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RESEARCH PAPER

Low genetic variability and strong differentiation among isolated populations of the rare steppe grass *Stipa capillata* L. in Central Europe

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ABSTRACT

***Stipa capillata* L. (Poaceae) is a rare grassland species in Central Europe that is thought to have once been widespread in post-glacial times. Such relict species are expected to show low genetic diversity within populations and high genetic differentiation between populations due to bottlenecks, long-term isolation and ongoing habitat fragmentation. These patterns should be particularly pronounced in selfing species. We analysed patterns of random amplified polymorphic DNA (RAPD) variation in the facultatively cleistogamous *S. capillata* to examine whether genetic diversity is associated with population size, and to draw initial conclusions on the migration history of this species in Central Europe. We analysed 31 *S. capillata* populations distributed in northeastern, central and western Germany, Switzerland and Slovakia. Estimates of genetic diversity at the population level were low and not related to population size. Among all populations, extraordinarily high levels of genetic differentiation (amova: ϕ_{ST} = 0.86; Bayesian analysis: θ^{B} = 0.758) and isolation-by-distance were detected. Hierarchical amova indicated that most of the variability was partitioned among geographic regions (59%), or among populations between regions when the genetically distinct Slovakian populations were excluded. These findings are supported by results of a multivariate ordination analysis. We also found two different groups in an UPGMA cluster analysis: one that contained the populations from Slovakia, and the other that combined the populations from Germany and Switzerland. Our findings imply that *S. capillata* is indeed a relict species that experienced strong bottlenecks in Central Europe, enhanced by isolation and selfing. Most likely, populations in Slovakia were not the main genetic source for the post-glacial colonization of Central Europe.**

INTRODUCTION

Central European steppe grasslands are thought to have been widespread in Central Germany during the earliest post-glacial periods (Lang 1994; Hewitt 1996; Frey & Lösch 2004). Post-glacial range expansion probably began with increasing climate warming about 10,000 years ago, when Eastern European steppe plants started to migrate westwards along dispersal routes provided by the spreading grasslands (Pott 1996; Frey & Lösch 2004). With increasing cooling and humidity, steppe grasslands were increasingly replaced by deciduous forests and became restricted to isolated sites where edaphic and climatic conditions excluded tree growth. In the last 2000 years, logging and grazing led to a re-expansion of these grassland fragments (Lang 1994). At present, however, intense agricultural management and abandonment have reduced grassland fragment sizes and enhanced isolation once again. Populations of steppe species in Central Europe have thereby experienced several phases of expansion and contraction since the Holocene.

Knowledge of genetic diversity patterns and migration history of steppe relict species in Europe is still scarce in comparison with other groups of plants (Petit *et al.* 1997; Franzke *et al.* 2004; Hensen *et al.* 2005; Wróblewska & Brzosko 2006). Arctic-alpine species, with their probably much longer colonization history in Central Europe, have frequently been shown to be characterized by high levels of genetic variation among populations and by strong isolation-by-distance patterns due to fragmentation and increasing isolation during post-glacial times (Tremblay & Schoen 1999; Reisch *et al.* 2003a; Pimentel *et al.* 2007; Reisch 2008; but see Reisch *et al.* 2002 for an exception). However, in addition to individual population history and possible fluctuations in range size, population size, longevity and mating system may also influence the genetic structure of relict populations, particularly at the margins of their distribution range, where

populations are often genetically isolated due to fragmentation (Durka 1999; Van Dyke 2003; Hensen *et al.* 2005). Smaller populations are known to be more susceptible to random genetic drift, increased selfing and mating among related individuals (Shaffer 1981; Boyce 1992; Honnay & Jacquemyn 2007). Genetic exchange between generations and among populations may additionally be influenced by the mating system (Hensen & Oberprieler 2005; Honnay & Jacquemyn 2007): it is hypothesized that, in selfing species, a larger proportion of genetic diversity is partitioned among populations, while in outbreeding species, within-population diversity is higher (Hamrick & Godt 1996; Michalski & Durka 2007).

Stipa capillata L. (Poaceae) is one of the most common Eurasian steppe grasses, with a wide distribution throughout Asia and Eastern Europe. In Central Europe, this species is at its northwestern distribution edge, but was probably more widespread in the early Quaternary (Fig. 1; Hensen 1995; Frey & Lösch 2004). It is likely that *Stipa* spp. grew in Central Europe in the rich vegetation of the interstadial phases, but it is unlikely that they occurred during the cold conditions of the stadials (Behre *et al.* 2005; Bieniek & Pokorný 2005). The few extant occurrences are concentrated mainly in three principal regions: western, central and northeastern Germany (Fig. 2). *Stipa capillata* is currently included in the German Red Lists as vulnerable, both at federal and national levels (Korneck *et al.* 1996). With respect to the highly fragmented occurrences, a better understanding of genetic diversity patterns is needed to facilitate any effective conservation strategy.

The present study aims to evaluate the genetic structure and diversity of *S. capillata* in Central Europe to examine whether long-term isolation, repeated range expansions and contractions, and small population size are reflected in the current genetic structure. In particular, we addressed the following questions: (i) What is the level of genetic diversity of populations, and is genetic diversity related to population size? (ii) How is genetic variation distributed among and within populations of *S. capillata*, and do patterns in variation provide evidence for selfing in this facultative cleistogamous species? (iii) Are levels of genetic similarity related to geographic distance between populations? (iv) Do molecular data support the hypothesis that *S. capillata* is a relict in Central Europe, and are possible colonization routes detectable?

