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Spatial Genetic Structure of the Sedge Carex nigra Reflects Hydrological Conditions in an Alpine Fen

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Abstract

Fine-scale genetic structure of plant populations depends on several ecological processes. In this study, we analyzed the impact of hydrological heterogeneity on the spatial genetic structure of the wind-pollinated black sedge *Carex nigra* in an alpine fen. We performed amplified fragment length polymorphisms (AFLPs) with 111 samples collected along a grid covering the whole area of the fen and studied fine-scale genetic structure using spatial autocorrelation and Bayesian cluster analyses. We observed a significant spatial genetic structure indicating isolation-by-distance, which can be ascribed to restricted seed dispersal. Bayesian cluster analysis revealed four groups of genetically related and patchy distributed samples within the fen. Two of these groups were distributed in the deeper and moister regions of the fen, while the two other groups were spatially restricted to the higher and drier regions of the fen. We think that the observed pattern of spatial genetic variation reflects hydrological heterogeneity within the fen and conclude that the four groups represent cohorts of individuals originating from different recruitment events in different parts of the fen. Genetic variation was much lower in the groups from the drier regions of the fen. Since *Carex* species require moist conditions for germination and establishment, the low level of genetic variation can most likely be ascribed to restricted seedling recruitment in the drier regions of the fen. Habitat heterogeneity affects, therefore, both spatial genetic structure and levels of genetic variation. This study clearly demonstrates that integrating fine-scale genetic analyses with complementary biological data can markedly improve the identification of processes that shape fine-scale genetic structure within plant populations.

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Introduction

In natural plant populations genetic variation normally is distributed in a nonrandom way (Vekemans and Hardy, 2004; Troupin et al., 2006). This fine-scale genetic structure can be related to a set of different factors (Chung et al., 2004; Nakagawa, 2010). Although microgeographical variation may principally also be caused by selection due to different environmental conditions (Hamrick and Allard, 1972), limited pollen and seed dispersal are considered as the most important factors (Hamrick and Nason, 1996; Vekemans and Hardy, 2004). The amount of gene dispersal especially via seeds is a major force shaping the spatial genetic structure within populations while patterns of pollen movement influence the degree of relatedness among individuals. Following the principle of isolation-by-distance, restricted gene dispersal results in the formation of local pedigree structures, which are the basic cause of the spatial genetic structure within populations (Vekemans and Hardy, 2004). The biological traits of plant species have a strong impact on gene dispersal. Consequently, it has been demonstrated that selfing, insect pollinated species, or animal dispersed plant species exhibit a stronger pattern of spatial genetic variation than outcrossing, wind pollinated species, or wind dispersed species (Vekemans and Hardy, 2004).

Besides limited gene dispersal, clonality (Reusch et al., 1999; Jacquemyn et al., 2005) and seedling recruitment (Parker et al., 2001; Cruse-Sanders and Hamrick, 2004; Shimono et al., 2006) have been identified as key factors influencing the fine-scale genetic structure of populations. In clonal species, the genetic similarity should be maximal at short distances as nearby ramets are expected to belong to the same genet. In contrast genetic distances between ramets located farther apart are expected to be larger, because they are likely to originate from different genets (Jacquemyn et al., 2005). Clonally reproducing species are, therefore, expected to have a stronger spatial genetic structure than mainly sexual reproducing species. Additionally it has been demonstrated that the level of clonality observed within populations is clearly affected by the environmental conditions within the habitat (Richards et al., 2004; Jacquemyn et al., 2005).

In recent studies, which linked demographic and spatial genetic data at the individual level to assess the effects of dispersal and establishment, the impact of seedling recruitment as a major demographical filter in the plant life cycle on the spatial genetic structure has been emphasized (Hampe et al., 2010). However, the recruitment of seedlings strongly depends on environmental conditions. Seeds need germination gaps and specific environmental conditions within these gaps to germinate successfully, and the establishment of seedlings also depends on these conditions (Klinkhamer and Jong, 1988; Bakker and Olff, 2003). The spatial arrangement of safe sites in the mostly heterogeneous habitats is, therefore, of outstanding importance for the fine-scale genetic structure within populations and has already been indirectly employed to explain patterns of fine-scale genetic structure (Cruse-Sanders and Hamrick, 2004; Shimono et al., 2006; Troupin et al., 2006). It has been demonstrated that the aggregation of recruits in safe sites often

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results in a marked fine-scale spatial genetic structure (Shimono et al., 2006).

