American Journal of Botany

American Journal of Botany 98(7): 1173–1179. 2011.

Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass[1]

Viktoria Wagner[2,4], Walter Durka[3], and Isabell Hensen[2]

[2]Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, D-06108 Halle, Germany; and [3]UFZ-Helmholtz Centre for Environmental Research UFZ, Department of Community Ecology (BZF), Theodor-Lieser-Strasse 4, D-06120 Halle, Germany

- *Premise of the Study:* Intraspecific genetic variation is essential for the performance and evolution of species. Populations at a species' geographic range periphery receive considerable attention in biogeography and conservation because they are smaller and spatially more isolated than central populations, a pattern expected to lead to higher genetic differentiation and lower within-population genetic diversity. We tested these predictions in central and peripheral populations of the Eurasian steppe grass *Stipa capillata*.
- *Methods:* We analyzed AFLP fingerprint patterns in 319 individuals from 20 large and abundant populations in the core, in Kazakhstan, and 23 small and isolated populations at the periphery, in Central Europe. We calculated different genetic diversity estimates and assessed genetic differentiation among populations by examining F_{ST} values, a neighbor-net network, and an AMOVA.
- *Key Results:* As expected, genetic differentiation among populations was significantly larger at the range periphery (F_{ST} = 0.415) than in the range core (F_{ST} = 0.164). In contrast to predictions, however, we found similarly low genetic diversity within central (proportion of polymorphic bands = 21.9%) and peripheral (20%) populations.
- *Conclusions:* Higher genetic differentiation in the small and spatially isolated peripheral populations is likely driven by genetic drift and reduced gene flow due to a complex landscape structure and the abandonment of traditional management regimes. With regard to unchanged genetic diversity, it appears that life-history traits like longevity or sufficiently large population sizes could allow *S. capillata* to escape deleterious effects at the range edge.

Key words: abundant center hypothesis; AFLP; dry grassland; fragmentation; genetic differentiation; genetic diversity; Poaceae; range periphery; steppe; *Stipa*.

A commonly observed pattern in biogeography is that populations become less abundant toward a species' range periphery (Stott, 1981; Sagarin and Gaines, 2002). This phenomenon has led to the formulation of the "abundant center hypothesis" (Brown, 1984), which explains that less favorable environmental conditions cause smaller population sizes and stronger spatial isolation at the range edge (Stott, 1981; Brown, 1984). Genetic diversity is crucial for the performance of a species and forms a basis for its evolution (Blows and Hoffmann, 2005; Leimu et al., 2006). Population genetic theories as well as empirical studies have suggested that small population size can create a loss of genetic diversity and fixation of alleles due to genetic drift and inbreeding (Ellstrand and Elam, 1993). These deleterious effects can drive peripheral populations close to extinction and make them a concern for conservation (Lesica and Allendorf, 1995). In addition, spatial isolation and genetic drift can contribute to a higher genetic differentiation among populations (Templeton et al., 1990). There is, therefore, a general consensus that the genetic consequences of the abundant center model are two-fold: lower genetic diversity within and higher genetic differentiation among peripheral populations compared with central populations (Eckert et al., 2008).

Despite these intuitive predictions, surprisingly many studies have failed to detect the expected patterns when comparing central and peripheral populations (Eckert et al., 2008). Some of these studies may have misleadingly chosen populations that did not have an abundant center distribution to begin with (Eckert et al., 2008; Yakimowski and Eckert, 2008). However, studies examining a true abundant center distribution and still not detecting the predicted patterns could point to the importance of additional factors that are strong enough to override basic mechanisms. Among these, biological properties are reasonable candidates (Loveless and Hamrick, 1984; Schiemann et al., 2000): longevity, outcrossing, and the ability for long distance gene flow might counteract a loss of genetic diversity and decrease genetic differentiation in isolated populations at the range periphery. In addition, historical mechanisms could play a decisive role at range edges, for example, in the case of slow colonization and extinction rates or long-distance dispersal events in the past (Schiemann et al., 2000; Gaston, 2009).

We tested the genetic predictions of the abundant center hypothesis using the steppe grass *Stipa capillata* L. as a model species. This species is known to follow an abundant center

[1]Manuscript received 29 September 2010; revision accepted 6 May 2011.

We are grateful to numerous people and institutions who were an enormous help during field work, laboratory analysis, and writing of the manuscript, especially T. Bragina, A. Fedulin, Kokchetau National Park, the Ministry of Agriculture of Kazakhstan, Naurzum Nature Reserve, and T. Sidorova (all Kazakhstan); and J. Danihelka, C. Eichberg, I. Geier, M. Hermann, H. Hirsch, C. Kilian, K. Micieta, B. Müller, M. Partzsch, A. Schwabe-Kratochwil, T. Spribille, E. Welk, K. Wesche, and W. Willner (all Europe). We thank three anonymous reviewers for their comments.

[4]Author for correspondence (e-mail: viktoria.wagner@botanik.uni-halle.de)

doi:10.3732/ajb.1000385

distribution in Asia and Europe (Fig. 1) . It is one of the most common plants in the vast dry grasslands of continental Asia (Lavrenko, 1970) but is so rare at its northwestern edge in Central Europe that it is red-listed in all respective countries (e.g., Ludwig and Schnittler, 1996). In examining *S. capillata* populations in Central Europe and Kazakhstan, we asked whether there are differences in (1) genetic differentiation and (2) genetic diversity among peripheral and central populations. On one hand, we expected genetic differentiation to be higher and genetic diversity to be lower at the range edge. On the other hand, life-history traits of the species, in particular its longevity, could have had the potential to distort these patterns.

MATERIALS AND METHODS

Study species—*Stipa capillata* L. (Poaceae, *Stipa* sect. *Leiostipa* Dum., according to Tsvelev, 1976) is a perennial and tetraploid grass (2n = 44, e.g., Skalińska et al., 1968). Tetraploid *Stipa* species are considered to be allopolyploid (Johnson, 1945; Tsvelev, 1977). The grass grows in a caespitose form (i.e., its tussocks are distinct genets). Its inflorescence bears one flower per floret with a characteristic long and naked awn. The breeding system is facultative cleistogamy (Ponomarev, 1961), but the self-fertilization rate is unknown. Flowering begins in late June to early August. Fruits ripen in August to September and are dispersed in animal fur or by wind.

