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Van Rossum, F; Prentice, Honor C

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Structure of allozyme variation in Nordic *Silene nutans* (Caryophyllaceae): population size, geographical position and immigration history

FABIENNE VAN ROSSUM* and HONOR C. PRENTICE

Systematic Botany, Department of Ecology, Lund University, Sölvegatan 37, S-223 62 Lund, Sweden

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We investigated allozyme variation in 34 populations of the perennial herb *Silene nutans* from Sweden and northern Finland, areas that were ice-covered during the last (Weichselian) glaciation. The present geographical structure of genetic variation in *S. nutans* in Sweden and northern Finland appears to have been mainly shaped by ancient historical processes. Patterns of variation in allele frequencies suggest two major postglacial immigration routes into Sweden, with populations entering the area from both the south and the east and forming a contact zone with admixed populations in central Sweden. While estimates of within-population genetic diversity and allelic richness are significantly correlated with present population size and geographical position (latitude), population size is not correlated with latitude. Low genetic diversity in the northern populations is more likely to have resulted from ancient stochastic events during the process of immigration than from recent population fragmentation. F_{IS} values are high and increase with latitude. Evidence of recent bottlenecks was detected in several southern Swedish populations: these can be interpreted in terms of population fragmentation as a result of anthropogenic disturbance. Soil pH is uncorrelated with population size and position. *Biological Journal of the Linnean Society,* 2004, **81**, 357–371.

ADDITIONAL KEYWORDS: bottlenecks – contact zone – habitat fragmentation – postglacial immigration – range periphery – soil pH.

INTRODUCTION

Levels of intrapopulation genetic diversity and the spatial structuring of intraspecific variation in plant species are determined by a range of ecological, demographic and historical factors (e.g. Loveless & Hamrick, 1984; Hamrick & Godt, 1990). In recent centuries, human activities have led to extensive modification and fragmentation of habitats, and anthropogenic activity may also have an important impact on the genetic structure of wild plant populations (e.g. Young, Boyle & Brown, 1996). Many plant species are currently undergoing a process of progressive range-disjunction. Local reductions in population size lead to the loss of genetic variation and to increased rates of inbreeding (e.g. Barrett & Kohn, 1991; Oostermeijer, Berholz & Poschlod, 1996).

On a larger geographical scale, the present structure of genetic variation may still reflect the long-term effects of past environmental changes. There is increasing evidence that the climatic changes associated with the repeated cycles of glaciation during the Quaternary not only reshaped the geographical distributions of most of the plant and animal taxa in Europe but have also had a lasting impact on the structuring of genetic variation within taxa (e.g. Hewitt, 1996; Taberlet *et al*., 1998). During the Quaternary glacial maxima, large areas of northern Europe were covered by ice or by open, steppe-like habitats. Temperate species survived the glacial periods in refugial areas to the south and east of the main European ice sheet and the refugial populations served as sources for northward and westward range expansion during intergla-

*Corresponding author. Current address: Plant Science and Nature Management (APNA), Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.
E-mail: fvrossum@vub.ac.be

cial periods. During successive refugial periods and phases of range-expansion, populations are likely to have experienced prolonged episodes of small size and genetic isolation (e.g. Hewitt, 1996; Comes & Kadereit, 1998; Taberlet *et al*., 1998).

Modes of migration and range expansion from refugial source populations are expected to have had a strong influence on the levels of genetic diversity and patterns of geographical variation that we can observe in widespread species at the present day. If range expansion proceeds via small and isolated founding populations, genetic diversity will be lost during the process of migration and repeated colonization. In this way, even large and genetically diverse source populations may give rise to geographically structured patterns of variation (Hewitt, 1996, 1999). Wholesale range expansions associated with climate change may also involve the spread of genetically distinct populations with different geographical origins or derived from different refugial areas (e.g. Hewitt, 1996; Taberlet *et al*., 1998). If these genetically distinct (and previously isolated) races are interfertile, intraspecific hybrid zones containing individuals of mixed ancestry may form in the contact zones where the races meet during the process of range expansion (Hewitt, 1996, 1999; Jiggins & Mallet, 2000).

Most of Fennoscandia was ice-covered during the last (Weichselian) glacial maximum [*c*. 22 000–17 000 cal year BP (Svendsen *et al*., 1999)]. The deglaciation of southernmost Sweden was complex. While some areas in the north-west of Scania were ice-free as early as 17 000 BP (Sandgren *et al*., 1999), the northern and central parts were not deglaciated until 14 000 BP (Lundqvist & Wohlfarth, 2001). Species-immigration to Sweden during the late Weichselian and early postglacial period was constrained by the position of the retreating ice margin and by accompanying changes in sea level that altered the positions of the coastlines and the extent of the land connection to Denmark (Berglund *et al*., 1994; Björck, 1995). Later immigration of plant species – in particular those that depend on open habitats – is also likely to have been constrained by the progressive development of closed forest communities in Fennoscandia from *c.* 9000 BP (e.g. Berglund *et al*., 1994). We can therefore predict that levels of genetic diversity and the structuring of genetic variation within many plant species in the Nordic area will have been strongly influenced by their histories of postglacial immigration. Evidence suggesting that Fennoscandia was colonized from separate geographical sources has been reported for a number of animal and plant species (e.g. Nordal & Jonsell, 1998; Taberlet *et al*., 1998; Nyberg Berglund & Westerbergh, 2001; Malm & Prentice, 2002).

Many European plant species reach their northern range limit in Fennoscandia. There is a steep, latitudinal, climatic gradient in Fennoscandia, with conditions becoming progressively more severe northwards (Sjörs, 1999). Range-periphery populations may also be ecologically marginal and on the limits of the species' overall physiological amplitude (cf. Hoffmann & Blows, 1994; Holt & Keitt, 2000). Individuals in ecologically marginal situations are expected to show reduced reproductive success and lower individual survival (Galen & Stanton, 1993; Hoffmann & Blows, 1994; Dorken & Eckert, 2001). Marginal populations may be sparsely distributed, small, or show pronounced temporal fluctuations (Pamilo & Savolainen, 1999). Levels of gene flow may decrease and levels of inbreeding increase as a result of a lack of suitable pollinators in marginal populations (Barrett & Kohn, 1991).

Over time, reduced levels of gene flow and increased levels of genetic drift and inbreeding are thus predicted to lead to low overall levels of genetic variation and relatively high levels of interpopulation differentiation in peripheral populations – particularly if the populations are also ecologically marginal (cf. Lönn & Prentice, 2002). Directional selection may also contribute to local adaptation, and to differentiation and divergence between populations that occupy ecological boundaries (e.g. Mayr, 1963; Soulé, 1973; Hoffmann & Blows, 1994; Pamilo & Savolainen, 1999). Populations that are on the northern limit of their European range in Fennoscandia may be expected to show a genetic structure and levels of variation that reflect their peripheral position. However, depending on the characters surveyed, it may be difficult to separate the effects of recent population processes (including anthropogenic disturbance) and longer-term historical events during range-extension (or contraction) on the current distribution of genetic variation in peripheral populations.

