

# Spatial analysis of genetic diversity as a tool for plant conservation

Adrián Escudero<sup>a,\*</sup>, José M. Iriondo<sup>b</sup>, M. Elena Torres<sup>b</sup>

<sup>a</sup>Area de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, C/ Tulipán s/n, E-28933 Móstoles, Spain

<sup>b</sup>Departamento de Biología Vegetal, Universidad Politécnica de Madrid, Ciudad Universitaria, E-28040 Madrid, Spain

## Abstract

Development of suitable approaches to the analysis of genetic diversity in a spatial context, where factors such as pollination, seed dispersal, breeding system, habitat heterogeneity and human influence are appropriately integrated, can provide new insights in the understanding of the mechanisms of maintenance and dynamics of populations. In this sense, it is important to recognise that patterns and processes may take place at different scales at the same time, and that the scales of a study must be chosen in accordance with the objectives pursued. Apart from conventional approaches to genetic structure, spatial autocorrelation and related techniques, such as Mantel test, correlograms, Mantel correlograms, join-counts, variograms and point pattern analysis, can detect and characterise the existence of spatial genetic structures and lead the way to discussing the environmental and biological factors responsible for them. An alternative way of including spatial variability in modelling approaches that deal with genetic patterns or processes is through the use of constrained ordinations. Although scarcely used at present, these methodologies have great applicability in conservation biology and can lead a way to an effective integration of genetic, demographic and ecological perspectives. © 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** Spatial autocorrelation; Correlograms; Variograms; Point pattern analysis; Constrained ordinations

## 1. Introduction

During the last few decades we have witnessed an intense academic debate between plant conservation biologists about which is the most appropriate way to approach a conservation problem—the so called ecological or the genetic approach (Schemske et al., 1994). In the course of this long debate, several authors have pointed out that not only is knowledge of the amount of genetic diversity critical for a correct diagnosis of the status, threats and viability of populations (Frankham, 1995), but also the spatial distribution of this diversity (Falk and Holsinger, 1991; Dunham et al., 1999). This recognition falls within a more general statement in which geneticists have recognized the importance of the interaction between genome and environment in time and space so as to better understand evolution (Berry, 1989), thus connecting genetics and ecology (Jelinski, 1997). Genetic diversity may appear spatially structured at different scales, such as population, subpopulation or among neighbouring individuals. This spatial distribu-

tion is necessarily a product of environmental influences, including human activities (Knowles et al., 1992), life story traits and demographic past history of the plant species (Loveless and Hamrick, 1984; Slatkin, 1985). Thus, knowledge of spatial genetic structures provides a valuable tool for inferring these causal factors and also the underlying genetic processes such as differential selective pressures, gene flow and drift (Nevo et al., 1986; Barbujani, 1987; Epperson 1993; Bjornstad et al., 1995). Consequently, information about dispersal, pollinator behaviour, breeding system, safe site availability for establishment and other processes operating and structuring populations at such scales can be derived from spatially explicit approaches (Peakall and Beattie, 1995). Knowledge of all these features represents a key priority for conservation managers. Surprisingly, however, most genetic studies on endangered plants either lack explicit spatial considerations or analyse them only at scales where biological interpretations at the population level are not straightforward.

Although there is considerable agreement that there is no single “correct” scale at which to describe populations, since the processes that originate the spatial patterns may operate at different scales (Levin, 1992), most

\* Corresponding author.

E-mail address: [a.escudero@escet.urjc.es](mailto:a.escudero@escet.urjc.es) (A. Escudero).

of the available numerical methods used in population genetics have been applied at medium and large scales (population or larger geographical approaches). The spatial association of environmental and genetic variables (Hedrick, 1986) and the spatial patterning of plant genetic diversity (Heywood, 1991) have been extensively studied at these medium or large scales. However, abundant evidence has been reported in which plant populations exhibit genetic micro-differentiation, spatially closer individuals being genetically more alike than individuals at some distance (Sokal et al., 1989; Epperson 1993). Even at extremely fine scales, spatial genetic structures have been detected in some plant populations (Turkington and Harper, 1979; Epperson and Clegg, 1986; Wagner et al., 1991; Tani et al., 1998), although, in other cases, random or near random distributions have been found (Waser, 1987; Epperson and Allard, 1989; Leonardi et al., 1995). Therefore, the challenge that arises when studying the genetic structure of populations of rare plants is not only choosing the scale of description, but rather recognising that change may be taking place at several scales at the same time (McCue et al., 1996).

There is an urgent need to integrate the knowledge derived from genetic, demographic and ecological approaches to species conservation in order to be able to formulate management strategies that take into account all the different considerations. Spatial analysis techniques are a meeting point for all these three approaches and thereby progress on this direction is likely to facilitate a much sought comprehensive and integrated outlook in conservation biology.

## 2. Objectives

The purpose of this work is to present methods that can be applied to the study of spatial genetic structures in endangered plants, both at broad and narrow scales, and that may be helpful in interpreting key biological processes which affect the viability of the populations. A second matter which is also posed is the treatment of genetic spatial variability in the context of hypothesis testing and its use as a tool in conservation biology.

Traditionally genetic concerns have been put on one side when developing conservation strategies or are included as vague rules of thumb in recovery plans that usually have not been satisfactorily tested (Fleishman et al., 2001). Nowadays, we are realising that empirical assessment of genetic variability is essential for a successful management of endangered plants (Fenster and Dudash, 1994; Knapp and Rice, 1996). Some of the specific issues in which spatial genetic knowledge may be crucial for plant conservation are the improvement of sampling strategies for seed stocks—*ex situ* conservation—and for decision-making on which popula-

tions should be protected or about the area size necessary for the conservation of a particular population (Maki and Yahara, 1997; Chung et al., 1998). Other aspects that can be added to this list are the development of viable strategies for the establishment of corridor populations, the reintroduction of extirpated populations or the foundation of new ones (Pavlik et al., 1993; Helenurm and Parsons, 1997), the design and development of breeding programs (inbreeding versus outbreeding depression balance) (Williams and Hamrick, 1996) or, finally, the evaluation of the implications of habitat fragmentation scenarios for species conservation (Young and Brown, 1996).

We do not intend to go too deeply into the debate about the capability of spatial analysis techniques to infer processes in population genetics (see Slatkin and Arter, 1991) but are interested in their value as management tools in plant conservation. In this sense, these authors recognised the interest of such type of techniques in exploratory analysis that can be useful in suggesting hypotheses which can be furtherly tested. Furthermore, spatial analysis techniques, such as autocorrelation methods, may be useful to draw inferences about underlying genetic processes of interest in conservation such as migration, dispersal, drift or mutation (Epperson, 1993; Sokal et al., 1997). Thus, Hardy and Vekemans (1999), exploring the gap between spatial autocorrelation analysis and population genetics models, have concluded that some autocorrelation statistics can be easily predicted from analytical theory.

In spite of the relevance of spatial analysis techniques, some limitations exist at present that are linked to the nature of molecular markers. Molecular markers are considered to provide essentially neutral genetic variation (Avise, 1994). Therefore, spatial genetic structures obtained from the genetic variation of molecular markers will only reflect isolation by distance processes and, since the markers are not affected by natural selection, will have no relation to spatial environmental heterogeneity.

The assumption underlying the use of molecular markers is that their levels and distribution of genetic variation are well correlated with the levels and distribution of variation for loci affecting traits of future adaptive importance. However, there are theoretical reasons for believing that this correlation does not always exist (Ennos et al., 1997). While some conservationists may be inclined to preserve all kinds of genetic diversity, others believe that only adaptive variation is worth the effort (see Heywood, 1991, Slatkin and Arter, 1991), and, thus, that conservation oriented genetic studies should only concentrate in the analysis of adaptive traits (Heywood, pers. commun.). Nevertheless, neutral variation may be used to infer geneflow which ultimately underlie any natural selection event (Hardy and Vekemans, 1999). Moreover, conservation

biologists are not only interested in interpreting the spatial genetic structure as an expression of genetic processes but are also concerned with the effect of ecological processes on such structure. For instance, Knowles et al. (1992) observed the existence of a clear spatial genetic structure in a forest originated from regeneration of a limited number of widely spaced trees left by foresters. Such a type of information is basic for any plant conservation manager.

### 3. Conventional approaches to genetic structure

Traditionally, population genetic structures within a species have been primarily studied by testing departures of allele frequencies from panmictic expectations or testing for heterogeneity in allele frequencies among populations or other spatial subdivisions (Heywood, 1991). This implies that the genetic information at the individual level is necessarily merged into clusters of a different spatial or biological nature (populations, sub-populations...) and that the relative frequencies are calculated before further analyses such as *G*-test,  $\chi^2$  test, or more accurately, exact tests (Goudet et al., 1996), are performed. In this sense, Loikart and England (1999) present a summary of recent statistical methods and computer programs for analysing allele frequency data and for estimating genetic processes such as effective population size, inbreeding, dispersal, migration and even relatedness and parentage.

The construction of hierarchical models based on the pioneer work of Wright (1943) and further developed by Weir and Cockerman (1984), in which the genetic variance is assigned to different levels, constituted a notorious improvement in this field. The Analysis of Molecular Variance (AMOVA) procedure developed by Excoffier et al. (1992) provided a method for such a type of analysis that can be easily implemented from inter-individual genetic distance matrices. These last techniques have provided plant conservationists with an efficient and flexible tool to explore a wide range of hypothesised genetic population structures. Other related approaches have used diversity statistics, some of them widely used in other scientific contexts such as the Shannon's index and others specifically developed in the context of genetic research such as Nei's (1973), to estimate diversity present within and among populations. An extension of these approaches based on similarity matrices has been the use of clustering techniques, mainly SAHN (Sequential Agglomerative Hierarchical and Non-overlapping) algorithms such as UPGMA (see Podani, 1989, for details). When the spatial units are not conspicuous (i.e. continuous populations), numerous methods have been carried out to study the spatial patterns of variation (Schaal, 1975; Knowles and Grant, 1985; Van Damme, 1986) but always limited by the

arbitrary selection of the sampling size and shape (Epperson and Clegg, 1986).

