

Spatiotemporal mating pattern variation in a wind-pollinated Mediterranean shrub

RAFAEL G. ALBALADEJO,* SANTIAGO C. GONZÁLEZ-MARTÍNEZ,† MYRIAM HEUERTZ,†,‡ GIOVANNI G. VENDRAMIN§ and ABELARDO APARICIO*

*Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Profesor García González 2, 41012 Seville, Spain,

†Department of Forest Systems and Resources, CIFOR-INIA, Carretera de La Coruña km 7.5, 28040 Madrid, Spain,

‡Evolutionary Biology & Ecology, Faculty of Science, Université Libre de Bruxelles, Av. F.D. Roosevelt, 50, CP 160/12, B-1050 Brussels, Belgium, §Istituto di Genetica Vegetale, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Firenze), Italy

Abstract

Spatiotemporal variation in mating patterns is poorly known in wind-pollinated plant species. Here, we analysed mating patterns of the wind-pollinated dioecious shrub *Pistacia lentiscus* by genotyping 904 seeds from 30 mother plants with eight microsatellite markers in a high-density population in two consecutive flowering seasons. We found significant differences in some mating system estimates between years, particularly in the levels of correlated paternity. Overall, within-mothers correlated paternity was higher in 2007 than in 2006 ($r_{pWM} = 0.085$ and 0.030), which translated into an effective number of fathers (N_{ep}) of 11.8 and 33.6 respectively. Using a smoothing interpolation technique, we show that the effective pollen cloud was spatially structured in patches of high- and low-genetic diversity, which do not remain constant from year to year. In 2006, the among-mothers correlated paternity (r_{pAM}) showed no trend with distance, suggesting no restriction of pollen dispersal. However, in 2007, r_{pAM} was greater than zero at short distances, revealing the existence of small-scale patterns of pollen dispersal. The fact that the studied seasons were climatically homogeneous during the flowering time suggested that the observed differences might be ascribed to between-year phenological variation of individuals in the studied population or other (unknown) factors. Numerical simulations, based on the real data set, indicated that the clumping of males and decreasing plant density, which is related to different types of pollen limitation, greatly increase correlated mating in this wind-pollinated species, which is of relevance under the frame of the continuous anthropogenic habitat disturbance suffered by Mediterranean ecosystems.

Keywords: correlated paternity, mating system, *Pistacia lentiscus*, spatial variation, temporal variation, wind-pollinated species

Received 3 June 2009; revision received 1 October 2009; accepted 3 October 2009

Introduction

Mating patterns of seed plant species rely not only on the reproductive traits of individual plants, but also on environmental factors (e.g. density and spatial distribution of pollen sources) that notoriously influence pollen availability (Franceschinelli & Bawa 2000). For example,

recent studies have shown a positive association between mating system parameters and the distance of individual plants to their closest neighbours (e.g. Robledo-Arnuncio *et al.* 2004; González-Varo *et al.* 2009) highlighting the role of the spatial distribution of pollen sources. However, compared with animal-pollinated species, our knowledge of the reproductive biology of wind-pollinated species is, in Friedman & Barrett's (2009) words, 'very rudimentary'. Until a few years ago, pollen limitation was widely believed not to affect

Correspondence: Rafael G. Albaladejo, Fax: +34 954556358; E-mail: albaladejo@us.es

wind-pollinated species (Koenig & Ashley 2003), but recent studies are showing that wind-pollinated trees growing at low density might be as affected by pollen limitation as many animal-pollinated species. For example, Sork *et al.* (2002) showed that the average effective number of fathers (N_{ep}) in the outcrossed *Quercus lobata* was less than four, a value typical of animal-pollinated plants. However, the number of studies is still scarce and it remains an open question whether pollen limitation could also happen in species growing at higher densities (Koenig & Ashley 2003).

Additionally, mating system studies in perennial plant species often rely on single-season estimates, despite the fact that they might not represent a comprehensive picture of the general mating pattern. Although the need for multi-year studies has been explicitly stressed several times (Smouse & Sork 2004; Robledo-Arnuncio & Gil 2005; Sork & Smouse 2006), few works have been conducted to evaluate the constancy of mating parameters over two or more years, and those focusing on wind-pollinated species produced mixed results (e.g. Nakanishi *et al.* 2005; Pakkad *et al.* 2008). Interestingly, interannual studies have revealed that at least for some species, the genetic diversity of the pollen pool received by seed plants may vary over time. In wind-pollinated plants, year-to-year changes in biotic (e.g. flowering phenology) as well as in abiotic (e.g. temperature, occurrence of storms) ecological factors may contribute to create temporal heterogeneity in the male gametic pool that will be available for females (Irwin *et al.* 2003). However, as a result of the scarcity of studies, it remains largely unknown whether temporal variability (or constancy) in mating patterns is a general feature of the wind-pollination syndrome (Friedman & Barrett 2009).

Correlated paternity (r_p), i.e. the proportion of full-sibs within maternal progeny arrays or the proportion of paternal half-sibs among maternal progeny arrays, and its inverse, the effective number of pollen donors (N_{ep}), are parameters of pivotal importance in the study of plant mating systems, allowing a better understanding of the pollination biology of plant species (Hardy *et al.* 2004; Verdú *et al.* 2006). For instance, it has been suggested that multiple-sired females might display an increased fitness as a genetically diverse progeny array could exploit an ecologically diverse environment more efficiently (Young 1981; Karron & Marshall 1990). Further, a high number of different pollen donors (i.e. low correlated paternity) imply high genetic diversity of the pollen cloud received by the seed plants, which, from a microevolutionary point of view, enlarges the genetic neighbourhoods of the species and consequently their effective population sizes (Smouse & Sork 2004). These mating parameters are also important because they

allow direct comparisons among species or habitats, which constitute the main body of evidence for the establishment of general patterns linking mating system characteristics to plant life history traits. It is relevant to notice that for out-crossing species, correlated paternity is more sensitive for detecting spatiotemporal patterns in mating system variation than the traditionally estimated out-crossing rates (de-Lucas *et al.* 2008).