In fens vegetation and population structure profoundly depend on hydrology (Ellenberg, 1988). Water level changes at even marginal differences in topography. This in turn affects ecological conditions, causes a mosaic-like structure of vegetation, and creates microsites, differing in their hydrological conditions, which are suitable or unsuitable gaps for germination of seeds and recruitment of seedlings. Plant species growing in fens are, therefore, particularly suitable to study the impact of heterogeneous environmental conditions on the spatial genetic structure of plant populations. So far, most studies on fine-scale genetic structure referred to trees or herbs (Kalisz et al., 2001; Chung et al., 2003; Jacquemyn et al., 2005; van Rossum and Triest, 2006) growing in more or less dry lowland habitats. In the study presented here, we analyzed for the first time the spatial genetic structure of a herbaceous, wind pollinated, clonal, and alpine species. The black sedge *Carex nigra* (L.) Reichard is widely distributed in European and Siberian mountains and one of the most common sedges in alpine fens. In the study presented here, we used amplified fragment length polymorphisms (AFLPs) combined with autocorrelation analysis and Bayesian clustering to study the impact of environmental heterogeneity at the individual level on the fine-scale genetic structure of *C. nigra*.

Methods

SPECIES DESCRIPTION, SAMPLING LOCATION AND DESIGN

The wind-pollinated and perennial sedge *Carex nigra* (L.) Reichard is native to European and Siberian wetlands (Tutin et al., 1964). Sedges are typically self-compatible (Bertin, 2007) and high selfing rates have recently been shown in several *Carex* species (Friedman and Barrett, 2009). Unfortunately, for our specific study species, the selfing rate has not yet been determined. Seed dispersal proceeds mainly by autochory, but partly by anemochory, hydrochory, or ornithochory (Bonn and Poschlod, 1998). *C. nigra* spreads clonally with rhizomes and grows in fens and wet meadows (Tutin et al., 1964; Adler et al., 1994). The maximum height of the species is about 20 cm. Data about the size of the clones and the seed set of the species are actually not available.

In the study presented here, we analyzed the fine-scale genetic structure of *C. nigra* in an alpine hanging fen, which extends on the flank of an alpine high valley. The fen is located in the central Alps (in the Valley of Stubai near Greitspitze, 47°03′18″N and 11°11′48″E, about 2400 m above sea level). It is dominated by *C. nigra* (about 70% coverage), *Carex canescens*, and *Eriophorum angustifolium*.

The fen faces southeast with a slope of about 8°. The length of the fen is about 35 m and the maximum width about 30 m. While detailed information concerning the water level is not available, we assume that the water level is relatively constant except during the annual snowmelt in early summer. Within the area of the fen topographical differences of about 20 cm occur, resulting in different hydrologic conditions in different parts of the fen, with lower regions being conspicuously moister than higher regions. To study the impact of clonal propagation, gene flow, and environmental heterogeneity on the fine-scale genetic structure, we collected fresh leaf material of 111 *C. nigra* samples along a 2 m vertical and 3 m horizontal grid, which covered the whole surface area of the fen (Fig. 1). Plant material was placed into plastic bags, which were kept in a cool box and later stored at −80 °C.

For each sample the relative topographical level was determined when collecting the leaf material. From these topographical data the surface relief of the fen was displayed cartographically and contour lines were interpolated and displayed using the program ArcGIS V 9.3.1 (Fig. 1).

AFLP ANALYSIS

DNA was isolated from 20 mg frozen leaf material using the CTAB (cetyltriammonium bromide) method (Rogers and Bendich, 1994) with minor variances (Reisch et al., 2005). DNA-concentration was standardized at a dilution of 7.8 ng/μl. AFLP procedure was performed according to the protocol from Beckman Coulter, adapted as described in former studies (Reisch, 2007). Restriction and ligation reaction was conducted with *Mse*I and *Eco*RI restriction enzymes and T4 DNA ligase (Fermentas) at 37 °C for 2 h. Polymerase chain reactions were run in volumes of 5 μl. Preselective primers had a single selective nucleotide (*Mse*I-C and *Eco*RI-A). Selective amplifications were conducted with triple-selective primers. The *Eco*RI primers were labeled black (Beckman dye D2), green (Beckman dye D3), and blue (Beckman dye D4). After a screening of 36 primer combinations performed on 4 samples, the combinations *Mse*I-CAT/*Eco*RI-AGC (D2), *Mse*I-CTA/*Eco*RI-AGG (D3), and *Mse*I-CAA/*Eco*RI-ACA (D4) were selected for further analyses. D3 fluorescence labeled PCR products were diluted 5×, and D4 fluorescence labeled products 6× with TE buffer (1×). The fluorescence labeled selective amplification products were separated according to fragment size by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analyzed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter) and then exported as synthetic gel files. These files were examined for strong and clearly defined bands in the program BIONUMERICS 3.6 (Applied Maths, Kortrijk, Belgium). Each band was scored across all samples as either present or absent. Both samples and fragment sizes that did not give clear and defined bands were excluded from the analysis, and AFLP procedure was repeated. For quality control of AFLPs we replicated PCR procedure for 10% of the samples and calculated a genotyping error rate (Bonin et al., 2004), which was 1.9%.