Although all these techniques give valuable information about the structure of genetic diversity, they do not provide information below the size of the smaller subdivision, so that lower scales cannot be explored. Neither do they account for the presence of spatial autocorrelation of the genetic structure, nor shed any light about the directionality and shape of the spatial relationships (Heywood, 1991). We will focus our review on techniques that can explore such scales—even the individual to individual scale—reveal shape and size of the spatial structures and, primarily have a potential value in the context of plant conservation biology. This implies that the above-mentioned array of techniques that provide information on the genetic structure has been deliberately excluded.

### 4. Molecular markers and nature of genetic data

A wide choice of genetic markers is now available to the conservation biologist (see review by Haig, 1998). Since the 1960s, allozymes have been successfully used to characterise genetic diversity of rare plant species (Hamrick and Godt, 1990), although sometimes they have not shown enough discriminatory power to distinguish between individuals (Brauner et al., 1992; Buso et al., 1998). In the past decade, DNA techniques have gained ground, especially those based on the polymerase chain reaction (PCR) such as microsatellites (or SSR), RAPD, ISSR and AFLP, in part because these molecular markers provide a larger number of potentially polymorphic loci than allozymes (Heun et al., 1994). Furthermore, they also require small amounts of tissue, an aspect which is especially interesting in plant conservation where the least destructive technique should be considered (Rossetto et al., 1995).

Each of these molecular markers exhibits different properties, but for the purpose of this review two major classes can be identified, based upon the type of expression: codominant markers and dominant markers. The information that may be extracted from them, and therefore, the numerical tools employed in each case are necessarily different. Allozymes and SSRs are codominant markers. This means that the two alleles present in a particular locus of a diploid organism are usually identifiable, and that heterozygotes can be distinguished from homozygotes, which is a prerequisite for estimation of allele frequencies in population genetic studies. Thus, these markers provide interval data (i.e. quantitative data such as allele frequency or genetic distances) and nominal data (i.e. qualitative data such as genotypes or alleles). When spatial genetic approaches are explored at the individual to individual scale, genotypic data are used. Usually, spatial analyses are conducted

on independent diallelic loci and the diploid genotype at each location is converted into the values 0, 0.5 and 1 according to the frequency (none, one, two) of a particular allele. Phenetic analyses of individuals using binary data from allozymes or microsatellites can be also undertaken (Ayres and Ryan, 1999) so that construction of genetic distance matrices or use of raw data for further analyses is feasible in both cases. When the spatial approach is at a larger scale, allele frequencies are used.

In contrast, RAPD, ISSR and AFLP segregate as dominant markers, and must be treated as phenotypic characters (presence/absence data). In this case, genetic matrices, composed of “1s” and “0s”, are used where each row shows the data of a particular individual and each column shows the presence or absence of a particular band. These data are usually converted into similarity matrices for calculation of genetic distances. They can also be explored using summary measures, which has the advantage that only a reduced number of statistics are necessary (Bertorelle and Barbujani, 1995) but the clear disadvantage that they can only rarely be related to very informative genetic coefficients (Epperson et al., 1999). Allele frequencies can also be indirectly estimated from these markers and results can be used to obtain the classical parameters of population genetic studies. Nevertheless, in order to do this, several assumptions must be made that do not always coincide with reality (Lynch and Milligan, 1994).

## 5. Measuring spatial autocorrelation of genetic data

Genetic data frequently present spatial autocorrelation, that is, the association of values of one geographically distributed variable with the values of the same variable at all other localities. In these cases, it is possible to predict the values of a genetic variable at some points of the space from the known values of the variable at other known sampling points.

### 5.1. Mantel test

The first approach, and probably the most widely used, to find a relationship between genetic data and space is the Mantel test (1967). This method requires two data matrices, a genetic distance matrix which can be obtained from allozymes or DNA products, and a Euclidean distance matrix which computes the distance between any two experimental units, from populations to individuals. This implies the measurement of the spatial coordinates (northing, easting and altitude) at every experimental unit. The Mantel test takes into account the correlation between these two distance matrices (Fortin and Gurevitch, 1993; Legendre and Legendre, 1998). The normalised Mantel statistic ( $r_M$ ) is

interpreted as a Pearson correlation coefficient varying between  $-1$  and  $1$ , and tested by means of a randomisation procedure of rows and columns in one of the two matrices. For instance, Martín et al. (1999) observed that geographical distance partially explained the genetic distance among populations in threatened narrow endemic *Erodium paularense*.

The main limitation of this technique in plant population genetics is that this test is only sensitive to linear relationships of spatial autocorrelation (Heywood, 1991). Ways round this constraint are the use of non-Euclidean metric distances (Heywood, 1991) or the transformation of data, such as ranking (Legendre and Legendre, 1998). For instance, McCue et al. (1996), working with the rare plant *Clarkia springvillensis*, evaluated the relationship between genetic distances and spatial distances among subpopulations by means of non-parametric Kendall's and Spearman's correlation tests as a way to overcome the above-listed difficulties.

### 5.2. Correlograms

Spatial genetic structures can be more efficiently described by spatial autocorrelation analysis (Sokal and Oden, 1978). This technique overcomes the limitations of Mantel's test and has the advantage of not having previous assumptions about the spatial scale of the structure (Heywood, 1991). Spatial autocorrelation coefficients indicate whether the values of a variable influence each other and measure the strength of their association. Population geneticists have preferably accessed this technique using Moran's  $I$  (1950) statistic, although other autocorrelation coefficients such as Geary's  $c$  (1954) or the coefficient of coancestry (Cockerham, 1969) have also been applied (see Appendix). Moran's  $I$  is usually evaluated under the assumption that the observations were random, independent samples of a population with an unknown distribution function.  $I$  values which are significantly greater than expected, occur when pairs within a distance class have scores more similar than it would be expected if the variable were randomly distributed. Conversely, values significantly lower than expected indicate that scores of the variable are more dissimilar than expected by chance. Results are usually shown as correlograms, graphic displays in which the values of the autocorrelation coefficients are plotted against distance classes. Tests of significance are obtained for each distance class by randomisation processes, whereas overall significance of correlogram is modified by correction methods, such as the rather conservative Bonferroni method ( $P < 0.05/k$ , where  $k$  is the number of considered lags) or Holm's method which is more permissive because it is sequential (Legendre and Legendre, 1998).

Surface pattern analysis techniques such as spatial autocorrelation coefficients require interval data

because they have been developed to study spatially continuous phenomena (Legendre and Fortin, 1989). The most frequent modelled variable arises when information for diallelic loci is computed as “0”, “0.5” or “1” values at each individual point at the individual to individual scale. Other alternatives at larger scales are simply allele frequencies or the number of alleles in common (Boshier et al., 1995). The problem arises when numerous loci are simultaneously tested because many times discrepancies are notorious as the spatial structure across loci is rarely consistent (Smouse and Peakall, 1999). The different patterns obtained from different loci may be due to sampling errors and stochastic processes in the population or because different forces may be acting on different loci (Slatkin and Arter, 1991). To summarise the information provided by all the loci and deal with the discrepancies among spatial structures among loci, mean correlograms can be calculated using the mean value of Moran’s  $I$  over all loci, for each distance class (e.g. Bacilieri et al., 1994; Williams, 1994; Gömöry and Paule, 2001a,b). Smouse and Peakall (1999) proposed another method to deal with codominant markers that overcomes these difficulties. This method is conceptually similar to a Mantel correlogram. The multivariate Mantel test (or Mantel correlogram) is an ingenious procedure to compute a correlogram for multivariate data using the normalised Mantel statistic ( $r_M$ ) and a permutation test for significance (see Oden and Sokal, 1986; Legendre and Legendre, 1998 for details). It is essentially a modification of the Mantel test where distance is divided into categories and it is tested whether the degree of autocorrelation exhibited by pairwise values within each category is greater than or less than the overall average autocorrelation between sites. The interest of these techniques is the capability to test multidimensional data for the departure from spatial independence both in its totality and for each lag class. Although scarcely used, they have shown power-

ful potentialities in population genetics (Bjornstad et al., 1995; Smouse and Peakall, 1999). A much simpler alternative is the use of the so-called genetic distogram which is a graph where mean genetic distances between pairs of individual belonging to a distance class are plotted against distance classes (Degen and Scholz, 1998; Degen et al., 2001). Statistical significance of such a distogram can be tested using permutation tests.

An alternative and promising way of dealing with multivariate data when using RAPDs or other dominant markers, or if allozyme variability is condensed into a distance genetic matrix, is to summarise the variability of genetic matrices by means of ordinations and to consider the value of each individual on the first extracted gradient as the variable of interest. Thus, the above-mentioned methods that deal with univariate data can be applied. Similar approaches of summing up spatial pattern of complex data sets, based on ordination techniques, have been conducted by other authors (Nesbitt et al., 1995; Le Corre et al., 1998). Such summary techniques reduce the stochastic allele-to-allele and locus-to-locus noise of other approaches.