The species' central range extends from eastern Romania to eastern Kazakhstan, and disjunct peripheral populations can be found as far west as Spain and as far east, north, and south as Yakutia, Siberia, and the deserts of Central Asia (Fig. 1). Whereas steppes in the range core are natural vegetation, dry grassland fragments in Central Europe depend mostly on an anthropogenic regime of mowing and grazing to prevent succession by mesophytic plants and shrubs (Ellenberg, 1996). Steppes in continental Eurasia date probably back to the Pliocene (Frenzel, 1968). By contrast, European dry grasslands were widely distributed in the warm climate shortly after the Pleistocene, but as the climate became more humid they were mostly replaced by forests and increasingly confined to small patches (Hensen, 1995; Ellenberg, 1996). Deforestation during the Middle Ages allowed steppe fragments to expand again, but the abandonment of traditional forms of grazing and mowing put steppe grasslands and their species under a new threat of extinction (Ellenberg, 1996). *Stipa capillata* is one of the many steppe species that is currently red-listed in the majority of the European countries (e.g., Ludwig and Schnittler, 1996).

Study sites—We collected fresh leaf material in 15 peripheral populations in Central Europe and 20 populations in Kazakhstan in 2007 and dried it in silica gel (Fig. 1, Table 1). In addition, we included DNA extractions from eight European populations studied by Hensen et al. (2010), resulting in a total of 23 peripheral populations. We defined populations as an entity of individuals that were at least 1 km apart from the next entity or, if less, isolated by forests or settlements. We estimated population size in the field as small (<1000 individuals), medium (1000–50 000), large (50 001–200 000), or very large (>200 000). European populations encompassed small to large population sizes and were clearly delimited by forests, agricultural fields, and settlements. In Asia, *S. capillata* formed extensive populations that were spread over the vast areas of steppe and forest steppe, with few settlements and agricultural fields in between. Thus, population sizes were rated uniformly as very large. Voucher specimens are deposited at HAL (Viktoria Wagner, Nr. V 1151–1189). We chose a sampling area of 50 × 50 m within populations. This sampling design might underestimate total genetic diversity in large populations, but it allows a comparison across the two regions on a similar spatial basis. Because we tried to capture among- and within-population diversity equally, our number of samples within populations (5–8, mean = 7) was sacrificed to the high number of populations (n = 43; for a similar strategy, see Prinz et al., 2009). In both study regions, populations were sampled at a similar spatial scale, the distance between the studied populations ranging between 1.6 and 808 km in Europe and between 4.1 and 485 km in Kazakhstan. The total sample size comprised 319 samples, 150 samples from Kazakhstan and 169 samples from Europe.

DNA extraction, AFLP analysis, and genotyping—We used amplified fragment length polymorphism (AFLP) markers for our study to create dominant

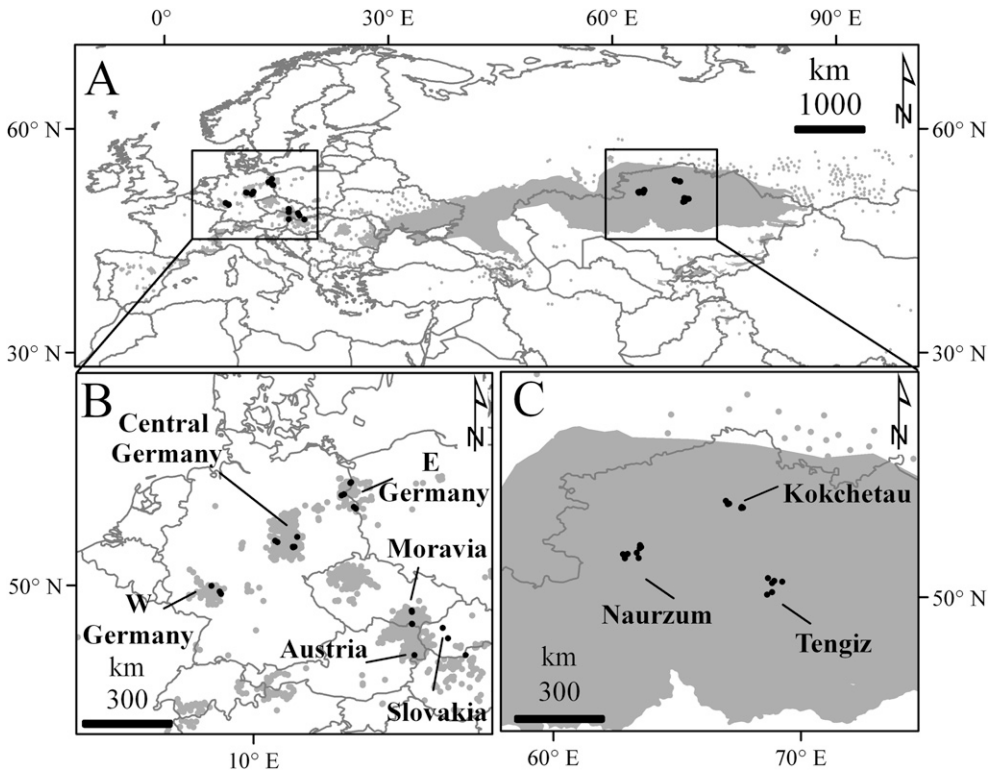

Fig. 1. (A) Distribution map of *Stipa capillata* based on literature, online data, and herbarium specimens. The core of the range is shown as a gray shaded area, whereas peripheral populations are presented as gray dots. Study populations (B) at the periphery and (C) in the core are indicated as black dots.

