Plant Syst. Evol. 242: 49–61 (2003)
DOI 10.1007/s00606-003-0049-3

Plant Systematics and Evolution
Printed in Austria

A comparative study of allozyme variation of peripheral and central populations of *Silene nutans* L. (Caryophyllaceae) from Western Europe: implications for conservation

F. Van Rossum[1,2], **X. Vekemans**[1,2], **E. Gratia**[1], and **P. Meerts**[1]

[1]Laboratoire de Génétique et Ecologie végétales, Université Libre de Bruxelles, Belgium
[2]Present address: Laboratoire de Génétique et Evolution des Populations Végétales, Université de Lille, Villeneuve d'Ascq, France

Received September 4, 2002; accepted June 11, 2003
Published online: November 6, 2003

Abstract. In Belgium, at the western border of its continental distribution range, the perennial herb *S. nutans* has evolved two parapatric ecotypes (calcicolous or silicicolous), which show contrasted allozyme, morphological and adaptive patterns, and isolating reproductive barriers. We examined allozyme variation in 21 populations close to Belgium (from France and Luxemburg) in relation to their peripheral or central geographical position, and investigated their genetic relationships with the Belgian ecotypes. Both peripheral and central populations showed high genetic variation. They were differentiated from each other, but not in relation to the substrate (calcareous or siliceous). The peripheral and central populations were related to the Belgian silicicolous and calcicolous ecotype, respectively. This suggests an ancient differentiation and different past histories for the Belgian ecotypes, and parapatric speciation processes. This study exemplifies the high evolutionary potential of populations at range peripheries and the need for considering them in conservation strategies.

Key words: Allozymes, Nottingham catchfly, parapatric speciation, phylogeography, population size, range periphery, *Silene nutans*.

Introduction

The preservation of the potential for evolutionary change and of evolutionary processes is an important goal of species conservation (e.g. Young et al. 1996, Crandall et al. 2000). Conservation strategies generally recommend to focus on the core populations and often neglect those at range periphery (Coates 2000, Channel and Lomolino 2000), because of small population sizes and sparse distribution, combined with low overall level of genetic variation resulting from lower levels of immigration and higher genetic drift effect (e.g. Mayr 1963, Soulé 1973, Hoffmann and Blows 1994).

However, peripheral populations can show complex patterns of genetic variation. Fragmented range limits can reflect species particular ecological requirements, and geographical periphery is often tied to ecologically marginal conditions (e.g. Lesica and Allendorf 1995). Presumably, the central populations occur in favourable, optimal environmental conditions, whereas at borders less suitable habitats are available, and populations are likely to experience more stressful conditions (e.g.

Hoffmann and Blows 1994, Holt and Keitt 2000). Directional selection pressures can therefore be expected in populations at boundaries, which can promote, in association with genetic drift due to isolation, local adaptation and genetic divergence (e.g. Soulé 1973, Hoffmann and Blows 1994). This may even lead to speciation events (e.g. Mayr 1963, Gavrilets et al. 2000). Due to their evolutionary potential and adaptation to stressful environmental conditions, populations at range limits may be important for long-term survival and evolution of species, especially in case of environmental changes, such as climate changes or lower habitat quality resulting from intensive agricultural land use and urbanisation. They may therefore have a high value for conservation (Millar and Libby 1991, Lesica and Allendorf 1995, Pamilo and Savolainen 1999).

Species history can also play an important role in their present-day geographical distribution and genetic structure (e.g. Hewitt 1996, Taberlet et al. 1998). Scattered populations may be remnants of a formerly continuous distribution range that was fragmented due to large-scale environmental changes or as a result of anthropogenic extinction forces (Lesica and Allendorf 1995, Channel and Lomolino 2000). The currently observed genetic patterns can be the result of past events, as processes of range-contraction/expansion and (re)colonisation (Hewitt 1996, Taberlet et al. 1998, Schiemann et al. 2000).

In this context, the study of genetic diversity and structure of populations from range peripheries can be quite valuable for understanding the processes that generate diversity and the possible evolution of declining species (Millar and Libby 1991, Lesica and Allendorf 1995). It can carry implications for conservation strategies as well for threatened as for widespread species, such as the delimitation of evolutionary units or the preservation of populations located in habitats, e.g. small landscape elements and ecological margins, that are often considered as of low conservation value (Millar and Libby 1991, Hogbin et al. 1998, Pamilo and Savolainen 1999, Jones et al. 2001).

Silene nutans L. (Caryophyllaceae) is a diploid ($2n = 22$), protandrous and predominantly outcrossed, long-lived perennial herb which occurs in xeric habitats, in particular on rock outcrops in dry, thermophilous grasslands, open forests and forest edges (Rameau et al. 1989, Van Rossum et al. 1999). It shows a wide continental distribution range, extending from north-western Europe to central Siberia and South-Caucasus (Hegi 1979). At its western border (Great Britain, NW France, Belgium and the Netherlands), *S. nutans* is rare, showing a patchy distribution with scattered, often small, populations (Debray 1965, Hepper 1956, Fitter 1978, Hendoux 2002). Previous studies on *S. nutans* populations from Belgium, where the ecological conditions can be considered as marginal (Van Rossum 2000), demonstrated a strong differentiation of populations into two parapatric ecotypes in relation to the substrate, calcareous or siliceous. These calcicolous and silicicolous ecotypes occur in different, contiguous but not overlapping ecological niches (Van Rossum et al. 1999), show contrasted morphological (De Bilde 1973, Van Rossum 2000) and adaptive traits (De Bilde 1977, 1978), and can be distinguished into two distinct gene pools based on allozyme variation. They maintained high levels of genetic variation despite small population sizes and ecologically marginal position (Van Rossum et al. 1997). The strong differentiation combined with pre- and post-zygotic isolating reproductive barriers (Van Rossum et al. 1996) suggests an ongoing speciation process. These results raised the question of a possibly different phylogeographic origin of these ecotypes and of the time of their differentiation, ancient or recent.

