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Chloroplast DNA haplotypes in Nordic *Silene dioica:* postglacial immigration from the east and the south*

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Abstract. Analysis of PCR/RFLP variation in 57 Nordic populations of the herb *Silene dioica*, revealed 13 composite chloroplast DNA (cpDNA) haplotypes. The geographic distribution of the haplotypes suggests that the postglacial colonization of Fennoscandia by *S. dioica* may have involved immigration of populations from two main directions. The commonest cpDNA haplotype dominates in populations throughout most of Finland and northern and central Sweden, but is absent from southern Sweden. The distribution of this haplotype is interpreted in terms of immigration from an eastern or northern direction. In contrast, eight haplotypes that are absent from northern Fennoscandia are represented in populations in southern Sweden and in Denmark, suggesting colonization by populations derived from one or several refugial areas further to the south in Europe. The overall NE-SW pattern of cpDNA haplotype variation is similar to, but less diffuse than, the pattern revealed by allozyme markers.

Key words: *Silene dioica*, red campion, cpDNA, postglacial history.

The glacial-interglacial cycles during the Quaternary have played a central role in shaping the present-day distributions of European plant and animal taxa (Andersen and Borns 1994, Hewitt 1996). Fennoscandia and parts of northern Europe were covered by ice during the maximum of the last (Weichselian) glaciation (c. 22 000 to 17 000 cal yrs BP). The Scandinavian ice sheet began to retreat northwards from parts of southern and western Sweden as early as 17 000 cal yrs BP (Sandgren et al. 1999) and the ice retreat proceeded rapidly after c. 10 200 BP (Berglund 1979, Björck 1995). The deglaciated areas of Fennoscandia became successively available for colonization by plant and animal populations that had survived the glacial period in areas to the south and east of the ice sheet (Tegelström 1987; Nordal and Jonsell 1998; Hewitt 1999, 2000).

It has been possible to reconstruct general directions of postglacial migration in many European tree species with the help of syntheses of data from pollen diagrams. Isopoll maps provide some support for the suggestion that trees colonized Fennoscandia from different geographic sources (Huntley and Birks 1983). However, while many trees are wind-pollinated and provide a reliable pollen record (e.g. Huntley and Birks 1983), herbaceous plants and animal-pollinated species are poorly

*cpDNA variation in Nordic *Silene dioica*.

represented in the subfossil record (Widmer and Lexer 2001). Molecular markers now provide us with an alternative way of inferring postglacial migrational history in both plants and animals. The geographic structuring of present day genetic variation can be used to reconstruct migration routes and to identify possible refugial areas (Comes and Kadereit 1998, Newton et al.1999).

Pollen data have indicated that there were refugial areas for trees in the eastern and western Mediterranean region (e.g. Bennett et al. 1991, Brewer et al. 2002). More recently, compilations of genetic data for both plants and animals have provided further evidence for glacial refugia in several parts of the Mediterranean region (Hewitt 1996, Comes and Kadereit 1998, Taberlet et al. 1998, Petit et al. 2002a) and also suggest that there were refugial areas to the east of the ice sheet in Russia (Kontula and Väinölä 2001, Palmé et al. 2003).

A study of allozyme variation in Nordic populations of the widespread European herb, *Silene dioica*, showed an overall, pattern of SW-NE genetic differentiation (Malm and Prentice 2002). This pattern could be detected by multivariate analysis of allele frequencies at eight polymorphic loci. However, inspection of maps of individual allele frequencies revealed a range of idiosyncratic and semi-congruent geographic patterns of variation. Although the allozyme data from *S. dioica* reveal a diffuse and locally complex picture of genetic differentiation, they provide support for a scenario in which the species immigrated into the region both from the southwest and from an eastern or northeastern direction.

Allozyme markers are coded for by nuclear genes and show biparental inheritance. Patterns of allozyme variation, therefore, reflect the effects of gene flow and dispersal by both seed and pollen (Ennos 1994, Schaal et al. 1998). Gene dispersal by pollen may occur over long distances, particularly in wind-pollinated trees. Paternity analyses and other types of pollen sink experiment also demonstrate that there may be appreciable levels of interpopulation gene dispersal by pollen, even in insect-pollinated taxa and on a short time scale (e.g. Ellstrand 1992, Dow and Ashley 1996, Schaal et al. 1998). The fact that allozyme markers often show diffuse and subtle patterns of geographic variation (e.g. Finkeldey and Mátyás 2003) is likely to reflect the homogenizing effects of gene dispersal by pollen on a long time scale.

In contrast to allozymes, both chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) markers are usually uniparentally transmitted and behave as single heritable units (Palmer 1985a, 1985b; Palmer et al. 1987; Weising et al. 1995). The fact that haploid mtDNA and cpDNA markers can be used to trace the spread of maternal lineages makes them particularly useful in the study of phylogeographic variation. Many studies have used variation in mtDNA markers to infer patterns of racial differentiation and migrational history in animals (e.g. Tegelström 1987, Taberlet et al. 1995, Boursot et al. 1996). In plants, maternally inherited plastid markers can be used to trace patterns of gene dispersal by seed (Ennos 1994) and may be expected to provide a more simplified reflection of migration processes than allozyme markers.

