Plant Syst Evol (2013) 299:259–269
DOI 10.1007/s00606-012-0719-0

ORIGINAL ARTICLE

Extreme genetic depauperation and differentiation of both populations and species in Eurasian feather grasses (*Stipa*)

Walter Durka · Constanze Nossol · Erik Welk · Eszter Ruprecht · Viktoria Wagner · Karsten Wesche · Isabell Hensen

Received: 22 July 2012 / Accepted: 9 October 2012 / Published online: 8 November 2012

Abstract A highly selfing breeding system affects gene flow, which may have consequences for patterns of genetic variation and differentiation on both the population and species level. Feather grasses (*Stipa* spp.) are dominant elements of Eurasian steppes that persist in Central Europe in scattered isolated populations that are of great conservation interest. Cleistogamy is common in the *Stipa pennata* group, the phylogeny of which is largely unresolved. Intraspecific patterns of genetic variation can be characterised by lack of gene flow due to selfing, but also by large-scale historical migrations and long-term isolation. We analysed both 5 species within the *S. pennata* group and 33 populations of *Stipa pulcherrima* sampled across a large part of its range. Using AFLP markers we assessed phylogenetic relationships of the *S. pennata* group and patterns of genetic variation within and among populations. The *S. pennata* group formed a consistent clade separated from *S. capillata*. *Stipa pulcherrima* was sister to *S. eriocaulis*, but the relationships among *S. pennata* s. str., *S. borysthenica.*, and *S. tirsa* remained unresolved. Within-population genetic variation was extremely low in all species of the *S. pennata* group ($H_e = 0.0$–0.013). In *S. pulcherrima*, genetic variation was consistently relatively high in the east (Romania, Russia) and declined toward western populations, with many populations at the western range edge lacking genetic variation entirely. Populations were strongly differentiated ($F_{ST} = 0.762$), and this differentiation did not follow a classical pattern of isolation by distance. Bayesian cluster analysis revealed nine gene pools in *S. pulcherrima*, which were mostly geographically clustered. Overall the results suggest that *S. pulcherrima* and species of the *S. pennata* group are characterised by a cleistogamous breeding system leading to extremely low levels of genetic variation and high levels of population differentiation at both the population and species level. Postglacial colonisation, current population isolation, and population bottlenecks at the western range periphery have further reduced genetic variation and obviated gene exchange. Thus, genetic variation can only be preserved by the conservation of multiple populations.

Keywords Genetic variation · Genetic drift · Habitat isolation · Cleistogamy · *Stipa pulcherrima*

Electronic supplementary material The online version of this article (doi:10.1007/s00606-012-0719-0) contains supplementary material, which is available to authorized users.

W. Durka (✉) · C. Nossol
Department Community Ecology (BZF), Helmholtz Centre for Environmental Research-UFZ, Theodor-Lieser-Str. 4, 06120 Halle, Germany
e-mail: walter.durka@ufz.de

C. Nossol · E. Welk · V. Wagner · I. Hensen
Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle, Am Kirchtor 1, 06108 Halle/Saale, Germany

E. Ruprecht
Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Str. Republicii 42, 400015 Cluj Napoca, Romania

K. Wesche
Senckenberg Museum for Natural History, PO Box 300154, 02806 Görlitz, Germany

Introduction

A central role in plant evolution is played by the mating system as it affects the level of gene flow among individuals and populations. Consequently, the patterns of genetic variation within and among populations are most strongly

affected by the mating system, with selfing species showing low levels of within- and high levels of among-population genetic variation (Hamrick and Godt 1990). At the same time, the effects of self-pollination also extend to species level evolution because selfing leads to reproductive isolation and affects the potential for local adaptation (Levin 2010). Although selfing has been hypothesised to represent an evolutionary dead end (Stebbins 1957), it may also have positive genomic effects such as purging of deleterious alleles or the reduction of genomic conflict (Wright et al. 2008). In concert, these effects may contribute to speciation in selfing lineages. Thus, similar patterns of low variation within both populations and species are expected as a result of selfing.

Apart from the mating system, demographic history has strong effects on the distribution of genetic variation within species. Across the distribution range, clines of genetic variation from the centre to the periphery are expected because of reduction of both habitat quality and quantity toward the periphery resulting in smaller and less well-connected populations suffering from genetic drift (Eckert et al. 2008). Similar clines result from historic colonisation processes, e.g. during postglacial range expansion, which lead to reduced genetic variation in more recently colonised areas because of bottlenecks and founder effects (Ibrahim et al. 1996). In addition, more recent processes such as human land use can affect genetic variation. A major distinction has been made between species that became rare because of recent land use changes ("new rare species") and species that have been rare for much longer times ("old rare species"; Huenneke 1991; Oostermeijer et al. 1996). The former should have undergone population fragmentation and demographic bottlenecks only quite recently and may show both, positive correlations of population size and genetic variation (Ellstrand and Elam 1993; Leimu et al. 2006) and patterns of isolation by distance with equilibrium of gene flow and drift governing population differentiation (Hutchison and Templeton 1999). Old rare species, in contrast, have persisted in isolated and relatively small populations for much longer times. In such relict species, demographic bottlenecks, genetic drift, inbreeding and restricted gene flow may have led to a general and more drastic reduction of genetic variation and more pronounced population differentiation, thus blurring expected equilibrium relationships by random effects and aggravating the interpretation of population genetic data (Peakall et al. 2003). However, rare and relict species have also been found to harbour similar levels of genetic variation as common species (Cole 2003; Hensen and Wesche 2007; Michalski and Durka 2007). Thus, predictions about levels of genetic variation and population differentiation remain difficult, although this knowledge is essential for conservation purposes.

Steppes dominate the natural vegetation in continental areas of Eurasia, which are characterised by hot summers and cold and dry winters. Steppe ecosystems are thought to date back to the Pliocene (Frenzel 1968), whereas in Central Europe steppe vegetation spread mainly in the warmer periods after the last glaciation (between 10,000 and 5,000 years ago; Pott 1996; Walter and Straka 1970). Some cold-tolerant species are, however, suspected to have migrated to Central Europe already during the latest glacial period (14,000 years ago; Ellenberg and Leuschner 2010). Due to subsequent climatic cooling and the establishment of dominant forests, natural open grasslands vanished in Central Europe except for the driest and warmest regions and habitats. Therefore, a number of steppe species that persisted as relics of the former steppe period reach their northwestern range edge in Central Europe. Here, they are confined to spatially isolated sites such as dry grasslands in south-facing slopes. These grasslands are particularly species rich and represent focal objects of current nature conservation efforts, e.g. as protected habitat types within the Natura 2,000 network. A major goal of such efforts is the conservation of intact stands and thus viable populations with sufficient genetic variation within populations and species to allow future evolution and adaptation (Frankham 1995). Therefore, knowledge about the level and hierarchical organisation of genetic variation within these species is indispensable.

