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Seed gene flow and fine-scale structure in a Mediterranean pine (*Pinus pinaster* Ait.) using nuclear microsatellite markers

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Abstract The Mediterranean populations of maritime pine (*Pinus pinaster* Ait.) are typically small and have a scattered distribution, being threatened by human activities and forest fires. In the framework of the genetic-resources conservation program of this species, a native multi-age stand located in a Mediterranean area (central Spain) was studied using three highly polymorphic nuclear microsatellites (SSRs). Spatial autocorrelation analysis was conducted using Moran's index in order to detect fine-scale structure in both natural regeneration and mature trees. The spatial pattern of seed flow based on dispersed progeny was studied using a highly reliable subset of parent-offspring matches obtained by means of parentage analysis and simulation-based calculation of statistical confidence. Maritime pine showed a fine-scale structure at the seedling stage. In natural regeneration, the autocorrelograms indicated a patch size of approximately 10 m. The fine-scale structure seems to be produced by a restricted seed gene flow. In fact, there was an excess of parent-offspring matches in a radius of 15 m from the parent trees. Pines with a heavy seed, such as *P. pinaster*, are expected to have a short dispersal distance, thus producing a fine-scale structure. However, the fine-scale structure did not persist in the mature trees. Within-population genetic structure in Mediterranean pines may be affected by a number of post-dispersal events (e.g. mortality due to the severity of the Mediterranean climate and animal-mediated secondary dispersal during the summer period). Thus, great alteration in the pattern produced by the initial seed rain and differences in genetic structure between tree cohorts are expected.

Keywords LOD-scores · SSRs · Genetic conservation · *Pinus pinaster* · Iberian Peninsula

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Introduction

Classical theoretical models indicate that restricted gene flow and preferential mating by proximity result in genetic isolation by distance and within-population genetic structure. Fine-scale genetic structure (i.e. spatial clustering of like genotypes in small patches) may become established after only a few generations (Epperson 1995). The evolutionary and ecological importance of fine-scale structure in plant populations has been highlighted because it influences effective population size and, consequently, population dynamics. Such a structure has a central role in adaptation to microenvironmental variation occurring within populations (Levin and Kerster 1974; Schnabel et al. 1998). In addition, within-population genetic structure has been shown to decrease the equilibrium frequencies of embryonic lethals in species that allow partial self-pollination (Ronfort and Couvet 1995; Hedrick et al. 1999), thus having significant evolutionary consequences (Charlesworth and Charlesworth 1987). The classical evidence of isolation by distance in continuous plant populations is generally expressed in terms of average or expected inbreeding coefficients, or by indirect measures of gene dispersal such as seed or pollen dispersion (Levin and Kerster 1974; Levin 1981; Campbell 1991). In addition, two main approaches have been developed based on directly observable results using molecular markers: (1) the study of spatial distri-

bution of alleles or genotypes (Heywood 1991; Epperson 1992), and (2) the direct estimation of gene movement by means of paternity or parentage analysis (Schnabel 1998; Sork et al. 1999). Many studies, both in temperate (Schnabel et al. 1991; Dow and Ashley 1996; Latta and Mitton 1997; Ledig 1998) and tropical forest species (Hamrick et al. 1993; Boshier et al. 1995), have reported that pollen dispersal is often the major contributor to gene flow, and that spatial genetic structure is mainly a result of limited seed dispersal. In particular, restricted seed flow is important in determining the population structure of wind-dispersed trees where the difference between seed and pollen mass is normally great.