Hence it appeared relevant to investigate genetic variation and structure using allozymes in *S. nutans* populations from France and Luxemburg. Indeed, because of their geographical proximity to Belgium, these populations might have experienced similar past histories. Moreover, S. *nutans* shows there a wide ecological amplitude (Rameau et al. 1989), as in Belgium (Van Rossum et al.

1999, Van Rossum 2000), and a contrasted distribution range. In north-western France, populations are sparsely distributed and are geographically peripheral (Debray 1965, Fitter 1978, Hendoux 2002). Populations from Luxemburg and south-western to eastern France form the western limit of the continuous, more central, part of the range (Fitter 1978, Hegi 1979). In this paper, we addressed the following questions: (1) Do populations from France and Luxemburg show differences in genetic variation and structure in relation to their geographical position (peripheral or central), to substrate type, and to population size? (2) What are the relationships between these populations and the Belgian ecotypes, and can this study give some enlightening on the origin and time (ancient or recent) of differentiation of the Belgian populations? We compared therefore the present data with the patterns of genetic variation and structure observed in Belgium (Van Rossum et al. 1997) and discussed the implication of our results for the conservation of the diversity and the evolutionary potential of *S. nutans* in western Europe.

Materials and methods

Studied sites and sampling procedure. A total of 337 individual plants (rosette cuttings) were sampled from 21 populations and transplanted into a garden: 20 populations originating from five regions in France and one population from Luxemburg Duchy (Fig. 1). Due to its rarity (Debray 1965, Hendoux 2002), only five populations of *S. nutans* could be sampled in NW France (Seine-Maritime and Boulonnais). These populations (M to Q) are peripheral compared to populations from Poitou (R to U), Lorraine (I to L), Jura (B to H) and Luxemburg (A) which are already part of the central, continuous range of the species (Fitter 1978, C. Lahondère personal communication). Description of populations and sites, including population size, soil pH_{water} (1 : 1 de-ionised water mixture) of the superficial horizon (0–20 cm in depth), and substrate type, is given in Table 1. When it was not possible to collect soil samples (too skeletal soil), the presence of $CaCO_3$ in the bedrock was tested with HCl (in case of effervescence, pH > 7). Within each sampled site, plant material was collected from throughout the reachable area (some plants were out of reach because they grew on vertical rock slopes) occupied by *S. nutans*. To avoid the sampling of close relatives, the sampled individuals were separated from each other by at least 2 metres.

Enzyme electrophoresis. Rosette leaf material from only 300 of the 337 individuals could be screened for allozyme variation due to some mortality occurring in the garden. Extraction and electrophoretic procedures followed those described in Van Rossum et al. (1997). Seven putative loci encoded by five enzyme systems revealed a clear pattern for genetic analyses: alcohol dehydrogenase (*Adh*, E.C.1.1.1.1), glutamate-oxaloacetate transaminase (*Got-1* and *-2*, E.C.2.6.1.1), phosphoglucomutase (*Pgm*, E.C.5.4.2.2), leucine aminopeptidase (*Lap*, E.C.3.4.11.1), and esterase (*Est-1* and *-2*, E.C.3.1.1.-).

Data analysis. All analyses of genetic data were performed using GEN-SURVEY version 1.0 (Vekemans and 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 and Petit (1996) and calculated using FSTAT version 2.9.3.2. (Goudet 2001), the observed heterozygosity (H_o) and the expected heterozygosity (H_e), corrected for small sample size (Nei 1978). Deviation of H_o from Hardy-Weinberg expectations (H_e) was tested for each population by randomisation tests using FSTAT (Goudet 2001) and sequential Bonferroni-type corrections (Rice 1989).

For each locus and geographic group (central-peripheral), averages of A, H_o and H_e over all populations were computed. A test of comparison among groups of populations was performed using FSTAT (Goudet 2001) to test for lower allozyme variation in the peripheral populations than in the central populations. The significance of the tests was assessed using a randomised permutation scheme of the populations among the groups (1000 permutations). Spearman's rank correlation coefficients were calculated between population size and A, H_o and H_e using STATISTICA.

Population genetic structure was investigated using Nei's genetic diversity analysis on polymorphic loci using corrections from small sample size according to Nei and Chesser (1983). Averages of