So far, large-scale European plant studies using haploid maternally inherited cp- or mtDNA markers have mostly focussed on patterns of geographic variation and immigration history in trees (e.g. Dumolin-Lapègue et al. 1997, Ferris et al. 1998, Petit et al. 2002a, Rendell and Ennos 2003) or shrubs (e.g. Rendell and Ennos 2002). There is a lack of information on large-scale patterns of geographic variation in maternally inherited markers in herbaceous plants. There are also, so far, few studies that have attempted to examine the migrational history of Nordic plant species. Ferris and co-workers included some Fennoscandian material in their study of cpDNA variation in two European oak species, and their results suggest that there were two separate immigration routes into southern Finland – one western and one eastern (Ferris et al. 1998). In contrast, Nordic populations of

the shrub *Calluna vulgaris* show high levels of cpDNA haplotype diversity and no clear geographic structure (Rendell and Ennos 2002).

In the present study, we investigated the geographic structure of cpDNA variation from throughout the Nordic range of a widespread herbaceous plant. We used universal primers developed by Taberlet et al. (1991) and Demesure et al. (1995) and PCR/RFLP analysis to characterize the pattern of cpDNA variation in populations of the dioecious herb, *S. dioica*. We investigated whether cpDNA markers provide evidence that Nordic populations of *S. dioica* have originated from different maternal lineages and we compared the geographic pattern revealed by cpDNA markers with that detected by biparentally inherited allozyme markers.

Materials and methods

Plant material. The natural range of the red campion, *Silene dioica* (L.) Clairv. (Caryophyllaceae), extends over most of northern and central Europe (Jalas and Suominen 1986). In Fennoscandia, the species grows in nutrient-rich meadows, along shores and on woodland margins. It also occurs as a weed, particularly on disturbed roadsides in Finland. *S. dioica* is a dioecious, diploid ($2n = 24$) (Löve 1944), short-lived perennial herb (Baker 1947). The species is pollinated by bumblebees, honeybees, butterflies and hoverflies (Kay et al. 1984). The seeds are mainly dispersed by gravity (Baker 1947, Giles and Goudet 1997). Levels of vegetative spread are low (Baker 1947).

Seed was collected from Nordic populations during 1996-1997 (see Fig. 1 and the Appendix). Within each locality, seeds were sampled from c. 25 female individuals when possible and the sampled individuals were usually separated from each other by more than 5 m. Four wild-collected bulk

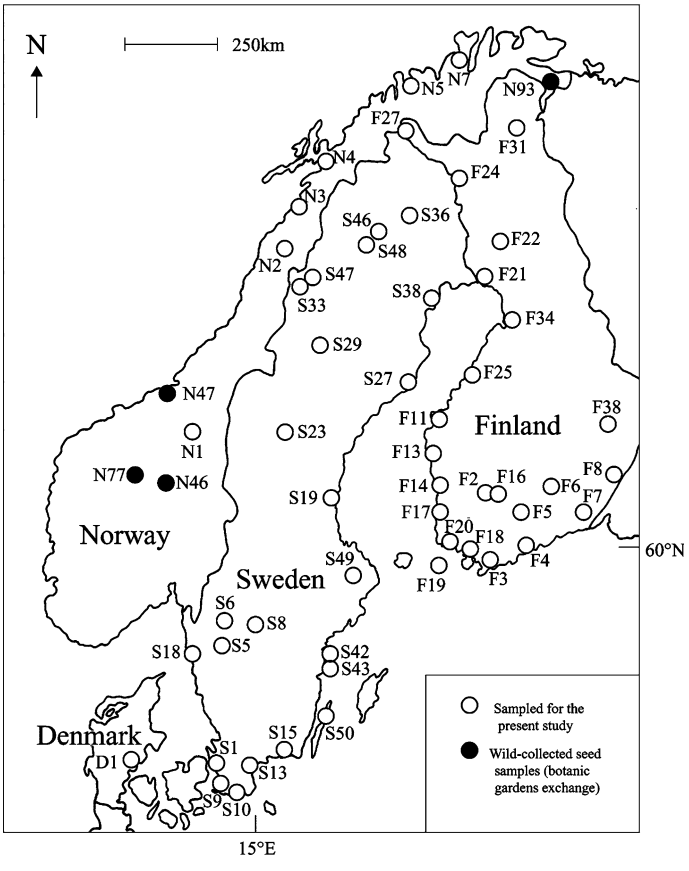

Fig. 1. Localities for the 57 sampled *Silene dioica* populations in Fennoscandia and Denmark (see Appendix for population codes)

samples from Norway were obtained through the botanic gardens' seed exchange service.

The seed samples were stored at 4°C for at least 1 month and were pretreated with 0.3% gibberellic acid (4% ProGibb®) before being sown in petri dishes. The seedlings were transferred to pots with soil c. five days after germination and the young plants were grown in a cool greenhouse.

PCR-RFLP analysis of cpDNA. One to five plants per population were sampled for the cpDNA analysis. Total DNA was extracted from approximately 50 mg fresh-weight of young leaf material following a modified (Thell et al. 1998) version of the CTAB method of Doyle and Doyle (1987). DNA concentrations were estimated using a Hoefer DyNA Quant 200 fluorometer or visually on 1.8% agarose (IBI, Shelton Scientific Inc.) gels with quantification markers (bacteriophage lambda DNA from Roche at a concentration of 0.25 μg per μL).

