RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

Clonality increases with snow depth in the arctic dwarf shrub *Empetrum hermaphroditum*[1]

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PREMISE OF THE STUDY: Vegetative reproduction and spread through clonal growth plays an important role in arctic-alpine ecosystems with short cool growing seasons. Local variation in winter snow accumulation leads to discrete habitat types that may provide divergent conditions for sexual and vegetative reproduction. Therefore, we studied variation in clonal structure of a dominant, evergreen, dwarf shrub (*Empetrum nigrum* s.l. with the two taxa *E. nigrum* L. s.s. and *E. hermaphroditum* Hagerup) along a snow cover gradient and compared clonal diversity and spatial genetic structure between habitats.

METHODS: We studied 374 individual shoots using 105 polymorphic AFLP markers and analyses based on hierarchical clustering, clonal diversity indices, and small-scale spatial genetic structure with pairwise kinship coefficient. We used two approaches to define a threshold of genotypic distance between two samples that are considered the same clone. Clonality was examined among three habitats (exposed ridges, sheltered depressions, birch forest) differing in snow conditions replicated in four study regions in Norway and Sweden.

KEY RESULTS: Clonality of *E. hermaphroditum* differed between habitats with an increase in clonal diversity with decreasing snow depth. Small-scale spatial genetic structure increased with decreasing clonal diversity and increasing clone size. In three study regions, *E. hermaphroditum* was the only species, whereas in one region *E. nigrum* also occurred, largely confined to exposed ridges.

CONCLUSIONS: Our results demonstrated that snow cover in conjunction with associated habitat conditions plays an important role for the mode of propagation of the dwarf shrub *E. hermaphroditum*.

KEY WORDS clonal structure; evergreen, dwarf shrub; Ericaceae; small-scale spatial genetic structure; snow cover gradient; subarctic

In arctic-alpine environments with cool growing seasons and cold, long winters, sexual reproduction through seeds is not very common (McGraw and Shaver, 1982; Boudreau et al., 2010; Graae et al., 2011). Instead vegetative reproduction and spread through clonal growth play an important role in many species (Bliss, 1971; Cook, 1983; Callaghan et al., 1992; Molau and Larsson, 2000). Flowering and seed production can be a risky mode of propagation at high latitudes facing the risk of recruitment failure in some years (Körner, 2003). Thus, clonal growth, which occurs parallel to flowering and fruiting during the growing season might help species to persist in communities independently of reproductive success (Körner, 2003). After establishment from seeds, clonal plants are able to rapidly occupy new habitats and space locally (Callaghan et al., 1992) through horizontal clonal growth (Cook, 1983) and may therefore often dominate tundra ecosystems (Tybirk et al., 2000). Benefits of clonal growth can include the better use of resources and reduced risk of genet mortality. Disadvantages of clonal propagation include reduction of resources available for flowering and seed production, the lack of sexual reproduction, and the dispersal of pathogens between ramets (Klimeš et al., 1997). Arctic clonal plant species and communities may be particularly vulnerable to global warming (Sala et al., 2000) since environmental change may force species either to adapt in situ or to colonize new sites to track their climatic niche. Although microhabitats may partly buffer macroclimatic change (e.g., De Frenne et al., 2013; Lenoir et al., 2013), sexual reproduction and long-distance dispersal of fruits and seeds for colonization of new habitats may then become crucial (Eriksson, 1989; Callaghan et al., 1992; Szmidt et al.,

[1] Manuscript received 14 June 2016; revision accepted 7 November 2016.

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doi:10.3732/ajb.1600229

2002). Thus, studies addressing local and regional patterns of sexual relative to clonal reproduction among habitats are particularly needed in tundra ecosystems.

Spatial variation in snow distribution in Arctic ecosystems, created by a combination of topography and wind, ranges from wind-exposed ridges with shallow snow cover to sheltered depressions with deep snow accumulation in the alpine tundra and mountain birch forest habitats with deep snow cover (Saarinen and Lundell, 2010). Snow cover provides a strong selection gradient within arctic and alpine landscapes, affecting growing season length and winter conditions (Jonas et al., 2008). Usually, habitats with different snow conditions contrast in the composition of the plant communities, characterized by species that prefer or avoid winter snow cover (Jonasson, 1981; Odland and Munkejord, 2008). However, some species occupy a wide range of habitats, and intraspecific differences in responses to variation in snow depth and duration can affect phenology and growth habit.

Empetrum nigrum s.l. (Ericaceae) with its segregates *E. nigrum* s.s. and *E. hermaphroditum* Hagerup is a dwarf shrub with small, evergreen, needle-like, dark green leaves, which is widely distributed in the northern hemisphere (Bell and Tallis, 1973). It occurs in several subarctic heath and mountain birch forest communities (Nilsson and Wardle, 2005), ranging from habitats with shallow (Jonasson, 1981; Odland and Munkejord, 2008) to relatively deep snow cover (Jonasson, 1981; Tybirk et al., 2000). It reproduces both through clonal growth and seeds. The species is able to form dense mats by horizontal creeping shoots and produces phenolic compounds (batatasin III) that have strong allelopathic effects on plants and soil organisms (Nilsson and Wardle, 2005). In the absence of wildfires, retrogressive succession driven by *E. hermaphroditum* leads to decreased abundance of *Vaccinium myrtillus* and *V. vitis-idaea*, an increase in abundance of feather mosses, a reduction in decomposer biomass, reduction of litter decomposition rate, and a decrease in N mineralization rate (Nilsson and Wardle, 2005). Hence, *Empetrum* is a dominant species and an ecosystem engineer of boreal and arctic ecosystems. We analyzed the clonal structure of this keystone species in three habitats differing in winter snow cover.