There have been few geographically comprehensive studies of genetic variation throughout the Fennoscandian ranges of plant species. Most such studies have focused either on large-scale geographical patterns and postglacial immigration (e.g. Nordal & Jonsell, 1998; Nyberg Berglund & Westerbergh, 2001; Malm & Prentice, 2002; Tyler, Prentice & Widén, 2002) or on the effects of recent population processes on levels of genetic variation within populations (Lammi, Siikamäki & Mustajävi, 1999). The contributions of both recent population processes and of immigration history to the present structure of genetic variation in Nordic species are examined by Rosquist & Prentice (2000) and Schiemann, Tyler & Widén (2000).

In the present study, we use allozymes to investigate genetic variation and population structure in the perennial herb *Silene nutans* in Fennoscandia. *Silene nutans* has a markedly disjunct distribution in northern Sweden and Finland, where it reaches the north-

ern limit of its European range, whereas it is more continuously distributed in southern Sweden (Hultén, 1971). Earlier studies of *S. nutans* in Belgium, on the western margin of its distributional range, showed that the Belgian populations maintain high levels of genetic diversity and are differentiated into two parapatric ecotypes that are associated with calcareous or siliceous substrates (Van Rossum *et al*., 1997, 1999), but which have also had different histories (Van Rossum *et al*., 2003). Edaphic adaptation (De Bilde, 1977, 1978), in combination with the partial isolation of the two ecotypes by pre- and postzygotic reproductive barriers (Van Rossum, De Bilde & Lefèbvre *et al.*, 1996), suggests a process of incipient parapatric speciation (Van Rossum *et al*., 1997).

In the study of Fennoscandian populations of *S. nutans*, we address the following questions. (1) What are the relationships between genetic variation, population position (peripheral or central location, defined by latitude), population size and substrate-type (calcareous or siliceous soils)? (2) Can levels of genetic diversity and the geographical structure of allelic variation be interpreted in terms of population processes associated with ancient historical events (i.e. the species' postglacial colonization of the Nordic region) and/or in terms of more recent population processes or adaptive differentiation? (3) Is there evidence that range fragmentation following anthropogenic disturbance has led to recent population bottlenecks?

MATERIAL AND METHODS

The species

Silene nutans L. (Caryophyllaceae) is a diploid ($2n = 22$), long-lived perennial herb. The species has a wide continental distribution, extending from northwestern Europe to central Siberia and the southern Caucasus (Hegi, 1979). It reaches its northern range limit in Fennoscandia, where it is widespread but locally distributed in southern and central Sweden and Finland (Hultén, 1971; Fig. 1). In the Nordic countries, *S. nutans* occurs in dry, open grassland and forest edge habitats. It has a relatively wide pH amplitude (Tyler, 1996; see also Table 1) and generally occurs on soils that are >10 cm deep. Nordic populations flower from June to August *Silene nutans* is protandrous, insect-pollinated and self-compatible, but shows strong maternal discrimination against selfing (De Bilde, 1984; Hauser & Siegismund, 2000). Inbreeding depression has been reported in Belgian (Van Rossum *et al*., 1996) and Danish populations (Hauser & Siegismund, 2000). The seeds are dispersed over relatively short distances by a simple censer mechanism (Hepper, 1956).

Sites and sampling

Rosette-leaf material was sampled from 737 individuals in 34 populations, representing the geographical extent of *S. nutans* in Sweden and northern Finland (Fig. 1, Table 1). Populations 1–27 are located within the species' main distributional range in Sweden while populations 28–34 represent disjunct occurrences on the species' northern range margin. The sampled populations and sites are described in Table 1. Population size (estimated as the number of flowering plants) ranged from 4 to 1200. To avoid the sampling of close relatives, the sampled individuals were separated from each other by at least 2 m and, depending on population size, 4–30 individuals were sampled from throughout the area occupied by the population. Soil pH (1 : 1 soil to de-ionized water suspension) was measured in pooled soil samples collected from the superficial horizon (0–20 cm in depth) in the vicinity of *S. nutans* plants within each population.

Allozyme electrophoresis

For each individual, approximately 20–30 mg of leaf material was ground, together with sand, in five drops of extraction buffer (Tris-HCl grinding buffer-PVP solution; Soltis *et al*., 1983). The leaf extracts were absorbed onto (4×6 mm) Whatman no. 3 filter paper wicks and stored at –80°C until they were used for electrophoresis. Electrophoresis was carried out in horizontal 11% starch gels ($5 \times 225 \times 150$ mm). Nine enzyme systems, representing 16 putative loci, were resolved: alcohol dehydrogenase (*Adh*, EC 1.1.1.1), esterase (*Est-1*, *Est-2*, *Est-3* and *Est-4*, EC 3.1.1.-), glucose-6-phosphate-isomerase (*Pgi-1* and *Pgi-2*, EC 5.1.3.9), glutamate-oxaloacetate transaminase (*Got-1* and *Got-2*, EC 2.6.1.1), phosphoglucomutase (*Pgm-1* and *Pgm-2*, EC 5.4.2.2), 6-phospho-D-gluconate (*Pgd*, EC 1.1.1.44), shikimate dehydrogenase (*Skd*, EC 1.1.1.25), triose phosphate isomerase (*Tpi-1*, EC 5.3.1.1), and uridine 5′ diphosphoglucose phosphorylase (*Ugpp-1* and *Ugpp-2*, EC 2.7.7.9).

Three buffer systems were used: lithium-borate pH 8.4/Tris-citrate pH 8.1 (Lönn & Prentice, 1990) for EST; sodium-borate pH 8.5/Tris-citrate pH 7.8 (Weidema, Siegismund & Philipp, 1996) for ADH, GOT, PGI, and TPI; and histidine-citrate pH 6.0 (Weidema *et al*., 1996) for PGM, PGD, SKD and UGPP. The staining recipes followed (with minor modifications) Wendel & Weeden (1989) for EST (modified according to Simonsen & Frydenberg, 1972) and GOT; Weidema, Magnussen & Philipp (2000) for UGPP, and Soltis *et al*. (1983) for PGD, PGI, PGM, SKD, and TPI. ADH was stained by adding 3% ethanol (98%) to the TPI staining solution. Loci and alleles were numbered in order of ascending mobility.