The use of the Mantel correlogram in the endangered snapdragon *Antirrhinum microphyllum* played a key role in interpreting the existence of patchy genetic structures in the studied populations (Fig. 1). Usually, the first  $x$ -intercept in the correlogram has been used as an estimate of the diameter of the patch. According to this, the size of the patch at the Bolarque population in *A. microphyllum* would be around 25 m. The estimation of the average size of genetic patches can be helpful in designing seed collection strategies for ex situ conservation. For instance, Chung et al. (1998), working on threatened populations of *Cymbidium goeringii*, recommended that the sampling of seeds could be conducted at 14–16 m intervals in order to optimise the genetic diversity in collected samples. This suggestion was based in the estimation of the genetic patch obtained from

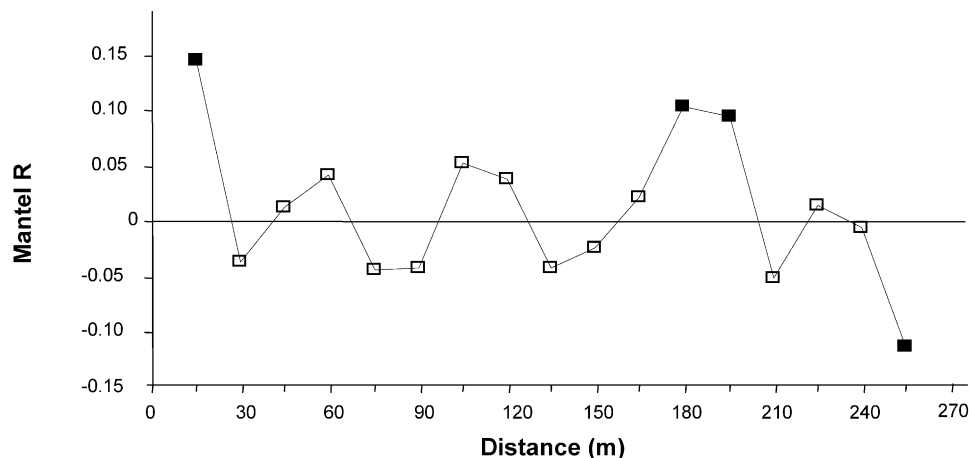


Fig. 1. Mantel correlogram of *Antirrhinum microphyllum* plants at Bolarque population obtained from RAPD data. Filled squares represent autocorrelation coefficients of the Mantel statistic that are significantly different from their expected value ( $P < 0.05$ ) (Torres, unpublished data).

Moran's *I* correlograms. Furthermore, as observed by He et al. (2000) in the endangered perennial *Ophiopogon xylorrhizus* in tropical rainforest, knowledge about the existence of a patchy genetic structure in a population implies that the population should be protected in situ as a whole. Since the population is not homogeneous, the conservation of part of the population would not guarantee the preservation of a representative sample of existing genetic diversity, as genotype distribution can greatly vary between patches.

Recent studies of genetic structure in plant populations have addressed differences in spatial distribution of genetic variation at various life stages or age classes and in different microenvironments of a particular population. This way of integrating genetics, demography and environment provides relevant information on how environment, mating system, seed dispersal and selection can interact. In *Neolitsea sericea*, Chung et al. (2000) found homogeneity of genetic structure among the five age classes considered. This suggests that reproductive events at the population were highly similar from year to year in genetic terms. Contrastingly, in *Cecropia obtusifolia*, seedlings showed marked microspatial structure (spaced out on a cm×cm scale), but spatial structure declined among saplings (spaced out on a m×m scale), and was absent among adults (spaced tens of metres apart) (Epperson and Alvarez-Buylla, 1997). Significant changes in genetic structure between life-cycle stages may provide evidence for population-wide selection based on degree of inbreeding, although it may also result from selection for or against more distantly dispersed offspring. Thus, this information might be useful in the design of population introductions. Furthermore, the effects of ongoing habitat fragmentation in populations can also be analysed through this approach.

A different methodological approach, which has also been used, is the use of joint count autocorrelation statistics that allow the use of nominal data (Sokal and Oden, 1978). In this case, the observed number of "like pairs" of genotypes, in which both individuals of a pair share the same genotype, are enumerated. These numbers are compared with expected numbers under the null hypothesis of a random distribution of genotypes. Autocorrelation statistics are usually computed as Standard Normal Deviates (SND) (Sokal and Oden, 1978). A significant positive value indicates that the pairs of individuals separated by the corresponding distance (lag interval) have more similar genotypes than expected by the null hypothesis, whereas a significant negative value indicates that they have dissimilar genotypes. According to Epperson et al. (1995) joint counts statistics for pairs are more sensitive than Moran's *I* statistic based on allele frequencies. He et al. (2000) used joint counts to show that plant populations of *Ophiopogon xylorrhizus* were subdivided in local neighbourhoods of related

individuals. These authors suggested that in this case limited gene flow due to short-distance dispersal of pollen and seed might account for the genetic clustering. This suggestion was backed by the nature of pollinators and the features of the species' seed dispersal.

Another useful application of spatial autocorrelation analysis is the assessment of the relative importance, in genetic and demographic terms, of clonal and sexual reproduction in species that both have alternative ways of propagation. Thus, both join-counts and Moran's *I* statistics have been used to separate the spatial genetic structure caused by clonal reproduction from that maintained in sexually reproduced individuals in populations of the endangered *Abeliophyllum distichum* (Chung and Chung, 1999) and the narrowly distributed *Adenophora grandiflora* (Chung and Epperson, 1999).

An important issue in all the above-mentioned techniques is the determination of the number and size of the distance intervals to be used. A recommended and classical rule of thumb to determine the range of the study is to consider only pairs separated by less than half the maximum distance observed (Le Corre et al., 1998). There is no consensus regarding the way of generating distance classes. Some authors suggest the use of uneven lags that comprise a constant number of individuals (equal frequencies), whereas others prefer using the same distance in all lags irrespective of the number of point pairs that fall in each class (equal intervals) (see Appendix). Under either of these criteria, each lag class should include at least 30 pairs of data (Legendre and Fortin, 1989). The first distance class is particularly critical, and Epperson and Chung (2001) recommended the establishment of its upper bound to be approximately equal to 1.5 times the square root of the inverse of the sample density. According to them, such a bound would insure that most pairs of near-neighbour individuals are included in distance class one. Following with other technical aspects, Rossi et al. (1992) have pointed out the importance of detecting outliers, because just one unusually large or small value may strongly influence the results. Hawkins's method (1980) provides an efficient tool for this purpose.

To compute spatial autocorrelation techniques, we may need to indicate connections among the localities because the use of raw spatial components may not always be straightforward. Under these circumstances, the Gabriel connected graph (Gabriel and Sokal, 1969), which is used to test for the lack of autocorrelation between nearest neighbours, has been the most used technique in population genetics (e.g. Dewey and Heywood, 1988; Schnabel et al., 1991; He et al., 2000). This algorithm pairs two individuals if no other individual lies on or within the circle whose diameter is the line connecting those individuals. Our experience suggests that the use of geographical distances is normally the best option. Only, when environmental constraints for

seed dispersal or pollination vectors are clearly identifiable (i.e. linear habitats, such as rivers or roads) the construction of connection graphs may be of interest.

### 5.3. Variograms

A semi-variogram (often called a variogram for simplicity) is a graph which shows the average semi-variance found in comparisons of samples (i.e. individuals) taken at increasing distances from one another, the lag interval (Legendre and Fortin, 1989). The underlying assumption, called the “intrinsic hypothesis” is that the difference between the values of two individuals is only a function of the distance between the points. This is a bit more flexible condition than the second order stationarity—existence of a dominant spatial structure—that is necessary to build up most of the known structure functions. As in the case of correlograms, variograms may be calculated for all directions in space, or for specific directions if anisotropy is suspected. In this case, only the pairs of points connected along a given direction are considered—taking a tolerance angle into consideration. The main interest of this technique is that theoretical variograms can be fitted to experimental ones, allowing for the comparison of observed structures with structures derived from hypothesised generating processes. Many models are available to conduct this adjustment, such as linear, Gaussian, exponential or spherical models and there are systems to evaluate their goodness of fit (Pannatier, 1996; Legendre and Legendre, 1998). Parameters of some of these theoretical variograms such as the range, which is the distance where the semi-variance stops increasing, or the sill, which is the ordinate value of the flat zone of the variogram, present undoubted interest in genetic conservation sense. For instance, these models may be used as a predictive tool for kriging contour maps (Burrough,

1987; Degen and Scholz, 1998) or variograms having a clear sill can be interpreted within the frame of isolation by distance models. According to these models, after a critical distance, little change in the semi-variance is encountered with increasing distances, so the total sample semi-variance found at all scales of sampling is essentially constant (Piazza et al., 1981; Rossi et al., 1992; Le Corre et al., 1998). Among the papers reviewed, only two used this technique (see Appendix). In Le Corre et al. (1998), allozymes were used and variograms were independently calculated for allele frequencies from different loci, so we again face the problem of interpreting divergent results. In Fig. 2, the results of a variogram approach on *A. microphyllum* in which the RAPDs variability was summarised by means of a PCA are shown. The first component was used to build up the variogram (Torres, 1999). Note that the results obtained are rather similar to those from Mantel correlogram (Fig. 1) (the graphs mirror each other because variograms plot semi-variances and correlograms plot correlation coefficients).

Because the shape of structure functions may not unambiguously correspond to a single type of spatial structure they must be completed with maps of the original variable or if possible, the theoretical model (Legendre, 1993). Consequently, along with the variogram patterns, we suggest the construction of contour plots of the consensus genetic variable by means of an interpolation procedure to characterise the pattern of the genetic structure. In *A. microphyllum* the interpolation map of the population clearly shows a patchy pattern with a reduced number of genetic vicinity units (Fig. 3). This patchy spatial genetic structure can be explained by a combined effect of short-distance pollen and seed dispersal, and by the existence of a patchy spatial structure of available safe sites for the establishment of this species, a chasmophyte.

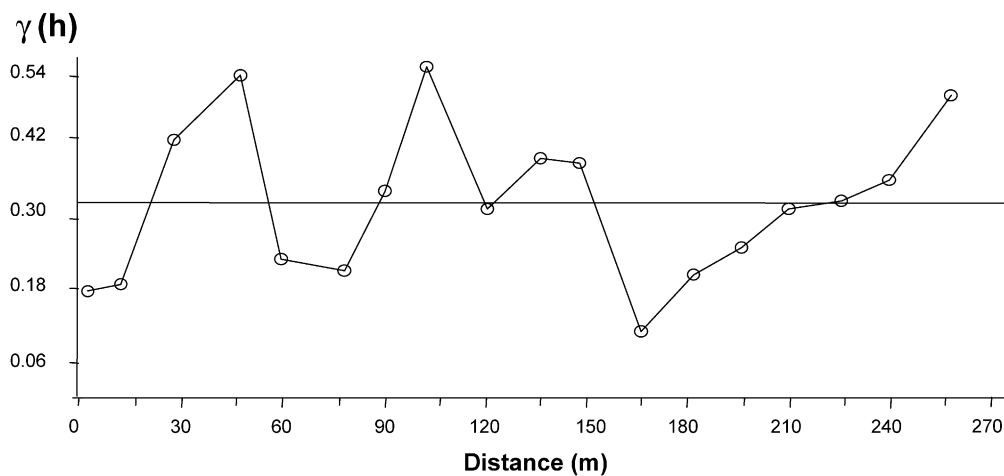


Fig. 2. Variogram for *Antirrhinum microphyllum* plants at Bolarque population. The first extracted component of a PCA obtained from RAPD data was used to build the variogram (Torres, unpublished data).