Feather grasses of the genus *Stipa* are the name-giving, dominant elements of steppe vegetation. The genus has diversified in the Eurasian dry grasslands. Cleistogamy and thus a selfing mating system are common in the feather grasses (Godron 1873; Ponomarev 1961) and were held responsible for fixation of new morphological forms that gave rise to the description of new taxa (Freitag 1985). In this study we analyse genetic variation within and among populations of the feathery awned *S. pulcherrima* and related species of the *S. pennata* group that are particularly abundant in western Eurasia. The phylogenetic relationships of the species within the *S. pennata* group are not well understood (Hamasha et al. 2012) and new species are being described (e.g. Korneck and Scholz 2007). *Stipa* species are known to harbour comparatively low levels of genetic variation, which additionally may be reduced towards the range edges in Central Europe (e.g. Hamasha et al. 2013; Hensen et al. 2010; Wagner et al. 2011, 2012). Based on the relict status of the species in the presumably long-term isolated populations of Central Europe, we hypothesise that genetic variation is lower than in the more contiguous eastern populations because of genetic drift, which also should have led to pronounced genetic differentiation among populations.

In particular, we ask (1) what are the phylogenetic relationships within the *S. pennata* group? (2) What is the level of

genetic variation within populations and how does it vary between feather grasses of the *S. pennata* group? For *S. pulcherrima*, we specifically ask (3) whether genetic variation within populations differs in central vs. marginal range positions; (4) we discuss the implications of the patterns of genetic variation for understanding the phylogeography and for the conservation of *Stipa* species in Central Europe.

Materials and methods

Study species

Stipa pulcherrima K.H.E. Koch 1848 (Poaceae) is a tetraploid ($2n = 4x = 44$) perennial tussock grass with characteristic feathery awns of up to 50 cm length. The distribution of *S. pulcherrima* is Eurasiatic (Submediterranean-Pannonic-Pontic-South Siberian), ranging from the Omsk region in western Siberia over the Pontic–Pannonic steppe region and the Balkan peninsula to Central Europe, where it reaches its western range edge (Fig. 1). Like many of its congeners, *S. pulcherrima* is a steppe grass able to endure the prolonged drought periods during summer and winter typical for continental areas. At its western range edge the species is confined predominantly to rocky or sandy south-facing slopes on alkaline soils in areas of low rainfall resembling the continental climate of the steppe regions. *Stipa pulcherrima* only reproduces by seed; however, large tussocks may occasionally break up into small ones, leading to clonal structures on very small scales. The breeding system of *Stipa* species has not been studied recently, but cleistogamy was described very early for the feather grasses and is widespread in the *S. pennata* group (personal observation, Godron 1873; Ponomarev 1961). Still, cleistogamy is facultative in most *Stipa* species and chasmogamous flowering has also been described in *S. pulcherrima* (Hackel 1906).

Stipa pulcherrima together with the morphologically and ecologically similar feather grass species *S. pennata* L. 1753 (syn. *S. joannis* Čelak.), *S. borysthenica* Klokov ex Prokudin 1951, *S. eriocaulis* Borbas 1878, *S. tirsa* Steven 1857 and others belongs to the *S. pennata* group in sect. Eriostipa Dumort., the largest section of the genus (Freitag 1985). All these species are perennials and most probably tetraploid (Sheidai et al. 2006; Tsvelev 1977). Depending on the species concept, these taxa are treated as true species (Wisskirchen and Haeupler 1998) or subspecies within a larger *S. pennata* (Freitag 1985). Thus, for comparison we additionally included populations of these taxa and additionally *S. capillata*, a member of the smooth-awned sect. Leiostipa Dumort., which served as outgroup in the phylogenetic analysis.

Sites and sampling

We sampled 33 populations of *S. pulcherrima* across large parts of the species range between Germany (hereafter referred to as Central Europe) and southwestern Russia (Table 1, Fig. 1), including two adjacent populations of *S. p.* subsp. *palatina* H. Scholz et Korneck (Leistadt, SPP_D_3 and SPP_D_4; see Korneck and Scholz 2007). Within each population, leaves from 2 to 30 (mean 9.7; Table 1) plants were sampled randomly, ensuring that potentially disintegrated tussocks were not sampled repeatedly. We additionally sampled 16 populations of other species within the *S. pennata* group (*S. pennata* s. str., *S. borysthenica*, *S. eriocaulis*, *S. tirsa*; 5–13 samples, mean 8.5) and two populations of *S. capillata*.

AFLP genotyping

Amplified fragment length polymorphisms (AFLP) were employed following the protocol of Lachmuth et al. (2010). After initial screening of 24 primer combinations, we selected six primer combinations for final analysis: ACT (FAM)-CAT, ACT (FAM)-CAG, ACA (VIC)-CAC, ACG (VIC)-CAT, AGG (PET)-CAG and AGC (PET)-CAT. Fragment analysis was performed on a 3130 Genetic Analyser (Applied Biosystems) with Genescan-LIZ-600 as internal size standard. AFLP bands between 50 to 600 bp were manually scored with GeneMapper 3.7 (Applied Biosystems). Care was taken to exclude homoplasious loci by checking the peak height frequency distribution for each putative locus and adjusting an individual peak height cutoff threshold. Markers that showed a multimodal peak height distribution across samples, potentially indicative of homoplasy, were skipped. Across all samples this procedure first resulted in 486 preliminarily defined putative loci, 366 of which were retained, ranging from 53 to 586 bp. Preliminary analyses had revealed that in several populations all individuals had the same multilocus AFLP phenotype across six primer combinations (see below), suggesting a very high reproducibility and low error rate.

Phylogenetic relationships

We analysed the relationships among species and populations using Nei's standard genetic distances among populations estimated in AFLP-SURV 1.0 (Vekemans 2002). This matrix was used to calculate neighbour-joining (NJ) trees with the NEIGHBOR and CONSENSUS programs in PHYLIP 3.65 (Felsenstein 1989). Support for individual nodes was assessed with 1,000 bootstrapped distance matrices.