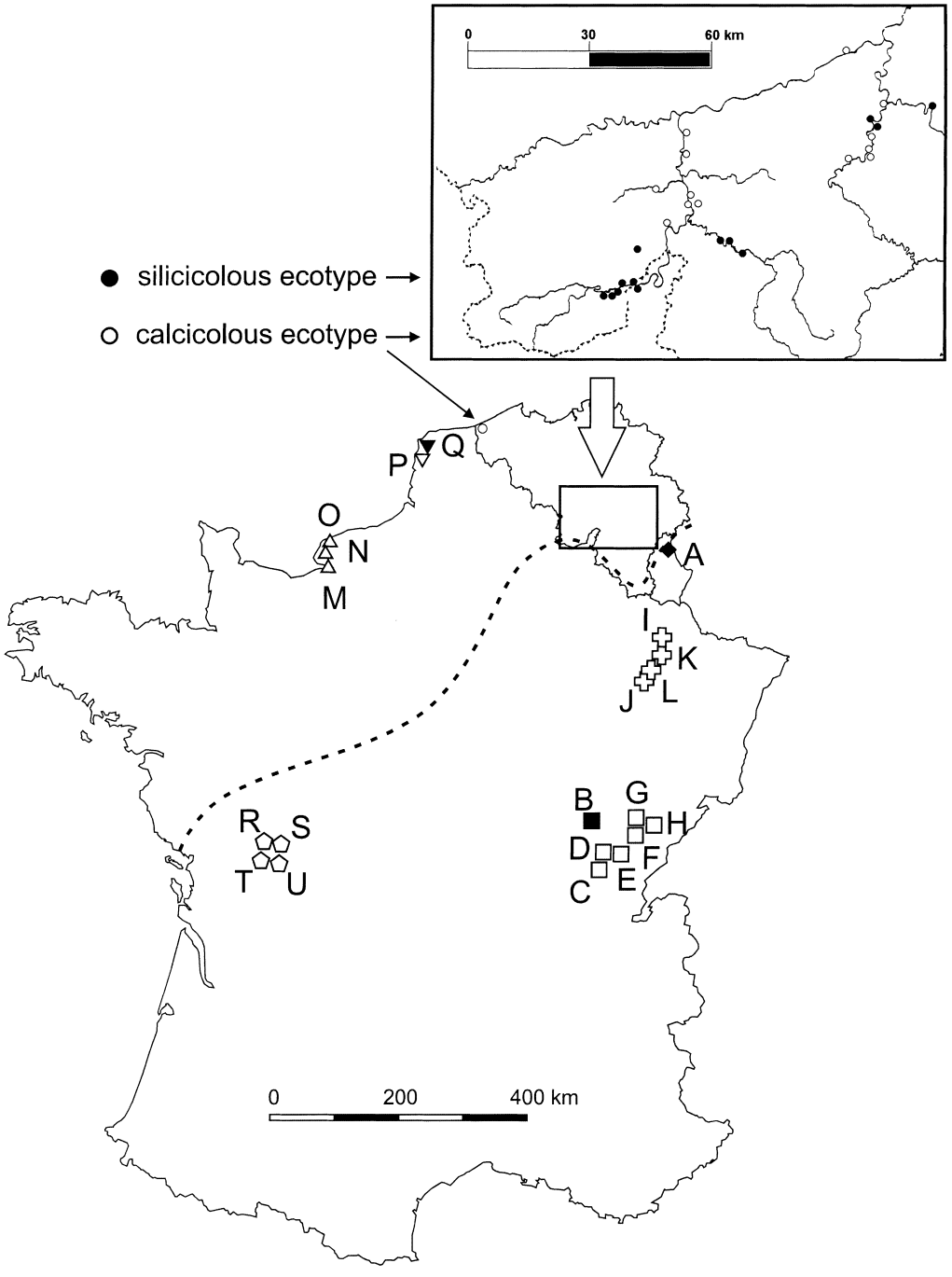

Fig. 1. Location of the studied populations of *Silene nutans* in France, Luxemburg and Belgium. ♦ Luxemburg Duchy; ✜, Lorraine; □, Jura; ▽, Boulonnais; △, Seine-Maritime; ⬠, Poitou; filled symbols, on siliceous soils; empty symbols, on calcareous soils. ○, Belgian calcicolous ecotype; ●, Belgian silicicolous ecotype. The dotted line shows the limit between the scattered (above) and the continuous (below) distribution in this part of the range (based on Fitter (1978), Hendoux (2002) and on data from the Société Botanique du Centre-Ouest for SW France)

Table 1. Site details and genetic variation for 21 populations of *Silene nutans* from France and Luxemburg, and for the Belgian calcicolous and silicicolous ecotypes (15 and 14 populations, respectively). N = total number of individuals, n = sample size, si = siliceous, ca = calcareous, A = mean allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity, SD = standard deviation

Region	Population		N	pH_{water}	Bedrock type	n	A	H_o	H_e
Central populations									
Luxemburg	Dirbach	A	50	5.7	si	14	1.56	0.170	0.275
Jura	Forêt de Serre	B	>100	5.0	si	26	1.95	0.365	0.453
	Poligny	C	15	7.9	ca	5	1.37	0.193	0.181
	Mesnayl	D	6	>7.0	ca	6	1.71	0.214	0.381
	Mesnay2	E	10	>7.0	ca	6	1.70	0.150	0.371
	Les Granges	F	>200	7.6	ca	26	1.67	0.257	0.332
	Pugey	G	6	>7.0	ca	6	1.50	0.381	0.286
	Bonneveaux	H	50	7.6	ca	15	1.60	0.145	0.285
Lorraine	Saulny	I	15	>7.0	ca	8	1.64	0.191	0.343
	Jaulny	J	100	7.4	ca	10	1.63	0.252	0.324
	Ars	K	20	>7.0	ca	14	1.86	0.337	0.413
	Waville	L	60	7.7	ca	21	1.75	0.279	0.365
Poitou	Saint Benoitl	R	>250	7.5	ca	25	1.78	0.358	0.397
	Saint Benoit2	S	80	7.3	ca	18	1.79	0.346	0.391
	Ligugél	T	80	7.4	ca	15	1.80	0.320	0.386
	Ligugé2	U	30	7.7	ca	15	1.74	0.391	0.377
Peripheral populations									
Seine-Maritime	Saint Vigor	M	200	7.1	ca	14	1.87	0.402	0.421
	Saint Jouin	N	43	7.6	ca	16	1.66	0.241	0.332
	Bruneval	O	54	7.4	ca	10	1.78	0.270	0.391
Boulonnais	Ambleteuse1	P	10	7.6	ca	8	1.26	0.196	0.146
	Ambleteuse2	Q	>3000	6.9	si	25	1.52	0.320	0.287
	Mean (France)					300	1.67	0.275	0.340
	SD						0.17	0.083	0.076
Belgian populations									
	Calcicolous group		7.2–8.0		ca		1.71	0.343	0.364
	SD						0.08	0.061	0.036
	Silicicolous group		3.8–7.2		si		1.70	0.342	0.354
	SD						0.16	0.061	0.071

Nei's statistics for all populations, and for the central and peripheral populations separately, and their standard errors over loci were calculated together with 95% confidence intervals obtained through bootstrapping over loci. Furthermore, hierarchical F-statistics were computed with the software ARLEQUIN (Schneider et al. 2000) for partitioning genetic differentiation into differentiation among populations within each group and among groups (peripheral versus central). The proportion of genetic variance was determined for the following components: among populations in the overall sample (F_{ST}); among populations within regions (F_{SR}) and among regions (F_{RT}). The F-statistics are related by the equation $(1-F_{ST}) = (1-F_{SR})(1-F_{RT})$ (Weir 1990). The significance of each variance component was tested with permutation tests (Excoffier et al. 1992).