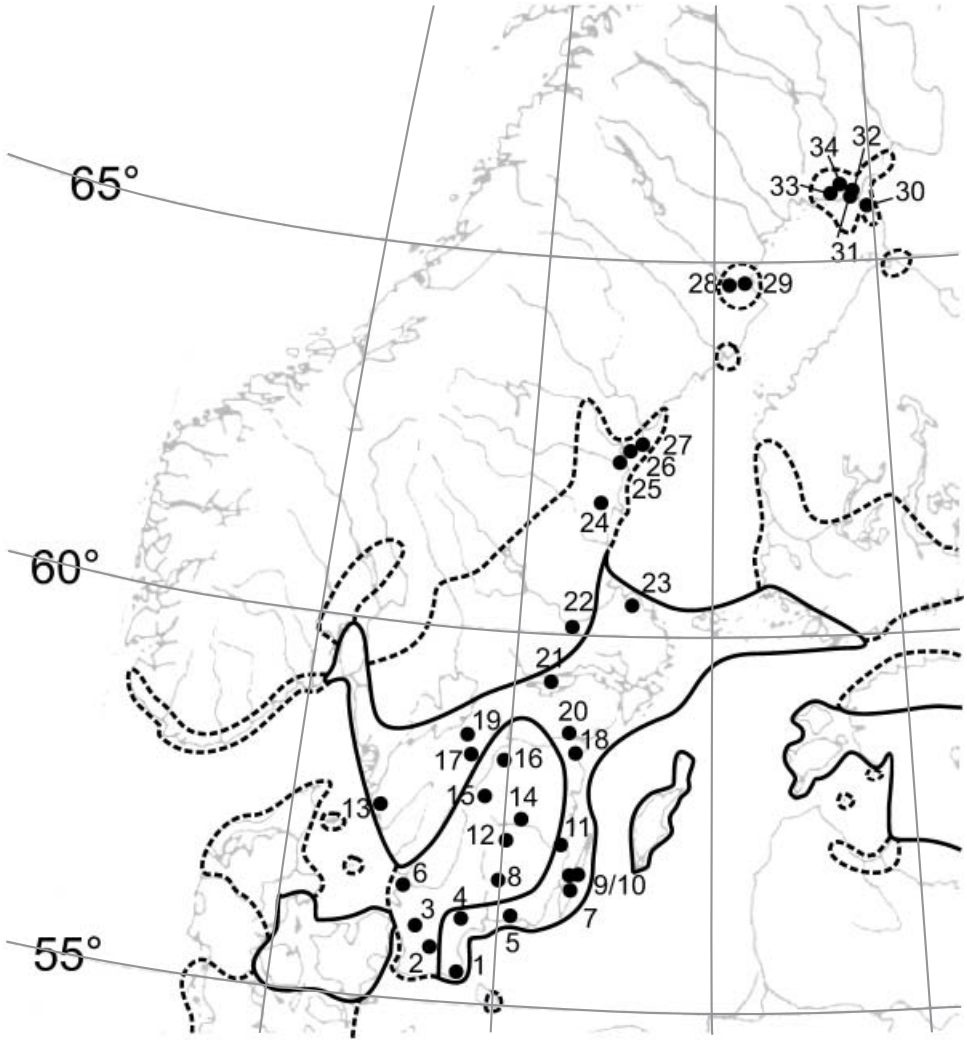

Figure 1. The Fennoscandian distribution of *S. nutans* (based on Hultén, 1971) and the locations of the 34 sampled populations in Sweden and northern Finland. The solid lines show the limits of the species' continuous distribution and the dotted lines the limits of areas with scattered populations. Population details are given in Table 1.

Data analysis

All the analyses of genetic data were performed using GEN-SURVEY ver.1.0 (Vekemans & Lefèbvre, 1997), except when otherwise specified. The following measures of genetic variation were calculated for each population: the mean allelic richness (A), based on the rarefaction method described in El Mousadik & Petit (1996) and calculated using FSTAT ver. 2.9.3.2. (Goudet, 2001), the observed heterozygosity (H_o), the expected heterozygosity (H_e), corrected for small sample size (Nei, 1978), and Wright's inbreeding coefficient (F_{IS}) corrected for small sample size (Kirby, 1975).

A test for genotypic disequilibrium between pairs of loci was performed using FSTAT. Because multiple tests were involved, the sequential Bonferroni-type correction was applied to test for significance (Rice, 1989).

Deviations from Hardy–Weinberg expectations were investigated by performing exact tests for each population and locus, using GENEPOP ver.3.2 (Raymond & Rousset, 1995). The significance of the F_{IS} values estimated for each population over all loci was tested by randomization tests using FSTAT and sequential Bonferroni-type correction.

To investigate whether there were relationships between within-population genetic variation (A, H_o, H_e and F_{IS}), population size, geographical position (latitude) and soil pH, we performed multiple stepwise regression analyses (with forward stepwise procedure) using STATISTICA. Multiple stepwise regression

Table 1. Population codes and site details for 34 populations of *Silene nutans* from Sweden and northern Finland: location, latitude, longitude, population size (N), soil pH_{water} and habitat type

Code	Location	Region	Latitude	Longitude	N	pH_{water}	Habitat
1	Löderup	Skåne	55°23′N	14°07′E	150	5.8	coastal sand-dune
2	Hällestad	Skåne	55°41′N	13°25′E	200	5.3	dry sandy grassland
3	Sireköpinge	Skåne	55°56′N	13°00′E	300	6.0	dry grassland
4	Lommarp	Skåne	56°06′N	13°51′E	150	7.7	dry, open to closed grassland
5	Våby	Blekinge	56°11′N	15°07′E	200	5.0	forest edge
6	Kullen	Skåne	56°18′N	12°27′E	350	4.4	scrub on coastal cliff
7	Gösslunda	Öland	56°29′N	16°32′E	193	7.5	dry limestone grassland and scrub
8	Tingsryd	Småland	56°39′N	14°52′E	670	4.3	dry, open to closed grassland
9	Jordtorp 1	Öland	56°41′N	16°35′E	20	6.5	dry, grazed limestone grassland
10	Jordtorp 2	Öland	56°41′N	16°36′E	28	5.5	dry, grazed limestone grassland and scrub
11	Ökno	Småland	57°00′N	16°32′E	240	4.9	coastal sand-dune
12	Ramkvilla	Småland	57°13′N	14°57′E	300	5.0	dry, open to closed grassland
13	Värö	Halland	57°15′N	12°08′E	175	4.1	dry, closed grassland
14	Sjunnen	Småland	57°26′N	15°11′E	11	5.0	forest edge and scrub
15	Tenhult	Småland	57°43′N	14°22′E	400	5.5	forest edge
16	Alvastra	Östergötland	58°18′N	14°40′E	266	7.3	forest edge and clearing
17	Skövde	Västergötland	58°19′N	13°49′E	1000	4.8	grassland in pine forest clearing
18	Linneberga	Östergötland	58°30′N	16°31′E	170	4.9	dry, open grassland
19	Mariestad	Västergötland	58°35′N	13°49′E	175	5.2	dry, closed grassland
20	Krokek	Östergötland	58°40′N	16°25′E	200	7.3	pine forest on limestone
21	Glanshammar	Närke	59°19′N	15°25′E	25	6.4	dry, open grassland
22	Grådö	Dalarna	60°14′N	16°02′E	200	5.2	birch forest and forest edge
23	Hållnäs	Uppland	60°32′N	17°50′E	90	7.4	dry, open limestone grassland
24	Njurunda	Medelpad	62°15′N	17°23′E	4	3.9	forest edge and scrub
25	Berghamn	Ångermanland	62°49′N	18°14′E	40	4.8	dry, open grassland
26	Bergdal	Ångermanland	62°51′N	18°15′E	800	4.9	dry to mesophilous grassland
27	Ådal	Ångermanland	62°52′N	18°21′E	1200	4.8	open to closed grassland
28	Skellefteå 1	Västerbotten	64°44′N	20°39′E	150	4.8	forest edge and open forest on river bank
29	Skellefteå 2	Västerbotten	64°45′N	20°46′E	85	5.1	grassland and forest edge on river bank
30	Kemi	Finland	65°47′N	24°38′E	34	5.9	open pine and birch steppic forest
31	Keminmaa 1	Finland	65°48′N	24°33′E	70	5.5	gravelly river bank
32	Keminmaa 2	Finland	65°48′N	24°33′E	16	4.6	forest clearing
33	Haparanda	Norrbotten	65°49′N	24°08′E	102	4.3	dry, open grassland
34	Keminmaa 3	Finland	65°49′N	24°28′E	100	6.6	forest edge, scrub and open forest

examines the relationship between a dependent variable and a single independent variable while the other independent variables in the model are (statistically) held constant (Sokal & Rohlf, 2000). Population size was log-transformed to achieve homoscedasticity and normality. Deviations from normality were not detected for the other variables. The relationships between the predictor variables (population size, latitude and soil pH) were also examined using Pearson's correlation coefficients.

