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THE APPLICATION OF SPATIAL AUTOCORRELATION METHODS TO THE STUDY OF *CALLUNA VULGARIS* POPULATION GENETICS

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ABSTRACT. — Spatial structure of *Calluna vulgaris* populations was investigated at various scales using autocorrelation statistical techniques. Intrapopulation structure has been studied by recording the genotypes at four polymorphic loci of 41 individual plants of *Calluna vulgaris* within a grid of 16 × 60 m, from the site of Sacrawé (High Ardenne, Belgium). The spatial correlograms gave no significant results of autocorrelation of genotypes within the grid. It is therefore concluded that at this scale there is panmictic mixing of genotypes. A short review of other similar studies show that patterns vary greatly between species. Interpopulation analyses were carried out by recording the allele frequencies of 18 populations from North Spain to Belgium in relation to their geographic position. This revealed different patterns for the different alleles studied including clines of frequencies, autocorrelation at regional scale or no detectable trends. It is concluded that autocorrelation usefully supplement other statistical methods, such a Wright's F_{ST}, by giving information on the scales and processes involved in intra- and inter-population spatial structuring.

KEY WORDS. — *Calluna vulgaris*, autocorrelation, allele frequency, spatial population structure, Moran's *I*.

INTRODUCTION

The distribution of genetic variation within and among plant populations has been widely documented by means of allozymes markers in the last 20 years (e.g. HAMRICK & GODT 1989). Most of the studies were concerned with widespread geographical zones and lead to the conclusion that most of the variation lies within rather than among populations, a feature particularly marked for perennial outcrossed plants. Genotypic structuring within populations is also likely to develop either as the result of limited dispersal of seed and pollen (LEVIN & KERSTER 1974), selection in a patchy environment (BRADSHAW 1984, EPPERSON 1990) or spread of vegetative clones (HANDEL 1983). The prevailing view of plant populations is that they are genetically heterogeneous in space (e.g. LEVIN 1981).

Inference on the genetic structure of populations may be done using different methods. Wright's F statistics are widely used to analyse both intra (F_{IS}) and inter (F_{ST}) population structure. These statistics are the results of comparisons with ideal panmictic populations, and thus tell if the observed population fits the panmictic

model ; but they give little information as to which factors may influence the observed genetic structure. Autocorrelation methods, first devised mainly by geographers, may be helpful in the study of spatial population structure. Autocorrelation methods allow to test whether the measurement of an object at a given location is independent from the measurements of other objects situated at given distances. The most widely used autocorrelation statistics is Moran's *I*, which computes the correlation of measurements according to the distance between them. The first biological applications of autocorrelation methods were put forward by SOKAL & ODEN (1978a), with an example of analysis of allozyme frequencies in *Helix aspersa* populations. Autocorrelation techniques have the advantage on F_{ST} statistics in identifying the scale of genetic structure without prior knowledge of the scale involved (HEYWOOD 1991).

During the last decade, autocorrelation techniques have been increasingly used to study within-population genetic structure of plant populations. Such studies are of special relevance to plants where the location of each individual within a population may be known (HEYWOOD 1991). These techniques can tell whether there are evidence for limited gene flow at local scale, and thus supplement ecological data on pollen and seed dispersal in conditions at which these cannot be assessed in the field. At the between-population level, autocorrelation methods allow the detection of trends across the study area. These may reveal processes of migration and colonisation, as well as processes affecting the distribution of individual alleles, such as selection pressure and genetic drift (SOKAL *et al.* 1989a). SOKAL *et al.* (1989b) suggested interpreting rules for inter-populations correlograms by stating that "Autocorrelation coefficients summarise important aspects of a spatial pattern such as whether the variation is regionally patchy, shows long distance differentiation, or is clinal". Surprisingly, if some papers mention autocorrelation analyses of allele frequencies in animal populations (e.g. cats : RUIZGARCIA 1993, *Drosophila* : SOKAL *et al.* 1987, humans : SOKAL *et al.* 1989, butterflies : NÈVE 1996), we could not find any paper of analysis of allele frequency autocorrelation in plant species.

The purposes of the present paper are to show how autocorrelation methods could be used to study the genetic structure both within and between populations of *Calluna vulgaris*, a well known widespread shrub, and to discuss both theoretical and empirical studies relative to autocorrelations.