In order to test for isolation by distance, $F_{ST}/(1-F_{ST})$ ratios were computed for each pair of populations and regressed on the logarithm of the geographic distance using SPAGeDI version 1.0 (Hardy and Vekemans 2002). The significance of the regression was tested by performing a 1000 permutations test.

Comparison with the Belgian populations. Twenty-nine Belgian populations (15 calcicolous and 14 silicicolous, 540 individuals) were added to the analyses for comparison with the present data (Fig. 1). For population genetic measures and site details, see Van Rossum et al. (1997, 1999) and Van Rossum (2000). Tests of comparison among groups of populations as described above were used to test for differences in A, H_o and H_e between two groups of populations (peripheral, central, Belgian calcicolous, and Belgian silicicolous populations). Averaged Nei's genetic diversity measures were calculated for the Belgian ecotypes taking all the 7 loci into account (including the monomorphic loci) for allowing comparison with the present data.

Nei's genetic distances, corrected for small sample size (Nei 1978) were computed for each pair of French, Luxemburg and Belgian populations. Cluster analysis using Ward's method was performed on Nei's distance data using STATISTICA. To test for genetic differentiation between the main clusters in the dendrogram, and between peripheral and central populations and the Belgian ecotypes, the statistical significance of the difference between the average Nei's distances within against average Nei's distances between groups of populations was investigated using a numerical resampling method as described in Vekemans and Lefèbvre (1997).

Results

Loci and alleles scored. All the seven loci surveyed showed variation in at least one population from France and Luxemburg. Thirty alleles were detected: *Adh* (2 alleles), *Got-1* (5 alleles), *Got-2* (3 alleles), *Pgm* (4 alleles), *Lap* (5 alleles), *Est-1* (5 alleles) and *Est-2* (6 alleles). Compared to the Belgian populations, five new alleles were found in the present data: *Got-1.5*, only detected in Lorraine (J, L) at low frequency (0.083 and 0.095, respectively); *Got-2.2*, detected in Poitou (R, S, T) with frequencies ranging from 0.026 to 0.115; *Got-2.3*, found in Luxemburg (A, frequency: 0.036) and Seine-Maritime (M, frequency: 0.077); *Pgm-4*, present in Lorraine (J, K, L), Seine-Maritime (M) and Poitou (R, S), with frequencies from 0.024 to 0.107; *Est-1.5*, only detected in Jura (D, frequency: 0.250). No allele was observed in the Belgian ecotypes only.

Within-population genetic variation. Mean allelic richness (A) ranged from 1.26 to 1.95 (Table 1). The observed and expected heterozygosities (H_o and H_e) ranged from 0.145 to 0.402, and from 0.146 to 0.453, respectively. Six out of 21 populations (A, B, F, H, K and N) showed a significant deviation of genotype frequencies from Hardy-Weinberg expectation with lower heterozygosity than expected ($P < 0.05$ after sequential Bonferroni corrections). No significant difference ($P > 0.05$) was found between the central and peripheral populations for the averages of A (1.69 and 1.62, respectively), H_o (0.295 and 0.296, respectively) and H_e (0.347 and 0.315, respectively). None of the paired comparisons between groups of populations from France and Belgium were significantly different ($P > 0.05$). No significant correlation was found between population size and A ($r_s = 0.389$, $P = 0.081$), H_o ($r_s = 0.407$, $P = 0.067$), and H_e ($r_s = 0.337$, $P = 0.135$).

Population genetic structure. The total genetic diversity (H_T) for all populations from France and Luxemburg was 0.433, and 0.401 for both peripheral and central populations (Table 2). The mean genetic diversity within populations (H_S) was 0.317 and 0.356 for the peripheral and central populations, respectively. The two groups showed overlapping 95 per cent confidence intervals for H_T and H_S. The values of H_T and H_S were similar to those of the Belgian calcicolous and silicicolous ecotypes (Table 2). The genetic differentiation among populations (G_{ST}) was 0.215 for all populations from France and Luxemburg. Mean G_{ST}-values tended to be higher among the peripheral populations (0.183) than among the central populations (0.079), although with overlapping 95 per cent confidence intervals.

Table 2. Averaged measures of genetic diversity at 7 loci for 21 populations of *S. nutans* from France and Luxembourg (for all populations, and for peripheral and central populations separately), and for the calcicolous (15 populations) and silicicolous (14 populations) ecotypes from Belgium. H_T = total gene diversity, H_S = mean gene diversity within populations, G_{ST} = proportion of interpopulation gene differentiation, SE = standard error, CI = confidence intervals

	H_T	H_S	G_{ST}
France + Lux.	0.433	0.346	0.215
SE	0.037	0.032	0.027
95% CI	0.248–0.589	0.185–0.486	0.100–0.365
Peripheral	0.401	0.317	0.183
95% CI	0.275–0.489	0.202–0.418	0.050–0.322
Central	0.401	0.356	0.079
95% CI	0.188–0.600	0.166–0.529	0.030–0.132
Belgian ecotypes			
Calcicolous	0.397	0.350	0.118
Silicicolous	0.424	0.370	0.127

Mean G_{ST}-values among the Belgian calcicolous and silicicolous ecotypes were found to be intermediate (0.118 and 0.127, respectively). The hierarchical analysis of molecular variance indicated that the differentiation between peripheral and central groups (F_{RT}) was 0.128, and 0.164 between populations within regions (F_{SR}), with an overall $F_{ST} = 0.271$. The F_{RT}, F_{SR} and F_{ST} values were significantly different from zero ($P < 0.001$) as tested by permutation tests.