MATERIAL AND METHODS

Study species

Stipa capillata (Poaceae, *Stipa* sect. *Leiostipa* Dumort. *sensu* Tzvelev 1976) is a long-lived perennial tussock grass. Its distributional range comprises vast areas in the continental regions of Asia and Eastern Europe, where it is common in dry grasslands (Fig. 1). In Central Europe, the species is restricted to highly fragmented, isolated sites where it often dominates dry nutrient-poor grasslands on calcareous soils. The species is also known to efficiently colonize secondary sites (Hensen 1995; Süß *et al.* 2004). Flowering takes place between July and August, while fruit dispersal occurs from August to September. *Stipa capillata* is pollinated by wind and, according to Ponomarev (1961), it is a facultative cleistogamous species, and produces both chasmogamous and cleistogamous flowers. Caryopses are characterized by a long hygroscopic awn that facilitates dispersal by wind and animals (Hensen & Müller 1997). As *S. capillata* grows as a clonal phalanx plant, vegetative reproduction is possible but restricted to the close vicinity of the mother plant (Hensen 1997).

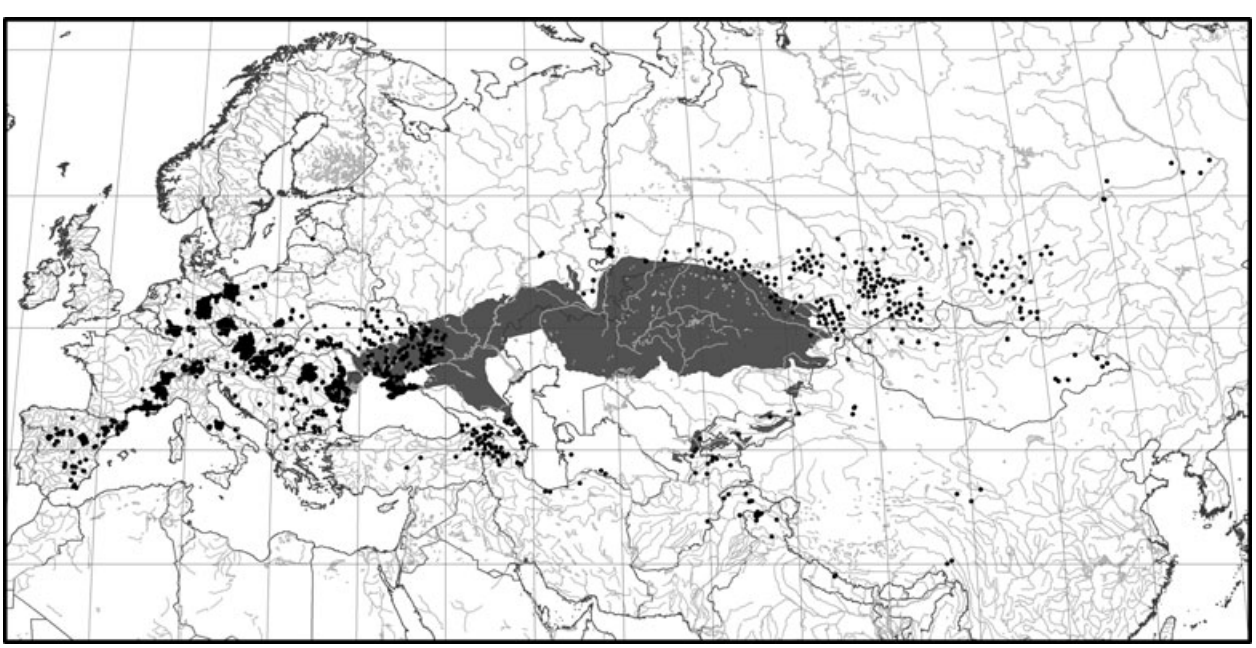

Fig. 1. Distribution map for *Stipa capillata* L. The map is based on Meusel *et al.* (1965), and was revised and completed with new data. The grey area represents the putative main distribution range where *S. capillata* is a dominant element of the zonal vegetation. Black dots outside of the main range denote more or less isolated occurrences and populations. A list of sources of the distribution data is available on request from the first author.

