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Spatial scales in the genetic diversity of allogamous *Antirrhinum microphyllum* Rothm. (*Scrophulariaceae*)

Abstract

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The relevance of spatially explicit studies of genetic diversity in threatened taxa along with the importance of understanding that biological proccesses and environmental factors that determine the genetic structure is examplified in the study case of *Antirrhinum microphyllum* Rothm. (*Scrophulariaceae*) an endangered narrow endemic of Central Spain. The approach at a regional scale including all known populations was based in allozyme analysis of 184 individuals (46 individuals per population) and showed little differentiation among populations. In a second step Bolarque population was approached through RAPD analysis. The study showed that the population was genetically structured in neighborhoods. Pollen transport may be responsible for the maintenance of genetic vicinities whereas sporadic long-distance seed dispersal is likely to explain the relatedness among certain patches. All these features carry out important implications for conservation.

Introduction

The knowledge of the spatial genetic structure of populations, that is, the amount and organization of genetic variation in space, is considered to be of prime interest by conservation biologists for the development of long-term conservation and management plans for rare or threatened species (Barrett & Kohn 1991; Fenster & Dudash 1994; Guerrant 1996; Dunham & al. 1999).

Spatial genetic structure results from the combined action of several factors such as microhabitat heterogeneity, mating system, seed and pollen dispersal, genetic drift or natural selection (Loveless & Hamrick 1984; Heywood 1991). Historical events such as founder events, occurrence of bottlenecks, or range expansions following climatic change are other possible sources of spatial patterns (Whitlock & McCauley 1990). Since there are so many environmental and biological processes that conform the spatial genetic structure, and they operate at different spatial scales, even in opposite directions, it becomes very difficult to predict under particular circumstances how genetic variation is distributed in

space. Therefore, as a first approach, it is advisable to study the spatial distribution of genetic diversity before implementing any conservation strategy.

Once pattern has been detected and described, it is possible to infer the cause of pattern, and the mechanisms that have generated and maintained it, through manipulative experiments (Legendre & Legendre 1998). The understanding of the mechanisms that control the genetic distribution of a particular species may provide insight on the genetic consequences of changes in the habitat of natural origin or due to human intervention, an aspect that is imposible to study just by correlation alone (Levin 1992). Moreover, this understanding is also of great relevance at the time of evaluating and adopting measures of conservation.

A first aspect in the study of spatial patterns is to choose the correct scale of description. What is an 'appropriate' scale depends in part on the questions one is searching for an answer, and thus on the underlying process (Wiens 1989). For instance, the effect of genetic drift can not be detected in an area of a few square meters, whereas it is possible find local genetic structure generated by limited seed dispersal at this narrow spatial scale. Therefore, firstly, it is neccesary to define the *sampling extent*, that is, the overall area included in a study, which must be comparable to the scale of the phenomenon under study. Secondly, the *grain size*, the size of the elementary sampling units, must also be adequate. It should be large enough to avoid the noise of stochasticity whereas it must be small enough so that structures and patterns do not get masked. In some cases, patchiness has been found at short distances, for instance 1-4 m in *Ipomopsis aggregata* (Campbell & Dooley 1992) and *Sanicula odorata* (Williams 1994), but in other cases, stochastic phenomena may make the systems of interest unpredictable at this fine spatial scale. In contrast, a greater grain size may mask the pattern. Differences among organisms (e.g. size, type of pollinators, ecological range, etc.) also affect the scale of investigation.

A second aspect is to realize that different processes in the same system may be taking place at different scales. Thus, it is important to have in mind that no single mechanism is likely to explain the pattern at all scales. This also means that conclusions derived from one spatial scale cannot be extrapolated to other scales and, that, therefore, it may not be sufficient to examine a particular system at only one spatial scale.

In this paper, we show how different patterns of genetic diversity in *Antirrhinum microphyllum* can be detected and how the action of different biological processes can be inferred depending on the scale of observation. Our main goal is to know whether the genetic diversity of this species appears structured, and to determine the spatial scale where this structure can be detected. Furthermore, if a spatial structure is present we are interested in describing the shape and directionality of existing patterns and in formulating hypothesis on the biological processes and environmental factors that may be operating.

Study Species

Antirrhinum microphyllum Rothm. (*Scrophulariaceae*) is a herbaceous perennial that grows on small crevices of calcareous and dolomite vertical cliffs, and constitutes part of chasmophytic communities of *Antirrhinetum microphylli* Fdez.-Casas (Fernández-Casas 1974). Flowering takes place over a 10-week period from mid-March to end-June, and

plants bear on average 47 capsules, each containing an average of 199 small seeds (Torres & al. in press). Manual crossings have shown that plants are self-incompatible (Torres & al. 2002), and observations in the field indicate the flowers are pollinated mainly by *Rhodanthidium sticticum*, a solitary bee (Torres & al. 2001).