In this study, we analysed the variation in mating patterns of a wind-pollinated Mediterranean shrub, *Pistacia lentiscus* L. (Anacardiaceae), at both spatial and temporal scales. To accomplish this goal, we genotyped adult plants and offspring arrays from two pollen dispersal seasons using recently developed microsatellite markers (Albaladejo *et al.* 2008). As plant density in the study population was high and the climatic conditions during the flowering time of *P. lentiscus* were homogeneous in the two study years (see below), we expected (i) no evidence of pollen limitation because of distance from males to females, and (ii) constant year-to-year levels of correlated paternity. As the pattern of pairing between gametes is influenced by the existence of spatial patches of interbreeding individuals, we also assessed fine-scale spatial genetic structure (SGS) in *P. lentiscus*. We predicted weak or no within-population SGS and low levels of biparental inbreeding (i.e. the mating between genetically related individuals) because of various reasons: (i) long-distance seed dispersal mediated by migratory birds (i.e. wintering species), (ii) species longevity and (iii) high density of plants in the study plot and the consequent overlapping of seed shadows (Kalisz *et al.* 2001; Vekemans & Hardy 2004; Dick *et al.* 2008).

Finally, we produced expected patterns of correlated paternity under different scenarios of spatial aggregation and density of pollen sources using numerical simulations based on the real data set. These analyses allowed us a better understanding of the effects of population density and spatial structure on mating patterns, which are crucial for predicting the consequences of management activities and human disturbance in forested ecosystems (Knapp *et al.* 2001).

Materials and methods

Study species, study site and sampling strategy

Pistacia lentiscus is a dioecious evergreen shrub or small tree (up to 4 m tall) distributed throughout the Mediterranean Basin at low altitudes (Quézel 1981). It blooms between mid-March and late April, and male and female plants exhibit synchronized flowering phenology (Jordano 1988; Correia *et al.* 1992; Verdú & García-Fayos 2002), without notable differences in

reproductive effort among sex morphs (as estimated by the number of inflorescences per branch and flowers per inflorescence; Martínez-Pallé & Aronne 2000). The drupes mature from September to December and are dispersed during winter and early spring by small and medium-sized birds (blackcaps, thrushes and robins, among others) (Herrera 1984; Jordano 1988, 1989). Fruit colour is associated to seed viability and normally only black mature fruits contain viable seeds (Jordano 1988, 1989; Verdú & García-Fayos 1998; but see Verdú & García-Fayos 2001). Red and white fruits contain parthenocarpic and aborted seeds respectively.

The study site was located in Chiclana (Cádiz province, southern Spain, coordinates 36°23'10" N 6°05'58" W) at 40 m a.s.l. It is a c. 200 ha dense stone pine (*Pinus pinea* L.) forest (Fig. 1) with *P. lentiscus* being the dominant species in the understory (total density of adult *P. lentiscus* plants was 287 ind/ha, and female and male density 164 ind/ha and 123 ind/ha respectively). Although slightly female-biased, the sex-ratio did not significantly differ from 1:1 in the study population ($\chi^2 = 2.23$; d.f. = 1; $P = 0.136$; $N = 203$, 42.86% males and 57.14% females). Climatic data gathered from the nearest meteorological station (at Conil de la Frontera, 6 km from the study site and at 26 m a.s.l.; data available at <http://www.juntadeandalucia.es/innovacioncienciayempresa/ifapa/ria/servlet/FrontController>) showed that the number of rainy days and total rainfall between mid-March and late April were slightly higher in 2006 than in 2007 (21 days and 80.2 mm and 16 days and 52.8 mm respectively), albeit they were not significantly different (Wilcoxon's matched pairs test; $Z = 0.319$, $P > 0.1$). Further, wind speed during flowering was low (0.8 m/s and 1.1 m/s average values for 2006 and 2007 respectively) and the distribution of wind direction was

not significantly different between years (average directions were 182° and 206° for 2006 and 2007 respectively; Watson U^2 -test = 0.171; $P > 0.05$).

To estimate genetic diversity and fine-scale spatial genetic structure (SGS), we sampled 56 *P. lentiscus* adult plants along three intersecting transects of 450 m each, disposed in a triangular fashion (Fig. 1). In this way, we maximized the inclusion of short- and long-distances among pairs of plants and optimized the power to detect spatial genetic structure (see Vekemans & Hardy 2004). Thirty out of the 56 plants sampled were females and we collected seed progenies from each of them in 2006 and 2007 to conduct mating system analyses.

Conspecific neighbourhood of mother plants and seed collections

We measured height (h) as well as major (R_1) and minor (R_2) radii of the vertical projection of the crown of each of the 30 selected mother plants. Plant size, as a surrogate of female fitness, was estimated as a revolution ellipsoid by $\pi R_1 R_2 h / 6$ (Verdú & García-Fayos 1998). The conspecific neighbourhood of each mother plant was characterized by (i) counting the number of *P. lentiscus* flowering males within a 15 m buffer radius around mother plants, and (ii) measuring the distance to the closest *P. lentiscus* flowering male. The shrub cover in the study site was typically dominated by *P. lentiscus*, and thus no attempts of characterizing the heterospecific neighbourhood were made.

In October 2006, between 32 and 222 (mean₂₀₀₆ \pm SD = 107.6 \pm 51.8; $N = 30$ mothers) fully mature black fruits were collected directly from the crown of each of the 30 mother plants (we avoided sampling red and white fruits) and, in October 2007, we repeated the sampling and collected between 31 and 170 fruits from the same plants (mean₂₀₀₇ \pm SD = 70.3 \pm 30.1; $N = 28$ mothers). Two mother plants died in 2006 and could not be sampled in 2007. Overall, 3227 fruits were collected in 2006 and 1967 fruits in 2007. The percentage of viable seeds (%VS) for each plant in the 2 years was recorded after removing the pulp (pericarp and mesocarp) from each fruit and testing its viability through the flotation/sink method (i.e. only pulp-less fruits that sink have a well-developed embryo inside; see Fig. 1 in Jordano 1989). The accuracy of the method was previously checked by manually opening sunken and floating fruits (50 each), and confirming that the former had a well-formed embryo inside, whereas the latter had an aborted one.