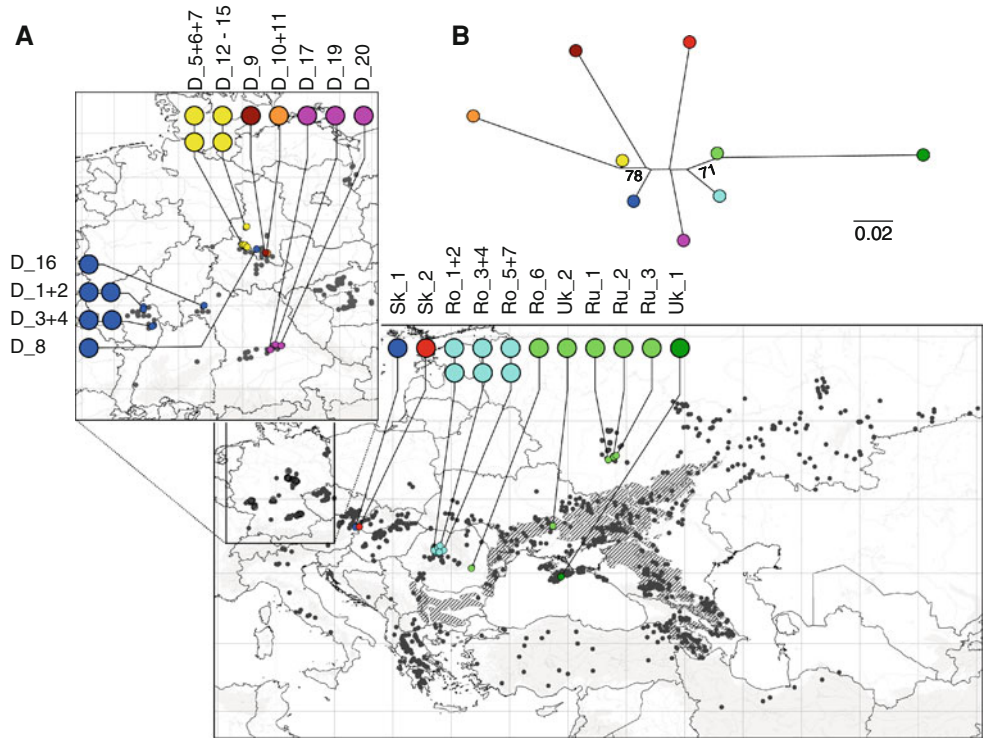

Fig. 1 **a** Distribution map of *Stipa pulcherrima* sampling sites and their affililation to nine genetic clusters according to Bayesian assignment analysis with BAPS (see Fig. S1). **b** Neighbour-joining tree of the nine genetic clusters based on Nei's distances of AFLPs with percentage boostrap support if >50 %

Genetic data analyses

A test for size homoplasy of the AFLP products was performed by correlating AFLP fragment sizes and frequencies with AFLP-SURV 1.0. We applied a band-based approach to AFLP data analysis because of polyploidy, which precludes the calculation of allele frequencies, and set the allele frequency equal to band frequency. We determined the percentage of polymorphic loci (PLP) and gene diversity (H_e) as parameters of genetic variation at the species and population level with the AFLP-SURV 1.0 programme. Additionally, we calculated genotypic diversity (D_g) with AFLPdat (Ehrich 2006).

Genetic differentiation among populations was calculated as Wright's F_{ST} (Lynch and Milligan 1994) in AFLP-SURV 1.0 using 1,000 permutations to test for significance. Hierarchical analysis of molecular variance (AMOVA) was performed to assign components of genetic variation to hierarchical sets of populations in Arlequin 3.5 (Excoffier & Lischer 2010). We applied a Bayesian approach to cluster individuals into 'panmictic' groups using BAPS 5.2 (Corander et al. 2008). We ran a population mixture analysis with the maximal number of groups, K, set to 20 with five replicates that identified one most probable partition with the highest marginal log likelihood. We tested for a pattern of isolation by distance by correlating pairwise F_{ST} with log geographic distances. However, due to nonequilibrium conditions, F_{ST} values may reach very high values in selfing populations, and we therefore also tested for isolation by distance with an evolutionary perspective with Nei's genetic distance. Significance was assessed with a Mantel test in *R*, package ade4 (Chessel et al. 2004).

Results

AFLP polymorphism

Across all species (473 samples) and six primer combinations, 366 AFLP loci were scored, 319 of which (87.6 %) were polymorphic and used for the species level analyses (data set 1, 319 loci). In *S. pulcherrima* (320 samples), 179 of the 366 loci were present, of which 134 were polymorphic (74.9 %) and used for the analyses involving only *S. pulcherrima* (data set 2, 134 loci). The correlation between fragment sizes and fragment frequencies was $r = -0.151$ ($p = 0.042$) for data set 1 and $r = -0.046$ ($p = 0.597$) in data set 2, indicating some size homoplasy in the former but none in the latter.

Phylogenetic relationships

Distance analysis showed that the *S. pennata* group forms a well-supported clade strongly separated from *S.*

Table 1 Sampled populations of *Stipa pulcherrima* and other *Stipa* spp., and descriptors of genetic variation

