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Evolution in *Helianthemum oelandicum* (Cistaceae) – evidence of Holocene differentiation in morphology on the Baltic island of Öland, south-eastern Sweden

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We use *Helianthemum oelandicum* subsp. *oelandicum* as a model for studies of mechanisms shaping the diversity in a rapidly radiating lineage of the *H. oelandicum* aggregate. Locally, correlations between drainage conditions (using GIS to measure variation in the micro-topography on the more or less horizontal bedrock) and frequency of alleles of Mendelian genes for pubescence indicated that hairs are markers of adaptation to the unique environmental conditions (drought and periodic flooding) on the alvar habitats of Öland. An allozyme study showed that 1 and 4% of the genetic diversity is partitioned among two varieties and natural populations, respectively. F_{ST} for hair alleles was up to ten time greater than F_{ST} for allozymes. Öland rose above sea level after the LGM, which makes it possible to date the onset of the post-glacial diversification on the island. Only two, mainly allopatric, plastid DNA haplotypes were detected, H1 and H2. The southern *Helianthemum oelandicum* subsp. *oelandicum* var. *canescens* has only H1, whereas the more widespread var. *oelandicum* possesses both haplotypes. A restricted occurrence of haplotype H1 in the core area of var. *oelandicum* in the central Öland, coincided with the highest elevation on the island, probably representing the post-glacial arrival of the species on the island.

ADDITIONAL KEYWORDS: allozymes – ecotypes – flowering phenology – GIS – hybrid zones – indumentum – micro-topography – PCR-RFLPs – plastid haplotypes.

INTRODUCTION

A significant outcome of species expansion and contraction during the cycles of Ice Ages in the Pleistocene may have been the formation of hybrid zones between species as well as contact zones between refugial populations within species (Nichols & Hewitt, 1994). Many animal and plant species had been split into genetically and morphologically distinct groups in their glacial refugia. When these lineages met, hybrid or contact zones could have been formed. Such zones could have been stable over time, possibly because the different migrant populations had adapted to different ecological niches in geographically separated refugial populations (Hewitt, 2000, 2001). Alternatively, gene flow between lineages created dynamic contact zones, which were not stabilized due to the short time in refuge areas during the climatic cycles, or the genetic admixture between lineages created novel adaptive gene combinations that became established (Havrdová *et al.*, 2015). Such dynamic cycles of expansion and retraction of the range of species may explain the rapid radiations found in many species-rich plant clades of temperate European plants (Guzmán, Lledó & Vargas, 2009; Valente, Savolainen & Vargas, 2010; Fior *et al.*, 2013; Moazzenti *et al.*, 2014; Martin-Hernanz *et al.*, 2019). The onset of the radiation often coincided with the climatic changes in the transition between the Pliocene and the cold and dry climate of the Pleistocene. Diversification rates accelerated

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during the glacial cycles of the Pleistocene (Valente *et al.*, 2010) and led to an intricate morphological diversity in many clades with notorious taxonomic complexity. Differentiation could be a consequence of geographical isolation per se (Edh, Widén & Ceplitis, 2007; Valente *et al.*, 2010), but in many clades radiation was adaptive, for example, in floral characters (Fior *et al.*, 2013) or leaf morphology (Guzmán *et al.*, 2009).

Variation in morphological traits used as markers in taxonomically complex species may have a Pleistocene origin, but the current structuring of the phenotypic differentiation is shaped by both historical and contemporary processes (Sork *et al.*, 1999). Mechanisms and processes creating the diversity in morphology during the climatic cycles of the Pleistocene are obscure and can only be indirectly inferred. This contrasts with the processes in Holocene where local adaptation during the ongoing climate change and human impact on vegetation (Mottl *et al.*, 2021) can be studied by various methods (e.g. Turesson, 1922; Clausen, Keck & Hiesey, 1940; Ågren & Schemske, 2012). Recent local adaptation of plants over various distances has been well documented (e.g. Kruckeberg, 1951; Jain & Bradshaw, 1966; Snaydon, 1970; Kärkkäinen, Løe & Ågren, 2004), and the identification of genes underlying a specific adaptation is a fundamental task for understanding evolution (Orr, 2005; Ellegren & Sheldon, 2008). The adaptive significance of different phenotypes or alleles of morphological markers governed by Mendelian genes (Widén, 2015, 2018a, b), can be tested directly in natural populations. By choosing geographical areas that were re-colonized after the Last Glacial Maximum (LGM), a starting point for the onset of local adaptation in the Holocene can be established (*tabula rasa*).

Here, we have chosen the Baltic island of Öland and *Helianthemum oelandicum* (L.) Dum. Cours. subsp. *oelandicum* as a model for studying differentiation in a rapidly radiating lineage (Martin-Hernanz *et al.*, 2019). First, *H. oelandicum* shows great morphological diversity throughout its distribution range, with a number of subspecies based on a few key characters (Janchen, 1907; Proctor & Heywood, 1968; Widén, 1980, 2018a, b). The diversity of the endemic subsp. *oelandicum* on the restricted area of the island of Öland covers variation in the main taxonomic key characters of the species complex (e.g. pubescence and flowering phenology), and recent studies have shown that simple Mendelian genes are involved in some of these characters (Widén, 2018a, b). Second, Scandinavia including Öland was covered by ice during the Last Ice Age, and the island rose above sea level after 12 000 BP. Pollen records of *H. oelandicum* (Berglund, 1966) give a starting point for the processes of differentiation of *H. oelandicum* on Öland after the LGM and show how the history of the species on the island (Königsson, 1968) has been influenced by climate and human impact on vegetation (cf. Mottl *et al.*, 2021).

We use plastid DNA to trace migration history, allozymes to quantify genetic diversity and morphology to study adaptation of the 'Öland rock rose' *H. oelandicum* subsp. *oelandicum* to the unique alvar habitats on Öland, i.e. dry calcareous grasslands more or less endemic to the Baltic Sea region (Bengtsson *et al.*, 1988; Reitalu *et al.*, 2014). We investigate the fine-scale morphological/genetic structure of the species and test whether the spatial pattern of diversity can be explained by adaptation to the local environment as suggested by Widén (1988, 2018a, b). We use GIS to record the topographical structure as a measure of the fine-scale drainage conditions of the bedrock of Öland. The aims of this paper are (1) to investigate the plastid DNA and nuclear gene variation (allozymes) of the species on Öland, (2) to compare this molecular diversity with the geographical distribution of adaptive genes for morphological traits, (3) to compare adaptive genes and environment and (4) to interpret the spatial distribution of plastid DNA and nuclear genes in the light of available pollen records, evolutionary processes and the Holocene history of the species on Öland.

MATERIAL AND METHODS

Study species

Helianthemum is a young monophyletic genus of Mediterranean origin in Cistaceae (Aparicio *et al.*, 2017). *Helianthemum oelandicum* is one of several species complexes originating in the Pliocene—Pleistocene (Aparicio *et al.*, 2017; Martin-Hernanz *et al.*, 2019). Indumentum is the morphological marker in much of the differentiation of *Helianthemum*, especially in two of the most widespread lineages, the *H. oelandicum* aggregate (agg.) and the *H. nummularium* (L.) Mill. agg. (Janchen, 1907, 1909; Soubani, Hedrén & Widén, 2014, 2015; Widén, 2015, 2018a, b; Volkova *et al.*, 2016).

Helianthemum oelandicum subsp. *oelandicum* is a young (cf. Aparicio *et al.*, 2017), diploid ($2n = 22$), self-incompatible, wind pollinated (Törnblom, 1908; Janzon, 1983) and short- to long-lived (cf. Fröberg *et al.*, 2009) dwarf shrub belonging to the *H. oelandicum* complex (Widén, 2010, 2018a, b). The species is represented by two endemic varieties, *H. oelandicum* subsp. *oelandicum* var. *oelandicum* and *H. oelandicum* subsp. *oelandicum* var. *canescens* (Hartm.) Fr., which differ in flowering phenology (Widén, 1980, 2010, 2018a, b). The type variety (var. *oelandicum* from hereon) has a short, concentrated flowering (CF) period in early June based on inflorescences borne on the previous year's growth. *Helianthemum oelandicum* subsp. *oelandicum*

var. *canescens* (var. *canescens* from hereon) has a protracted flowering (PF) period from June till October with one flowering peak in June (inflorescences borne on the previous year's growth) and one flowering peak in July–August (inflorescences borne on the current year's growth) (Widén, 1980). Both varieties show variation in indumentum, from glabrous plants to plants with bristles in var. *oelandicum* and plants with only bristles to plants with a dense cover of stellate hairs on the abaxial surface of the leaves in var. *canescens* (Widén, 1988, 2018a, b).

The distribution of the two varieties is allopatric; var. *canescens* is restricted to the southernmost part of Öland and var. *oelandicum* has a wide distribution on the Great Alvar and on isolated alvars in the central and northern part of the island (Fig. 1). In the southernmost part of the Great Alvar, where the two varieties meet, narrow hybrid zones have been established (Widén, 1980, 1988). In the following, we use CF and PF plants synonymously with var. *oelandicum* and var. *canescens*, respectively.

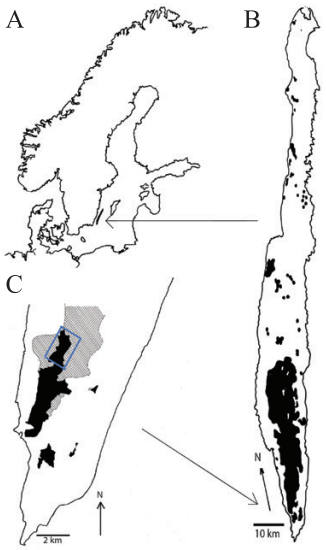

Figure 1. Distribution of *Helianthemum oelandicum* subsp. *oelandicum* on the Baltic island of Öland, SE Sweden. A, The Baltic area. B, The Baltic island of Öland. The distribution of *H. oelandicum* (black) according to Sterner (1936). The Great Alvar coincides with the continuous distribution of *H. oelandicum* on the southern third of Öland. C, A simplified representation of the distribution of *H. oelandicum* on the southernmost part of Öland. Black indicates the area where > 90% of the plants belong to var. *canescens* and stripes indicate the distribution of var. *oelandicum*. The rectangle shows the hybrid zones discussed in Figure 6D, E (see also Supporting Information, S5). The area marked with black in the southernmost part of Öland represents the outer border of several small, more or less isolated patches of var. *canescens* south of the Great Alvar.

The study area

During the Last Ice Age (maximum 22–20 000 years BP), Fennoscandia was covered with a thick ice sheet (Svendsen *et al.*, 2004; Binney *et al.*, 2017). As the main ice sheet retreated from Fennoscandia (starting *c.* 15 000 years BP), plants and animals re-colonized the region from different directions and source areas (Hewitt, 1999; cf. Soubani, 2010, for references).

The bedrock of Öland is made up of Ordovician limestone pavements. The southern half of the island is dominated by the Great Alvar, a more or less treeless steppe covering about 250 km^2 characterized by an extremely flat limestone plateau (Fig. 2), which is mostly completely exposed or covered by a thin layer of weathered soil or glaciofluvial deposits (Königsson, 1968; Rosén, 1982).

The Great Alvar and a number of restricted alvar areas outside the Great Alvar on Öland are often subjected to extreme drought in summer, frost perturbation in winter and waterlogging in autumn and spring. Most summers have periods of low precipitation and severe drought occurs at intervals of approximately ten years (Prentice *et al.*, 1995), the most extreme year during the last 50 years being 2018 (B. Widén, in prep.).

The present-day distribution of *H. oelandicum* on Öland is restricted to a mosaic of habitats in the open grasslands, either on horizontal bedrocks with thin layers of weathered soils or on shallow gravel deposits, or on open bedrocks with fissures and cracks, filled with soil (Sterner, 1936; Albertsson, 1950; Königsson, 1968; Bengtsson *et al.*, 1988). The patchy distribution of such habitats on the Great Alvar promotes the growth and colonization of more or less continuous and large populations of *H. oelandicum* over thousands of square metres, making it one of the most conspicuous and important plants on the Great Alvar today (Fig. 2; Widén, 2018b).

Data collection

This study is a synthesis, partly based on a recompilation of data collected for *H. oelandicum* on Öland over decades. The sampling of sites originally took place during the mid-1970s in a randomized procedure, described in Widén (2018a, b). The successive sampling was intended to be representative for the detected diversity. In the following, all plants from a site are considered a 'local population', although it should be observed that the distribution of *H. oelandicum* subsp. *oelandicum* in many alvar areas on Öland is patchy, with more or less continuous stands.