Finally, to conduct mating system analyses using molecular markers, we randomly selected 465 pulp-less fruits (hereafter seeds) in 2006 (mean number of seeds

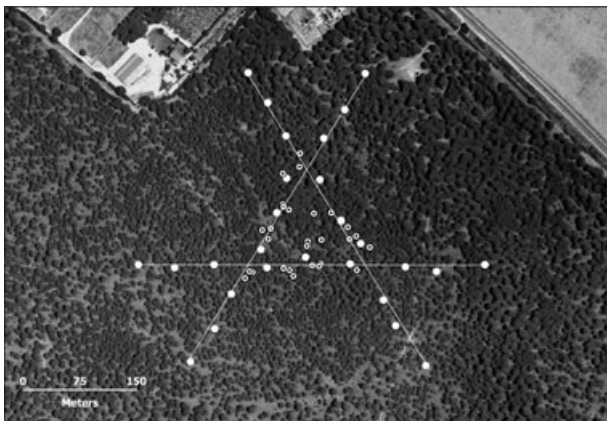


Fig. 1 Aerial photograph of the study plot and location of the sampled *Pistacia lentiscus* individuals (white circles). Mother plants collected for parent-offspring genetic analyses are also shown (white circles with a black spot).

per mother in 2006 \pm SD = 15.5 \pm 3.7; range 8–20 seeds; $N = 30$ mothers), and 439 seeds in 2007 (mean \pm SD = 16.9 \pm 1.6; range 11–20 seeds; $N = 26$ mothers), and stored them in silica-gel until DNA isolation. Despite intensive sampling, we recovered only two and three seeds respectively, from two of the mother plants in 2007 and consequently, these were discarded from the genetic analyses that year.

DNA isolation and microsatellite markers

Total genomic DNA was isolated from young leaves of the 56 adult plants and from a total of 904 seeds with the Invisorb DNA Plant HTS 96 Kit (Invitek) according to the manufacturer's protocol with some slight modifications for seeds; seeds were soaked for 24 h, the hard endocarp was carefully removed along with the maternal endospermic tissue and fresh embryos were ground in a mill (Retsch MM200) with 50 μ L of lysis buffer. We amplified eight polymorphic nuclear microsatellite loci (*Pislen 20B*, *Pislen 21*, *Pislen 114*, *Pislen 333*, *Pislen 501*, *Pislen 510*, *Pislen 526* and *Pislen R05*) recently developed for *P. lentiscus* (Albaladejo *et al.* 2008), and only samples successfully genotyped with at least five loci were considered in genetic analyses. Details about amplification conditions and PCR cycle profiles can be found in Albaladejo *et al.* (2008). Amplified products were labelled with fluorescent dyes (6-FAM, NED, VIC or PET) and analysed on an ABI 3730 DNA Analyzer (PE Applied Biosystems, Foster City, CA, US) at the 'Unidad de Genómica' (Universidad Complutense). Fingerprint profiles were scored with the software GeneMapper 3.7 (PE Applied Biosystems).

Genetic diversity and mating system analyses

Standard genetic diversity parameters were computed at each locus using *STAT* 2.9.3.2 (Goudet 2002): the number of alleles per locus (A), expected heterozygosity (H_E) and Weir & Cockerham's (1984) inbreeding coefficient (f) were computed for adults; pollen pool number of alleles and gene diversity were determined in the 2 years after subtracting the maternal contribution to each offspring genotype following Hardy *et al.* (2004). For adults, departure from Hardy–Weinberg (HW) equilibrium ($f = 0$) was tested by permutation (1000 replicates). G -tests were used to test for linkage disequilibrium (LD) between any pair of loci in the adult population using *STAT* 2.9.3.2 (Goudet 2002).

Mating system parameters, including multilocus (t_m) and single-locus (t_s) out-crossing rates, were calculated each year at both population and family levels with MLTR 3.3 (Ritland 2002). Maximum likelihood estimators were used at the population level, whereas a

moment-based procedure was used at the family level because it is more appropriate when dealing with small sample sizes (Ritland 2002). As *P. lentiscus* is dioecious, the multilocus out-crossing rate, t_m , must equal one; this quantity being used here only to estimate biparental inbreeding ($t_m - t_s$; the mating between genetically related individuals). At the population level, pollen and ovules were constrained to have the same gene frequencies and as missing data were not frequent in our data set (less than 5%), analyses were run with the Newton-Raphson algorithm. Standard errors and confidence intervals were assessed by bootstrap (1000 replicates) using families as the resampling unit.

Within-mothers correlated paternity (r_{pWM}), i.e. the proportion of the progeny of a mother sired by the same father, was calculated as twice the average kinship coefficient ($2F_{ij}$) between paternal genes of offspring pairs within maternal sibships following Hardy *et al.* (2004). This method provides more accurate estimates of correlated paternity than Ritland's estimator, which has been reported to provide downward biased estimates (Hardy *et al.* 2004; Verdú *et al.* 2006; de-Lucas *et al.* 2008).

Differences in mating system variables (%VS, $t_m - t_s$ and r_{pWM}) between years were assessed with Student's paired t -tests; homogeneity of mating system variables for mothers in both years was assessed using Pearson's correlations. Pearson's correlations were also used to check the association of mating system parameters with plant size and conspecific maternal neighbourhood variables (number of males within 15 m buffer radius and distance to the nearest pollen source) in the 2 years.

Fine-scale spatial genetic structure of adults and pollen pools

Spatial genetic structure in the adult population was detected by regressing pairwise kinship coefficients (F_{ij} ; Loiselle *et al.* 1995) on the logarithm of geographical distance between individuals. Significance of SGS was obtained by comparing the observed regression slope (b) with those obtained from randomly permuting (1000 permutations) individual locations among individuals (Vekemans & Hardy 2004). SGS analyses were performed with the software SPAGeDI 1.2 (Hardy & Vekemans 2002).

Apart from the spatial distribution of adults, it is of interest to analyse the spatial patterns of the pollen pools and their correlation across years. Thus, we computed gene diversity (H) based on male gametes for each mother plant and constructed interpolated contour plots for each year with a local smoothing technique through fitting a third-degree polynomial function. Further, to gain a more detailed picture about the

patterns of effective pollen movement, we also averaged correlated paternity values between paternal genes of offspring pairs among maternal sibships (r_{pAM}) in each of seven distance classes and computed confidence intervals for each distance class by permutation (1000 permutations of locations among mother plants). If pollen limitation because of distance from males to females exists in *P. lentiscus*, it would be expected, given restricted pollen dispersal, that the progenies of nearby mothers share more sires than the progenies of mothers located far away (as is the case, for example, in *Centaurea corymbosa*, Hardy *et al.* 2004).