STATISTICAL ANALYSIS

The statistical analysis comprised 117 AFLP fragments (D2: 51, D3:33, D4:33), of which 94.87% were polymorphic. The presence of a band was scored as 1, the absence of the band was coded as 0 in a binary (0/1) matrix. We used the program GENOTYPE to detect clonality within our data set (Meirmans and van Tienderen, 2004). The software considers individuals with exactly the same multilocus genotypes as representatives of the same clonal lineage. However, genotyping errors may cause small differences between samples from the same clonal lineage, which are then erroneously assigned to different clonal lineages. To avoid this bias a clonal threshold based on the genetic distance between the replicates and the original samples amplified for the calculation of the genotyping

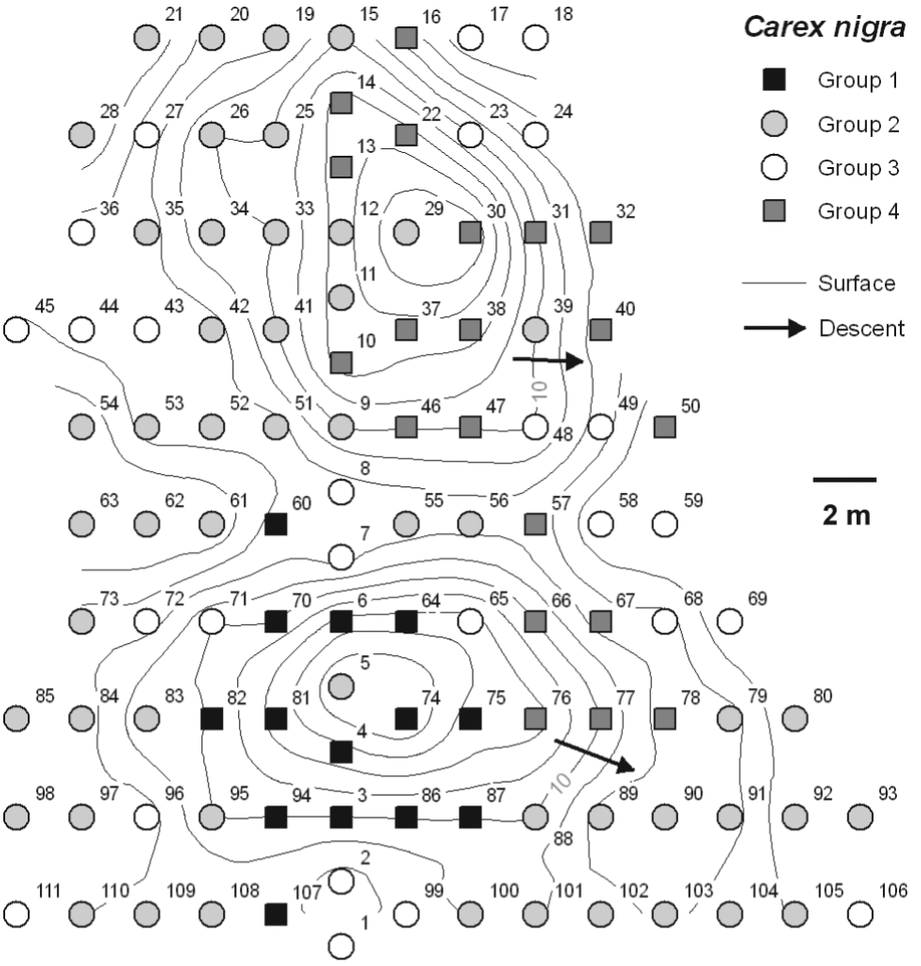

FIGURE 1. Spatial position of the analyzed *Carex nigra* samples and affiliation of the samples (numbered 1–111) to the observed four Bayesian groups, labeled after Table 1. Contour lines indicate topographical differences of 2 cm. Samples were collected along a 2 m vertical and 3 m horizontal grid. In the central strip samples were also collected in 2 m distances, resulting in a shifted spatial position.