The extent of clonality within populations does have a strong influence on spatial genetic structure (SGS), with neighboring ramets being more closely related than more distant ones (Reusch et al., 1999; Pluess and Stöcklin, 2004). In alpine tundra habitats, populations tend to be genetically strongly differentiated because suitable habitats are isolated from each other and recruitment within a habitat or from neighboring habitats is more likely than colonization from other habitats by long-distance seed dispersal, despite suitable dispersal mechanisms (Pluess and Stöcklin, 2004). In clonal plant species, the spatial distribution of clones will also depend on the clonal growth strategy (Escaravage et al., 1998). Two clonal growth forms can be distinguished; the guerilla form has longer internodes and more widely spaced ramets, which can infiltrate the surrounding vegetation, and the phalanx form has tightly packed ramets, which exclude other plants from their growing space (Doust, 1981). The growth habit of *Empetrum* varies between different habitats, with a more guerilla-like growth on sites with intermediate and deep snow cover and a more phalanx-like growth on sites with shallow snow cover (Bienau et al., 2014). Furthermore, we observed more berries per *Empetrum* shoot, which likely leads to higher local seed rain in the shallow snow cover habitat than in habitats with deep snow cover (Bienau et al., 2014). The higher seed production might be an effect of the open habitat, where germination of *Empetrum* seeds might be promoted by soil disturbance (Graae et al., 2011), less competition from surrounding vegetation (Szmidt et al., 2002), and an earlier start of the growing season (Körner, 2003). Higher germination rates might promote higher clonal diversity resulting from a higher number of individuals.

Using a molecular marker approach in the present study, we compared patterns of clonal structure of *Empetrum* along a natural snow cover gradient with shallow snow cover (alpine tundra on wind-exposed ridges) and habitats with deep snow cover (alpine tundra in wind-sheltered depressions and subalpine birch forest). We addressed the following questions: (1) Does clonal diversity and clonal size vary between habitats? We expected higher clonal diversity and smaller clones in shallow snow cover habitats than in deep snow cover habitats of alpine tundra and birch forest from growth habit and better conditions for seedling recruitment. (2) Because the identification of clones may be problematic, we asked whether small-scale spatial genetic structure, which is assessed independently of the delineation of clones, differs between habitats. We expected higher genetic autocorrelation in the alpine tundra with deep snow cover and birch forest because conditions are better for vegetative growth with longer internodes and therefore more widely spaced ramets.

MATERIALS AND METHODS

Study regions and habitats—To test for general patterns between habitats, we selected four study regions with steep local climatic gradients described in detail by Bienau et al. (2014). In short, the study regions were situated at two different latitudes (Fig. 1A), i.e., northern Sweden (Abisko [68°2′N 18°49′E; mean annual precipitation: 304 mm; mean annual temperature: −0.8°C] and Vassijaure [68°2′N 18°10′E; 844 mm; −1.7°C]) and Central Norway (Kongsvoll [62°18′N 09°36′E; 450 mm; −0.4°C] and Samsjøen [63°05′N 10°38′E; 830 mm; 3.9°C]). The regions at each latitude represent a steep gradient between subcontinental (Abisko and Kongsvoll) and suboceanic climate (Vassijaure and Samsjøen), with low and high winter precipitation, respectively. At each of the four regions, we distinguished three habitat types differing in snow depth and covarying abiotic factors (for details, see Bienau et al., 2014) based on topography, community type, and indicator species of contrasting snow cover conditions (Jonasson, 1981; Odland and Munkejord, 2008): birch forest (hereafter, birch), alpine tundra with deep snow cover (hereafter, deep), and alpine tundra with shallow snow cover (hereafter, shallow). In the birch and deep habitats, snow height is >50 cm and 30–50 cm, respectively, because vegetation traps snow, whereas in shallow with sparse vegetation cover, snow height is only 5–10 cm (Bienau et al., 2014). Depending on snow depth, dates of snow melt timing in spring are different between the habitats. Snow melt occurred at the end of March and the beginning of April in the shallow habitat and about 40 d later in the deep and birch habitats (Bienau et al., 2015).

Study species—Within *Empetrum nigrum* s.l. (Ericaceae), two taxa are commonly distinguished: the diploid, dioecious *E. nigrum* L. s.s. and the tetraploid, hermaphrodite *E. hermaphroditum* Hagerup, which can only be distinguished visually when flowering. They are evergreen, dwarf shrubs able to expand vegetatively by rooting of horizontal aboveground and epigeogenous stems (Klimešová and de Bello, 2009). In both species, fruit and seed production by