The dendrogram based on Nei's genetic distances revealed that populations clustered in two main, significantly differentiated, branches (average Nei's genetic distance between clusters = 0.223, $P < 0.001$). Populations from France and Luxemburg clustered according to their geographical position, peripheral or central (Fig. 2). They also tended to be grouped in "subregions" for Jura and Poitou. The central populations (Luxemburg, Jura, Lorraine, Poitou) clustered with the Belgian calcicolous populations, and the peripheral populations (Seine-Maritime, Boulonnais) with the populations of the Belgian silicicolous ecotype. Only population I (Lorraine) and one Belgian calcicolous population (Comblain-au-Pont) did not cluster with populations of the same origin (Fig. 2). Average Nei's genetic distance between groups was significantly higher ($P < 0.05$) than the average pairwise distance within groups for all groups (Table 3), except between the peripheral and the Belgian silicicolous populations ($P > 0.05$). Average distance values between groups were the highest between the peripheral populations, and the central and the Belgian calcicolous populations (0.277 and 0.270, $P < 0.001$ and 0.01, respectively). Average genetic distance between the Belgian calcicolous ecotype and the central populations was low (0.096), but significantly higher than the average values within groups ($P < 0.05$). A pattern of isolation by distance was found for populations from France and Luxemburg, as $F_{ST}/(1 - F_{ST})$ and geographic distances matrices were positively correlated ($r = 0.267$, $P < 0.001$). The observed differentiation between populations from France and Luxemburg was not related to the substrate (calcareous or siliceous) as found for the Belgian populations (Fig. 2).

Discussion

Low levels of genetic diversity can be expected in populations at range limits as a result of low levels of immigration and genetic drift (e.g. Soulé 1973, Hoffmann and Blows 1994), as reported for *Lychnis viscaria* (Lammi et al. 1999). Our results do not support

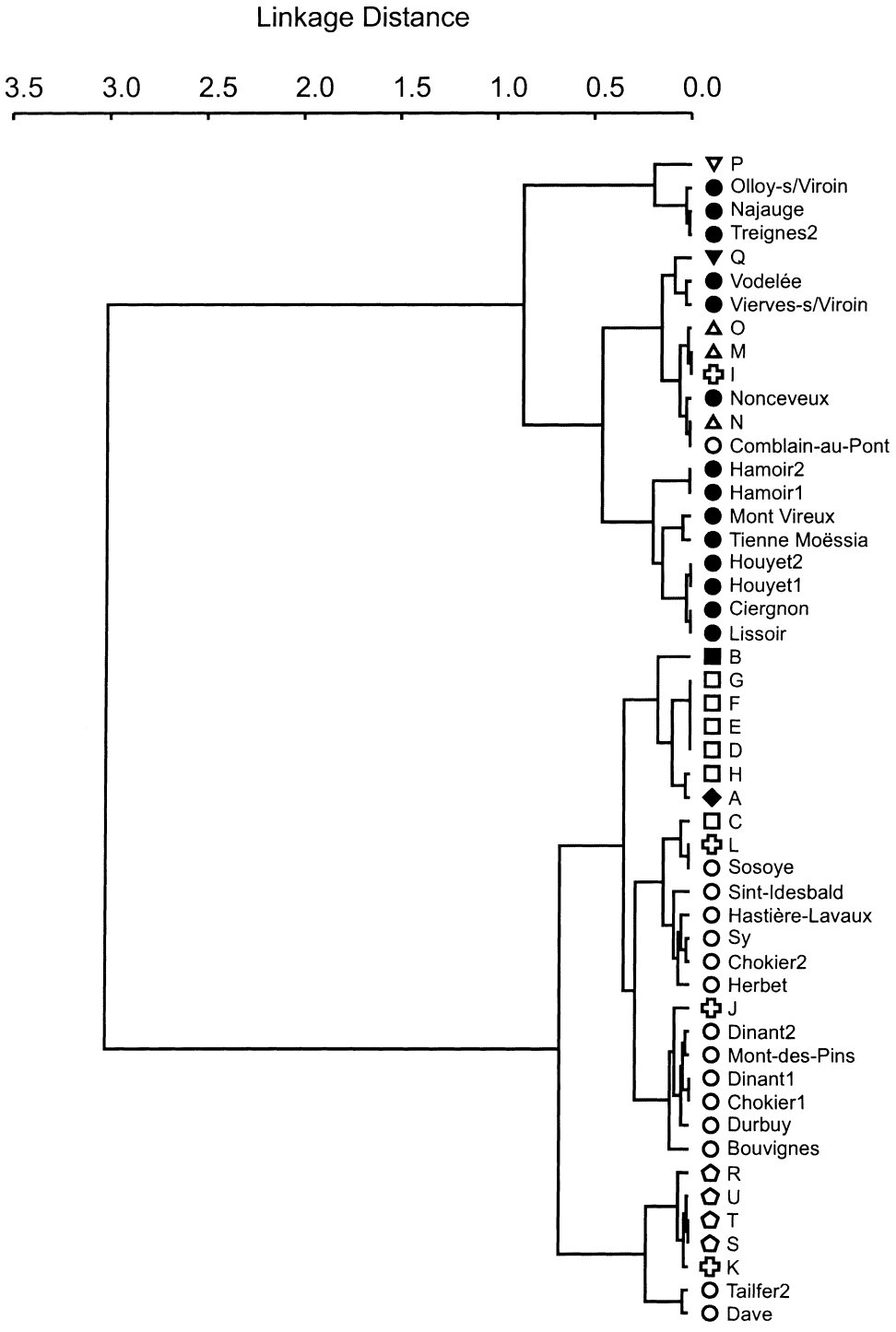

Fig. 2. Cluster analysis of 50 populations of *Silene nutans* from France, Luxemburg and Belgium based on Nei's genetic distances (Nei 1978). ♦, Luxemburg Duchy; ✜, Lorraine; □, Jura; ▽, Boulonnais; △, Seine-Maritime; ⬠, Poitou (filled symbols, on siliceous soils; empty symbols, on calcareous soils). ○, Belgian calcicolous ecotype; ●, Belgian silicicolous ecotype