MATERIALS AND METHODS

SAMPLING

Local genetic structure of a *Calluna* population was assessed in a peat bog of Upper Ardenne, Belgium (Sacrawé, 5°45′ E, 50° 15′ N). A patch of 16 × 60 m, where *Calluna vulgaris* forms the dominant species and covers more than 75% of the surface, was arbitrarily chosen. Ramets were sampled exactly to the points of a regularly spaced lattice of 4 × 4 m. Forty-one ramets were sampled and distance between adjacent sampling points ranged from 4 to 8 m. Large scale genetic structure was studied by sampling 18 populations from the south-western range of *Calluna vulgaris*. Twenty eight to 35 individuals were sampled in each studied population. These were grouped into five regions (fig. 1) : Ardenne (Belgium), Massif-Central, Atlantic Pyrénées, Landes (France), North Spain. Detailed description of genetic variation and genetic structure of the 18 populations will be given elsewhere (MAHY *et al.*, submitted)

ELECTROPHORESIS

Ramets were collected and grown in a greenhouse until vegetative buds flushed. Enzymes were extracted from young tissues with an extraction buffer consisting of 0.1 M phosphate buffer (pH 7.5) with 10% (w/v) PVP and 1% (v/v) 2-mercaptoethanol. Electrophoresis was conducted onto 12% starch gels. Malate dehydrogenase (MDH, E.C. 1.1.1.44) and phosphoglucomutase (PGM, E.C. 2.7.5.1) were resolved on a histidine citrate electrode and gel buffer system (system 9, SOLTIS *et al.* 1983) with 12 hr migration. Phosphoglucoisomerase (PGI, E.C 5.3.1.9), menadione reductase (MNR, E.C. 1.6.99.2) and isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) were resolved on a citric acid electrode buffer / histidine HCl gel buffer (system 1, SOLTIS *et al.* 1983). Staining methods generally followed SOLTIS *et al.* (1983) and VALLEJOS (1983). The alleles are numbered first by locus, then by alleles.