Most published studies to-date have used allozyme markers for gene dispersal estimation (e.g. Meagher 1986; Boshier et al. 1995; Schnabel and Hamrick 1995). However, the recent development of codominant, highly polymorphic microsatellite markers (Morgante and Olivieri 1993; Powell et al. 1996) and the high resolution obtained with these markers in the estimation of relationships between individuals (Blouin et al. 1996; Gerber et al. 2000), have recently produced several studies of gene dispersal in both animal (Jones and Avise 1997; Taylor et al. 1997; Fontaine and Dodson 1999) and plant species (Chase et al. 1996; Dow and Ashley 1998; Sampson 1998; Streiff et al. 1999). For plant species, genetic markers have mostly been used to measure effective pollen dispersal and relative male fertilities by means of paternity analysis (Meagher 1991; Adams et al. 1992; Smouse and Meagher 1994; Streiff et al. 1999; Schuster and Mitton 2000). However, some authors have suggested that successful pollination by a foreign gamete may not be equivalent to successful establishment of that gamete's genes in the local gene pool (Schnabel and Hamrick 1995; Dow and Ashley 1996). In addition, seeds resulting from gene flow could be less inbred than those produced by local mating and, therefore, may be favoured in establishment (Levin 1981). In plant species, published reports of gene dispersal based on established individuals are restricted to only a few species, some of them dioecious and insect-pollinated (*Chamaelirium luteum*, Meagher and Thompson 1987; *Gleditsia triacanthos*, Schnabel and Hamrick 1995) and others monoecious and wind-pollinated (*Pinus sylvestris*, Yazdani et al. 1989; *Quercus macrocarpa*, Dow and Ashley 1996). Using a parentage approach based on dispersed progeny to study gene dispersal may be particularly interesting in *Pinus* species where seed production is usually great and strong selection at early stages of development is expected (Lanner 1998).

Maritime pine (*Pinus pinaster* Ait.) is a highly outcrossed conifer with a wide distribution in the western Mediterranean Basin. Maritime pine has high economical value in its Atlantic area of distribution (Portugal, Galicia and Landes) and has been successfully cultivated worldwide (Australia, South Africa and New Zealand, mainly). The Mediterranean populations of this species are typically small and have a scattered distribution, being threatened by human activities and forest fires. In the Iberian Peninsula, seed production and the life-history strategy of maritime pine are related to forest fire regimes (intensity, frequency, size and spatial distribution). Stands with recurrent fires show serotinous cones and an important canopy seed bank, whereas serotinous cones and seed production are scarce in non-burned areas (Tapias et al. 2001). Genetic variation patterns across the native range of the species are quite complex (Baradat and Marpeau 1988; Vendramin et al. 1998; Salvador et al. 2000). In the Iberian Peninsula, seven geographical races included in three main groups (Atlantic, Mediterranean and Maghrebian) have been distinguished (Baradat and Marpeau 1988). In addition, genetic variation is arrayed clinally in the southeastern portion of the Iberian Peninsula, while a lack of genetic structure in the northwestern portion of the Peninsula has been reported (Salvador et al. 2000; Ribeiro et al. 2001). No geographic genetic pattern was found in the Portuguese populations of maritime pine, as a consequence of human activities during the last century and extensive gene flow among populations (Ribeiro et al. 2001). Although the geographic patterns of gene variation in this species are well studied, gene dispersal and the fine-scale structure of gene variation within populations are not well understood.

The aim of this work is to investigate gene flow via seed within a maritime pine central-range population and its influence on fine-scale genetic structure. The study of dispersal patterns in maritime pine is also useful in understanding the wide-range geographic structure of the species, since they influence the process of genetic differentiation due to drift or differential selection within populations.

Materials and methods

Plant material

The study was situated in a native stand of maritime pine located in Coca, central Spain (Fig. 1). The climate here is dry Mediterranean, with an annual average rainfall of 432 mm (only 72 mm in summer) and an average annual temperature of 12.3 °C. The floristic community within the stand is composed of typical Mediterranean grasses [*Corynephorus canescens* (L.) P. Beauv. and *Stipa* spp.], patches of shrubs [*Retama sphaerocarpa* (L.) Boiss., *Lavandula stoechas* L. and *Thymus mastichina* L.] and isolated individuals of stone pine (*Pinus pinea* L.). In the study plot, seed dispersion is concentrated in the summer period (from June to September) and a low number of serotinous cones has been found (approximately 2%). The study plot, 50 m in radius, was located in a flat sandy area, that had not been disturbed by silviculture treatments. The spatial position of all mature maritime pine trees within the plot (n = 76), including two old-growth individuals (<120 years), was recorded. In addition, all seedlings and saplings taller than 20 cm within a central subplot of 25 m were recorded (n = 132) (Fig. 1).