Table 3. Average Nei's genetic distances among pairs of populations within groups (in brackets) and belonging to different groups of *S. nutans* populations. Between-group differentiation significantly higher than within-group differentiation: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, ns = not significant

	Peripheral	Central	Calcicolous	Silicicolous
Peripheral	(0.166)			
Central	0.277***	(0.092)		
Calcicolous	0.270**	0.096*	(0.077)	
Silicicolous	0.151 ns	0.212***	0.214***	(0.092)

this hypothesis, since the peripheral populations from NW France exhibit high levels of genetic variation. These are similar to the values found for the central populations, and for the Belgian calcicolous and silicicolous ecotypes. The allozyme variation found in France and Luxemburg covers the range of variation in Belgium, since no private allele was found for the Belgian populations.

The maintenance of high levels of genetic variation in populations of *S. nutans* despite the relatively small population sizes (mostly less than 100 individuals) over the whole studied range, including Belgium (Van Rossum et al. 1997), may be attributed to its particular life-history traits: outcrossing breeding system and pollination by moths combined with high lifetime fecundities because of its long-lived perennial trait. Furthermore, it occurs in a wide range of habitats, from coastal cliffs and sand-dunes to dry grasslands and open forests, along a wide pH gradient (Rameau et al. 1989, Van Rossum et al. 1999, Van Rossum 2000). High levels of genetic diversity in natural populations can be associated with a wide ecological amplitude and niche variation (Babbel and Selander 1974, Prentice et al. 1995). Genetic variation and structure can also reflect past events (Taberlet et al. 1998, Pamilo and Savolainen 1999, Schiemann et al. 2000). For instance, higher levels of genetic variation have been reported in populations located in or near Pleistocene glacial refugia than in populations from previously glaciated regions for many species (Hewitt 1996, Wallace and Case 2000).

Populations from Belgium have evolved two parapatric ecotypes, differentiated into two gene pools with evidence of correlated differences in allozyme and ecological traits (Van Rossum et al. 1999) and of adaptive traits, e.g. aluminium tolerance only for the silicicolous ecotype and a higher competitive ability for the calcicolous ecotype (De Bilde 1977, 1978; De Bilde and Lefèbvre 1990). By contrast, in France and Luxemburg, genetic differentiation was found among central and peripheral populations, but was not related to the substrate type (calcareous or siliceous) as found in Belgium. Unfortunately, only a few populations could be found on siliceous soils because the main bedrock types in this part of the distribution range are limestone and chalk, which contrasts with the high geological diversity on a small scale in Belgium (Lombard 1957). Regional differences were also found, suggesting limited gene flow between the groups.

NW France, which corresponds to the range periphery, shows different climatic conditions compared to the continuous distribution range of *S. nutans* in France: it has a temperate, oceanic climate, which is less favourable for this continental species; the regions in eastern France are associated with a semi-continental climate and in SW France (Poitou) with a warmer climate (Visset et al. 1996). The particular ecological requirements of the species (dry and warm habitats) and ecologically marginal conditions could explain its scattered distribution at the western border, and these differences might have promoted genetic divergence.

The slightly lower differentiation found among central populations compared to

among peripheral ones can be the result of the more continuous distribution of the populations in this part of the range, favouring gene flow, whereas peripheral populations show a more scattered distribution (Fitter 1978, Hendoux 2002). Seeds of *S. nutans* are mainly dispersed over short distances by a simple censer mechanism: seeds are merely shaken out of the capsules by the wind (Hepper 1956). However, gene flow through long distance seed-dispersal might have been promoted by transportation in wild mammals and sheep fur, especially during traditional transhumant shepherding that was formerly widespread on dry calcareous grasslands in France and southern Belgium (Willems 1990). Long-distance seed-dispersal by sheep was shown for several species, e.g. *Silene vulgaris* (Fisher et al. 1996), a species closely related to *S. nutans* which shows a similar dispersal mechanism. The abandonment of these practices is recent (during the 20th century) (Willems 1990, Fisher et al. 1996) and therefore the number of successive generations might have been too low for genetic drift and differentiation to occur among central populations.

Phylogeographic comparisons between peripheral and central populations from France and Luxemburg and the Belgian ecotypes indicate that the silicicolous ecotype is closely related to the peripheral group located in NW France (Seine-Maritime, Boulonnais). The calcicolous ecotype is closer, yet significantly differentiated, to the central populations (Table 3). The level of genetic differentiation between central and peripheral populations (10%) is similar to that found between the Belgian ecotypes (8.4%) (Van Rossum et al. 1997). Moreover, morphological studies (including amongst others leaf shape and fruit and seed size) of a larger sampling of populations from France and Europe (De Bilde et al. 1977, Van Rossum and Lefèbvre, unpublished results) indicated the existence of four different morphological types: a western morphotype with large capsules, comprising populations from W France and SE England (= var. *salmoniana* in Hepper (1951) and in Debray (1965)) and the Belgian silicicolous ecotype; a second one consisting of the Belgian calcicolous ecotype, showing small capsules; a continental morphotype showing intermediate-sized capsules (to which can be ascribed the populations from eastern France and Luxemburg); and a Nordic morphotype. Although investigating populations from more geographic origins (e.g. The Netherlands, Germany, Great Britain), including populations from putative glacial refugial areas, would be necessary to resolve the issue, we suggest that the two Belgian ecotypes and the geographical groups from France and Luxemburg might have some common evolutionary histories, and that the differentiation between the two ecotypes is ancient. This was also hypothesised by the lack of contrasted patterns in root organic acids exudation and seed phosphorus content observed between the two Belgian ecotypes, which are usually found between calcifugous and calcicolous species (Bruun et al. 2001). We may hypothesise that the genetically differentiated patterns of *S. nutans* in western Europe might be the result of (re)colonisation of the area from two different, geographically isolated, origins where populations have genetically diverged from each other. Our results, together with morphological data (Van Rossum, unpublished results), suggest the hypothesis of a western and an eastern origin. Moreover, it seems that the two different groups of populations made contact in Belgium. They might have entered into competition, and as a result of different initial traits, have developed ecological specialisation in relation to edaphic properties (De Bilde 1977, 1978), even within ecotype (Bruun et al. 2001). This may have been promoted by the very particular geological context in southern Belgium: a narrow mosaic of siliceous and calcareous bedrocks from different stratigraphic stages (Lombard 1957), providing suitable – but heterogeneous – ecological conditions for the species (Van Rossum 2000). Strong reproductive barriers were found at the prezygotic (pollen-stigma incompatibility) and postzygotic (F1 hybrids