Dispersal kernels

We also fitted probability density functions for pollen dispersal (i.e. dispersal kernels) based on progeny arrays following a two-parameter exponential-power function (Clark 1998; Austerlitz *et al.* 2004) in the two study years:

$$p(a, b; x, y) = \frac{b}{2\pi a^2 \Gamma(2/b)} \exp\left(-\left(\frac{\sqrt{x^2 + y^2}}{a}\right)^b\right), \quad (\text{eqn 1})$$

where x and y are spatial coordinates, Γ is the gamma function, and a and b are scale and shape parameters fitted by the algorithm. This function has become popular in pollen flow studies because it accommodates normal and exponential functions and allows for fat tails. The function was fitted to the parent-offspring genotypic data using the KinDist approach. This approach is based on the expected decay of correlated paternity among maternal pairs with distance (see details in Robledo-Arnuncio *et al.* 2006).

Numerical simulations

Further information on the effects of individual trait variables and spatial configuration of pollen sources on the patterns of correlated paternity (r_{pWM} and r_{pAM}) was gained through numerical simulations. We generated a 500 m × 500 m population comprised of individual male plants (i.e. potential fathers), imposing a realistic minimum distance of 2 m between plants (see below), and of 26 mother plants located in the centre of the population in a triangular fashion (following our real sampling design in 2007). In our simulations, the probability of the i th male to mate with the j th mother plant is given by the function π_{ij} :

$$\pi_{ij} = \frac{\lambda_i p_{ij}}{\sum_{k \in N} \lambda_k p_{kj}}, \quad (\text{eqn 2})$$

which depends on the effective pollen dispersal kernel (p_{ij} ; eqn 1), the number of potential fathers (N) and the

relative fecundity of the potential fathers (λ_i) (see Devlin *et al.* 1992; Robledo-Arnuncio *et al.* 2004; Robledo-Arnuncio & Gil 2005). For the 26 mother plants, we generated 399 seeds (as in our real data set in 2007, see Results) and recorded the proportion of full-sib pairs within each maternal progeny array (r_{pWM}). We conducted 1000 runs for each simulation and recorded average values and standard deviations. Further, r_{pAM} values were obtained and averaged in each of seven distance classes of the same sizes as in the real data sets.

We simulated several scenarios (detailed in Table S1, provided as Supplementary Material) by sequentially modifying mating parameters. As we were unable to fit a reliable dispersal kernel in any of the two study years (see Results), we simulated pollen dispersal using three different bivariate power-exponential kernels by fixing the scale parameter (a) and changing the shape parameter (b) as follows. The first kernel was a thin-tailed leptokurtic kernel, which simulated restricted pollen dispersal ($a = 3.637$, $b = 0.907$ and $\delta = 9$ m). The second kernel was less L-shaped allowing for longer pollen dispersal distances ($a = 3.637$, $b = 0.670$ and $\delta = 21$ m). The last one was a fat-tailed kernel that allowed for even longer pollen dispersal distances ($a = 3.637$, $b = 0.450$ and $\delta = 131$ m), which is the norm in most wind-pollinated forest trees (e.g. *Araucaria*, Bittencourt & Sebbenn 2007; *Fraxinus*, Bacles & Ennos 2008; *Pinus sylvestris*, Robledo-Arnuncio & Gil 2005; *Pinus pinaster*, de-Lucas *et al.* 2008). Males were assumed to have equal fecundity ($\lambda_i = 1$) or, alternatively, male fecundity was modelled making it proportional to plant size ($\lambda_i \neq 1$) using either a linear or exponential function (Burczyk *et al.* 1996; de-Lucas *et al.* 2008). Plant size was drawn from a Weibull distribution obtained by fitting plant size of the 30 mother plants measured in the study site. We are not aware of any reports on patterns of variation in male fecundity for *P. lentiscus*, so we assumed that the males in a population may display patterns of variability comparable to those of females (see Jordano 1989; Martínez-Pallé & Aronne 2000) and fitted linear and exponential functions to the observed female fertility data. The exponential fit resulted in a leptokurtic fecundity distribution with a wide coefficient of variation for male fecundity ($CV \approx 120\%$), similar to those assumed in forest trees (Robledo-Arnuncio *et al.* 2004; de-Lucas *et al.* 2008).

It has been suggested that pollen limitation may occur in *P. lentiscus* at male densities below 100 ind/ha (Verdú & García-Fayos 1998). Consequently, we assessed different pollen limitation scenarios by decreasing the global density of potential fathers through setting N in the simulations to 3000 (density of 120 ind/ha; close to the real density of males in the population, $d = 123$ ind/ha),

1500 (simulated $d = 60$ ind/ha) or 750 (simulated $d = 30$ ind/ha). Finally, to test for the effects of spatial aggregation of potential pollen donors on correlated paternity (Robledo-Arnuncio & Austerlitz 2006), we constructed populations with different spatial configurations of males: (i) spatially homogeneous populations where the spatial coordinates of males were generated randomly and (ii) heterogeneous populations where males were clustered in 30 patches of 64 m² to reduce the global density to 60 or 30 ind/ha (as in simulated scenarios described above), but keeping the local (within-cluster) density of males constant at 120 ind/ha or 60 ind/ha respectively.

All simulations were run in Microsoft Excel 2003 using Visual Basic macros based on earlier code provided by O. Hardy (Université Libre de Bruxelles, Belgium).

Results

Genetic diversity and mating system analyses

The final data set was based on seven loci, which resolved 77 alleles overall; locus *Pislen 20B* failed to amplify in c. 60% of the samples and was therefore removed. Standard genetic information for each locus in the adults and the pollen pools is detailed in Table S2. The study population did not depart from HW proportions and no linkage disequilibria between loci were detected. Levels of gene diversity in the pollen pool were very similar in the two study seasons ($H = 0.676$ in 2006 and $H = 0.671$ in 2007); however, the number of alleles was higher in 2006 than in 2007 (76 vs. 66 alleles).