In order to infer recent (within the past few dozen generations) population bottlenecks from within-population allozyme allele frequencies, we performed Wilcoxon signed-ranks tests (with 1000 simulations iterations) under the Infinite Allele Model (IAM), using BOTTLENECK, which tests for population heterozygosity excess (Cornuet & Luikart, 1996; Piry, Luikart & Cornuet, 1999). The tests conducted by this software are based on the theoretical expectation that populations that have experienced a recent reduction in effective size will have undergone a more rapid reduction in the number of alleles than of the level of expected heterozygosity (= H_e = genetic diversity) at polymorphic loci. Hence, in a recently bottlenecked population, the expected heterozygosity (H_e, calculated from the sum of squared allele frequencies) will be higher than the equilibrium heterozygosity (H_{eq}) estimated from the observed number of alleles under the assumption of mutation-drift equilibrium (Cornuet & Luikart, 1996; Luikart & Cornuet, 1998). As recommended by Piry *et al*. (1999) for allozyme data, we used IAM and Wilcoxon signed-rank tests because less than 20 polymor-

phic loci were involved (4–15). Population 24 was not analysed because of its small sample size ($n = 4$).

Population genetic structure at the polymorphic loci was investigated using Nei's total genetic diversity (H_T) and mean within-population diversity (H_S) with correction for small sample size according to Nei & Chesser (1983), and using Weir & Cockerham's (1984) estimate of the between-population component of diversity (F_{ST}). Averages of these statistics for all populations (with over-locus standard errors and 95% confidence intervals) were obtained by bootstrapping over loci.

In order to test for isolation by distance, $F_{ST}/(1–F_{ST})$ ratios were computed for all pairs of populations and regressed on the logarithm of the geographical distance using SPAGeDI ver. 1.0 (Hardy & Vekemans, 2002). The significance of the regression was tested by performing a 1000-permutation Mantel test. The F_{ST} values were computed according to Weir & Cockerham (1984).

Nei's genetic distances, corrected for small sample size (Nei, 1978), were computed between all pairs of populations. To summarize the patterns of differentiation between populations, a cluster analysis using Ward's (1963) method was performed on the matrix of Nei's distances using STATISTICA.

To identify possible routes of immigration into Sweden and northern Finland, we used STRUCTURE ver. 2 (Pritchard, Stephens & Donnelly, 2000) to infer population structure. STRUCTURE implements a model-based clustering method (fully Bayesian approach) using genotype data. The method attempts to identify K populations or clusters (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. The sampled individuals are (probabilistically) assigned to populations, or jointly to two or more populations if their genotypes indicate that they are admixed. The proportion of membership in each of the K clusters is estimated for each individual and a mean individual membership for each cluster is calculated for the predefined populations. When the location of the individuals (i.e. the sampled predefined populations) is known, it is possible to identify hybrid zones, migrants and hybrid individuals (Pritchard *et al*., 2000). In order to interpret the relationships between the sampled individuals and between populations in terms of possible immigration history, we examined the clustering results for a range of values of K, starting from 2. Our analyses were based on an admixture ancestry model with correlated allele frequencies (because of high F_{IS} values in most populations), using a burn-in period and a run length of 10^5 and 10^6 iterations, respectively, so that approximate stabilization of the summary statistics could be reached (Pritchard *et al*., 2000). Mean F_{ST} values (corresponding to divergence from the hypothetical ancestral population) were calculated for each of the K clusters (see Pritchard & Wen, 2002 for details).

To examine the effect of substrate type (calcareous = $pH_{soil} > 7.0$; siliceous = $pH_{soil} < 7.0$) on genetic structure, an analysis of molecular variance was performed using ARLEQUIN (Schneider, Roessli & Excoffier, 2000). The analysis included populations 1–23 (five populations with $pH_{soil} > 7.0$ and 18 populations with $pH_{soil} < 7.0$, Table 1) but excluded the disjunct regions where no populations were found on calcareous substrates (Table 1). The between-population component of the total genetic diversity is given by F_{ST}. F-statistics were also used to partition the genetic diversity into its between-substrate-category component (= F_{RT}) and its between-population (within-substrate-category) component (= F_{SR}) (Excoffier, 2001). The F statistics are related by the equation $(1 – F_{ST}) = (1 – F_{SR})\ (1 – F_{RT})$ (Weir, 1990). The significance of the F_{ST}, F_{SR} and F_{RT} values was tested using a non-parametric permutation approach (Excoffier, Smouse & Quattro, 1992).

RESULTS

Loci and alleles

Fifteen out of the 16 surveyed loci showed variation in at least one population. Only *Pgi-2* was monomorphic in all the populations. The number of polymorphic loci per population ranged from 4 to 13. A total of 59 alleles was detected: *Adh* (3 alleles), *Est-1* (3 alleles), *Est-2* (6 alleles), *Est-3* (4 alleles), *Est-4* (4 alleles), *Got-1* (6 alleles), *Got-2* (3 alleles), *Pgd* (5 alleles), *Pgi-1* (4 alleles), *Pgm-1* (5 alleles), *Pgm-2* (4 alleles), *Skd* (3 alleles), *Tpi-1* (2 alleles), *Ugpp-1* (4 alleles), and *Ugpp-2* (2 alleles). No linkage disequilibrium was found between pairs of loci after sequential Bonferroni correction.

The occurrences of several of the less common alleles are geographically structured. The alleles *Got-1-3*, *Pgm-2-1*, *Pgm-1-2*, *Skd-3* and *Ugpp-1-3* were restricted to southern and central Sweden and absent from northern Sweden, while *Est-2-1* and *Est-2-4* were only found in central and northern Sweden. *Pgm-2-2* was only recorded in central Sweden.

Genetic variation within populations

Mean values for the estimates of within-population genetic variation are given in Table 2. Allelic richness (A) ranged from 1.21 to 1.86, observed heterozygosity (H_o) from 0.033 to 0.209, expected heterozygosity (H_e, corrected for small sample size) from 0.083 to 0.305, and Wright's inbreeding coefficient (F_{IS}) from –0.124 to 0.609.