sterility) stages (Van Rossum et al. 1996). These could prevent recombination of co-adapted traits, resulting in two Belgian parapatric ecotypes that may be considered as two incipient biological species, but as subspecies if we consider the rest of the distribution, since reproductive barriers between the Belgian ecotypes on the one hand and populations from other parts of Europe on the other hand are weaker than between the two ecotypes themselves (Van Rossum et al. 1996). These ecotypes exemplify adaptive radiation and parapatric speciation processes, as a consequence of migration history and particular ecological conditions (Gavrilets et al. 2000).

This study also carries implications for the conservation of *S. nutans* in western Europe. At this border of its distribution range, our results indicate that peripheral populations of *S. nutans* have a high conservation value as they retain a high evolutionary potential and show particular evolutionary processes, such as incipient speciation combined with local adaptation. The preservation of the evolutionary potential and evolutionary processes in this taxon requires therefore the maintenance of populations from throughout its distribution range, including the peripheral populations. The particular patterns observed in Belgium should hold a special attention and be considered as separate conservation units.

In conclusion, this study, as previously reported for boreal species (Pamilo and Savolainen 1999), and for the widespread *Lloydia serotina* (Jones et al. 2001), exemplifies the need for considering differentiation processes that can occur at borders of distribution range and for evaluating the conservation value of peripheral populations, in order to preserve species evolutionary potential. We have to insure that rare, but also widespread species can face the environmental changes that could lead to less suitable habitat conditions. The protection of populations at boundaries or occurring in ecologically, more stressful habitats conditions, must be considered in the conservation strategies.

We would like to thank C. Lahondère (Société Botanique du Centre-Ouest) for providing data on the distribution of *S. nutans* in Poitou-Charente (SW France), H. C. Prentice for constructive discussions and T. Hauser and anonymous referees for comments on the manuscript. This study was financially supported by the Fonds de la Recherche Fondamentale Collective (F.R.F.C.). F.V.R. was a research fellow of the Belgian National Fund for Scientific Research (F.N.R.S.).

References

Babbel G. R., Selander R. K. (1974) Genetic variability in edaphically restricted and widespread plant species. Evolution 28: 619–630.

Bruun H. H., Van Rossum F., Ström L. (2001) Exudation of low molecular weight organic acids by germinating seeds of two edaphic ecotypes of *Silene nutans*. Acta Oecol. 22: 285–291.

Channell R., Lomolino M. V. (2000) Dynamic biogeography and conservation of endangered species. Nature 403: 84–86.

Coates D. J. (2000) Defining conservation units in a rich and fragmented flora: implications for the management of genetic resources and evolutionary processes in south-west Australian plants. Austral. J. Bot. 48: 329–339.

Crandrall K. A., Bininda-Emonds O. R. P., Mace G. M., Wayne R. K. (2000) Considering evolutionary processes in conservation biology. Trends Ecol. Evol. 15: 290–295.

De Bilde J. (1973) Etude génécologique du *Silene nutans* L. en Belgique: populations du *Silene nutans* L. sur substrats siliceux et calcaires. Rev. Gén. Bot. 80: 161–176.

De Bilde J. (1977) Effet de l'aluminium et du calcium sur l'activité phosphatasique acide des racines d'écotypes calcicole et silicicole de *Silene nutans* L. (Caryophyllaceae). Bull. Soc. Roy. Bot. Belg. 110: 151–160.

De Bilde J. (1978) Nutrient adaptation in native and experimental calcicolous and silicicolous populations of *Silene nutans*. Oikos 31: 383–391.

De Bilde J., Gorenflot R., Briane J.-P. (1977) Traitement numérique de populations du *Silene nutans* L. d'Europe nord-occidentale et centrale. Rev. Gén. Bot. 84: 341–354.

De Bilde J., Lefèbvre C. (1990) The strategy of *Silene nutans* on calcareous and siliceous soils. Acta Oecol. 11: 399–408.

Debray M. (1965) Sur une variété franco-britannique de *Silene nutans* L. Rev. Soc. Savantes Haute Normandie–Sciences- 39: 21–24.

El Mousadik A., Petit R. J. (1996) High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. Theor. Appl. Genet. 92: 832–839.

Excoffier L., Smouse P., Quattro J. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Fisher S. F., Poschlod P., Beinlich B. (1996) Experimental studies on the dispersal of plants and animals on sheep in calcareous grasslands. J. Appl. Ecol. 33: 1206–1222.

Fitter A. (1978) An atlas of the wild flowers of Britain and Northern Europe. Collins Sons and Co, Glasgow.

Gavrilets S., Li H., Vose M. D. (2000) Patterns of parapatric speciation. Evolution 54: 1126–1134.

Goudet J. (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Free program distributed by the authors at http://www.unil.ch/izea/softwares/fstat.html.

Hardy O. J., Vekemans X. (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2: 618–620.

Hegi G. (1979) Illustrierte Flora von Mittel-Europa, Band III, Teil 2. Parey, Berlin.