The percentage of black fruits bearing viable seeds (%VS) was (surprisingly) low in both years (average values were 35% in 2006 and 48% in 2007) and significantly higher in 2007 (Table 1). %VS was significantly correlated for mothers over years, indicating a remark-

able stability in female fecundity. As expected for a dioecious species, the multilocus out-crossing rate, t_m did not differ from one (Table 1 and S2). Biparental inbreeding was low, but significantly different from zero in both years ($t_m - t_s$ 2006 = 0.082, 95% CI = 0.059–0.259; $t_m - t_s$ 2007 = 0.074, 95% CI = 0.034–0.233), indicating that at least some seeds were sired by related males. Biparental inbreeding did not differ globally between years, but it was heterogeneous for the same mothers over years (mothers displaying the highest levels of biparental inbreeding in 2006 were different from those in 2007; Table 1 and S3). Overall, r_{pWM} was moderately low, but interestingly, three times higher in 2007 than in 2006, and there were significant differences among families across years. Accordingly, the effective number of fathers was much lower in 2007 ($N_{ep} = 1/r_{pWM} = 11.8$) than in 2006 ($N_{ep} = 33.6$).

Neither plant size nor the number of *P. lentiscus* males surrounding females was significantly correlated with any mating system variable in any year (Table 2). However, the distance of females to their nearest flowering male was negatively correlated with $t_m - t_s$ and r_{pWM} (although it was significant or marginally significant only in 2007), reflecting lower biparental inbreeding and correlated paternity in the most isolated females.

Fine-scale spatial genetic structure of adults and pollen pools

The overall regression slope between pairwise kinship coefficients and the logarithm of spatial distance was slightly negative, albeit non-significant ($b = -0.002$; $P = 0.582$), indicating the absence of SGS in the study population.

The interpolated contour plots for gene diversity of the pollen cloud suggested spatial and temporal patterns of variation. The pollen cloud was structured in small patches of high or low genetic diversity and inter-

Table 1 Mating system parameters of the *P. lentiscus* population in the two study years (standard errors in brackets). Differences between years were assessed using Student's *t*-paired tests and correlations in mating system parameters across mothers using Pearson's statistics. *N*, number of families; %VS, percentage of viable seeds; t_m , multilocus out-crossing rates; t_s , single-locus out-crossing rates; $t_m - t_s$, biparental inbreeding; r_{pWM} , within-mothers correlated paternity; N_{ep} , effective number of fathers

	<i>N</i>	%VS	t_m	t_s	$t_m - t_s$	r_{pWM}	N_{ep}
Year 2006	30	35.4 (4.7)	0.997 (0.061)	0.915 (0.014)	0.082	0.030 (0.013)	33.63
Year 2007	26	48.8 (5.2)	0.996 (0.050)	0.922 (0.022)	0.074	0.085 (0.024)	11.75
Student's <i>t</i> -paired test	26	-2.239*	—	—	0.233 ns	-2.215*	—
Pearson's <i>r</i> correlation coefficient	26	0.516**	—	—	0.088 ns	-0.032 ns	—

* $P < 0.05$, ** $P < 0.01$.

ns, not significant.

Mating system variables	Plant size	Nearest-male distance	Number of males in 15 m buffer
2006			
%VS ₂₀₀₆	0.001 (<i>P</i> = 0.994)	-0.018 (<i>P</i> = 0.931)	-0.148 (<i>P</i> = 0.472)
$t_m - t_s$ ₂₀₀₆	0.129 (<i>P</i> = 0.530)	-0.126 (<i>P</i> = 0.540)	-0.042 (<i>P</i> = 0.840)
r_{p2006}	-0.148 (<i>P</i> = 0.470)	-0.196 (<i>P</i> = 0.337)	0.158 (<i>P</i> = 0.442)
2007			
%VS ₂₀₀₇	0.152 (<i>P</i> = 0.459)	-0.257 (<i>P</i> = 0.205)	-0.069 (<i>P</i> = 0.738)
$t_m - t_s$ ₂₀₀₇	0.318 (<i>P</i> = 0.113)	-0.558 (<i>P</i> = 0.003)	0.067 (<i>P</i> = 0.746)
r_{p2007}	0.090 (<i>P</i> = 0.664)	-0.351 (<i>P</i> = 0.079)	-0.052 (<i>P</i> = 0.800)

Table 2 Pearson's correlation coefficients between maternal plant size or conspecific neighbourhood variables and individual mating system variables in 2 years in a *P. lentiscus* population. Acronyms as in Table 2. Significant (*P* < 0.05) and marginally significant (*P* < 0.1) correlations are highlighted in bold face

estingly, these areas did not remain constant from year to year (Fig. 2). In fact, the gene diversity of the pollen pool sampled by the mother plants was significantly different between years (Student's paired *t*-test, $t = 2.005$, *P* = 0.05). Heterogeneity across years was also detected in the frequency of correlated matings among-mothers (r_{pAM}) with distance. In 2006, r_{pAM} showed no trend with distance (Fig. 3), suggesting the absence of pollen limitation because of distance from males to females, whereas in 2007, r_{pAM} was greater than zero in the first two distance classes, indicating that a portion of the progeny of mothers occurring less than 30 m apart shared some fathers. This result, to some extent, revealed the existence of small-scale patterns of pollen dispersal in the population during 2007.

Dispersal kernels

Unfortunately, we could not reliably fit a dispersal kernel for *P. lentiscus* based on progeny arrays in any of the 2 years. Although slightly negative, Spearman's rank correlation coefficient between correlated paternity among maternal pairs and inter-mother spatial distance ($r_s = -0.016$ in 2006 and $r_s = -0.015$ in 2007) did not reach the advisable level ($r_s < -0.1$) to fit a dispersal kernel using this method (for technical details see Robledo-Annuncio *et al.* 2007) suggesting very fat-tailed pollen dispersal kernels in this population (J.J. Robledo-Annuncio, personal communication).