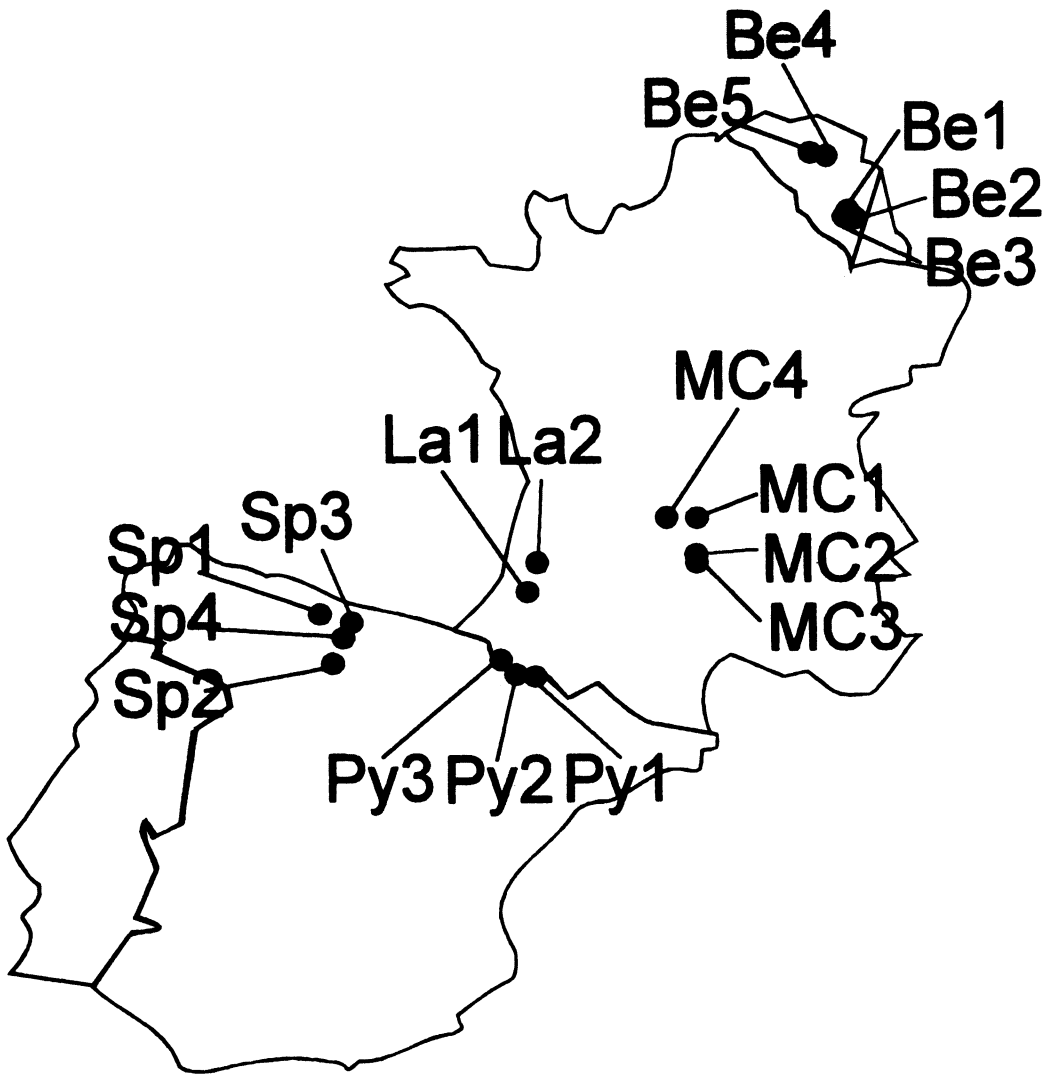

FIG. 1. — Location of the 18 *Calluna vulgaris* populations sampled for autocorrelation analysis at the European scale.

DATA ANALYSIS

Within the population of Sacrawé, a matrix of geographic distances between individual ramets was computed from their x and y coordinates obtained in the field. At the European level, geographic distances were assessed for each pair of populations belonging to the same region while geographic distances between central points of relevant regions were used for populations from different regions. Distances between populations ranged from 2 to 1040 km. The distances between populations were grouped into six equidistant classes, each of which with a 115 km range, centred respectively at 57, 173, 404, 519, 866 and 982 km. Classes for 288, 635 and 751 km are not represented in the data set, due to the nested sampling.

For the analysis of genetic structure within population, individuals were characterised, for each allele, by allele frequencies of 0.0, 0.5 or 1.0 according to whether they carry respectively, 0, 1 or 2 copies of the allele in their genotype, as used by BACILIERI *et al.* (1994). At the European level, populations were considered as sampling points and were characterised by their allele frequencies. To compute Moran's *I*, the geographic distances between samples are divided into a series of classes, and the coefficient is then calculated for each class as follows :

$$I(D) = \frac{\frac{1}{W}\sum_{i=1}^{p}\sum_{j=1}^{p} w_{ij}(y_i - \bar{y})(y_j - \bar{y})}{\frac{1}{p}\sum_{i=1}^{p}(y_i - \bar{y})^2} \quad \text{for each } i \neq j$$

with D the distance class considered, W the number of sample pairs in the distance class, p the number of sample points, $w_{ij} = 1$ if the distance between the considered samples i and j is within D, and $w_{ij} = 0$ otherwise (Upton & Fingleton 1985); for inter-population analysis y_i and y_j are the allele frequencies in the i^{th} and j^{th} population respectively and $\bar{y}$ is the mean allele frequencies over all populations while for intra-population analysis y_i and y_j are the transformed genotype scores for individual i and j respectively and $\bar{y}$ is the mean score for all the individuals sampled. The autocorrelation coefficient Moran's I was computed using the R package (Legendre & Vaudor 1991). Spatial autocorrelation was performed on the most common allele of each polymorphic locus. A locus was considered polymorphic when the frequency of the most common allele was less than 0.95. When more than two alleles were present at a single locus, analysis was also performed on all alleles whose frequencies exceeded 10%. Correlograms for each allele for each locus were drawn plotting Moran's I against distance classes. To summarise the information provided by all loci, under the hypothesis of neutrality of alleles, we calculated the mean of Moran's I over alleles, for each distance class (Bacilieri *et al.* 1994). Spatial autocorrelation was analysed independently at the local scale (within population) and at the European scale.