Twelve fragments of cpDNA were amplified using the universal primers of Taberlet et al. (1991) and Demesure et al. (1995) (cf. Grivet et al. 2001). The PCR mix was as follows: c. 30 ng of template DNA, 0.6U *Taq* DNA polymerase (Roche or Merck), 1 × PCR buffer (Roche or Merck), 3.5 mM $MgCl_2$ (Roche or Merck), 100 μM of each of dATP, dCTP, dGTP, dTTP (Roche)), 0.2 μM of each primer (Operon) and ddH_2O to make up the volume. The PCR mix was based on a modification of recipes from K Wolff and NJ Ouborg (personal communication). Amplifications were performed using an MJ Research PTC-1197 thermal cycler. An initial denaturation of 95°C for 4 min was followed by 40 cycles at 92°C for 45 sec, annealing at primer-specific temperatures for 45 sec and elongation at 72°C for primer- specific times. The PCR reaction was ended by a 10 min elongation step at 72°C. The annealing temperatures and elongation times followed King and Ferris (1998) or NJ Ouborg (personal communication).

Test runs were carried out on one individual from each of eight geographically separated Fennoscandian and European populations to identify variable primer/restriction enzyme combinations. In the initial exploratory runs, 100 μL PCR reaction volumes were used. After variable primer-pair/enzyme combinations had been identified, 25 μL reaction volumes were used on the remaining samples. A 5 μL subsample of the PCR product was used to check for successful amplification and the rest of the amplification product was divided into subsamples of 10 μL. Each of the subsamples was digested separately at 37°C for at least 2 h (or overnight) using 7U of each of seven different 4bp-cutting (*Alu*I, *Cfo*I, *Hae*III, *Hinf*I, *Msp*I, *Nde*II, *Rsa*I) and two 6bp-cutting (*Eco*RI, *Hind*III) restriction enzymes, giving a total of 108 amplification fragment/enzyme combinations. The resulting restriction fragments were separated in 1.8% or 4% agarose (IBI, Shelton Scientific Inc.) gels. Four base-pair ladders ranging in size from 19-1114bp to 500-5000bp (nos. VI, VIII, XVI and XVII from Roche) were used as size markers. The gels were stained with ethidium bromide and visualized and photographed under UV light. If different restriction enzymes revealed similar polymorphisms for a particular primer-pair, it was assumed that the band-pattern represented a single length polymorphism and data were collected for only one of the restriction enzymes. The polymorphic cpDNA regions (containing length or site mutations) were used to identify composite haplotypes.

Maternal inheritance of cpDNA has been shown in *Silene latifolia* (= *S. alba*) (McCauley 1994), a species that is closely related to *S. dioica*, and in *S. hifacensis* (Prentice et al. 2003).

Analysis of cpDNA data. The program Arlequin 2.000 (Schneider et al. 2000) was used to calculate a matrix of squared Euclidean distances between pairs of haplotypes, and the relationships between the haplotypes were summarized in a minimum spanning network (MSN). In the MSN analysis, the polymorphic DNA fragments (''markers'') were treated as characters and the individual bands for each marker scored as presence/absence character states. The MSN was drawn with the help of the program TREEVIEW (Page 1996).

Results

Chloroplast DNA polymorphisms in Nordic *Silene dioica*. Preliminary screening of eight European populations of *Silene dioica* identified eight primer-pair/restriction enzyme combinations (*trn*L5'-*trn*L3'/*Rsa*I, *trn*H-*trn*K/*Hae*III, *trn*K^1-*trn*K^2/*Hinf*I, *trn*T-*trn*L/*Hinf*I, *trn*T-*trn*L/*Rsa*I, *trn*L-*trn*F/*Rsa*I, *trn*M-*rbc*L/*Nde*II and *psb*C-*trn*S/*Hind*III) that detected polymorphic cpDNA regions (Table 1). Seven

Table 1. Variable primer-pair/restriction enzyme combinations in Nordic *Silene dioica*. Primer sequences are from Taberlet et al. (1991)* and Demesure et al. (1995)**. Roman numerals indicate the variable bands (markers)

Primer pair	Restriction enzyme	Marker
*trn*L5'-*trn*L3'*	*Rsa*I	I, II, III
*trn*H-*trn*K**	*Hae*III	IV, V
*trn*K^1-*trn*K^2**	*Hinf*I	VI, VII
*trn*L-*trn*F*	*Rsa*I	VIII, IX, X
*trn*T-*trn*L*	*Rsa*I	XI, XII
*trn*M-*rbc*L**	*Nde*II	XIII, XIV

of these polymorphic primer-pair/restriction enzyme combinations were used in the analysis of the full set of Nordic populations (the *psb*C-*trn*S fragment was excluded from the analyses because of problems with incomplete digestion when cutting with *Hind*III). Four of the seven amplified cpDNA fragments contained length mutations and two fragments contained site mutations. One fragment (*trn*T-*trn*L/*Hinf*I) was monomorphic in the Nordic populations.

Three different length variants were detected in both the *trn*L5'-*trn*L3' and *trn*L-*trn*F fragments, while the *trn*H-*trn*K and the *trn*K^1-*trn*K^2 fragments each showed two length variants. The remaining two fragments (*trn*T-*trn*L and *trn*M-*rbc*L) each contained site mutations and showed two band phenotypes.