Exact tests for Hardy–Weinberg proportions, for each population and locus, showed significant positive deviations ($P < 0.001$) in 45 out of 280 tests (16.1%) for

F_{IS} values (i.e. a deficiency of heterozygotes). Thirty-two out of 34 populations showed significant (positive) F_{IS} values. No test showed significant negative F_{IS} values (heterozygote excess). Populations 14 and 23 had non-significant F_{IS} values (Table 2). The mean F_{IS} value over all populations was also significant (0.352, $P < 0.001$).

Effects of geographic position (latitude), population size and soil pH on genetic variation within populations

There was no significant correlation between population size and latitude ($r = –0.266$, $P > 0.05$), between soil pH and latitude ($r = –0.194$, $P > 0.05$), or between soil pH and population size ($r = –0.012$, $P > 0.05$). Significant ($P < 0.05$) multiple stepwise regression models (after forward selection) were found for all the response variables (A, H_o, H_e and F_{IS}) (Table 3). The explanatory variables that showed a significant effect after forward selection were 'latitude' and 'population size'. There were no significant relationships involving the variable 'soil pH', and this predictor variable was therefore not selected in the forward stepwise procedure for any of the models. The standard partial regression (β) coefficient indicated that A, H_o, and H_e were negatively related to latitude and that F_{IS} was positively related to latitude (Fig. 2). Significant positive relationships were found between population size and A and population size and H_e ($\beta = 0.278$, $P = 0.012$ and $\beta = 0.254$, $P = 0.014$, respectively) (Table 3, Fig. 3).

Detection of recent bottlenecks

Evidence of recent bottlenecks was found in six out of 33 populations (nos. 6, 8, 12, 14, 18 and 20). These southern Swedish populations (Fig. 1) showed significant ($P < 0.05$) excesses of heterozygosity ($H_e > H_{eq}$) under IAM when tested by Wilcoxon signed rank tests (Table 4).

Structure of genetic diversity

The total (mean over loci) genetic diversity (H_T) for all populations was 0.250 and the mean (over populations and loci) within-population genetic diversity (H_S) was 0.211 (Table 5). The mean (over loci) between-population component of diversity (F_{ST}) was 0.134, and F_{ST} ranged from 0.059 (*Adh*) to 0.251 (*Got-2*). A pattern of isolation by distance is indicated by the positive correlation between the matrix of $F_{ST}/(1 - F_{ST})$ values between populations and the matrix of between-population geographical distances ($r = 0.332$, $P < 0.001$).

The overall pattern of between-population differentiation, based on Nei's genetic distances, is summarized in the dendrogram in Figure 4. Populations tended to cluster according to their geographical origin. The populations from the disjunct northern regions (with the exception of no. 33) were separated at a high level from the populations from the species' main range in southern and central Sweden. Although there was less pronounced geographical structure within the main group of populations, three of the northern populations (nos. 24, 26 and 27) were included in the same intermediate-level cluster.

Table 2. Estimates of genetic variation for 34 populations of *Silene nutans* from Sweden and northern Finland. n = sample size, A = mean allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = Wright's inbreeding coefficient, SD = standard deviation. Significance level of F_{IS} values: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant

Code	Population	n	A	H_o	H_e	F_{IS}
1	Löderup	23	1.60	0.126	0.228	0.446***
2	Hällestad	22	1.77	0.185	0.264	0.301***
3	Sireköpinge	28	1.69	0.205	0.251	0.180***
4	Lommarp	22	1.59	0.139	0.223	0.375***
5	Våby	22	1.67	0.149	0.221	0.320***
6	Kullen	22	1.86	0.181	0.305	0.408***
7	Gösslunda	24	1.60	0.129	0.218	0.404***
8	Tingsryd	24	1.80	0.209	0.292	0.290***
9	Jordtorp 1	15	1.65	0.163	0.233	0.296***
10	Jordtorp 2	19	1.52	0.133	0.181	0.251***
11	Ökno	23	1.60	0.124	0.207	0.412***
12	Ramkvilla	23	1.72	0.140	0.263	0.458***
13	Värö	22	1.63	0.136	0.236	0.410***
14	Sjunnen	12	1.43	0.207	0.183	–0.124 ns
15	Tenhult	22	1.50	0.149	0.180	0.174**
16	Alvastra	22	1.45	0.108	0.144	0.252***
17	Skövde	20	1.71	0.138	0.250	0.440***
18	Linneberga	20	1.65	0.139	0.226	0.388***
19	Mariestad	22	1.52	0.178	0.208	0.129*
20	Krokek	23	1.72	0.144	0.244	0.407***
21	Glanshammar	23	1.69	0.147	0.223	0.338***
22	Grådö	24	1.76	0.173	0.248	0.305***
23	Hållnäs	22	1.47	0.168	0.186	0.099 ns
24	Njurunda	4	1.31	0.047	0.114	0.582**
25	Berghamn	21	1.55	0.163	0.203	0.198**
26	Bergdal	22	1.58	0.122	0.198	0.387***
27	Ådal	30	1.49	0.104	0.174	0.401***
28	Skellefteå 1	29	1.30	0.069	0.123	0.441***
29	Skellefteå 2	25	1.21	0.033	0.083	0.609***
30	Kemi	22	1.32	0.046	0.100	0.541***
31	Keminmaa 1	21	1.32	0.064	0.106	0.392***
32	Keminmaa 2	15	1.29	0.067	0.117	0.431***
33	Haparanda	24	1.34	0.048	0.122	0.599***
34	Keminmaa 3	20	1.21	0.053	0.090	0.429***
	Mean		1.54	0.129	0.196	0.352***
	SD		0.18	0.050	0.060	0.150

Table 3. Multiple stepwise regression analyses (forward selection) of latitude, population size (log-transformed) and soil pH on measures of within-population genetic variation for 34 populations of *S. nutans* from Sweden and northern Finland. Only the significant models and the significant predictor variables, after forward selection, are shown. *A* = mean allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = Wright's inbreeding coefficient; *R* = multiple correlation coefficient; β = standardized partial regression coefficient; *t* = *t*-statistic (two-tailed test of whether the partial regression coefficient differs from zero); d.f. = degrees of freedom; *MS* = mean square; *P* = significance probability

			ANOVA					
Variable	β	*t*	*P*	Source	d.f.	*MS*	*F* ratio	*P*
A (*R* = 0.827)								
Latitude	–0.708	–6.76	<0.001	Regression	2	0.3522	33.50	<0.001
Population size	0.278	2.65	0.012	Residual	31	0.0105		
H_o (*R* = 0.778)								
Latitude	–0.778	–7.00	<0.001	Regression	1	0.0496	48.98	<0.001
				Residual	32	0.0010		
H_e (*R* = 0.851)								
Latitude	–0.748	–7.64	<0.001	Regression	2	0.0427	40.65	<0.001
Population size	0.254	2.59	0.014	Residual	31	0.0010		
F_{IS} (*R* = 0.420)								
Latitude	0.420	2.62	0.013	Regression	1	0.1314	6.86	0.013
				Residual	32	0.0192		