Code	Site	N	E	N	N_{gt}	D_g	N_{bands_319}	PLP_{319}	H_{e_319}	PLP_{134}	H_{e_134}
S. pulcherrima											
SP_D_1	Dorsheim, Burgberg	49.9321	7.8798	8	2	0.25	96.1	0.3	0.001	0.7	0.002
SP_D_2	Fichtekopf	49.9079	7.8806	9	1	0.00	97.0	0	0.000	0	0.000
SPP_D_3	Leistadt, Annaberg	49.4874	8.1651	2	1	0.00	95.0	0	0.000	0	0.000
SPP_D_4	Leistadt, Großer Höbel	49.5000	8.1639	2	2	1.00	99.5	0.3	0.002	0.7	0.006
SP_D_5	Badraer Lehde	51.4067	11.0015	10	1	0.00	94.0	0	0.000	0	0.000
SP_D_6	Harslebener Berge	51.8298	11.0764	10	2	0.20	96.5	1.6	0.003	3.7	0.007
SP_D_7	Mittelberg	51.4174	10.9812	9	1	0.00	94.0	0	0.000	0	0.000
SP_D_8	Bottendorf	51.3121	11.4081	10	1	0.00	98.0	0	0.000	0	0.000
SP_D_9	Dorndorf	51.2434	11.6826	10	6	0.84	102.5	1.9	0.010	4.5	0.023
SP_D_10	Schafberg	51.2161	11.7202	16	6	0.68	97.5	3.8	0.009	9	0.022
SP_D_11	Schafberg/Schießstand	51.2206	11.7168	30	2	0.13	98.9	0.3	0.000	0.7	0.001
SP_D_12	Kosakenberg	51.3683	11.0820	10	2	0.36	96.4	4.1	0.014	9.7	0.034
SP_D_13	Barbarossa-Höhle	51.3766	11.0398	10	2	0.20	97.1	0.3	0.001	0.7	0.001
SP_D_14	Kyffhäuser	51.3806	11.0389	8	6	0.93	95.6	5.3	0.019	12.7	0.044
SP_D_15	Vatersberg	51.3735	11.0444	10	2	0.20	99.1	0.3	0.001	0.7	0.001
SP_D_16	Karlstadt	49.9893	9.7646	4	2	0.50	97.0	5	0.024	11.9	0.056
SP_D_17	Ebenwies	49.0425	11.9950	9	4	0.58	91.1	2.2	0.005	5.2	0.013
SP_D_19	Fellinger Berg, Keilstein	49.0298	12.1547	19	11	0.87	94.7	5.3	0.016	12.7	0.037
SP_D_20	Schulerloch	48.9279	11.8199	10	1	0.00	91.0	0	0.000	0	0.000
SP_Sk_1	Devinsca kob./Mervice	48.1839	16.9875	10	5	0.76	96.2	6.3	0.021	14.9	0.050
SP_Sk_2	Devinsca kobyla	48.1861	16.9839	9	3	0.56	101.0	0.9	0.002	2.2	0.006
SP_Ro_1	Suatu	46.7915	23.9666	9	7	0.94	95.8	7.5	0.026	17.9	0.061
SP_Ro_2	Sucutard	46.9086	24.0411	10	6	0.87	97.7	8.5	0.027	20.1	0.064
SP_Ro_3	Viişoara	46.5502	23.9125	10	10	1.00	103.2	8.2	0.024	19.4	0.058
SP_Ro_4	Turda, Cheile Turzii	46.5692	23.6704	9	6	0.83	96.4	9.4	0.029	22.4	0.069
SP_Ro_5	Căian	46.7659	23.9008	10	9	0.98	98.5	9.4	0.032	22.4	0.076
SP_Ro_6	Buzău	45.3607	26.7159	7	4	0.80	96.5	5.3	0.014	12.7	0.034
SP_Ro_7	Band	46.6124	24.3326	4	4	1.00	98.0	6	0.028	14.2	0.068
SP_Uk_1	Krimia, Angarskiy Pereval	44.7773	34.3817	9	4	0.69	98.0	9.4	0.028	22.4	0.068
SP_Uk_2	Novomykhalivka	48.2433	33.6952	8	8	1.00	100.9	6.9	0.028	16.4	0.066
SP_Ru_1	Bykovaya Sheya	52.7678	39.0540	16	12	0.96	96.0	8.2	0.026	19.4	0.062
SP_Ru_2	Balki Korytnya	52.7170	38.8543	6	4	0.87	94.8	4.1	0.015	9.7	0.035
SP_Ru_3	Dolina ruchya Pazhen	52.5374	38.4511	7	5	0.86	95.7	4.4	0.013	10.4	0.031
S. eriocaulis											
SE_D_26	Isteiner Klotz	47.6620	7.5301	7	4	0.71	99.9	1.3	0.004		
SE_Sk_2	Devinsca kobyla	48.1861	16.9839	9	8	0.97	100.1	5.3	0.022		
SE_Sk_3	Tematinske Kopce	48.6795	17.9015	9	7	0.94	94.9	2.8	0.010		
SE_Sw_1	Wallis			8	2	0.25	102.5	2.5	0.006		
S. tirsa											
ST_D_21	Badra	51.4067	11.0015	9	2	0.22	100.8	1.9	0.004		
ST_D_7	Mittelberg	51.4174	10.9812	9	2	0.22	100.9	0.6	0.001		
S. pennata											
SJ_D_16	Karlstadt	49.9893	9.7646	9	5	0.81	100.4	1.9	0.006		
SJ_D_22	Börnecke	51.8336	11.0328	8	3	0.61	101.6	0.6	0.002		
SJ_D_23	Norheim	49.8121	7.7969	6	4	0.87	94.8	1.3	0.006		
SJ_D_24	Napptal, Kyffhäuser	51.3672	11.1016	7	5	0.86	105.1	6.9	0.021		
SJ_D_25	Rothenburg	51.6518	11.7579	9	3	0.64	99.4	0.6	0.002		

Table 1 continued

Code	Site	N	E	*N*	N_{gt}	D_g	N_{bands_319}	PLP_{319}	H_{e_319}	PLP_{134}	H_{e_134}
SJ_Ro_7	Band	46.6124	24.3326	5	3	0.70	102.6	2.2	0.008		
S. borysthenica											
SB_D_27	Biesdorfer Kehlen	52.7229	14.0806	13	1	0.00	119.0	0	0.000		
SB_D_28	Gartz	53.2170	14.3778	10	1	0.00	113.0	0	0.000		
SB_D_29	Geesow	53.2408	14.3857	10	1	0.00	118.0	0	0.000		
SB_Sk_4	Ostrov Kopac	48.0387	17.4878	10	1	0.00	114.0	0	0.000		
S. capillata											
SC_D_23	Norheim	49.8121	7.7969	7	7	1.00	110.9	6.9	0.030		
SC_Sk_2	Devinsca kobyla	48.1770	17.0143	8	8	1.00	108.5	14.1	0.054		
S. pulcherrima	Mean					**0.57**	**97.0**	**3.8**	**0.013**	**9.0**	**0.030**
S. eriocaulis						**0.72**	**99.3**	**3.0**	**0.011**		
S. tirsa						**0.22**	**100.8**	**1.3**	**0.003**		
S. pennata						**0.75**	**100.7**	**2.3**	**0.008**		
S. borysthenica						**0.00**	**116.0**	**0.0**	**0.000**		
S. capillata						**1.00**	**109.7**	**10.5**	**0.042**		

Bold values indicate species level mean

No. number of samples, N_{gt} number of genotypes, D_g genotype diversity, N_{bands_319} mean number of bands per genotype in the 319 loci data set, PLP_{319}, PLP_{134}, percentage polymorphic loci in the 319 and 134 loci data sets, respectively; H_{e_319}, H_{e_134}, gene diversity in the two data sets

capillata by a mean Nei's distance of 0.49 (Fig. 2). All species formed well-separated clades with *S. pulcherrima* and *S. eriocaulis* being sister groups (Fig. 2). Within *S. pulcherrima*, in the NJ tree, the most eastern populations from Romania and Ukraine took a basal position. However, hardly any further structure was apparent with only two pairs of spatially close populations clustering together with high bootstrap support (D3, D4 and D10, D11), one of which was the recently described subspecies *S. pulcherrima palatina*. All these groupings were in line with the Bayesian cluster analysis of *S. pulcherrima* (see below).

Genetic variation at species and population scale

Across all 320 individuals of *S. pulcherrima*, 222 multilocus AFLP genotypes were distinguished. Six and eight populations consisted of a single or two multilocus genotypes, respectively (Table 1). None of the genotypes occurred in more than one population. Very low genetic variation of *S. pulcherrima* was also indicated by the mean percentage of polymorphic loci at the population level ($PLP_{134} = 9.0$; $PLP_{319} = 3.8$; Table 1) and by expected heterozygosity on the species and population level with $H_{T\_134} = 0.127$ and mean $H_{e\_134} = 0.030$ (SE 0.005) in the 134 locus data set and $H_{T\_319} = 0.054$ and mean $H_{e\_319} = 0.013$ (SE 0.002) in the 319 locus data set. Genetic variation at the population level was consistently relatively high in the eastern populations (Fig. 3), whereas in the Central European populations, both high and low values were found. Overall genetic variation was significantly higher in the eastern (>20°) populations than in the west (H_{e_134} and H_{e_319}; t test $p < 0.0001$).

