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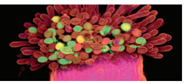

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RESEARCH PAPER

Holocene re-colonisation, central–marginal distribution and habitat specialisation shape population genetic patterns within an Atlantic European grass species

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ABSTRACT

Corynephorus canescens **(L.) P.Beauv. is an outbreeding, short-lived and wind-dispersed grass species, highly specialised on scattered and disturbance-dependent habitats of open sandy sites. Its distribution ranges from the Iberian Peninsula over Atlantic regions of Western and Central Europe, but excludes the two other classical European glacial refuge regions on the Apennine and Balkan Peninsulas. To investigate genetic patterns of this uncommon combination of ecological and biogeographic species characteristics, we analysed AFLP variation among 49 populations throughout the European distribution range, expecting (i) patterns of SW European glacial refugia and post-glacial expansion to the NE; (ii) decreasing genetic diversity from central to marginal populations; and (iii) interacting effects of high gene flow and disturbance-driven genetic drift. Decreasing genetic diversity from SW to NE and distinct gene pool clustering imply refugia on the Iberian Peninsula and in western France, from where range expansion originated towards the NE. High genetic diversity within and moderate genetic differentiation among populations, and a significant pattern of isolation-by-distance indicate a gene flow drift equilibrium within *C. canescens*, probably due to its restriction to scattered and dynamic habitats and limited dispersal distances. These features, as well as the re-colonisation history, were found to affect genetic diversity gradients from central to marginal populations. Our study emphasises the need for including the specific ecology into analyses of species (re–)colonisation histories and range centre–margin analyses. To account for discontinuous distributions, new indices of marginality were tested for their suitability in studies of centre–periphery gradients.**

INTRODUCTION

Past and current species distribution ranges and their genetic consequences in Europe are strongly shaped by the Pleistocene climate oscillations and re-colonisation processes from the last glacial refugia (Taberlet *et al.* 1998; Hewitt 1999, 2000). Repeated climatic oscillations alternately led to distribution range contractions during glacials, when most temperate species were forced into one or more southern refugia for thousands of years, and to (re-)colonisations during interstadials, interglacials or the Postglacial (Hewitt 2000, 2004; Cheddadi *et al.* 2005). Refugial regions for European species predominantly existed on the Iberian, Apennine and Balkan Peninsulas, but also in areas of SE Europe and in northern Turkey (Comes & Kadereit 1998; Taberlet *et al.* 1998; Hewitt 1999, 2004). Additionally, more recent evidence from phylogeographic and palynological data suggest that also some smaller refugia beyond these regions existed, allowing some species to persist in more northern locations (Magri *et al.* 2006; Bhagwat & Willis 2008; Kelly *et al.* 2010; Stewart *et al.* 2010).

When a species survived in several isolated refugia, this vicariance often caused isolated intraspecific microevolution, and led to genetic differentiation between refugia (*e.g.* Hewitt 1996; Magri *et al.* 2006; Naydenov *et al.* 2007). Thus, subsequent re-colonisations can result in intraspecific hybrid zones in regions of secondary contact, with significant genetic mixture of individuals and admixture within individuals (*e.g.* Taberlet *et al.* 1998; Petit *et al.* 2003; Hewitt 2004). Additionally, along routes of range expansion, genetic diversity is in general expected to decrease from refugial areas to the colonisation front due to sequential founder events of leading edge populations (Hewitt 1996; Ibrahim *et al.* 1996; Comps *et al.* 2001).

A number of theoretical and empirical studies have shown that from the centre to the margin of a species range decreasing environmental suitability can lead to reduced individual fitness and population density (*e.g.* Hengeveld & Haeck 1982; Brown 1984; Lawton 1993; Vucetich & Waite 2003 and references therein). In turn, this can decrease gene flow, increase effects of genetic drift and thus limit the accumulation of genetic diversity but enhance genetic differentiation (Holt *et al.* 2005; Eckert

et al. 2008; Sexton *et al.* 2009; Lira-Noriega & Manthey 2014). Additionally, environmental and demographic stochasticity increase local extinction and re-colonisation (Eckert *et al.* 2008; Gerst *et al.* 2011). Potential founder effects should therefore be more recent in marginal populations. As a consequence, genetic variation within populations is expected to decrease towards the range periphery (Eckert *et al.* 2008). However, assuming dynamic species ranges, leading edge areas can be assumed to have been colonised later than persistent rear edge areas (Hampe & Petit 2005). The latter likely have acted as re-colonisation sources, and their current peripheral status is in Europe typically the result of past unidirectional northward rather than centrifugal range expansion. Such differences between range periphery regions due to the biogeographic history are rarely considered in existing central–peripheral analyses. Additionally, often only broad categories of 'central' and 'peripheral' are used rather than assessing explicitly the degree of marginality.

Here, we analyse intraspecific genetic patterns of grey hair-grass, *Corynephorus canescens* (L.) P.Beauv. (Poaceae), which is a disturbance-dependent habitat specialist. This species is widespread throughout large parts of Western and Central Europe, being an element of the Atlantic floristic region, with additional areas in boreal and sub-continental Central European regions. Among the potential Mediterranean refugial regions, only the Iberian Peninsula is currently populated (Fig. 1), making *C. canescens* a model for post-glacial biogeographic history of Atlantic floristic elements. The distribution is discontinuous, including large exclaves and outposts, resulting in large proportions of marginal areas. Moreover, the distribution of *C. canescens* is scattered, due to its strong association with habitats of dry, acidic and nutrient-poor open sandy soils (Rychnovská 1963; Quinger & Meyer 1995; Jentsch & Beyschlag 2003). It depends on substrate dynamics and frequent soil disturbance, *e.g.* on open sand dunes and drought- and animal-driven vegetation gaps or anthropogenic sand mines and military training areas (Marshall 1965; Jentsch *et al.* 2002, 2009; Tschöpe & Tielbörger 2010). When disturbance ceases, such habitats are prone to succession and concomitant soil formation, which suppresses the poorly competitive *C. canescens* (Jentsch & Beyschlag 2003; Ellenberg & Leuschner 2010; Ujházy *et al.* 2011). Thus, the existence of populations is often temporally limited. This fact, together with its limited dispersal capacity (Böger 2007), potentially promotes genetic drift and population differentiation. In contrast, reproduction is by seeds, resulting from wind pollination and predominant outcrossing (Böger 2007), potentially allowing for high gene flow within and between populations.

We focus on the following three hypotheses: (i) *C. canescens* survived the last glacial maximum in refugia in SW Europe, from which it re-colonised Central and Eastern Europe to its recent distribution. This would be reflected in differentiated gene pools, private alleles in potential refugial areas and decreasing genetic diversity from SW to NE; (ii) marginal NE populations in areas of re-colonisation underwent founder events and genetic drift and thus are genetically less diverse; and (iii) as an outcrossing, wind-pollinated but short-lived and disturbance-dependent species, both gene flow and genetic drift can play a significant role in structuring genetic variation among populations. We therefore expect high genetic variation within populations, moderate population differentiation and a pattern of isolation-by-distance (IBD).