error rate, was defined. Therefore, we used the genetic distance following Dice ($D(i_1, i_2) = 2a/2a + b + c$), where a is the number of positions with shared bands for both individuals, b is the number of positions where individual i_1 has a band but i_2 does not, and c is the number of positions where individual i_2 has a band but i_1 does not. In the analyses of clonality using GENOTYPE, samples with a genetic distance less than the threshold (0.06) were considered as belonging to the same clonal lineage. In GENOTYPE histograms of clonal differences were drawn based on Dice distance measures for dominant markers to detect clonality.

Fine-scale spatial genetic structure was assessed in a spatial autocorrelation analysis using the SPAGEDI software (Spatial Genetic Distance) (Hardy and Vekemans, 2002). For autocorrelation analysis we used the estimator of the kinship coefficient for dominant markers Fd_{ij} (Hardy, 2003). When isolation by distance occurs, the multilocus kinship coefficient should decrease approximately linearly with the physical distance between individuals. Average multilocus kinship coefficients were calculated for 11 distance classes (Table 2) and were plotted against distance. Standard errors for the multilocus estimates of the kinship coefficients were estimated using a jackknife procedure over the loci. Jackknife standard errors (SE) provided approximate confidence intervals as ± 2SE (Hardy and Vekemans, 2002). To test for significant spatial genetic structure, the observed regression slope of Fd_{ij} on ln (r_{ij}), b_F was compared with those obtained after 10,000 random permutations of individuals. In this formula ln (r_{ij}) is the natural logarithm of the physical distance between the samples i and j. Since inbreeding levels for *C. nigra* have not yet been reported in other studies, we calculated the inbreeding coefficient (0.22) used for this analysis with the program FAFLPcalc (Dasmahapatra et al., 2008).

Spatial genetic structure was also quantified by the '*Sp*'-statistics, which is calculated as $-b_F/(1 - F_{(1)})$, where $F_{(1)}$ is the mean $F_{(ij)}$ between individuals belonging to the first distance interval, and b_F is the regression slope of $F_{(ij)}$ on ln (r_{ij}) (Hardy, 2003). Group assignment was tested with Bayesian clustering in the program structure version 2.2 (Pritchard et al., 2000). The program implements a model-based clustering method for inferring the structure of a data set using genotype data consisting of unlinked markers. Briefly it is assumed that the data set consists of an unknown number of K groups, each of which is characterized by a set of allele frequencies at each locus. Samples from the data set are assigned probabilistically to groups, or jointly to two or more groups. In this way it can be analyzed with STRUCTURE of how many groups the data set consists.

Allele frequencies were used as correlated within the population in a model of no population admixture (Colling et al., 2010). For each predefined number of K (1–9), 20 iterations were run, and 10^4 iterations for estimating the number of groups with a burn-

TABLE 1

Genetic variation of *Carex nigra* in total and in the four groups obtained from Bayesian clustering measured as percentage of polymorphic loci (*P*), Nei's Gene Diversity (*H*), Shannon's Information Index (*I*), and the symbols used to label the affiliation of the samples to one of the four groups (*sym*). For each group averaged Nei's Gene Diversity (H_{14}) and Shannon Index (I_{14}) based on 6 calculations of 14 randomly selected individuals were calculated. Additionally the topographical level *(TL)* and the water regime *(WR)* are given.

	TL	*WR*	*n*	*P*	*H*	*I*	H_{14}	I_{14}	*sym*
Group 1	11.8	Dry	14	41.88	0.1566	0.2337	0.1566	0.2337	■
Group 2	5.6	Moist	51	77.78	0.3203	0.4693	0.2890	0.4187	●
Group 3	4.2	Moist	26	93.16	0.3924	0.5721	0.3718	0.5396	○
Group 4	10.3	Dry	20	45.30	0.1749	0.2590	0.1608	0.2363	■
All samples	—	—	111	94.87	0.3767	0.5545	—	—	
averages	—	—	—	64.53	0.2611	0.3835	0.2446	0.3571	

in-period of 10^4 were set. Group assignment was further tested in an ad hoc quantity procedure calculating ΔK (Evanno et al., 2005).