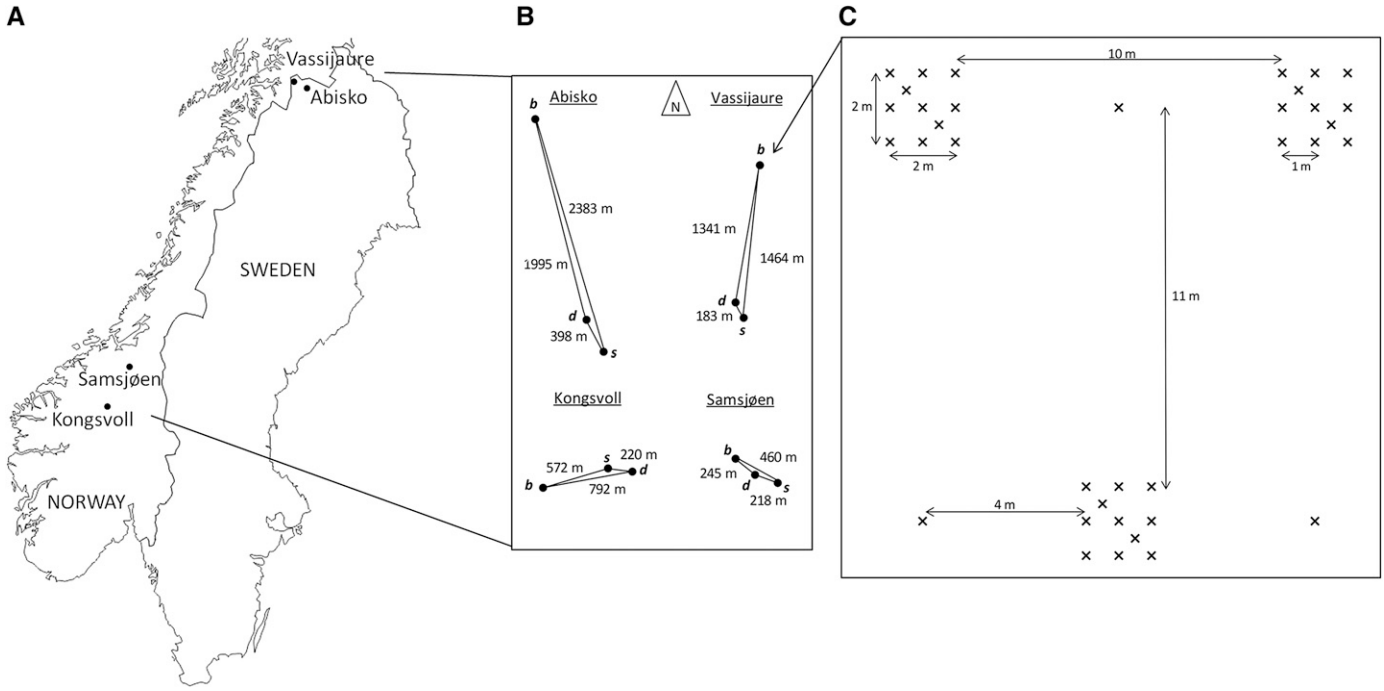

FIGURE 1 (A) Position of study areas Abisko and Vassijaure in northern Sweden and Kongsvoll and Samsjøen in central Norway; (B) schematic representation of distances between the three habitats, birch forest (birch), alpine tundra with deep snow cover (deep), and alpine tundra with shallow snow cover (shallow) in the four study regions; and (C) sampling scheme of *Empetrum* shoots (N = 36) within each plot for analysis of clonal structure.

wind-pollinated flowers may be frequent (Bell and Tallis, 1973). Whereas *E. nigrum* is outcrossing, *E. hermaphroditum* seems to be predominantly self-pollinating as evidenced by an autodeposition efficiency of 0.9 (Tikhmenev, 1984, cited in the supplement of Alsos et al., 2012; see also Bell and Tallis, 1973; Callaghan and Emanuelsson, 1985). Seeds of *Empetrum* are dispersed by birds (Bell and Tallis, 1973) and the arctic fox (Graae et al., 2004).

In three of our study regions (Abisko, Vassijaure, and Samsjøen), only *E. hermaphroditum* (Suda, 2002) occurred, whereas in the Dovrefjell National Park (study region Kongsvoll), we also found *E. nigrum* in unexpectedly high abundance (see also Suda, 2002). We distinguished both taxa post hoc based on their AFLP profiles (described later).

Sampling design—We established one 14 × 14 m plot per habitat in each of the four study regions in summer 2012. Plots were selected according to topography and indicator species for snow conditions on sites where *Empetrum* was present. The distance between the different habitats ranged between 218 and 2383 m. However, the distance between the birch forest habitat and the two alpine tundra habitats (deep and shallow) was larger than between the deep and shallow habitats (Fig. 1B) because the habitats represent an altitudinal gradient with birch forest habitats below the treeline and alpine tundra habitats above the treeline. Within each plot, we aimed to sample 36 individual *Empetrum* shoots using a systematic grid (Fig. 1C), which creates both small and large pairwise distances for analysis of clonality and enables appropriate comparisons between different plots (cf. Szmidt et al., 2002). If there was no shoot at a predefined sampling point, we sampled a shoot and recorded exact coordinates within a distance of maximum 1 m, or omitted that sampling point. Shoots were stored in teabags and within 3 to 4 h, the samples were brought in a cooling room (5°C) to keep the material fresh. They were stored no longer than 3 d before they were freeze-dried for 48 h.

Molecular analysis—We investigated a total of 374 shoots (24 to 36 per plot; for data set, see Appendix S1 in Supplemental Data with the online version of this article) for the clonal structure of *Empetrum* with AFLP markers (Vos et al., 1995). Genomic DNA was extracted from 10 mg freeze-dried leaf material from each sample and from 20 randomly chosen replicate samples with the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the manufacturer's manual. Details of the AFLP protocol are given in Appendix S2 (see online Supplemental Data). After an initial screening of 16 primer combinations, four primer pairs (FAM-ACT-CAG, VIC-ACA-CTC, NED-AAG-CAG, PET-AGC-CAC) were chosen for AFLP analyses of the 374 samples and 104 duplicates (=27.8%). Fragments were analyzed on an ABI 3130xx Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with the Genescan 500 LIZ size standard. We used the program GeneMapper version 5.0 (Applied Biosystems) to analyze the AFLP profiles. We manually binned the fragments of all samples in one batch using peak height thresholds of 5 rfu. These data were exported, then for each fragment a specific peak height threshold was manually determined based on the peak height distribution to score fragment presence and absence. Monomorphic or bands with an individual error rate >5% were discarded, resulting in 105 polymorphic AFLP loci. Preliminary analyses had shown that a number of samples had strongly divergent AFLP genotypes, which proved to be diploid *E. nigrum* (M. J. Bienau and J. Bielke, unpublished

flow cytometry data). We distinguished *E. nigrum* from *E. hermaphroditum* based on the total number of bands per individual which was <34 (mean: 25, range 21–33) for *E. nigrum* and >34 (mean: 46, range: 35–59) for *E. hermaphroditum*. *E. nigrum* was confined to the Kongsvoll region where one individual occurred in the birch forest where it was the only cytotype in the shallow habitat. *Empetrum nigrum* was treated separately in the definition of clones.