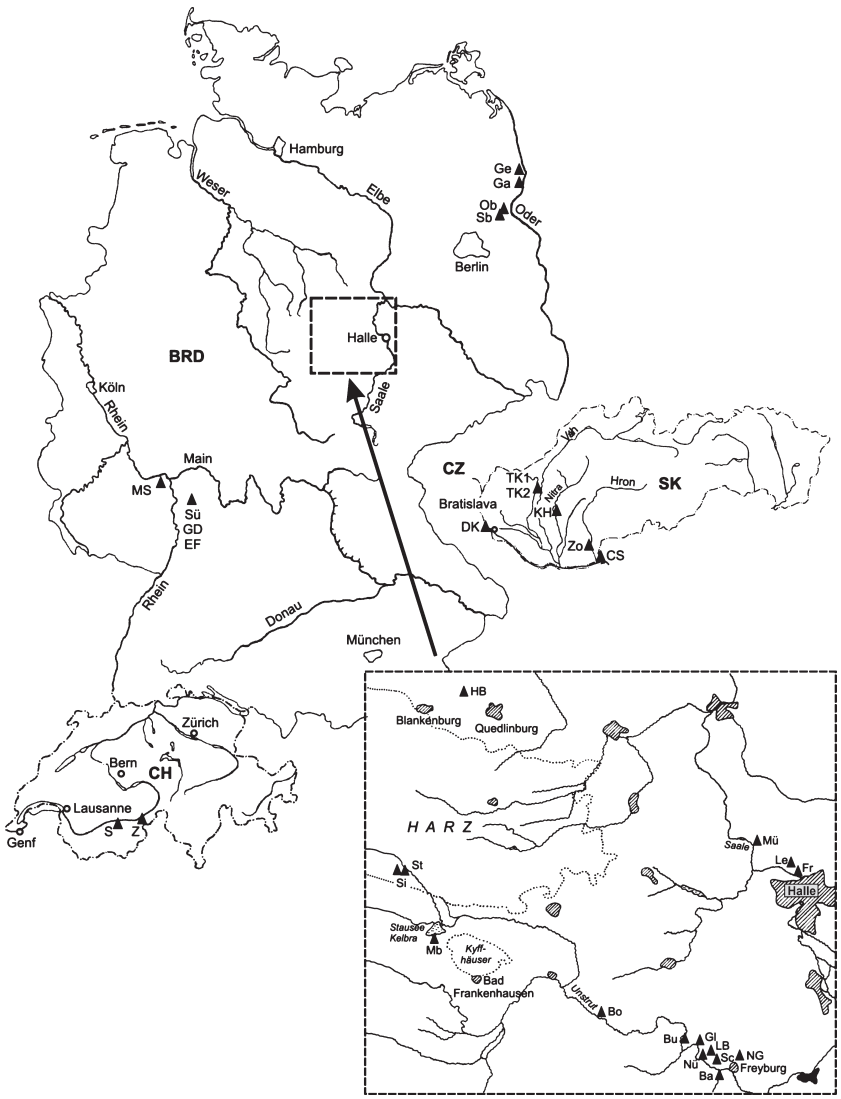

Fig. 2. Locations of the sampled populations (for abbreviations refer to Table 1).

Study regions and sampling of populations

For the present study, 31 *S. capillata* populations were sampled in northeastern (4), central (15) and western Germany (4), Switzerland (2) and Slovakia (6) (Fig. 2; Table 1), at altitudes ranging from 10 m (northeastern Germany) to 1450 m asl. (Zeneggen, Switzerland). A population was defined as a group of plants separated from their closest conspecifics by more than 250 m. The minimum distance between two locations was 350 m, with the maximum distance being 912 km. Population sizes were examined by estimating the area covered by *S. capillata* tussocks – and usually varied between 16 and 8000 m^2 (Table 1). For the RAPD analysis, we sampled leaves of 8–12 individual tussocks per population between May 1997 and October 2005. Distances between individual tussocks were always >1 m, and the samples were collected from areas no larger than 400 m^2.

RAPD-PCR

Genomic DNA was extracted from 25 mg of silica gel dried leaf material following the protocol described by Qiagen (DNeasy Plant Mini Kit, 2000: 18). DNA was amplified in reaction volumes of 10 µl containing 0.8 µl DNA (10 ng·µl^{-1}), 0.6 µl primer (10 pmol·µl^{-1}; Roth), 1.0 µl of each dNTP (2 mM; peqlab), 1.0 µl 10× buffer (Qbiogene), 0.1 µl Taq polymerase (5 U·µl^{-1}, Qbiogene) and 6.5 µl H_2O. PCR was carried out in an Eppendorf Mastercycler gradient, which was set to one cycle of 2 min at 94 °C followed by 36 cycles of 12 s at 94 °C, 45 s at 36 °C and 120 s at 72 °C, with a final cycle of 7 min at 72 °C. DNA fragments were separated by electrophoresis in 2% agarose gels with a Tris–acetate–EDTA (TAE) buffer system at 150 V for 150 min, and then stained with ethidium bromide. DNA bands were visualized with UV light and documented using a video camera. To ensure reproducibility, each sample was run in at least three independent RAPD-PCR amplification reactions. Based on an initial screening of 40 RAPD primers (Roth Random-Primer-Kits for RAPD-PCR, Kits D and N, Fa; Carl Roth, Germany), nine primers were chosen for the analysis that showed polymorphism, readability and reproducibility: D05: TGAGCGGACA; D08: GTGTGCCCCA; D11: AGCGCCATTG; D18: GAGAGCCAAC; D20: ACCCGGTCAC; N05: ACTGAACGCC; N12: CACAGACACC; N16: AAGCGACCTG; and N18: GGTGAGGTCA.

Table 1. Regions and localities of sampled populations, population sizes measured as area (m^2), sample numbers, genetic diversity and percentage of polymorphic loci of *Stipa capillata* stands.

region	location	pop. – size (m^2)	no. of samples	gene diversity	mean Sørensen dissimil.	no. of bands present	no. of polymorphic bands	% of polymorphic bands[a]
central Germany	Neue Göhle (NG)	2500	12	0.043	0.036	54	10	18.5
	Schafberg (Sc)	8000	12	0.097	0.080	62	20	32.3
	Langer Berg (LB)	200	12	0.036	0.031	52	10	19.2
	Burgscheidungen (Bu)	400	12	0.076	0.061	59	17	28.8
	Nüssenberg (Nü)	600	12	0.033	0.028	53	7	13.2
	Balgstädt (Ba)	150	12	0.060	0.052	53	12	22.6
	Gleina (Gl)	16	12	0.072	0.060	56	18	32.2
	Bottendorf (Bo)	2000	12	0.101	0.080	63	22	34.9
	Mittelberg (Mb)	600	12	0.042	0.034	57	12	21.0
	Singerberg (Si)	25	12	0.020	0.016	53	6	11.3
	Steinberg (St)	50	12	0.006	0.005	51	2	3.9
	Mücheln (Mü)	1000	12	0.106	0.088	65	24	37.0
	Franzigmark (Fr)	50	8	0.111	0.086	65	23	35.3
	Lerchenhügel (Le)	100	11	0.054	0.044	57	11	19.3
	Harslebener Berge (HB)	2000	12	0.037	0.029	56	7	12.4
northeastern Germany	Oderberg (Ob)	100	12	0.083	0.064	62	17	27.4
	Struwenberg (Sb)	50	12	0.054	0.044	58	12	20.6
	Geesow (Ge)	150	12	0.007	0.006	50	1	2.0
	Gartz (Ga)	200	12	0.016	0.014	51	5	9.8
western Germany	Mainzer Sand (MS)	600	12	0.038	0.031	55	11	20.0
	Standortübungsplatz (Sü)	200	12	0.011	0.009	51	4	7.9
	Griesheimer Düne (GD)	20000	12	0.014	0.012	52	4	7.7
	Euler Flugplatz (EF)	30000	12	0.010	0.008	52	3	5.8
Slovakia	Tematinske Kop. 1 (TK1)	1600	11	0.087	0.053	52	6	11.5
	Tematinske Kop. 2 (TK2)	400	12	0.060	0.073	56	24	42.9
	Kamenica n. Hronom (KH)	200	12	0.012	0.010	48	16	33.3
	Devinsca kobyla (DK)	2000	12	0.074	0.066	55	3	5.5
	Zobor (Zo)	150	12	0.076	0.064	55	16	29.1
	Cenkovska Stepp (CS)	2500	9	0.031	0.030	46	17	36.9
Switzerland	Sierre (S)	100	10	0.051	0.041	59	13	22.1
	Zeneggen (Z)	400	10	0.024	0.023	47	7	14.8