Hendoux F. (2002) Liste rouge synoptique de la flore vasculaire du Nord/Pas-de-Calais. Centre Régional de Phytosociologie/Conservatoire Botanique National de Bailleul, Bailleul.

Hepper F. N. (1951) The variations of *Silene nutans* L. in Great Britain. Watsonia 2: 80–90.

Hepper F. N. (1956) *Silene nutans* L. Biological Flora of the British Isles. J. Ecol. 44: 693–700.

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

Hoffmann A. A., Blows M. W. (1994) Species borders: ecological and evolutionary perspectives. Trends Ecol. Evol. 9: 223–227.

Hogbin P. M., Ayre D. J., Whelan R. J. (1998) Genetic variation and reproductive success of road verge populations of the rare shrub *Grevillea barklyana* (Proteaceae). Heredity 80: 180–186.

Holt D. H., Keitt T. H. (2000) Alternative causes for range limits: a metapopulation perspective. Ecol. Lett. 3: 41–47.

Jones B., Gliddon C., Good J. E. G. (2001) The conservation of variation in geographically peripheral populations: *Lloydia serotina* (Liliaceae) in Britain. Biol. Conserv. 101: 147–156.

Lammi A., Siikamäki P., Mustajärvi K. (1999) Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. Conserv. Biol. 13: 1069–1078.

Lesica P., Allendorf F. W. (1995) When are peripheral populations valuable for conservation? Conserv. Biol. 9: 753–760.

Lombard A. (1957) Géologie de la Belgique. Les Naturalistes Belges, Bruxelles.

Mayr E. (1963) Animal species and evolution. Harvard University Press, Cambridge.

Millar C. I., Libby W. J. (1991) Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. In: Falk D. A., Holsinger K. E. (eds.) Genetics and conservation of rare plants. Oxford University Press, New York, pp. 149–170.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

Nei M., Chesser R. K. (1983) Estimation of fixation indices and diversities. Ann. Hum. Genet. 47: 253–259.

Pamilo P., Savolainen O. (1999) Postglacial colonization, drift, local selection and conservation of populations: a northern perspective. Hereditas 130: 229–238.

Prentice H. C., Lönn M., Lefkovitch L. P., Runyeon H. (1995) Associations between allele frequencies in *Festuca ovina* and habitat variation in the alvar grasslands on the Baltic island of Öland. J. Ecol. 83: 391–402.

Rameau J. C., Mansion D., Dumé G., Timbal J., Lecointe A., Dupont P., Keller R. (1989) Flore forestière française. 1. Plaines et collines. Institut pour le développement forestier, Dijon-Quetigny.

Rice W. R. (1989) Analysing tables of statistical tests. Evolution 43: 223–225.

Schiemann K., Tyler T., Widén B. (2000) Allozyme diversity in relation to geographic distribution and population size in *Lathyrus vernus* (L.) Bernh. (Fabaceae). Plant Syst. Evol. 225: 119–132.

Schneider S., Roessli D., Excoffier L. (2000) Arlequin version 2000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Soulé M. (1973) The epistasis cycle: a theory of marginal populations. Annu. Rev. Ecol. Syst. 4: 165–187.

Taberlet P., Fugamalli L., Wust-Saucy A.-G., Cosson J.-F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. Molec. Ecol. 7: 453–464.

Van Rossum F. (2000) Amplitude synécologique de *Silene nutans* L. (Caryophyllaceae) en Belgique. Dumortiera 75: 11–24.

Van Rossum F., De Bilde J., Lefèbvre C. (1996) Barriers to hybridization in calcicolous and silicicolous populations of *Silene nutans* from Belgium. Belg. J. Bot. 129: 13–18.

Van Rossum F., Vekemans X., Meerts P., Gratia E., Lefèbvre C. (1997) Allozyme variation in relation to ecotypic differentiation and population size in marginal populations of *Silene nutans*. Heredity 78: 552–560.

Van Rossum F., Meerts P., Gratia E., Tanghe M. (1999) Ecological amplitude in relation to allozyme variation in *Silene nutans* at the western margin of its distribution. J. Veg. Sci. 10: 253–260.

Vekemans X., Lefèbvre C. (1997) On the evolution of heavy-metal tolerant populations in *Armeria maritima*: evidence for allozyme variation and reproductive barriers. J. Evol. Biol. 10: 175–191.

Visset L., Aubert S., Belet J. M., David F., Fontugne M., Galop D., Jalut G., Janssen C. R., Voeltzel D., Huault M. F. (1996) 17. France. In: Berglund B. E., Birks H. J. B., Ralska-Jasiewiczowa M., Wright H. E. (eds.) Paleological events during the last 15000 years, Regional syntheses of paleological studies of lakes and mires in Europe. Wiley and Sons, Chichester, pp. 575–645.

Wallace L. E., Case M. E. (2000) Contrasting allozyme diversity between northern and southern populations of *Cypripedium parviflorum* (Orchidaceae): implications for Pleistocene refugia and taxonomic boundaries. Syst. Bot. 25: 281–296.

Weir B. S. (1990) Genetic data analysis. Sinauer, Sunderland, Massachussets.

Willems J. H. (1990) Calcareous grasslands in continental Europe. In: Hillier S. H., Walton D. W. H., Wells D. A. (eds.) Calcareous grasslands: ecology and management. Bluntisham Book, Bluntisham, pp. 3–10.

Young A., Boyle T., Brown T. (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. 11: 413–418.

Addresses of the authors: Fabienne Van Rossum* (e-mail: fvrossum@vub.ac.be), Xavier Vekemans*, Laboratoire de Génétique et Ecologie végétales, Université Libre de Bruxelles, chée de Wavre 1850, B-l160 Brussels, Belgium. **Present address:* Laboratoire de Génétique et Evolution des Populations Végétales, UMR CNRS 8016, Université des Sciences et Technologies de Lille, Bâtiment SN2, F-59655 Villeneuve d'Ascq Cedex, France.