Data analysis—Defining clones using molecular markers is notoriously difficult because of somatic mutations (Douhovnikoff and Dodd, 2003) and genotyping errors (Bonin et al., 2004), which necessitates defining a threshold of genotypic distance between two samples that are considered the same clone. Two different approaches have been taken. The first method (hereafter, mean threshold) uses the mean error rate or mean genotypic distance of replicate samples as the threshold (e.g., Vonlanthen et al., 2010), assuming that on average, clonal samples differ that much. However, the observed number of differences between replicate samples follows a Poisson distribution (Fig. 2A, B), and the maximum observed error is larger than the mean. Thus, using the mean threshold might falsely split clones and thus overestimate the number of clones. The second method, hereafter called bimodal threshold, defines the threshold using the minimum of the bimodal frequency distribution of pairwise differences between all samples, including clones and replicates (De Witte et al., 2012). Ideally, this threshold is derived by comparing pairwise differences between replicate samples with those between full sibs, which represent the most closely related sexually derived genotypes that need to be distinguished from clones (Schleuning et al., 2011; Douhovnikoff and Dodd, 2003). The bimodal threshold approach potentially underestimates clone number, in particular when distributions of replicates and nonclonal genotypes overlap. In line with reports of a very low germination rate for *E. hermaphroditum* (Graae et al., 2011), we were not able to grow full-sib plants from seeds and thus cannot quantify their genetic distance. We therefore used both the mean threshold and the bimodal threshold method for identification of clones, thus setting lower and upper levels of clonality. Mean error rate was 1.12%, based on 112 errors in 95 replicate samples across 105 loci for *E. hermaphroditum* and 0.32% (3 errors in 9 replicate samples across 105 loci) for *E. nigrum*. Consequently, a difference of 1 and 0 was used as the mean threshold for *E. hermaphroditum* and *E. nigrum*, respectively. Frequency distributions of pairwise differences were used to define the bimodal thresholds, which was 7 for *E. hermaphroditum* and 1 for *E. nigrum* (Fig. 2C, D).

Individual clones were defined by hierarchical clustering using the hclust function in the program R 3.2.3 (R Core Team, 2015) based on the number of differences between AFLP genotypes (Manhattan distance) and using complete linkage agglomeration. Complete linkage assures that within a clone the maximal distance does not exceed the threshold. Clones were defined using the cutree function with the respective threshold values. Using the identified clones, we calculated six indices to estimate the clonal diversity: (1) clone number (G) as number of different multilocus genotypes, (2) clonal diversity (R) as $R = (G - 1) / (N - 1)$, where N is the sample size (Ellstrand and Roose, 1987; Dorken and Eckert, 2001; Arnaud-Haond et al., 2007). (3) Simpson's index of diversity (D; complement) modified for finite population size (Pielou, 1969) as $D = 1 - \Sigma\{[G(G - 1)] / [N(N - 1)]\}$ and (4) Simpson's evenness (E) index as $E = (D - \min D)/(\max D - \min D)$. D expresses the probability that two randomly selected individuals from a sample will belong to the same clone, and E is the relative abundance of clones (Arnaud-Haond et al., 2007). To allow comparison with Szmidt et al. (2002), we also calculated (5) Shannon's diversity index (H) as $H = \Sigma(p_i \cdot \ln p_i / \ln N)$ where p_i is the proportion of genotypes and (6) clonal fraction (CF) as $CF = (N - G) / N$, which is close to $1 - R$. Furthermore, we calculated maximum clone size (Clmax) as

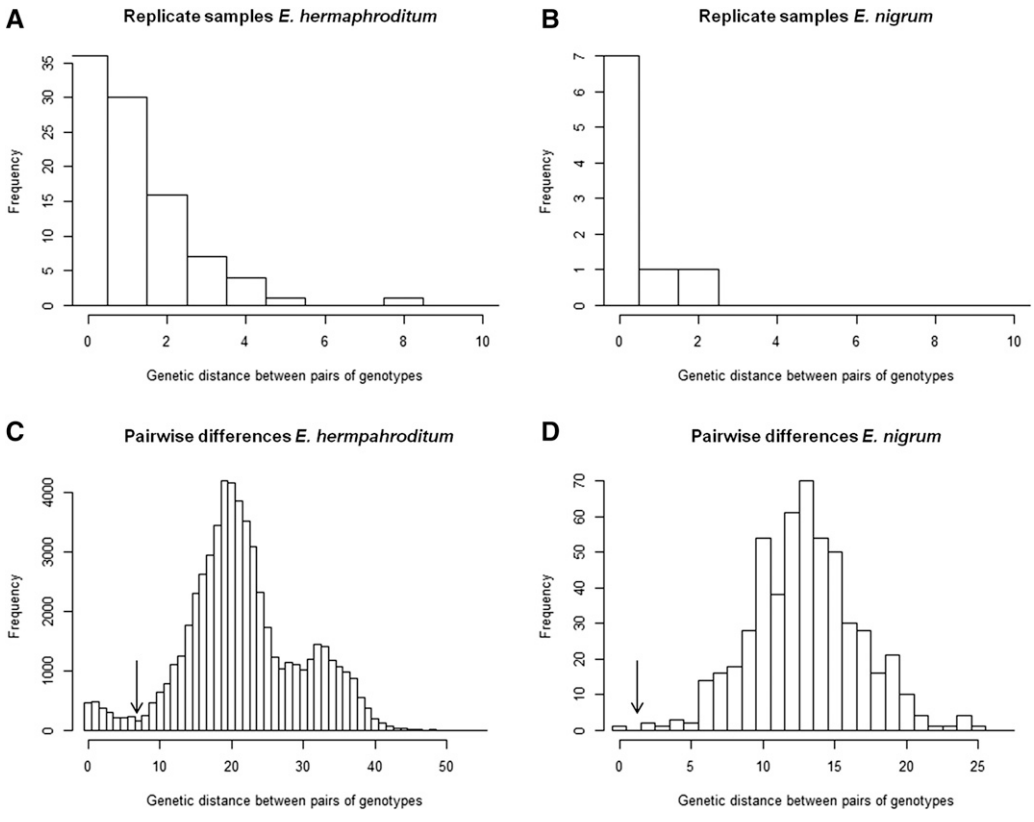

FIGURE 2 Frequency distributions of the number of pairwise differences among AFLP genotypes (Manhattan distance). Shown are results for comparison of pairs of replicate samples of (A) tetraploid *E. hermaphroditum* and of (B) diploid *E. nigrum*, and comparisons of all samples of (C) *E. hermaphroditum* and (D) *E. nigrum*. Bimodal thresholds are indicated (arrow) at the frequency minimum, which was 7 for *E. hermaphroditum* and 1 for *E. nigrum*.

the maximum Euclidian distance among all sampling coordinates of a clone per plot.