RESULTS

Within population structure

Spatial autocorrelation analysis yielded 72 (6 alleles × 12 distances classes of equal width) Moran's I statistics, of which seven differed significantly from zero at the 0.05 level. Correlograms based on individual alleles and the correlogram of the mean values suggested random fluctuations between positive and negative values with no clear tendency for I to be more positive over short distance as opposed to longer distances (fig. 2). In addition mean Moran's I were close to zero over all classes ($0.090 <$ mean Moran's $I < 0.142$).

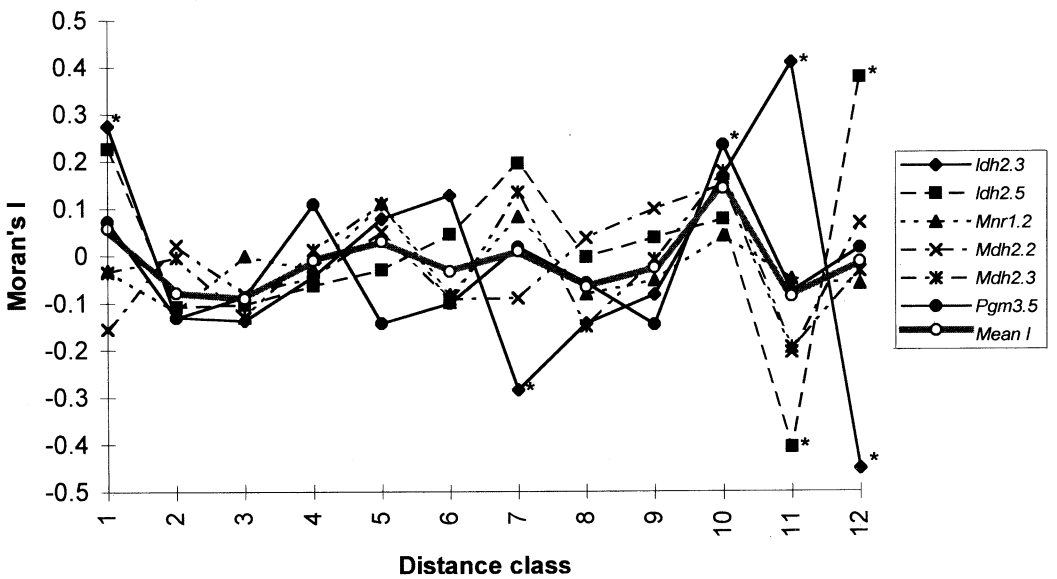

Fig. 2. — Autocorrelation analysis within a *Calluna vulgaris* population. Correlogram of Moran's I for six alleles and correlogram of mean values of Moran's I. Distance between maximum and minimum value of each class is 5.2 m. (* : Moran's I significantly different from zero at $P < 0.05$).