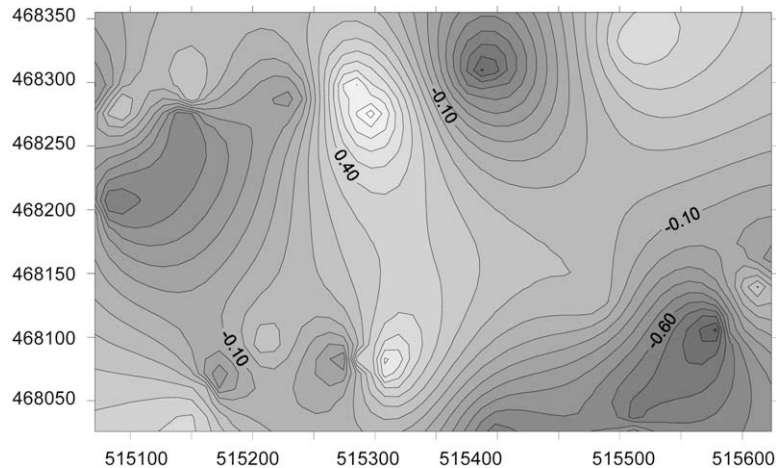


Fig. 3. Contour map depicting the isolines and the associated grey scale of the synthetic genetic variable obtained from RAPDs data with the first extracted component of a PCA for the Bolarque population of *Antirrhinum microphyllum* (Torres, unpublished data).

#### 5.4. Point pattern analysis

Point pattern analysis is a complete battery of methods to establish whether the distribution of data points is random, that can be applied to model the spatial pattern of a particular genotype. Surprisingly, the use of point pattern analysis has not been implemented in population genetics nor in conservation biology, despite some indexes based on counts and specifically developed to evaluate spatial patterns having been used in these contexts, such as the Morisita index (Ueno et al., 2000). The development of density functions using the second moment, i.e. the variance of all point–point distances, offers a powerful analytical tool for the study of distribution patterns (Diggle, 1983; Haase, 1995). Probably the most interesting methods are those based on Ripley's (1976) *K*-function, which is being widely used in plant ecology. The function is calculated from the data and then tested against the null hypothesis of complete spatial randomness. Usually the statistical significance is based on Monte Carlo methods. Univariate and bivariate approaches are feasible. In the latter case, the repulsion or aggregation of different genotypes for any distance from a target individual can be studied for any known radius around the individual. Recently, Podani and Czarán (1997) have developed a multivariate approach in a vegetation context which seems to be largely applicable to population genetics.

#### 6. Hypothesis testing and partialling out spatial information

Theoretical and empirical work is rapidly advancing a set of hypothesis tests that use spatial patterns to study some genetic processes such as gene flow or natural selection (Epperson, 1990). The evaluation of the effect of environmental conditions on spatial genetic structure can be viewed as a problem of spatial covariation

(Legendre and Fortin, 1989). This type of covariation can efficiently be approached by means of constrained ordinations considering the spatial location of each individual as a variable upon which statistical analyses are performed. As suggested by McCune (1997), these techniques (ter Braak, 1986; ter Braak and Prentice, 1988) can be used as tools for hypothesis testing (ter Braak and Prentice, 1988; Palmer, 1993; Legendre and Anderson, 1999). Genetic distance matrices, such as those obtained from allozyme or RAPD data, constitute the target genetic matrix.

In order to select the appropriate ordination constraining technique, the target genetic matrix must be submitted to a Detrended Correspondence Analysis (DCA) with detrending by segments and non-linear rescaling of the axes, which has the property that the extracted axes are scaled in units of average standard deviation (Gauch, 1982). Values above 3 SD units suggest the use of techniques assuming unimodal responses such as Canonical Correspondence Analysis (CCA) or other related techniques (ter Braak, 1986; Legendre and Anderson, 1999). Linear techniques such as those related to redundancy analysis should be conducted when the extracted gradient is smaller. Both spatial variables (northing, easting and altitude) and other explanatory sets (i.e. environmental variables) can be used to constrain the genetic matrix. Total Variation Explained (TVE) by each data set can be calculated as the sum of all canonical extracted axes (Borcard et al., 1992). A Monte Carlo permutation test is performed to determine the accuracy of the relationship. The sum of all canonical eigenvalues or trace is used to build the *F*-ratio statistic (ter Braak, 1990; Verdonschot and ter Braak, 1994; Legendre and Anderson, 1999). Bonferroni correction is usually used after multiple comparisons (Legendre and Legendre, 1998).

A forward step is achieved by means of partial constraining ordinations (ter Braak, 1987). These proce-



dures evaluate the relative importance of any constraining matrix (spatial, environmental...) after adjusting the variability of other data sets which are considered as covariables (Borcard et al., 1992; Borcard and Legendre, 1994; Okland and Eilertsen, 1994; Legendre and Legendre, 1998). Partial ordinations allow one to determine the variation that can be explained by the variables that remain after the variation associated with the covariable data set has been removed. In this case, the question is not only whether a correspondence between the data sets exists but, what fraction of the seed counts information is explained by the covariable data set and how much by the constraining matrix.

Epperson (1990) suggested the use of regression models to account for such a type of hypothesis testing approaches. However, the difficulties of these methods in hypothesis testing were summarised by Legendre (1993). On the other hand, Bjørnstad et al. (1995) suggested the use of a conceptually similar approach, the so-called Partial Mantel test. Moreover, they proposed four models of causal relationships between three different data sets (space, genetics and environment). Thus, they showed that the genetic pattern at the smaller scales is largely due to spatial constraints, which they related to isolation by distance, whereas selective forces (related to environmental variability) may generate the genetic pattern at larger scales. One of the main restrictions of this technique, also recognised by the authors, is that the relationships among the considered data set are assumed to be linear. This is worth noting because evidence suggesting other type of relationships between distance and genetic sets has been reported (Epperson, 1993).

The results of a constrained ordination performed in *A. microphyllum* are shown in Table 1, where the relevance of the spatial components (including some interaction terms) in explaining a fraction of the genetic variability in a population is assessed. A forward stepwise procedure was subsequently carried out to select a reduced model including only significant variables (geo-

graphical in this case). We incorporated explanatory variables one at a time and step by step in the order of their decreasing eigenvalues after partialling out the variation accounted for the already included variables. The process stopped when the new variable was not significant ( $P > 0.05$ ). In this case, northing and easting components were selected as variables that significantly explained part of the genetic variation in the Bolarque population of *A. microphyllum*. Reduced models incorporating only these variables implied a very small drop of TVE. The results obtained are supported by the particular geomorphologic structure of the cliff environments where the population appears (Torres, 1999). This approach may be extended in such a way that environmental, morphological, experimental or theoretically emerged classifications, and other type of data sets can be simultaneously evaluated.

## 7. Past and present applications and future possibilities

Spatial statistical methods provide plant conservation biologists with powerful tools for measuring the structure of genetic diversity of target plants. However, a quick review of current literature shows that the use of these techniques is low among genetic conservationists and even among population geneticists. We have reviewed papers approaching spatial genetic structures in plant species and limited our search to articles dealing with spatial autocorrelation and related techniques. The papers that simply used the Mantel test have not been considered. The results are shown in the Appendix. Sixty-one papers were found in which 67 species were analysed. In only 15 cases the studies referred to rare or endangered species. Almost all papers (87%) used allozymes to estimate the amount of genetic diversity. Similarly, most works calculated the Moran's autocorrelation coefficient  $I$  (77%) and joint count statistics (15%). There is a very reduced set of papers that have explored spatial genetic structures by other means, such as variograms or Mantel correlograms.

Nevertheless, the range of possible practical applications of these approaches is very large and so an effort should be made to spread the use of these techniques in conservation. Although many tools have been proposed to quantify patterns, their development is far from being completed (Gustafson, 1998), so future tools will be necessarily be tested and will probably give new insights into plant conservation concerns. The potential of density functions (point pattern analysis) is enormous, so we predict their wide use among population geneticists and, especially plant conservationists. The recognition of univariate patterns of individuals and bivariate patterns comprising different age classes or individuals of different species, will become of prime interest in restoration plans for endangered plants. From here, the

Table 1

Results of a stepwise RDA forward selection including the spatial coordinates that significantly explain the total variation of the basic genetic matrix, and the order in which they are selected for the Bolarque population of *Antirrhinum microphyllum* (data from Torres, 1999)<sup>a</sup>

Population	Variable	$F$	$P$
Bolarque	$x$	1.79	0.005
	$y$	1.50	0.031
	$z$		n.s.

<sup>a</sup>  $x$  is the spatial coordinate in the east–west direction;  $y$  is the spatial coordinate in the north–south direction;  $z$  is the altitude;  $F$  is the  $F$ -ratio statistic and  $P$  is the level of significance of the selected variable in the reduced model (1000 randomisations).

spread to other interests of genetic plant conservation is really foreseeable.

The promising value of matrix approaches to partialling out spatial information from genetic data sets needs to be explored. Approaches, like in Bjørnstad et al. (1995), suggest a wide range of possibilities through the use of the partial Mantel test and constrained ordination techniques. Efforts must be done to build efficient theoretical models to constraint the basic genetic information. These models should comprise not only different natural genetic scenarios but also diverse management situations.