TABLE 1. Overview of the studied *Stipa capillata* populations. Population size: small: <1000 individuals, medium: 1000–50 000, large: 50 001–200 000, and very large: >200 000. *n*: number of individuals. Estimates of genetic diversity per population: BCD: mean Bray Curtis dissimilarity, PPB%: proportion of polymorphic bands, H_E: gene diversity, PrB: number of private bands, NLCB: number of bands that occurred in less than 25% of the data set.

Code	Country/subregion	°N	°E	Population size	*n*	BCD	PPB%	H_E	PrB	NLCB
1. Peripheral populations										
AU1	Austria	47.841	16.643	Small	7	0.108	16.3	0.071	0	7
CZ2	Czech Republic	48.819	16.640	Medium	7	0.133	20.8	0.090	0	9
CZ3		48.806	16.647	Medium	7	0.139	22.9	0.094	2	12
CZ4		49.190	16.673	Medium	8	0.101	16.0	0.063	0	5
CZ5		49.226	16.664	Small	8	0.068	12.5	0.044	0	6
SLO_KNH	Slovakia	47.829	18.752	Large	7	0.071	14.6	0.051	0	7
SLO_TK		48.679	17.908	Large	6	0.135	22.2	0.097	1	11
SLO_ZN		48.350	18.094	Medium	8	0.138	26.0	0.096	1	19
D_DAR1	Western Germany	49.843	8.567	Large	5	0.145	21.2	0.095	9	20
D_DAR2		49.774	8.626	Medium	8	0.100	19.8	0.070	2	16
D_MS		50.015	8.209	Medium	7	0.072	11.8	0.050	0	7
D_FRY1	Central Germany	51.233	11.784	Medium	8	0.103	18.4	0.072	0	7
D_FRY2		51.228	11.711	Medium	7	0.174	29.2	0.115	1	14
D_FRY3		51.214	11.722	Medium	8	0.165	30.2	0.119	4	19
D_HAL1		51.543	11.917	Small	8	0.147	24.7	0.100	1	16
D_KYF1		51.420	10.947	Small	8	0.129	20.5	0.083	1	8
D_KYF2		51.377	11.044	Medium	7	0.132	22.9	0.086	1	11
D_ODE1	Eastern Germany	52.413	14.536	Large	7	0.153	25.0	0.105	2	15
D_ODE2		52.472	14.463	Medium	7	0.129	22.6	0.087	0	16
D_OB		52.866	14.043	Small	8	0.088	16.0	0.063	0	12
D_STR		52.828	13.936	Small	8	0.066	13.2	0.049	0	6
D_GA		53.215	14.360	Small	8	0.040	8.7	0.029	0	9
D_GE		53.240	14.388	Small	7	0.133	24.7	0.100	1	17
				Peripheral region, mean:		0.116	20.0	0.079	1.1	11.7
				Peripheral region, variance:		0.001	31.6	0.001	3.9	22.9
2. Central populations										
KZ_KOK1	Kazakhstan, Kokchetau region	52.945	69.038	Very large	8	0.108	19.1	0.075	0	6
KZ_KOK2		52.911	69.010	Very large	8	0.116	21.9	0.081	0	8
KZ_KOK3		52.918	69.080	Very large	8	0.103	18.8	0.071	0	6
KZ_KOK4		53.141	68.365	Very large	6	0.102	17.0	0.069	1	6
KZ_KOK5		53.039	68.429	Very large	6	0.107	17.7	0.074	0	10
KZ_KOK6		53.053	68.493	Very large	8	0.132	22.9	0.094	0	10
KZ_NAU1	Kazakhstan, Naurzum region	51.560	64.099	Very large	8	0.164	27.4	0.109	0	8
KZ_NAU2		51.390	64.154	Very large	7	0.122	22.9	0.082	1	7
KZ_NAU3		51.392	63.549	Very large	8	0.136	22.9	0.091	1	6
KZ_NAU4		51.529	63.692	Very large	8	0.094	17.7	0.065	0	7
KZ_NAU5		51.797	64.278	Very large	8	0.121	20.5	0.080	0	4
KZ_NAU6		51.734	64.318	Very large	8	0.112	18.4	0.073	0	1
KZ_NAU7		51.705	64.248	Very large	8	0.127	21.9	0.086	0	5
KZ_NAU8		51.518	63.484	Very large	8	0.106	20.1	0.073	0	6
KZ_TEN1	Kazakhstan, Tengiz region	50.541	69.801	Very large	8	0.129	26.7	0.093	0	10
KZ_TEN2		50.192	69.503	Very large	8	0.132	22.9	0.090	0	12
KZ_TEN3		50.269	69.734	Very large	7	0.122	21.9	0.086	0	9
KZ_TEN4		50.580	70.251	Very large	7	0.138	27.4	0.102	2	20
KZ_TEN5		50.696	69.657	Very large	6	0.147	24.0	0.102	0	9
KZ_TEN6		50.612	69.912	Very large	7	0.136	26.0	0.094	2	12
				Central region, mean:		0.123	21.9	0.085	0.4	8.1
				Central region, variance:		0.0003	10.7	0.0002	0.5	15.0
				Total, mean:		0.119	20.9	0.082	0.8	10.0