Although not locally rare, its area of distribution is very reduced, aproximately 30 Km^2. Thus it has been classified as "vulnerable" (Gómez-Campo 1987; VVAA 2000) according to IUCN criteria, and it is protected by the regional legislation of Junta de Castilla-La Mancha (DOCM 1998). To date, only four populations are known, all of them located in the northern half of Sierra de Altomira (Central Spain). The most distant populations, Entrepeñas and Bolarque, are just 15 km apart. The nearest population of *A. pulverulentum*, which is a related taxon widely distributed in the eastern half of the Iberian Peninsula, appears at Durón, a narrow gorge located 20 Km north of the Entrepeñas population.

Spatial genetic structure at a regional scale

In order to know whether the genetic diversity of *A. microphyllum* was structured at a regional scale including all known populations, we used allozyme markers. Specifically, we sampled a total of 184 individuals from all four populations, and evaluated 13 allozyme loci (details in Torres 1999), six of which were polymorphic using the 0,95 criterion. Firstly, we used a χ^2 test to detect heterogeneity in allele frequency distribution among populations for each polymorphic locus (Workman & Niswander 1970). Secondly, we calculated Wright's F statistics to compare the rate of inbreeding at the individual level with regard to the population (F_{IS}) and that of the populations with respect to the species (F_{ST}). F_{ST} statistic which is a measure of population differentiation, ranges between 0 and 1. In general, F_{ST} values of 0,05-0,15 indicate moderate differentiation whereas values > 0.15 indicate great genetic differentiation between populations (Wright 1965). It is neccessary to take into account that F_{ST} is a parameter which summarizes the evolutionary history of the populations under study. So it yields insights about the relative importance of gene flow and genetic drift in an evolutionary time scale. Finally, genetic divergence among populations was estimated by calculating Nei´s genetic distance (Nei 1972) for each pair of populations.

Populations tended to share most alleles observed for most of the polymorphic loci. However, there were significant differences in allele frequencies among populations for four of the six tested loci. Similarly, mean F_{ST} value (0,069) and the range of genetic distances (0,013-0,054) also evidence moderate genetic structure at the species level. Taking into account that the mean distance among populations (8,5 Km) is too large when compared to average flight distances from the main pollinator and mean distances of seed dispersal, we expected greater differentiation among them, at least between Entrepeñas and Bolarque (the most distant populations). A possible explanation is that the populations have become isolated relatively short time ago, and that there has not been enough time for differentiation through genetic drift.

On the other hand, the analysis of F_{IS} statistic for each polymorphic locus in each population showed that in 78% of the cases genotype frequencies did not depart from Hardy-Weinberg equilibrium (value of F was not significantly different from 0). In all cases were

there was departure from equilibrium the value of F was greater than 0, indicating a deficiency of heterozygotes. These results suggest that populations of *A. microphyllum* are genetically structured.

Spatial genetic structure within populations

Spatial inferences based on F_{ST} are limited because structure cannot be detected at spatial scales smaller than the subdivision size (in our case below population level) (Heywood 1991). Therefore, in order to obtain more detailed information about the genetic structure within populations, we studied the distribution of genetic diversity in Bolarque population through the use of semi-variograms, a spatial autocorrelation technique. In this case we characterized the individuals of the population through the random amplified polymorphic DNA (RAPD) technique (Williams & al. 1990) since we were not be able to discern each individual with allozymes. DNA extraction and amplification conditions are described in Torres (1999). RAPD data were interpreted as a phenotype. A matrix was built, where in each row one individual was characterized by a vector of 1s and 0s which indicated the presence or absence respectively of a set of molecular markers studied. The multidimensional genetic information of each individual (basic genetic matrix) was summarized in new consensus and continuous variables generated by means of a Principal Component Analysis (PCA). The values for each individual of the first extracted axis were used to build up omnidireccional semi-variograms (Legendre & Fortin 1989). The semi-variogram represents the values of semi-variance between all pairs of individuals belonging to a given spatial distance class. In order to test the effects of spatial scale several variograms were produced which differed in the extent and the lag size.