Implementation of recovery plans following suggestions from spatial genetic approaches will require monitoring programmes to control the results in terms of total genetic diversity and inbreeding and outbreeding constraints. This experience will enable the formulation of general rules for the management of endangered plants (Pavlik, 1996). Guerrant (1996), discussing whether or not founding populations should come from single or multiple sources in restoration projects, clearly points out that arguments in favour or against may be oversteering how genetic diversity appears structured within populations (Huenneke, 1991; Barrett and Kohn, 1991; Hol-

singer and Gottlieb, 1991). Furthermore, outbreeding and inbreeding paradigms of restoration ecology must be revisited in the light of new realities, such as the existence of genetic neighbourhoods at very small scales. These may be responsible for the failure of many reintroduction projects dealing with endangered or rare plants.

There is an urgent need to integrate the knowledge derived from genetic, demographic and ecological approaches to species conservation in order to be able to formulate management strategies that take into account all different considerations. Spatial analysis techniques are a meeting point for all these three approaches and thereby progress on this direction is likely to facilitate a much sought after comprehensive and integrated outlook in conservation biology.

### Acknowledgements

We are grateful to J. Heywood and D. Gömöry for their very useful comments and suggestions. This work has been partially financed by REN 2000-0254-P4-03 project of the Spanish Ministry of Science and Technology.

### Appendix. Literature survey of spatial genetic studies at within-population level or among-population level

For each species, the molecular marker used, the method of spatial analysis used, the upper bound for the first distance class and the criteria followed to define classes is given. Underlined species correspond to rare or endangered taxa.

Reference	Scientific name	Level	Molecular marker	Method	First class (m)	Criteria for defining class boundaries <sup>a</sup>
Bacilieri et al., 1994	<i>Quercus petraea</i>	Within	Allozymes	Moran's <i>I</i>	10	EI
Bacilieri et al., 1994	<i>Quercus robur</i>	Within	Allozymes	Moran's <i>I</i>	10	EI
Berg and Hamrick, 1995	<i>Quercus laevis</i>	Within	Allozymes	Moran's <i>I</i> ; join-counts	1	EI
Bjornstad et al., 1995	<i>Poa alpina</i>	Within	Allozymes	Mantel correlogram; partial Mantel test	10	d.b.a.
Bonnin et al., 2001	<i>Medicago truncatula</i>	Within	SSRs	Coancestry coefficient	0.5	EI
Bouza et al., 2002	<i>Dorycnium spectabile</i>	Within	RAPD	Moran's <i>I</i>	3/5	d.b.a.
Bouza et al., 2002	<i>Isoplexis chalcantha</i>	Within	RAPD	Moran's <i>I</i>	3	d.b.a.
Bucci and Menozzi, 1995	<i>Picea abies</i>	Within	RAPD	Join-counts	28.5	EF
Caujapé-Castells and Pedrola-Monfort, 1997	<i>Androcymbium gramineum</i>	Within	Allozymes	Moran's <i>I</i>	2.6/3.9/4.6	d.b.a.
Caujapé-Castells et al., 1999	<i>Androcymbium bellum</i>	Within	Allozymes	Moran's <i>I</i>	1	d.b.a.
Caujapé-Castells et al., 1999	<i>Androcymbium guttatum</i>	Within	Allozymes	Moran's <i>I</i>	0.25	d.b.a.
Caujapé-Castells et al., 1999	<i>Androcymbium pulchrum</i>	Within	Allozymes	Moran's <i>I</i>	0.3/1	d.b.a.
Coates, 1992	<i>Styloidium coroniforme</i>	Within	Allozymes	Moran's <i>I</i>	–	–
Chung and Chung, 1999b	<i>Abeliophyllum distichum</i>	Within	Allozymes	Moran's <i>I</i> ; join-counts	4/5	EF
Chung and Epperson, 1999	<i>Adenophora grandiflora</i>	Within	Allozymes	Moran's <i>I</i> ; join-counts	7/8	EF
Chung and Park, 1998	<i>Hosta capitata</i>	Within	Allozymes	Moran's <i>I</i>	2.4/2.9	EF
Chung and Park, 1998	<i>Hosta minor</i>	Within	Allozymes	Moran's <i>I</i>	2.3/2.5	EF
Chung et al., 1998	<i>Cymbidium goeringii</i>	Within	Allozymes	Moran's <i>I</i>	3/4	EF
Chung et al., 1999a	<i>Rhus trichocarpa</i>	Within	Allozymes	Moran's <i>I</i>	9/14	EF
Chung et al., 2000	<i>Neolitsea sericea</i>	Within	Allozymes	Moran's <i>I</i>	10	EF
Dewey and Heywood, 1988	<i>Psychotria nervosa</i>	Within	Allozymes	Moran's <i>I</i>	3.5	EF
Doliguez and Joly, 1997	<i>Carapa procera</i>	Within	Allozymes	Moran's <i>I</i>	50/100	EI

(continued on next page)

## Appendix (continued)

Reference	Scientific name	Level	Molecular marker	Method	First class (m)	Criteria for defining class boundaries <sup>a</sup>
Epperson and Allard, 1989	<i>Pinus contorta</i> subsp. <i>latifolia</i>	Within	Allozymes	Join-counts	15	EI
Epperson and Chung, 2001	<i>Pinus ponderosa</i>	Within	Allozymes	Moran's <i>I</i>	15	EI
Epperson et al., 2003	<i>Pinus arizonica</i>	Within	Allozymes	Moran's <i>I</i>	100	EI
Epperson et al., 2003	<i>Pinus ponderosa</i>	Within	Allozymes	Moran's <i>I</i>	100	EI
Fleishnan et al., 2001	<i>Cordylanthus palmatus</i>	Within	Allozymes	Moran's <i>I</i>	–	–
Foster and Sork, 1997	<i>Ancistrocladus korupensis</i>	Within	Allozymes	Coancestry coefficient	10	EI
Garnier et al., 2002	<i>Hyparrhenia diplandra</i>	Within	SSRs	Moran's <i>I</i>	0.5	d.b.a.
Geburek and Tripp-Knowles, 1994	<i>Quercus macrocarpa</i>	Within	Allozymes	Moran's <i>I</i>	5	EI
Gömöry et al., 1998	<i>Fagus sylvatica</i>	Among	Allozymes	Variogram	–	–
Gömöry and Paule, 2001a	<i>Alnus glutinosa</i>	Within	Allozymes	Moran's <i>I</i>	70	EI
Gömöry and Paule, 2001b	<i>Prunus avium</i>	Within	Allozymes	Moran's <i>I</i>	36.8	EF
He et al., 2000	<i>Ophiopogon xylorrhizus</i>	Within	Allozymes	Moran's <i>I</i> ; join-counts	5	EI
Hossaert-Mckey et al., 1996	<i>Lathyrus sylvestris</i>	Within	Allozymes	Moran's <i>I</i> ; Geary's <i>c</i>	0.5	EF
Kalisz et al., 2001	<i>Trillium grandiflorum</i>	Within	Allozymes	Coancestry coefficient	0.125/0.25	EI
Kang and Chung, 1997	<i>Chimaphila japonica</i>	Within	Allozymes	Moran's <i>I</i>	2.5	EF
Kang and Chung, 1997	<i>Pyrola japonica</i>	Within	Allozymes	Moran's <i>I</i>	2	EF
Kang and Chung, 2000	<i>Hemerocallis hakuunensis</i>	Within	Allozymes	Moran's <i>I</i>	3	EF
Kang and Chung, 2000	<i>Hemerocallis thunbergii</i>	Within	Allozymes	Moran's <i>I</i>	3	EF
Knowles, 1991	<i>Picea mariana</i>	Within	Allozymes	Moran's <i>I</i>	7.5/24	EF
Knowles et al., 1992	<i>Larix laricina</i>	Within	Allozymes	Moran's <i>I</i>	5	EI
Kudoh and Whigham, 1997	<i>Hibiscus moscheutos</i>	Within	Allozymes	Moran's <i>I</i>	3	EI
Le Corre et al., 1998	<i>Quercus petraea</i>	Among	Allozymes	Variograms	–	–
Loiselle et al., 1995	<i>Psychotria officinalis</i>	Within	Allozymes	Coancestry coefficient	5	EI
Maki and Masuda, 1993	<i>Chionographis japonica</i> var. <i>japonica</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Maki and Masuda, 1994	<i>Chionographis japonica</i> var. <i>kurohimensis</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Maki and Yahara, 1997	<i>Cerastium fischerianum</i> var. <i>molle</i>	Within	Allozymes	Moran's <i>I</i>	2.3	EF
McCue et al., 1996	<i>Clarkia springvillensis</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Merzeau et al., 1994	<i>Fagus sylvatica</i>	Within	Allozymes	Moran's <i>I</i> ; join-counts	3	EI
Michaud et al., 1995	<i>Quercus ilex</i>	Among	Allozymes	Moran's <i>I</i>	400k	EI
Montalvo et al., 1997	<i>Quercus chrysolepis</i>	Within	Allozymes	Coancestry coefficient	1	EI
Peakall and Beattie, 1995	<i>Sclerolaena diacantha</i>	Within	Allozymes	Moran's <i>I</i> ; coancestry coefficient	3	EI
Perry and Knowles, 1991	<i>Acer saccharum</i>	Within	Allozymes	Moran's <i>I</i>	11.8/11.9/17.7	EF
Pigliucci and Barbuiani, 1991	<i>Ornithogalum montanum</i>	Among	Allozymes	Moran's <i>I</i>	104.6k	EF
Reusch et al., 1999	<i>Zostera marina</i>	Within	SSRs	Moran's <i>I</i> ; join-counts	3	EI
Sarthou et al., 2001	<i>Pitcairnia geyskesii</i>	Within	Allozymes	Mantel correlogram	45	EF
Schnabel and Hamrick, 1990	<i>Gleditsia triacanthos</i>	Within	Allozymes	Moran's <i>I</i>	–	–
Schnabel et al., 1991	<i>Gleditsia triacanthos</i>	Within	Allozymes	Moran's <i>I</i>	4	EF
Schnabel et al., 1991	<i>Maclura pomifera</i>	Within	Allozymes	Moran's <i>I</i>	10	EF
Shapcott, 1995	<i>Atherosperma moschatum</i>	Within	Allozymes	Join-counts	10	EI
Smouse and Peakall, 1999	<i>Caladenia tentaculata</i>	Within	Allozymes	Smouse and Peakall	1	EI
Stehlik and Holderegger, 2000	<i>Anemone nemorosa</i>	Within	Allozymes	Moran's <i>I</i>	0.5	EI
Streiff et al., 1998	<i>Quercus petraea</i>	Within	Allozymes/SSRs	Moran's <i>I</i>	10	EI
Streiff et al., 1998	<i>Quercus robur</i>	Within	Allozymes/SSRs	Moran's <i>I</i>	10	EI
Torres, 1999	<i>Antirrhinum microphyllum</i>	Within	RAPD	Variogram	15	EI
Ueno et al., 2000	<i>Camellia japonica</i>	Within	SSRs	Moran's <i>I</i>	10	EI
Waser, 1987	<i>Delphinium nelsonii</i>	Within	Allozymes	Moran's <i>I</i>	20	EI
Williams and Waser, 1999	<i>Delphinium nuttallianum</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Williams, 1994	<i>Cryptotaenia canadensis</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Williams, 1994	<i>Osmorhiza claytonii</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Williams, 1994	<i>Sanicula odorata</i>	Within	Allozymes	Moran's <i>I</i>	1	EI
Xie and Knowles, 1991	<i>Pinus banksiana</i>	Within	Allozymes	Moran's <i>I</i>	9.6/12.1/13	EF