Because sample sizes differed between plots from $N = 24$ to 36, we performed rarefaction. We randomly drew, for 100 times, n samples ($n = 10$ to N) and calculated indices of clonal diversity, finally averaging across randomizations. There was a clear sample size effect leading, in general, to an increase of the expected number genotypes and a decrease of expected clonal diversity with increasing sample size n (online Appendix S3). We therefore used rarified values of all indices of clonal diversity at the smallest sample size ($n = 24$) for all further analyses. However, these were highly correlated with nonrarified estimates ($r = 0.902$ for G and $r = 0.951$ for R). For analysis of clonal diversity and rarefaction, convenient R functions were developed (online Appendix S4).

Linear mixed effect models were applied using the R package lme4 (Bates et al., 2015) to test for significant differences among habitats (Pinheiro et al., 2015), taking regions into account as random factor. In case of significant differences between habitats, pairwise comparisons between habitat types were done using least square means on the estimates of the linear mixed models and significance of the random effect tested through a likelihood ratio test, using the R package lmerTest (Kuznetsova et al., 2016). All analyses were done in R version 3.2.3 (R Core Team, 2015).

We used spatial autocorrelation methods to examine the small-scale spatial genetic structure (SGS), which is a measure of the genetic relatedness between pairs of individuals in relation to their spatial distance (Vekemans and Hardy, 2004). We combined samples across regions within habitats but focused on plot-scale relationships by restricting the distance range to the within-plot scale. To cover all possible distances of the sampling design and to ensure sufficient distribution of pairs among distance classes, distance class limits were set at 1, 2, 4, 8, 12, 25, and >25 m, the latter representing comparisons between habitats and regions. Within the program SPAGeDi 1.5a (Hardy and Vekemans, 2002), we used the pairwise kinship coefficient for dominant markers F_{ij} (Hardy, 2003) with an inbreeding coefficient of 0.5 and tested for significance of mean F_{ij} with 9999 permutations. To quantify the degree of SGS, we calculated the Sp statistic as $Sp = -b_{log} / (1 - F_{(1)})$, where b_{log} is the regression slope of mean F_{ij} on log geographic distance and $F_{(1)}$ is the mean F_{ij} of the first distance class (Vekemans and Hardy, 2004). Furthermore, we used the program GenAlEx 6.5 (Peakall and Smouse, 2012) to assess SGS and to test for differences in SGS between habitats by heterogeneity tests (Smouse et al., 2008). Number of permutations and bootstraps was 9999, respectively. Differences in the heterogeneity tests were considered significant if $p < 0.01$ (Banks and Peakall, 2012).

RESULTS

Clonal diversity—Estimates of clonal diversity were strongly affected by the choice of threshold (Fig. 3, Table 1). As expected, estimates of clonal diversity (G, R, D, E, H) were much higher and clonal fraction was lower for the mean threshold than for the bimodal threshold.

For *E. hermaphroditum*, mean values per habitat showed lowest clonal diversity in the birch, intermediate in the deep, and highest in the shallow habitat. However, comparison among habitats revealed

FIGURE 3 Spatial distribution of clones of *Empetrum* in different habitats in the four study regions Abisko, Vassijaure, Kongsvoll, and Samsjøen at bimodal threshold and mean threshold. Each gray dot indicates a different unique genotype; dots sharing a color belong to the same clone within one row (region). Note that in Kongsvoll, both *E. hermaphroditum* (squares) and *E. nigrum* (triangles) occurred, with *E. nigrum* mainly restricted to shallow habitat.

slightly different results between the two thresholds. For the bimodal threshold, clonal diversity (*G*, *R*, *D*, *CF*, *H*) was significantly lower in the birch habitat than in the shallow habitat, and *D* and *H* were also lower in the birch than in the deep habitat (Table 1). In contrast, for the mean threshold, no significant differences between habitats were found. However, patterns for habitats in regions were not totally consistent. The region effect was not significant ($p > 0.3$).

In all regions and at both threshold levels, we observed putative clones that spread across habitats (Fig. 3). When restricting the estimation within plots, maximal clone size ranged from 9.7 to 19.4 m for mean threshold and from 13.4 to 19.5 m for the bimodal threshold and did not differ between habitats (Table 1).

The single plot consisting solely of *E. nigrum*, i.e., the shallow habitat in Kongsvoll, had very high clonal diversity ($R = 0.98$ for both thresholds, Table 1) and small clone size (0.5 m), in contrast to *E. hermaphroditum*, which had on average lower clonal diversity ($R = 0.65$ and 0.31 for mean and bimodal threshold, respectively) and much larger clones (Clmax = 14.4 and 16.2 m).