MATERIAL AND METHODS

Study species

Corynephorus canescens is a tussock-building pioneer grass species, with life span of 2–6 years (Marshall 1967; Böger 2007). It is diploid (2n = 14; Albers 1980), wind-pollinated and predominantly outcrossing (Böger 2007). Reproduction is almost exclusively by seed (Marshall 1967), although clonal growth

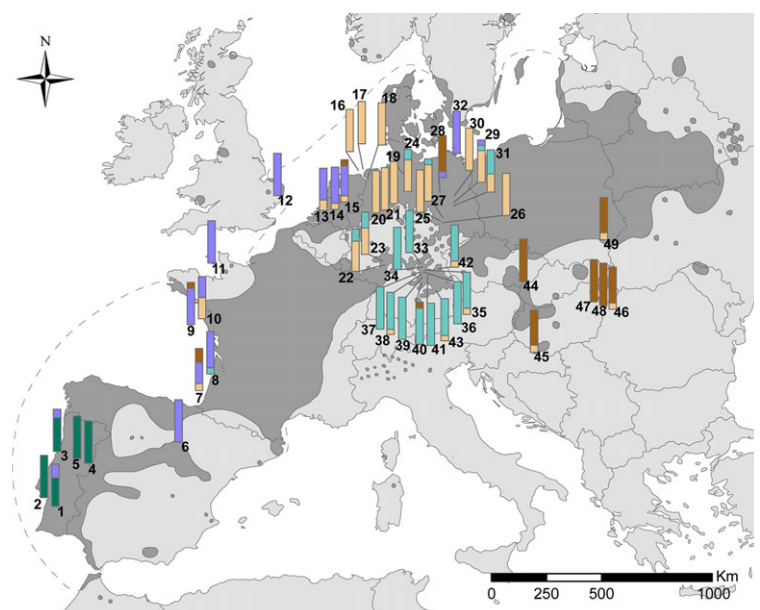

Fig. 1. Distribution range of *C. canescens* across Europe (dark grey shading) and sampled populations. Bars represent the portions of individual assignments to the five clusters found in the *k*-means cluster analysis implemented in the DAPC. Colours of the clusters correspond with colouring of points in the DAPC ordination plots (Fig. 2) and with the other cluster analyses (Figs S2–S4). Dashed lines represent the assumed climatic borders off the coast following Hegi & Conert (1998).

has occasionally been reported (Schulz 1950; Frey & Hensen 1995). Although seed production is high, seed viability and dispersal capacity of *C. canescens* are limited, as typical diaspore dispersal distances lie within only a few meters (Marshall 1968; Böger 2007). The habitat of *C. canescens* has been widespread during the last centuries, because forest logging and livestock grazing maintained dry acidic grasslands on coastal and inland dunes (Quinger & Meyer 1995; Jentsch & Beyschlag 2003). However, land-use change, in particular afforestation, land consumption and atmospheric nitrogen deposition have led to habitat loss and fragmentation (Quinger & Meyer 1995; Jentsch & Beyschlag 2003; Riecken *et al.* 2006).

Plant sampling and DNA extraction

Forty-nine populations were sampled for the current study, covering most parts of the distribution range of *C. canescens* (Fig. 1, Table 1, Table S1). In each population eight vital and healthy tussocks were arbitrarily chosen, resulting in 384 sampled individuals in total. Leaf samples were dried and stored in silica gel, and genomic DNA was extracted with the DNeasy® 96 Plant Kit following the protocol of the manufacturer (Qiagen, Venlo, The Netherlands).

Amplified fragment length polymorphism (AFLP) analyses and genotyping

We used AFLP (Vos *et al.* 1995) following the protocol of Kloss *et al.* (2011). Three primer combinations were used: $EcoRI_{FAM}$-ACT – CTA-MseI, $EcoRI_{JOE}$-AGG – CAG-MseI, $EcoRI_{TAMRA}$-ACC – CAG-MseI. Genotyping was done with GeneMapper software (Applied Biosystems, Carlsbad, CA, USA) by manual binning to produce preliminary peak height matrices. These were processed manually, specifying a threshold for minimum individual peak height based on the individual frequency distributions of peak heights, as well as thresholds for minimum peak number and minimum mean peak height for individual samples. Loci and samples that did not reach the respective thresholds were stringently discarded, resulting in a final binary presence/absence matrix of 326 samples and 137 distinct and polymorphic AFLP loci.

Data analysis

To analyse gene pool differentiation, we applied three methods of non-hierarchical genetic cluster analysis. First, we used the Bayesian clustering approach implemented in *Structure* version 2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2003, 2007), which accounts for admixture of gene pools within individuals and dominant markers. Accordingly, the admixture model with correlated allele frequencies was calculated with K ranging from 1 to 20. For each K 20 runs were performed with 100,000 replicates after a burn-in period of 100,000 runs. The outputs were processed and analysed using *Structure Harvester* (Earl & vonHoldt 2012) implementing the method of Evanno *et al.* (2005). The results were averaged for a particular K using *Clumpp* (Jakobsson & Rosenberg 2007) and visualised using *Distruct* (Rosenberg 2004).

Second, we conducted a Discriminant Analysis of Principal Components (DAPC; Jombart *et al.* 2010) implemented in the *R*-package *adegenet* (v. 1.3-5; Jombart 2008), which allows assessment of the genetic relationships among gene pools. DAPC uses a *k*-means clustering approach to identify individual assignments to genetic clusters, which are then analysed in a two-step way. First, the genetic data are transformed and summarised in a principal components analysis (PCA), and then a discriminant analysis (DA) uses the PCA variables to maximise group differentiation based on *k*-means clustering. Cluster numbers from 1 to 50 were tested with the *k*-means analysis and the most likely number of clusters was inferred by comparing the Bayesian information criterion. The optimal number of principal components (n = 13) was detected using the *a*-score optimisation (5000 iterations) to avoid over-fitted discrimination of clusters.

Third, we applied a Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in *Geneland* version 4.0.3 (Guillot *et al.* 2005a,b, 2012), which also takes the spatial origin into account to assign individuals to different genetic clusters. Twenty independent MCMC runs were made using 10 million iterations, with a thinning of 1000 to save 10,000 iterations under the spatial model with uncorrelated allele frequencies among populations. The number of genetic clusters was set to unknown, but the maximum possible number of clusters was

Table 1. List of inferred gene pools from *Geneland* results (according to Fig. S4) with mean values ± SD of descriptive parameters over assigned populations and population differentiation (F_{ST}) within gene pools. Population numbers are according to Table S1 and Fig. 1. H_j = Nei's gene diversity; *Br* = Band richness, $PLP_{5\%}$ = Proportion of polymorphic loci without alleles of frequencies below 5%; *Br* and $PLP_{5\%}$ were standardised for different sample sizes of populations by rarefaction to n = 4. Equal lowercase letters behind mean values for H_j and *Br* indicate homogeneous groups, as detected by Tukey's *post-hoc* test subsequent to significant results of an ANOVA (H_j: F = 6.544, P = 0.0003, *Br*: F = 8.214, P = 0.0005) based on gene pools as grouping factors. ANOVA on $PLP_{5\%}$ revealed no significant differences.

inferred gene pool	abbreviation	populations assigned (No.)	H_j	*Br* (4)	$PLP_{5\%}$ (4)	number of private alleles	marginality M_{min}	F_{ST} within gene pool	*P*-value of F_{ST}
Iberian Peninsula	IP	1–5	$0.278^{a} \pm 0.017$	$1.426^{a} \pm 0.028$	0.480 ± 0.058	0.4 ± 0.5	0.14 ± 0.04	0.1128	<0.0001
Coastal areas	CA	6–15, 28, 32	$0.279^{a} \pm 0.012$	$1.409^{a} \pm 0.033$	0.486 ± 0.077	0	0.33 ± 0.23	0.0693	<0.0001
Northern Germany	NG	16–21, 24–27, 29–31	$0.241^{b} \pm 0.022$	$1.336^{b} \pm 0.044$	0.453 ± 0.054	0	0.33 ± 0.19	0.0808	<0.0001
Southern Germany	SG	22–23, 33–43	$0.249^{ab} \pm 0.028$	$1.348^{b} \pm 0.050$	0.456 ± 0.068	0	0.55 ± 0.06	0.0644	<0.0001
Eastern Europe	EE	44–49	$0.240^{ab} \pm 0.032$	$1.336^{b} \pm 0.054$	0.424 ± 0.059	0	0.65 ± 0.09	0.0745	<0.0001

limited to 12 to offer a large enough search space for the MCMC algorithm. As no individual spatial data were available, we set an uncertainty level of 50 m to account for realistic extents of sampling sites and to allow for individual mixture within populations. MCMC post-processing was done with a burn-in of 2000 iterations, and the average posterior probability was used to select the best-suited run.