Molecular markers

Genomic DNA was extracted from needles using a modified protocol from Dellaporta et al. (1983), and assayed using three nucle-

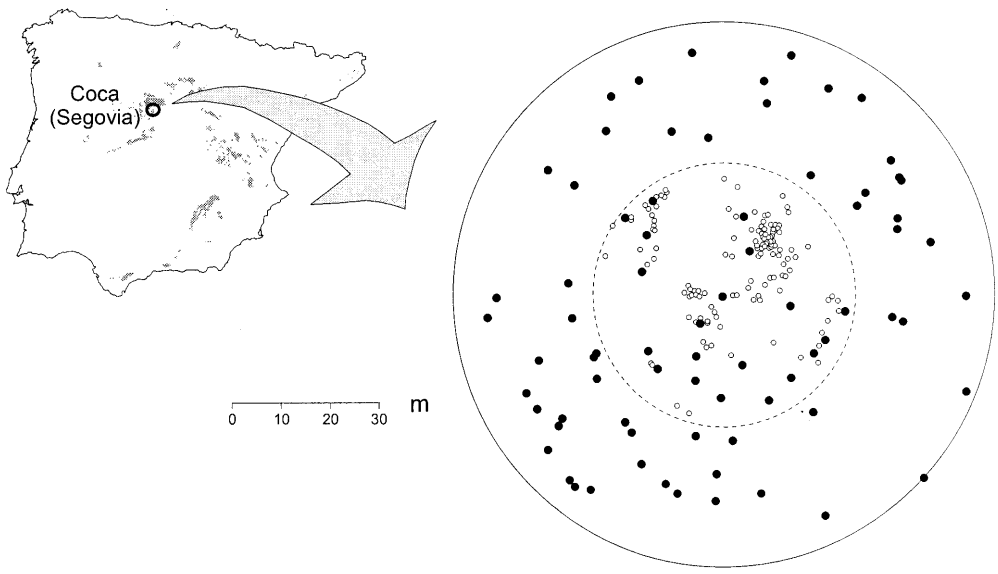

Fig. 1 Location of the study plot and spatial distribution of mature trees (*circles*) and offspring (*open circles*) within the plot

Table 1 Description of the three nuclear SSRs used in this study (n = 208). H_e and H_o are the expected and observed heterozygosities, respectively

Locus	Primers (5′→ 3′)	SSR sequence	Length of PCR product	Number of alleles	H_e	H_o
FRPP91	F:GTACTCCCACATAAAATGAGACTT R:CCGAAATACATTGCAGGTTA	$(CT)_{20}$	168	22	0.910	0.918
FRPP94	F:GGCAAACCTCTTTTAGAGTGC R:TTTGTCGATTTTTCTTGAAATCTAA	$(GT)_{22}$	162	8	0.730	0.716
ITPH4516	F:TGATGCAAACAAGTTCCATG R:AGCACTCGCTAAACTATGAAGG	$(CT)_{27}$	159	13	0.891	0.904

ar microsatellites (SSRs; Table 1). One primer pair (ITPH4516) was transferred from *Pinus halepensis* Mill. whereas the other two (FRPP91 and FRPP94) were specifically developed for *P. pinaster* by Mariette et al. (2001). One of the loci (FRPP94) was scored using a LI-COR 4000 automatic sequencer (LI-COR Inc., Nebraska, USA) following protocols extensively described in Mariette et al. (2001). The other two SSRs (FRPP91 and ITPH4516) were analysed using the following protocol. The amplification was carried out in a Perkin Elmer GeneAmp PCR system 9600, using 0.5 U of *Taq* Polymerase (Perkin Elmer), and 20 ng of DNA in a final volume of 10 µl containing 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 0.2 mM of each dNTP, 2 µM of each primer and 5% DMSO to enhance amplification. The concentrations of $MgCl_2$ were 1.5 mM for ITPH4516 and 2 mM for FRPP91. Forward primers were labelled with $^{33}\gamma$P-ATP. PCR amplifications were carried out using the following cycling parameters: 5 min at 94 °C, followed by one cycle of: 30 s at 94 °C, 30 s at 63 °C, 45 s at 72 °C, then 19 cycles in which the annealing temperature decreases 0.1 °C per cycle, followed by 15 cycles of 30 s at 94 °C, 30 s at 61 °C and 45 s at 72 °C, and a final extension of 5 min at 72 °C. The amplified fragments were denatured by adding 20 µl of formamide buffer (98% formamide, 10 mM of EDTA pH 8.0, 0.05% bromophenol blue and 0.05% xylene cyanol), heated for 3 min at 94 °C, and 1.5 µl of each sample were loaded on 6% acrylamide/bisacrylamide (19:1), 7.5 M urea and 1 × TBE gels. Gels were dried and exposed to X-ray films for 1–3 days. Allele sizes were determined by comparison to known sequences as well as to a 100-bp standard marker (Gibco BRL). Additionally, four previously scored individuals were run on all gels to ensure gel-to-gel consistency. The three nuclear SSRs included in this work are located in different linkage groups (FRPP91 in group 9, FRPP94 in group 5 and ITPH4516 in group 3) according to the genetic linkage map constructed by Costa et al. (2000).