Small-scale spatial genetic structure—All plots of *E. hermaphroditum* in the three habitats across the four regions showed significant SGS (online Appendix S5). In all populations of *E. hermaphroditum*, autocorrelation was highest in the first distance class. Significant autocorrelation was found up to 4 m in both birch and deep

TABLE 1. Indices of clonal diversity of *Empetrum hermaphroditum* populations (*E. nigrum* in the shallow habitat of Kongsvoll) in different habitats in the four study regions. Values are means across 100 times randomly drawing sets of $N = 24$ samples per plot and thus represent expected values (see Appendix S3 for nonrarified estimates). Significant differences between habitats revealed by least square means on the estimates of the linear mixed models are indicated by differing letters, and a significant effect of habitat is indicated by asterisks (**$p < 0.05$, *$p < 0.1$). df = 8. *N* = sample size, *G* = number of genotypes, *R* = clonal diversity, *D* = Simpsons complement, *E* = Simpson's evenness, *H* = Shannon diversity index, CF = clonal fraction, Clmax = maximal clone size. Average values were calculated without *E. nigrum*. For clonal identification, we used either the mean threshold of AFLP mismatches (1 for *E. hermaphroditum* and 0 for *E. nigrum)* or the bimodal threshold (7 for *E. hermaphroditum* and 1 for *E. nigrum)*. birch = birch forest, deep = alpine tundra with deep snow cover, shallow = alpine tundra with shallow snow cover.

Region/habitat	*N*	*G*	*R*	*D*	*E*	CF	*H*	Clmax (m)
Mean threshold								
Abisko								
birch	24	14.9	0.605	0.930	0.692	0.378	1.780	15.7
deep	24	16.6	0.677	0.959	0.764	0.309	1.841	9.7
shallow	24	18.3	0.751	0.972	0.743	0.239	1.883	13.7
Vassijaure								
birch	24	18.0	0.739	0.942	0.294	0.250	1.841	14.5
deep	24	18.2	0.748	0.962	0.588	0.241	1.867	15.6
shallow	24	15.4	0.625	0.937	0.695	0.359	1.792	15.3
Kongsvoll								
birch	24	15.3	0.621	0.929	0.645	0.363	1.787	16.6
deep	24	18.1	0.741	0.963	0.623	0.248	1.867	12.8
shallow (*E. nigrum*)	24	23.6	0.981	0.998	NA	0.019	1.992	0.5
Samsjøen								
birch	24	13.1	0.527	0.927	0.816	0.454	1.746	13.3
deep	24	11.5	0.455	0.872	0.695	0.522	1.664	19.4
shallow	24	16.8	0.687	0.958	0.737	0.300	1.841	12.5
Average								
birch		15.3 a	0.623 a	0.932 a	0.612 a	0.361 a	1.789 a	15.0a
deep		16.1 a	0.655 a	0.939 a	0.668 a	0.330 a	1.810 a	14.4a
shallow		16.8 a	0.688 a	0.956 a	0.725 a	0.299 a	1.839 a	13.8a
Bimodal threshold								
Abisko								
birch	24	4.7	0.162	0.601	0.594	0.803	1.332	17.2
deep	24	8.9	0.344	0.871	0.851	0.629	1.620	13.4
shallow	24	8.2	0.314	0.869	0.882	0.658	1.605	14.3
Vassijaure								
birch	24	8.0	0.304	0.732	0.554	0.667	1.500	14.5
deep	24	10.3	0.403	0.810	0.575	0.572	1.591	16.1
shallow	24	7.5	0.283	0.814	0.789	0.687	1.533	18.9
Kongsvoll								
birch	24	7.4	0.278	0.810	0.789	0.692	1.537	17.0
deep	24	8.4	0.322	0.859	0.847	0.650	1.600	16.6
shallow (*E. nigrum*)	24	23.4	0.975	0.998	NA	0.024	1.990	0.4
Samsjøen								
birch	24	5.8	0.210	0.820	0.910	0.757	1.509	15.3
deep	24	6.7	0.246	0.784	0.782	0.722	1.502	19.5
shallow	24	12.2	0.487	0.919	0.830	0.491	1.722	15.1
Average								
birch		6.5 a	0.213 a	0.741 a	0.712 a	0.730 a	1.470 a	16.0 a
deep		8.7 ab	0.329 ab	0.831 b	0.764 a	0.643 ab	1.578 b	16.4 a
shallow		9.3 b*	0.361 b*	0.867 b**	0.834 a	0.612 b*	1.620 b**	16.1 a

but only up to 2 m in the shallow habitats. In *E. nigrum* only the first distance class (1 m) showed significant values. When combining data sets per habitat across regions for *E. hermaphroditum*, the heterogeneity tests revealed significant differences in SGS between all three habitats ($p < 0.007$). In particular, deep and shallow had a stronger decline in autocorrelation in the first three distance classes than birch (Fig. 4). The *Sp* values decreased from birch (0.153) to deep (0.128) to shallow (0.118), indicating strongest SGS in the birch forest. Also, the diploid E. nigrum in the shallow-habitat in Kongsvoll showed significant SGS, with *Sp* = 0.026, indicating much weaker SGS than in *E. hermaphroditum*.

DISCUSSION

We found differences in clonal diversity between habitats that were confirmed through analyses of spatial genetic structure. The method used for clonal identification strongly influenced the absolute measures of clonal diversity. For both methods, clone mates were identified in different habitats up to 2 km apart, which we consider biologically impossible to achieve by vegetative spread as discussed below.

Snow-related habitat effects on clonality and spatial genetic structure—Concerning our first study question, and excluding *E. nigrum*, we overall observed lower clonal diversity in the birch forest habitat than in the alpine tundra with deep and shallow snow cover for the bimodal threshold, although not significant for all pairwise observations. In the birch habitat, snow depth is highest and snow melt in the spring is latest, whereas in the shallow habitat snow depth is lowest and snow melt occurs earlier; the snow conditions in deep are intermediate (Bienau et al., 2014, 2015). Szmidt et al. (2002) investigated the genetic structure of *E. hermaphroditum* in three regions with different successional stages in northern Sweden. The results of their study are comparable to ours for the indices of clonal diversity. Also, both studies revealed that sexual reproduction plays an important role in the slow-growing *E. hermaphroditum*. In particular, the low proportion of clonal reproduction in the shallow habitat implies a higher reproduction by seedlings under such conditions (Eriksson, 1992). Within the shallow habitat, competition is lower, and the proportion of open soil is higher, which likely facilitates recruitment from seeds (Callaghan et al., 1992; Szmidt et al., 2002). This result was also confirmed by a study of Boudreau et al. (2010), who found that most individuals in a subarctic sand dune ecosystem originated from seeds instead of clonal spread on early successional sites, where conditions were comparable to the shallow habitat in our study regarding the amount of open soil.