fingerprints across the cellular genome (Vos et al., 1995). Genomic DNA was extracted with the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) using 20 mg of dried leaf material. Restriction and ligation was performed for 12 h at 20°C in an 11-μL reaction volume using 6 μL DNA extract, 0.55 μL BSA (1 mg/mL), 1.1 μL 10 × T4 DNA ligase buffer, 1.1 μL NaCl-solution (0.5 M), 0.1 μL each of the enzymes EcoR I (100 000 u/mL) and Mse I (10 000 u/mL), 0.05 μL T4 DNA ligase (2 000 000 u/mL), 1 μL each of Mse I adapter (50 pmol/μL) and EcoR I adapter (5 pmol/μL). Preselective amplification was carried out in a 21-μL reaction volume, using 4 μL of the restriction/ligation product, 1 μL of each EcoR I and Mse I preselective primers (30 ng/μL each), 2 μL 10 × dNTPs (2 mM), 2 μL 10 × PCR buffer with $(NH_4)_2SO_4$, 1.2 μL $MgCl_2$ solution (25 mM), 0.16 μL Taq polymerase (5 u/μL) and 9.64 μL H_20. The reaction was run within a cycler program of 2 min at 72°C, 20 cycles of: 20 s 94°C, 30 s 56°C, 2 min 72°C, and a step of 30 min at 60°. Selective amplification was performed by using 1 μL preselective amplification product (1:5 dilution), 0.6 μL of a combination of EcoR I (5 ng/μL) and Mse I (30 ng/μL) primers, and 5μL AFLP amplification core mix (Applied Biosystems, Foster City, California, USA). We used four primer combinations: FAM-AAC/CTA, VIC-ACA/CAG, NED-AAG/CAC, and PET-AGG/CAT starting with 2 min at 94°C, and 10 cycles of: 20 s at 94°C, 30 s at 66°C, 2 min at 72°C, followed by 20 cycles of: 20 s 94°C, 30 s 56°C, 2 min at 72°C, and finally 30 min at 65°C. A solution of 2 μL selective amplification products, 14.7 μL deionized formamide, and 0.3 μL Gene Scan 500 LIZ size standard was denaturated for 5 min at 95°C, cooled on ice, and analyzed by automatic electrophoresis in the ABI Prism 3130xl Genetic Sequencer (Applied Biosystems). We scored peaks in GeneMapper version 3.7 (Applied Biosystems) ranging in length between 50 and 500 base pairs and exported data on peak height of all scored fragments >25 relative fluorescence units (rfu). To exclude nonreliable bands, we set

individual thresholds for peaks at 40 or 50 rfu, discarded those with mostly low-intensity peaks (0–100 rfu), and eliminated monomorphic ones. The four primer pairs thus yielded 288 polymorphic bands (92% of total fragments). We transformed the data into a 1/0 matrix for further analysis. Reproducibility of data were assessed by running 10 replicate samples from extraction to electrophoresis and calculating the ratio of observed phenotypic differences between original and repeated samples divided by the total number of comparisons (Bonin at al., 2004). The overall error rate was 0.45% for 2880 compared AFLP-phenotypes.

Genetic data analysis—Because our study species was polyploid, it was not possible to unambiguously estimate allele frequencies. Instead, we employed a conservative approach to analyze our AFLP data: we used both the band-based and fragment-frequency-based approaches throughout our analysis (Bonin et al., 2007). In the latter approach, allele frequency was set equal to fragment frequency.

Genetic differentiation—Genetic differentiation among populations was analyzed by pairwise Nei's genetic distances calculated in the program AFLP-SURV (Vekemans, 2002) and visualized in a neighbor-net network using SPLITSTREE (Huson and Bryant, 2006). In these networks, ambiguities of tree topology are represented by a network structure rather than by bootstrap values. To quantify the distribution of genetic variation at different hierarchical levels, we used analysis of molecular variance (AMOVA) in GENALEX (Peakall and Smouse, 2006), treating data as binary diploid and employing a Euclidean distance measure as defined by Huff et al. (1993). Significance of the variance components was based on 999 permutations generated in GENALEX. Genetic differentiation was also assessed by inspecting F_{ST} distances calculated in AFLP-SURV. We used permutation tests ($n = 5000$) to compare mean and variance of F_{ST} values between the two regions. In the case of the mean, a P value was calculated as the proportion of randomized runs in which simulated t values exceeded or were equal to observed ones (t values were obtained by Welch's approximation to account for unequal variance between regions), whereas in the case of the variance, a P value was determined as the proportion of randomized runs in which the region was found to yield a higher or equal variance than the other region. We tested for an isolation-by-distance relationship between pairwise linearized F_{ST} distance ($F_{ST,Lin} = F_{ST}/[1 - F_{ST}]$; Rousset, 1997) and geographic distance with a Mantel test using the package Vegan (Oksanen et al., 2010) in the program R (R Development Core Team, 2010). The slope and intercept of the regression were compared with a permutation test, in which the P value was calculated as the proportion of randomized runs that had a higher or equal difference in slope or intercept than the observed runs.

Genetic diversity—We calculated the number of bands confined to the range core and edge regions using the program FAMD (Schlüter and Harris, 2006). Genetic diversity at the population level was assessed as proportion of polymorphic bands (PPB) and, to facilitate comparisons with other studies, as Nei's gene diversity (H_E) in AFLP-SURV. Bray-Curtis dissimilarity (BCD) among individuals (equivalent to Nei and Li's genetic distance) was determined using the Vegan package in R. Additionally, the number of private bands per population (PrB) and the number of less common bands (NLCB, defined as bands occurring in <25% of the data set) were calculated in GENALEX. Because our samples were spatially nested and thus nonindependent (Fig. 1), we compared diversity values between regions (central vs. peripheral), among subregions (see Table 1), and among populations with variance components analysis using linear mixed-effects models (LMM), treating spatial levels as random factors, with subregions nested in regions (Crawley, 2007). Populations from Austria, the Czech Republic, and Slovakia were combined into one subregion for the analysis because of their spatial proximity. To meet assumptions of normality, NLCB was first calculated as proportion data and then arcsine–square root transformed. We used the lmer models with a Gaussian distribution family and the Restricted Maximum Likelihood Estimator in the lme4 package of R (Bates and Maechler, 2010). Significance of random factors for our LMMs was assessed by a likelihood ratio test as described by Zuur et al. (2009). Because the number of private bands followed a zero-inflated distribution, we analyzed this variable using a two-step hurdle approach with generalized mixed-effects models (GLMM; Zuur et al., 2009; Seifert et al., 2010) using the glmmPQL command and the penalized quasi-likelihood estimator in the MASS package (Venables and Ripley, 2002). Likelihood ratio tests are not possible when using this estimator because only quasi-likelihoods are calculated (Bolker et al., 2009).