Data analysis

Spatial autocorrelation

Spatial autocorrelation analysis was used to detect within-population structure in both offspring and parent populations. Moran's index was computed for diploid multilocus genotypes based on unordered alleles (identity in state). Microsatellites interpreted as unordered alleles have been shown to exhibit a stronger spatial structure than microsatellites interpreted as ordered alleles (Streiff et al. 1998). In addition, Moran's index computed at the individual level provides an estimator of Wright's coefficient of relationship as shown by Hardy and Vekemans (1999). Distance classes (k) with an interval of 5 m were chosen in order to allow more than 30 pairs of data in each class. Ten classes of distance were used for offspring, 2.5 (0–5 m) to 47.5 (45-50 m), and 20 for parent trees, 2.5 (0–5 m) to 97.5 (95–100 m). For each allele with a frequency higher than 5%, Moran's index, I_k, was calculated as follows:

$$I_k = \frac{\mathbf{n}\sum_{i=1}^{n}\sum_{j\neq i}^{n} W_{ij}(x_i-\bar{x})(x_j-\bar{x})}{W\sum_{i=1}^{n}(x_j-\bar{x})^2} \qquad W=\sum_{i=1}^{n}\sum_{j\neq i}^{n} W_{ij},$$

where n is the total number of individuals; w_{ij} equals 1 if the individuals i and j are both in the spatial class k, otherwise being equal to 0; the x_i value is 1 or 0.5 if the tree i is homozygous or heterozygous for that allele, respectively, otherwise x_i = 0 and $\bar{x}$ is the overall mean value of x_i. Autocorrelation over all the loci was calculated summing the numerator and denominator of the first equation over the total number of alleles (Streiff et al. 1998). The values of I_k for each k class were compared with a random distri-

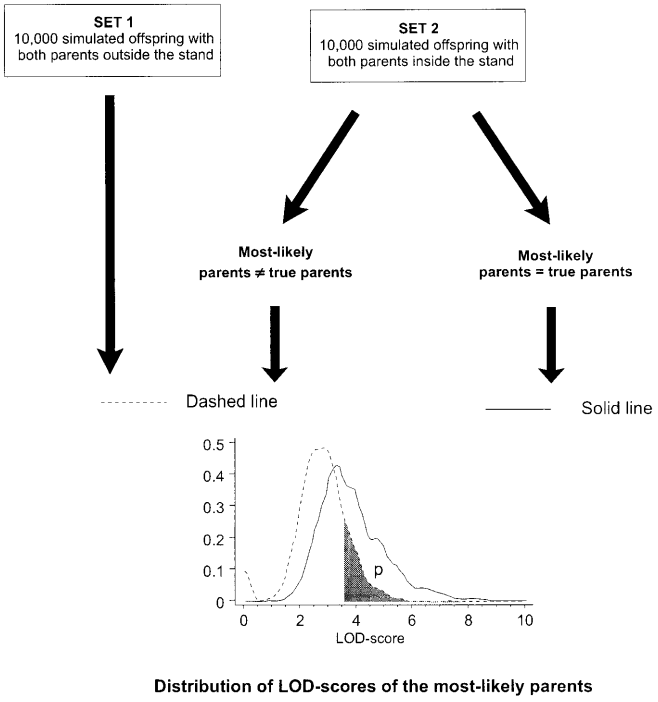

Fig. 2 Flow-chart illustrating the simulations conducted in order to compute probability levels for each single parent-offspring match

bution obtained by 1,000 permutations of the spatial co-ordinates of the trees in order to test for significant spatial structure.