Furthermore, the probability of seed dispersal to the shallow habitat by bird droppings may be high because birds often roost on exposed, elevated rocks around which a lot of bird droppings can be found (M. J. Bienau, personal observations). This interpretation is also corroborated by the results of our previous finding of higher fruit and seed production in the shallow habitat, i.e., larger seed rain, than in deep and birch (Bienau et al., 2014). In addition to long-distance seed dispersal, seedling recruitment in the surrounding of the mother plant might also be important for clonal diversity of *E. hermaphroditum*.

Clonal growth is expected to promote spatial autocorrelation (SGS), i.e., increasing genetic similarity with decreasing spatial distance (Reusch et al., 1999). The mean *Sp* value of 0.14 in *E. hermaphroditum* ranges among the largest values listed by Vekemans and Hardy (2004) for selfing species, whereas *Sp* in the single plot of *E. nigrum* was 0.02, similar to other outcrossing dwarf shrubs like *Calluna vulgaris*. Thus, the interspecific pattern closely follows the known effect of the breeding system on SGS (Vekemans and Hardy, 2004). For populations of *E. hermaphroditum*, we observed higher SGS in the birch habitat than in the deep and shallow habitat. Thus, SGS increased, as expected, with decreasing clonal diversity and increasing clone size. We observed that *E. hermaphroditum* in the birch habitat grows more like a guerilla species and in the shallow habitat more like a phalanx species, whereas in the deep habitat its growth was intermediate. In a former study, we investigated shoot growth of *E. hermaphroditum* (Bienau et al., 2014) and found significantly lower ramet heights, shorter annual shoot segments, fewer lateral shoots and lower total biomass in the shallow than in the deep and birch habitats. In contrast, leaf density and relative leaf mass were highest in shallow habitats, intermediate in deep, and lowest in birch habitats. The more procumbent growth form in the shallow habitat had lower ramet heights and shorter shoot segments, but higher leaf density protects the species during periods of unstable snow cover from cold winter temperatures and strong winds and reduces freezing and desiccation (Körner, 2003). In the birch and deep habitats, protection by snow in the winter (Körner, 2003) and surrounding vegetation (Sturm et al., 2001; Fletcher et al., 2010) during the growing season is higher, promoting shoot growth. Further

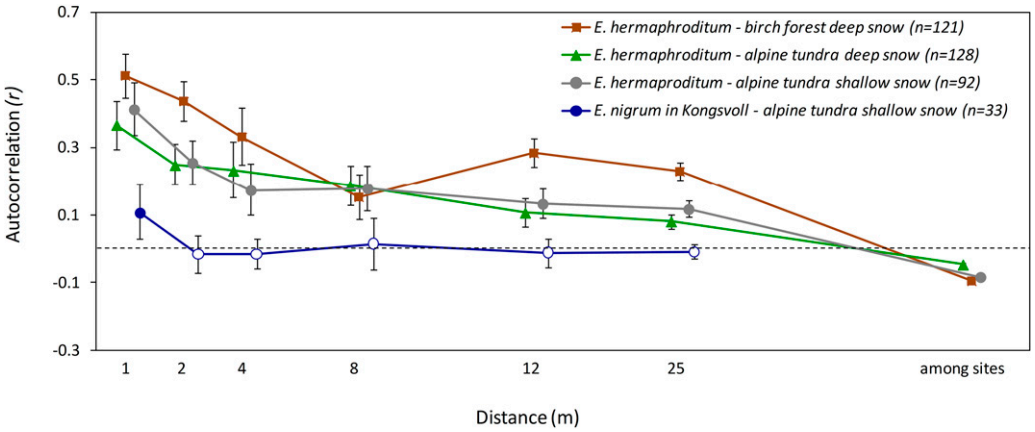

FIGURE 4 Spatial genetic autocorrelation in populations of *Empetrum hermaphroditum* in the three habitats (birch forest, alpine tundra with deep snow cover, and alpine tundra with shallow snow cover), combining data of four study regions, and of *E. nigrum* in the shallow habitat in Kongsvoll. Filled symbols indicate significant genetic autocorrelation for single distance classes. SGS was significant in all habitats. Individual analyses for the regions are shown in Appendix S5.

aspects promoting growth of *E. hermaphroditum* in sheltered habitats may be higher water (Sturm et al., 2001; Fletcher et al., 2010) and nutrient availability after spring-melt of a deep snowpack, which stores deposited inorganic nitrogen (Bowman, 1992; Weih, 1998).

Nevertheless, the more phalanx growth form in the shallow habitat might facilitate a high density of flowers within a particular clone, thus increasing the likelihood of selfing (cf. Handel, 1985) and reducing the extent of genetic autocorrelation. However, effective sexual propagation does not only depend on pollen flow; seed dispersal, seed germination, and seedling establishment are also of high importance (Alberto et al., 2005). Thus, the shallow habitat might present the most favorable environment for seedling establishment due to large seed rain, high seed dispersal into this habitat by bird droppings, and a higher amount of open soil and less competition as than in the birch and deep habitat.