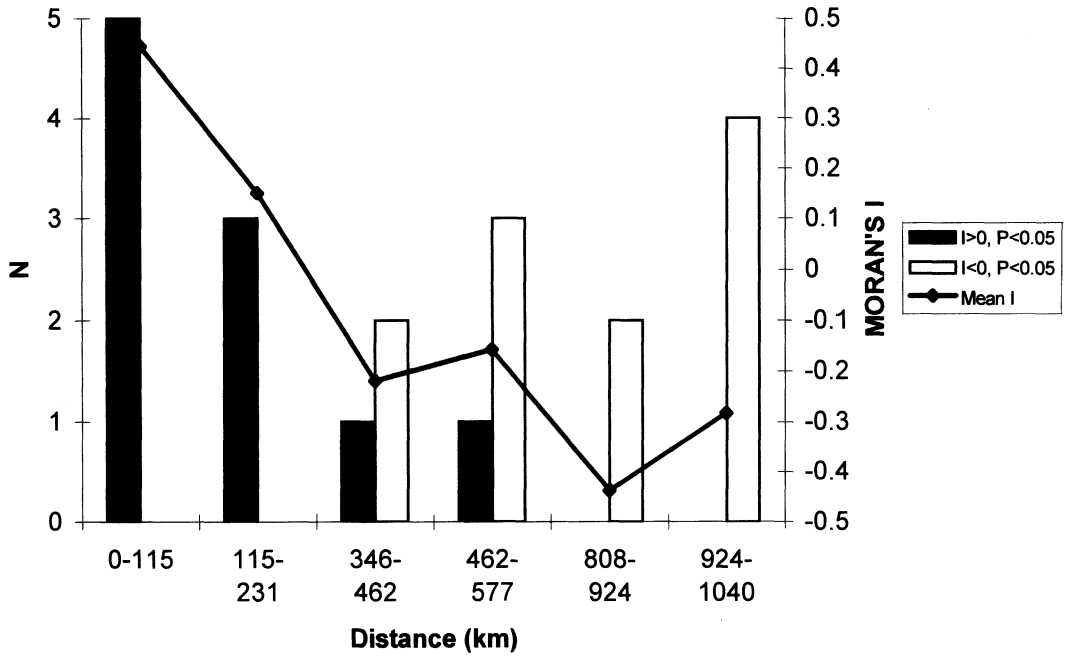

FIG. 3. — Autocorrelation analysis among 18 European *Calluna vulgaris* populations. Correlogram of mean values of Moran's *I* for 7 alleles and number of positive and negative values of *I* significantly different from zero ($P < 0.05$) for each distance class (N). For each class the total number of tests is seven.

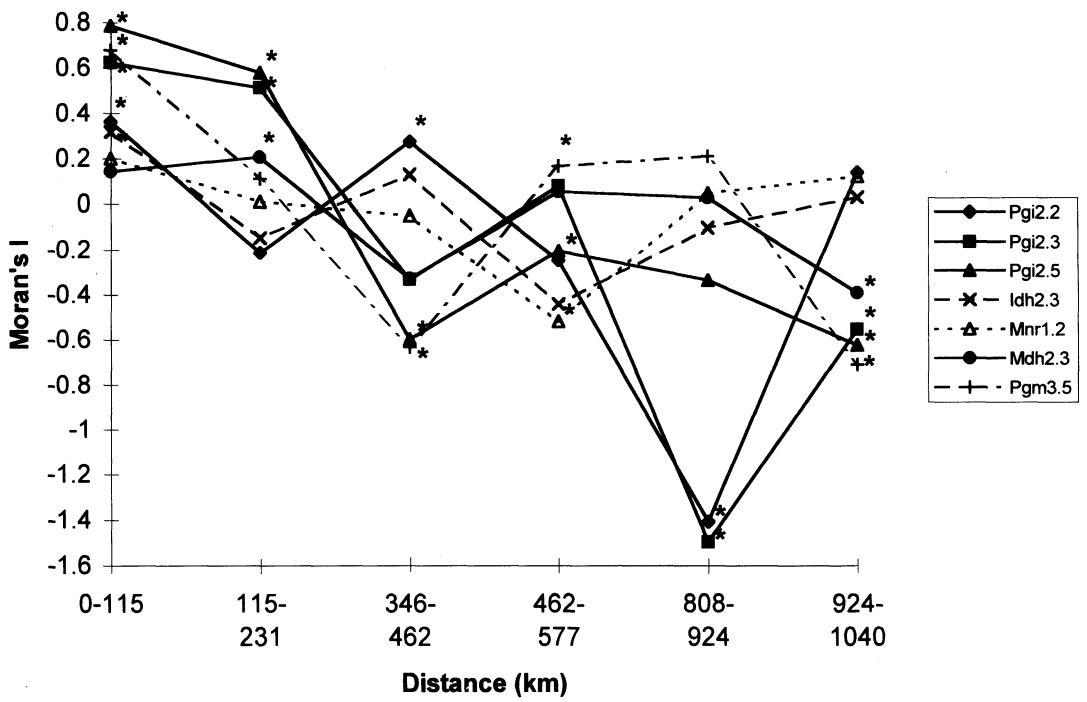

FIG. 4. — Autocorrelation analysis among 18 European *Calluna vulgaris* populations : Correlogram of Moran's *I* for 7 alleles. (* : Moran's *I* significantly different from zero at $P < 0.05$).