Parentage analysis

The most-likely parents and parent pairs were detected by means of log-likelihood ratios or LOD-scores (Meagher and Thompson 1986; Gerber et al. 2000) using population allele frequencies estimated from the whole set of data (n = 208). Genetic data from offspring and potential parents were pooled to estimate population allele frequencies because no significant differences were found between them when tested with Fisher's exact test (Weir 1990), and LOD scores are robust under small fluctuations in allele frequencies (Meagher and Thompson 1987). Because the three SSR loci were unlinked, overall LOD-scores were obtained by adding LOD-scores calculated individually for each locus (Thompson 1991; Thompson and Meagher 1998). Using LOD-scores, parent-offspring relationships between mature trees in main plots and natural regeneration (seedlings and saplings) were inferred. Thompson and Meagher (1987) pointed out that in a population the full sibs of the offspring whose parentage is being tested have, on average, a higher likelihood than the true parent. The analysis of individuals grouped into natural regeneration (seedlings and saplings) and mature trees mostly avoided this statistical pitfall.

As the significance levels for LOD-scores cannot be properly derived analytically in the present case, a simulation approach to evaluate the significance of LOD-scores in the parentage analysis was used (Taylor et al. 1997; Marshall et al. 1998; Gerber et al. 2000). Simulations were performed generating 10,000 offspring randomly with both parents outside (set 1) or inside the stand (set 2). Offspring with both parents outside the plot were generated by picking both alleles at each locus at random, according to their frequencies in the whole population. Offspring with parents from inside the plot were generated by randomly choosing pairs of parents among the 76 adults available, selfing allowed, and by randomly generating a gamete from each parent according to Mendelian inheritance. For each offspring, the two potential parents and the parent pair giving the highest LOD-scores were recorded (most-likely parents). The probability, p, of wrongly inferring a tree as the parent of a given offspring when the most-likely parent is assumed to be the true parent was computed following the scheme in Fig. 2. With respect to the parent-pair analysis, a similar simulation scheme to calculate probability levels was used but including only trees that performed well as single parents. This was statistically justifiable because there is a high correlation between the single parent LOD-scores and the LOD-scores for the parent pairs in which they occur (Meagher and Thompson 1986, 1987).

Spatial patterns of seed dispersal

Parent-offspring matches significant at the 15% level (LOD-score > 3.5 for single parents and LOD-score > 7.5 for parent pairs) were used to study the spatial patterns of seed dispersal within the stand. Following Dow and Ashley (1996), two assumptions were made: (1) if offspring matched only one parent in the stand, it was assumed to be the seed parent; and (2) if a parent pair was found, the closest parent was assumed to be the seed parent and the other the pollen parent. Two parameters were evaluated: the distance between each offspring and its assumed seed parent and the angle of the line joining seed parents and offspring with respect to the east-west horizontal line. The distribution of distances and directions between seed parents and putative offspring were compared with a random distribution obtained by permutation of the spatial co-ordinates of the trees. A Kolmogorov-Smirnov test was used to test for differences between the two distributions. Then, the permutation of the co-ordinates was repeated 10,000 times, computing the average distance and angle after each step. The distribution of random average distances and angles were compared with the average distance and angle of seed parent-offspring matches to test for spatial structure and preferential directions of seed dispersion.

Home-made C programs (Gerber et al. 2000) were used for log-likelihood calculations and simulations. Most of them were based on earlier programs written by E. A. Thompson (University of Washington, USA).

Results

Spatial autocorrelation

The within-population genetic structure was different in natural regeneration and mature trees. Seedlings and saplings showed a significant autocorrelation of alleles on a short scale whereas fine-scale structure was not present in mature trees (Fig. 3). In natural regeneration, the autocorrelograms indicated a patch size of approximately 10 m (intercept with the x axis). Autocorrelograms showed the same trend for the three markers assayed, thus indicating the absence of locus-specific selection processes affecting within-population genetic structure.

Parentage analysis and spatial patterns of seed dispersal

The microsatellite markers analysed were highly polymorphic in the studied stand (average number of alleles = 14.33, average Nei's expected heterozygosity = 0.85; see Table 1), thus resulting in high exclusion probabilities for parentage analysis (0.922 and 0.999 for single parents and parent pairs, respectively). Because unambiguous assignment also depends on the number of potential parents in the population, the markers detected few seedlings with only one non-excluded potential single parent