RESULTS

Genetic differentiation—The neighbor-net network showed a distinct clustering of the two study regions (Fig. 2). Populations from Kazakhstan formed a tight cluster with short branches indicating high genetic similarity among them whereas populations from Europe had much longer branches pointing to higher genetic differentiation. When peripheral and central populations were combined in a single data set, the AMOVA showed that 61.0% of the genetic variation was found within populations and 26.6% among populations (Table 2). However, when the two study regions were analyzed separately, the core showed a higher genetic variation within (85.1%) than among (14.9%; AMOVA $\Phi_{ST} = 0.149$) populations. In comparison, the peripheral region had less variation within populations (61.4%) but more variation among populations (38.6%, $\Phi_{ST} = 0.386$). In both regions, genetic distance was positively correlated with geographic distance (Fig. 3). Pairwise F_{ST} values among peripheral populations were, on average, significantly larger (mean: 0.415 vs. 0.164, $P < 0.001$) and significantly more variable (range: 0.108–0.733 vs. 0–0.345; variance: 0.017 vs. 0.005; $P < 0.001$) than among central populations. In the linear regressions between linearized F_{ST} and geographic distance, there was a significant difference in the intercept (0.568 vs. 0.118, $P < 0.001$) but not in the slope ($0.661 \cdot 10^{-3}$ *vs.* $0.329 \cdot 10^{-3}$, $P = 0.196$) between the periphery and the core.

Genetic diversity—When the central and peripheral regions were compared, 207 (72%) of all polymorphic bands were shared between core and edge, 17 (6%) were found only in the core, and 63 (22%) only at the periphery. The number of bands occurring in either the range core or periphery, but in more than one population, was 10 (3%) and 37 (13%), respectively. At the population level, variance components analysis showed that most of the variation in all calculated genetic diversity parameters was not found among regions but among populations or subregions within regions (Table 3). Thus, central and peripheral populations did not differ in genetic diversity. We found no effect of population size on genetic diversity among European populations (analysis of variance permutation test, df = 2 and 20, $P > 0.05$ in all cases).

DISCUSSION

The maintenance of genetic diversity is a central aim of conservation, given its positive role for a species' performance and evolution (Reed and Frankham, 2003). It is assumed that genetic variation is not uniformly distributed across its geographic range (Eckert et al., 2008). Populations at the range periphery are expected to be genetically more differentiated because of strong spatial isolation and enhanced genetic drift. Our results confirmed this hypothesis and showed that peripheral populations of *Stipa capillata* in Central Europe are genetically more differentiated (AMOVA $\Phi_{ST} = 0.386$) than central populations in Kazakhstan ($\Phi_{ST} = 0.149$). This finding is in line with Eckert et al.'s (2008) finding that 70% of all reviewed studies showed a stronger genetic isolation at the range edge. Such a pattern was documented, for example, for *Cirsium acaule* (Jump et al., 2003), *Corylus avellana* (Persson et al., 2004), *Neotinea maculata* (Duffy et al., 2009), and *Viola* species (Eckstein et al., 2006). Contrary to our expectations, however, we did not find any difference in mean genetic diversity between central and