Population samples

In the original *field sample 1* (cf. Widen, 2018b), 106 sites of var. *oelandicum*, 29 sites of var. *canescens* (Widén, 2018a, b) and 19 sites from the narrow zones

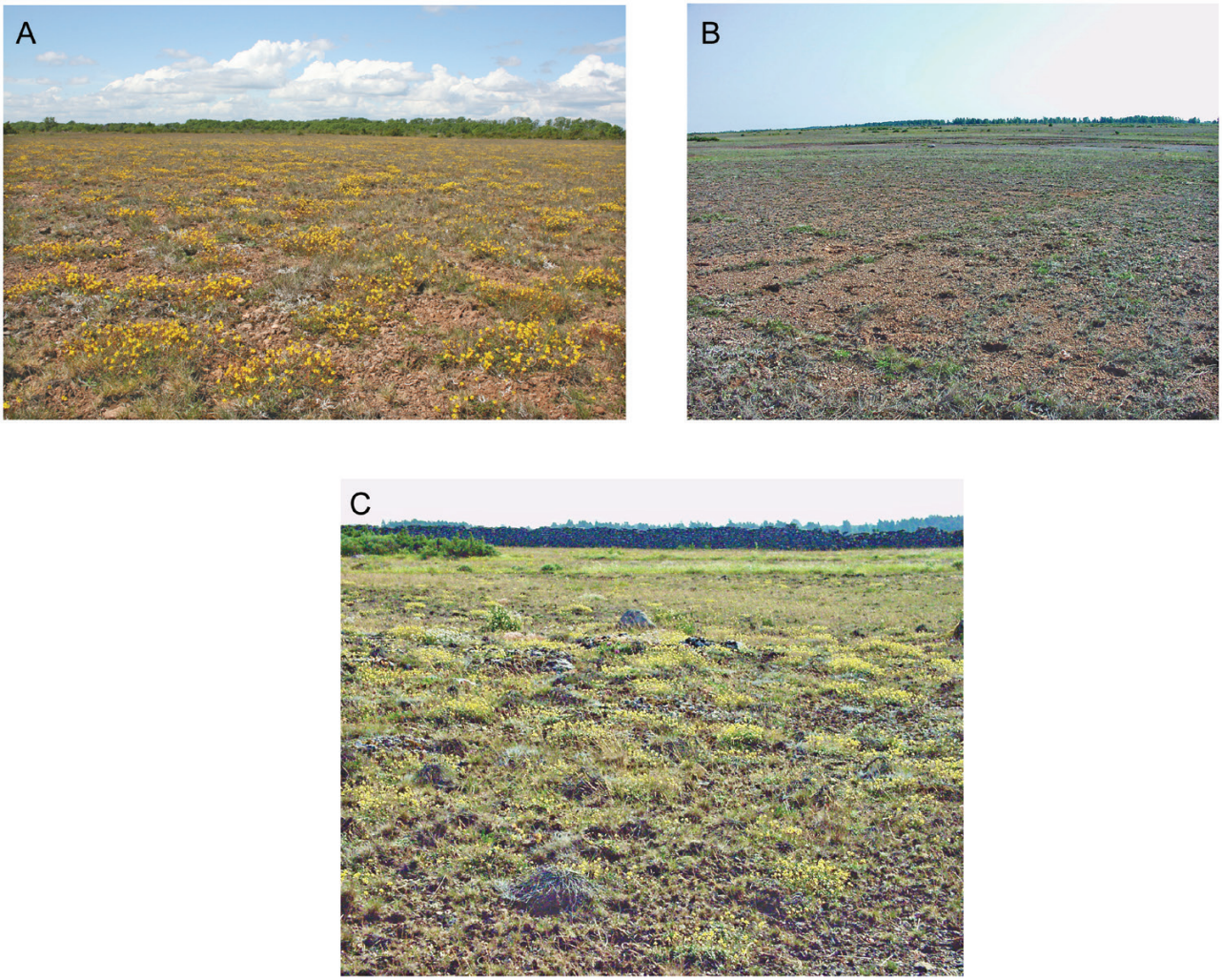

Figure 2. The Great Alvar is coloured yellow by the flowering of *Helianthemum oelandicum* subsp. *oelandicum*. A, Flowering of var. *oelandicum* during a few weeks in early summer: a south-east view just southeast of Vickleby, 13 June 2010. Photograph: E. Rosén. B, The colour of the Great Alvar dominated by *H. oelandicum* turns dark after the flowering of var. *oelandicum*. An eastwards view along the section C in the Albrunna transect in Figure 5C, 15 July 2003. C, The yellow colour of the Great Alvar occupied by var. *canescens* often has a pale intensity but a longer duration than in var. *oelandicum*. The peak flowering of var. *canescens* often occurs in mid-late July. A south-east view from the core area of section PF in Figure 5C, 3 July 2001.

between the two varieties were sampled (cf. Widén, 1988). Some of the sites in the zone were difficult to locate accurately and have been discarded (cf. Widén, 1988). We allocated the 12 sites in the zones included in this study to var. *oelandicum,* if the proportion of CF plants at the site was > 0.5, otherwise to var. *canescens* (Supporting Information, S1).

Plastid samples

Based on earlier knowledge of the spatial pattern of morph distribution of *H. oelandicum* subsp. *oelandicum* on Öland (Widén, 1980, 1988), 123 sites were sampled on a regional scale in the early 2000s. All sampled sites could be referred to a distinct variety. The sampling was particularly dense in areas that had shown variation in morphology in previous studies. Sites were usually > 100 m apart. At each site (within a radius of 10 m), leaves (from at least two plants) were stored in silica gel until used for DNA extraction and five adult plants (at flowering or post-flowering stage) were sampled and pressed for morphological analysis (Supporting Information, S2).

Allozyme samples

The sites, sampled in the early 1990s (Fig. 3), constituted a proportion of the sites in the 'population sample', representing diversity and geographical

range (cf. Widén, 2018a, b). Cultivated plants used for the allozyme study were derived from cuttings of plants in permanent plots or an offspring derived from seeds sampled from individual plants within the original sites and cultivated 1992-1996 in the common garden at Lund University. Plants from 14 sites were classified as var. *oelandicum*, and plants from 12 sites as var. *canescens*.

Hybrid zones

The distribution areas of the two varieties are often separated by habitats not suitable for *H. oelandicum* [e.g. wet meadows, temporal pools or dense stands of the shrub *Dasiphora fruticosa* (L.) Rydb.]. Populations consisting of a mixture of the two varieties and various intermediates frequently occur when the distribution of continuous populations of the two varieties meet in narrow zones (hereafter called hybrid zones). Scattered individuals of var. *canescens* (i.e. individuals with at least a few inflorescences borne on the current year's growth and probably of hybrid origin) can be found in continuous areas of var. *oelandicum* at a distance of several hundred metres from the border between the distributions of the two varieties (cf. Widén, 1980). However, no individuals of var. *oelandicum* (i.e. individuals with only inflorescences borne on the previous year's growth) have been ascertained within continuous areas of var. *canescens* (Widén, 1980).

Ignoring outliers of var. *canescens* in the areas of var. *oelandicum*, the spatial extent of the zones between the two varieties was estimated in mid-July to August of 2004 (and some in 2011) by the following procedure. Walking across the zones (based on knowledge acquired since the early 1970s, cf. fig. 13 in Widén, 1980), the coordinates of the most marginal patch (the mid-point of a plot with a radius of 10 m) of pure populations of var. *canescens* (PF sites) and var. *oelandicum* (CF sites), respectively, were determined with a GPS device. Coordinates for patches with both varieties (mixed sites) were recorded and the patches (radius 10 m) were roughly classified as (1) dominated by CF plants, (2) no dominance of any variety and (3) dominated by PF plants (a more detailed examination of hybrid zones will be published elsewhere).

Transects

One W-E transect across the alvar in the northern, central and southern part of the Great Alvar, respectively, was used to estimate (1) the frequency of dead plants after the catastrophic drought in 2018 (see fig. 2 in Widén, 2018b) and (2) to record pubescence, especially the allele frequency for hair markers (see

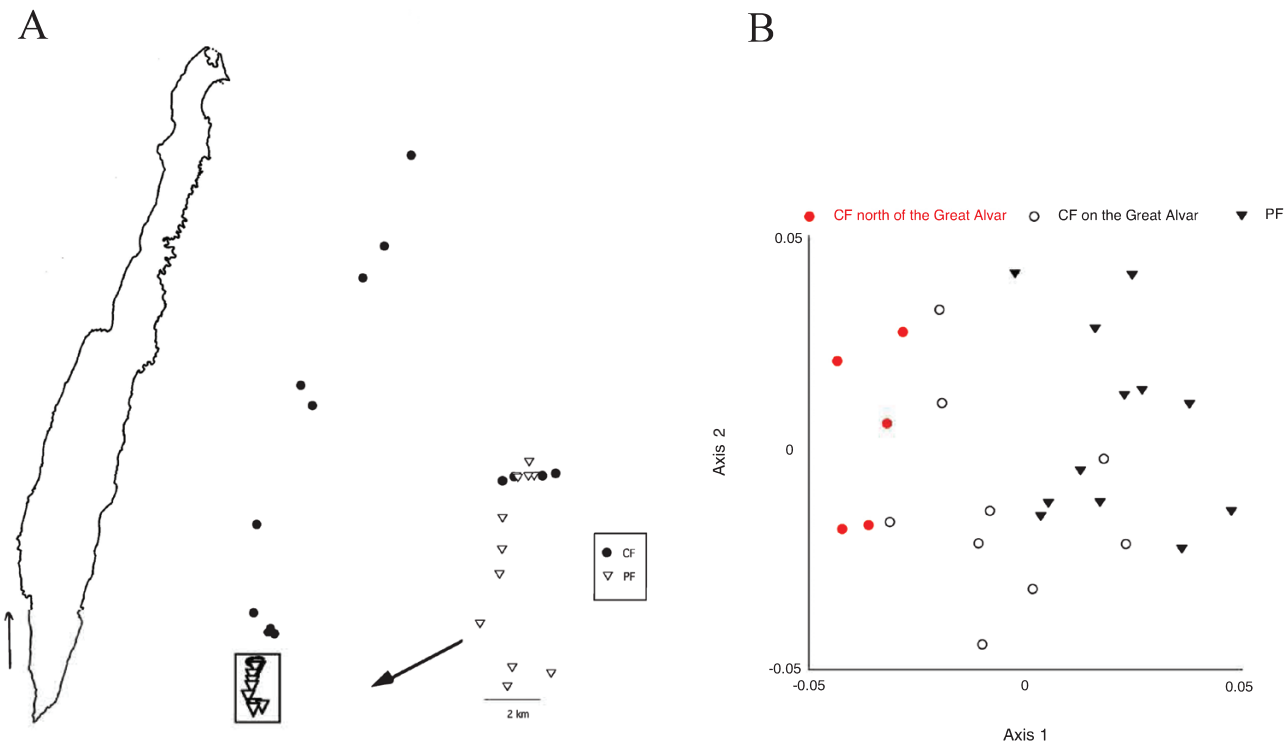

Figure 3. The allozyme samples of *Helianthemum oelandicum* subsp. *oelandicum* on the Baltic island of Öland, SE Sweden. A, The distribution of samples. Variety *oelandicum* (CF) and var. *canescens* (PF). B, Principal coordinates analysis of allozymes. The first and second axes explained 23.8 and 18.2%, respectively, of the variation.

next). The northern transect (established in 2019) comprised *c.* 1 km of the westernmost edge of the alvar, just south of the village of Vickleby (the Vickleby region). The middle transect (established in 2019 in the Storåsen region), and the southern transect made between the villages of Albrunna and Kvinnsgröta in 2020 (the Albrunna region, cf. Widén, 1980, 2018a, b), extended across the entire width of the Great Alvar.

If any *H. oelandicum* was present (dead or alive) within a radius of 10 m at each 50-m interval (GPS determined) along a transect, the distance (a site) was recorded as occupied by the species, and two samples (1-m^2) were chosen. To estimate the death rate at each site, a first plot was sampled by throwing a frame to the nearest patch with at least five plants of *H. oelandicum* (dead or alive). A second plot was subjectively chosen within the site to include at least five living plants (if possible). The position of the frame was adjusted to include a maximum number of individuals. A sample of plants alive within the plots (excluding seedlings with only cotyledons and fewer than three pairs of leaves) was classified according to hair phenotype and to flowering phenotype (if possible). To obtain a rough estimate of the population continuity along transects we calculated the proportion of 50-m intervals that contained any plants (dead or alive) along different section of a transect (population continuity = the number of intervals with plants/ the total number of 50-m intervals).

Topography

The spatial distribution of sites was estimated using the national grid system (SW99TM). The position of most sites in the population sample (cf. Widén, 2018b) was estimated from the national topographical maps (Topografisk karta över Sverige) in the 1970s. The coordinates for sites used for population studies after 2003 (the plastid samples, the hybrid zones and transects) were determined by using a GPS device (Magellan). The coordinates in the population sample were therefore less accurate than in other samples. We used the digital maps from Lantmäteriet to obtain an estimation of the topographical variation at each sampled site. The altitude was recorded with an accuracy of 0.1 m in a grid of 1 m. We collected data from each grid in three/four areas around each sampling point: within a radius of 10 m (N = 80), 20 m (N = 310), 30 m (N = 710) and 50 m (N = 1968), respectively. The more variation in elevation among sampling points in an area, the more heterogeneous the topography. We assumed that the local drainage conditions were correlated with heterogeneity of the topography at each site. As a measure of the heterogeneity of topography we used the range and SD of the altitudes for each sampled radius.

DATA PROCESSING

DNA extraction and PCR-RFLP analysis

Three hundred and two individuals representing 123 sites (usually N = 2 individuals/site, but sometimes as many as ten plants/site) were used for DNA extraction and PCR-RFLPs analysis (see Supporting Information, S2). Total genomic DNA was extracted following the CTAB-based extraction protocol (Doyle & Doyle, 1990), with some minor adjustments according to Lodhi *et al.* (1994) to optimize the protocol for *Helianthemum*. Based on Soubani (2010), four primer pairs that showed considerable variation in the PCR-RFLPs were used for screening of the entire material (see Soubani (2010) and Supporting Information, S2 for details).