A total of 259 individuals from 57 populations (Fig. 1) was analyzed, and 13 composite cpDNA haplotypes (Table 2) were distinguished on the basis of the presence/absence of the ten length and four site variants. Complete data from all six polymorphic primer-pair/restriction enzyme combinations were obtained from most of the populations. However, repeated attempts failed to produce amplification products for the *trn*M-*rbc*L primer-pair in a subset of individuals from several populations. Individuals with missing data from more than one primer-pair/enzyme combination are not represented in the haplotype map in Fig. 2. Some of the individuals that lacked data for the *trn*M-*rbc*L/*Nde*II primer/enzyme combination could have belonged to either haplotype J or to haplotype K (Tables 1 and 2; Fig. 2).

Patterns of chloroplast haplotype variation. Haplotype A (Table 2) is the commonest haplotype in the Nordic populations of *S. dioica*. It is found in the majority of the Norwegian and Finnish populations and the northern and central Swedish populations. It

Table 2. Thirteen composite cpDNA haplotypes detected in Nordic *Silene dioica*. The haplotypes are based on the polymorphic markers in Table 1. The marker states are the presence (1) or absence (0) of bands. Missing data are indicated by question marks (?)

Marker	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Haplotype	Marker state													
A	0	0	1	1	0	1	0	0	1	0	1	0	1	0
B	0	0	1	1	0	1	0	0	0	1	1	0	?	?
C	0	0	1	1	0	0	1	0	1	0	1	0	1	0
D	0	0	1	1	0	1	0	1	0	0	1	0	1	0
E	0	0	1	1	0	0	1	1	0	0	1	0	1	0
G	0	0	1	1	0	0	1	0	1	0	1	0	0	1
H	0	0	1	1	0	0	1	1	0	0	1	0	0	1
J	1	0	0	1	0	0	1	0	1	0	0	1	1	0
K	1	0	0	1	0	0	1	0	1	0	0	1	0	1
N	1	0	0	0	1	0	1	1	0	0	0	1	0	1
S	0	1	0	1	0	0	1	1	0	0	1	0	1	0
T	0	1	0	1	0	0	1	0	1	0	1	0	0	1
U	0	1	0	1	0	1	0	0	1	0	1	0	?	?

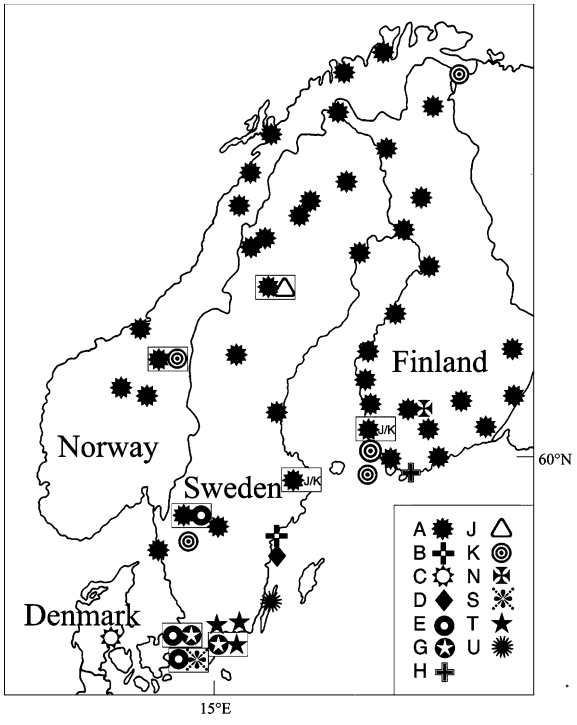

Fig. 2. The distribution of the cpDNA haplotypes detected in *Silene dioica* in the Fennoscandia and Denmark. Haplotype symbols for the eight polymorphic populations (N1, S1, S6, S9, S10, S29, S49 and F17) are enclosed in boxes (see Appendix for population codes). The haplotypes with missing data for one primer-pair/enzyme combination are represented by letters for the haplotypes to which they may belong

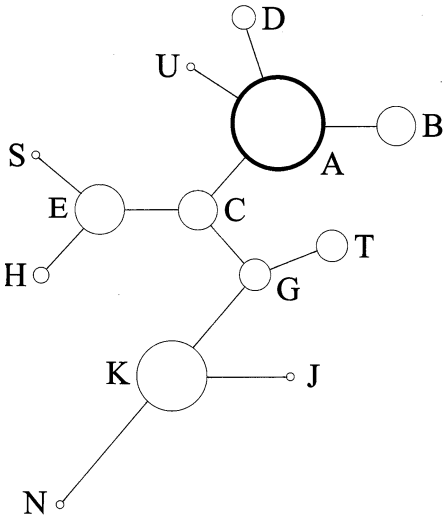

Fig. 3. Minimum spanning network between the 13 cpDNA haplotypes found in Nordic *Silene dioica*. The areas of the circles are proportional to the frequencies of the haplotypes, with the exception of the circle for haplotype A which is drawn with a × 10 reduction. There are alternative links between the following pairs of haplotypes: B – D, D – E, G – H, T – U. Haplotype codes are given in Table 2

is, however, absent from southern Sweden (Fig. 2). The southern Swedish part of the species' range is, instead, characterized by the presence of several different haplotypes. The southwestern tip of Finland also contains a concentration of different haplotypes. Haplotype K is the second most common Nordic haplotype and occurs in five widely separated populations (Fig. 2). Of the remaining haplotypes, only three occur in more than one population. Haplotype G is restricted to two populations on the south Swedish coast, haplotype T is found in three southeastern Swedish populations and haplotype E occurs in three populations in west and south Sweden. Haplotypes B, D, S and U are unique to separate populations along the south and southeastern coast of Sweden and haplotype J to a north-central Swedish population (Fig. 2). Haplotype C is present in the only Danish population that was included in the study, and haplotypes H and N are restricted to single populations in southwestern Finland. Eight populations (Fig. 2), mostly from south Sweden, contained more than one haplotype (Fig. 2). The relationships between the 13 Nordic haplotypes in *S. dioica* are summarized in the minimum spanning network in Fig. 3.