<sup>a</sup>Criteria for defining class boundaries: EF = equal frequencies; EI = equal intervals; d.b.a. = defined by author.

## References

- Avise, J.C., 1994. *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.
- Ayres, D.R., Ryan, F.J., 1999. Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. *American Journal of Botany* 86, 344–353.
- Bacilieri, R., Labbe, T., Kremer, A., 1994. Intraspecific genetic structure in a mixed population of *Quercus petraea* (Matt.) Leibl and *Q. robur* L. *Heredity* 73, 130–141.
- Barbujani, G., 1987. Autocorrelation of gene frequencies under isolation by distance. *Genetics* 117, 777–782.
- Barrett, S.C.H., Kohn, R., 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A., Holsinger, K.E. (Eds.), *Genetics Conservation of Rare Plants*. Oxford University Press, Oxford, pp. 1–30.
- Berg, E.E., Hamrick, J.L., 1995. Fine-scale genetic structure of a Turkey oak forest. *Evolution* 49, 110–120.
- Berry, R.J., 1989. Ecology: where genes and geography meet. *Journal of Animal Ecology* 58, 733–759.
- Bertorelle, G., Barbujani, G., 1995. Analysis of DNA diversity by spatial autocorrelation. *Genetics* 140, 811–819.
- Bjørnstad, O.N., Iversen, A., Hansen, M., 1995. The spatial structure of the gene pool of a viviparous population of *Poa alpina*—environmental controls and spatial constraints. *Nordic Journal of Botany* 15, 347–354.
- Bonnin, I., Ronfort, J., Wozniak, F., Olivieri, I., 2001. Spatial effects and rare outcrossing events in *Medicago trunculata* (Fabaceae). *Molecular Ecology* 10, 1371–1383.
- Borcard, D., Legendre, P., 1994. Environmental control and spatial structure in ecological communities: an example using oribatid mites (Acari, Oribatei). *Environmental and Ecological Statistics* 1, 37–53.
- Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological variation. *Ecology* 73, 1045–1055.
- Boshier, D.H., Chase, M.R., Bawa, K.S., 1995. Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 3. Gene flow, neighborhood, and population substructure. *American Journal of Botany* 82, 484–490.
- Bouza, N., Caujapé-Castells, J., González-Pérez, M.A., Batista, F., Sosa, P.A., 2002. Population structure and genetic diversity of two endangered endemic species of the Canarian laurel forest: *Dorycnium spectabile* (Fabaceae) and *Isooplexis chalcantha* (Scrophulariaceae). *International Journal of Plant Science* 163, 619–630.
- Brauner, S., Crawford, D.J., Stuessy, T.F., 1992. Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. *American Journal of Botany* 79, 1436–1439.
- Bucci, G., Menozzi, P., 1995. Genetic variation of RAPD markers in a *Picea abies* Karst. Population. *Heredity* 75, 188–197.
- Burrough, P.A., 1987. Spatial aspects of ecological data. In: Jongman, R.H.G., ter Braak, C.J.F., van Tongeren, O.F.R. (Eds.), *Data Analysis in Community and Landscape Ecology*. Pudoc, Wageningen, pp. 213–248.
- Buso, G.S.C., Rangel, P.H., Ferreira, M.E., 1998. Analysis of genetic variability in South American wild rice populations (*Oryza glumapatula*) with isozymes and RAPD markers. *Molecular Ecology* 7, 107–117.
- Caujapé-Castells, J., Pedrola-Monfort, J., 1997. Space-time patterns of genetic structure within a stand of *Androcymbium gramineum* (Cav.) McBride (Colchicaceae). *Heredity* 79, 341–349.
- Caujapé-Castells, J., Pedrola-Monfort, J., Membrives, N., 1999. Contrasting patterns of genetic structure in the South African species *Androcymbium bellum*, *A. guttatum* and *A. pulchrum* (Colchicaceae). *Biochemical Systematics and Ecology* 27, 591–605.
- Coates, D.J., 1992. Genetic consequences of a bottleneck and spatial genetic structure in the triggerplant *Stylidium coroniforme* (Stylidiaceae). *Heredity* 69, 512–520.
- Cockerham, C.C., 1969. Variance of gene frequencies. *Evolution* 23, 72–84.
- Chung, M.G., Chung, J.M., Epperson, B.K., 1999. Spatial genetic structure of allozyme polymorphism within populations of *Rhus trichocarpa* (Anacardiaceae). *Silvae Genetica* 48, 223–227.
- Chung, M.G., Chung, M.Y., 1999. Spatial genetic structure of clonal and sexual reproduction in a population of *Abeliophyllum distichum* (Oleaceae), an endangered monotypic genus. *Genes and Genetics Systems* 74, 9–14.
- Chung, M.G., Chung, M.Y., Oh, G.S., Epperson, B.K., 2000. Spatial genetic structure in a *Neolitsea sericea* population (Lauraceae). *Heredity* 85, 490–497.
- Chung, M.G., Epperson, B.K., 1999. Spatial genetic structure of clonal and sexual reproduction in populations of *Adenophora grandiflora* (Campanulaceae). *Evolution* 53, 1068–1078.
- Chung, M.G., Park, K.B., 1998. Spatial genetic structure in populations of *Hosta capitata* and *Hosta minor* (Liliaceae). *Israel Journal of Plant Sciences* 46, 181–187.
- Chung, M.Y., Chung, G.M., Chung, M.G., 1998. Spatial genetic structure in populations of *Cymbidium goeringii* (Orchidaceae). *Genes and Genetics Systems* 73, 281–285.
- Degen, B., Caron, H., Bandou, E., Maggia, L., Chevallerier, M.H., Leveau, A., Kremer, E., 2001. Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. *Heredity* 87, 497–507.
- Degen, B., Scholz, F., 1998. Spatial genetic differentiation among populations of European beech (*Fagus sylvatica* L.) in western Germany as identified by geostatistical analysis. *Forest Genetics* 5, 191–199.
- Dewey, S.E., Heywood, J.S., 1988. Spatial genetic structure in a population of *Psychotria nervosa*. I. Distribution of genotypes. *Evolution* 42, 834–838.
- Diggle, P.J., 1983. *Statistical Analysis of Spatial Point Pattern*. Academic Press, London.
- Doliguez, A., Joly, H.I., 1997. Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guinea. *Heredity* 79, 72–82.
- Dunham, J., Peacock, M., Tracy, C.R., Nielsen, J., Vinyard, G., 1999. Assessing extinction risk: integrating genetic information. *Conservation Ecology* 3 (1), 2.
- Ennos, R.A., Cowie, N.R., Legg, C.J., Sydes, C., 1997. Which measures of genetic variation are relevant in plant conservation? A case study of *Primula scotica*. In: Tew, T.E., Crawford, T.J., Spencer, J.W., Stevens, D.P., Usher, M.B., Warren, J. (Eds.), *The Role of Genetics in Conserving Small Populations*. Proceedings of a British Ecological Society Symposium, 18–19th September 1995, York. JNCC, Peterborough, pp. 73–79.
- Epperson, B.K., 1990. Spatial patterns of genetic variation within plant populations. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), *Plant Population Genetics, Breeding, and Genetic Resources*. Sinauer, Sunderland, pp. 229–253.
- Epperson, B.K., 1993. Spatial and Space-time correlations in systems of subpopulations with genetic drift and migration. *Genetics* 133, 711–727.
- Epperson, B.K., 1995. Fine-scale spatial structure—correlations for individual genotypes differ from those for local gene frequencies. *Evolution* 49, 1022–1026.
- Epperson, B.K., Alvarez-Buylla, E.R., 1997. Limited seed dispersal and genetic structure in life stages of *Cecropia obtusifolia*. *Evolution* 51, 275–282.
- Epperson, B.K., Allard, R.W., 1989. Spatial autocorrelation analysis of the distribution of genotypes within populations of Lodgepole pine. *Genetics* 121, 369–377.
- Epperson, B.K., Clegg, M.T., 1986. Spatial-autocorrelation analysis of flower color polymorphisms within substructured populations of morning glory (*Ipomoea purpurea*). *The American Naturalist* 128, 840–858.