Allozyme extraction and electrophoresis

Trials with different extraction methods using leaves gave mucilaginous extracts with bad resolutions in subsequent electrophoresis. To avoid the problem of resolution we extracted only flower buds (petals, stamens and ovaries). The details of extraction and electrophoresis are given in Supporting Information, S3.

Classification of indumentum

Widén (1988) introduced a protocol to describe the complex variation of hairs in *H. oelandicum* agg. (cf. Janchen, 1907). The density of hairs on different parts of the plant was classified in hair scores ($Hair_{(x)}$ score, x = type of hairs on specific part of the plant) according to a template, increasing from 0 to 3 in intervals of 0.5 (Widén, 2018a, b). Here we combined hair scores in different parts of the plant in a hair index, which is the average of the hair scores for (1) stellate hairs on the abaxial surface of the leaves ($Hair_{(stab)}$ score), (2) bristles on leaves ($Hair_{(brl)}$ score), (3) hairs on inflorescences, (4) stellate hairs on sepals, (5) bristles on sepals and (6) glandular hairs on inflorescences (cf. Widén, 1988).

Hair phenotypes in the transects were classified in the field using a hand lens, and applying a simplified version of the hair scores (0, 1, 2 and 3) for $Hair_{(stab)}$ and $Hair_{(brl)}$ (see previously), summarized in the hair index $HI_{(2)}$. Plants in all other samples were phenotyped under a dissecting microscope in the lab using the original protocol. For the population sample, six hair scores were included in the hair index $HI_{(6)}$. In the plastid sample, $Hair_{(brl)}$ and $Hair_{(stab)}$ scores (see previously) were included in the hair index $HI_{(2)}$.

Allele frequencies for indumentum

The overall density of hairs on leaves is determined by quantitative genes, whereas a threshold density is determined by recessive alleles of Mendelian genes

in both varieties (Widén, 2018a, b). Provisionally, we treat the analysed indumentum characters on leaves of *H. oelandicum* as representing separate genes in the two varieties. Glabrous plants ($Hair_{(brl)}$ score = 0 and $Hair_{(stab)}$ score = 0) of var. *oelandicum* were classified as homozygous for the *glab* allele (cf. Widén, 2018b) in the locus GENE1. Plants of var. *canescens* were classified as homozygous for the *can* allele in the locus GENE2 if the density of stellate hairs on abaxial surface of the leaves ($Hair_{(stab)}$ score) was ≥ 2 on the scale 0 to 3 (see Widén, 2018a, for more information). Interaction between GENE1 and GENE2 in hybrids between var. *oelandicum* and var. *canescens* is complex and not yet fully understood (B. Widén, unpublished). Therefore, we simplified the calculation of gene frequencies in the few mixed sites by referring individual sites to either var. *oelandicum* or var. *canescens*.

Data analysis

Plastid DNA

The molecular data was scored as multistate characters where each primer–enzyme combination was considered a character and the different banding patterns (as a result of differences in the size and numbers of fragments) as character states (Supporting Information, S2).

Allozymes

The genetic analysis was based on seven polymorphic allozyme loci (Supporting Information, S2), and performed using the GenAlEx v.6.5 program (Peakall & Smouse, 2006, 2012). It has been argued that allozymes provide a poor estimation of neutral population differentiation, since allozyme loci may be subjected to natural selection (Riginos, Sukhedo & Cunningham, 2002; Dhuyvetter, Gaublomme & Desender, 2004; Li *et al.*, 2015). We tested for any association between allele frequency and topography (the environmental factor in focus of the present study). The total genetic diversity (mean over loci) was shown by H_T, and the within-population genetic diversity by H_S (Nei, 1973). The between-population genetic diversity was estimated by F_{ST} ($F_{ST} = (H_T - H_S)/H_T$). The deviation from the Hardy–Weinberg equilibrium tested (Chi-square test) for each locus and population (Nei, 1973) was based on the observed number of heterozygotes (H_{obs}) and the expected number of heterozygotes under the Hardy–Weinberg equilibrium (H_{exp}). AMOVA was used to partition the genetic diversity between the two varieties and among population (SPSS 27). A Mantel test, implemented in PAST v.4.04 software (Hammer & Harper, 2006) based on pair-wise F_{ST}, was performed to test for correlation between genetic and geographical distances.

GIS data

The raw data was scrutinized to exclude sampled radii where obstacles in the bedrock, such as escarpments, man-made cavities or stone walls could increase the heterogeneity measures despite an underlying smooth bedrock. Large erratic boulders in some areas with glacial deposits could also increase the recorded heterogeneity. We therefore excluded a few data representing radii of 30 to 50 m when the ranges in elevation exceeded 2 m, and radii of 10 to 20 m when the ranges of elevation exceeded 1 m.

RESULTS

Allozyme diversity

We found no significant correlation between allele frequencies and topography after Bonferroni correction (see Supporting Information, S3). Ninety percent of alleles did not deviate from Hardy–Weinberg equilibrium (Supporting Information, S3). The total diversity $H_T = 0.377$, $F_{ST} = 0.054$ and 1 and 4% of the diversity were partitioned among varieties and populations, respectively (Table 1). The PCA analysis showed a geographical structure in the genetic diversity (Fig. 3), with populations of var. *oelandicum* north of the Great Alvar found to the left on axis 1 and var. *canescens* to the right (the correlation between axis 1 and latitude is significant, $r = 0.74$, $P < 0.01$). A Mantel test showed significant positive correlation between genetic and geographical distances (Table 1).

Distribution of plastid haplotypes

Two plastid haplotypes were recorded on Öland, H1 and H2 (Supporting Information, S2). The 13 populations of var. *canescens* were fixed for H1. Of the 110 populations of var. *oelandicum*, 49 populations contained H1, 57 populations contained H2 and four showed a mixture of the two plastid haplotypes (Supporting Information, S2).

The distribution of the two haplotypes was mainly allopatric with H1 covering the southern part of Öland and a disjunct area in the northern part of the Great Alvar (Vickleby region), whereas H2 was found in the rest of the Great Alvar and further to the north (Fig. 4A). A Mantel test showed significant positive correlation between haplotypes and geographic distance (Table 1). Mixture of the two plastid haplotypes (within sampling plots) was only found in the northern Great Alvar, in four sites along the border between the distribution of the two haplotypes (Fig. 4B). A narrow contact zone between the two haplotypes across the alvar was found in the southern part of the Great Alvar in the Storåsen region (Fig. 4B). In the southernmost part of

Table 1. Population genetics of *Helianthemum oelandicum* subsp. *oelandicum*

A, Partitioning of variation in the allozyme study

Source	Percentage of variation
Between varieties	1
Between populations	4
Between individuals	4
Within individuals	91

B, Population genetics based on the allozyme data

	Mean over loci and populations	SE
F_{IS}	0.012	0.030
F_{IT}	0.066	0.029
F_{ST}	0.054	0.007
H_o	0.366	0.016
H_e	0.373	0.015

C, Distance matrices

Distance matrix	Correlation coefficient	*P* value	Number of populations
Allozymes vs. geography	0.573	0.01	26
Haplotypes vs. geography	0.093	0.000	123
$HI_{(6)}$ vs. geography†	0.011	0.37	147
$HI_{(2)}$ vs. geography*	0.001	0.33	123

D, F_{ST} for hair alleles**

Allele	Sample	Variety	F_{ST}	*P* value	Number of populations
can	Population sample	*canescens*	0.306	0.000	28
can	Plastid sample	*canescens*	0.356	0.000	13
glab	Population sample	*oelandicum*	0.509	0.000	107
glab	Plastid sample	*oelandicum*	0.567	0.000	110

†Population sample.
*Plastid sample.
**F_{ST} was calculated as AMOVA according to Weir and Cockerham (1984) with significance test based on 10 100 permutations. Sites with both varieties in population sample were excluded from the calculations (see text).

Öland, all plants had the H1 haplotype, irrespective of taxon (Fig. 4B).

Helianthemum oelandicum var. *oelandicum* has a continuous distribution in the central part of the Great Alvar around the contact zone between the two haplotypes in Storåsen region. The central part of the contact zone was often distinct; populations displaying different haplotypes were sometimes separated by only 100 m, but were often intermixed with habitats without *H. oelandicum*. The south-easternmost part of the contact zone, on the other hand, was more diffuse with large areas without *H. oelandicum*.

The bedrock of the Great Alvar dips slightly to the south-east (Supporting Information, S4). The highest elevation on Öland (55.0 m a.s.l.) is found in the centre of the island, just north of the Great Alvar. We recorded both haplotypes in var. *oelandicum* at elevations > 45 m in the northern part of the Great Alvar in the Vickleby region, close to the village of Resmo, eight sites with haplotype H2, three sites with haplotype H1 and one site with both haplotypes (Fig. 4A, B). Haplotype H2 in var. *oelandicum* just north of the Great Alvar was recorded at 35 m, whereas H1 in var. *oelandicum* was recorded at all elevation intervals on the Great Alvar.

The most northern record of H1 in var. *canescens* was at an elevation interval > 15 m. The highest elevational record of H1 in var. *canescens* south of the Great Alvar was at an elevation below 15 m a.s.l. Haplotype H2 in var. *oelandicum* in the northern half of Öland was also recorded < 15 m (Fig. 4A).

Variation in hair index

Variation in pubescence showed significantly higher values in var. *canescens* than in var. *oelandicum*, when measured as $HI_{(6)}$ in the population sample, as

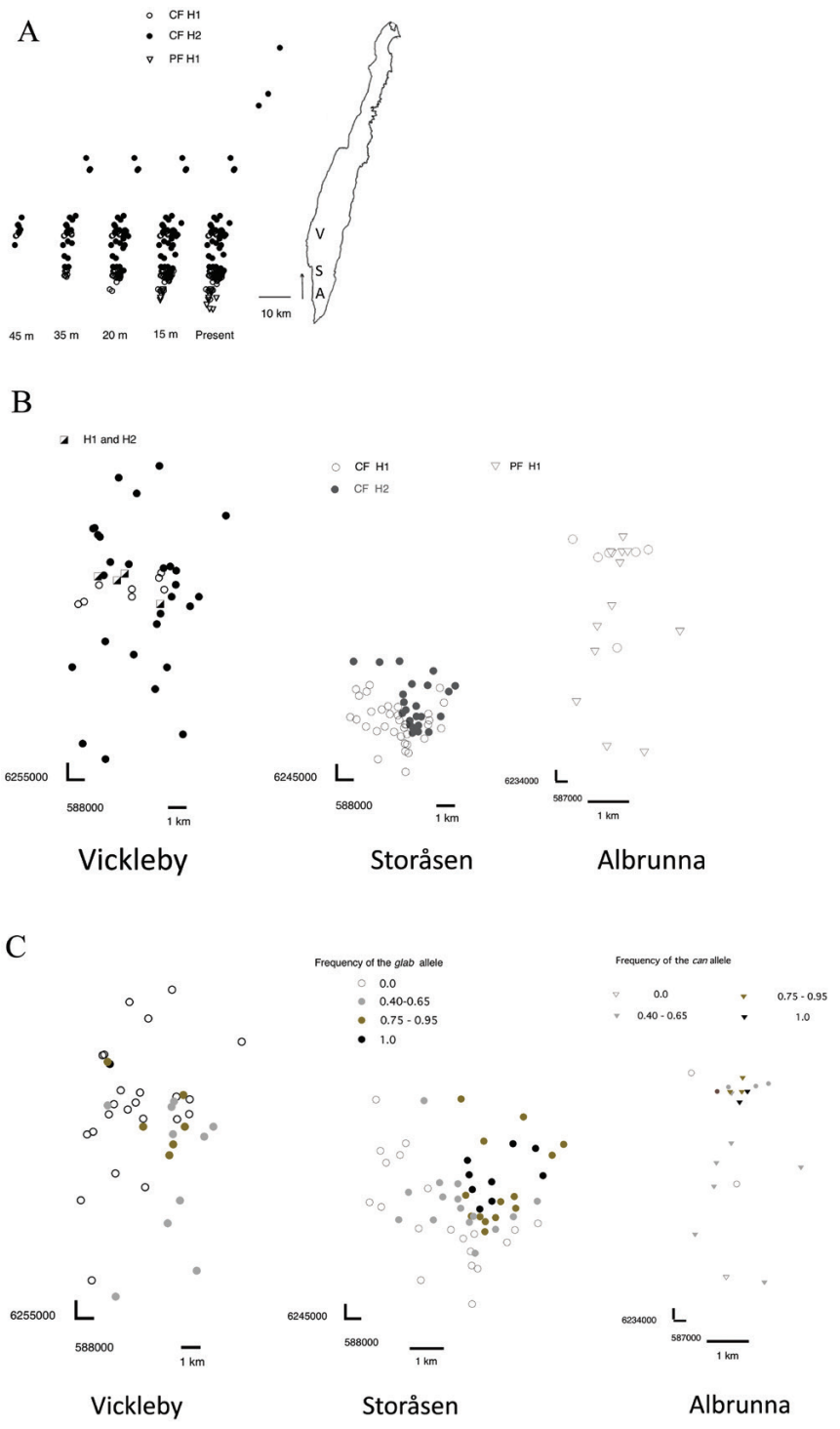

Figure 4. The haploid sample of *Helianthemum oelandicum* subsp. *oelandicum* on the Baltic island of Öland. A, The distribution of the two plastid haplotypes (H1 and H2) in *H. oelandicum* on Öland. CF = var. *oelandicum* and PF = var. *canescens*. The distribution of samples above different altitudes (indicated on the horizontal axis). V = Vickleby region. S = Storåsen region. A = Albrunna region. The area north of V (outside the Great Alvar) is denoted Northern Öland in Table 2C. B, Plastid haplotype distribution in enlarged areas in Vickleby, Storåsen and Albrunna regions. The figures indicate the origin of *x* and *y* coordinates for the area in the Swedish coordinate system (SW99TM). C, Frequency of the *glab* allele in the plastid sample of *H. oelandicum* in the Vickleby, Storåsen and Albrunna regions, as well as the frequency of the *can* alleles in the Albrunna region. The origins of *x* and *y* coordinates in the Swedish grid system (SW99TM) are shown.

well as for $HI_{(2)}$ in the plastid sample (Table 2A, B). A Mantel test showed no significant correlation between pubescence [$HI_{(6)}$ or $HI_{(2)}$] and geographical distance (Table 1). No differences in $HI_{(2)}$ between regions within the distribution of var. *oelandicum* could be established in the plastid sample (Table 2C), although diversity in the Storåsen region was heterogeneous in the plastid sample (Table 2D). The heterogeneity of pubescence in the Storåsen and Albrunna regions revealed by the transect sample is shown in Table 3.