Discussion

An early study of chloroplast variation (Sandbrink et al. 1990) hinted at the existence of a NE/SW pattern in Nordic *Silene dioica*. The study was based on classic RFLP markers, detected low levels of variation and included only nine Nordic populations. Nevertheless, one Danish and one south Norwegian population showed the same restriction fragment

pattern as populations further south in Europe, while material from Sweden and Finland showed a different restriction pattern (Sandbrink et al. 1990). The distribution of cpDNA haplotypes in Nordic populations of *S. dioica* in the present study reveals a clear geographic pattern that can be interpreted in terms of immigration history. The populations fall into two main geographic groups – a haplotypically uniform eastern/northern group and a variable southern group (Fig. 2).

Haplotype A dominates over much of the region (Fig. 2) and is widespread in all but the extreme south of Sweden. Preliminary results also suggest that haplotype A is absent in samples from the rest of Europe (J.U. Malm and H.C. Prentice, unpublished data). Few other haplotypes co-occur with haplotype A in the northern and eastern parts of Fennoscandia and most populations in the north and east contain only haplotype A. The distribution of this haplotype suggests that northern and eastern Fennoscandia were colonized after the last glaciation by populations of *S. dioica* that originated from an eastern refugial area. It is possible that haplotype A also occurred in southern refugial areas but was lost at some stage during the species' expansion into northern Europe. However, data from nuclear (allozyme) markers in Fennoscandia (Malm and Prentice 2002) and preliminary data from cpDNA markers in the rest of Europe (J.U. Malm and H.C. Prentice, unpublished data) further support the suggestion that the main group of Nordic *S. dioica* populations had an eastern or northern origin, entering the region through Finland and then spreading westwards and southwards through northern Sweden (Malm and Prentice 2002). A few earlier genetic studies have also indicated eastern or northeastern origins for population-groups within Nordic plant or animal species (e.g. Ferris et al. 1998, Nordal and Jonsell 1998, Jaarola et al. 1999, Kontula and Väinölä 2001, Palmé et al. 2003).

While northern Fennoscandia is dominated by a single haplotype (haplotype A), there are eight haplotypes (B, D, E, G, K, S, T and U), in addition to haplotype A, present in the southern third of Sweden (Fig. 2). The Danish population contains a further haplotype (C). Haplotype A is completely absent from Denmark and the extreme south of Sweden. Denmark and south Sweden appear to have been colonized by a suite of haplotypes derived from populations that originated from one or several refugial areas somewhere in southern or eastern Europe. It is possible that some of the rare haplotypes, both in southern Sweden and elsewhere in the Nordic countries, represent local mutations. For example, haplotype D differs from the widespread haplotype A by only one length mutation in the *trn*L2-*trn*F fragment (Tables 1 and 2). Rare haplotypes may also represent marginal occurrences of haplotypes that are more widely distributed further to the south or east in Europe: preliminary data from the rest of the species' European distribution confirm this prediction for some of the haplotypes (J.U. Malm and H.C. Prentice, unpublished data).

The southwestern tip of Finland contains four haplotypes (including haplotype A). Haplotype K is found in both south Sweden and southwestern Finland. This haplotype may have entered Fennoscandia from both the southwest (into Sweden) and the southeast (into Finland). Alternatively, haplotype K may have entered Finland from Estonia (cf. Kontula and Väinölä 2001), or from southern Sweden via the Åland archipelago. Similarities between allozyme frequencies in populations in southern Sweden and southwestern Finland also suggest that the Åland archipelago may have functioned as a route for gene flow by pollen or seed dispersal across the Baltic (Malm and Prentice 2002). Studies in the Skeppsvik archipelago, an area of isostatic land uplift further to the north in the Gulf of Bothnia, have followed the colonization of newly-formed islands by S. dioica and show that seed can be dispersed between islands in water-borne drift material (Giles and Goudet 1997). Long-distance seed dispersal may also occur across open areas of ice or frozen snow.

The scattered distribution of the other occurrences of haplotype K (Fig. 2) also suggests long-distance seed dispersal. Although Nordic *S. dioica* is mostly found in its natural coastal, meadow or woodland-margin habitats, it also occurs as a roadside weed, particularly in eastern and northern Finland (Jonsell 2001). Anthropogenic disturbance (for example, during road construction) is likely to have led to occasional, recent, long-distance dispersal of *S. dioica* seed. Long-distance seed dispersal may also occur as the result of the escape of cultivated *S. dioica* from gardens.

As predicted, cpDNA markers reveal a less diffuse pattern of geographic differentiation than the nuclear (allozyme) markers used in the earlier study of Nordic *S. dioica* (Malm and Prentice 2002). While the northern/eastern populations are characterized by the more-or-less universal and uniform presence of the chloroplast haplotype A, allozyme data reveal complex trends of population differentiation within the same northeastern group of populations. Allozyme data also detect a more continuous pattern of intergradation between the two main groups of populations (Malm and Prentice 2002).