- Epperson, B.K., Chung, M.G., 2001. Spatial genetic structure of allozyme polymorphisms within populations of *Pinus strobus* (Pinaceae). *American Journal of Botany* 88, 1006–1010.
- Epperson, B.K., Huang, Z., Li, T.Q., 1999. Measures of spatial structure in samples of genotypes for multiallelic loci. *Genetical Research* 73, 251–261.
- Epperson, B.K., Chung, M.G., Telewski, F.W., 2003. Spatial pattern of allozyme variation in a contact zone of *Pinus ponderosa* and *P. arizonica* (Pinaceae). *American Journal of Botany* 90, 25–31.
- Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131, 479–491.
- Falk, D.A., Holsinger, K.E., 1991. *Genetics and Conservation of Rare Plants*. Oxford University Press, Oxford.
- Fenster, C.B., Dudash, M.R., 1994. Genetic considerations for plant population restoration and conservation. In: Bowles, M.L., Whelan, C.J. (Eds.), *Restoration of Endangered Species: Conceptual Issues, Planning, and Implementation*. Cambridge University Press, Cambridge, pp. 34–62.
- Fleishman, E., Launer, A.E., Switky, K.R., Yandell, U., Heywood, J., Murphy, D.D., 2001. Rules and exceptions in conservation genetics: genetic assessment of the endangered plant *Cordylanthus palmatus* and its implications for management planning. *Biological Conservation* 98, 45–53.
- Fortin, M.-J., Gurevitch, J., 1993. Mantel tests: spatial structure in field experiments. In: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*. Chapman, New York.
- Foster, P.F., Sork, V.L., 1997. Population and genetic structure of the West African rain forest liana *Ancistrocladus korupensis* (Ancistrocladaceae). *American Journal of Botany* 84, 1078–1091.
- Frankham, R., 1995. Inbreeding and conservation: a threshold effect. *Conservation Biology* 9, 792–799.
- Gabriel, K.R., Sokal, R.R., 1969. A new statistical approach to geographic variation analysis. *Systematic Zoology* 18, 259–278.
- Garnier, L.K.M., Durand, J., Dajoz, I., 2002. Limited seed dispersal and microspatial population structure of an agamosperous grass of West African savannahs, *Hyparrhenia diplandra* (Poaceae). *American Journal of Botany* 89, 1785–1791.
- Gauch, H.G., 1982. *Multivariate Analysis in Community Ecology*. Cambridge University Press, Cambridge.
- Geary, R.C., 1954. The contiguity ratio and statistical mapping. *Incorp.Statist.* 5, 115–145.
- Geburek, T., Tripp-Knowles, P., 1994. Genetic architecture in bur oak, *Quercus macrocarpa* (Fagaceae), inferred by means of spatial autocorrelation analysis. *Plant Systematics and Evolution* 189, 63–74.
- Gömöry, D., Hynek, V., Paule, L., 1998. Delineation of seed zones for European beech (*Fagus sylvatica* L.) in the Czech Republic based on isozyme gene markers. *Ann. Sci. For.* 55, 425–436.
- Gömöry, D., Paule, L., 2001a. Spatial and microgeographical genetic differentiation of black alder (*Alnus glutinosa* Gaertn.) populations. *Forest Ecology and Management* 5529, 1–7.
- Gömöry, D., Paule, L., 2001b. Spatial structure and mating system in wild cherry (*Prunus avium*) population. *Biologia, Bratislava* 56, 117–123.
- Goudet, J., Raymond, T., de Mees, T., Rousset, F., 1996. Testing differentiation in diploid populations. *Genetics* 144, 1933–1940.
- Guerrant, E.O., 1996. Designing populations: demographic, genetic and horticultural dimensions. In: Falk, D.A., Millar, C.I., Olwell, M. (Eds.), *Restoring Diversity. Strategies for Reintroduction of Endangered Plants*. Island Press, Washington, pp. 171–207.
- Gustafson, E.J., 1998. Quantifying landscape spatial pattern: what is the state of the art? *Ecosystems* 1, 143–156.
- Haase, P., 1995. Spatial pattern analysis in ecology based on Ripley's k-function: introduction and methods of edge correction. *Journal of Vegetation Science* 6, 575–582.
- Haig, S.M., 1998. Molecular contributions to conservation. *Ecology* 79, 413–425.
- Hamrick, J.L., Godt, M.J.W., 1990. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), *Plant Population Genetics, Breeding and Genetic Resources*. Sinauer Associates, Sunderland, pp. 43–63.
- Hardy, O.J., Vekemans, X., 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetic models. *Heredity* 83, 145–154.
- Hawkins, D.M., 1980. *Identification of Outliers*. Chapman and Hall, London.
- He, T., Rao, G., You, R., Ge, S., Hong, D., 2000. Spatial autocorrelation of genetic variation in three stands of *Ophiopogon xylorrhizus* (Liliaceae s.l.). *Annals of Botany* 86, 113–121.
- Hedrick, P.W., 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annual Review of Ecology and Systematics* 17, 535–566.
- Helenum, K., Parsons, L.S., 1997. Genetic variation and the reintroduction of *Cordylanthus maritimus* ssp. *maritimus* to Sweetwater Marsh, California. *Restoration Ecology* 5, 236–244.
- Heun, M., Murphy, J.P., Phillips, T.D., 1994. A comparison of RAPD and isozyme for determining the genetic relationships among *Avena sterilis* L. accessions. *Theoretical and Applied Genetics* 87, 689–696.
- Heywood, J.S., 1991. Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* 22, 335–355.
- Holsinger, K.E., Gottlieb, L.D., 1991. Conservation of rare and endangered plants: principles and prospects. In: Falk, D.A., Holsinger, K.E. (Eds.), *Genetics and Conservation of Rare Plants*. Oxford University Press, New York, pp. 195–208.
- Hossaert-McKey, M., Valero, M., Magda, D., Jarry, M., Cuguen, J., Vernet, P., 1996. The evolving genetic history population of *Lathyrus sylvestris*: evidence from temporal and spatial genetic structure. *Evolution* 50, 1808–1821.
- Huenneke, L.F., 1991. Ecological implications of genetic variation in plant populations. In: Falk, D.A., Holsinger, K.E. (Eds.), *Genetics and Conservation of Rare Plants*. Oxford University Press, Nueva York.
- Jelinski, D.E., 1997. On genes and geography: a landscape perspective on genetic variation in natural plant populations. *Landscape and Urban Planning* 39, 11–23.
- Kalisz, S., Nason, J., Hanzawa, F.M., Tonsor, S.J., 2001. Spatial population genetic structure in *Trillium grandiflorum*: the roles of dispersal, mating, history, and selection. *Evolution* 55, 1560–1568.
- Kang, S.S., Chung, M.G., 1997. Spatial genetic structure in populations of *Chimaphila japonica* and *Pyrola japonica* (Pyrolaceae). *Annales Botanici Fennici* 34, 15–20.
- Kang, S.S., Chung, M.G., 2000. High levels of allozyme variation within populations and low allozyme divergence within and among species of *Hemerocallis* (Liliaceae). *American Journal of Botany* 87, 1634–1646.
- Knapp, E.E., Rice, K.J., 1996. Genetic structure and gene flow in *Elymus glaucus* (blue wildrye): implications for native grassland research. *Restoration Ecology* 4, 1–10.
- Knowles, P., 1991. Spatial genetic structure within two natural stands of black spruce (*Picea mariana* (Mill.) B.S.P.). *Silvae Genetica* 40, 13–19.
- Knowles, P., Grant, M.C., 1985. Genetic variation of lodgepole pine over time and microgeographical space. *Canadian Journal of Botany* 63, 722–784.
- Knowles, P., Perry, D.J., Foster, H.A., 1992. Spatial genetic structure in two tamarack (*Larix laricina* Du Roi K.Koch) populations with different establishment histories. *Evolution* 46, 572–576.
- Kudoh, H., Whigham, D.F., 1997. Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* (Malvaceae) populations. *American Journal of Botany* 84, 1285–1293.
- Le Corre, V., Roussel, G., Zanetto, A.K.A., 1998. Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. III.