Frequency of hair alleles

Variation in pubescence within sites in the transect sample was homogeneous. The correlation between the two samples within sites in the transect sample was significant for the frequency of the *glab* allele in the Storåsen transect ($r = 0.912$, $P < 0.001$) and for CF sites in the Albrunna transect ($r = 0.517$, $P < 0.001$), but not in the Vickleby transect. The correlation of the *can* allele for the PF sites in the Albrunna transect was also significant ($r = 0.734$, $P < 0.05$).

The spatial pattern of the *glab*-allele frequency differed among regions (Fig. 4C) and transects as well as between sections within transects (Fig. 5, Table 3A). The frequency was zero at the western margin of the Vickleby transect and increased after 350 m along the transect (Fig. 5A, Table 3B). The frequency of the *glab* allele differed significantly between the western and eastern half of the Storåsen transect, with an increasing frequency eastward in the western half to close to 1.0 in the eastern part (Fig. 5B, Table 3C, see also Fig. 4C). The Albrunna transect cuts across the northern part of the distribution of var. *canescens* in the centre of the Great Alvar (cf. Figs 1 and 5C, Table 3D), and the shift between the two varieties is abrupt, and it was only at the eastern edge of the distribution of var. *canescens* that a mixed population was encountered (Fig. 5C). The frequency of the *glab* allele shows a remarkably similar and symmetric pattern on each side of the distribution area of var. *canescens* (Fig. 5C). Few PF plants (with inflorescences borne on the current year's growth) were found outside the core area of var. *canescens* (Fig. 5C), only a few at the easternmost part of the Albrunna transect. In contrast, scattered plants with a dense cover of stellate hairs on the abaxial surface of the leaves ($Hair_{(stab)}$ score ≥ 2) in var. *oelandicum* were found outside the core area of var. *canescens* (Fig. 5C). Only one individual with $Hair_{(stab)}$ score = 3 was found in var. *oelandicum* in the easternmost part of the Storåsen transect (a CF plant). The differentiation between local populations with respect to the recessive alleles was pronounced; $F_{ST} = 0.502$ for the *glab* allele and $F_{ST} = 0.282$ for the *can* allele in the population sample, and $F_{ST} = 0.567$ for the *glab* allele and $F_{ST} = 0.356$ for the *can* allele in the plastid sample (Table 1).

Population continuity along transects across the Great Alvar

The estimated population continuity varied among transects and sections within transects (Table 3B–D). The western section of the Storåsen transect showed an especially low value for the estimated population continuity (Table 3C). The distribution of *H. oelandicum* is more or less continuous along the western and central part of the Albrunna transect, with estimated population continuity between 0.82 and 0.89, but the distribution is interrupted by a mosaic of habitats not suitable for the species in the easternmost part of the transect with a low value for the estimated population continuity (0.43) (Fig. 5C, Table 3D).

Effects of drought

Drought in 2018 had a great impact on the survival of *H. oelandicum*, with large heterogeneity among sites. The survival of plants in the Albrunna transect averaged only ten percent and was slightly higher in the other transects (Fig. 5C, Table 3A) based on the random sample at each site. Survival was especially low at the western margin of the Vickleby transect (Fig. 5A, Table 3B), whereas survival showed more heterogeneity along the Storåsen and Albrunna transects (Fig. 5B, Table 3C, D).

There was no overall correlation between the frequency of hair alleles and survival of the drought in 2018, but different trends among and within transects (Table 3A). A significant negative correlation between survival and frequency of the *glab* allele was found in var. *oelandicum* in the Albrunna transect (Table 3A). Glabrous plants also seem to have a disadvantage over pubescent plants at the margin of the Vickleby transect, since the frequency of the *glab* allele decreased between 1975 and 2019 (Table 3B). The same trend was found in the western section of the Storåsen transect (Table 3C). The differences between 1975 and 2019 in other sections of the transects at Vickleby and Storåsen were non-significant.

Distribution of varieties across hybrid zones

The border between the distribution areas of var. *oelandicum* and var. *canescens* is sharp in the northern third of the distribution of var. *canescens* on the Great Alvar (marked in Fig. 1, Supporting Information, S5). Narrow hybrid zones < 100 m wide can be identified

Table 2. Pubescence and topography in *Helianthemum oelandicum* subsp. *oelandicum*. Mean (SD) sample size. Topography was measured as range and SD of altitude measured with an accuracy of 0.1 m in a grid of 1 m around the mid-point at each plot

A, Hair index (see the text) and topography in the population samples in *H. oelandicum*.

Taxon	Hair index $HI_{(6)}$	Average range of altitude within a radius of 10 m	Average range of altitude within a radius of 30 m	Average range of altitude within a radius of 50 m
var. *oelandicum*	0.56 (0.265) 114	0.33 (0.204) 111	0.72 (0.322) 110	0.99 (0.379) 105
var. *canescens*	1.47 (0.328) 32	0.34 (0.179) 32	0.80 (0.320) 32	1.04 (0.368) 32
P	0.000	0.631	0.231	0.572
Taxon	Average SD of altitude within a radius of 10 m		Average SD of altitude within a radius of 30 m	Average SD of altitude within a radius of 50 m
var. *oelandicum*	0.08 (0.053) 111		0.14 (0.079) 110	0.19 (0.081) 105
var. *canescens*	0.08 (0.034) 32		0.15 (0.050) 32	0.19 (0.060) 32
P	0.951		0.735	0.943

B, Hair index in *H. oelandicum* (see the text) in the plastid sample.

Taxon	Average $HI_{(2)}$	SD	*N*	*P* = 0.000
var. *oelandicum*	0.49	0.295	110	
var. *canescens*	1.69	0.553	13	

C, Hair index in var. *oelandicum* in the plastid sample.

Region	Average $HI_{(2)}$	SD	*N*	*P* = ns
Northern Öland	0.42	0.306	6	
Vickleby	0.57	0.285	40	
Storåsen	0.45	0.301	58	
Albrunna	0.53	0.234	6	

D, Pubescence of var. *oelandicum* and topography in Storåsen region (the plastid sample).

Trait	Haplotype 1	Haplotype 2	*P*
$HI_{(2)}$	0.58 (0.247) 35	0.25 (0.268) 23	0.000
Frequency of the recessive allele for glabrous plants (*glab*)	0.29 (0.336) 35	0.78 (0.284) 23	< 0.001
Range in altitude (m) within a 10 m radius	0.27 (0.128) 35	0.21 (0.082) 23	0.061
SD within a 10 m radius	0.064 (0.0336) 35	0.048 (0.0206) 23	0.056
Range in altitude (m) within a 20 m radius	0.56 (0.249) 35	0.42 (0.131) 23	0.013
SD within a 20 m radius	0.111 (0.0546) 35	0.085 (0.0332) 23	0.043
Range in altitude (m) within a 30 m radius	0.77 (0.294) 35	0.56 (0.152) 23	0.002
SD within a 30 m radius	0.152 (0.0680) 35	0.114 (0.0391) 23	0.018
Range in altitude (m) within a 50 m radius	1.05 (0.340) 33	0.88 (0.287) 23	0.044
SD within a 50 m radius	0.213 (0.0898) 33	0.173 (0.0583) 23	0.060

N = sample size, ns = not significant.

Table 3. Variation in *Helianthemum oelandicum* subsp. *oelandicum* in transects across the Great Alvar. The survival was calculated based on one random plot (1 m^2) sampled at each 50 m distance, number of living plants was based on two plots and included both adult plants and non-reproductive plants. The number of plants in 2018 was based on the sum of surviving and dead plants counted after the drought in 2018. Population continuity is the proportion of 50-m distances in a section of the transect that contains any individuals of *H. oelandicum* (dead or alive after the drought in 2018) within a radius of 10 m. $HI_{(2)}$ = hair index for $Hair_{(brl)}$ and $Hair_{(stab)}$ (see the text). Mean (SD) and number of sites, ns = non-significant

A,Variation in the three transects across the Great Alvar (cf. Fig. 5).

Transect	Year of census	Position of the most western plot with *H. oelandicum*†	Number of 50-m distances with *H. oelandicum*	Average number of plants per m^2 in 2018	Survival of the drought in 2018	Average number of plants per m^2 after the drought	Correlation between survival and frequency of the *glab* allele {*can* allele}
Vickleby	2019	589542/6270275	13	18.1 (8.19)	0.13 (0.167)	13.3 (8.56)	0.411 ns
Storåsen	2019	588406/6249661	64	27.7 (9.58)	0.13 (0.146)	16.3 (10.08)	0.224 ns
Albrunna	2020	587664/6242870	60	15.1 (7.43)	0.10 (0.170)	9.8 (7.59)	−0.343* {−0.091 ns}

B, Variation in var. *oelandicum* along the Vickleby transect 2019 and Vickleby transect 3 in 1975 (Widén, 2018b).

Section of the 2019 transect and 1975 transect 3 (from western edge)	Population continuity	Survival of the drought in 2018	Average $HI_{(2)}$ in 2019	Frequency of the *glab* allele in 2019	Frequency of the *glab* allele in 1975
west 0–300 m	0.67	0.0 (0.00) 4	0.64 (0.101) 3	0.0 (0.00) 3	0.36 (0.449) 4
east 350–1100 m	0.69	0.19 (0.171) 9	0.41 (0.174) 9	0.58 (0.173) 9	0.56 (0.216) 8
P		0.055	ns	0.000	ns

C, Variation in var. *oelandicum* along the Storåsen transect. The border between western and eastern part of the transect was set at the eastern edge of the Storåsen ridge in the middle of the Great Alvar. For transects 1 and 2 in 1976 see table 7 in Widén 2018b (see the text). The differences in allele frequencies between years were significant ($P < 0.05$) in the western section of the Great Alvar for both 1976 transects, but not in the eastern section.

Section of the transect	Population continuity	Survival of the drought in 2018	Average $HI_{(2)}$ in 2019	Frequency of the *glab* allele in 2019	Frequency of the *glab* allele in transect 1 1976	Frequency of the *glab* allele in transect 2 1976
west	0.38	0.07 (0.109) 25	0.61 (0.227) 23	0.23 (0.314) 23	0.27 (0.193) 18	0.34 (0.198) 18
east	0.68	0.17 (0.158) 39	0.06 (0.116) 39	0.96 (0.073) 39	0.91 (0.088) 41	0.90 (0.145) 36
P		0.012	0.000	0.000	0.000	0.000

D, Population continuity, survival, hair indexes ($HI_{(2)}$) and allele frequencies along the Albrunna transect. Section of transect see Fig. 5. The frequency of the *can* allele is based on plants with $Hair_{(stab)}$ score ≥ 2 (see the text). Frequency of PF plants is based on plants with inflorescences born on the current year's growth.

Section of the transect	Population continuity	Survival of the drought in 2018	Average $HI_{(2)}$ in 2020	Frequency of the *glab* allele in 2020	Frequency of the *can* allele in 2020	Frequency of PF plants in 2020
west	0.89	0.11 (0.196) 24	0.77 (0.274) 24	0.35 (0.307) 24	0.03 (0.096) 24	0.00 (0.000) 24
PF	0.87	0.10 (0.171)12	2.29 (0.330) 13	0.02 (0.062) 13	0.86 (0.163) 13	1.00 (0.000) 13
central	0.82	0.02 (0.043) 12	0.60 (0.523) 14	0.55 (0.260) 14	0.08 (0.213) 14	0.02 (0.088) 14
east	0.43	0.18 (0.173) 9	1.14 (0.169) 9	0.06 (0.088) 9	0.09 (0.135) 9	0.01 (0.031) 9
P		ns	0.000	0.000	0.000	0.000

†Coordinates in Swe99 (x/y).
*significant at $P < 0.05$, ns non-significant.