Allozyme markers reflect gene flow by pollen as well as dispersal by seed, and the complex, semi-congruent patterns shown by individual allozymes in *S. dioica* are consistent with extensive gene flow by pollen, over a prolonged period of time (Malm and Prentice 2002). In contrast, maternally inherited chloroplast markers detect only the more conservative dynamics of population establishment and dispersal by seed. The fact that uniparentally inherited haploid markers are more susceptible than diploid nuclear markers to the effects of genetic drift in small populations (Ennos 1994, Schaal et al. 1998) is likely to promote the sorting of haplotype lineages and the development of geographically-structured haplotype distributions. Haplotype sorting, as a result of bottlenecks in refugial populations or founder events during range-expansion, is likely to be particularly pronounced in dioecious species such as *S. dioica*, where the effective population size for the chloroplast genome is about one quarter of that for the nuclear genome (Schaal et al. 1998).

The present study was designed to investigate the geographic distribution of cpDNA haplotypes rather than focussing on levels of intrapopulation haplotype diversity, and we gave wide geographic coverage priority over within-population replication. However, despite the small sample sizes (≤ 5 individuals per population), there is a geographic trend in the levels of within-population haplotype polymorphism. Only eight populations contained more than one haplotype, and all but two of the polymorphic populations were from the extreme south of Finland or from the southern third of Sweden (Fig. 2). Only five of the populations that contained haplotype A also contained a second haplotype, and the majority of the northern and eastern populations appear to be fixed for haplotype A.

The fixation of *S. dioica* for a single haplotype over much of the Nordic region differs from the situation observed in *Calluna vulgaris*, where the majority of the investigated populations in the region contained two to four haplotypes (Rendell and Ennos 2002). In contrast to the situation for temperate trees, both palaeoecological data and the structure of cpDNA variation suggest that *Calluna* was not restricted to small, southern or eastern glacial refugia with long migration distances into northern Europe. There is little phylogeographic pattern across Europe, and northern European *Calluna* populations have retained a high haplotypic diversity (Rendell and Ennos 2002). Nordic populations of *Betula pendula* are also characterized by an admixture of cpDNA haplotypes and populations typically contain more than one haplotype (Palmé et al. 2003). In contrast, the tendency for different regions of Europe to be dominated by particular haplotypes (as is the case with the *S. dioica* A-haplotype), or groups of haplotypes, is consistent with a scenario of postglacial spread from separate eastern and/or southern refugial populations (e.g. Demesure et al. 1996, Dumolin-Lapègue et al. 1997, Palmé and Vendramin 2002, Petit et al. 2002b).

Regional sorting of haplotype lineages and a general northward and westward decline in haplotype diversity can be interpreted as a consequence of genetic bottlenecks in small refugial populations, or founder effects and the progressive loss of haplotypes during range expansion (Petit et al. 2002b).

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Appendix

Population numbers and collecting localities for Fennoscandian *Silene dioica*. The seed samples were collected from wild populations by the authors except for populations N46-N93 (obtained via the European botanical gardens exchange service) and populations F25, S42-S50 and D1 (collected by colleagues from wild populations). Pooled samples from more than one year are indicated by #. S = Sweden, F = Finland, N = Norway, D = Denmark. The numbers of progeny individuals included in the cpDNA analyses are given in parentheses.