- Patterns of variation identified by geostatistical analyses. *Heredity* 80, 464–473.
- Legendre, P., 1993. Spatial autocorrelation: trouble or new paradigm? *Ecology* 74, 1659–1673.
- Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69, 1–24.
- Legendre, P., Fortin, M.-J., 1989. Spatial patterns and ecological analysis. *Vegetatio* 80, 107–138.
- Legendre, P., Legendre, L., 1998. Numerical ecology. In: Anonymous. *Developments in Environmental Ecology*. Elsevier, Amsterdam.
- Leonardi, S., Raddi, S., Borghetti, M., 1995. Spatial autocorrelation of allozyme traits in a Norway spruce (*Picea abies*) population. *Canadian Journal of Forests Research* 26, 63–71.
- Levin, S.A., 1992. The problem of pattern and scale in ecology. *Ecology* 73, 1943–1967.
- Loikart, G., England, P.R., 1999. Statistical analysis of microsatellite DNA data. *Trends in Ecology and Evolution* 14, 253–256.
- Loiselle, B.A., Sork, V.L., Nason, J., Graham, C., 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82, 1420–1425.
- Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15, 65–95.
- Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3, 91–99.
- Maki, M., Masuda, M., 1993. Spatial autocorrelation of genotypes in a gynodioecious population of *Chionographis japonica* var. *kurohimensis* (Liliaceae). *International Journal of Plant Science* 154, 467–472.
- Maki, M., Masuda, M., 1994. Spatial genetic structure within two populations of a self-incompatible perennial, *Chionographis japonica* var. *japonica* (Liliaceae). *Journal of Plant Research* 107, 283–287.
- Maki, M., Yahara, T., 1997. Spatial structure of genetic variation in a population of the endangered plant *Cerastium fischerianum* var. *molle* (Caryophyllaceae). *Genes and Genetics Systems* 72, 239–242.
- Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27, 209–220.
- Martín, C., González-Benito, M.E., Iriondo, J.M., 1999. The use of genetic markers in the identification and characterization of three recently discovered populations of a threatened plant species. *Molecular Ecology* 8, S31–S40.
- McCue, K.A., Buckler, E.S., Holtzford, T.P., 1996. A hierarchical view of genetic structure in the rare annual plant *Clarkia springvillensis*. *Conservation Biology* 10, 1425–1434.
- McCune, B., 1997. Influence of noisy environmental data on canonical correspondence analysis. *Ecology* 78, 2617–2623.
- Merzeau, D., Comps, B., Thiébaud, B., Cuguen, J., Letouzey, J., 1994. Genetic structure of natural stands of *Fagus sylvatica* L. (beech). *Heredity* 72, 269–277.
- Michaud, H., Toumi, R., Lumaret, R., Li, T.X., Romane, F., Di Giusto, F., 1995. Effect of geographical discontinuity on genetic variation in *Quercus ilex* L. (holm oak). Evidence from enzyme polymorphism. *Heredity* 74, 590–606.
- Montalvo, A.M., Conard, S.G., Conkle, M.T., Hodgskiss, P.D., 1997. Population structure, genetic diversity, and clone formation in *Quercus chrysolepis* (Fagaceae). *American Journal of Botany* 84, 1553–1564.
- Moran, P.A.P., 1950. Notes on continuous stochastic phenomena. *Biometrika* 37, 17–23.
- Nei, M., 1973. Analysis of gene in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* 70, 3321–3323.
- Nesbitt, K.A., Potts, B.M., Vaillancourt, R.E., West, A.K., Reid, J.B., 1995. Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtaceae). *Heredity* 74, 628–637.
- Nevo, E., Beiles, A., Kaplan, D., Golenberg, E.M., Olsvig-Whittaker, L., Naveh, Z., 1986. Natural selection of allozyme polymorphisms: a microsite test revealing ecological genetic differentiation in wild barley. *Evolution* 40, 13–20.
- Oden, N.L., Sokal, R.R., 1986. Directional autocorrelation: an extension of spatial correlograms to two dimensions. *Systematic Zoology* 35, 608–617.
- Okland, R.H., Eilertsen, O., 1994. Canonical correspondence analysis with variation partitioning: some comments and an application. *Journal of Vegetation Science* 5, 117–126.
- Palmer, M.W., 1993. Putting things in even better order, the advantages of canonical correspondence analysis. *Ecology* 74, 2215–2230.
- Pannatier, Y., 1996. *VARIOWIN: Software for spatial Data Analysis in 2D*. Springer-Verlag, New York.
- Pavlik, B.M., 1996. Defining and measuring success. In: Falk, D.A., Millar, C.I., Olwell, M. (Eds.), *Restoring Diversity. Strategies for Reintroduction of Endangered Plants*. Island Press, Washington.
- Pavlik, B.M., Nickrent, D.L., Howald, A.M., 1993. The recovery of an endangered plant. I. Creating a new population of *Amsinckia grandiflora*. *Conservation Biology* 7, 510–526.
- Peakall, R., Beattie, A.J., 1995. Does ant dispersal of seeds in *Sclerolaena diacantha* (Chenopodiaceae) generate local spatial structure? *Heredity* 75, 351–361.
- Perry, D.J., Knowles, P., 1991. Spatial genetic structure within three sugar maple (*Acer saccharum* Marsh.) stands. *Heredity* 66, 137–142.
- Piazza, A., Menozzi, P., Cavalli-Sforza, L., 1981. The making and testing of geographic gene-frequency maps. *Biometrics* 37, 635–659.
- Pigliucci, M., Barbujani, G., 1991. Geographical patterns of gene frequencies in Italian populations of *Ornithogalum montanum* (Liliaceae). *Genetical Research* 58, 95–104.
- Podani, J., 1989. New combinatorial clustering methods. *Vegetatio* 81, 61–77.
- Podani, J., Czarán, T., 1997. Individual-centered analysis of mapped point patterns representing multispecies assemblages. *Journal of Vegetation Science* 8, 259–270.
- Reusch, T.B.H., Hukriede, W., Stam, W.T., Olsen, J.L., 1999. Differentiation between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity* 83, 120–126.
- Ripley, B.D., 1976. The second-order analysis of stationary processes. *Journal of Applied Probability* 13, 255–266.
- Rossetto, M., Weaver, P.K., Dixon, K.W., 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). *Molecular Ecology* 4, 321–329.
- Rossi, R.E., Mulla, D.J., Journal, A.G., Franz, E.H., 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. *Ecological Monographs* 62, 277–314.
- Sarthou, C., Samadi, S., Boisselier-Dubayle, M.-C., 2001. Genetic structure of the saxicole *Pitcairnia geyskesii* (Bromeliaceae) on inselbergs in French Guiana. *American Journal of Botany* 88, 861–868.
- Schaal, B.A., 1975. Population structure and local differentiation in *Liatris cylindracea*. *The American Naturalist* 109, 511–528.
- Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M., Bishop, J.G., 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75, 584–606.
- Schnabel, A., Hamrick, J.L., 1990. Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* 77, 1060–1069.
- Schnabel, A., Laushman, R.H., Hamrick, J.L., 1991. Comparative genetic structure of two cooccurring tree species, *Maclura pomifera* (Moraceae) and *Gleditsia triacanthos* (Leguminosae). *Heredity* 67, 357–364.
- Shapcott, A., 1995. The spatial genetic structure in natural populations of the Australian temperate rainforest tree *Atherosperma moschatum* (Labill.) (Monimiaceae). *Heredity* 74, 28–38.
- Slatkin, M., 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16, 393–430.
- Slatkin, M., Arter, H.E., 1991. Spatial autocorrelation methods in population genetics. *The American Naturalist* 138, 499–517.

- Smouse, P.E., Peakall, R., 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* 82, 561–573.
- Sokal, R.R., Jacquez, G.M., Wooten, M.C., 1989. Spatial autocorrelation analysis of migration and selection. *Genetics* 121, 845–855.
- Sokal, R.R., Oden, N.L., 1978. Spatial autocorrelation in biology. I. Methodology. *Biological Journal of the Linnean Society* 10, 199–228.
- Sokal, R.R., Oden, N.L., Thomson, B.A., 1997. A simulation study of microevolutionary inferences by spatial autocorrelation analysis. *Biological Journal of the Linnean Society* 60, 73–93.
- Stehlik, I., Holderegger, R., 2000. Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of Central Europe. *Journal of Ecology* 88, 424–435.
- Streiff, R., Labbe, T., Bacilieri, R., Steinkellner, H., Gloessl, J., Kremer, A., 1998. Within-population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assessed with isozymes and microsatellites. *Molecular Ecology* 7, 317–328.
- Tani, N., Tomaru, N., Tsumura, Y., Araki, M., Ohba, K., 1998. Genetic structure within a Japanese stone pine (*Pinus pumila* Regel) population on Mt. Aino-Dake in central Honshu, Japan. *Journal of Plant Research* 111, 7–15.
- ter Braak, C.J.F., 1986. Canonical correspondence analysis, a new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67, 1167–1179.
- ter Braak, C.J.F., 1987. CANOCO—a FORTRAN program for canonical community analysis by correspondence analysis, principal components analysis, and redundancy analysis. (2.1). Agricultural Mathematics Group, Wageningen.
- ter Braak, C.J.F., 1990. Update notes: CANOCO version 3.1. Microcomputer Power, Ithaca.
- ter Braak, C.J.F., Prentice, I., 1988. A theory of gradient analysis. *Advances in Ecological Research* 18, 271–317.
- Torres, M.E., 1999. Estudios de la autoecología, biología reproductiva y estructura genética de *Antirrhinum microphyllum* Rothm. (*Scrophulariaceae*). Evaluación de su estado de conservación. PhD Thesis, Universidad Politécnica de Madrid, Madrid.
- Turkington, R., Harper, J.L., 1979. The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture: IV: Fine scale biotic differentiation. *Journal of Ecology* 67, 245–254.
- Ueno, S., Tomaru, N., Yoshimaru, H., Manabe, T., Yamamoto, S., 2000. Genetic structure of *Camellia japonica* L. in an old-growth evergreen forest, Tsushina, Japan. *Molecular Ecology* 9, 647–656.
- Van Damme, J.M.A., 1986. Gynodioecy in *Plantago lanceolata* L. V. Frequencies and distribution of nuclear and cytoplasmic genes. *Heredity* 56, 355–364.
- Verdonschot, P.F.M., ter Braak, C.J.F., 1994. An experimental manipulation of oligochaete communities in mesocosms treated with chloropyrifos or nutrients additions: multivariate analyses with Monte Carlo permutation tests. *Hydrobiologia* 278, 251–266.
- Wagner, D.B., Sun, Z.X., Govindaraju, D.R., Dancik, B.P., 1991. Spatial patterns of chloroplast DNA and cone morphology variation within populations of a *Pinus banksiana*-*Pinus contorta* sympatric region. *The American Naturalist* 138, 156–170.
- Waser, N.M., 1987. Spatial heterogeneity in a population of the montane perennial plant *Delphinium nelsonii*. *Heredity* 58, 249–256.
- Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1358–1370.
- Williams, C.F., 1994. Genetic consequences of seed dispersal in three sympatric forest herbs. II. Microspatial genetic structure within populations. *Evolution* 48, 1959–1972.
- Williams, C.F., Waser, N.M., 1999. Spatial genetic structure of *Delphinium nuttallianum* populations: inferences about gene flow. *Heredity* 83, 541–550.
- Williams, C.G., Hamrick, J.L., 1996. Elite populations for conifer breeding and gene conservation. *Canadian Journal of Forests Research* 26, 453–461.
- Wright, S., 1943. Isolation by distance. *Genetics* 28, 114–138.
- Xie, C.Y., Knwoles, P., 1991. Spatial genetic substructure within natural populations of jack pine (*Pinus banksiana*). *Canadian Journal of Botany* 69, 547–551.
- Young, A.G., Brown, A.H.D., 1996. Comparative population genetic structure of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Conservation Biology* 10, 1220–1228.