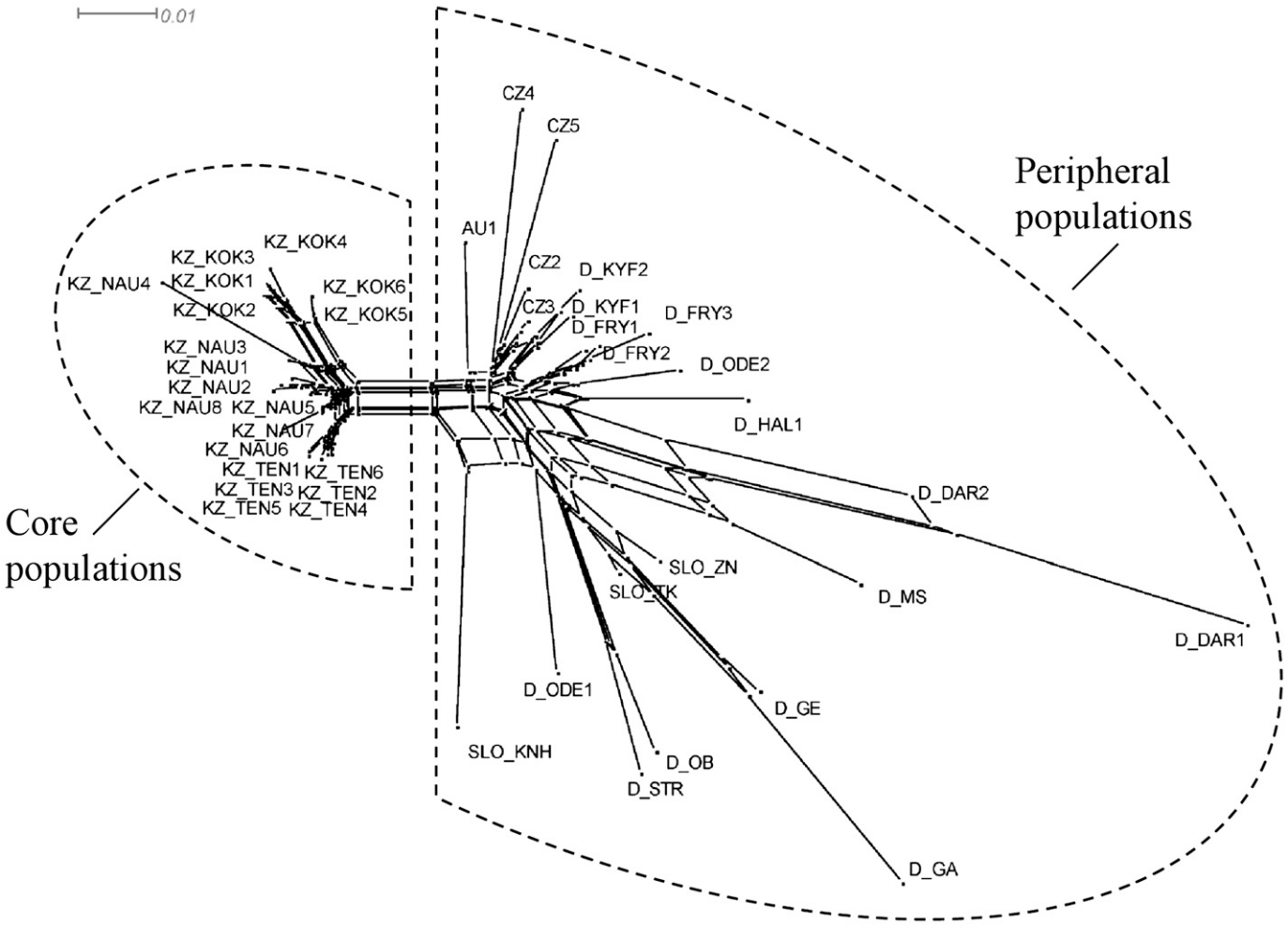

Fig. 2. Neighbor-net network based on Nei's pairwise genetic distance among *Stipa capillata* populations.

peripheral populations of *S. capillata.* Eckert et al. (2008) showed that roughly a third of all reviewed studies similarly failed to detect the expected decline in genetic diversity at the range edge, as exemplified by results on *Atriplex tatarica* (Mandák et al., 2005), *Lathyrus vernus* (Schiemann et al., 2000), and *Uniola paniculata* (Franks et al., 2004).

TABLE 2. Analysis of molecular variance for the total data set and each of the two studied regions, respectively. *** $P < 0.001$; ** $0.001 < P < 0.01$.

	df	SS	MS	Est. Var.	%
Total data set					
Between regions	1	497.3	497.3	2.8	13.4***
Among populations	41	2149.8	52.4	5.3	26.6***
Within populations	276	3528.5	12.8	12.8	61.1***
Total	318	6175.6		20.9	100.0
Peripheral populations, only					
Among populations	22	1593.0	72.4	8.1	38.6***
Within populations	146	1880.1	12.9	12.9	61.4***
Total	168	3473.0		21.0	100.0
Central populations, only					
Among populations	19	556.8	29.3	2.2	14.9**
Within populations	130	1648.4	12.7	12.7	85.1***
Total	149	2205.2		14.9	100.0

The high genetic differentiation among peripheral populations of *S. capillata* is not unusual for a mixed-mating species (Loveless and Hamrick, 1984) and is in the range of what other AFLP studies have reported for mixed-mating nonclonal grasses (Φ_{ST} = 0.446, $n = 6$; Appendix S1: see Supplemental Data online at http://www.amjbot.org/content/98/7/1173/suppl/DC1). By contrast, central populations had much lower genetic differentiation than could be expected for a mixed-mating grass (Appendix S1). In fact, central populations had the lowest genetic differentiation among all nonclonal grasses. The breeding system is an essential factor shaping genetic diversity (Loveless and Hamrick, 1984). In that respect, the low diversity in *S. capillata* (PPB = 21%, H_E = 0.082) was in agreement with its facultative selfing pollination and even more similar to genetic diversity in fully self pollinating species (PPB = 26%, $n = 6$; H_E = 0.072, $n = 3$) than in outcrossing and mixed mating species (PPB = 60%, $n = 13$, H_E = 0.180, $n = 6$; Appendix S1). Low genetic diversity in European *S. capillata* populations was also detected by two previous RAPD studies (Krzakowa and Michalak, 2007; Hensen et al., 2010). However, absolute genetic diversity estimates in our study species should be interpreted with some caution, given that the low within-population sample size could have led to imprecise and underestimated diversity values.

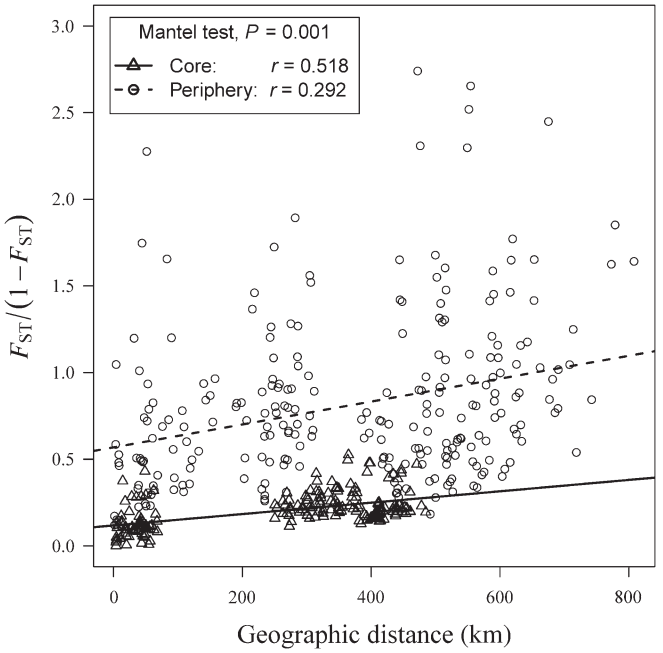

Fig. 3. Relationship between pairwise linearized F_{ST} distance and geographic distance in central and peripheral populations of *Stipa capillata*.

Apart from the breeding system, high genetic differentiation among peripheral populations can be best explained by the strong spatial isolation of populations in Central Europe. This process was most likely accelerated by the complex landscape structure in this region, in which forests, roads, and dense settlements form barriers for pollen and seed flow. With the abandonment of traditional pastoralism in the past century, seed dispersal can be expected to have declined even further (Picó and Groenendael, 2007; Ozinga et al., 2009). The large variance of pairwise linearized F_{ST} values among peripheral populations indicates that genetic drift is also contributing to differentiation (Hutchison and Templeton, 1999; Eckstein et al., 2006). Theoretically, divergence at the periphery could be attributed to different extinction/colonization dynamics in the past (Hewitt, 1999) and to a more heterogeneous environment, but this possibility needs to be evaluated by more detailed studies. In the core of the distribution range, low genetic differentiation is likely the result of large population sizes and extensive gene flow that counteract divergence through selfing and genetic drift. Unlike in Europe, the open landscape structure with steppe vegetation and the past nomadic activities of Kazakhs have likely enabled a continuous exchange of pollen and seeds.