Numerical simulations

The results obtained from numerical simulations were similar irrespective of the method used –linear or exponential– to model relative male fecundity, thus only the latter is presented here (results from the linear models for r_{pAM} can be found in Fig. S1, provided as Supplementary Material). For all scenarios, the thin-tailed dispersal kernel provided unrealistically high levels of r_{pWM} (Fig. 4). Similar values to those observed in 2006 and 2007 were obtained when we used intermediate-distance or fat-tailed dispersal kernels to model pollen

dispersal behaviour respectively. Unequal male fecundity related to plant size (scenario B, Fig. 4), moderate pollen limitation modelled through reducing adult

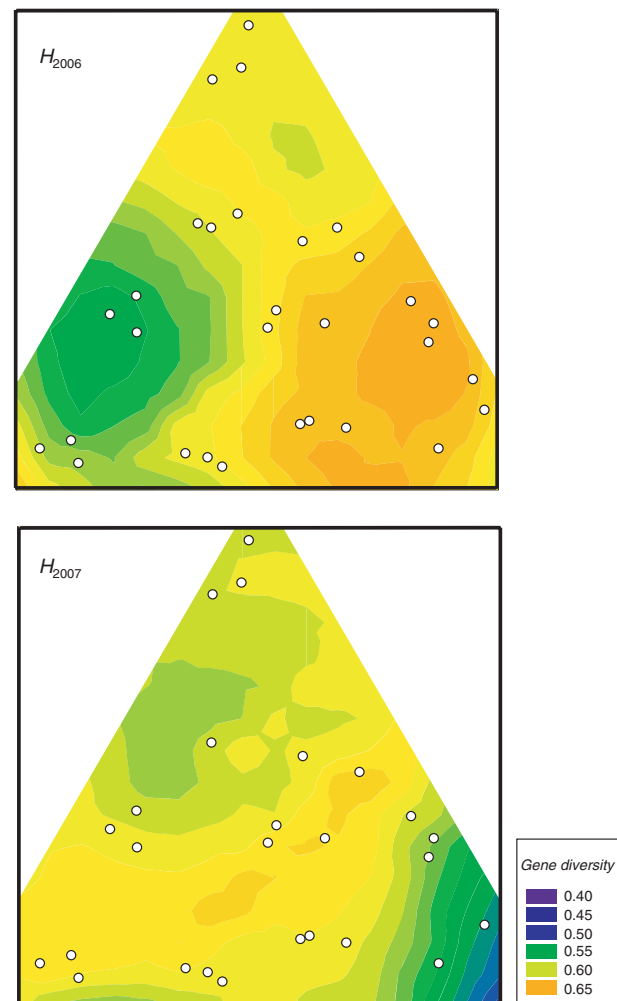


Fig. 2 Contour plots showing rich (orange) and poor (blue) areas of gene diversity of the pollen cloud available for the sampled mother plants (white dots). Interpolation was conducted through fitting a third-degree polynomial function. Data from 30 and 26 mothers were available for the years 2006 and 2007 respectively.

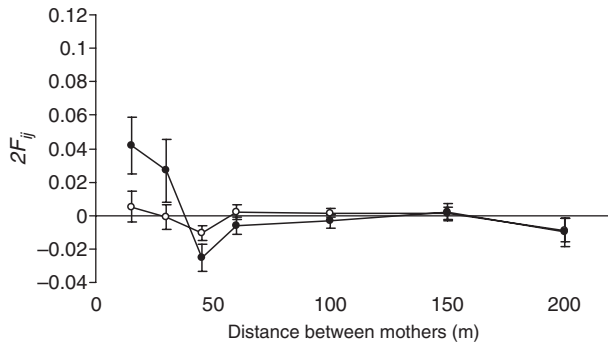


Fig. 3 Correlograms showing levels of correlated paternity, estimated as $2F_{ij}$ following Hardy *et al.* (2004), between pairs of *P. lentiscus* offspring in 2006 (empty circles) and 2007 (filled circles) over the distance separating mother plants. Vertical bars are standard errors.

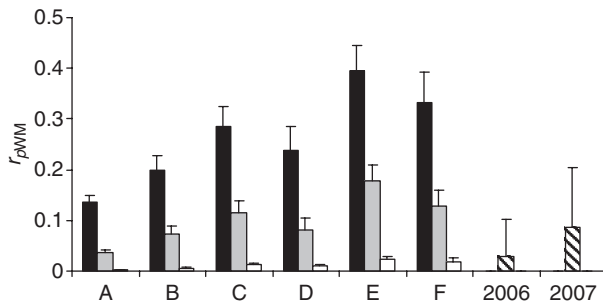


Fig. 4 Average values of within-mothers correlated paternity (r_{pWM}) based on simulated progeny arrays (see text for details). Values are based on 1000 independent runs and error bars are the standard deviations. Bar colours correspond to different bivariate power-exponential pollen dispersal kernels used in the simulations: (i) a thin-tailed kernel (black bars), (ii) an intermediate kernel (grey bars), and (iii) a fat-tailed kernel (white bars). Scenario A simulated the real population of *P. lentiscus* with a high global density (120 ind./ha) of randomly distributed males. Scenario B included non-equal relative male fecundity. Scenarios C and E simulated the population of *P. lentiscus* with pollen limitation via reduction in the global density of males to 60 and 30 ind./ha respectively. Scenarios D and F included the spatial aggregation of males in the population (see text and Table S1 for details). Observed r_{pWM} (hatched bars) in the population are also provided for comparative purposes.

density (C, E) or some spatial aggregation of males (or its flowering, D, F) would be sufficient to explain the observed values in 2007. Nevertheless, it is noteworthy that the standard deviations of the observed r_{pWM} estimates were much higher than those obtained through simulations, suggesting that some parameters increasing differences in correlated paternity across mothers were not accounted for in our simulations.