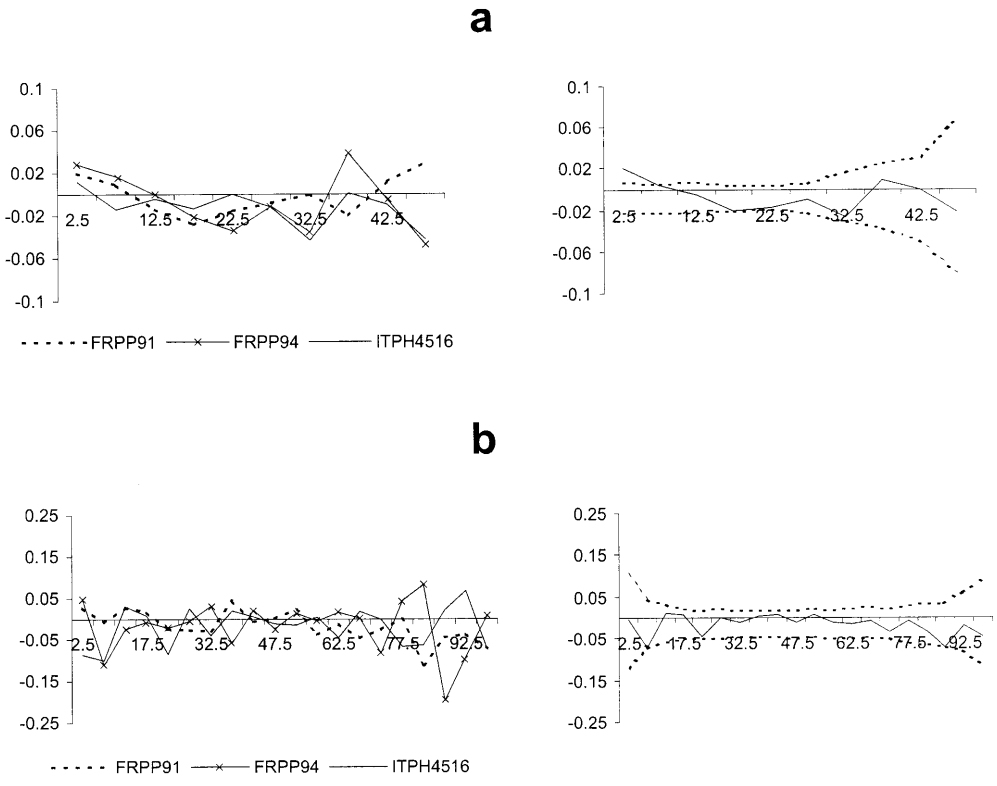

Fig. 3 Variation of Moran's index for each locus at different spatial subdivisions for natural regeneration (**a**) and mature trees (**b**). The graphs on the right show pooled autocorrelograms and 95% confidence intervals obtained by 1,000 permutations of the spatial co-ordinates of the trees

Table 2 Number of single parent-offspring and parent pair-offspring relationships inferred in the study plot; *p* stands for the probability of wrongly inferring a tree as the parent of a given offspring when the most-likely parent is assumed to be the true parent

Type	Not possible parent/couple[a]	Matches by probability level		
		$p < 0.15$	$p < 0.10$	$p < 0.05$
Single parent	1	54	40	16
Parent pair	41	12	11	4

[a] Number of offspring which genotype can not be generated by any single parent or parent pair within the plot

($n = 8$) or parent pair ($n = 13$). However, even with only three microsatellite markers, the computation of LOD-scores and the simulation procedure allowed us to infer which offspring had a significant probability of having one or both of its parents inside the plot (Table 2). Meagher (1986) showed a bias in the most-likely methods towards the identification of parents that carry less common alleles due to ties between plants that had identical genotypes. However, although the subset of plants that had a higher chance of being distinguished as parents were not random with respect to their genotypes, they were a random subset with respect to ecological properties such as spatial location and size (Meagher 1986; Meagher and Thompson 1987). In our study, two ties were found and dropped from the analysis but were not associated with identical genotypes of the potential parent trees.