Genetic diversity was assessed as band richness (*Br*) and the percentage of polymorphic loci without alleles of frequencies below 5% ($PLP_{5\%}$) using a rarefaction method implied in *AFLPdiv* version 1.1 (Coart *et al.* 2005) to account for different sample sizes. Analyses of Nei's gene diversity (H_j) and global and pair-wise differentiation indices (F_{ST}) were performed with *AFLP-surv* version 1.0 (Vekemans 2002; Vekemans *et al.* 2002), using the Bayesian method with non-uniform prior allele frequency distribution and assuming Hardy–Weinberg equilibrium ($F_{IS} = 0$) to account for negligible selfing in *C. canescens* (Böger 2007). Significance of the global F_{ST} was tested with 10,000 permutations. Finally, numbers of private bands were determined with *GenAlEx* version 6.4 (Peakall & Smouse 2006). To test for isolation-by-distance patterns, a Mantel test was performed between matrices of geographic distance and linearised F_{ST} (Rousset 1997) using *GenAlEx* version 6.4 (Peakall & Smouse 2006) with 9999 permutations. The same software served for an analysis of molecular variance (AMOVA), which was done based on Φ statistics, a Euclidian distance measure (Huff *et al.* 1993) and 9999 permutations.

We tested whether genetic variation is related to geographic location, marginality and continentality. For the analysis of marginality, we digitised the distribution range of *C. canescens* from Hegi & Conert (1998) and FloraWeb (Bundesamt für Naturschutz 2006) and displayed it with ArcInfo version 10.0 (ESRI Inc., Redlands, CA, USA). The placement of distribution borders is not trivial for *C. canescens* as the current range extends to the Atlantic coastlines. However, recent range maps place the distribution borders off the coast following assumed bioclimatic limitations (Marshall 1968; Hegi & Conert 1998; see Fig. 1). For the calculations of marginality we therefore took these sea areas into account. The discontinuous distribution range of *C. canescens* precluded the use of centre-based centrality–marginality indices, *e.g.* distance from an assumed centre approximated by average latitude/longitude coordinates (Dixon *et al.* 2013). Instead, we calculated three new indices of the marginality of populations within the species' distribution range.

First, we calculated Areal Marginality (M_r) as the percentage area outside the distribution range of circular buffers constructed around each location:

$$M_r = \frac{A_{out}}{\pi r^2} \cdot 100 \quad (1)$$

where A_{out} is the proportion of area outside the distribution range, and r is the buffer radius. We used three radii (50, 100 and 200 km) resulting in three measures of Areal Marginality (M_{r50}, M_{r100}, M_{r200}).

Second, we calculated Minimum Distance Marginality (M_{min}) as the minimum log distance of a location to the range boundary. Normalised values (0–1) were obtained by dividing absolute distances by the maximum possible minimum distance within the distribution range:

$$M_{min} = 1 - \frac{\log d_{min}}{\log d_{max\,min}} \quad (2)$$

with d_{min} is the minimum distance of a location to the distribution range boundary, and d_{maxmin} is the maximum possible distance within the distribution range to the nearest boundary (352 km, referring to a location in northern Poland: 4856047 E, 3395747 N in ETRS89).

Third, we calculated Average Distance Marginality (M_{av}) as the average log distance of a location to the range boundary in multiple geographic directions, normalised to the maximum possible average distance within the distribution range:

$$M_{av} = 1 - \log \frac{\sum_{i=1}^{n} d_{(i \cdot 360^\circ/n)}}{n} / \log d_{max\,av} \quad (3)$$

where n is the desired number of directions, d is the distance of the sampling point of interest to the distribution range boundary at a specified angle, and d_{maxav} is the maximum possible average distance of any given location within the distribution range (597 km, referring to location 4623018 E, 3398409 N in northwest Poland). We calculated M_{av} using $n = 16$ geographic directions, *i.e.* 22.5° increments starting from 0° (North).

The degree of continentality was calculated for every location following (Gorczyński 1922) as

$$c = \frac{1.7 \cdot A}{\sin \theta} - 20.4 \quad (4)$$

where A is the annual temperature range and θ the latitude, using temperature data (1950–2000) from WorldClim (Hijmans *et al.* 2005). Calculations of M_r, M_{min} and c were done with ArcInfo version 10.0 (ESRI Inc.), and the calculations of M_{av} were carried out with the statistical software environment *R* version 2.15.2 (R Core Team 2012) with packages *sp* (Pebesma *et al.* 2013) and *rgeos* (Bivand *et al.* 2013).

We also used *R* to test for differences in genetic diversity between gene pools, performing an analysis of variance (ANOVA) and Tukey's *post-hoc* test for pair-wise group differences, as well as for all linear regression analyses throughout the study. Normal distribution of residuals was tested and model fits of regression analyses were verified visually in each analysis. To test the marginality indices for their consistency, a correlation matrix (*R* package fAssets; Würtz 2012) was analysed. Additionally, regression models with genetic diversity (*Br*) were calculated and compared.

RESULTS

Large-scale differentiation patterns

The three types of cluster analyses yielded largely congruent results, indicating the existence of four or five gene pools. The Bayesian *Structure* analysis revealed $K = 2$ gene pools (Fig. S1), with all populations of Central Europe, except two populations from NE Germany (#28, #32), forming one cluster and all others the second cluster (Fig. S2). However, local maxima of $L'(K)$ and ΔK (Fig. S1) suggested the

presence of four gene pools ($K = 4$), which had high prevalence in Iberian populations, in S Germany, in N Germany and in Eastern Europe (Fig. S3). A high level of individual admixture was detected, especially in populations from the Atlantic Coast and some N German populations, where no distinct assignment to a regional cluster was obvious (Figs S2, S3).

The *k*-means analysis resulted in five clusters that best described genetic structure of *C. canescens* (Fig. 1). The clusters resemble those inferred by *Structure*; however, an additional cluster was identified along the Atlantic Coast. In the DAPC analysis the 13 retained PCs accounted for 37.4% conserved variation out of the AFLP dataset. A clear association between individuals of the N German and the S German populations was obvious in the scatterplots, as well as between individuals from Eastern European and Atlantic Coast populations (Fig. 2). The individuals of the Portuguese cluster were the most differentiated, with only slight association to the Atlantic Coast individuals.

The *Geneland* analyses revealed mean posterior probabilities for the 20 runs from $P = –27,113$ to $P = –28,078$. In the most likely solution, the posterior density distribution indicated five clusters (Fig. S4), which resembled the results of the *Structure* and *k*-means approaches. Again, the Portuguese populations formed cluster IP – Iberian Peninsula, the populations along the Atlantic Coast region plus the population from Spain made up cluster CA – Coastal Areas, N German (cluster NG) and S German (cluster SG) populations were clearly separated, and finally the eastern populations formed cluster EE – Eastern Europe. In contrast to previous analyses, populations 28 and 32 from N Germany were included in the CA cluster.