Some of the semi-variograms obtained are presented in Fig. 1. Figs 1a and 1b show the spatial pattern with a 300 m extent and 50 and 15 m lag intervals respectively, whereas in Fig. 1c a 25-m extent and a 5 m lag interval has been used. It can be observed that when the extent is kept constant, an increase in the lag size decreases spatial variance and maskes finer spatial structure (Figs 1a and 1b). Thus, using an extent of 300 m, we detected a weak spatial genetic structuring when 50 m intervals were considered (Fig. 1a), but evidence of patchiness derived from a bumping pattern is detected at 15 m intervals (Fig. 1b). Bolarque and likely the rest of the populations are structured in patches within which mating is approximately random but between which mating is less frequent. This pattern, which was previously suggested by the results obtained with allozymes, is consistent with the observations of the flying movements of *Rhodanthidium sticticum*, the main pollinator of *A. microphyllum.* Movements of female individuals are mainly between close plants. In adittion, males of *R. sticticum* have a territorial behavior. They occupy areas of a few square meters and patrol to keep them free from other males and insects while females collect pollen. Moreover, limited gene flow due to predominant short-distance seed dispersal is an additional factor that contributes to the patchy structure.

According to Fig. 1b individuals separated between 82,5 m and 97,5 m (the 90 m lag) are in average genetically as similar between them as those individuals separated less than 22,5 m. The same thing happens to individuals separated between 152,5 m and 167,5 m (the 160 m lag). This suggests that gene flow is at certain occasions less limited than pre-

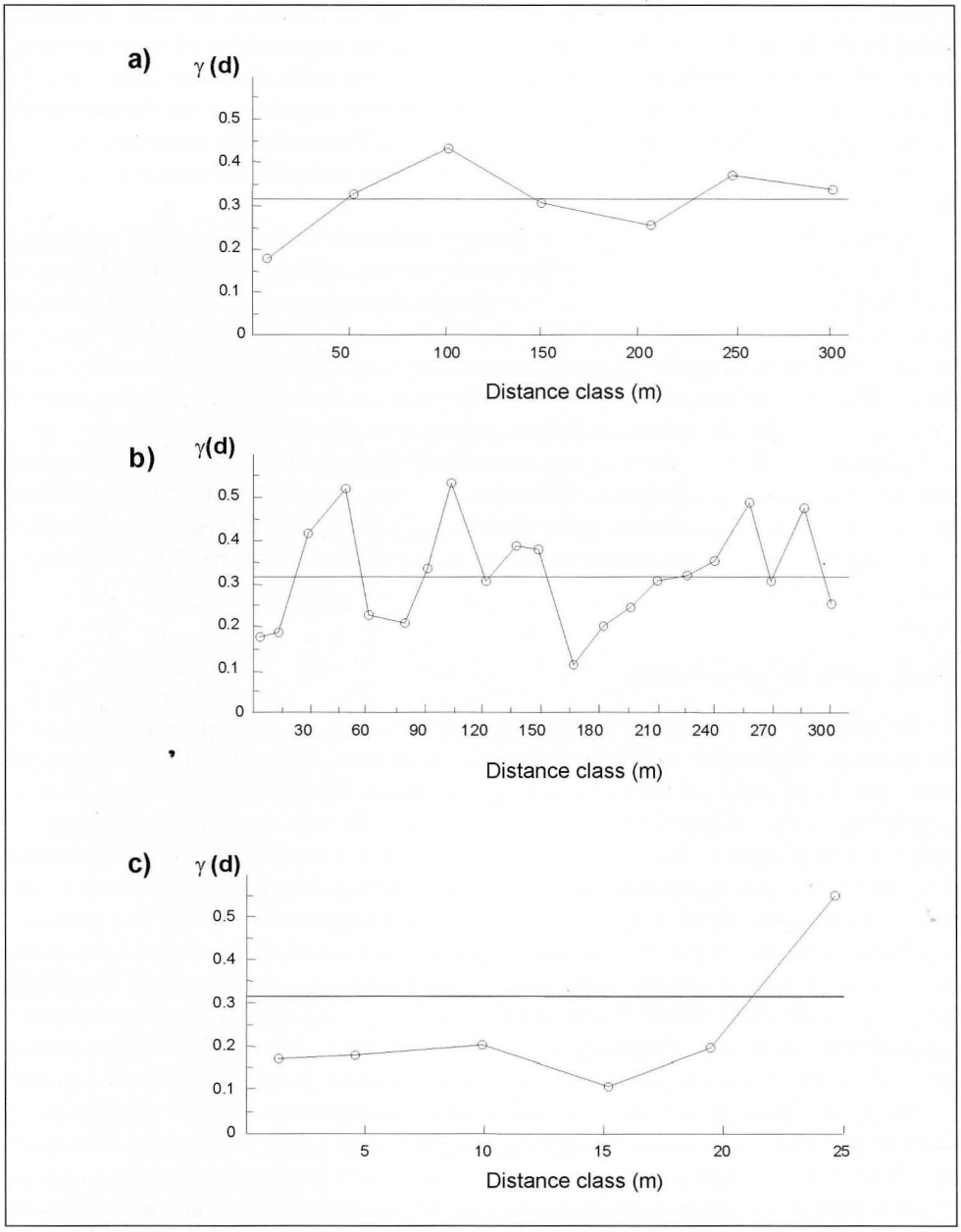

Fig. 1. Semivariograms showing spatial genetic structure of *Antirrhinum microphyllum* at the Bolarque population at different scales. a) Extent: 300 m and lag size: 50 m; b) Extent: 300 m and lag size: 15 m; c) Extent: 25 m and lag size: 5 m. **γ (d)** represents semivariance of the synthetic genetic variable obtained from RAPD data with the first extracted component of a PCA for each distance class. Data are plotted at the midpoint of distance classes. Horizontal dotted line indicates average semivariance.