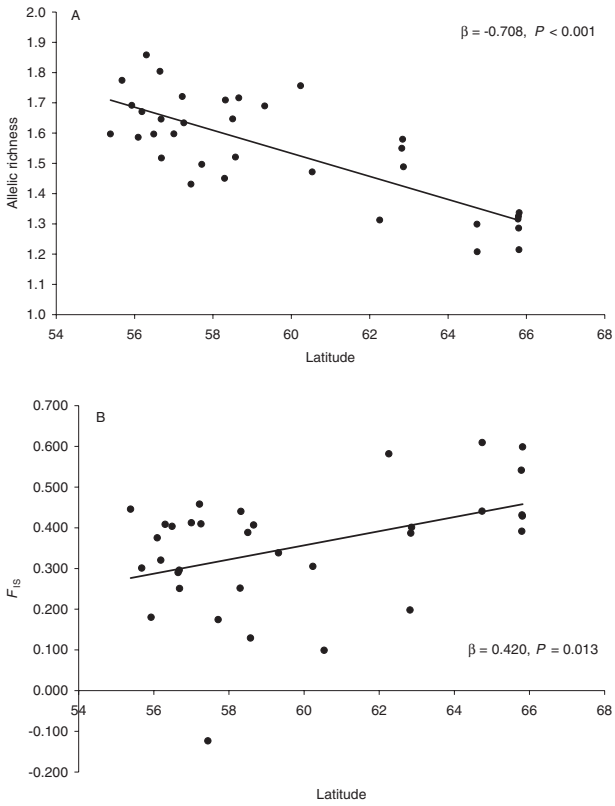

Figure 2. Relationships between latitude and (A) mean allelic richness, (B) Wright's inbreeding coefficient (F_{IS}) in Fennoscandian populations of *S. nutans*. β = standardized partial regression coefficient.

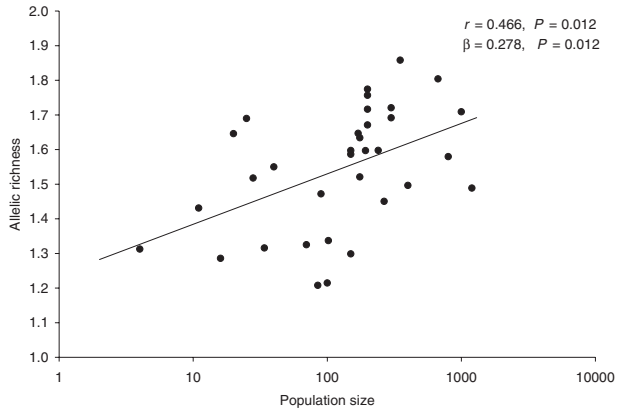

Figure 3. Relationship between population size (log-scale) and allelic richness in 34 Fennoscandian populations of *S. nutans*. *r* = Pearson's correlation coefficient; β = standardized partial regression coefficient.

Analysis of the relationships between individuals, using STRUCTURE (Pritchard *et al*., 2000), revealed a geographical structure (related to latitude) when *K* = 2 (Fig. 5). The southern populations showed a higher proportion (>60%) of mean individual membership in cluster 1, while the populations that showed a higher proportion (>60%) of mean individual membership in cluster 2 were located in the north (and southwards to population 15). Populations with intermediate (*c*. 50%) cluster-membership values (e.g. nos. 13, 17, 19 and 21) were found in south-central

Table 4. Number of loci showing deficiency/excess of heterozygosity under IAM and significance level (*P*) of the Wilcoxon signed rank tests for bottleneck detection in six populations of *S. nutans*

	Population code					
	6	8	12	14	18	20
Deficiency/excess of heterozygosity	1/10	3/8	3/9	1/7	2/6	3/6
P	0.003	0.006	0.046	0.020	0.020	0.024

Table 5. Estimates of genetic diversity at 15 polymorphic loci for 34 populations of *S. nutans* from Sweden and northern Finland. H_T = total gene diversity, H_S = mean gene diversity within populations, F_{ST} = between-population component of diversity, SD = standard deviation, 95% CI = 95% confidence intervals

Locus	H_T	H_S	F_{ST}
Adh	0.010	0.010	0.059
Est-1	0.345	0.309	0.106
Est-2	0.535	0.454	0.151
Est-3	0.558	0.500	0.104
Est-4	0.645	0.488	0.243
Got-1	0.130	0.106	0.188
Got-2	0.021	0.016	0.251
Pgd	0.101	0.093	0.075
Pgi-2	0.064	0.058	0.085
Pgi-1	–	–	–
Pgm-1	0.051	0.048	0.071
Pgm-2	0.313	0.279	0.109
Skd	0.070	0.060	0.133
Tpi-1	0.331	0.272	0.178
Ugpp-1	0.506	0.409	0.190
Ugpp-2	0.066	0.062	0.066
Mean	0.250	0.211	0.134
SD	0.224	0.184	0.063
95%CI	0.142; 0.372	0.123; 0.313	0.104; 0.167

Sweden (Fig. 5). The mean F_{ST} values (relative to the hypothetical ancestral population) estimated for clusters 1 and 2, respectively, were 0.251 and 0.202. Increasing *K* provided no further interpretable results.

The analysis of molecular variance gave a value of 0.003 for the between-substrate-category component of genetic diversity (F_{RT}) and 0.109 for F_{SR} (between-populations within substrate categories). The value for the overall F_{ST} was 0.112. The permutation test showed significance ($P < 0.001$) for the F_{SR} and F_{ST} values, while F_{RT} was non-significant ($P > 0.10$). The majority of the total variance (88.9%) was attributable to the within-population component of variation (covariance = 1.828). A further 10.9% of the total variance (covariance = 0.223) was explained by variation between populations within substrate-groups, and 0.2% by variation between substrate-groups (covariance = 0.005).

DISCUSSION

GENETIC VARIABILITY: POPULATION SIZE, POPULATION POSITION AND SOIL PH

The levels of within-population genetic diversity (H_e = 0.083–0.305) in Nordic *S. nutans* fall within the range of those reported by Hamrick & Godt (1990) for species with similar life-history traits (i.e. long-lived perennial, widespread, insect-pollinated outcrossers: H_e = 0.084–0.159). However, the values of H_e in the Nordic populations are lower than those reported for populations of *S. nutans* from Belgium (mean H_e = 0.343) and France (mean H_e = 0.275) (Van Rossum *et al*., 1997, 2003). The overall diversity in Nordic *S. nutans* (H_T = 0.250) is lower than that reported by Hamrick & Godt (1990) for comparable species (H_T = 0.310–0.347) and is also lower than that in Belgian and French *S. nutans* (H_T = 0.397–0.433). Not only are levels of diversity lower in Nordic than in French/Belgian *S. nutans*, but there is also a northward decline in genetic diversity (H_e), observed heterozygosity (H_o) and allelic richness (*A*) within Sweden and northern Finland. The peripheral, disjunct, northern populations are genetically depauperate compared with the southern populations.

Similar trends of decreasing genetic variability with latitude or in northern range-periphery populations have been reported from several European plant species (Comps *et al*., 1990; Michaud *et al*., 1995; Lammi *et al*., 1999). Such trends may reflect the results of selection along large-scale climatic gradients (e.g. Pamilo & Savolainen, 1999; Comps *et al*., 1990), of recent demographic fluctuations in peripheral populations (cf. Soulé, 1973; Lesica & Allendorf, 1995; Lönn & Prentice, 2002) or of stochastic processes during the process of postglacial range expansion from southern, refugial populations (e.g. Sage & Wolff, 1986; Hewitt, 1996).