Genetic differentiation was moderate with $F_{ST} = 0.136$ ($P < 0.0001$) among populations and $F_{ST} = 0.105$ ($P < 0.0001$) among the five gene pools revealed in the *Geneland* analysis. Population differentiation among populations was highest within cluster IP, intermediate in NG and EE, and lowest in CA and SG (Table 1). Hierarchical partitioning of molecular variance (AMOVA) revealed the highest proportion of variance within populations (75.2%; $P < 0.0001$) and lower proportions among populations (11.8%; $P < 0.0001$) or among gene pools (13.0%; $P \leq 0.0001$; see Table S2). Pair-wise genetic differentiation was significantly correlated to geographic distance, as revealed in the Mantel test for all sites ($R = 0.574$, $P = 0.0001$; Fig. 3), demonstrating a large-scale isolation-by-distance pattern among *C. canescens* populations.

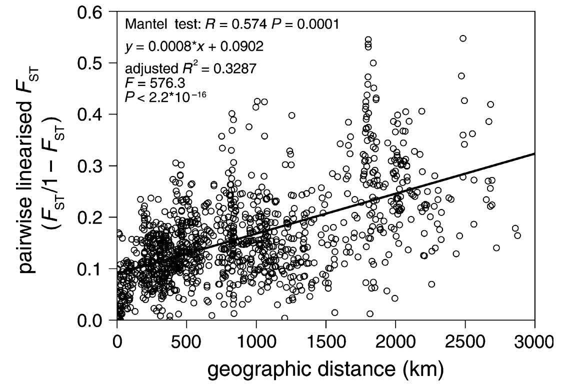

Fig. 3. Correlation of pair-wise geographic distance and pair-wise linearised F_{ST} between populations.

Genetic variation and marginality

Measures of genetic diversity at the population level are given in Table S1. Nei's gene diversity (H_j) ranged from 0.182 (population #47, Martinka, Hungary) to 0.305 (#40, Mandlesmühle, S Germany). However, even though the two highest values of H_j were found in south German populations, the majority of higher values were found in populations of the western parts of the sampling range. A similar geographic trend was observed for Band richness (*Br*), which ranged from 1.234 (#47, Martinka, Hungary) to 1.470 (#7, Contis Plage, France) and was negatively correlated both to longitude and latitude (Fig. 4). Likewise, percentage of polymorphic loci was lowest in a Hungarian population (#47, Martinka: $PLP_{5\%} = 0.307$) and highest in a French population (#10, Ile de Normoutier: 0.620). The only populations with private alleles were Mira (#3) and Figueira da Foz (#4) in Portugal.

When populations were grouped into clusters, genetic diversity was significantly higher in the western gene pools (IP and CA) than in the more eastern ones (NG, SG and EE), which was more obvious for *Br* than for H_j and not true for $PLP_{5\%}$ (Table 1).

The indices of marginality were highly correlated to each other (Pearson's $r \geq 0.8$, $P < 0.001$; Fig. S5), and all of them were significantly negatively correlated to genetic diversity *Br* ($r \geq -0.33$, $P < 0.05$), with M_{r100} and M_{min} showing the highest correlations (both: $r = 0.381$ and $P < 0.01$). Genetic variation was not related to continentality (adj. $R^2 = -0.006$, $F = 0.74$, $P = 0.395$).

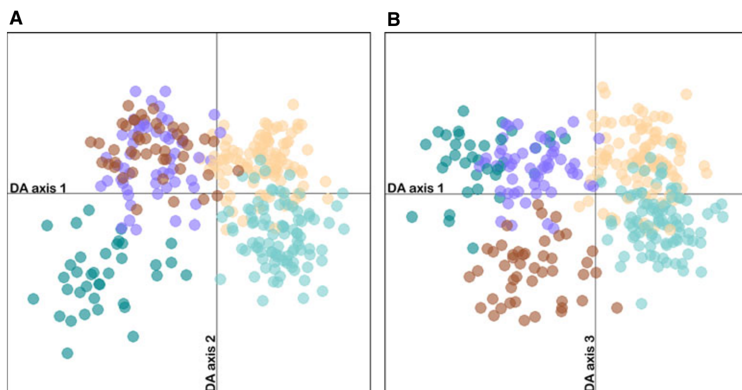

Fig. 2. Ordination plots from the Discriminant Analysis of Principal Components (DAPC) corresponding to Fig. 1. The first two (A) and the first and third (B) axes of discriminant functions are shown. Each point represents one individual distributed along the two linear discriminants. Thirteen principal components were retained to obtain adequate discrimination of clusters, based on *a*-score optimisation.

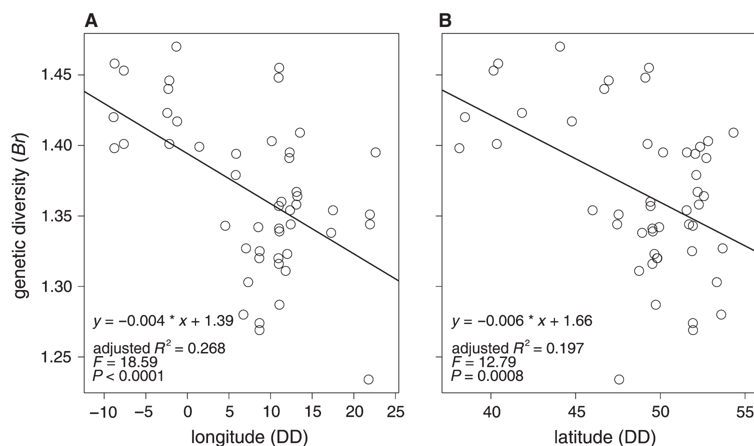

Fig. 4. Relationship between genetic diversity of populations measured as band richness (*Br*) and their (A) longitude and (B) latitude coordinates shown in decimal degrees (DD).

In simple linear regression analyses, genetic diversity *Br* was weakly related to marginality indices M_{r50} (adj. $R^2 = 0.087$, $P = 0.022$), M_{r200} (adj. $R^2 = 0.090$, $P = 0.021$) and M_{av} (adj. $R^2 = 0.092$, $P = 0.019$), and more strongly to M_{r100} (adj. $R^2 = 0.127$, $P = 0.0069$) and M_{min} (adj. $R^2 = 0.127$, $P = 0.0069$; see Fig. 5). In a multiple linear regression including longitude, latitude and marginality M_{min} and their interactions as predictors of genetic diversity, *Br* yielded a significant correlation (adj. $R^2 = 0.424$, $F = 6.898$, $P < 0.0001$) with all variables and interactions significant (Table S3). In particular, marginality (M_{min}) had a negative effect on genetic diversity after accounting for longitude and latitude. In similar multiple regressions analyses additionally including the other marginality indices, none of the latter were included in the respective final models after accounting for longitude and latitude (data not shown).

DISCUSSION

Re-colonisation patterns

We found a moderate but clear genetic differentiation between gene pools of the Iberian Peninsula, French Atlantic Coast, N Germany, S Germany and Eastern Europe. Genetic diversity was highest in populations on the Iberian Peninsula and along the Atlantic Coast and showed a significant decrease from the SW to the NE of Europe. This genetic structure suggests that *C. canescens* persisted in SW Europe during the last glacial maximum and that re-colonisation took place from these refugia into E and NE regions of Europe during Holocene climate warming, supporting our first hypothesis. Similar genetic structure and post-glacial re-colonisations from the Iberian Peninsula towards Central and Northern Europe were also suggested for other plant species with Atlantic distributions (*Festuca pratensis* Fjellheim *et al.* 2006; *Corylus avellana* Palmé & Vendramin 2002; *Hedera* sp. Grivet & Petit 2002; *Ilex aquifolium* Rendell & Ennos 2003; *Ceratocapnos claviculata* Voss *et al.* 2012).