[a]The percentage of polymorphic bands was calculated with respect to the number of bands present in a given population.

Statistical analysis

RAPD bands that could be scored were entered as presence (1) and absence (0) data into a matrix. Considering that with dominant markers the application of standard measures of genetic diversity relies on several assumptions – including the presence of a Hardy–Weinberg equilibrium – several approaches are usually combined to analyse them (*e.g.* Schönswetter *et al.* 2005). We chose three approaches for our statistical analysis, one band-based and two allele frequency-based methods (Bonin *et al.* 2007). We calculated dissimilarities using the asymmetric Sørensen coefficient (equivalent to the coefficient of Nei & Li 1979), which is based on a similar reasoning as the Jaccard coefficient but places higher emphasis on shared bands. We obtained average Sørensen dissimilarity among samples of a given population as a robust measure of within-population diversity. The numbers of private and polymorphic bands and the percentage of polymorphic bands of all bands present in a given population were used as additional band-based estimates of population diversity. Sørensen dissimilarities among individual plant samples were subjected to UPGMA clustering; a bootstrap analysis was performed to assess stability of the branching pattern (50 resamples). The dendrogram was pruned at a dissimilarity level of 0.1, and redrawn to show the main patterns. We also assessed the genetic pattern in higher dimensional space by subjecting the Sørensen dissimilarity matrix to a Principal Coordinate Analysis (PCoA). Sørensen values were square-root transformed to improve metric properties (Legendre & Legendre 1998). Analyses were performed with MVSP 3.12 (Kovach 1998), NTSYSPC (Exeter Software) and CANOCO 4.5 (ter Braak & Smilauer 2002).

The second approach was based on symmetric measures of genetic diversity using squared Euclidean distances among RAPD phenotypes (Excoffier *et al.* 1992). Genetic diversity of populations was assessed from the average gene diversity over loci among all members of the population using the software ARLEQUIN version 3.2 (Excoffier & Schneider 2006). The same software was used to assess the partitioning of genetic variance within populations, among populations, and among groups of populations by conducting an analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) in which ϕ statistics (analogues of F statistics) were extracted. Populations examined in the AMOVA procedure were initially assigned to five

different groups based on their geographic origins (northeastern, central and western Germany, Slovakia and Switzerland; Fig. 1, Table 1). To assess the level of differentiation at different spatial scales, we calculated another three AMOVAS based on the following reduced datasets: (i) only the populations from Germany and Switzerland, (ii) only the populations from Germany, (iii) only the populations from central Germany (six groups based on geographic location: Freyburg NG, Sc, LB, Bu, Nü, Ba, Gl; Bottendorf Bo; Kyffhaeuser Mb; Harz Si, St; Halle Mü, Fr, Le; and Quedlinburg HB; Fig. 1, Table 1). Considering that for six of the 31 populations <12 specimens were sampled, we additionally checked whether unbalanced sampling affected the results of our AMOVA. To do this, we randomly removed samples from the larger datasets to obtain a balanced sampling scheme with n = 8 throughout. In all four geographical groupings, differences between the ϕ_{ST} values of the complete dataset (Table 1) and those of the reduced datasets were <0.01, confirming that RAPD-based assessments here are relatively insensitive to sampling intensity (Nybom & Bartish 2000). Thus, we only report results that are based on the complete dataset (Table 1). Significance levels of variance components, of global and of pairwise ϕ_{ST} values were calculated by conducting 1000 permutations for each analysis.

Third, we calculated θ^B, a Bayesian estimator of population structure with the program HICKORY, version 1.1 (Holsinger & Wallace 2004), as this approach does not assume Hardy–Weinberg equilibrium or a fixed value of f. Values for burn-in, sampling and thinning were 5000, 100,000 and 20, respectively. Based on the deviance information criterion (DIC) we compared model fit of alternative models and choose the full model (Holsinger & Lewis 2003–2007). We used $\theta^{(I)}$, which is the best estimate of Wright's F_{ST} (Holsinger & Lewis 2003–2007).

Mantel tests (Mantel 1967) were used to examine whether genetic differentiation among populations (pairwise ϕ_{ST} values, Excoffier *et al.* 1992) is related to geographical distances. Global Mantel tests were used to describe the overall spatial genetic structure. The approach is rather crude but methods that allow a more fine-scale analysis (*e.g.* Mantel correlograms, Diniz-Filho & Telles 2002) are sensitive to sampling scheme, and results need to be interpreted with caution (Vekemans & Hardy 2004). We thus calculated Mantel statistics only for the four geographic groupings mentioned above. Because Mantel tests assume a linear model, we also compared tests based on raw and rank-transformed distance matrices, which produced similar results. Differences in r_M were always <0.05, and P values remained identical, except for the second grouping (only Slovakian populations excluded). Here, r_M for the rank transformed data was 0.1 units higher than for the Mantel test based on a linear model. Again, significances were not affected, so we only provide data for the standard Mantel tests (all calculated with Arlequin, 1000 permutations, Excoffier & Schneider 2006).