The offspring and the assumed seed parents included in the spatial analysis are shown in Fig. 4. A significant

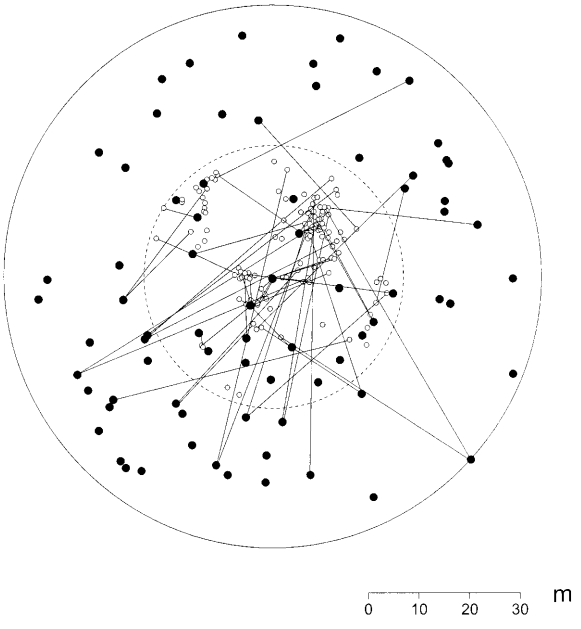

Fig. 4 Offspring (*open circles*) and assumed seed parents (*circles*) included in the analysis of the spatial pattern of seed gene flow

spatial pattern of effective seed dispersal within the stand was found in distances but not in angles (Fig. 5 and Table 3). Seedlings were closer, on average, to their parents than to randomly selected adult trees. In fact, there were more parent-offspring matches in a radius of 15 m from the parent trees, and less from greater than 55 m away than expected with random seed dispersal.

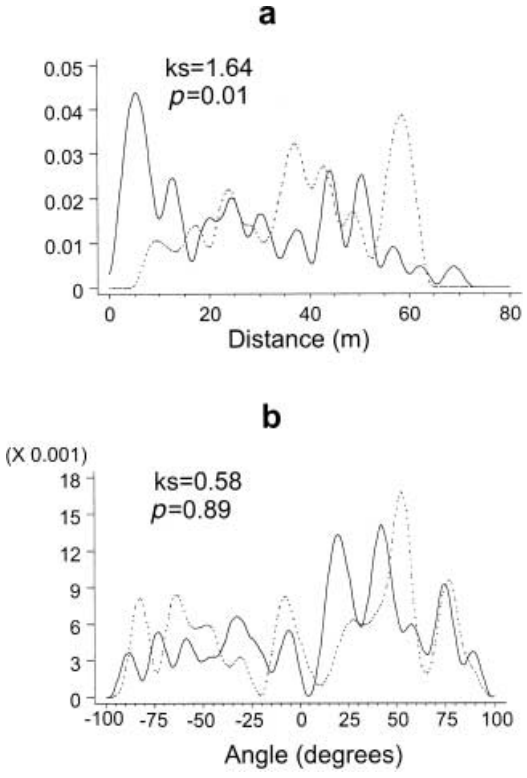

Fig. 5 Comparison between the distributions of inferred seed dispersal (*solid lines*) distances (**a**) and angles (**b**) and random distributions (*dashed lines*) obtained by permutation of the spatial co-ordinates of the trees. Also given are the Kolmogorov-Smirnov statistic (ks) for testing the deviation of the inferred seed dispersal distribution from the expected distribution assuming random dispersal, and the associated probability (*p*)

Table 3 Average distance (m) and angle (degrees) between assumed seed parents and offspring. Low95 and Up95 are the lower and upper 95% confidence intervals, respectively, of a random distribution of average distances and angles between mature trees and natural regeneration, obtained by 10,000 permutations of the co-ordinates of the individuals

Item	Average in the field	Random distribution		
		Average	Low95	Up95
Distance	26.53	37.40	34.00	40.91
Angle	11.91	10.82	–1.35	32.49

No preferential direction of dispersal within the stand was detected. Although there were more-positive and less-negative angles observed with respect to the reference horizontal line (east-west direction), no significant difference in angles from those expected under the random permutation of tree co-ordinates was found.