Between population structure

Spatial autocorrelations yielded 42 (7 alleles × 6 distance classes of equal width) Moran's *I* statistics, of which 21 (50%) differed significantly from zero at the 0.05 level. Values of Moran's *I* are generally positive at the short and negative at long distances ; the average curve shows a slight downward curve, implying a general trend towards a cline structure (fig. 3). However, individual correlograms computed for the seven studied alleles show a variety of patterns. Three alleles show a significant clinal trend, with significantly positive values of Moran's *I* at short distances and negative values at longer distances (*MDH2.3*, *PGI2.5*, *PGI2.3* ; fig. 4). *IDH2.3* shows a complex pattern, similar to the one called "regional patch" by Sokal *et al.* (1989b), which implies positive correlation at short distance (the size of the regional patch of populations), then a negative correlation (neighbouring patches tend to have different frequencies) then a levelling at higher distance, implying no long distance trend. *PGM3.5* shows a similar pattern, superimposed with a long distance cline, suggested by a negative value of Moran's I at distances over 900 km.

DISCUSSION

Within population structure

A lack of significant autocorrelation suggests that the distribution of sample genotypes is random or nearly random at all loci within the *C. vulgaris* population investigated. This result was not expected due to the reproductive characteristics of *Calluna* : clonality, insect pollinated and limited seed dispersal.

Positive autocorrelation of plants with the same genotype at short distance is predicted when reproduction occurs vegetatively (Sokal & Oden 1978b). In such case, clusters of clones would result. Our results show that despite a high frequency of layering in the population investigated, individual clones have limited spatial extension. Nevertheless, the data set does not allow structure on a scale smaller than four meters to be directly assessed ; thus it is still possible that a genetic structure would be revealed at a smaller scale. *C. vulgaris* is mainly insect-pollinated although wind may play a role (Gimingham 1960, Herrera 1987, pers. obs.). Insect pollination often results in limited dispersal of pollen and movement of wind-dispersed pollen is probably limited due to the low growth habit of *C. vulgaris*. *Calluna*'s seeds have no obvious mean of long-distance dispersal. Most of the wind-dispersed tiny seeds fall within a couple of meters of the mother plant (Legg *et al.* 1992). Nevertheless, the most likely explanation for the lack of spatial structure at local scale is that gene flow has been sufficiently extensive to prevent the emergence of subpopulation spatial structure by genetic drift or selection. Thus, our results suggest that dispersion of pollen or/and seeds may be more extensive than previously reported. Discrepancy between observation of genetic structure by mean of spatial autocorrelation and expected gene flow based on ecological observations have been reported in previous studies (Campbell & Dooley 1982, Waser 1987). Campbell & Dooley (1992) stated that occasional long distance gene flow, difficult to observe, may at least partially encountered for such discrepancy. Occasional wind-dispersion of *Calluna* for 100 m to 25 km has been reported by Beijerinck (1940). Viable seeds and young seedlings have also been found in the dungs of herbivores (Welch 1985). Occasional seed dispersal due to wind or animal ingestion is likely to result in long-distance gene flow. However, influence of occasional long distance dispersion on the genetic structure at local scale is still debated. Some authors have reported that uncommon long-distance dispersal events have little effect on the development of family structure (Epperson 1990, Schnabel *et al.* 1991). In addition, temporal dispersal of seeds may also account for a lack of local genetic structure. *C. vulgaris* produce large quantities of small seeds that remain viable in a seed bank for many years (up to 150 years ; Cumming & Legg 1995). The period of viability of seeds largely exceeds the viability of individuals (25 years), then a germination site may contain the production of seeds from numerous individuals. Due to the temporal succession of genetically different individuals at the same locality, random spatial distri-

bution of seed-grown individual genotypes may be expected, giving a panmictic-like structure. This pattern will be reflected in the structure of adult populations which grow from a seed bank. Our results did not preclude that local structuring may develop in other populations of the species with different histories. Populations of *Pinus banksiana*, for example, have been found to differ in their extent of genetic substructuring (XIE & KNOWLES 1990).