The other species of the *S. pennata* group had similarly low or even lower levels of genetic variation compared to *S. pulcherrima* (Table 1). *Stipa borysthenica* lacked within-population variation and each of the four sampled populations consisted of a different genotype. In contrast, both populations of *S. capillata* showed much higher values of genetic and genotypic variation.

Population structure

The BAPS analysis of *S. pulcherrima* identified nine clusters, most of which formed geographically coherent groups (Fig. 1, Fig. S1). Cluster A (green) and B (dark green) were closely related, as shown by high bootstrap support in the NJ analysis, and included all steppe regions in Russia, Ukraine and trans-Carpathian Romania. Cluster C (blue) comprised the cis-Carpathian Romanian populations. In the western part of the range, the populations along the Danube formed cluster E (pink), whereas populations from central Germany were assigned to four different clusters (F, G, H, I). Finally, cluster I (dark blue) encompassed populations from relatively distant regions including the range edge in Western German, Central Germany and Slovakia. This cluster was consistently retrieved when reducing K (data not shown). Despite this grouping into nine clusters, the relationships among these clusters were not resolved in NJ analysis (Fig. 1b).

Populations of *S. pulcherrima* were strongly differentiated with an overall F_{ST} of 0.762 (SE 0.041). This

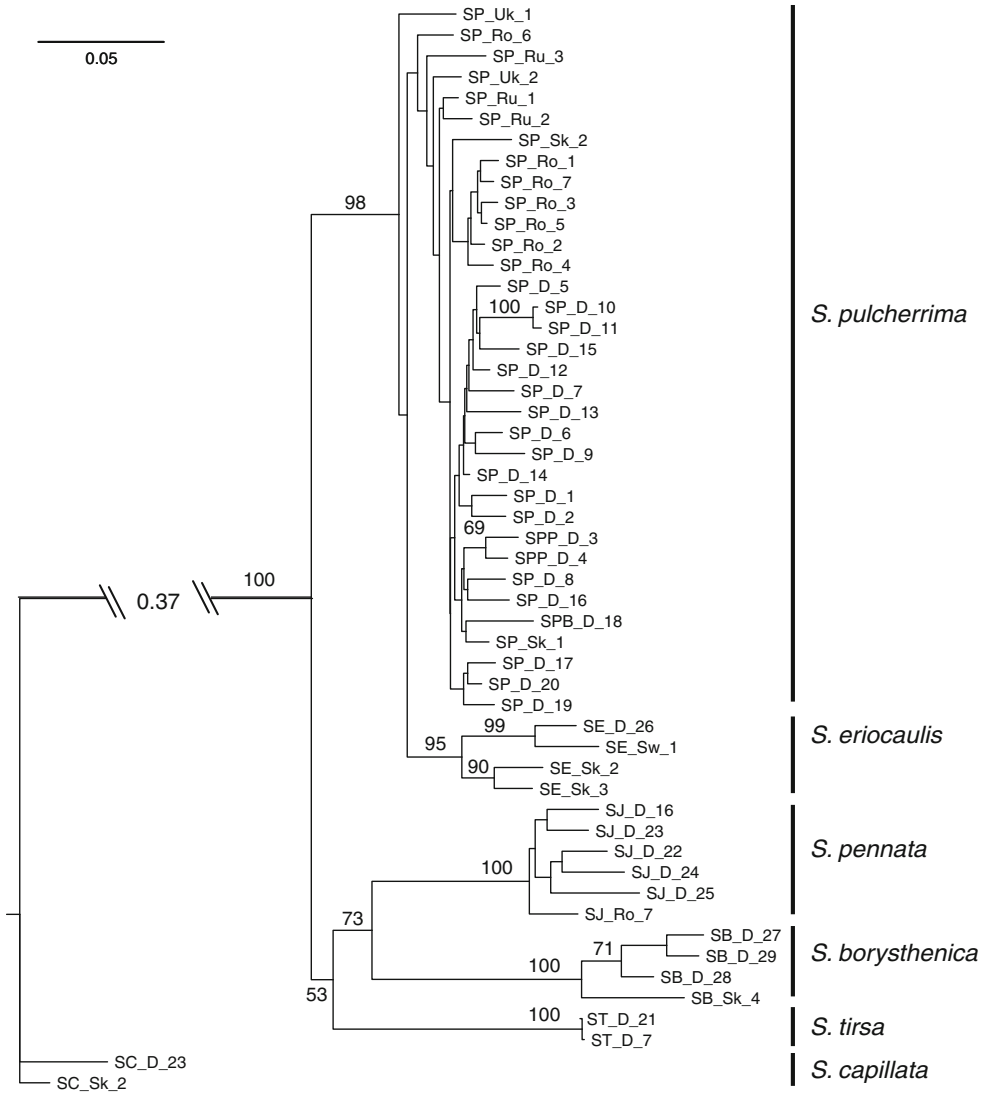

Fig. 2 Relationships among *Stipa* species and populations (Table 1) based on Nei's genetic distance and neighbour joining. Numbers above branches indicate percentage bootstrap support if >50 %

differentiation was only partly due to differentiation between geographic regions, as shown by the hierarchical AMOVA (Table 2), which resulted in 20.3 % of variation residing among geographic groups and 57.8 % residing among populations within groups. When the AMOVA was performed on the nine clusters identified in the Bayesian cluster analysis, 33.9 and 44 % of variation were found among clusters and among populations within clusters, respectively. Population differentiation of *S. pulcherrima* did not conform to isolation by distance, since extremely high F_{ST} values were observed across most distances, indicating the predominant role of genetic drift (Fig. 4). However, a weak but significant increase of pairwise Nei's genetic distance with geographic distance was found, indicating that genotypes become more different with geographic distance (Fig. 4).

The other species of the *S. pennata* group showed similarly strong population differentiation as *S. pulcherrima*, with $F_{ST} = 0.875$ (SE 0.048, $n = 6$) in *S. pennata s. str.*, $F_{ST} = 1$ (SE 0.0, $n = 4$) in *S. borysthenica* and $F_{ST} = 0.857$ (SE 0.049, $n = 4$) in *S. eriocaulis*. For *S. tirsa* two nearby populations were analysed, which were hardly differentiated at $F_{ST} = 0.009$ (SE 0.414, $n = 2$).