Historical gene flow was roughly estimated using the formula $N_em = ¼ (1/\phi_{ST} - 1)$ (Wright 1951), based on ϕ statistics with the necessary, but possibly unrealistic, assumptions of demographic and genetic equilibrium, equal population size, random migration and absence of selection and mutation (Whitlock & McCauley 1999).

Simple comparisons of mean genetic diversity between different regions (ANOVA) and Pearson correlation tests were performed with SPSS 15.0 (SPSS Inc., Chicago, IL).

RESULTS

The nine primers used in the RAPD analysis of 358 plants from 31 populations of *Stipa capillata* yielded 99 DNA bands that could be scored. Among these, 82 bands (83%) were polymorphic and were included in the analysis. Their size ranged from 290 to 2200 bp. The total number of amplified fragments per primer varied between five (D 05) and 14 (N 16), and the percentage of polymorphic bands per primer ranged from 62.5% (D 05) to 100% (N 12 and N 16). We detected a total of 233 different RAPD phenotypes. In some populations, several (two to nine) individuals had the same RAPD phenotype. RAPD phenotypes shared between populations were only found in populations sampled in western Germany (Sü and GD, Fig. 2).

All measures of within-population diversity yielded low values (Table 1). Among the simple band-based methods, the proportion of polymorphic RAPD loci per population varied between 2.0% and 42.9%, and values for mean Sørensen distance among samples from a given population ranged from 0.005 to 0.088. These estimates correlated closely (Pearson r = 0.97) with values of average gene diversity (based on allele frequency), which ranged between 0.006 and 0.111 (Table 1). Mean diversity was lowest in the populations from western Germany (gene diversity 0.018/% polymorphic bands 10.3), intermediate in northeastern Germany and Switzerland (0.040/0.049 and 14.9/20.6%, respectively), and highest in central Germany and Slovakia (0.060/0.057 and 22.8/26.5%, respectively). These differences between regions were, however, not significant (ANOVA, P = 0.174 for gene diversity, and 0.165 for polymorphic loci). None of the populations had private bands, and even on the level of regions only Slovakia had bands that did not also occur in other areas. We found no relationship between genetic diversity and population size ($r < 0.1$, $P > 0.25$ for all measures) and sample size ($r < 0.30$, $P > 0.15$).

The first axis of the PCoA ordination explained 40.5%, and the first four axes accounted for 61.9% of the overall genetic variance. Specimens from the Slovakian populations were separated from the other populations along the first axis of the scatter plot (Fig. 3A). The populations from central and northeastern Germany and from Switzerland largely overlapped, while those from western Germany formed two subgroups that where either clearly separated from this central group or clustered close to it. Along the third axis, some samples from northeastern Germany were separated from the rest. In a closer inspection of only the German samples, the ordination explained 19.7% along the first axis and 52.7% summed over the first four axes (Fig. 3B). Here, the same patterns were found as before, and within the western German populations, populations Sü, GD and EF were separated from the Mainzer Sand (MS), and within northeastern German populations, Ge and Ga were separated.

The UPGMA dendrogram (Fig. 4) supported the ordination results and partly reflected geographic relationships. Samples from Slovakia formed an heterogeneous, but well supported group that was separated from the rest. One of the