Discussion

Maritime pine showed a fine-scale structure at the seedling stage and an excess of seed parent-offspring matches in a radius of 15 m from the parent trees, within a native stand under severe Mediterranean conditions (this study). However, the fine-scale structure did not persist with age as shown by autocorrelation analysis of alleles in mature trees. A generally weak within-population structure has been described for most temperate forest species (Perry and Knowles 1991; Schnabel et al. 1991; Shapcott 1995; Leonardi and Menozzi 1996; Leonardi et al. 1996; Streiff et al. 1998). The presence of fine-scale structure is also uncommon in *Pinus* species. Epperson and Allard (1989), studying the spatial pattern of allozyme alleles within *Pinus contorta* ssp. *latifolia* stands, found a lack of structure in the distributions of most genotypes. Tracking dispersal of rare alleles from individual mother trees in a 2-hectare seed stand of *P. sylvestris*, Yazdani et al. (1989) estimated that less than 10% of the seedlings within 10 m from mother trees came from those trees. In contrast, Linhart et al. (1981) found intercluster differences within a population of *Pinus ponderosa* (ponderosa pine) of a magnitude comparable to differences among populations of this species at the regional scale. The spatial structure is also tighter in *Quercus* species. In a stand containing 62 mature bur oaks (*Quercus macrocarpa*), approximately 75% of the saplings were found in clusters of half-sibs around one of their parents, and four mother trees accounted for 86% of the unambiguous parent-offspring matches (Dow and Ashley 1996). Berg and Hamrick (1995) found a genetic structure on a scale of 5–10 m within an old-growth stand of turkey oak (*Quercus laevis*).

The differences in within-population genetic structure among wind-dispersed forest trees could be explained by differences in seed dispersion capability. The seed mass of samaras is inversely proportional to the distance that seeds disperse by wind (Greene and Johnson 1993; see Benkman 1995 for the genus *Pinus*). Maritime pine seeds have a mass 11-fold that of *P. contorta* ssp. *latifolia* and from 4 to 8-fold (depending on the provenance) the mass of *P. sylvestris* seeds, and are similar to those of ponderosa pine. Pines with a heavy seed (like *P. pinaster* and *P. ponderosa*) are expected to have a short dispersal distance by seed, thus producing a fine-scale structure. In ponderosa pine, the spatial distribution of maternally inherited mitochondrial DNA was clustered within populations whereas paternally inherited chloroplast DNA polymorphisms were not, indicating a limited seed dispersal that creates matrilineal clusters in space (Latta et al. 1998). In the case of Mediterranean stands of *P. pinaster* the fine-scale structure seems restricted to young stands whereas in *P. ponderosa*, under more mesic conditions, the fine-scale structure is present whatever the age of the trees (Linhart et al. 1981). Genetic structure at the seedling stage in maritime pine populations could be lost when the stands grow older due to strong local mortality. As full clusters of seedlings usually die, fine-scale structure at short distances (10–15 m) may disappear, in particular when intraspecific competence between mature trees and seedlings is high. In the study area, summer drought is pronounced (soil temperatures up to 60 °C have been recorded on the patches

without forest cover) and the soils are low-fertility continental dunes. The competition of grasses and shrubs, and the Mediterranean climate, severely limit the survival of seedlings (for instance, in the 1999 summer period, only 35% of the seedlings survived) and poorly regenerated stands are common.

In contrast with well-established theories of dispersion from a point source and the experimental evidence from seed trapping (Higgins and Richardson 1999), directionality in the establishment of maritime pine seedlings was not found in the present study. In central Spanish forests of this species, wind patterns are expected to be highly variable during the seed-dispersion period (from June to September, mainly), due to low tree density and their situation in a flat area. On the other hand, effective gene flow (seedlings and saplings) is especially complex because it represents two levels of dispersal (pollen and seed), and seeds are potentially subject to many post-dispersal influences. Two facts related to Mediterranean environments could strongly affect the within-population genetic structure of *Pinus* species: the severity of the climate (as previously discussed) and animal-mediated secondary dispersal. The role of seed-foraging animals seems important in the studied stand as 75% of the maritime pine seeds were removed from their initial position in a period of a week (unpublished results). Seed predation and animal-mediated secondary dispersal by birds and rodents have important effects on the spatial distribution of seeds of *Pinus* species after the initial seed rain (Lanner 1998; Vander-Wall 1992; Castro et al. 1999).

In conclusion, the study of parentage relationships using microsatellite markers revealed fine-scale genetic structure in the natural regeneration resulting from limited seed dispersal. Within-population genetic structure in Mediterranean forests is most likely affected by a number of post-dispersal events, each of which can modify the pattern produced by the initial seed rain. The analysis conducted also provided some useful information for gene management in this species. Sampling from the crown of trees for conservation purposes seems adequate as a wide sample of the local gene pool is obtained. Because fine-scale structure could persist from seedling to mature stages in some stands, a minimum distance between sampled trees of 15 m and seed collection from different plots within each population are recommended. However, extension of the study to other ecological and management conditions and more-precise estimates of dispersal patterns would be needed to optimize conservation and breeding strategies in this species.

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