Three genetic patterns typically arise in such refugium–re-colonisation systems. First, diversity and population differentiation was highest in the putative refugial Iberian gene pool. This indicates accumulation of genetic population divergence among different micro-refugia over long periods of local persistence, which can be assumed for glacial refugia (Hewitt 1996; Petit *et al.* 2003; Schmitt 2007). The Iberian Peninsula as a glacial refugium has been shown for other plant species as facilitative for local population differentiation (*e.g.* Hampe *et al.* 2003; Jiménez-Mejías *et al.* 2012). However, for *C. canescens* it is not clear whether the Iberian Peninsula represents the only refugium. The French Atlantic Coast CA gene pool was clearly separated in some analyses, and showed similar or higher genetic diversity than the more southern IP gene pool. This potentially indicates that the French Atlantic Coast served as refugium and as source for re-colonisation, similar to *Himantoglossum hircinum* (Pfeifer *et al.* 2009). The presence and local spread of the CA gene pool during Holocene climate warming might have prevented a northward expansion of Iberian populations. Additionally, individuals of S Germany were differentiated from all but the north German gene pool and showed disproportionally high local genetic diversity. This may point to a discontinuous pattern of gene pool relatedness from the SW to the NE of Europe and contradicts the proposed unidirectional re-colonisation, and thus might indicate an additional refugium in S Germany. Palynological evidence from Central Europe suggests long-term persistence of herbaceous temperate species between northern and alpine ice sheets throughout the last glaciation (*e.g.* Bos *et al.* 2001; Müller *et al.* 2003). However, definite inferences on the origin of the south German gene pool cannot be made due to a lack of information from south and central France, which also might have acted as source regions for Central European populations.

Second, decreasing genetic diversity towards areas of re-colonisation is a common pattern of stepping stone-like or leptokurtic range expansions (Ibrahim *et al.* 1996) characteristic for species with narrow habitat requirements (like *C. canescens*) and species capable of long-distance dispersal (Schmitt 2007). Decreasing genetic diversity along colonisation routes was accordingly found in plant species like *Fagus sylvatica* (Comps *et al.* 2001), *Fraxinus excelsior* (Heuertz *et al.* 2004), *Corrigiola litoralis* (Durka 1999) and *Ceratocapnos claviculata* (Voss *et al.* 2012).

Third, the differentiation into regional gene pools found in *C. canescens* potentially reflects sequential founder effects and genetic drift along the re-colonisation routes (Hewitt 1996; Ibrahim *et al.* 1996). The range of *C. canescens* is structured into larger areas due to physical barriers (*e.g.* Pyrenean mountains) and geological conditions like the absence of suitable sandy habitats. Thus, gene flow will likely be restricted across such barriers, facilitating the build-up of regional gene

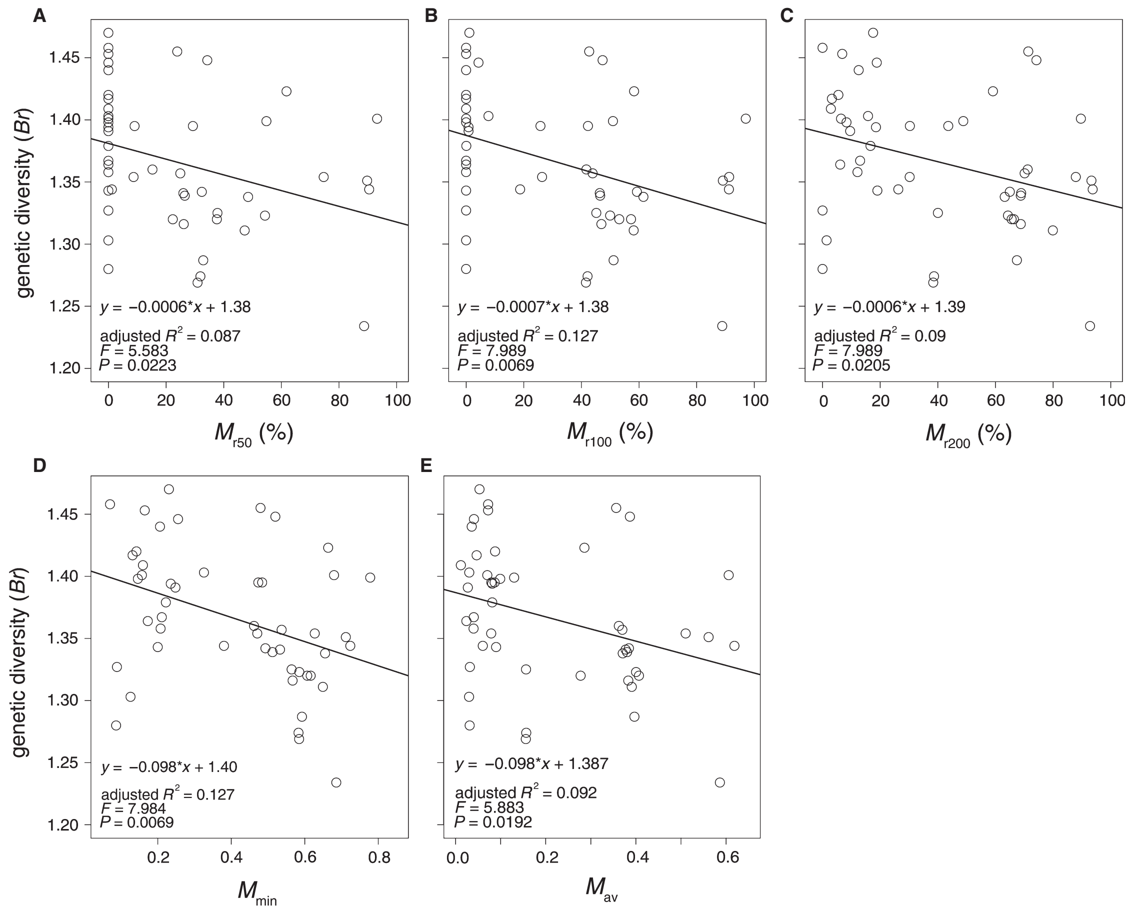

Fig. 5. Relationship between genetic diversity of populations measured as band richness (*Br*) and different marginality indices: areal marginality M_r for radii (A) 50 km, (B) 100 km, (C) 200 km, (D) minimum distance marginality M_{min}, (E) average distance marginality M_{av}.

pools. Potentially also mutations arising in the leading edge of advancing populations may contribute to the generation of new lineages during range expansions through effects of gene surfing, due to their preferential contribution in subsequently colonised areas (Klopfstein *et al.* 2005; Hallatschek & Nelson 2008; Excoffier *et al.* 2009). In addition to neutral processes, different selection pressures in different biogeographic and phylogeographic areas are expected to lead to regional genetic adaptation, which will eventually be detectable using molecular markers (Frei *et al.* 2012). Although gene pools form rather coherent areas across the range of *C. canescens*, some populations, notably in N Germany (#28, #32) are assigned to geographically more distant clusters, which might indicate possible long-distance dispersal.