Discussion

Phylogeny of the *Stipa pennata* group

In a recent phylogenetic analysis of Stipeae, species of the sections *Stipa* and *Barbatae*, including all species treated in this article, proved to be closely related, whereas their

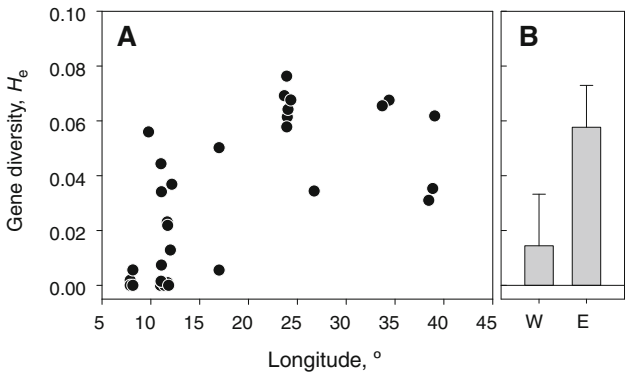

Fig. 3 Gene diversity of *Stipa pulcherrima*. **a** Population level estimates as a function of longitude. **b** Population mean ± SD for western (<20°) and eastern (>20°) populations ($p < 0.001$)

Table 2 Results of analyses of molecular variance (AMOVA) of *S. pulcherrima*

	df.	Variation (%)	Variance component	Fixation index
Six geographic groups				
Among groups	5	20.3	1.797	$\Phi_{CT} = 0.203$
Among populations within groups	27	57.8	5.118	$\Phi_{SC} = 0.724$
Within populations	295	22.0	1.948	$\Phi_{ST} = 0.780$
Total	327		8.864	
Nine BAPS clusters				
Among groups	8	33.9	2.981	$\Phi_{CT} = 0.339$
Among populations within groups	24	44.0	3.876	$\Phi_{SC} = 0.666$
Within populations	295	22.1	1.948	$\Phi_{ST} = 0.779$
Total	327		8.805	

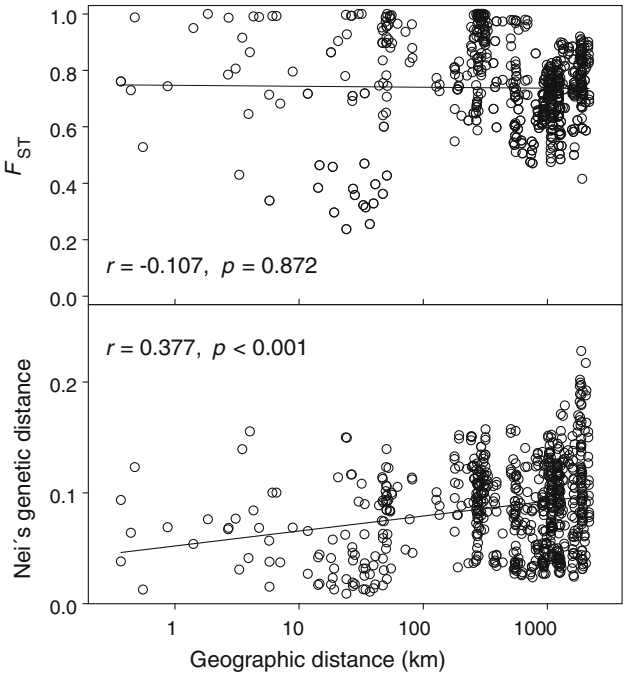

Fig. 4 Pairwise F_{ST} values and pairwise Nei's genetic distances as a function of log geographical distances among populations of *Stipa pulcherrima*. Mantel test with 2,000 permutations

phylogeny was largely unresolved based on chloroplast and nuclear ribosomal DNA sequences (Hamasha et al. 2012). Only a few members of the *S. pennata* group formed a weakly supported clade, which however did not include *S. pulcherrima* and *S. eriocaulis* (Hamasha et al. 2012). In our analysis, in contrast, all analysed species of the *S. pennata* group were clearly separated from *S. capillata* (sect. Leiostipa), forming a highly supported clade. Additionally, a sister-group relationship between *S. pulcherrima* and *S. eriocaulis* was found. Thus, AFLP analysis is a promising tool to analyse relationships within this species-rich group.

The species concept for the taxa in the Central European *Stipa pennata* group has changed repeatedly in the last decades, with current views recognising several local species (Scholz in Jäger 2011). This rather narrow species concept is also supported by our AFLP analysis, which distinguished all main taxa. Note, however, that considerable parts of the range, e.g. in the Caucasus and the Mediterranean, were not sampled. Thus, a conclusive picture is only possible with samples covering the total range of the investigated species because of strong spatio-genetic population differentiation (see below). Within *S. pulcherrima* three subspecies are recognised in Central Europe (Scholz in Jäger 2011). However, although the two populations of *Stipa pulcherrima* subsp. *palatina* (Korneck and Scholz 2007) clustered together (populations SPP_D_3/4 in Figs. 2, S1), this was also the case for other groups of spatially adjacent populations in our analysis. Recently, it was also shown that *S. pulcherrima* ssp. *bavarica* was not distinguishable from other *S. pulcherrima* populations (Meindl 2012). Thus, the relevance of these suggested endemic subspecific taxa is questionable. Central European populations of the *S. pennata* group are often both spatially and genetically isolated and numerically small. Thus, any morphologically visible mutation may quickly become fixed within a population because of the selfing mating system. These genetic and ecological processes have to be taken into account when interpreting morphological variation in *Stipa* (Freitag 1985).

Genetic variation within populations

Genetic variation within populations of all studied feather grass species was extraordinarily low. Six out of the 33 *Stipa pulcherrima* stands and all 4 *Stipa borysthenica* stands were composed of a single AFLP phenotype. Being an example from another sections of the genus, *S. capillata*

was found to show higher values of genetic variation, using both AFLP markers (this study, Wagner et al. 2011) and RAPDs (Hensen et al. 2010; Krzakowa and Michalak 2007). Similarly, *S. purpurea* from Tibet and four Jordanian *Stipa* species were also found to have higher genetic variation ($H_e = 0.13$–0.18; Hamasha et al. 2013; Liu et al. 2009). The low genetic variation found in our *Stipa* species indeed is exceptional because non-clonal grass species with a mixed mating system typically are characterised by much higher levels of genetic variation (mean PLP = 38 %, mean $H_e = 0.188$; see Wagner et al. 2011 for an AFLP data compilation). Genetic variation in our study species was even lower than that reported for non-clonal selfing grass species (PLP = 10–50 %, mean 26 %, mean $H_e = 0.072$; Wagner et al. 2011).