F2 (4) Etelä-Häme, Sääksmäki. **F3** (5) Varsinais-Suomi, Kisko, SW Toija, Härkähaka. **F4** (3) Uusimaa, Helsinki, Niinisaari, Niinilahti. **F5** (2) Etelä-Häme, Lammi, Kinnala, Reväsvuori. **F6** (1) Etelä-Häme, Hartola, Ruskealan tila, Seppälänmäki. **F7** (1) Etelä-Karjala, Luumäki, Haimiala, Kankaan hautuumaa. **F8** (2) Laatokan Karjala, Parikkala, Rasvaniemi. **F11** (3) Vaasa, Vaskiluoto. **F13** (4) Etelä-Pohjanmaa, Teuva, Teuvajoki. **F14** (1) Satakunta, Pori, Yyteri, Herrainpäiväin niemi. **F16** (2) Etelä-Häme, Valkeakoski, Tallinmäki. **F17** (3) Satakunta, Rauma, Petäjäs. **F18** (4) Varsinais-Suomi, Piikkiö, Puosta, Puostantie. **F19** (4) Varsinais-Suomi, Korppoo. **F20** (1) Varsinais-Suomi, Turku, Ruissalo. **F21** (3) Pohjois-Pohjanmaa, Ala-Tornio. **F22** (3) Pohjois-Pohjanmaa, Rovaniemen maalaiskunta, Pekkala. **F24** (5) Kemin Lappi, Kangosjoki. **F25** (5) Keski-Pohjanmaa, Öja, Kokkola, Bodö, Knitsund. **F27** (4) Enontekiön Lappi, Kilpisjärvi, Saana. **F31** (5) Inarin Lappi, Ivalo, Tolonen. **F34** (5) Pohjois-Pohjanmaa, Kiiminki. **F38** (4) Pohjois-Karjala, Joensuu – Varkaus region. **S1** (5) Skåne, Härslöv parish. **S5** (1) Västergötland, Västra Tunhem parish. **S6** (4) Dalsland, Edsleskog parish. **S8** (5) Västergötland, Amnehärad parish. **S9** (2) Skåne, Bara kommun, Förtuna. **S10** (5) Skåne, Hörte. **S13** (1) Skåne, Degeberga. **S15** (1) Blekinge, Ronneby, W Göholm castle. **S18** (2) Bohuslän, Fiskebäckskil, Skaftö, Vägeröd. **S19** (5) Hälsingland, Enånger, Gamla kyrka. **S23** (4) Jämtland, Pilgrimstad. **S27** (4) Västerbotten, Umeå, Skeppsvik. **S29** (3) Åsele Lappmark, Vilhelmina. **S33** (5) Lycksele Lappmark, Mosekälla. **S36** (5) Lule Lappmark, Gällivare. **S38** (5) Norrbotten, Luleå, Björkön, Björkvägen. **S42** (5) Östergötland, Harstena. **S43** (5) Östergötland, Eknön. **S46** (5) Lule lappmark, Saltolouhta area. **S47** (3) Lycksele Lappmark, Ammarnäs, Ammarfjället. **S48** (4) Lule Lappmark, Rapaälven, S Vassja mountain. **S49** (5) Uppland, Uppsala, Morga hage. **S50** (1) Öland, Högby. **N1** (5) Sör-Tröndelag, Åle. **N2** (5) Nordland, Storforshei, Skogly. **N3** (5) Nordland, Skjersta. **N4** (4) Nordland, Efjord. **N5** (3) Troms, Rotsund. **N7** (4) Finnmark, Kralsund, Neverfjord. **N46**# (4) Oppland, Ringebu, Venabygdsfjellet, Olavbu, Trabelia. **N47**# (5) Sör-Trondelag, Örland, Storfosna, Storhaugen. **N77**# (5) Oppland, Vågå, Gjendesheim. **N93** (5) Finnmark, Neiden, Skoltebyen. **D1** (5) Jutland, Horsens, S Bygholm's lake.

References

Andersen B. G., Borns H. W. (1994) The Ice Age World. Scandinavian University Press, Oslo.

Baker H. G. (1947) Accounts of *Melandrium dioicum* and *M. album* for the Biological Flora of the British Isles. J. Ecol. 35: 271–292.

Bennett K. D., Tzedakis P. C., Willis K. J. (1991) Quaternary refugia of north European trees. J. Biogeogr. 18: 103–115.

Berglund B. E. (1979) The deglaciation of southern Sweden 13,500-10,000 B.P. Boreas 8: 89–117.

Björck S. (1995) A review of the history of the Baltic sea, 13.0–8.0 ka B.P. Quaternary International 27: 19–40.

Boursot P., Din W., Anand R., Darviche D., Dod B., Von Deimling F., Talwar G. P., Bonhomme F. (1996) Origin and radiation of the house mouse: mitochondrial DNA phylogeny. J. Evol. Biol. 9: 391–415.

Brewer S., Cheddadi R., de Beaulieu J. L., Reille M. (2002) The spread of deciduous *Quercus* throughout Europe since the last glacial period. Forest Ecology and Management 156: 27–48.

Comes H. P., Kadereit J. W. (1998) The effects of Quaternary climatic changes on plant distribution and evolution. Trends Plant Sci. 3: 432–438.

Demesure B., Sodzi N., Petit R. J. (1995). A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molec. Ecol. 4: 129–131.

Demesure B., Comps B., Petit R. J. (1996) Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica*) in Europe. Evolution 50: 2115–2520.

Dow B. D., Ashley M. V. (1996) Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. Molec. Ecol. 5: 615–627.

Doyle J. J., Doyle J. L. (1987) A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Dumolin-Lapègue S., Demesure B., Fineschi S., Le Corre V., Petit R. J. (1997) Phylogeographic structure of white oaks throughout the European continent. Genetics 146: 1475–1487.

Ellstrand N. C. (1992) Gene flow by pollen: implications for plant conservation genetics. Oikos 63: 77–86.

Ennos R. A. (1994) Estimating the relative rates of pollen and seed migration among plant populations. Heredity 72: 250–259.

Ferris C., King R. A., Väinölä R., Hewitt G. M. (1998) Chloroplast DNA recognizes three refugial sources of European oaks and suggests independent eastern and western immigrations to Finland. Heredity 80: 584–593.

Finkeldey R., Mátyás G. (2003) Genetic variation of oaks (*Quercus* spp.) in Switzerland. 3. Lack of impact of postglacial recolonization history on nuclear gene loci. Theor. Appl. Genet. 106: 346–352.

Giles B. E., Goudet J. (1997) Genetic differentiation in *Silene dioica* Amer. Naturalist 149: 507–526.

Grivet D., Heinze B., Vendramin G. G., Petit R. J. (2001) Genome walking with consensus primers: application to the large single copy region of chloroplast DNA. Molec. Ecol. Notes 1: 345–349.

Hewitt G. M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58: 247–276.

Hewitt G. M. (1999) Post-glacial re-colonization of European biota. Biol. J. Linn. Soc. 68: 87–112.

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

Huntley B., Birks H. J. B. (1983) An Atlas of past and present pollen maps for Europe: 0–13,000 Years Ago. Cambridge University Press, Cambridge.