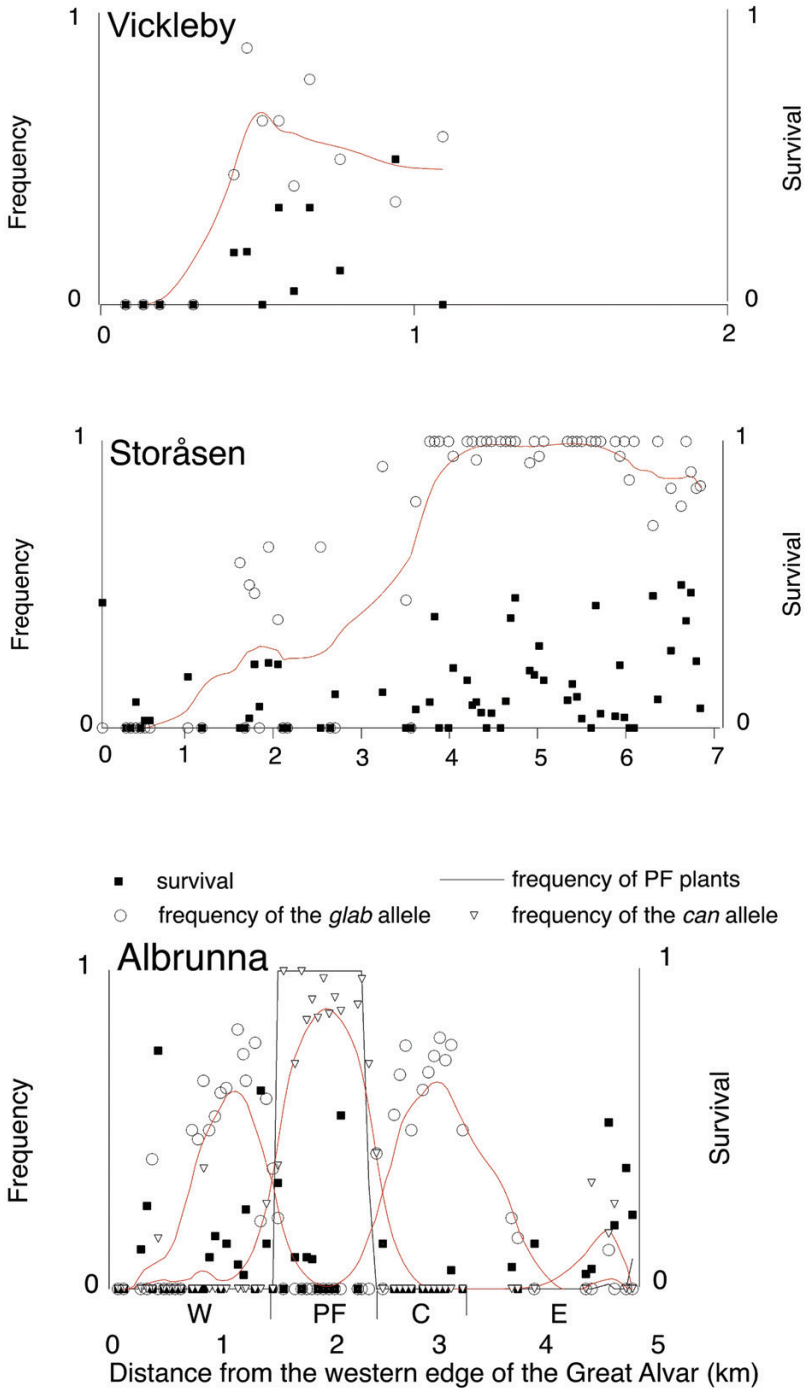

Figure 5. The distribution of *Helianthemum oelandicum* subsp. *oelandicum* in transects across the Great Alvar in the Vickleby, Storåsen and Albrunna regions (cf. Fig. 4). Survival of plants after the drought in 2018 was based on one randomly 1 m² plot sampled at 50-m intervals with *Helianthemum* and the frequency of alleles was based on two plots (see text). The red curves are produced by the option smooth in the Curve Fit of the KaleiaGraph program and are based on average of allele frequencies in three neighbouring samples. The letters on the *x*-axis at Albrunna indicate sections of the transect; var. *oelandicum* occupies the western (W), central (C) and eastern (E) sections, and var. *canescens* occupies the PF section (see Table 3D).

on both the western and eastern edges of the northern part of the distribution of var. *canescens* (cf. Fig. 5C), although scattered individuals of var. *canescens* can be found in the area of var. *oelandicum* far from the border. The hybrid zones become more diffuse further to the south at the southern edge of the continuous distribution of var. *oelandicum* (Supporting Information, S5).

Heterogeneity of topography

The average ranges of differences in altitude at each site increased from 0.3 m at a radius of 10 to 1.0 m at a radius of 50 m around the mid-point in the population samples (Table 2A), and from 0.3 to 1.1 m in the haplotype samples (Table 4). Neither the range nor the standard deviation of the elevations around the mid-point of each site were significantly different between the two varieties at any radius in the population samples (Table 2A).

The heterogeneity of the bedrock measured both as the range and standard deviation of altitudes around the mid-point of each sampled plot was significantly greater in radii between 30 to 50 m at sites with haplotype H1 than at sites with haplotype H2 (Table 4).

The heterogeneity of the bedrock showed significant differences along the transects at both Storåsen and Albrunna (Fig. 6), with the greatest heterogeneity in topography in the western parts of transects.

Topography across hybrid zones

The topography across the distinct hybrid zones in the northern part of the distribution area of var. *canescens* (marked in Fig. 1; Supporting Information, S5) differed between CF, PF and mixed sites, with the greatest heterogeneity in sites occupied by PF plants (Table 5A, B, Fig. 6). The topography was more heterogeneous at the eastern than at the western borders between the distributions of the two varieties ($P < 0.001$) for all radii (cf. Table 5A, B). The differences in heterogeneity of the bedrock between PF, CF and mixed sites in the more diffuse hybrid zones in the southernmost part differed from the northern hybrid zones (Table 5C). Here, PF sites tended to be less heterogeneous than CF sites, but the differences were only significant at a radius of 10 m (Table 5C). The differences between the three types of site did not change if mixed sites dominated by CF plants (category 1) were re-classified as CF sites, and mixed sites dominated by PF plants (category 3) re-classified as PF sites (data not shown).

Topography and pubescence

Hair indices increased with heterogeneity of the topography, measured as the standard deviation at different radii around sampling points when the positions of the sites were determined with a GPS device (Fig. 7), but not with the less accurate position determination in the population sample (see the Supporting Information, S6). The increase was significant for the radii 10 to 30 m in var. *oelandicum*, and for radii 30 and 50 m in var. *canescens* (Fig. 7). The relationship between the heterogeneity of topography and the frequency of the two recessive alleles varied in different directions. The frequency of the *glab* allele in var. *oelandicum* decreased with the heterogeneity of the topography (significant at all distances around the sampling point, except the 50 m radius). The *can* allele in var. *canescens* first decreased and then increased with the heterogeneity at different distances, though not statistically

Table 4. Topography in the plastid samples of *H. oelandicum* subsp. *oelandicum*. Topography was measured as range and SD of altitude measured with an accuracy of 0.1 m in a grid of 1 m around the mid-point at each site

Haplotype	Number of sites	Average range of altitude within a radius of 10 m	Average range of altitude within a radius of 20 m	Average range of altitude within a radius of 30 m	Average range of altitude within a radius of 50 m
H1	62	0.305	0.553	0.770	1.058
H2	57	0.316	0.529	0.658	0.908
P		0.745	0.595	0.031	0.012
Haplotype	Number of sites	Average SD within a radius of 10 m	Average SD within a radius of 20 m	Average SD within a radius of 30 m	Average SD within a radius of 50 m
H1	62	0.072	0.113	0.152	0.207
H2	57	0.070	0.103	0.122	0.169
P		0.822	0.277	0.006	0.006

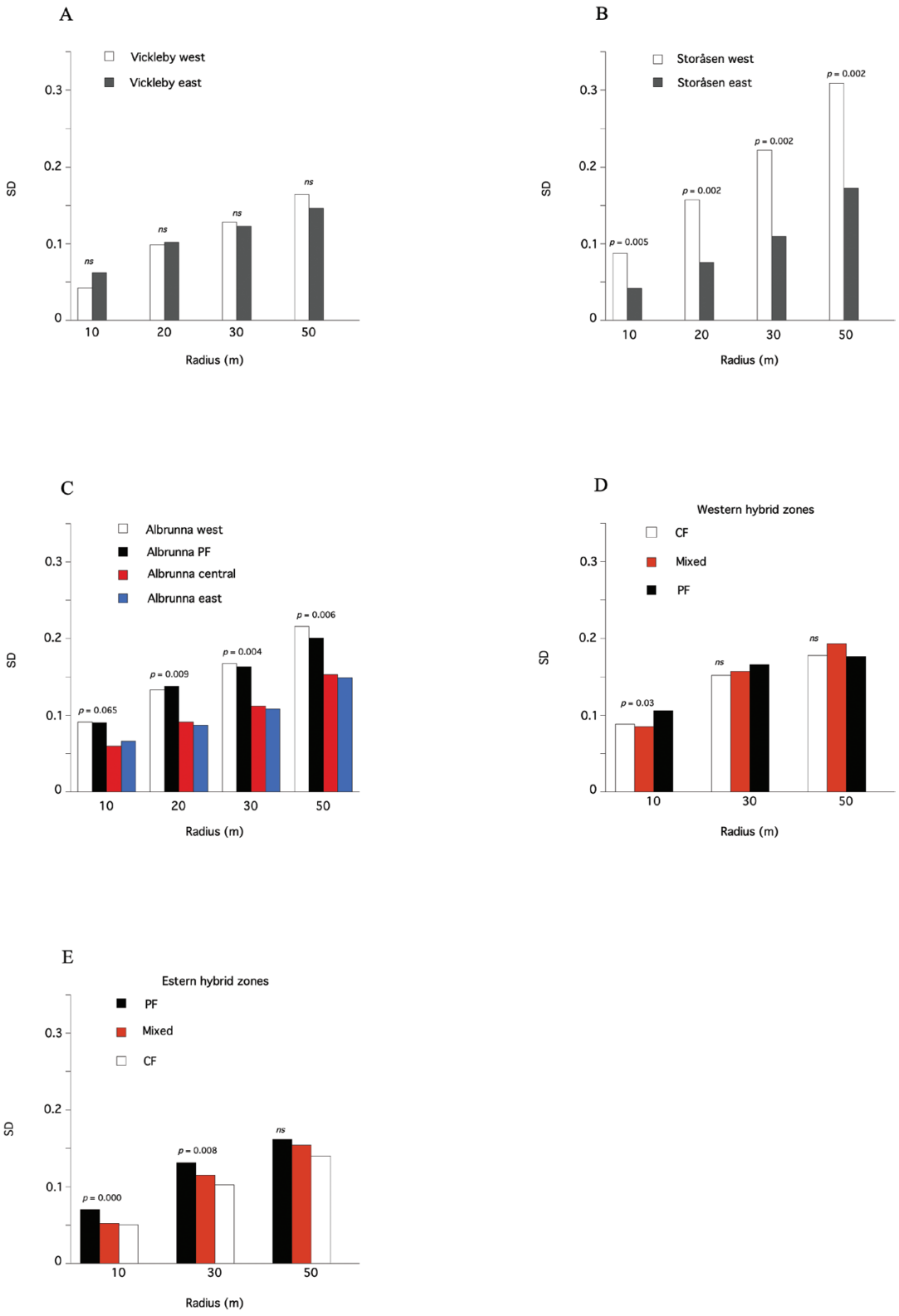

Figure 6. Heterogeneity of the alvar plain with *Helianthemum oelandicum* subsp. *oelandicum* in different sections of transects across the Great Alvar (Table 3) and the northern hybrid zones marked in Fig. 1 (see also Supporting Information, S5), measured as standard deviation of altitudes in areas (radius) around sampling points; see text. A, Vickleby transect, B, Storåsen transect, C, Albrunna transect and D, E, the hybrid zones.

Table 5. Topography in hybrid zones between the distribution of *H. oelandicum* subsp. *oelandicum* var. *oelandicum* and var. *canescens* in the southern part of the Great Alvar (cf. Fig. 1, Supporting Information, S5). Height around each sampling point was measured in a grid of 1 m within three radii (see the text). Mean (SD) and number of plants. CF = var. *oelandicum,* PF = var. *canescens* and Mixed = both varieties

A, The north-western part of the hybrid zone between the distribution of *H. oelandicum* var. *oelandicum* and var. *canescens* (see Fig. 1 and Supporting Information, S5).

Flowering phenology	Average range of altitude within a radius of 10 m	Average range of altitude within a radius of 30 m	Average range of altitude within a radius of 50 m
CF	0.35 (0.138) 52	0.75 (0.224) 51	0.97 (0.220) 51
PF	0.44 (0.184) 55	0.78 (0.259) 53	0.94 (0.197) 51
Mixed	0.36 (0.136) 53	0.78 (0.254) 50	1.00 (0.277) 49
P	0.007	0.811	0.452
Flowering phenology	Average SD within a radius of 10 m	Average SD within a radius of 30 m	Average SD within a radius of 50 m
CF	0.09 (0.038) 52	0.15 (0.053) 51	0.18 (0.052) 51
PF	0.11 (0.056) 55	0.17 (0.082) 53	0.18 (0.059) 51
Mixed	0.08 (0.031) 53	0.16 (0.050) 50	0.19 (0.061) 49
P	0.03	0.555	0.277

B, The north-eastern part of the hybrid zone between the distribution of *H. oelandicum* var. *canescens* and var. *oelandicum* (see Fig. 1 and Supporting Information, S5).