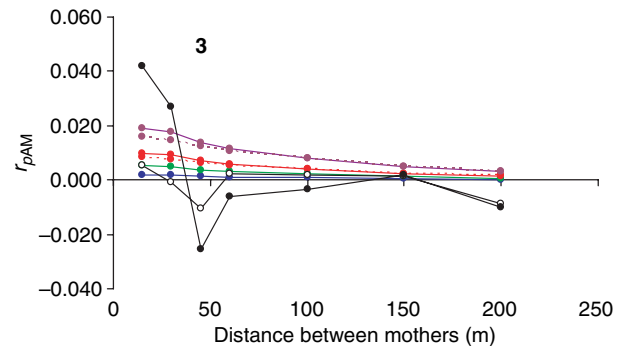
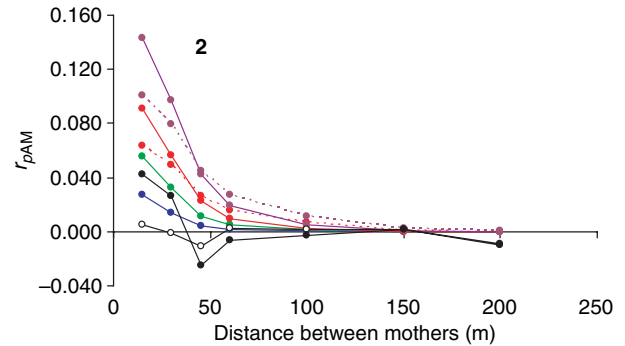
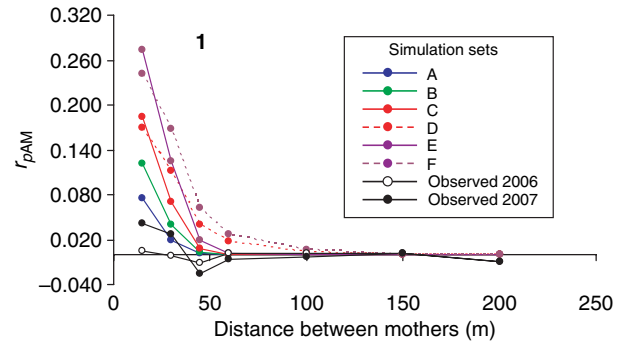


Fig. 5 Average values of among-mothers correlated paternity (r_{pAM}) based on simulated progeny arrays. Correlograms in 1, 2 and 3 were obtained through fitting pollen dispersal to thin-tailed, intermediate and fat-tailed kernels respectively. Simulation sets as in Fig. 4 (see text and Table S1 for details on the parameters used in the different simulation sets). Values are based on 1000 independent runs and averaged over seven distance classes. Observed values in 2006 and 2007 are also shown for comparative purposes.

Levels of among-mothers correlated paternity, r_{pAM} , were also chiefly influenced by the pollen dispersal kernel used in the simulations (Fig. 5). For a thin-tailed leptokurtic kernel (scenario 1A), the frequency of correlated matings at short distances was higher than that observed in the real population. This trend was enhanced when other parameters such as unequal male fecundity or spatial aggregation were included in the simulations. An intermediate-distance dispersal kernel, however, provided similar values to those observed in

2007 (scenarios 2A-B). Finally, the fat-tailed pollen dispersal kernel provided very low levels of correlated paternity with distance, similar to those recorded in 2006, especially at the first distance class (scenario 3B). For all dispersal kernels, the inclusion of pollen limitation through decreasing male density (scenarios C and E) increased the levels of correlated paternity especially at short distances (including r_{pWM}). On the other hand, the inclusion of some pattern of spatial aggregation of males (scenarios D and F) had variable effects; at short distances (including r_{pWM} , see Fig. 4), it tended to diminish the proportion of correlated matings compared with the case with random distribution of males, whereas at long distances it resulted in higher levels of r_p . This reversal trend occurred at higher spatial scales as fatter-tailed dispersal kernels were used (i.e. when increasing the average effective pollination distance).

Discussion

Genetic diversity and mating system

Pistacia lentiscus showed high levels of genetic diversity, typical of out-crossing wind-pollinated woody plant species (Nybom 2004). Biparental inbreeding (t_m-t_s) and within-mother correlated paternity (r_{pWM}) were relatively low in the study population, implying that *P. lentiscus* females growing at high conspecific density and balanced sex-ratio received genetically diverse pollen clouds from a relatively high number of fathers ($N_{ep} = 33.6$ and 11.8 in 2006 and 2007 respectively) [see Hardy *et al.* (2004) and Smouse & Sork (2004) for reviews of N_{ep} values in plants]. However, the observed N_{ep} values were generally lower (especially in 2007) than those reported in wind-pollinated tree species growing at high conspecific densities (e.g. $N_{ep} = 71-125$ in *Pinus sylvestris*, Robledo-Arnuncio *et al.* 2004; $N_{ep} = 63-143$ in *Picea glauca*, O'Connell *et al.* 2006). The shrubby growth habit of *P. lentiscus* and the lack of adaptations in the pollen grains to air flotation could decrease pollen dispersal distances compared with coniferous trees.

The percentage of viable seeds (%VS) was low overall, but significantly different between years. Water availability during the period of fruit maturation has been suggested as a determinant factor of *P. lentiscus* fruit-set (Jordano 1988). Accordingly, we found higher %VS in 2007, which was the wetter of the two studied years (49 mm vs. 159 mm in 2006 and 2007 respectively, from May to September). At the individual plant level, %VS was very variable (Table S3) and contrary to previous studies (Jordano 1988), not related to plant size. This high inter-individual variation suggests that female fitness in *P. lentiscus* is tightly linked to the individual plant's stochastic history (i.e. predation rates, resource

accumulation, microenvironment, etc.), as suggested by Verdú & García-Fayos (1998, 2002).

Interestingly, we found that biparental inbreeding and within-mother correlated paternity (r_{pWM}) were negatively correlated to the distance between females and their closest flowering male, despite unavoidable stochasticity resulting in wide confidence intervals (Ivey & Wyatt 1999). Thus, the most isolated mother plants were pollinated by the most genetically diverse and unrelated pollen clouds, especially in 2007. Preferential matings with neighbouring individuals have been frequently reported for wind-pollinated species (Burczyk *et al.* 1996; Dow & Ashley 1998), however, this effective pollen contribution from spatially close individuals is expected to be conspicuous only when the total number of potential pollen donors in the population is small and/or plant density is low (Levin & Kerster 1974; Loveless & Hamrick 1984). For instance, Robledo-Arnuncio *et al.* (2004) found a significant relationship between r_{pWM} and the distance to the nearest tree within a small, isolated and sparse population of Scots pine of only 2.4 trees/ha. Our results extend this view and show that even in situations of high global population density, individual-plant mating systems may display variation at small spatial scales, in our case, probably influenced by subtle changes in the conspecific male distribution.