Genetic differentiation among assumed groups obtained from the Bayesian clustering was studied by the program GENALEX version 5 (Peakall and Smouse, 2001) with a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992). Genetic distances among pairwise comparisons of Bayesian groups were calculated as PhiPT values. Significance tests were based on 1000 permutations. Genetic variation measures within the detected Bayesian groups were calculated using POPGENE version 1.32 (Yeh et al., 1997): percentage of polymorphic bands (*P*), Nei's Gene Diversity ($H = 1 - \Sigma\, p_i$), and Shannon's Information Index ($I = \Sigma\, p_i\, ln\, p_i$), where p_i is the frequency of a fragment at the locus *i*.

Additionally we repeated the Bayesian cluster analysis and the spatial autocorrelation analysis with an equalized sample size of 14, which corresponds to the size of the smallest group. All calculations were repeated six times with randomly selected individuals, but the results were comparable. In the results section we, therefore, present the full data set analysis. We also calculated the values of genetic variation (H_{14},I_{14}) with 14 randomly selected individuals of each group and iterated the procedure six times.

A neighbor-joining tree was calculated using Cavalli-Sforza & Edward chord distances with the program FAMD (Schlüter and Harris, 2006). The assignment of the analyzed samples to the groups obtained from the Bayesian clustering was displayed graphically to infer the spatial relationship between samples from the same group (Fig. 1). Topographical levels of the individuals within the four groups obtained from the Bayesian cluster analysis were compared using a nonparametric Kruskal-Wallis test in SPSS for Windows.

Results

In this study every analyzed sample of *C. nigra* exhibited a unique pattern of fragments, which indicated even after the application of the clonality threshold a lack of clonality at the spatial level analyzed here. The spatial autocorrelation analysis revealed significant spatial genetic structure. We observed significant positive autocorrelation at the 3 m, 5 m, and 7 m distance intervals. At the 9 m and 11 m distance intervals no significant Fd_{ij} values could be detected. Beyond 13 m a significant negative autocorrelation was found (Fig. 2, Table 2). The slope of the regression between the kinship coefficient and the natural logarithm of the distance between individuals in the first distance class of 3 m, *b*, was 0.0047 with a significance of $p < 0.005$. The kinship coefficient between neighbors ($F_{(1)}$) was 0.144. The *Sp* statistic was 0.0055.

Bayesian cluster analysis resulted in the most likely assignment of the studied samples to four groups with a standard deviation of 6.32 (Fig. 3). Individuals were predominantly assigned to the same group during the 20 iterations (Fig. 4).

The *Sp* statistics for single groups resulted in higher SGS for groups one and four (0.0138 and 0.0133, respectively), lower SGS for groups two and three (0.0083 and 0.0035, respectively).

The analysis of molecular variance (AMOVA) revealed a clear differentiation of 20% among these four groups, while 80% of genetic variation was found within the groups ($p < 0.001$). Phi_{PT}

TABLE 2

Spatial genetic structure of *Carex nigra*. For each of the 11 distance classes maximal distance (m), number of pairwise comparisons, and kinship coefficients *F*(*ij*) are given. Significant correlation ($p < 0.001$) between geographic and genetic distance is marked with an asterisk.

	Maximum distance (m)										
	3	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**	**23**	**35**
No. of pairs	220	437	489	598	666	633	587	634	513	706	622
F(*ij*)	0.144	0.091	0.038	0.008	−0.008	−0.020	−0.026	−0.035	−0.036	−0.031	−0.019
p	*	*	*			*	*	*	*	*	*

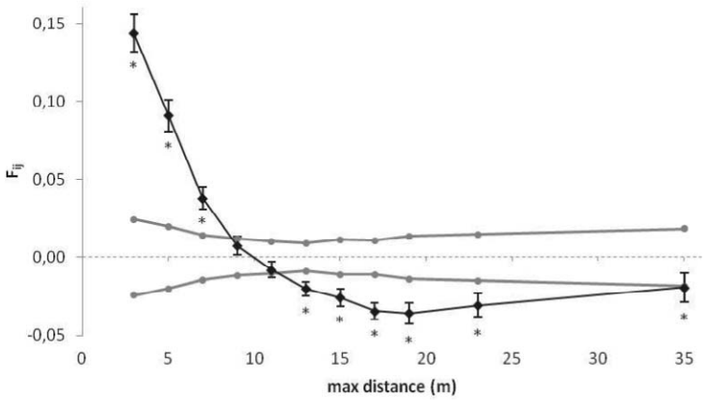

FIGURE 2. Pairwise average kinship and distance between analyzed samples of *C. nigra*. Asterisks indicate significant deviation ($p < 0.001$) from the expectation of random mating according to a randomization test. Approximate 95% confidence intervals based on standard errors of jackknifing procedure over loci are shown for each given distance interval.