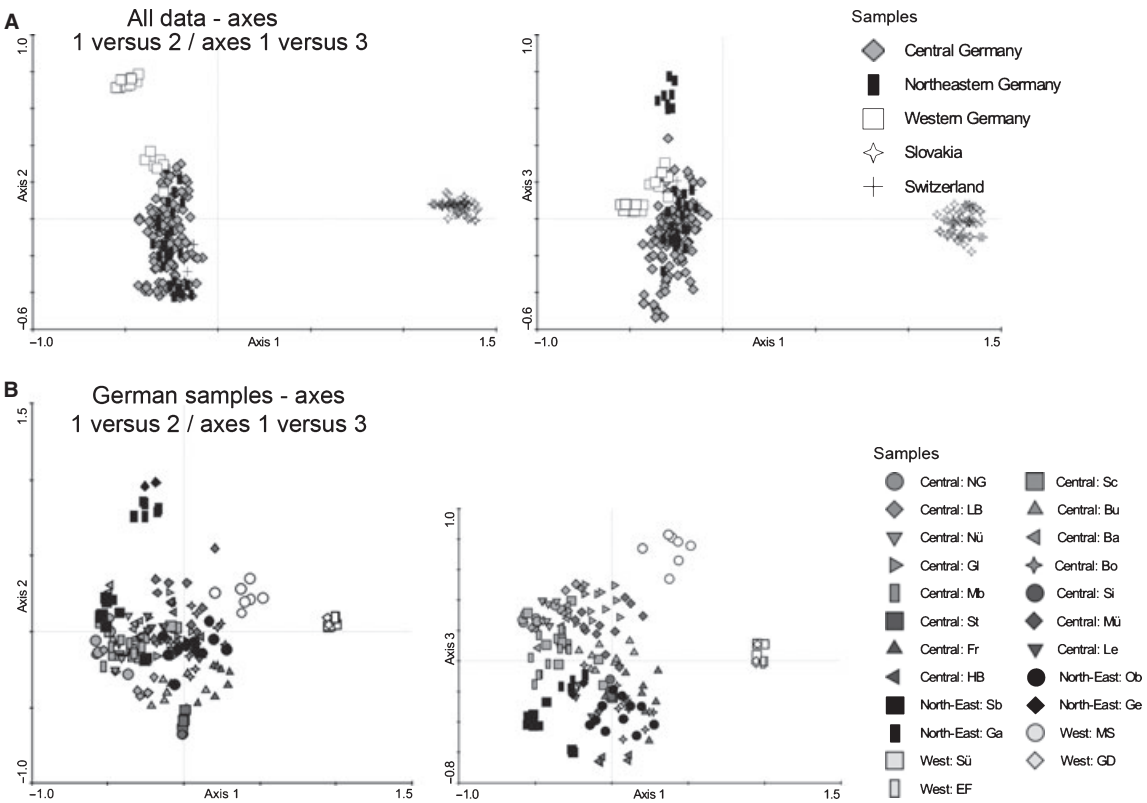

Fig. 3. Principal Coordinate Analysis (PCoA) of genetic data based on square-root transformed Sørensen dissimilarity given for axes 1 and 2 and 1 and 3, respectively. (A) All data, symbols indicate regions of origin (explained variances axis 1: 37.9%; axis 2: 14.3%; axis 3: 5.6%); (B) only German populations, the three main regions are indicated by different colours, symbols indicate populations (explained variances axis 1: 19.7%; axis 2: 13.9%; axis 3: 10.8%; for abbreviations refer to Table 1).

Swiss populations (Z) formed a distinct subgroup, while the other (S) clustered among a large group containing samples from central Germany. Groupings among the remaining populations were not very pronounced, except for population MS from western Germany, which clustered separately. Samples from central Germany formed one large subgroup with

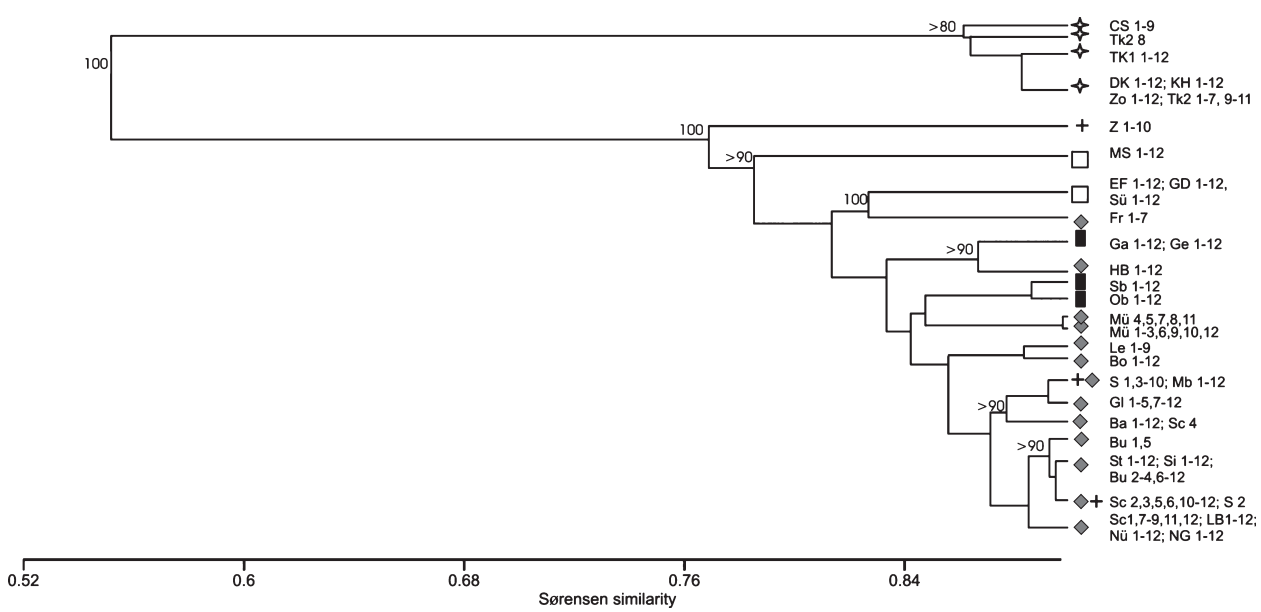

Fig. 4. Simplified UPGMA dendrogram indicating relationships between samples of individual plants originating from different regions. The dendrogram was pruned at a Sørensen similarity level of 0.9, bootstrap values for major groupings are only indicated if >70% (regions are coded with the same symbols used in Fig. 3A: ◆ central Germany, ■ northeastern Germany, □ western Germany, ✧Slovakia, + Switzerland).

Table 2. Results of the analysis of molecular variance (AMOVA) for A) 358 *Stipa capillata* individuals grouped into 31 populations and five regions; B) 290 *Stipa capillata* individuals grouped into 25 populations and four regions; C) 270 *Stipa capillata* individuals grouped into 23 populations and three regions; D) 174 *Stipa capillata* individuals grouped into 15 populations and six regions (P based on 1000 permutations).

	df	sum of squares	variance	% total	ϕ statistics	P
A) all populations						
among regions	4	2288.42	8.447	57.0	ϕ_{CT} = 0.57	<0.0001
among populations	26	1361.94	4.352	29.4	ϕ_{SC} = 0.68	<0.0001
within populations	327	660.42	2.020	13.6	–	–
total	351	4310.77	14.818	–	ϕ_{ST} = 0.86	<0.0001
B) populations in Germany and Switzerland						
among regions	3	598.55	2.57	28.0	ϕ_{CT} = 0.28	<0.0001
among populations	21	1185.93	4.69	50.1	ϕ_{SC} = 0.71	<0.0001
within populations	265	514.29	1.94	21.1	–	–
total	289	2298.77	9.20	–	ϕ_{ST} = 0.79	<0.0001
C) populations in Germany						
among regions	2	497.08	2.73	29.4	ϕ_{CT} = 0.29	<0.0001
among populations	20	1109.53	4.57	49.3	ϕ_{SC} = 0.70	<0.0001
within populations	247	486.29	1.97	21.3	–	–
total	269	2092.89	9.26	–	ϕ_{ST} = 0.79	<0.0001
D) populations in central Germany						
among regions	5	325.98	0.886	12.7	ϕ_{CT} = 0.13	<0.0001
among populations	9	411.76	3.720	53.1	ϕ_{SC} = 0.61	<0.0001
within populations	159	381.13	2.397	34.2	–	–
total	173	1118.86	7.002	–	ϕ_{ST} = 0.66	<0.0001

Ga, Ge, Ob and Sb from northeastern Germany, and the remaining western German populations EF, GD and Sü clustered with the central German population Fr as sister group. However, the latter groupings had no bootstrap support.