Flowering phenology	Average range of altitude within a radius of 10 m	Average range of altitude within a radius of 30 m	Average range of altitude within a radius of 50 m
CF	0.22 (0.066) 30	0.57 (0.139) 30	0.77 (0.186) 30
PF	0.29 (0.096) 42	0.63 (0.123) 42	0.84 (0.177) 42
Mixed	0.22 (0.076) 28	0.60 (0.178) 28	0.83 (0.205) 28
P	0.001	0.177	0.278
Flowering phenology	Average SD within a radius of 10 m	Average SD within a radius of 30 m	Average SD within a radius of 50 m
CF	0.05 (0.018) 30	0.10 (0.037) 30	0.14 (0.045) 30
PF	0.07 (0.023) 42	0.13 (0.037) 42	0.16 (0.043) 42
Mixed	0.05 (0.020) 28	0.12 (0.042) 28	0.15 (0.046) 28
P	0.001	0.008	0.132

C, Southern part of the hybrid zones (cf. Fig. 1 and Supporting Information, S5)

Flowering phenology	Average range of altitude within a radius of 10 m	Average range of altitude within a radius of 30 m	Average range of altitude within a radius of 50 m
CF	0.31 (0.181) 83	0.68 (0.295) 82	0.93 (0.337) 80
PF	0.28 (0.120) 124	0.62 (0.234) 123	0.86 (0.268) 122
Mixed	0.26 (0.125) 156	0.62 (0.216) 154	0.87 (0.260) 153
P	0.019	0.169	0.136
Flowering phenology	Average SD within a radius of 10 m	Average SD within a radius of 30 m	Average SD within a radius of 50 m
CF	0.07 (0.044) 83	0.14 (0.058) 82	0.18 (0.065) 80
PF	0.06 (0.035)124	0.14 (0.052) 123	0.17 (0.061) 122
Mixed	0.06 (0.034) 156	0.13 (0.048) 154	0.17 (0.056) 153
P	0.043	0.189	0.406

significant (Fig. 7, Supporting Information, S6). The average ranges in elevation around sampling points showed the same trends as standard deviations (data not shown).

DISCUSSION

Helianthemum oelandicum subsp. *oelandicum* showed a pronounced spatial structure in molecular and morphological markers on Öland. The association

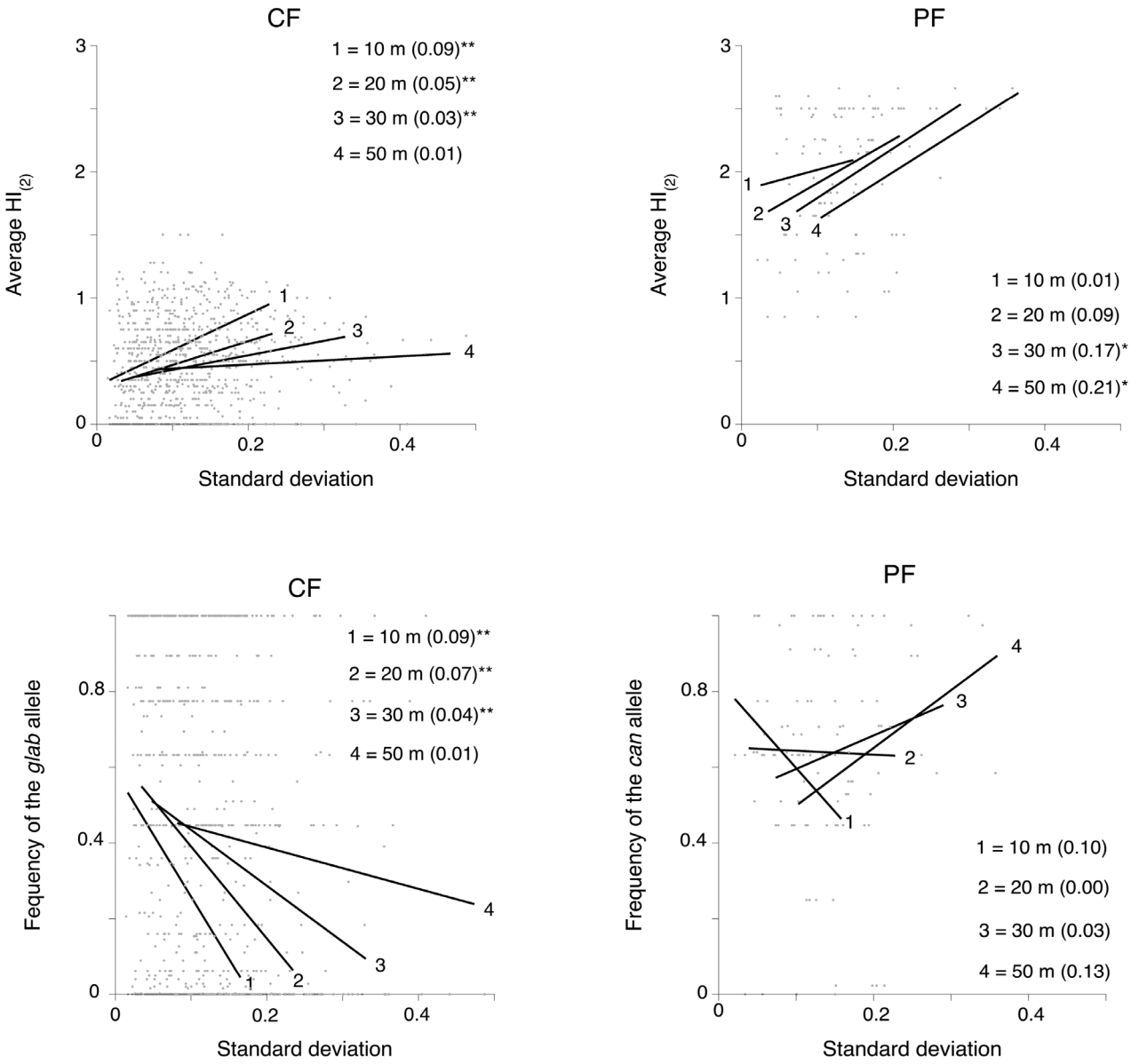

Figure 7. The relationship between pubescence in *Helianthemum oelandicum* subsp. *oelandicum* and heterogeneity in the topography of the alvar plain (measured as standard deviation of altitudes in areas within radii 10, 20, 30 and 50 m around sampling points) in samples where site position was accurately established with GPS (the haploid and transect samples). Pubescence is represented by the hair indexes [$HI_{(2)}$] and the frequency of the *glab* allele in var. *oelandicum* (CF) and the frequency of the can allele in var. *canescens* (PF). The R^2 of the regression line is shown within brackets for each radius. * $P < 0.05$, ** $P < 0.01$.

between topography on a larger geographical scale (elevation a.s.l.) and distribution of plastid haplotypes (Fig. 4) and allozymes (Fig. 3) suggested plausible post-glacial migration routes, whereas correlation between topography at a local scale and allele frequencies for morphology indicated local adaptation (cf. Figs 5 and 7). Thus, current patterns of diversity on Öland in the neutral markers (allozymes and plastid haplotypes) reflect historical processes (migration and gene flow), and the patterns in morphological diversity reflect the action of natural selection (historical and/or present-day) on *H. oelandicum* (cf. Widén, 1980, 1988, 2018a, b). These conclusions were supported by the significant distance effects on plastid and allozyme markers and non-significant distance effects on morphological markers revealed by the Mantel tests.

We did not find any significant correlation between allozyme and topography and assume that allozymes

are neutral with respect to topography. Allozyme diversity showed little differentiation between the two varieties (1%) and between populations (4%) on Öland. Our study, thus, indicated extensive historical gene flow among populations. The between-population differentiation in allozymes of *H. oelandicum* is of the same magnitude as in other investigated outbreeding species on Öland (Prentice, 1992; Rosquist & Prentice, 2000).

Post-glacial history of *Helianthemum oelandicum* subsp. *oelandicum*

We found two plastid haplotypes (H1 and H2) at elevations > 45 m (Fig. 4) close to the highest elevation on Öland, indicating the same arrival route for both lineages. Soubani (2010) found a pronounced geographical structure in the distribution of plastid haplotypes across Europe, with no correlation between morphology (taxonomy) and haplotypes. Two plastid lineages reached Öland after LGM (Soubani, 2010): H1 (the west European lineage) and H2 (the east European lineage). We suggest, based on microfossils and the current distribution of plastid haplotypes, that var. *canescens* with the western plastid lineage (H1) arrived first during the Younger Dryas period to the northern part of the present Great Alvar and expanded southwards as more of the island rose above sea level. Variety *oelandicum* with the eastern haplotype (H2) must have arrived in the middle of Öland soon after the first migration wave. The H2 lineage (cf. Fig. 4A) was the only one to expand northwards when northern Öland rose above the sea level (Björck, 1995). Pollen records show that after the initial periods of open, treeless vegetation, a forest period prevailed on Öland during the wetter Boreal and Atlantic periods (Königsson, 1968). The vegetation on the Great Alvar was more closed (forest dominance) than today, and the records of pollen of *Helianthemum* Mill. declined. We therefore suggest that *H. oelandicum* survived the Boreal and Atlantic periods in small isolated populations restricted to areas with well-drained, open bedrock.

We have no direct evidence of which variety (var. *oelandicum* or var. *canescens*) or phenotype survived the last glacial period and immigrated to Öland, since extant populations close to Scandinavia deviate from the character combination found in subsp. *oelandicum* (for a more detailed discussion see Soubani, 2010). Available data suggest that *H. oelandicum* with H1 survived the last glacial period in the periglacial landscape northwest of the Alpine and south-west of the Scandinavian ice sheets (Soubani, 2010). Potential glacial refugia of *H. oelandicum* with the plastid haplotype H2 (Soubani, 2010) were located south-east of the Scandinavian ice sheet (cf. Hirsch *et al.*, 2015) or in the Penega region in northern Russia (Yuzepchuk, 1974; Meusel *et al.*, 1978, Widén *et al*., in prep.).

Pollen records demonstrate that *Helianthemum* was abundant in Denmark and in the southernmost part of Sweden (Blekinge) in the first ice-free areas in the Older Dryas. (Iversen, 1944; Berglund, 1966; Mortensen *et al.*, 2011). At that time, most of Öland was still submerged under the Baltic Ice Lake (Björck, 1995). The first records of *Helianthemum* pollen from Öland are from the Younger Dryas (Berglund, 1966) and the Preboreal period (Königsson, 1968), at which time central Öland had risen above sea level. Königsson (1968) reported abundant pollen records from central Öland during Preboreal period, when northern Öland was still submerged under the Baltic Ice Lake (Björck, 1995). According to Königsson (1968), pollen of *Helianthemum* was recorded later (during the Boreal period) on the southern part of Öland.

The pollen records of *Helianthemum* continued to be low in the central part of Öland (Königsson, 1968), where contemporary populations of *H. oelandicum* are restricted to a few small alvar areas in a mosaic of forests, grazed pastures and arable fields. On the Great Alvar, pollen records show that *Helianthemum* increased dramatically in the Sub-Boreal period and has become abundant since then (Königsson, 1968). This increase in pollen frequency coincided with an increased human impact on the landscape, which gave rise to more open vegetation on the Great Alvar (Königsson, 1968; cf. Mottl *et al.*, 2021). The distribution of *H. oelandicum* expanded and the present-day geographical pattern of plastid haplotype/flowering morphs became established.

Recently, Martin-Hernanz *et al.* (2019, 2021) showed that *H. oelandicum* radiated rapidly in the Pleistocene. They used five taxa of *H. oelandicum* in a phylogenetic reconstruction of *Helianthemum* based on genotyping-by-sequencing and found a geographical structure among the five subspecies of *H. oelandicum*. Two geographical clusters, one in Spain and one south of the Alps, comprised the range of key morphological traits in the taxonomy of the *H. oelandicum* agg. The diversity within these clusters mirrors the diversity of *H. oelandicum* subsp. *oelandicum* on Öland, and the morphological diversity both in southern Europe and on Öland could, therefore be the result of adaptive radiation, but at different time scales (Pleistocene and Holocene, respectively).

Habitat heterogeneity and pubescence

We found a positive correlation between hairiness and topography in both varieties; plants of *H. oelandicum* characterized by high values of hair indexes were usually more frequent in heterogenous habitats. When variation in pubescence was partitioned into the two

phenotypes governed by simple recessive Mendelian alleles, it was obvious that natural selection acts in two contrasting directions (Fig. 7). The phenotype governed by the *glab* allele in var. *oelandicum* decreased with heterogeneity of the habitat. The correlation between allele frequency and habitat heterogeneity was more complicated in var. *canescens* (probably due to the small sample size), where the *can* allele decreased with heterogeneity within narrow radii at the site and then increased at radii of 30–50 m (Fig. 7).