Table 3. Variance components analysis using linear mixed-effects (LMM) or generalized linear mixed-effects (GLMM) models to compare variation of genetic diversity at the regional and subregional scales. BCD: mean Bray Curtis dissimilarity; H_E, gene diversity; NLCB, number of bands that occurred in less than 25% of the data set; PPB, proportion of polymorphic bands; PrB, number of private bands. Residual variation represents variation among populations. Subregions and regions were treated as random factors with subregions nested within regions. Asterisks indicate whether the predictors significantly improved the model (* $0.01 < P < 0.05$; no asterisk: $0.1 < P < 1$). Note that P values for spatial levels cannot be computed using the likelihood ratio test in GLMM models.

Variable	Region	Subregion	Residuals
BCD (LMM)	0	11.4	88.6
PPB (LMM)	$1.3 \cdot 10^{-17}$	19.9	80.1
H_E (LMM)	0	9.4	90.6
NLCB (LMM)	18.6	20.3*	61.1
PrB (binomial GLMM)	27.3	$0.2 \cdot 10^{-1}$	72.7
PrB (zero-truncated quasi-Poisson GLMM)	$0.1 \cdot 10^{-2}$	0.01	99.9

Although peripheral populations were smaller and spatially more isolated, they did not show lower genetic diversity than central populations. This unexpected pattern could be due to still sufficiently large population sizes that have counteracted random loss of genetic diversity in the past (Yakimowski and Eckert, 2008). In fact, most peripheral populations had census sizes of >1000 individuals, which reduced the risk of genetic drift. The longevity of the species is certainly another important factor in maintaining genetic diversity. In long-lived species, overlapping generations and reduced temporal variation of population size help to maintain effective population size and reduce the effect of drift (Ellstrand and Elam, 1993; Nybom and Bartish, 2000).

In conclusion, our study confirmed the expectation that populations at the range periphery are genetically more isolated than populations in the range core. This pattern can be best explained by the differences in spatial isolation, landscape structure, and land use between the range core and edge. However, the unexpected lack of any differences in genetic diversity between the two regions points to the possibility that longevity and sufficiently large population sizes can reduce negative effects of genetic drift and isolation in peripheral populations.

LITERATURE CITED

Bates, D., and M. Maechler. 2010. Lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-37. Available at http://CRAN.R-project.org/package=lme4.

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86: 1371–1384.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24: 127–135.

Bonin, A., E. Bellemain, P. Bronken Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetic studies. *Molecular Ecology* 13: 3261–3273.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* 16: 3737–3758.

Brown, J. H. 1984. On the relationship between abundance and distribution of species. *American Naturalist* 124: 255–279.

Crawley, M. J. 2007. The R Book. Wiley, Chichester, United Kingdom.

Duffy, K. J., G. Scopece, S. Cozzolino, M. F. Fay, R. J. Smith, and J. C. Stout. 2009. Ecology and genetic diversity of the dense-flowered orchid, *Neotinea maculata*, at the centre and edge of its range. *Annals of Botany* 104: 507–516.

Eckert, C. G., K. E. Samis, and S. C. Lougheed. 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. *Molecular Ecology* 17: 1170–1188.

Eckstein, R. L., R. A. O'Neill, J. Danihelka, A. Otte, and W. Köhler. 2006. Genetic structure among and within peripheral and central populations of three endangered floodplain violets. *Molecular Ecology* 15: 2367–2379.

ELLENBERG, H. 1996. Vegetation Mitteleuropas mit den Alpen: in ökologischer, dynamischer und historischer Sicht. Ulmer, Stuttgart, Germany.

ELLSTRAND, N. C., AND D. R. ELAM. 1993. Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.

FRANKS, S. J., C. L. RICHARDS, E. GONZALES, J. E. COUSINS, AND J. L. HAMRICK. 2004. Multi-scale genetic analysis of *Uniola paniculata* (Poaceae): A coastal species with a linear, fragmented distribution. *American Journal of Botany* 91: 1345–1351.

FRENZEL, B. 1968. The pleistocene vegetation of northern Eurasia. *Science* 161: 637–649.

GASTON, K. J. 2009. Geographic range limits: Achieving synthesis. *Proceedings of the Royal Society of London, Series B* 276: 1395–1406.

HENSEN, I. 1995. Die kontinentalen *Stipa*-Steppenrasen der mittel- und nordost-deutschen Trockengebiete. *Gleditschia* 23: 3–24.

HENSEN, I., C. KILIAN, V. WAGNER, W. DURKA, J. PUSCH, AND K. WESCHE. 2010. Low genetic variability and strong differentiation among isolated populations of the rare steppe grass *Stipa capillata* L. in central Europe. *Plant Biology* 12: 526–536.

HEWITT, G. M. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87–112.

HUFF, D. R., R. PEAKALL, AND P. E. SMOUSE. 1993. RAPD variation within and among natural populations of outcrossing buffalograss. [*Buchloë dactyloides* (Nutt.) Engelm.] *Theoretical and Applied Genetics* 86: 927–934.

HUSON, D. H., AND D. BRYANT. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.

HUTCHISON, D. W., AND A. R. TEMPLETON. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53: 1898–1914.

JOHNSON, B. L. 1945. Cyto-taxonomic studies in *Oryzopsis. Botanical Gazette (Chicago, Illinois)* 107: 1–32.

JUMP, A. S., F. I. WOODWARD, AND T. BURKE. 2003. *Cirsium* species show disparity in patterns of genetic variation at their range-edge, despite similar patterns of reproduction and isolation. *New Phytologist* 160: 359–370.