Marginality

Populations of *C. canescens* in marginal areas showed reduced genetic diversity (*Br*), generally confirming our hypothesis of genetic drift and founder effects in marginal populations. This result is in accordance with the large majority of other studied species (see Eckert *et al.* 2008). However, although a consensus emerged that continuous measures of marginality are preferable to categorical definitions (Eckert *et al.* 2008; Abeli *et al.* 2014), various parameters have been used. In many studies, marginality is measured as distance to the range centre (*e.g.* Pfeifer *et al.* 2009; Holliday *et al.* 2012; Dixon *et al.* 2013; Lira-Noriega & Manthey 2014). However, for species with discontinuous and scattered ranges, like *C. canescens*, centrality-related measures may be misleading. We used three marginality indices taking into account distance to, or area outside, the range border. The marginality indices were highly correlated with each other and with genetic diversity, suggesting that they are useful for marginality analyses in discontinuous range distributions. Nevertheless, the different marginality measures may fit to specific cases depending on the shape and size of the distributional range. Distance to next range border (equivalent to M_{min}) has also been used in other studies (*e.g.* Schwartz *et al.* 2003; Hoban *et al.* 2010) and was preferred here due to its simple calculation without any assumptions. However, M_{min} may overestimate marginality when small, unoccupied enclaves or narrow range gaps in the proximity of a study site represent unsuitable azonal or extrazonal environments rather than large-scale, *i.e.* mainly climatic, range limits. In such cases the unoccupied area in the vicinity of a site (M_r) may represent a suitable estimate of marginality for discontinuous distributions. However, there is no straightforward value of the buffer radius around a sampling site used to calculate M_r without prior analyses of specific gene flow distances. Lastly, we employed the standardised mean distance to the range edges in multiple geographic directions (M_{av}) to avoid potential biases of the other marginality measures. This includes unbiased

distances without any pre-assumptions and should account well for complex range shapes. Nevertheless, M_{av} showed a weaker association to genetic diversity than M_{min} and M_{r100}. This may be caused by a dominating effect of the size of the hinterland, resulting in different M_{av} values for sites at the same distance to the edge.

Marginality, however, had only a moderate effect on genetic diversity in univariate analyses compared to a model including marginality, longitude, latitude and their interactions. This model accounts for both marginality and the direction and distance of range expansion. Including historic, *e.g.* post-glacial, colonisation patterns into studies of genetic diversity patterns along marginality gradients has been suggested previously (*e.g.* Eckert *et al.* 2008; Pfeifer *et al.* 2009; Hoban *et al.* 2010; Guo 2012). Thus, a more realistic model of genetic diversity along the core–periphery gradient is obtained by integrating effects of large-scale, long-term re-colonisation and of locally acting processes among spatially structured populations.

The proposed effect of continentality on genetic diversity within populations of *C. canescens* was not found. Probably, large-scale colonisation histories and local availability of open sandy substrate override potential gradual effects of continentality on environmental suitability.

Gene flow and disturbance

We found moderate genetic differentiation and a significant pattern of isolation-by-distance among populations of *C. canescens*, which reflects a gene flow–drift equilibrium. This is in contrast to other outcrossing grass species with more common and continuous habitat types, in which isolation-by-distance is weak (*e.g. Elymus athericus* Bockelmann *et al.* 2003; *Arrhenatherum elatius* Michalski *et al.* 2010) or lacking (*e.g. Festuca arundinacea* ssp. *arundinacea* Sharifi Tehrani *et al.* 2009; *Alopecurus myosuroides* Menchari *et al.* 2007; but see also Larson *et al.* 2004). Gene flow is expected to be high within and among populations of *C. canescens* as it is wind-pollinated and outcrossing with non-specialised seed dispersal. However, its life history and habitat requirements make it susceptible to genetic drift. First, its populations are spatially isolated from each other due to scattered distribution of sandy habitat. This limits gene flow and facilitates differentiation between populations, as has been found for other habitat specialist grasses in Central Europe (Wagner *et al.* 2011; Durka *et al.* 2013). Second, due to the dynamic character of its habitats, *C. canescens* may undergo metapopulation dynamics with disturbance-driven opening of sandy substrate sites, colonisation by founder propagules, followed by vegetation succession, competition-driven declines and population bottlenecks or local extinctions (Jentsch *et al.* 2002; Tschöpe & Tielbörger 2010) resulting in drift effects (Banks *et al.* 2013). Moreover, this species is a short-lived perennial, having a short-lived soil seed bank (Jentsch 2001), both contributing to high temporal population dynamics potentially resulting in reduction of effective population size. However, any such drift effects had only minor effects as, despite a W–E decline in genetic variation, high genetic variation was maintained in at least some populations throughout the species range.

A few shortcomings to our analysis must not be neglected. First, the geographic sample did not include central and S France and parts of NE Europe. While additional sampling would not fundamentally change the overall pattern, such additions would *e.g.* allow drawing a more detailed picture of the relationships of the south German gene pool to France. Second, we used AFLP as a marker system, which typically has error rates of about 3% (Bonin *et al.* 2004). Genotyping errors might partly account for both observed levels of genetic variation within and between populations. Third, homoplasy cannot be totally excluded in AFLP (Mechanda *et al.* 2004), which likely would lead to an underestimation of genetic differentiation between populations. Thus, additional analyses with alternative marker systems such as chloroplast DNA and/or co-dominant nuclear SNPs (McCormack *et al.* 2013; Senn *et al.* 2014) will improve our understanding of the phylogeography of *Corynephorus canescens*.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Assessment of the most likely number of clusters of *Structure* runs using the method of Evanno *et al.* (2005).

Figure S2. Results of the *Structure* analyses (barplot).

Figure S3. Geographical map showing the results of the Structure analysis.

Figure S4. Results of the Geneland analysis.

Figure S5. Correlation matrix for marginality measures and Band richness.

Table S1. List of analysed populations with sampling information and descriptive parameters of genetic variation.

Table S2. Hierarchical partitioning of molecular variance (AMOVA) with gene pool assignments based on the *Geneland* clustering results.

Table S3. Result table of the regression analysis (linear model) for the dependence of genetic diversity (*Br*) on the population location latitude, longitude and areal marginality, as well as their interactions, done with the statistical software *R*.

REFERENCES

Abeli T., Gentili R., Mondoni A., Orsenigo S., Rossi G., Burns K.C. (2014) Effects of marginality on plant population performance. *Journal of Biogeography*, **41**, 239–249.

Albers F. (1980) Comparative karyology of the grass subtribes aristaveninae and airinae (Poaceae – Aveneae). *Plant Systematics and Evolution*, **136**, 137–167.

Banks S.C., Cary G.J., Smith A.L., Davies I.D., Driscoll D.A., Gill A.M., Lindenmayer D.B., Peakall R. (2013) How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, **28**, 670–679.

Bhagwat S.A., Willis K.J. (2008) Species persistence in northerly glacial refugia of Europe: a matter of chance or biogeographical traits? *Journal of Biogeography*, **35**, 464–482.

Bivand R., Rundel C., Pebesma E., Hufthammer K.O. (2013) rgeos: Interface to Geometry Engine – Open Source (GEOS) version 0.3-6. (Available on-line)

Bockelmann A.-C., Reusch T.B.H., Bijlsma R., Bakker J.P. (2003) Habitat differentiation vs. isolation-by-distance: the genetic population structure of *Elymus athericus* in European salt marshes. *Molecular Ecology*, **12**, 505–515.

Böger S. (2007) Ausbreitung, Etablierung und Populationsgenetik des Silbergrases (*Corynephorus canescens* (L.) P.Beauv.) als Grundlage zur Konzeption eines überregionalen Biotopverbundes für Sandmagerrasen in Deutschland. Dissertation (not published), Bayreuth, Germany: 217 pp.

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

Bos J.A.A., Bohncke S.J.P., Kasse C., Vandenberghe J. (2001) Vegetation and climate during the Weichselian Early Glacial and Pleniglacial in the Niederlausitz, eastern Germany – macrofossil and pollen evidence. *Journal of Quaternary Science*, **16**, 269–289.