Organizing the populations into five groups corresponding to their geographic origins revealed that the highest molecular variance was found among regions (57.0%), while the variance within populations was 13.6% and among the populations within regions was 29.4% (Table 2A). When the populations from Slovakia were excluded, most variance was found among populations (around 50%, Table 2B, C, D). Within-population diversity was the lowest among the three levels considered in three out of four AMOVA analyses, with the exception being those containing only populations from central Germany (34%, Table 2D). The overall ϕ_{ST} value of 0.86 indicates extraordinarily high levels of genetic differentiation among populations, and is similar to the output of the Bayesian analysis (θ^B = 0.758, SD 0.004). In the reduced datasets, the overall ϕ_{ST} value decreased but remained at 0.66 for the central German populations. Pairwise ϕ_{ST} values between populations ranged from 0.149 (EF-Sü/western Germany; 0.4 km) to 0.984 (KH/Slovakia–Si/central Germany; 700 km). Genetic differentiation in the AMOVA ($P < 0.0001$; Table 2) as well as in all of the 465 pairwise genetic distances was highly significant, even after Bonferroni correction.

Genetic differentiation among populations (pairwise ϕ_{ST} values) was related to geographic distance: the matrix of 465 pairwise genetic distances among all 31 populations significantly correlated with the corresponding matrix of geographic distances according to a Mantel test ($r_M = 0.64$ ($P < 0.0001$; Fig. 5). Isolation-by-distance was also present at a smaller scale: the respective Mantel test still yielded a correlation coefficient of $r_M = 0.44$ when the Slovakian populations were excluded, an $r_M = 0.62$ when calculated for the German populations only, and an $r_M = 0.52$ when calculated for the 15 populations from central Germany (all: $P < 0.0001$).

DISCUSSION

The results of our study of *Stipa capillata* populations in Central Europe all indicated low within-population diversity, relatively high variability among regions and extraordinarily high genetic differentiation among populations that was significantly correlated with the geographical distance separating them.

At the population level, genetic diversity was mostly very low in *S. capillata*. This cannot be explained by clonal growth because this is a tussock grass with very limited capacity for spreading by stolons or runners (Hensen 1997). In addition, there was no relationship between within-population genetic diversity and population size. This result is in accordance

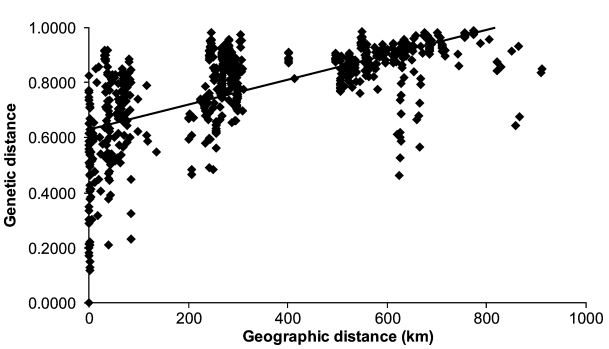

Fig. 5. Relationships between pairwise geographic and genetic distances (pairwise ϕ_{ST} values) among 31 populations of *Stipa capillata* in Central Europe.

with Honnay & Jacquemyn (2007), who found genetic diversity of self-compatible species to be less affected by decreasing population size than that of mainly outcrossing species. We found that 20.6% of RAPD bands were polymorphic at the population level, in line with published data for clonal *Calamagrostis* species (0–17.5%; Esselman *et al.* 1999; Schiebold *et al.* 2009). This is, however, clearly lower than data for other Poaceae taxa, such as *Stipa krylovii* Roshev. (41.4–61.2%; Zhao *et al.* 2006), *Stipa grandis* P. Smirn. (56.8–83.6%; Dan *et al.* 2006; Zhao *et al.* 2008), *Poa badensis* Haenke ex Willd. (63.2%; Hensen & Wesche 2007) or *Sesleria albicans* Kit. ex Schult. (29.7–56.7%; Reisch *et al.* 2002, 2003b). Equally low values based on iso- and allozyme variation have also been reported for several predominantly self-pollinating *Elymus* species (*e.g. Elymus caninus* (L.) L. 4.7%, Sun *et al.* 2001; *Elymus fibrosus* (Schrenk) Tzvelev 4.8%, Díaz *et al.* 2000; *Elymus canadensis* L. 9.2%, Sanders *et al.* 1979), which is remarkable as levels of variation are generally lower in allo- and isozyme studies compared with other types of marker. Furthermore, Schönswetter *et al.* (2006) found similar low genetic diversity values (between 0 and 0.053) for the sedge *Carex atrofusca* Schkuhr in the Alps, which they interpreted to be a consequence of founder events during colonization or due to subsequent reduction of population size in post-glacial times. The low genetic diversity found here within *S. capillata* populations in Central Europe implies that this species likely experienced strong bottlenecks in the past.