Empetrum nigrum—In Scandinavia, *E. hermaphroditum* and *E. nigrum* are partly allopatric with the latter being confined to more southern and coastal areas, where the former is missing (Hulten and Fries, 1986). Both species occurred in our Kongsvoll study region. Analyses of additional samples in the three habitats in Kongsvoll proved that *E. hermaphroditum* made up 99% of all investigated shoots in the birch habitat, whereas *E. nigrum* made up 45% in the deep and 73% in the shallow (M. J. Bienau, J. Bielke, unpublished flow cytometry data). Nearly all sampled ramets of *E. nigrum* were different genotypes, also reflected in SGS, which was spatially restricted to the first distance class. Therefore, we expect that most of the ramets of *E. nigrum* in the shallow habitat resulted from sexual reproduction. Moreover, the dioecious *E. nigrum* is obligatorily outcrossing (Bell and Tallis, 1973), and thus seed-derived offspring will be genetically heterogeneous. In contrast, *E. hermaphroditum* has strong vegetative reproduction and is selfing, resulting in genetically more homogenous offspring (Barrett, 2003). Both *E. nigrum* and *E. hermaphroditum* build up a perennial seed bank (Klimešová and de Bello, 2009). Even though germination of both species is rarely observed in the field (*E. hermaphroditum*: Graae et al., 2011, *E. nigrum*: Mallik and Gimingham, 1985), sexual reproduction clearly has been the major mode of reproduction in *E. nigrum* in this population.

Threshold definition, clone identification, and the role of selfing—To address the methodological difficulties inherent in the identification of clones, we used two different thresholds of genotypic difference above which ramets were considered to belong to the same genet. The mean threshold determined from doubly analyzed samples (e.g., Vonlanthen et al., 2010) neglects the fact that the error rate of multiple replicates follows a Poisson distribution and thus likely overestimates clonality. The bimodal threshold, in contrast, takes into account the whole distribution of genotypic differences (de Witte et al., 2012) but depends on a clear separation of the two modal distributions. In our case, the minimum of the bimodal distributions coincided with the upper range of distances observed between replicates. Therefore, individual clones are smaller and clonal diversity consequently is higher using the mean threshold than the bimodal threshold. However, significant differences for clonal diversity indices were only found for the bimodal threshold. This result might be an effect of the more conservative bimodal threshold, which underestimates clone number, in particular when distributions of replicates and nonclonal genotypes overlap. However, this threshold seems to split the indices clonal diversity between the habitats better because it takes into account the whole distribution of genotypic differences between the populations.

However, irrespective of the method used, in all regions we detected clones that apparently spread across different habitats up to 2 km apart, which we consider biologically impossible to achieve by vegetative spread. In a former study, we measured mean shoot elongation of 2.2 $cm \cdot a^{-1}$ (range: 0.5–5.9 $cm \cdot a^{-1}$ across all habitats in the four study regions) (Bienau et al., 2014). Burges (1951) observed similar growth rates of 2 $cm \cdot a^{-1}$. With 6 and 1 $cm \cdot a^{-1}$ as the upper and lower bounds, the age of a clone with a size of 14 to 16 m—the mean clone size calculated by the mean threshold and bimodal threshold, respectively, in our plots—would be between 125 and 750 yr assuming continuous bidirectional horizontal growth. A clone of 2 km extent as observed here would thus be between ~17,000 and 100,000 yr old. Such ages are at odds with both age estimates and history, since only around 8000 yr have passed after the latest glaciation (Weichselian) (Backéus, 1999). But how can the apparent occurrence of identical genotypes in distant plots be explained? With clonal spread, the probability of geitanogamous selfing might increase (Handel, 1985), which is in line with the reported high level of selfing in *E. hermaphroditum* (Tikhmenev, 1984). We therefore hypothesize that selfed seeds of a particular genotype might not be distinguishable unequivocally from vegetatively propagated clones. If maternal plants are highly homozygous as a result of previous cycles of selfing, then also sexually derived offspring may be genetically virtually identical with the mother plant, making it impossible to distinguish sexual from vegetative offspring. In this case, only demographic observations can unequivocally determine the origin of a particular ramet. With respect to the threshold of genetic distance allowed within a clone, the importance of studying half-sib families was underlined (Schleuning et al., 2011; Douhovnikoff and Dodd, 2003).

If the presence of distant identical clones is the result of sexually derived seeds after selfing of a highly homozygous mother plant, long-distance seed dispersal becomes an important process determining the genotypic structure of *E. hermaphroditum*. Important vectors for *Empetrum* seeds are birds and the arctic fox (*Alopex lagopus*; Graae et al., 2004) and red fox (*Vulpes vulpes*; Boudreau et al., 2010). However, the inability to unequivocally distinguish between selfed seeds and vegetative clones means that apparent clones within plots may also potentially be the result of sexual reproduction. Consequently, the proportion of sexual reproduction may be underestimated, and the results of our study should be interpreted with due caution.

CONCLUSIONS

Our study indicates that the clonal structure of *Empetrum* is affected by prevailing local habitat conditions. We observed an overall increase of clonal diversity from birch forest with deep snow cover to the alpine tundra with deep and shallow snow cover, although not consistently for all pairwise comparisons. High clonal diversity was found on exposed ridges with lower snow depth during winter, earlier snowmelt, higher proportion of open soil, and less competition than in the depression and the birch forest habitats (Callaghan et al., 1992; Szmidt et al., 2002). The low proportion of clonal reproduction implies relatively higher reproduction by seeds under such conditions (Eriksson, 1992; Boudreau et al., 2010).

It should be noted though that selfed seeds of some genotypes may not be distinguished unequivocally from vegetatively propagated clones. Our study showed that in *E. hermaphroditum* both clonal and sexual reproduction are important and that their relative proportions may be affected by habitat conditions.

ACKNOWLEDGEMENTS

Financial support was obtained from the Deutsche Forschungsgemeinschaft (DFG, grant EC209/9-1) and the European Union Seventh Framework Program [FP7/2007-2013] under grant agreement n_262693 [INTERACT]. Field assistance was provided by Josef Scholz-vom Hofe and laboratory assistance by Ina Geier, Martina Herrmann, and Michael Jager. We also thank Bente J. Graae, Ann Milbau, Wolf L. Eiserhardt, and the director and staff of the Abisko Scientific Research Station for climate data, logistic support, and accommodation. We are very grateful to Pamela K. Diggle and two anonymous reviewers for fruitful comments on an earlier draft of this manuscript. All help is gratefully acknowledged.

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