values among groups obtained from the Bayesian cluster values were all highly significant with the strongest differentiation among groups one and four and the lowest differentiation among groups two and three (Table 3). This result was affirmed by the Neighbor-joining tree (Fig. 5). Genetic variation within the four groups measured as a percentage of polymorphic bands, Nei's Gene diversity, and Shannon's Information Index was nearly twice as high in groups two and three as in groups one and four (Table 1).Values for whole data set were comparable with the values calculated for the equalized sample size (Table 1).

Plotting the samples on the sampling grid according to their assignment to the four groups obtained in the Bayesian cluster analysis revealed clear differences between these groups. Groups two and three were distributed in the deeper and moister regions of the fen, while groups one and four were spatially restricted to the highest and driest regions of the fen (Fig. 1). Topographical levels of individuals within the four groups differed significantly ($p < 0.001$) in a Kruskal-Wallis test. Mean topographical level in groups two and three were 5.6 ($\pm$ 0.70) cm and 4.2 ($\pm$ 0.76) cm, while the mean topographical level in groups one and four were 11.8 ($\pm$ 1.03) cm and 10.3 ($\pm$ 1.17) cm.

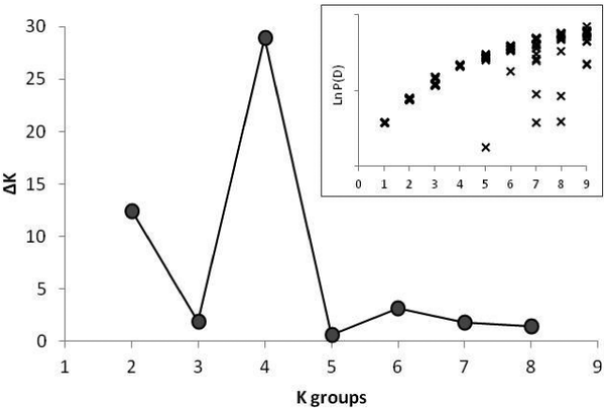

FIGURE 3. Results of 20 runs for 1–15 possible groups to infer population structure with Bayesian clustering in STRUCTURE. ΔK are shown for each of the tested groups $K = 1–9$. The embedded graph shows Ln P(D) variance for each of the tested groups.

Discussion

Sedges like *C. nigra* occurring in wetland and fen habitats typically spread clonally via rhizomes and the occurrence of clonal lineages within sedge populations has already been demonstrated in several investigations (McKlintock and Waterway, 1993; Jonsson et al., 1996; Stenström et al., 2001). In our study, we observed a complete lack of clonality, since every sample exhibited a unique pattern of fragments. This is most likely due to the sampling strategy we applied to collect plant material for molecular analyses. The detection of clonality always depends on the sampling scale, which means that clonal lineages can only be detected when the sampling distance corresponds to the range of clonal propagation with rhizomes or runners (Reisch and Kellermeier, 2007; Reisch et al., 2007). In the case of *C. nigra* samples were taken at a distance of 2 m, which may be too distant from each other to detect clonal lineages. It is, however, likely that clonality occurs at a finer spatial scale.

In this study *C. nigra* exhibited a significant fine-scale genetic structure, indicating isolation-by-distance based on local pedigree structures. The observed kinship coefficient of 0.144 is very close to the expected value of 0.125 for half-sibs (Kalisz et al., 2001). The calculated *Sp* value is comparable to that observed on average for six wind-pollinated species (including trees) in a previous study (Vekemans and Hardy, 2004), although comparisons should be handled with care since the level of self pollination and the life-form can influence *Sp* values. Empirical data indicate that the development of a spatial genetic structure within populations is mainly influenced by seed dispersal (Hamrick and Nason, 1996; Kalisz et al., 2001; Chung et al., 2004) although it is shaped by multiple factors that include colonization history of the population, clonal reproduction, mating system, density of maternal individuals, and thus degree of seed shadow overlap (Hamrick et al., 1993), and successional stage (Troupin et al., 2006; Chung et al., 2007). However, seed dispersal during population founding, as well as subsequent seed dispersal within established populations, are generally the most important determinants of within-population spatial genetic structure (Nason and Hamrick, 1997) and the distance over which spatial genetic structure occurs (Dyer, 2007).