A few studies have shown associations between patterns of variation in allozyme markers in plant species and large-scale environmental or climatic gradients,

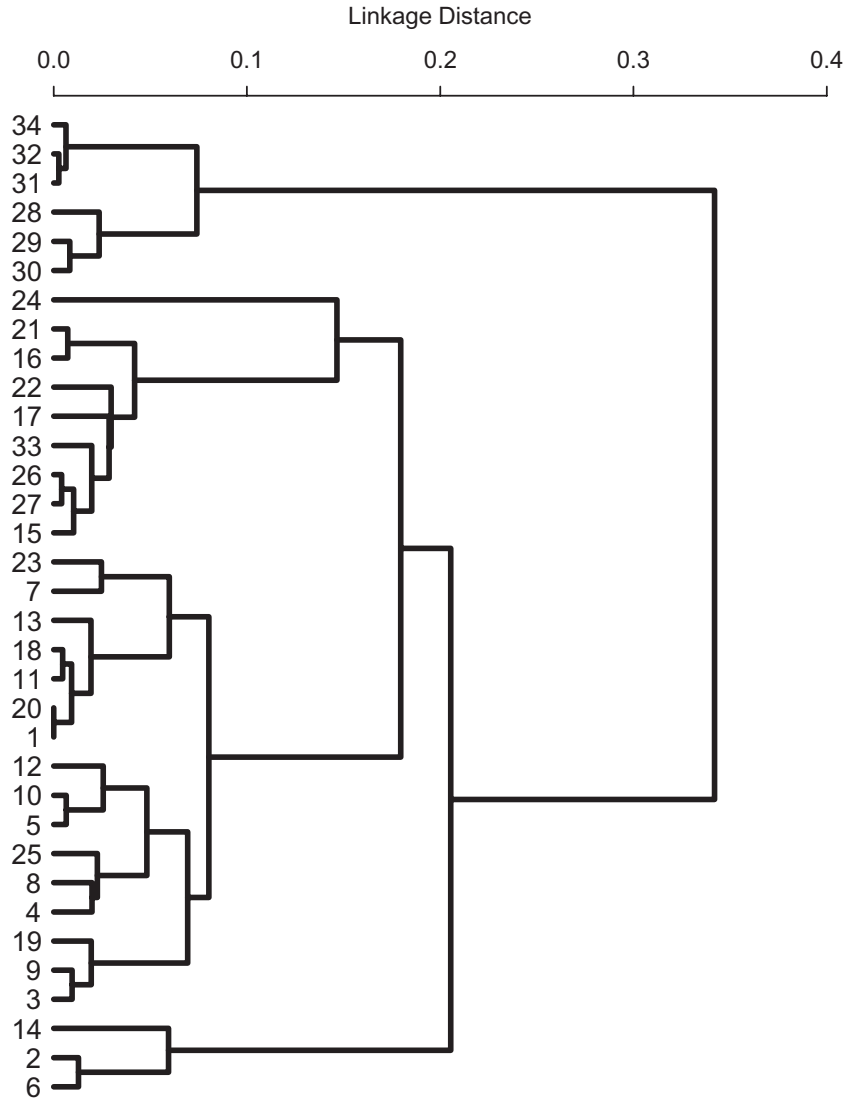

Figure 4. Ward's cluster analysis of 34 populations of *S. nutans* from Sweden and northern Finland based on Nei's genetic distances. Population codes are given in Table 1.

for example in *Bromus lanceolatus* (Ainouche, Misset & Huon, 1996), *Dactylis glomerata* (Roy & Lumaret, 1987) and *Fagus sylvatica* (Comps *et al*., 1990). Levels of genetic diversity have also been shown to be related to climatic gradients in *F. sylvatica* (Comps *et al*., 1990) and *Quercus ilex* (Michaud *et al*., 1995). Because there is evidence suggesting that the overall distribution of *S. nutans* may be limited by summer temperature (the species is absent from areas with a July mean of less than 15°C) (De Bilde, 1984) we cannot exclude the possibility that selection along large-scale climatic or environmental gradients may have made (at least a partial) contribution to the geographical structure of genetic variation within the species. *Silene nutans* also occurs in different habitats and vegetation types in the southern and northern parts of its distributional range in Sweden and northern Finland (see Table 1, F. Van Rossum & H.C. Prentice, unpubl. data).

Because latitude is not correlated with population size in Nordic *S. nutans* and because the peripheral, disjunct northern populations are not smaller than populations within the species' more continuous southern Swedish distribution, the lower variability in the northern populations is unlikely to reflect recent stochastic processes in small peripheral populations. Evidence of recent bottlenecks was only found in six of the Nordic populations. All six populations were from habitats with high levels of anthropogenic disturbance in southern Sweden. The sizes of these populations are