Jaarola M., Tegelström H., Fredga K. (1999) Colonization history in Fennoscandian rodents. Biol. J. Linn. Soc. 68: 113–127.

Jalas J., Suominen J. (1986) Atlas Florae Europaeae, vol. 7 Caryophyllaceae (Silenoideae). The Committee for the Mapping of the Flora of Europe and Societas Biologica Fennica Vanamo, Helsinki.

Jonsell B. (2001) In: Jonsell B. (ed.) Flora Nordica, vol. 2., Bergius Foundation, Stockholm, pp. 196–198.

Kay Q. O. N., Lack A. J., Bamber F. C., Davies C. R. (1984) Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species *Silene dioica*. New Phytol. 98: 515–530.

King R. A., Ferris C. (1998) Chloroplast DNA phylogeography of *Alnus glutinosa* (L.) Gaertn. Molec. Ecol. 7: 1151–1162.

Kontula T., Väinölä R. (2001) Postglacial colonization of Northern Europe by distinct phylogeographic lineages of the bullhead, *Cottus gobio*. Molec. Ecol. 10: 1983–2002.

Löve D. (1944) Cytogenetic studies on dioecious *Melandrium* Bot. Not. 1944: 125.

Malm J. U., Prentice H. C. (2002) Immigration history and gene dispersal: allozyme variation in Nordic populations of the red campion, *Silene dioica* (Caryophyllaceae). Biol. J. Linn. Soc. 77: 23–24.

McCauley D. E. (1994) Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: implications for studies of gene flow in plants. Proc. Natl. Acad. Sci. USA 91: 8127–8131.

Newton A. C., Allnutt T. R., Gillies A. C. M., Lowe A. J., Ennos R. A. (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol. Evol. 14: 140–145.

Nordal I., Jonsell B. (1998) A phylogeographic analysis of *Viola rupestris*: three post-glacial immigration routes into the Nordic area? Bot. J. Linn. Soc. 128: 105–122.

Page R. D. M. (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Applic. Biosci. 12: 357–358.

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

Palmé A. E., Su Q., Rautenberg A., Manni F., Lascoux M. (2003) Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula*. Molec. Ecol. 12: 201–212.

Palmer J. D. (1985a) Evolution of chloroplast and mitochondrial DNA in plants and algae. In: MacIntyre R. J. (ed.) Monographs in evolutionary biology: molecular evolutionary genetics. Plenum, New York, pp. 131–140.

Palmer J. D. (1985b) Comparative organization of chloroplast genomes. Annual Rev. Genet. 19: 325–354.

Palmer J. D., Osorio B., Aldrich J., Thompson W. F. (1987) Chloroplast DNA evolution among legumes: loss of a large inverted repeat occurred prior to other sequence rearrangements. Current Genetics 11: 275–286.

Petit R. J., Brewer S., Bordács S., et al. (2002a) Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. Forest Ecology and Management 156: 49–74.

Petit R. J., Csaikl U. M., Bordács S., et al. (2002b) Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. Forest Ecology and Management 156: 5–26.

Prentice H. C., Malm J. U., Mateu-Andres I., Segarra-Moragues J. G. (2003) Allozyme and chloroplast DNA variation in island and mainland populations of the rare Spanish endemic, *Silene hifacensis* (Caryophyllaceae). Conservation Genetics 4: 543–555.

Rendell S., Ennos R. A. (2002) Chloroplast DNA diversity in *Calluna vulgaris* (heather) populations in Europe. Molec. Ecol. 11: 69–78.

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

Sandbrink J. M., Bruggen A. C. van, Brederode J. van (1990) Patterns of infraspecific chloroplast DNA variation in species of *Silene* section *Elisanthe*. Biochem. Syst. Ecol. 18: 233–238.

Sandgren P., Snowball I., Hammarlund D., Risberg J. (1999) Stratigraphic evidence for a high marine shore-line during the Late Weichselian deglaciation of the Kullen Peninsula. Journal of Quaternary Science 14: 223–237.

Schaal B. A., Hayworth D. A., Olsen K. M., Rauscher J. T., Smith W. A. (1998) Phylogeographic studies in plants: problems and prospects. Molec. Ecol. 7: 465–474.

Schneider S., Roessli D., Excoffier L. (2000) Arlequin: A Software for Population Genetics Data Analysis. Version 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Taberlet P., Gielly L., Patou G., Bouvet J. (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. Plant Molec. Biol. 17: 1105–1109.

Taberlet P., Swenson J. E., Sandegren F., Bjärvall A. (1995) Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear *Ursus arctos* in Scandinavia. Conserv. Biol. 9: 1255–1261.

Taberlet P., Fumagalli L., Wust-Saucy A. G., Cosson J. F. (1998) Comparative phylogeogra-

phy and postglacial colonization routes in Europe. Molec. Ecol. 7: 453–464.

Tegelström H. (1987) Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). J. Mol. Evol. 24: 218–227.

Thell A., Berbee M., Miao V. (1998) Phylogeny of the genus *Platismatia* based on rDNA ITS sequences (Lichenized Ascomycotina). Cryptogamie, Bryologie, Lichénologie 19: 307–319.

Weising K., Nybom H., Wolff K., Mayer W. (1995) DNA Fingerprinting in Plants and Fungi. CRC Press Inc., Boca Raton.

Widmer A., Lexer C. (2001) Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends Ecol. Evol. 16: 267–269.

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