The pattern observed in this *Calluna* population is not consistent with the prediction that plant populations are subdivided into local demes or neighbourhoods of related individuals (LEVIN & KERSTER 1974, LEVIN 1981, BRADSHAW 1984, EPPERSON 1990). Comparisons among spatial autocorrelation studies are not easily made because of various sampling schemes (nearest neighbours, regular grids, irregular grids) and various scales of studies. Nevertheless, previous studies have reported localised structure over short distances (generally less than 10 m) by means of autocorrelation analysis : e.g. *Ipomoea purpurea* (EPPERSON & CLEGG 1986), *Pinus banksiana* (XIE & KNOWLES 1990), *Acer saccharum* (PERRY & KNOWLES 1991), *Maclura pomifera* and *Gledistia triacanthos* (SCHNABEL *et al.* 1991), *Quercus petrea* and *Quercus robur* (BACILIERI *et al.* 1994), *Psychotria officinalis* (LOISELLE *et al.* 1995), *Atherosperma moschatum* (SHAPCOTT 1995). On the other hand random genetic structure has been found in other species : e.g. *Delphinium nelsoni* (WASER 1987) ; *Psychotria nervosa* (DEWEY & HEYWOOD 1988) ; *Pinus contorta* ssp. *latifolia* (EPPERSON & ALLARD 1989) ; *Stylidium coroniforme* (COATES 1992). Together with the present data on *Calluna*, these latter studies suggest that local genetic structure in plant populations may not be as common as usually thought. An examination of the life-history characteristics of the species for whom the intrapopulation structure was studied revealed a large bias of life-form and mating system status. From the 14 species reported, 13 were woody perennial plants and only one was an annual (*Ipomea purpurea*, EPPERSON & CLEGG 1986) ; similarly most of the species (10) were highly outcrossed. Woody perennial outcrossed species are likely to experiment greater level of gene flow than annual and inbred species (HAMRICK & GODT 1989). This indicates the need to evaluate genetic structure from a range of populations and species in order to gain a better understanding of the genetic structure in relation with the life histories of the species and populations involved.

BETWEEN POPULATION STRUCTURE

Most correlograms show significant positive correlation up to 115 km, the upper limits of distance between populations belonging to the same region, suggesting that regions are genetically homogeneous as compared with the whole sampled area. Negative values of Moran's I at high distances suggest that populations coming from regions situated far away from each other are more different then populations from nearby regions. These two latter results, and regular decrease of mean Moran's I with increasing geographic distance, are consistent with the finding that isolation by distance has played a role in shaping genetic structure of *Calluna*'s populations at the European scale (MAHY *et al.*, submitted). G_{ST} values for the studied loci are quite similar, as they vary from 0.026 to 0.096 (MAHY *et al.*, submitted), which suggests that similar factors affect the distribution of the different allele frequencies ; whereas autocorrelation techniques seem to indicate that this is not the case. Indeed, at the European level, the latter techniques revealed distinct spatial variation patterns for the different alleles. Different patterns can be due to sampling errors and stochastic processes (SLATKIN & ARTER 1991). Nevertheless, a high proportion of Moran's I tests were found to be significant. This suggests that various factors, such as random genetic drift, selection or colonisation from different areas, may affect their local frequencies (MANLY 1985). Furthermore the G_{ST} analysis give little information as to the differences between the spatial distributions of the allele frequencies. Autocorrelation analysis showed that for some alleles there is an aggregative distribution (Sokal's "regional patches"), whereas for other alleles there is a cline structure. Our results suggest that, despite the role played by isolation by distance, the

patterns of gene frequencies in European populations are diverse and complex. This diversity of patterns may reflect the variety of events that have taken place over time on this continent, which may have affected the various loci in different ways. Further studies are needed to asses the identity of the processes involved.

CONCLUSIONS

The last decade has seen a lot of debate about the possible use of autocorrelation analysis in population genetics (see SLATKIN & ARTER 1991, SOKAL & ODEN 1991). The former authors point out that these methods relate to exploratory analysis rather than being inferential tools to test the processes involved. On the other hand, SOKAL & ODEN (1991) support the view that "autocorrelation techniques will be of considerable use to population genetics". At the intrapopulation level, the structure of many plant studies (regular sampling, known coordinates of each individual) are the ideal situation for the use of autocorrelation analysis. If the autocorrelation methods are clearly not a universal panacea, we nevertheless think that these methods usefully supplement other statistical techniques applied to spatial data.

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