viously suggested and that seed dispersal may not be restricted to short distances. Considering the small size of seeds (0,5-0,8 mm) and the large number of seeds produced by each plant (circa 9000), it is highly probable that a few seeds, dispersed relatively long distances by strong winds, originate new patches. Further experiments for the estimation of seed dispersal distance and the establishment of seedlings, and the characterization of pollen transport may help discriminate the importance of each of these processes at different scales.

Figure1c shows the pattern of genetic diversity at short distances (below 27,5 m). In this case, two ideas are worthy to note. First, the semi-variance of individuals separated by less than 22,5 m is lower than the average indicating that plants located in close spatial proximity tend to be genetically more alike than individuals separated at greater distance. Second, there is no evidence of spatial autocorrelation between plants separated less than 20 m. Thus, we can consider this distance is the average diameter of the existing genetic neighborhoods or patches at the Bolarque population of *A. microphyllum*.

Therefore, both pollen and seed flow condition spatial genetic patterns in *A. microphyllum* but their influence extends to different scales: the genetic similarity within patches seems to be mainly controlled by pollen flow whereas long-distance seed dispersal is likely to be responsible for the genetic similarity detected among some patches at a broader scale.

Implications for conservation

The study of spatial genetic variation at different scales has multiple implications for conservation. Depending on the scale of observation used, different conclusions may be inferred and as a consequence, different strategies may be adopted. The degree of isolation among populations may be important in deciding upon the appropiate geographic scale at which to pursue conservation efforts. The weak genetic differentiation among populations detected by allozyme analysis implies that no particular considerations can be made to any of the existing populations and that it is best to treat the species as a whole. Furthermore, in genetic terms the extinction of one of the populations would only represent a comparatively minor loss with respect to the total diversity of the species. However, the strong genetic differentiation found within populations shows the existence of characteristic genetic vicinities in each of the studied populations. The low genetic differentiation among populations found at the regional scale could lead us to think that there is an intense genetic flux among populations. However, the genetic structure found within populations is indicating the existence of restrictions in genetic flux even within populations. Therefore, the small differentiation among populations must be taking place through genetic drift. Conservation and management actions taken at the population level should be well aware of the presence of a genetic structure. For instance, seed collection in Bolarque population of *A. microphyllum* should be made at least at 25 m intervals (the average size of the genetic vicinity) in order to avoid the sampling of close relatives and to maximize the genetic diversity of the sample. Under these highly structured populations *in situ* management plans should take measures to preserve all different patches or microhabitats that make up the population. Contrarily to what was observed at the species level, at the within-popula-

tion scale, the loss of particular patches could seriously decrease the total genetic diversity of the population.

In *A. microphyllum* most pollination events take place among individuals of the same genetic vicinity and thus this situation could be thought to lead to inbreeding depression. However, the patchy structure of genetic diversity does not seem to affect reproductive success in a significant way, since present reproductive output in all populations studied is very high. The existence of a genetic self-incompatibility mechanism with a rich S-allele system may be responsible for maintaining the genetic vicinities away from inbreeding depression. The presence of a patchy genetic structure in small populations can limit reproductive success in other species and consequently may be one of the key factors conditioning the viability of their populations. Nevertheless, serious considerations must be made before any disruption or intervention of the current genetic structure of a population is approached. It has been shown that the existence of spatial structuring in large populations facilitates the generation and maintenance of high levels of variability with which to survive stochastic events or adjust to novel fluctuating environments (Huenneke 1991). Therefore, the recommended conservative approach is to preserve the existing structure of the populations unless evident signs of demographic collapse (inbreeding depression, lack of regeneration) are present in depauperated populations.

Concluding remarks

Although we have concentrated our attention in this paper on one particular example, the discussions that are presented here have wide application in plant conservation genetics. The main thought is that the study of genetic diversity should ideally be approached at different scales, in order to detect all existing spatial genetic structures and be able to interpret the underlying biological processes and environmental factors responsible for the structures. When this is not possible or when the genetic studies are oriented to find answers to a particular problem, great care should be taken to set the study at the appropriate scale, defining thoughtfully both the overall area included and the size of the elementary sampling units.

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