Within grasses, the level of genetic variation depends on the life form, geographic distribution and rarity as well as on seed dispersal mechanisms, but most prominently on the breeding system (Cole 2003; Godt and Hamrick 1998). As our *Stipa* species are perennial and have at least moderate range sizes, the extremely low level of diversity is unexpected. Clonality can be excluded as responsible, as can apomixis, because results of flow cytometry were consistent with sexual fertilisation of reduced sperm cells (Nossol, unpublished data). We suggest that a combination of several factors has contributed to extreme genetic depauperation: self-pollination, population bottlenecks and lack of gene exchange due to spatial isolation. First, self-pollination may be brought about by facultative cleistogamy, which is known in feather grasses and appears to be strongly related to water availability. *Stipa* species produce a higher percentage of cleistogamous flowers with increasing drought (Brown 1952; Ponomarev 1961) and may totally avoid opening flowers in the driest conditions (Ronnenberg et al. 2011). *S. pulcherrima* has a high potential for outcrossing by wind pollination, based on a mean pollen-ovule ratio of 10,750 (SD 3045, $n = 22$; Nossol, unpublished data) and considering other wind pollinated taxa and the principle correlation of P/O ratios and outcrossing rate (Michalski and Durka 2009, 2010). However, the species obviously relies mostly on cleistogamy, although relatively high levels of precipitation in Central European may suggest higher outcrossing rates compared to eastern steppe regions that receive less precipitation, presumably leading to cleistogamy. Second, however, toward the range periphery, population bottlenecks, spatial isolation and the lack of gene exchange between populations are expected (Eckert et al. 2008). Cleistogamy can explain low genetic variation in general, but it does not account for the further reduction of variation and the complete lack of variation in many populations of the western range edge. Range edge populations in *S. pulcherrima* are much smaller and more spatially isolated than in the centre. Thus, small population size resulting in demographic bottlenecks likely has contributed to a further loss of genetic variation due to genetic drift, and the strong spatial isolation has prevented gene flow and replenishment of variation between populations, as typically found in marginal populations (Eckert et al. 2008). In addition, recurrent population extinctions and (re)colonisations could have contributed to a decline in genetic variation in the periphery, which is particularly relevant for species where pollen migration is largely absent (Noel et al. 2007; Voss et al. 2012), as seems to be the case for our species.

Although our data set is comprehensive only for *S. pulcherrima*, the pattern of extremely low variation within populations is consistent across the other species, such as *S. eriocaulis*, *S. pennata*, *S. borysthemica* and *S. tirsa*, suggesting that predominant cleistogamy and lack of gene flow among isolated populations form a general pattern in the *S. pennata* group. As genetic diversity is also low in phylogenetically more distant *Stipa* species (e.g. Hamasha et al. 2013), low genetic variation in *Stipa* seems to represent a general pattern owing to cleistogamy. Massive production of viable seeds from cleistogamous flowering still ensures population persistence and dispersal, as shown for *S. krylovii* (Ronnenberg et al. 2011). The vigour of Central European populations of *S. capillata*, which expand despite low genetic diversity (Hensen et al. 2010), also shows that low diversity may not necessarily result in low fertility.

Genetic population structure and implications for phylogeography and conservation

In line with low variation within populations, the populations were extremely differentiated and did not follow a model of isolation by distance based on pairwise F_{ST}-values. However, Nei's genetic distances between populations increased with spatial distance. Taken together, this strongly suggests that populations are not connected by current gene flow but that mutations have accumulated and mostly became fixed in the course of historic range expansions or colonisations. Within *S. pulcherrima*, several population groups could be distinguished that may testify to multiple stepwise colonisation events. The most eastern populations from Russia and Ukraine took a basal position within the NJ tree and had the highest levels of genetic variation. This suggests that the species' evolutionary centre is located in, and that the westward colonisations started from, the Eastern steppe regions. However, the dating of the westward colonisation is not yet possible because Stipoid grasses are not distinguishable in palynological samples. There are no macro-remains of *Stipa* from the last glacial maximum (LGM), and the earliest definite *Stipa* caryopses in Central Europe date from the Bronze age (2600–1300 BC; Bieniek and Pokorny 2005). Still, it

has been suggested that Stipa species may well have occurred in Central Europe during the LGM (Kunes et al. 2008). In fact, when considering the level of genetic variation, several of the Central European populations (e.g. Karlstadt) contained similar diversity as those in the eastern steppes, which might indicate a long-term continuous history. However, S. *capillata* is a much more likely component of cold steppes during the LGM than the western Eurasian S. *pennata* group given the current distribution of the former, which reaches much farther into continental Central Asia (Hensen et al. 2010). Thus, it cannot be stated with any certainty when S. *pulcherrima* colonised Central Europe.

Overall, our results thus imply that, due to the dominating influence of the predominantly selfing mating system, low levels of genetic diversity and genetic exchange are typical for *Stipa* species. From a conservation perspective, the extremely low levels of both genetic diversity and genetic exchange observed in the peripheral populations of *S. pulcherrima* suggest, however, that these should nonetheless receive special attention in order to ensure their conservation, as in this case genetic variation can only be preserved in multiple populations.

Acknowledgments We thank D. Korneck and L. Skol'zneva for providing plant samples; K.-E. Behre, W. Frey and F. Schlütz kindly gave advice on the vegetation history of Central Europe.

References

Bieniek A, Pokorny P (2005) A new find of macrofossils of feather grass (*Stipa*) in an Early Bronze Age storage pit at Vlineves, Czech Republic: local implications and possible interpretation in a Central European context. Veget Hist Archaeobot 14:295–302

Brown WV (1952) The relation of soil moisture to cleistogamy ina *Stipa leucotricha*. Bot Gaz 113:438–444

Chessel D, Dafour A-B, Thioulouse J (2004) The ade4 package. I. One-table methods. R-news 4:5–10

Cole CT (2003) Genetic variation in rare and common plants. Annu Rev Ecol Evol Syst 34:213–237

Corander J, Marttinen P, Siren J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics 9:539

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Mol Ecol 17:1170–1188

Ehrich D (2006) AFLPDAT: a collection of R functions for convenient handling of AFLP data. Mol Ecol Notes 6:603–604

Ellenberg H, Leuschner C (2010) Vegetation Mitteleuropas mit den Alpen, 6th edn. Ulmer, Stuttgart

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size—implications for plant conservation. Annu Rev Ecol Syst 24:217–242

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res 10:564–567

Felsenstein J (1989) PHYLIP—Phylogeny Inference Package (Version 3.2). Cladistics 5:164–166

Frankham R (1995) Conservation genetics. Annu Rev Genet 29:305–327

Freitag H (1985) The genus *Stipa* (Gramineae) in southwest and south Asia. Notes Roy Bot Gard Edinburgh 42:355–489