Seed dispersal may, however, not only be conceived as the simple transport of diaspores. Seed dispersal has only an effect on

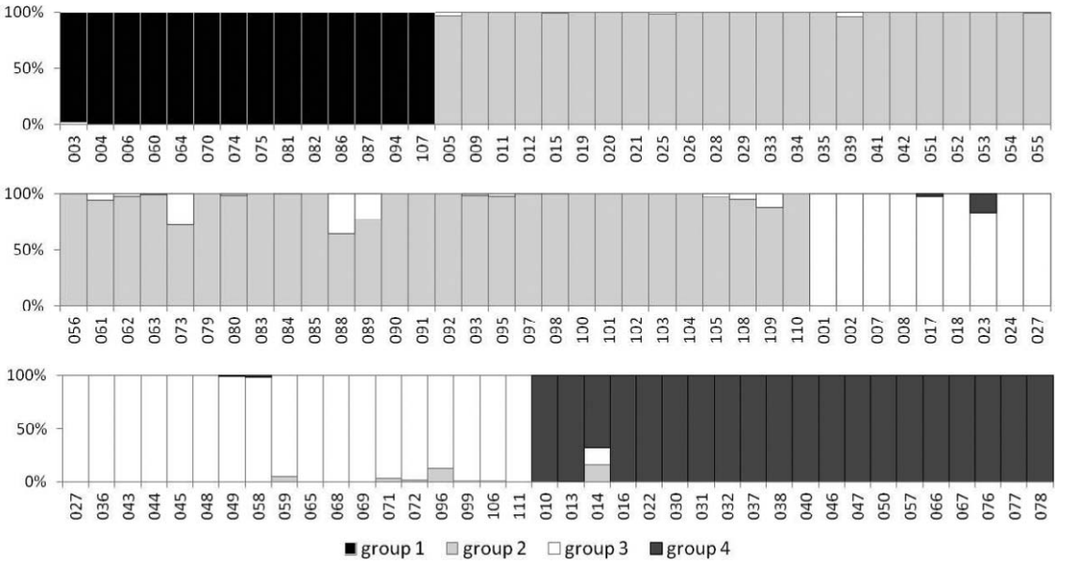

FIGURE 4. Assignment of the analyzed *Carex nigra* samples (1–111) to four groups obtained from Bayesian clustering, labeled after Table 1.

the spatial genetic structure, when germination and establishment have also been successful, since seedling recruitment is a major demographical filter in a plant's life cycle (Hampe et al., 2010). The spatial distribution of microsites with environmental conditions favorable for seed germination and seedling establishment has, therefore, a strong impact on spatial genetic structure and often results in a clumped distribution of genetically related individuals, as reported in several previous studies (Chung et al., 2003; Cruse-Sanders and Hamrick, 2004; Trapnell et al., 2004; Shimono et al., 2006; Troupin et al., 2006; Walker et al., 2009). It has been demonstrated that even minor discontinuities may have far-reaching consequences for the spatial genetic structure of populations (Jacquemyn et al., 2005; Vandepitte et al., 2009).

As pointed out in previous studies (Hampe et al., 2010) environmental data at the individual level can markedly improve the identification of processes that shape fine-scale genetic patterns. In the case of *C. nigra*, the patchy distribution and the different levels of genetic variation within the observed patchy groups may be a result of differing environmental conditions. The analyzed samples formed four genetically differing groups. Two of these groups were distributed in the moister area of the fen, while the other two groups were restricted to the drier parts of the fen depending on the topographical level. The four genetically differing groups of *C. nigra* observed in the fen may, therefore, represent cohorts of individuals originating from different successful recruitment events in different parts of the fen.