Our analysis of the genetic structure of *S. capillata* populations from Central Europe and from the Alps revealed extraordinarily high genetic differentiation among regions or, in the reduced datasets, among populations between regions. The ϕ_{ST} values calculated for *S. capillata* (*e.g.* 0.86 for all populations, 0.66 for those in central Germany only) are clearly higher than the mean ϕ_{ST} values of 0.38 calculated for monocotyledons, 0.25 for long-lived perennials, 0.42 for widespread species, and 0.27 for species with a mixed mating system, as presented by Nybom & Bartish (2000). These figures must be treated with caution as no phylogenetic corrections were applied. They nonetheless differ conspicuously from our estimate. Only the mean ϕ_{ST} value calculated for selfing species in this review is of a similar magnitude (0.7). The estimate of the Bayesian approach ($\theta^B = 0.758$) was similarly high. In fact, such extraordinarily high ϕ_{ST} values (or equivalents) have rarely been detected in grass species, although there are a few exceptions: G_{ST} values of between 0.55 and 0.95 were reported for selfing *Elymus* species (Knapp & Rice 1996; Díaz *et al.* 1999, 2000; Sun *et al.* 2001; Yan *et al.* 2007), and equally high genetic differentiation was found in the selfing *Melica transsilvanica* Schur in Poland ($\phi_{ST} > 0.86$; Szczepaniak & Cieslak 2007). For *S. krylovii* in China (Inner Mongolia), Zhao *et al.* (2006) report a G_{ST} value of 0.382. This species is closely related to *S. capillata* (Tzvelev 1976), and is also facultatively cleistogamous (K. Wesche, unpublished results). Populations of the equally closely related *S. grandis* in Inner Mongolia had an overall G_{ST} value of 0.199 (Dan *et al.* 2006), while other populations from the same region had an overall ϕ_{ST} value of 0.26 (Zhao *et al.* 2008). In both Inner Mongolian species, genetic structure and diversity was influenced by land use and/or climate (Dan *et al.* 2006; Zhao *et al.* 2006). Thus, the high ϕ_{ST} value found in our study appears to be atypical for *Stipa* species in particular, at least those that have been examined from this perspective.

In view of the ϕ_{ST} values reported for selfing species, patterns in *S. capillata* are likely to be the result of a high selfing rate in this species (Ponomarev 1961). In selfing species, genetic diversity is usually low at the population level (but see Michalski & Durka 2007) and, as gene flow between populations is reduced, a larger proportion of genetic diversity should be partitioned among the populations (Hamrick & Godt 1989; Honnay & Jacquemyn 2007). The values of N_em estimated using Wright's rule-of-thumb relationship between gene flow and ϕ_{ST} were only *ca*. 0.04 for the largest scale and *ca*. 0.13 for the smallest spatial scale, which indicates that genetic exchange among *S. capillata* populations, even within central Germany, must be extremely rare. Our estimate is much lower than that calculated for the wind-pollinated grass *Poa badensis* growing at similar sites (1.42; Hensen & Wesche 2007) and for insect-pollinated species collected in the same area and analysed using the same methodology (0.62, *Dictamnus albus* L. (Rutaceae); Hensen & Oberprieler 2005; 1.22, *Pulsatilla vulgaris* Mill. (Ranunculaceae); Hensen *et al.* 2005). This implies that seed dispersal in our study species has not been sufficiently effective to ensure genetic exchange among sampled populations. Experimental studies indeed point to a lack of pollen flow and a limited dispersal capacity of *S. capillata* caryopses (Hensen & Müller 1997), which leads to increasing genetic isolation with increasing geographic distance among study populations. Isolation-by-distance has also been reported for other grass species, *e.g.* by Bockelmann *et al.* (2003) for the salt marsh species *Elymus athericus* (Link) Kerguelen and by Kreivi *et al.* (2005) for *Arctophila fulva* var. *pendulina* (Laest.) A. and D. Löve endangered in Finland. In contrast, Reisch *et al.* (2002) found no correlation between spatial and genetic distances among populations of *Sesleria albicans*, and interpreted this finding as a lack of evidence for glacial relict endemism.

The differences in the RAPD phenotypes between Slovakia and the other study regions are surprising given that post-glacial expansion of many steppe species is thought to have occurred via Pannonia (Pott 1996) or the Balkans, which are among the main southern sources for the tree recolonization in Central Europe (Hewitt 2001). This scenario seems unlikely for *S. capillata* in view of the genetic structure detected here (Fig. 4). One of the populations from the Alps was nested within the central German populations, while the other was only weakly separated, suggesting a close relationship despite the relatively large geographic distance. This may indicate that the dry inner alpine valleys of the Swiss Alps were colonized by individuals from the lowlands, but final conclusions cannot be drawn without analysing material from other potential source regions, such as the French Rhone valley. The alternative hypothesis, that alpine populations acted as source areas for the colonization of central Germany, seems highly unlikely given the glacial history of the Alps. These were ice-covered during the last glacial maximum (Huijzer & Vandenberghe 1998; Carr *et al.* 2002) and all modern *S. capillata* populations occur in areas that were glaciated during the last glacial maximum. As the species avoids upper montane and alpine sites (Lauber & Wagner 1996), it cannot be expected to have survived at high nunatakers. The sand dune habitats of the Mainzer Sand (MS; west Germany,

Fig. 2) are also believed to be of young origin, as they were formed by drifting sand after the last ice age (Korneck & Pretscher 1984). Colonization of these sites could indeed have occurred via central Germany, as shown by the cluster dendrogram and previously suspected by Pott (1996). Founder effects and/or subsequent bottlenecks may have resulted in genetic depauperation, which is shown by the relatively low diversity levels of the westernmost populations (Table 1) and the separation of one population from that region (MS).

Implications

The extremely low levels of genetic variation found within populations of *S. capillata* in Central Europe have important implications for the conservation of this species. Low genetic diversity is likely to reduce fitness and to restrict a species ability to respond to changing environmental conditions through adaptation and selection (Booy *et al.* 2000; Honnay & Jacquemyn 2007). Further studies including populations from within the main distribution area in Middle Asia using molecular approaches should follow in order to elucidate the main post-glacial recolonization routes and to check whether the low genetic diversity and the assumed high selfing rate are in fact a result of the location of our study populations at the outmost periphery of the species distribution area.

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