The bedrock of Öland dips to the south-east, and shallow glaciofluvial deposits hinder runoff after rain and snow melting, contributing to the heterogeneity of habitats on the Great Alvar with both local and regional differences (Fig. 6). We assume that the drainage of the alvar plain is correlated with the local heterogeneity of the habitats. Widén (2018b) made a subjective estimation of the topography in the mid-1970s in both Vickleby and Storåsen regions and found significant differences along transects. The differences corresponded with our more objective measures of topography using GIS data. Well-drained habitats with *H. oelandicum* (Bengtsson *et al.*, 1988), such as the shallow glaciofluvial deposits, dry out early in the summer, but are normally not flooded during wet seasons. Areas with weathered soil on flat bedrock are often submerged after heavy rain or snow melt. Inundation frequency was one of the most important factors for species composition in bryophyte communities on the Great Alvar (Tyler *et al.*, 2018). Plants of *H. oelandicum* in water-saturated soil are often uplifted by frost heaving during the winter (Sterner, 1936), and seedlings are especially sensitive. Our findings of correlations between allele frequencies for hairs and topography (Fig. 7) correspond with the suggestion of Widén (2018b) that glabrous plants of var. *oelandicum* are better adapted to frost heaving than hairy plants and that a dense cover of pubescence is favourable in dry habitats (Widén, 2018a). However, the low R^2 values in our regression analysis (Fig. 7) showed that only part of variation in pubescence was explained by topography. Alternatively, our method of measuring heterogeneity of the topography has to be improved, for instance by removing more obstacles (stone walls and erratic boulders) in the bedrock from our data.

The correlations between pubescence and topography were not significant in the population sample. One reason could be the low accuracy of the spatial coordinates of the sites in the population sample. The coordinates were determined by using a topographical map in the 1970s (a GPS device was used for establishing site positions in the other samples). Given the mosaic distribution of habitats (cf. Bengtsson *et al.*, 1988; Tyler *et al.*, 2018) occupied by *H. oelandicum,* a small deviation from the exact position of a sample can distort the correlation between pubescence and topography.

Common to sites with a high frequency of glabrous plants are high values of population continuity and habitats with flat topography over large areas resulting in long periods of inundation. Large areas with a flat topography are more or less lacking in the distribution area of var. *canescens*, which may explain the absence of the *glab* allele in this taxon. Only a restricted area south of the Great Alvar is dominated by flat, sometimes inundated topography. Here, the *can* allele is rare or absent (Widén, 2018a).

Local differentiation of *H. oelandicum* across the hybrid zones on southern Öland (Fig. 5) resembles population differentiation found as response to edaphic conditions such as serpentine outcrops and mine-tailings (Kruckeberg, 1951; Jain & Bradshaw, 1966; Antonovics & Bradshaw, 1970; Snaydon, 1970). The most obvious edaphic transitions can be observed in the northern part along the eastern border between the two varieties (Fig. 6E). Here var. *canescens* occurs on coarse grey weathered soil in a rugged topography, whereas var. *oelandicum* inhabits more fine-grained red soil on flat pavements (cf. Fig. 2C and Fig. 2B, respectively), indicating different geological origins (B. Widén, unpublished). The mosaic distribution of edaphic conditions in other parts of the Great Alvar can create selection gradients over distances of metres or even tens of centimetres (Prentice *et al.*, 1995, 2000). The homogeneous within-site variation of pubescence that was found in the transect samples as well as in the population samples in Widén (2018a, b) indicates that selection gradients affecting *H. oelandicum* usually cover distances of > 50 m.

Pubescence as a morphological marker

The use of hair scores for indumentum in different parts of the plant and summarizing the overall pubescence in hair indexes are handy methods for describing population differentiation in *Helianthemum* (Widén, 1988, 2015, 2018a, b). Repeated samples within the local population (site) gave stable results (the present study and Widén, 1988, 2018a, b) and each population was characterized by its own 'finger print', i.e. distribution of plants with different hair scores or hair indexes (see figure 5 in Widén, 1988, and figure 7 in Widén, 2018b). This 'finger print' in pubescence seems to be ubiquitous throughout the *H. oelandicum* agg. (cf. Proctor, 1957; B. Widén, in prep.).

Hairs are often considered to be adaptive, such as protection against herbivores (e.g. Westerbergh & Nyberg, 1995; Kärkkäinen *et al.*, 2004; Kivimäki *et al.*, 2007) and UV radiation (Espigares & Peco, 1995), and for control of water and temperature regulation (Wagner, Wang & Shepherd, 2004). Hairs may have several functions and can be part of an adaptive

strategy together with other traits; plants with low HI values in *H. oelandicum* subsp. *oelandicum* are often darkly coloured by anthocyanins, whereas high HI values usually imply less anthocyanin colour (cf. fig. 4 in Widén, 2018b). A number of studies have shown simple genetic background of variation in pubescence (e.g. Westerbergh, 1992; Kärkkäinen & Ågren, 2002; Widén, 2018a, b). Kärkkäinen *et al.* (2004) studied discrete populations of the insect pollinated, self-incompatible *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz, polymorphic for trichome production in an area covered by the ice during the last glaciation in central Sweden. They found F_{st} = 0.45 for glabrousness (a recessive allele of a Mendelian locus) and F_{st} = 0.133 for eight allozyme loci, indicating more local genetic differentiation for allozymes, but similar differentiation for hairiness as in *H. oelandicum* on a comparable geographical scale (cf. Table 1).

Climatic impact

Öland is situated in the rain shadow of the mainland of southern Sweden, which explains an average low precipitation during summer (Sterner, 1936; Widén, 1980; Rosén, 1982). The death rate of *H. oelandicum* can be substantial during years with severe drought, which has occurred at approximately ten-year intervals during the last 50 years (Prentice *et al.*, 1995; B. Widén, personal observation). The average death rate in *H. oelandicum* over large areas in 2018 (Fig. 5) has never before been so high (0.85–0.90) during the last half-century, a possible consequence of climate change impact (cf. Sévellec & Drijfhout, 2018; B. Widén, in prep.).

Changes in allele frequencies

Changes over time in allele frequencies can be estimated for the *glab* allele in two transects (Vickleby and Storåsen), based on data in Widén (2018b). The frequency of the *glab* allele at the western edge of the Vickleby transect (0–300 m) changed from 0.4 to 0.0 between the mid-1970s and the late 2010s (Table 3B). This section of the transect coincided with high death rate during the 2018 drought, but it should be noticed that estimation of allele frequencies was based on few surviving plants. The same trend was found in the western section of the Storåsen transect, with significantly higher death rate in the 2018 drought compared with the eastern section of the transect and a lower frequency of the *glab* allele in 2019 than in 1976 in the western section (Table 3C). Other changes in pubescence between the 1970s and the 2010s were marginal and a comparison over time has to wait until the population structures have been restored after the 2018 drought and the sampling design in the two periods can be the same. The effects of the extreme 2018 drought showed pronounced regional variation irrespective of pubescence. To fully understand how drought as well as drainage of the habitat acts as selection agencies, other stages of the life cycle than survival of adults, for instance, seed production and seedling establishment, have to be studied (B. Widén, in prep.).

The Great Alvar – a hotspot for ecotypic diversity

According to Turesson (1922), 'the term ecotype is used as an ecological sub-unit to cover the product arising as a result of the genotypical response' to the environment. The diversity of pubescence in *H. oelandicum* on Öland and its correlation with topography can be seen as an example of classical edaphic ecotypic differentiation. In this respect, the species corresponds well with many taxa that have developed putative ecotypes in the unique habitats on Öland. Several taxa belong to species complexes with marginal populations on Öland, and they are recognized as endemic species [e.g. *Festuca oelandica* (Hack.) K.Richt. in the *F. rubra* L. agg., *Galium oelandicum* (Sterner & Hylander) Ehrend. in the *G. pumilum* Murray agg. and *Artemisia oelandica* (Besser) Krasch. close to *Artemisia laciniata* Willd.] or suggested as endemic subspecies [e.g. *Crepis tectorum* L. subsp. *pumila* (Liljeblad) Sterner in *C. tectorum*] (Jonsell & Karlsson, 2004; Jonsell, 2011). The Holocene origin of diversity in *H. oelandicum* subsp. *oelandicum* on Öland is probably representative of that for several endemic taxa with marginal distributions in alvar habitats on the island.

CONCLUSIONS

The present geographical pattern of diversity in molecular markers, pubescence and flowering phenology in *H. oelandicum* subsp. *oelandicum* on Öland reflects migration history, a period of restricted distribution on the island and natural selection in different habitats during the Holocene. Genetic admixture between two post-glacial migration lineages through hybridization and introgression of nuclear genes produced three morphological/cytoplasmic lineages: var. *canescens* (with haplotype H1), var. *oelandicum* (with haplotype H1) and var. *oelandicum* (with haplotype H2) with sometimes sharp borders between the distribution of haplotypes (100–200 m). Natural selection probably during the last 5000 years (after the Neolithic opening of the landscape) has created the present-day spatial structure in morphology with sometimes remarkably

sharp borders (50 m) between the distribution of morphs. The morphological differentiation rests mainly on a few putative Mendelian genes and a number of interacting quantitative genes (two loci determining pubescence and a few loci determining flowering phenology) (Widén, 1980, 2018a, b; B. Widén, in prep.). *Helianthemum oelandicum* subsp. *oelandicum* adds to a number of taxa with ecotypic morphs in marginal populations on the Baltic island of Öland.

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AUTHOR CONTRIBUTIONS

BW was responsible for the study design, sampling in the field, the allozyme study and the overall analysis of the data. MW took part in the allozyme study and sampling field data in 2019 and 2020. ES and MH were responsible for the laboratory work and analysis of the plastid data. OL was responsible for the GIS data. The main text was written by BW and ES. All authors have read and improved the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material. Herbarium specimens are deposited in Lund University Botanical Museum (LD). Further information is available from the corresponding author.

REFERENCES

Ågren J, **Schemske DW. 2012**. Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. *New Phytologist* **194**: 1112–1122.

Albertsson N. 1950. Das grosse südliche Alvar der Insel Öland. *Svensk Botanisk Tidskrift* **44**: 269–331.

Antonovics J, **Bradshaw AD. 1970**. Evolution in closely adjacent plant populations. 8. Clinal patterns at a mine boundary. *Heredity* **25**: 349–362.

Aparicio A, **Martin-Hernanz S**, **Parejo-Farnés C**, **Arroyo J**, **Lavergne S**, **Yesilyurt EB**, **Zhang M-L**, **Rubio E**, **Albaladejo RG. 2017**. Phylogenetic reconstruction of the genus *Helianthemum* (Cistaceae) using plastid and nuclear DNA-sequences: systematic and evolutionary inferences. *Taxon* **66**: 868–885.

Bengtsson K, **Prentice HC**, **Rosén E**, **Moberg R**, **Sjögren E. 1988**. The dry alvar grasslands of Öland: ecological amplitudes of plant species in relation to vegetation composition. *Acta Phytogeographica Suecica* **76**: 21–46.

Berglund B. 1966. Late-quaternary vegetation in Eastern Blekinge, southeastern Sweden. A pollen-analytical study. I. Late-glacial time. *Opera Botanica* **12**: 1149–1151.

Binney H, **Edwards M**, **Macias-Fauria M**, **Lozhkin A**, **Anderson P**, **Kaplan JO**, **Andreev A**, **Bezrukova E**, **Blyakharchuk T**, **Jankovska V**, **Khazina I**, **Krivonogov S**, **Kremenetski K**, **Nield J**, **Novenko E**, **Ryabogina N**, **Solovieva N**, **Willis K**, **Zernitskaya V. 2017**. Vegetation of Eurasia from the last glacial maximum to present: Key biogeographic patterns. *Quaternary Science Reviews* **157**: 80–97.

Björck S. 1995. A review of the history of the Baltic Sea, 13.0-8.0 ka BP. *Quarternary International* **27**. 19–40.

Clausen J, **Keck DD**, **Hiesey WM. 1940**. *Experimental studies on the nature of species. I. Effects of varied environments on western North American plants*. Washington: Carnegie Institute.

Dhuyvetter H, **Gaublomme E**, **Desender K. 2004**. Genetic differentiation and local adaptation in the salt-marsh beetle *Pogonus chalceus*: a comparison between allozyme and microsatellite loci. *Molecular Ecology* **13**: 1065–1074.

Doyle JJ, **Doyle JL. 1990**. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–15.

Edh K, **Widén B**, **Ceplitis A. 2007**. Nuclear and chloroplast microsatellites reveal extreme population differentiation and limited gene flow in the Aegean endemic *Brassica cretica* (Brassicaceae). *Molecular Ecology* **16**: 4972–4983.

Ellegren H, **Sheldon BC. 2008**. Genetic basis of fitness differences in natural populations. *Nature* **452**: 169–175.

Espigares T, **Peco B. 1995**. Mediterranean annual pasture dynamics: impact of autumn drought. *Journal of Ecology* **83**: 135–142.

Fior S, **Li M**, **Oxelman B**, **Viola R**, **Hodges SA**, **Ometto L**, **Varotto C. 2013**. Spatiotemporal reconstruction of the *Aquilegia* rapid radiation through next-generation sequencing of rapidly evolving cpDNA regions. *New Phytologist* **198**: 579–592.

Fröberg L, **Niklasson M**, **Paltto H**, **Knutsson T**, **Johansson T. 2009**. Age and epiphytic lichen diversity of the dwarf shrub *Helianthemum oelandicum* on the island of Öland, Sweden. *Lichenologist* **41**: 537–545.

Guzmán B, **Lledó MD**, **Vargas P. 2009**. Adaptive radiation in Mediterranean *Cistus* (Cistaceae). *PloS ONE* **4**: e6362.

Hammer Ø, **Harper DAT. 2006**. *Paleontological data analysis*. Oxford: Blackwell.

Havrdová A, **Douda J**, **Krak K**, **Vít P**, **Hadincová V**, **Zákravský P**, **Mándak B. 2015**. Higher genetic diversity in recolonized areas than in refugia of *Alnus glutinosa* triggered by continent-wide lineage admixture. *Molecular Ecology* **24**: 4759–4777.