Frenzel B (1968) The Pleistocene vegetation of Northern Eurasia. Science 161:637–649

Godron DA (1873) De la floraison des graminées et spécialement des céréales. Mémoires de la Société Nationale des Sciences Naturelles de Cherbourg 17:105–197

Godt MJW, Hamrick JL (1998) Allozyme diversity in the grasses. In: Cheplick GP (ed) Populations biology of grasses. Cambridge University Press, Cambridge, pp 11–29

Hackel E (1906) Über Kleistogamie bei den Gräsern. Österr Bot Z 56:81–88 143–154,180–186

Hamasha HR, von Hagen KB, Röser M (2012) *Stipa* (Poaceae) and allies in the Old World: molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages. Plant Syst Evol 298:351–367

Hamasha HR, Schmidt-Lebuhn AN, Durka W, Schleuning M, Hensen I (2013) Bioclimatic regions influence genetic structure of four Jordanian *Stipa* species. Plant Biol. doi:10.1111/j.1438-8677.2012.00689.x

Hamrick JL, Godt MJ (1990) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer Associates Inc., Sunderland, pp 43–63

Hensen I, Wesche K (2007) Genetic structure of the rare *Poa badensis* (Poaceae) in central Germany. Nova Hedwig Beih 131:177–186

Hensen I, Kilian C, Wagner V, Durka W, Pusch J, Wesche K (2010) Low genetic variability and strong differentiation among isolated populations of the rare steppe grass *Stipa capillata* L. in central Europe. Plant Biol 12:526–536

Huenneke LF (1991) Ecological implications of genetic variation in plant populations. In: Falk DA, Holsinger KE (eds) Genetics and conservation of rare plants. Oxford University Press, Oxford, pp 31–44

Hutchison DW, Templeton AR (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

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77:282–291

Jäger EJ (2011) Rothmaler–Exkursionsflora von Deutschland, Bd. 2, Grundband: Gefäßpflanzen. Elsevier, Heidelberg, p 930

Korneck D, Scholz H (2007) *Stipa pulcherrima* subsp. *palatina*, eine neue Federgras-Sippe aus der Pfalz. Kochia 2:1–7

Krzakowa M, Michalak M (2007) Genetic variability of selected marginal populations of *Stipa capillata* L. Biol Lett 44:127–135

Kunes P, Pelankova B, Chytry M, Jankovska V, Pokorny P, Petr L (2008) Interpretation of the last-glacial vegetation of eastern-central Europe using modern analogues from southern Siberia. J Biogeogr 35:2223–2236

Lachmuth S, Durka W, Schurr F (2010) The making of a rapid plant invader: genetic diversity and differentiation in the native and invaded range of *Senecio inaequidens*. Mol Ecol 19:3952–3967

Leimu R, Mutikainen P, Koricheva J, Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? J Ecol 94:942–952

Levin DA (2010) Environment-enhanced self-fertilization: implications for niche shifts in adjacent populations. J Ecol 98:1276–1283

Liu WS, Dong M, Song ZP, Wei W (2009) Genetic diversity pattern of *Stipa purpurea* populations in the hinterland of Qinghai-Tibet Plateau. Ann Appl Biol 154:57–65

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. Mol Ecol 3:91–99

Meindl C (2012) New aspects in plant conservation—Phylogeography, population dynamics, genetics and management of steppe plants in Bavaria. PhD thesis. Universität Regensburg

Michalski SG, Durka W (2007) High selfing and high inbreeding depression in peripheral populations of *Juncus atratus*. Mol Ecol 16:4715–4727

Michalski SG, Durka W (2009) Pollination mode and life form strongly affect the relation between mating system and pollen to ovule ratios. New Phytol 183:470–479

Michalski SG, Durka W (2010) Pollen and ovule production in wind-pollinated species with special reference to *Juncus*. Plant Syst Evol 286:191–197

Noel F, Machon N, Porcher E (2007) No genetic diversity at molecular markers and strong phenotypic plasticity in populations of *Ranunculus nodiflorus*, an endangered plant species in France. Ann Bot 99:1203–1212

Oostermeijer JGB, Berholz A, Poschlod P (1996) Genetical aspects of fragmented plant populations. In: Settele J, Margules CR, Poschlod P, Henle K (eds) Species survival in fragmented landscapes. Kluwer Academic Publishers, Dordrecht, pp 93–101

Peakall R, Ebert D, Scott LJ, Meagher PF, Offord CA (2003) Comparative genetic study confirms exceptionally low genetic variation in the ancient and endangered relictual conifer, *Wollemia nobilis* (Araucariaceae). Mol Ecol 12:2331–2343

Ponomarev AN (1961) Klejstogomiya u Kovylej [Cleistogamy in the feather grasses (*Stipa* spp.)]. Bot Zh 46:1229–1236

Pott R (1996) Die Entwicklungsgeschichte und Verbreitung xerothermer Vegetationseinheiten in Mitteleuropa unter dem Einfluß des Menschen. Tuexenia 16:337–369

Ronnenberg K, Hensen I, Wesche K (2011) Contrasting effects of precipitation and fertilization on seed viability and production of *Stipa krylovii* in Mongolia. Basic Appl Ecol 12:141–151

Sheidai M, Attaei S, Khosravi-Reineh M (2006) Cytology of some Iranian *Stipa* (Poaceae) species and populations. Acta Bot Croat 65:1–11

Stebbins GL (1957) Self fertilization and population variability in the higher plants. Am Nat 91:337–354

Tsvelev NN (1977) O proiskhozhdenie i évolyutsii kovyleĭ (On the origin and evolution of the feathergrasses). In: Problemii Ekologii, Geobotaniki, and Botaniicheskoi Geografii i Floristickii. Nauka, Leningrad (St. Petersburg), Russia, pp 139–150

Vekemans X (2002) AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium

Voss N, Eckstein RL, Durka W (2012) Range expansion of a selfing polyploid plant despite genetic uniformity. Ann Bot 110: 585–593

Wagner V, Durka W, Hensen I (2011) Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass. Am J Bot 98:1173–1179

Wagner V, Treiber J, Danihelka J, Ruprecht E, Wesche K, Hensen I (2012) Declining genetic diversity and increasing genetic isolation towards the range periphery of *Stipa pennata*, a Eurasian feather grass. Int J Plant Sci 173:802–811

Walter H, Straka H (1970) Arealkunde. Floristisch-historische Geobotanik. Verlag Eugen Ulmer, Stuttgart, p 478

Wisskirchen R, Haeupler H (1998) Standardliste der Farn- und Blütenpflanzen Deutschlands. Ulmer, Stuttgart, p 765

Wright SI, Ness RW, Foxe JP, Barrett SCH (2008) Genomic consequences of outcrossing and selfing in plants. Int J Plant Sci 169:105–118