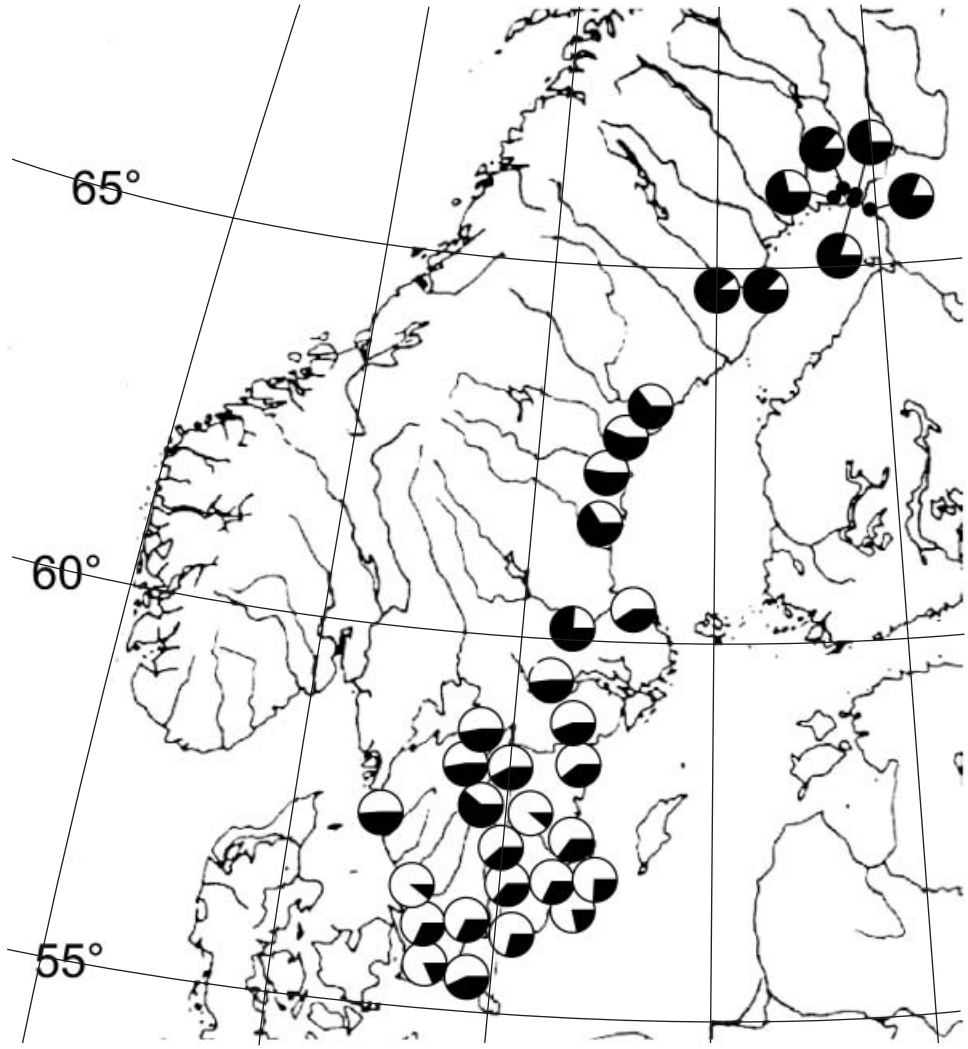

Figure 5. Map of the mean individual membership in cluster 1 (white) and cluster 2 (black) for each of the 34 sampled *S. nutans* populations. Results from a Bayesian model-based cluster analysis (with $K = 2$) in which individuals were assigned to two clusters.

likely to have been recently affected by human activities (such as road construction). In contrast, the lack of bottleneck signatures in the northern populations is consistent with the generally lower level of human activity in northern Fennoscandia, where seminatural grassland habitats are less fragmented (Rosén & Borgegård, 1999). The low levels of genetic variation in the northern Swedish and Finnish populations are more likely to reflect the results of ancient stochastic processes, such as founder events during postglacial range expansion (Comes & Kadereit, 1998; Hewitt, 1999), than to be a consequence of recent fluctuations in population size.

Significant departures from Hardy–Weinberg equilibrium and F_{IS} values that were generally higher than 0.250 were observed within most populations, indicating heterozygote deficiencies. F_{IS} increases with latitude, but is not related to population size in Nordic *S. nutans*. High F_{IS} values may result from selfing, inbreeding, restricted pollen and/or seed dispersal, clonality and/or the spatial effects of selection in different habitats (Linhart & Premoli, 1994; Berg & Hamrick, 1997; Tyler *et al*., 2002; Van Rossum *et al*., 2002). In contrast to the high values of F_{IS} in Nordic *S. nutans*, mean (over populations) F_{IS} values that are near to Hardy–Weinberg equilibrium have been reported for the silicicolous and calcicolous ecotypes (–0.009 and 0.023, respectively) of *S. nutans* in Belgium (Van Rossum *et al*., 1997). Whereas the Belgian populations show high outcrossing rates (E. Gratia, unpubl. data), it is possible that pollinator availability is limited by the relatively severe climatic conditions

in the northern Nordic region, and that poor pollinator-service results in increased levels of selfing and inbreeding (cf. Linhart & Premoli, 1994; Pamilo & Savolainen, 1999) in the Nordic populations.

However, strong maternal discrimination against selfing has been reported from both Belgian and Danish populations of *S. nutans* (De Bilde, 1984; Van Rossum *et al*., 1996; Hauser & Siegismund, 2000). *Silene nutans* lacks specialized mechanisms for seed dispersal (Hepper, 1956) and local seed dispersal in combination with poor pollen dispersal might lead to consanguineous matings and population substructuring. Although clonal propagation has been reported to increase with latitude and may be advantageous in areas with severe climatic conditions (Pamilo & Savolainen, 1999; Dorken & Eckert, 2001), vegetative spread is spatially restricted in *S. nutans* (Hepper, 1956; F. Van Rossum, pers. observ.). The sampling strategy in the present study was designed to avoid the sampling of close relatives or the repeated sampling of particular clones.

Selection in a mosaic of different habitats may lead to local differentiation within populations (e.g. Prentice *et al*., 1995). Although allozymes have been generally considered as neutral genetic markers (cf. Prentice *et al*., 2000), several studies of Nordic plant populations reveal fine scale, intrapopulation differentiation that is associated with habitat variation (e.g. Prentice *et al*., 1995; Lönn, Prentice & Bengtsson, 1996). It is possible that fine scale adaptive differentiation may be implicated in the larger heterozygote deficiencies observed in the northernmost populations of *S. nutans*. However, while significant associations were found between allozyme frequencies and edaphic factors in Belgian *S. nutans* (Van Rossum *et al*., 1999), the present study detected no association between overall geographical variation in Nordic *S. nutans* and substrate type (soil pH).

Genetic structure and history

Analysis of genetic structure reveals a geographical pattern of allozyme differentiation between the southern and the north-eastern Nordic populations, with populations from central Sweden characterized by allozyme frequencies that are intermediate to those in the northern and southern populations. This geographical pattern is consistent with a scenario of postglacial immigration into Sweden from both eastern and southern sources, with a zone of admixed populations in the centre of Sweden. Intraspecific contact zones in central Sweden, interpreted in terms of postglacial immigration from separate southern and northern/eastern origins, have been reported for several animal species (e.g. Jaarola & Tegelström, 1995; Pamilo & Savolainen, 1999) and for a few plant species (e.g. Malm & Prentice, 2002). However, while a number of recent studies using maternally inherited, haploid molecular markers reveal clear phylogeographical patterns in European plant species (e.g. Ferris *et al*., 1998; Palmé & Vendramin, 2002; Palmé *et al*., 2003), there are few reports of contact zones (such as that suggested by the present study) detected by (nuclear-encoded) allozyme markers (Malm & Prentice, 2002).

Despite the homogenizing effect of gene flow by pollen on nuclear allozyme markers, a large-scale geographical pattern with a clear S–NE differentiation of populations was detected in Nordic *S. nutans*. One direction of immigration may have been from Denmark into southern Sweden. These two regions were connected by a land-bridge until around 8200 BP (Björck, 1995). Immigrants following this route are likely to have a southern origin. The other major direction of colonization appears to have been via Finland, with immigrants that had a more easterly origin. The fact that some of the less common alleles (e.g. *Pgm2–2*) are only present in central Sweden suggests the possibility that the eastern immigrants might have entered Sweden from southern Finland, across the Baltic Sea via the Åland archipelago, rather than having migrated southwards from northern Finland to central Sweden. Gene dispersal via the Åland archipelago has also been suggested as an explanation for patterns of genetic variation in *Melica nutans* (Tyler *et al*., 2002) and *Silene dioica* (Malm & Prentice, 2002; unpublished results). At the present day, *Silene nutans* only occurs in the extreme south of Finland and in the coastal region of the northern Gulf of Bothnia, near to the border with Sweden (cf. Fig. 1). Despite the greater interpopulation distances and the high degree of population disjunction in the northern group of populations, cluster 2 shows a lower degree of differentiation than cluster 1 from the (hypothetical) ancestral gene pool in the STRUCTURE analysis (F_{ST} values related to divergence from a hypothetical ancestral population = 0.202 and 0.251, respectively), suggesting more recent immigration from the east than from the south.

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