Hewitt GM. 1999. Post-glacial recolonization of European biota. *Biological Journal of the Linnean Society* **68**: 87–112.

Hewitt GH. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Hewitt GH. 2001. Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology* **10**: 537–549.

Hirsch F, **Schneider A**, **Nicolay A**, **Blaszkiewicz M**, **Kordowski J**, **Noryskiewicz AM**, **Tyszkowski S**, **Raab A**, **Raab T. 2015**. Late quaternary landscape development at the margin of the Pomeranian phase (MIS 2) near Lake Wygonin (Northern Poland). *Catena* **124**: 28–44.

Iversen J. 1944. *Helianthemum* som fossil Glacialplante i Danmark. *Geologiska Föreningens i Stockholm Förhandlingar* **66**: 774–776.

Jain SK, **Bradshaw AD. 1966**. Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis. *Heredity* **21**: 407–441.

Janchen E. 1907. *Helianthemum canum* (L.) Baumg. und seine nächsten verwandten. *Abhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien* **4**: 1–67.

Janchen E. 1909. Die Cistaceen Österreich-Ungarns. *Mitteilungen des Naturwissenschaftlichen Vereines an der Universität Wien* **7**: 1–124.

Janzon L-A. 1983. Aculeate Hymenoptera and other flower visiting insects on the Great Alvar of Öland (in Swedish). *Entomologisk Tidskrift* **104**: 169–182.

Jonsell B. 2011. Endemism in the Swedish vascular flora (in Swedish). *Svensk Botanisk Tidskrift* **105**: 14–24.

Jonsell B, **Karlsson T. 2004**. Endemic vascular plants in Norden. In: Jonsell B, ed. *Flora Nordica. General Volume*. Stockholm: The Bergius Foundation, The Royal Swedish Academy of Sciences, 139–159.

Kärkkäinen K, **Ågren J. 2002**. Genetic basis of trichome production in *Arabidopsis lyrata*. *Hereditas* **136**: 219–226.

Kärkkäinen K, **Løe G**, **Ågren J. 2004**. Population structure in *Arabidopsis lyrata*: evidence for divergent selection on trichome production. *Evolution* **58**: 2831–2836.

Kivimäki M, **Kärkkäinen K**, **Gaudeul M**, **Løe G**, **Ågren J. 2007**. Gene, phenotype and function: GLABROUS1 and resistance to herbivory in natural populations of *Arabidopsis lyrata*. *Molecular Ecology* **16**: 453–462.

Königsson LK. 1968. The Holocene history of the Great Alvar of Öland. *Acta Phytogeographica Suecica* **55**: 1–172.

Kruckeberg AR. 1951. Intraspecific variability in the response of certain native species to serpentine soil. *American Journal of Botany* **38**: 408–419.

Li Y, **Canbäck B**, **Johansson T**, **Tunlid A**, **Prentie HC. 2015**. Evidence of positive selection within the PgiC1 locus in the grass *Festuca ovina*. *PLoS ONE* **10**: e0125831.

Lodhi MA, **Guang-Ning Y**, **Norman FW**, **Bruce IR. 1994**. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. *Plant Molecular Biology Reporter* **12**: 6–13.

Martin-Hernanz S, **Aparicio A**, **Fernández-Mazuecos M**, **Rubio E**, **Reyes-Betancort JA**, **Santos-Guerra A**, **Olangua-Corral M**, **Albaladejo RG. 2019**. Maximize resolution or minimize error? Using genotyping-by-sequencing to investigate the recent diversification of *Helianthemum* (Cistaceae). *Frontiers in Plant Science* **10**: 1416.

Martin-Hernanz S, **Velayos M**, **Albaladejo RG**, **Aparicio A. 2021**. Systematic implications from a robust phylogenetic reconstruction of the genus *Helianthemum* (Cistaceae) based on genotyping-by-sequencing (GBS) data. *Anales del Jardin Botanico de Madrid* **78**: e113.

Meusel H, **Jäger E**, **Rauschert S**, **Weinert E. 1978**. *Vergleichende Chorologie der Zentraleuropäischen Flora. 2. Karten*. Jena: Veb Gustav Fischer Verlag, 288–289.

Moazzenti H, **Zarre S**, **Pfeil BE**, **Bertrand YJK**, **German DA**, **Al-Shehbaz IA**, **Mummenhoff K**, **Oxelman B. 2014**. Phylogenetic perspectives on diversification and character evolution in the species-rich genus *Erysimum* (Erysimeae; Brassicaceae) based on densely sampled ITS approach. *Botanical Journal of the Linnean Society* **175**: 497–522.

Mortensen MF, **Birks HH**, **Christensen C**, **Kolm J**, **Noe-Nygaard N**, **Odgaard BV**, **Olsen J**, **Rasmussen KL. 2011**. Late glacial vegetation development in Denmark – new evidence based on macrofossils and pollen from Slotseng, a small-scale site in southern Jutland. *Quaternary Science Reviews* **30**: 2534–2550.

Mottl O, **Flantua SGA**, **Bhatta KP**, **Felde VA**, **Giesecke T**, **Goring S**, **Grimm EC**, **Haberele S**, **Hooghiemstra H**, **Ivory S**, **Kunes P**, **Wolters S**, **Seddon AWR**, **Williams JW. 2021**. Global acceleration in rates of vegetation change over the past 18 000 years. *Science* **372**: 860–864.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* **70**:3321–3323.

Nichols RA, **Hewitt GM. 1994**. The genetic consequences of long-distance dispersal during colonization. *Heredity* **72**: 312–317.

Orr HA. 2005. The genetic theory of adaptation: a brief history. *Nature Reviews Genetics* **6**: 119–127.

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

Peakall R, **Smouse PE. 2012**. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**: 2537–2539.

Prentice HC. 1992. The structure of morphometric and allozyme variation in relict populations of *Gypsophila*

fastigiata (Caryophyllaceae) in Sweden. *Biological Journal of the Linnean Society* **47**: 197–216.

Prentice HC, **Lönn M**, **Lager H**, **Rosén E**, **van der Maarel E. 2000**. Changes in allozyme frequencies in *Festuca ovina* populations after a 9-year nutrient/water experiment. *Journal of Ecology* **88**: 331–347.

Prentice HC, **Lönn M**, **Lefkovith LP**, **Runyeon H. 1995**. Associations between allele frequencies in *Festuca ovina* and habitat variation in the alvar grasslands on the Baltic island of Öland. *Journal of Ecology* **82**: 391–402.

Proctor MCF. 1957. Variation in *Helianthemum canum* (L.) Baumg. in Britain. *Watsonia* **4**: 28–41.

Proctor MCF, **Heywood VH. 1968**. *Helianthemum*. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea*, *Vol.* **2**. Cambridge: Cambridge University Press, 286–291.

Reitalu T, **Helm A**, **Pärtel M**, **Bengtsson K**, **Gerhold P**, **Rosén E**, **Takkis K**, **Znamenskiy S**, **Prentice HC. 2014**. Determinants of fine-scale plant diversity in dry calcareous grasslands within the Baltic Sea region. *Agriculture, Ecosystems and Environment* **182**: 59–68.

Riginos C, **Sukhedo K**, **Cunningham CW. 2002**. Evidence for selection at multiple allozyme loci across a mussel hybrid zone. *Molecular Biology and Evolution* **19**: 347–351.

Rosén E. 1982. Vegetation development and sheep grazing in limestone grasslands of south Öland, Sweden. *Acta Phytogeographica Suecica* **72**: 1–104.

Rosquist G, **Prentice HC. 2000**. Habitat fragmentation and structure of genetic diversity within disjunct isolates of *Anthericum ramosum* L. (Anthericaceae) in Scandinavia. *Biological Journal of the Linnean Society* **69**: 193–212.

Sévellec F, **Drijfhout SS. 2018**. A novel probabilistic forecast system predicting anomalously warm 2018-2022 reinforcing the long-term global warming trend. *Nature Communication* **9**: 3024.

Snaydon RW. 1970. Rapid population differentiation in a mosaic environment. I Response of *Anthoxanthum odoratum* populations to soils. *Evolution* **24**: 257–269.

Sork VL, **Nason J**, **Campbell DR**, **Fernandez JF. 1999**. Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution* **14**: 219–224.

Soubani E. 2010. *Systematics, phylogeography and multiple orgins of morphs in two species complexes belonging to Cistaceae, Helianthemum oelandicum and H. nummularium*. *PhD Thesis*, Lund University, Sweden.

Soubani E, **Hedrén M**, **Widén B. 2014**. Phylogeography of the European rock rose *Helianthemum nummularium* (Cistaceae): incongruent patterns of differentiation in plastid DNA and morphology. *Botanical Journal of the Linnean Society* **176**: 311–331.

Soubani E, **Hedrén M**, **Widén B. 2015**. Genetic and morphological differentiation across a contact zone between two postglacial immigration lineages of *Helianthemum nummularium* (Cistaceae) in southern Scandinavia. *Plant Systematic and Evolution* **301**: 1499–1508.

Sterner R. 1936. Ekologiska iakttagelser over *Helianthemum oelandicum* (L.) Willd. *Meddelanden från Göteborgs Botaniska Trädgård* **10**: 183–208.

Svendsen JI, **Alexanderson H**, **Astakhov VI**, **Demidov I**, **Dowdeswell JA**, **Funder S**, **Gataullin V**, **Henriksen M**, **Hjort C**, **Houmark-Nielsen M**, **Hubberten HW**, **Ingólfsson O**, **Jakobsson M**, **Kjær KH**, **Larsen E**, **Lokrantz H**, **Lunkka JP**, **Lyså A**, **Mangerud J**, **Matiouchkov A**, **Murray A**, **Möller P**, **Niessen F**, **Nikolskaya O**, **Polyak L**, **Saarnisto M**, **Siegert C**, **Siegert MJ**, **Spielhagen RF**, **Stein R. 2004**. Late Quarternary ice sheet history of northern Eurasia. *Quaternary Science Reviews* **23**: 1229–1271.

Törnblom G. 1908. **Iakttagelser** övfer *Helianthemum canum* (L.) Baumg. och *Helianthemum oelandicum* (L.) Willd. på Ölands alvar. (in Swedish). *Svensk Botanisk Tidskrift* **2**: 32–37.

Turesson G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* **3**: 211–350.

Tyler T, **Bengtsson F**, **Dahlberg CJ**, **Lönnell N**, **Hallingbäck T**, **Reitalu T. 2018**. Determinants of bryophyte species composition and diversity on the Great Alvar of Öland, Sweden. *Journal of Bryology* **40**: 12–30.

Valente LM, **Savolainen V**, **Vargas P. 2010**. Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1489–1496.

Volkova PA, **Schanzer IA**, **Soubani E**, **Meschersky IG**, **Widén B. 2016**. Phylogeography of the European rock rose *Helianthemum nummularium* (Cistaceae): western richness and eastern poverty. *Plant Systematics and Evolution* **302**: 781–794.

Wagner GJ, **Wang E**, **Shepherd RW. 2004**. New approaches for studying and exploiting an old protuberance, the plant trichome. *Annals of Botany* **93**: 3–11.

Weir BS, **Cockerham CC. 1984**. Estimating F-statistics for analysis of population structure. *Evolution* **38**: 1358–1370.

Westerbergh A. 1992. The genetic basis of hairlessness in *Silene dioica* (Caryophyllaceae). *Hereditas* **117**: 287–291.

Westerbergh A, **Nyberg A-B. 1995**. Selective grazing of hairless *Silene dioica* plants by land gastropods. *Oikos* **73**: 289–298.

Widén B. 1980. Flowering strategy in the *Helianthemum oelandicum* (Cistaceae) complex on Öland, Sweden. *Botaniska Notiser* **133**: 99–115.

Widén B. 1988. Partitioning of variation in pubescence of a dwarf shrub, *Helianthemum oelandicum*. *Acta Phytogeographica Suecica* **76**: 133–156.

Widén B. 2010. *Cistaceae*. In: Jonsell B, Karlsson T, eds. *Flora Nordica 6.* Stockholm: Swedish Museum of Natural History.

Widén B. 2015. Genetic basis of a key character in *Helianthemum nummularium*. *Plant Systematics and Evolution* **301**: 1851–1862.

Widén B. 2018a. Inheritance of a hair character in *Helianthemum oelandicum* var. *canescens* and allele frequencies in natural populations. *Plant Systematics and Evolution* **304**: 145–161.

Widén B. 2018b. Inheritance of glabrous plants in *Helianthemum oelandicum* var. *oelandicum* and patterns of allele frequencies in local populations. *Plant Systematics and Evolution* **304**: 1199–1219.

Yuzepchuk SV. 1974. *Helianthemum* Adans. In: Shishkin BK, ed. *Flora of the USSR*, *Vol.* **15** (translated from Russian). Jerusalem: Program for Scientific Translations, 248–260.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

S1. Population samples – pubescence (allele frequency and hair index) and location.
S2. Plastid samples – methods, pubescence, location, primers and banding patterns.
S3. Allozyme samples – methods, allele frequencies, Hardy-Weinberg equilibrium and correlation between allele frequency and topography.
S4. Transect samples – altitudes along transects.
S5. Hybrid zones – borders between the distribution of var. *oelandicum* and var. *canescens*.
S6. Regression coefficients between pubescence (allele frequency and hair index) and topography in the plastid, population and transect samples.