from each other with regard to the availability of nutrients, light and water as well as the type of land use (Table 1). As known from former investigations, genetic variation within and among populations is strongly affected by ecological factors (Nevo et al, 1988; Nevo and Beiles, 1989; Kölliker et al, 1998). The ecotypic

Figure 2 Bootstrapped cluster analysis (UPGMA) of 25 populations of *S. albicans* (CE = central Europe, NA = northern Alps, SA = southern Alps). Population abbreviations follow Table 2.

Table 5 Summary of analysis of molecular variance (three-level AMOVA)

Level of variation	SS	MS	%	P
<i>25 populations grouped together in six types of habitat</i>				
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
<i>Populations from southwest Germany grouped together in five types of habitats</i>				
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
<i>Populations from west and central Germany grouped together in five types of habitats</i>				
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
<i>Populations from the Swabian Alb grouped together in three types of habitats</i>				
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
<i>Populations from the Eifel grouped together in two types of habitats</i>				
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

In all, 25 populations of *S. albicans* were investigated from six different types of habitat (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, %: proportion of genetic variability, P: level of significance).

development of populations depends on climatic and edaphic conditions (Nevo *et al.*, 1988) and differences among populations referring to these factors can cause microgeographic differentiation, as reported for many grass species (Hamrick and Allard, 1972; Hsao and Rieseberg, 1994; Nevo *et al.*, 1994; Owuor *et al.*, 1997). Furthermore, different types of land use are thought to make a contribution to the differentiation among and within populations (Poschlod and Jackel, 1993; Poschlod *et al.*, 2000). Ecological differences among meadows and pastures in fertilization and defoliation can cause microenvironmental adaptations of plant populations (van Tienderen and van der Toorn, 1991; Sweeney and Danneberger, 1995) and development of ecotypic variants (Zopfi, 1993, 1998).

Considering these facts, an ecotypic differentiation among the populations of *S. albicans*, which grew in different habitats under various ecological conditions, could have been assumed. However, except for the southwest German lowland rocky ridge populations in an UPGMA cluster analysis, the populations were not grouped according to their origin from the same habitat. On a very large scale, the dendrogram showed a geographical pattern, since the south alpine population was separated from all other populations north of the Alps. On a smaller scale, populations were, however, not grouped geographically. This finding was corroborated by a Mantel test, which also showed no significant correlation between geographic and genetic distances. Despite colonizing ecologically differing habitats, a specific differentiation of *S. albicans* could not be resolved by cluster analysis based on RAPD data.

The results of the AMOVA also give only weak evidence for the ecotypic differentiation among popula-

tions from different habitats. In the AMOVA, 4.58% variation was observed among the habitats, which is comparable to investigations of further grass species colonizing ecologically different habitats. In an analysis of *Yushania niitakayamensis* from grassland and forest undergrowth, 2.91–7.99% variation among populations from different habitats was found (Hsao and Lee, 1999), and for *Schizachyrium scoparium* 3.18% variation among populations from different soil fertility levels was observed (Huff *et al.*, 1998). Considering the genetic variation among populations from different habitats, however, the natural distribution of *S. albicans* in central Europe must be taken into account. Since not all types of habitat could be found in all geographic regions of central Europe, it is likely that habitat-specific and geographic effects both contribute to the observed variation among populations from different habitats in central Europe.

Considering the results of our investigation, there is only little evidence for a genetic or even ecotypic differentiation. It could, however, be possible that there is a differentiation, which is not related to the investigated RAPD loci. Such an ecotypic differentiation could possibly be detected in further investigations using different molecular methods.

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