KRZAKOWA, M., AND M. MICHALAK. 2007. Genetic variability of selected marginal populations of *Stipa capillata* L. *Biological Letters* 44: 127–135.

LAVRENKO, E. M. 1970. Provinzial'noe razdelenie prichernomorsko—kazakhstanskoy podoblasti stepnoy oblasti evrazii. (The division of the Black Sea—Kazakhstan subregion of the Eurasian steppe region). *Botanicheskii Zhurnal* 55: 609–625.

LEIMU, R., P. MUTIKAINEN, J. KORICHEVA, AND M. FISCHER. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94: 942–952.

LESICA, P., AND F. W. ALLENDORF. 1995. When are peripheral populations valuable for conservation? *Conservation Biology* 9: 753–760.

LOVELESS, M. D., AND J. L. HAMRICK. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

LUDWIG, G., AND M. SCHNITTLER. 1996. Rote Liste gefährdeter Pflanzen Deutschlands. Schriftenreihe für Vegetationskunde 28. Landwirtschaftsverlag, Münster, Germany.

MANDÁK, B., K. BÍMOVÁ, I. PLAČKOVÁ, V. MAHELKA, AND J. CHRTEK. 2005. Loss of genetic variation in geographically marginal populations of *Atriplex tatarica* (Chenopodiaceae). *Annals of Botany* 96: 901–912.

NYBOM, H., AND I. V. BARTISH. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3: 93–114.

OKSANEN, J., F. G. BLANCHET, R. KINDT, P. LEGENDRE, R. B. O'HARA, G. L. SIMPSON, P. SOLYMOS, ET AL. 2010. Vegan: Community ecology package, version 1.17-4. Available at http://CRAN.R-project.org/package=vegan.

OZINGA, W. A., C. RÖMERMANN, R. M. BEKKER, A. PRINZING, W. L. M. TAMIS, J. H. J. SCHAMINÉE, S. M. HENNEKENS, ET AL. 2009. Dispersal failure contributes to plant losses in NW Europe. *Ecology Letters* 12: 66–74.

PEAKALL, R., AND P. E. SMOUSE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

PERSSON, H., B. WIDÉN, S. ANDERSSON, AND L. SVENSSON. 2004. Allozyme diversity and genetic structure of marginal and central populations of *Corylus avellana* L. (Betulaceae) in Europe. *Plant Systematics and Evolution* 244: 157–179.

PICÓ, F. X., AND J. VAN GROENENDAEL. 2007. Large-scale plant conservation in European semi-natural grasslands: a population genetic perspective. *Diversity & Distributions* 13: 920–926.

PONOMAREV, A. N. 1961. Kleystogamiya u kovyley. (Cleistogamy in feather grasses). *Botanicheskii Zhurnal* 46: 1229–1236.

PRINZ, K., K. WEISING, AND I. HENSEN. 2009. Genetic structure of coastal and inland populations of the annual halophyte *Suaeda maritima* (L.) Dumort. in Central Europe, inferred from amplified fragment length polymorphism (AFLP) markers. *Plant Biology* 11: 812–820.

R DEVELOPMENT CORE TEAM. 2010. R: A language and environment for statistical computing, version 2.12.0. R Foundation for Statistical Computing, Vienna, Austria.

REED, D. H., AND R. FRANKHAM. 2003. Correlation between fitness and genetic diversity. *Conservation Biology* 17: 230–237.

ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145: 1219–1228.

SAGARIN, R. D., AND S. D. GAINES. 2002. The 'abundant centre' distribution: To what extent is it a biogeographical rule? *Ecology Letters* 5: 137–147.

SCHIEMANN, K., T. TYLER, AND B. WIDÉN. 2000. Allozyme diversity in relation to geographic distribution and population size in *Lathyrus vernus* (L.) Bernh. (Fabaceae). *Plant Systematics and Evolution* 225: 119–132.

SCHLÜTER, P. M., AND S. A. HARRIS. 2006. Analysis of multilocus fingerprinting data sets containing missing data. *Molecular Ecology Notes* 6: 569–572.

SEIFERT, T., J. BREIBECK, S. SEIFERT, AND P. BIBER. 2010. Resin pocket occurrence in Norway spruce depending on tree and climate variables. *Forest Ecology and Management* 260: 302–312.

SKALIŃSKA, M., E. POGAN, AND A. JANKUN. 1968. Further studies in chromosome numbers of Polish angiosperms. Seventh Contribution. *Acta Biologica Cracoviensia. Series Botanica* XI: 199–224.

STOTT, P. A. 1981. Historical plant geography: An introduction. Allen & Unwin, London, United Kingdom.

TEMPLETON, A. R., K. SHAW, E. ROUTMAN, AND S. K. DAVIS. 1990. The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden* 77: 13–27.

TSVELEV, N. N. 1976. Zlaki SSSR. (Grasses of the USSR). Nauka, Leningrad, USSR.

TSVELEV, N. N. 1977. O proiskhozhdenie i evolyutsii kovyley. [On the origin and evolution of the feather grasses.] *In* Z. V. Karamysheva [ed.], Problemy ekologii, geobotaniki, botanicheskoy geografii i floristiki, 139–150. Nauka, Leningrad, USSR.

VEKEMANS, X. 2002. AFLP-SURV: A program for genetic diversity analysis with AFLP (and RAPD) population data. Distributed by the author, version 1.0. Université Libre de Bruxelles, Bruxelles, Belgium.

VENABLES, W. N., AND B. D. RIPLEY. 2002. Modern applied statistics with S, 4th ed. Springer, New York, New York, USA.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRIJTERS, ET AL. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.

YAKIMOWSKI, S. B., AND C. G. ECKERT. 2008. Populations do not become less genetically diverse or more differentiated towards the northern limit of the geographical range in clonal *Vaccinium stamineum* (Ericaceae). *New Phytologist* 180: 534–544.

ZUUR, A. F., E. N. IENO, N. J. WALKER, A. A. SAVELIEV, AND G. M. SMITH. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, New York, USA.