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

Bundesamt für Naturschutz (2006) FloraWeb: Daten und Informationen zu Wildpflanzen und zur Vegetation Deutschlands. Available from http://www.floraweb.de (accessed 25th April 2013).

Cheddadi R., de Beaulieu J.-L., Jouzel J., Andrieu-Ponel V., Laurent J.-M., Reille M., Raynaud D., Bar-Hen A. (2005) Similarity of vegetation dynamics during interglacial periods. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 13939–13943.

Coart E., van Glabeke S., Petit R.J., van Bockstaele E., Roldán-Ruiz I. (2005) Range wide versus local patterns of genetic diversity in hornbeam (*Carpinus betulus* L.). *Conservation Genetics*, **6**, 259–273.

Comes H.P., Kadereit J.W. (1998) The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science*, **3**, 432–438.

Comps B., Gömöry D., Letouzey J., Thiébaut B., Petit R.J. (2001) Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European Beech. *Genetics*, **157**, 389–397.

Dixon A.L., Herlihy C.R., Busch J.W. (2013) Demographic and population-genetic tests provide mixed support for the abundant centre hypothesis in the endemic plant *Leavenworthia stylosa*. *Molecular Ecology*, **22**, 1777–1791.

Durka W. (1999) Genetic diversity in peripheral and subcentral populations of *Corrigiola litoralis* L. (Illecebraceae). *Heredity*, **83**, 476–484.

Durka W., Nossol C., Welk E., Ruprecht E., Wagner V., Wesche K., Hensen I. (2013) Extreme genetic depauperation and differentiation of both populations and species in Eurasian feather grasses (*Stipa*). *Plant Systematics and Evolution*, **299**, 259–269.

Earl D.A., vonHoldt B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

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

Ellenberg H., Leuschner C. (2010) *Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht*, 6th edition. Ulmer, Stuttgart, Germany, 1333 pp.

Evanno G., Regnaut S., Goudet J. (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L., Foll M., Petit R.J. (2009) Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 481–501.

Falush D., Stephens M., Pritchard J.K. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Falush D., Stephens M., Pritchard J.K. (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.

Fjellheim S., Rognli O.A., Fosnes K., Brochmann C. (2006) Phylogeographical history of the widespread meadow fescue (*Festuca pratensis* Huds.) inferred from chloroplast DNA sequences. *Journal of Biogeography*, **33**, 1470–1478.

Frei E.S., Scheepens J.F., Armbruster G.F.J., Stöcklin J. (2012) Phenotypic differentiation in a common garden reflects the phylogeography of a widespread Alpine plant. *Journal of Ecology*, **100**, 297–308.

Frey W., Hensen I. (1995) *Spergulo morisonii-Corynephoretum canescentis* (Frühlingsspark-Silbergrasflur) – Lebensstrategien von Binnendünen- und Lockersandbesiedlern. *Feddes Repertorium*, **106**, 533–553.

Gerst K.L., Angert A.L., Venable D.L. (2011) The effect of geographic range position on demographic variability in annual plants. *Journal of Ecology*, **99**, 591–599.

Gorczyński L. (1922) The calculation of the degree of continentality. *Monthly Weather Review*, **50**, 370.

Grivet D., Petit R.J. (2002) Phylogeography of the common ivy (*Hedera* sp.) in Europe: genetic differentiation through space and time. *Molecular Ecology*, **11**, 1351–1362.

Guillot G., Estoup A., Mortier F., Cosson J.F. (2005a) A spatial statistical model for landscape genetics. *Genetics*, **170**, 1261–1280.

Guillot G., Mortier F., Estoup A. (2005b) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Guillot G., Renaud S., Ledevin R., Michaux J.R., Claude J. (2012) A unifying model for the analysis of phenotypic, genetic, and geographic data. *Systematic Biology*, **61**, 897–911.

Guo Q. (2012) Incorporating latitudinal and central–marginal trends in assessing genetic variation across species ranges. *Molecular Ecology*, **21**, 5396–5403.

Hallatschek O., Nelson D.R. (2008) Gene surfing in expanding populations. *Theoretical Population Biology*, **73**, 158–170.

Hampe A., Petit R.J. (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**, 461–467.

Hampe A., Arroyo J., Jordano P., Petit R.J. (2003) Rangewide phylogeography of a bird-dispersed Eurasian shrub: contrasting Mediterranean and temperate glacial refugia. *Molecular Ecology*, **12**, 3415–3426.

Hegi G., Conert H.J. (1998) *Illustrierte Flora von Mitteleuropa: Spermatophyta: angiospermae: monocotyledones 1 (2)*, 3rd edition, Band 1 Teil 3. Weissdorn, Jena, Germany, 982 pp.

Hengeveld R., Haeck J. (1982) The distribution of abundance. I. Measurements. *Journal of Biogeography*, **9**, 303–316.

Heuertz M., Fineschi S., Anzidei M., Pastorelli R., Salvini D., Paule L., Frascaria-Lacoste N., Hardy O.J., Vekemans X., Vendramin G.G. (2004) Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology*, **13**, 3437–3452.

Hewitt G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

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

Hewitt G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hewitt G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **359**, 183–195.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hoban S.M., Borkowski D.S., Brosi S.L., McCleary T.S., Thompson L.M., McLachlan J.S., Pereira M.A., Schlarbaum S.E., Romero-Severson J. (2010) Range-wide distribution of genetic diversity in the North American tree *Juglans cinerea*: a product of range shifts, not ecological marginality or recent population decline. *Molecular Ecology*, **19**, 4876–4891.

Holliday J.A., Suren H., Aitken S.N. (2012) Divergent selection and heterogeneous migration rates across the range of Sitka spruce (*Picea sitchensis*). *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1675–1683.

Holt R.D., Keitt T.H., Lewis M.A., Maurer B.A., Taper M.L. (2005) Theoretical models of species' borders: single species approaches. *Oikos*, **108**, 18–27.

Huff D., Peakall R., Smouse P. (1993) RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. *Theoretical and Applied Genetics*, **86**, 927–934.

Ibrahim K.M., Nichols R.A., Hewitt G.M. (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.

Jakobsson M., Rosenberg N.A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Jentsch A. (2001) The significance of disturbance for vegetation dynamics: a case study in dry acidic grasslands. Dissertation, Bielefeld, Germany: 199 pp.

Jentsch A., Beyschlag W. (2003) Vegetation ecology of dry acidic grasslands in the lowland area of Central Europe. *Flora*, **198**, 3–25.

Jentsch A., Friedrich S., Beyschlag W., Nezadal W. (2002) Significance of ant and rabbit disturbances for seedling establishment in dry acidic grasslands dominated by *Corynephorus canescens*. *Phytocoenologia*, **32**, 553–580.

Jentsch A., Friedrich S., Steinlein T., Beyschlag W., Nezadal W. (2009) Assessing conservation action for substitution of missing dynamics on former military training areas in Central Europe. *Restoration Ecology*, **17**, 107–116.

Jiménez-Mejías P., Luceño M., Lye K.A., Brochmann C., Gussarova G. (2012) Genetically diverse but

with surprisingly little geographical structure: the complex history of the widespread herb *Carex nigra* (Cyperaceae). *Journal of Biogeography*, **39**, 2279–2291.

Jombart T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.

Jombart T., Devillard S., Balloux F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94–108.

Kelly A., Charman D.J., Newnham R.M. (2010) A Last Glacial Maximum pollen record from Bodmin Moor showing a possible cryptic northern refugium in southwest England. *Journal of Quaternary Science*, **25**, 296–308.

Klopfstein S., Currat M., Excoffier L. (2005) The fate of mutations surfing on the wave of a range expansion. *Molecular Biology and Evolution*, **23**, 482–490.