Moreover, genetic variation was considerably lower in the two groups from the drier parts of the fen, both for the whole data set and the analysis with 14 individuals. Generally, sexual reproduction and seedling recruitment are accompanied by genetic recombination and hence by the addition of new genotypes. Therefore, sexual reproduction and even low levels of seedling recruitment may positively affect the level of genetic variation, whereas in the absence of these processes, genetic variation can only decline (Watkinson and Powell, 1993). Several reasons can be offered for the observed decline of genetic variation in the two groups from the drier areas of the fen. First, it is well conceivable that the plants in the moister areas had more reproductive events and undergone more outcrossing while plants in the drier areas have been more stressed and perhaps had less sexual reproduction. It is already known that fecundity is reduced under suboptimal ecological conditions (Jump and Woodward, 2003), which could then lead to the observed lower levels of genetic variation in the drier regions of the fen. Second, it is possible that the seeds from the higher topographical areas routinely get washed down to lower topographical sites where they then germinate. This would increase the number of seeds from different plants in the moister areas of the fen and finally increase the genetic variation in the lower topographical groups. Third, recruitment in the drier areas of the fen may principally be hindered due to more unsuitable germination conditions. It has already been demonstrated that patterns of seedling recruitment may be different depending on environmental conditions (Jacquemyn et al., 2005) and that especially the early establishment of seedlings is triggered by abiotic conditions rather than by direct competition or density dependent antagonists (Hampe et al., 2010). This means that seed-

TABLE 3

Genetic distances (PhiPT) among the individual groups from the Bayesian clustering obtained in the AMOVA calculated with GenAlEx. All results were significant with $p < 0.001$ indicated by asterisks.

	Group 1	Group 2	Group 3	Group 4
Group 1		*	*	*
Group 2	0.276		*	*
Group 3	0.221	0.103		*
Group 4	0.450	0.213	0.181	

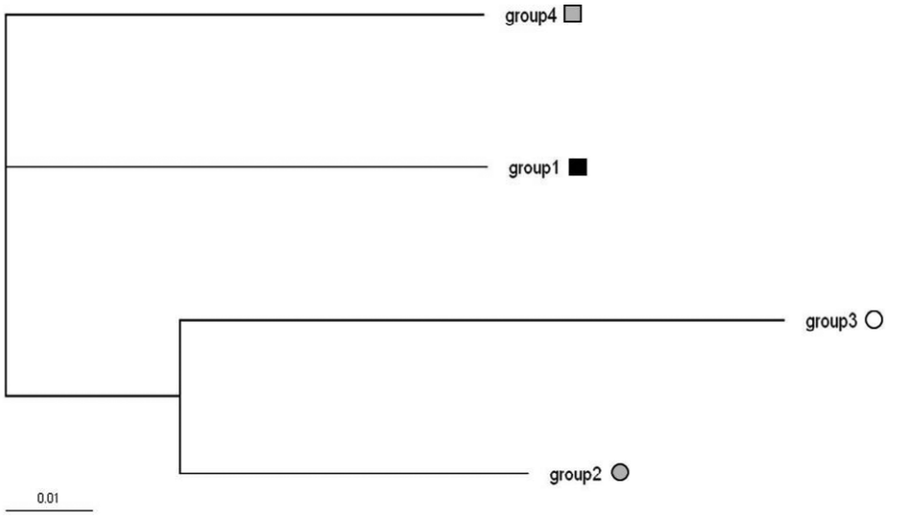

FIGURE 5. Neighbor-joining tree based on Cavalli-Sforza & Edward chord distances for the four groups from the Bayesian analysis labeled after Table 1.

ling recruitment indirectly affects genetic variation. Wetland species in general (Baskin et al., 2002) and particularly *Carex* species strongly depend on moisture and light for successful germination and establishment (van der Valk et al., 1999; Kettenring et al., 2006). Studies on germination ecology of numerous *Carex* species clearly revealed that germination rate increases with water and light availability (Budelsky and Galatowitsch, 1999). Consequently, germination conditions are most likely better in the deeper and moister parts of the fen than in higher and drier parts. Seedling recruitment could, therefore, be limited in these regions of the fen, which could result in the observed lower levels of genetic variation within the two groups from the drier parts of the fen.

Conclusions

The study presented here provides first evidence for the strong impact of environmental heterogeneity on the fine-scale genetic variation of plant populations within alpine fens. Existing studies mainly focused on the influence of biotic factors such as vegetative and generative reproduction or pollen and seed dispersal on spatial genetic structure. Based upon the results of our study it can be stated that the relationship between abiotic factors and fine-scale genetic variation deserves closer attention. Moreover, since previous studies mainly concentrated on lowland forest or grassland ecosystems, further research should aim to analyze more species from more alpine fens or wetlands. In these studies, information about the fine-scale genetic structure should be linked with a larger data set about the fine-scale ecological structure, such as e.g. temperature profile, availability of water and nutrients, or proportion of bare ground and mosses, to allow more detailed conclusions, especially about the impact of environmental heterogeneity on the spatial genetic structure of plant populations.

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