Kloss L., Fischer M., Durka W. (2011) Land-use effects on genetic structure of a common grassland herb: a matter of scale. *Basic and Applied Ecology*, **12**, 440–448.

Larson S.R., Jones T.A., Jensen K.B. (2004) Population structure in *Pseudoroegneria spicata* (Poaceae: Triticeae) modeled by Bayesian clustering of AFLP genotypes. *American Journal of Botany*, **91**, 1789–1801.

Lawton J.H. (1993) Range, population abundance and conservation. *Trends in Ecology & Evolution*, **8**, 409–413.

Lira-Noriega A., Manthey J.D. (2014) Relationship of genetic diversity and niche centrality: a survey and analysis. *Evolution*, **68**, 1082–1093.

Magri D., Vendramin G.G., Comps B., Dupanloup I., Geburek T., Gömöry D., Latalowa M., Litt T., Paule L., Roure J.M., Tantau I., van der Knaap W.O., Petit R.J., de Beaulieu J.-L. (2006) A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist*, **171**, 199–221.

Marshall J.K. (1965) *Corynephorus canescens* (L.) P. Beauv. as a model for the *Ammophila* problem. *Journal of Ecology*, **53**, 447–463.

Marshall J.K. (1967) Biological flora of the British Isles – *Corynephorus canescens* (L.) Beauv. *Journal of Ecology*, **55**, 207–220.

Marshall J.K. (1968) Factors limiting the survival of *Corynephorus canescens* (L.) Beauv. in Great Britain at the northern edge of its distribution. *Oikos*, **19**, 206–216.

McCormack J.E., Hird S.M., Zellmer A.J., Carstens B.C., Brumfield R.T. (2013) Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution*, **66**, 526–538.

Mechanda S.M., Baum B.R., Johnson D.A., Arnason J.T. (2004) Sequence assessment of co-migrating AFLP™ bands in Echinacea – implications for comparative biological studies. *Genome*, **47**, 15–25.

Menchari Y., Délye C., Le Corre V. (2007) Genetic variation and population structure in black-grass (*Alopecurus myosuroides* Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. *Molecular Ecology*, **16**, 3161–3172.

Michalski S.G., Durka W., Jentsch A., Kreyling J., Pompe S., Schweiger O., Willner E., Beierkuhnlein C. (2010) Evidence for genetic differentiation and divergent selection in an autotetraploid forage grass (*Arrhenatherum elatius*). *Theoretical and Applied Genetics*, **120**, 1151–1162.

Müller U.C., Pross J., Bibus E. (2003) Vegetation response to rapid climate change in Central Europe during the past 140,000 yr based on evidence from the Füramoos pollen record. *Quaternary Research*, **59**, 235–245.

Naydenov K., Senneville S., Beaulieu J., Tremblay F., Bousquet J. (2007) Glacial vicariance in Eurasia: mitochondrial DNA evidence from Scots pine for a complex heritage involving genetically distinct refugia at mid-northern latitudes and in Asia Minor. *BMC Evolutionary Biology*, **7**, 233–244.

Palmé A., Vendramin G.G. (2002) Chloroplast DNA variation, postglacial recolonization and hybridization in hazel, *Corylus avellana*. *Molecular Ecology*, **11**, 1769–1779.

Peakall R., Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pebesma E., Bivand R., Rowlingson B., Gomez-Rubio V. (2013) sp: Classes and methods for spatial data version 1.0-15. (Available on-line)

Petit R.J., Aguinagalde I., de Beaulieu J.-L., Bittkau C., Brewer S., Cheddadi R., Ennos R.A., Fineschi S., Grivet D., Lascoux M., Mohanty A., Müller-Starck G.M., Demesure-Musch B., Palmé A., Martín J.P., Rendell S., Vendramin G.G. (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

Pfeifer M., Schatz B., Picó F.X., Passalacqua N.G., Fay M.F., Carey P.D., Jeltsch F. (2009) Phylogeography and genetic structure of the orchid *Himantoglossum hircinum* (L.) Spreng. across its European central–marginal gradient. *Journal of Biogeography*, **36**, 2353–2365.

Pritchard J.K., Stephens M., Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Quinger B., Meyer N. (1995) *Lebensraumtyp Sandrasen*. Landschaftspflegekonzept Bayern, München, Germany, 253 pp.

R Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rendell S., Ennos R.A. (2003) Chloroplast DNA diversity of the dioecious European tree *Ilex aquifolium* L. (English holly). *Molecular Ecology*, **12**, 2681–2688.

Riecken U., Finck P., Raths U., Schröder E., Ssymank A. (2006) *Rote Liste der gefährdeten Biotoptypen Deutschlands: zweite fortgeschriebene Fassung 2006*. Naturschutz und Biologische Vielfalt, Germany, 318 pp.

Rosenberg N.A. (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

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

Rychnovská M. (1963) An outpost site of *Corynephorus canescens* in the region between the Danube and the Tisza and its causal explanation. *Acta Biologica Hungarica*, **14**, 57–66.

Schmitt T. (2007) Molecular biogeography of Europe: pleistocene cycles and postglacial trends. *Frontiers in Zoology*, **4**, 11–23.

Schulz I. (1950) *Studien zur Gattung* Corynephorus. Dissertation (not published), Berlin, Germany: 106 pp.

Schwartz M.K., Mills L.S., Ortega Y., Ruggiero L.F., Allendorf F.W. (2003) Landscape location affects genetic variation of Canada lynx (*Lynx canadensis*). *Molecular Ecology*, **12**, 1807–1816.

Senn H., Ogden R., Frosch C., Syrůčková A., Campbell-Palmer R., Munclinger P., Durka W., Kraus R.H.S., Saveljev A.P., Nowak C., Stubbe A., Stubbe M., Michaux J., Lavrov V., Samiya R., Ulevicius A., Rosell F. (2014) Nuclear and mitochondrial genetic structure in the Eurasian beaver (*Castor fiber*) – implications for future reintroductions. *Evolutionary Applications*, **7**, 645–662.

Sexton J.P., McIntyre P.J., Angert A.L., Rice K.J. (2009) Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 415–436.

Sharifi Tehrani M., Mardi M., Sahebi J., Catalán P., Díaz-Pérez A. (2009) Genetic diversity and structure among Iranian tall fescue populations based on genomic-SSR and EST-SSR marker analysis. *Plant Systematics and Evolution*, **282**, 57–70.

Stewart J.R., Lister A.M., Barnes I., Dalén L. (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 661–671.

Taberlet P., Fumagalli L., Wust-Saucy A.-G., Cosson J.-F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Tschöpe O., Tielbörger K. (2010) The role of successional stage and small-scale disturbance for establishment of pioneer grass *Corynephorus canescens*. *Applied Vegetation Science*, **13**, 326–335.

Ujházy K., Fanta J., Prach K. (2011) Two centuries of vegetation succession in an inland sand dune area, central Netherlands. *Applied Vegetation Science*, **14**, 316–325.

Vekemans X. (2002) *AFLP-SURV*. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Vekemans X., Beauwens T., Lemaire M., Roldán-Ruiz I. (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*, **11**, 139–151.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Voss N., Eckstein R.L., Durka W. (2012) Range expansion of a selfing polyploid plant despite widespread genetic uniformity. *Annals of Botany*, **110**, 585–593.

Vucetich J.A., Waite T.A. (2003) Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics*, **4**, 639–645.

Wagner V., Durka W., Hensen I. (2011) Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass. *American Journal of Botany*, **98**, 1173–1179.

Würtz D. (2012) fAssets: Rmetrics – Assets selection and modelling: R package version 2110.79. (Available on-line)