Spatial genetic structure at different scales

The lack of fine-scale SGS in adults was in agreement with low levels of biparental inbreeding in the *P. lentiscus* population. It is consistent also with unrestricted gene movement across the study site, as predicted based on life history traits (Kalisz *et al.* 2001; Vekemans & Hardy 2004). Long-distance pollen dispersal contributes to lessen the degree of within-population SGS (e.g. Streiff *et al.* 1998; González-Martínez *et al.* 2002), and several lines of evidence suggested that extensive pollen flow is common in the *P. lentiscus* population studied. First, the pollen cloud received by the females showed a weak spatial genetic structure, which suggested very fat-tailed pollen dispersal kernels. Second, the low levels of among-mothers correlated paternity (r_{pAM}), especially in 2006, suggested no pollen limitation because of distance from males to females (Verdú *et al.* 2006). Finally, numerical simulations using intermediate- and long-distance effective dispersal kernels provided the best matches to the observed values of r_{pWM} and r_{pAM} (see below).

Seed dispersal also shapes patterns of SGS within plant populations. High rates of long-distance seed dispersal are linked to the absence or weak patterns of SGS in plants (Vekemans & Hardy 2004; Hardy *et al.*

2006; Dick *et al.* 2008). *Pistacia lentiscus* fulfils the prerequisites for its seeds to move extensively across the landscape and it shows high fruit removal rates (ranging from 42% to 91% depending on the population, see Table 2 in Verdú & García-Fayos 2002). Some of the *P. lentiscus* regular seed dispersers (thrushes, black-caps, warblers and robins) winter in the study area (Herrera 1984) and are well-suited for efficient seed transport over long distances along their migratory routes.

Temporal variation of the mating system

One of the salient results from our study was the significant differences found in the levels of correlated paternity across years. Although relatively low, the within-mothers correlated paternity (r_{pWM}) was three times higher in 2007 than in 2006, which translated into a higher number of effective pollen donors in 2006. Interestingly, this variation in the frequency of correlated matings occurred even though the gene diversity of the pollen cloud was very similar during the 2 years. However, the contour plots showed a spatial pattern of variation in the gene diversity of the pollen cloud, with patches of high and low gene diversity that did not remain constant from year to year. Patches of high gene diversity were larger in 2006 than in 2007. This spatial heterogeneity translated to mothers located in close proximity (up to 30 m) that shared a detectable portion of fathers in 2007, but not in 2006. The fact that both r_{pWM} and r_{pAM} followed the same trend also suggests that, while most pollen was widely dispersed across the population, a detectable fraction of effective pollen was spatially restricted in 2007.

The fact that the two study seasons were climatically homogeneous during the flowering period suggests that the observed differences might be because of intrinsic individual factors such as interannual variation in phenological patterns (among others). Interannual variation in the length of the flowering time of males (of up to 10 days) has been reported in *P. lentiscus* (Jordano 1988) and could be responsible for the observed differences because of the modification of the effective average distance between individuals from year to year (Hall *et al.* 1996). Whatever the cause, interannual variation in the genetic composition of the pollen sampled by seed plants may have strong evolutionary implications (Hall *et al.* 1996; Irwin *et al.* 2003), particularly if subsequent seedling establishment is not constant from year to year and strongly depends on the unstable climate conditions associated with Mediterranean ecosystems (Lloret *et al.* 2005). Further, this can be enhanced in species, which do not form a permanent soil seed bank such as *P. lentiscus* (Verdú & García-Fayos 2002).

Numerical simulations

Numerical simulations showed that spatial clumping of males (or their flowering) and/or a decrease in the males' global density (i.e. below the suggested threshold for pollen limitation of 100 males/ha; Verdú & García-Fayos 1998), increased the rate of correlated matings within and among progeny arrays at short distances, exceeding the observed values in some cases. This density effect was proportionally stronger to the average pollen dispersal distance (i.e. simulating flat dispersal kernels). This result agreed with the empirical assessment of high levels of correlated paternity in low density populations of wind-pollinated species (Robledo-Arnuncio *et al.* 2004). Thus, our numerical simulations suggest that anthropogenic habitat disturbance, through the breaking of large woodland patches into small discrete remnant patches and by decreasing the density within patches, may have a great impact in the mating system of wind-pollinated species, which may be especially important in dioecious species such as *P. lentiscus* (Smouse & Sork 2004).

In conclusion, we have shown that despite extensive overall pollen flow, significant differences in mating system parameters could be detected even between apparently climatically homogeneous seasons in a wind-pollinated species. Thus, our study joins the growing body of evidence (e.g. Nakanishi *et al.* 2005; Pakkad *et al.* 2008) demonstrating that single-season dispersal and gene flow studies, although undoubtedly useful, might not represent a comprehensive view of the mating system in perennial organisms.

Acknowledgements

The authors thank L. Fernández and JP González-Varo for field assistance, Dr. C. de Vega and two anonymous referees for comments in previous drafts of the manuscript, John Muddeman for reviewing the English style and the landowners for permission to work in the area. Laboratory work was conducted at the Molecular Biology Service of the 'Centro de Investigación, Tecnología e Innovación de la Universidad de Sevilla (CITUSY). This study has been funded by grants of the Andalusian Regional Government (Proyecto de Excelencia P06-RNM-01499) to AA. RGA was supported by a postdoctoral grant associated to the same project. MH acknowledges a postdoctoral contract of the National Fund for Scientific Research of Belgium (FRS-FNRS) and an FNRS-funded scientific visit to CIFOR-INIA.

References

- Albaladejo RG, Sebastiani F, Aparicio A, Buonamici A, González-Martínez SC, Vendramin GG (2008) Development and characterization of eight polymorphic microsatellite loci from *Pistacia lentiscus* L. (Anacardiaceae). *Molecular Ecology Resources*, **9**, 904–906.

- Austerlitz F, Dick CW, Dutech C *et al.* (2004) Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology*, **13**, 937–954.
- Bacles CFE, Ennos RA (2008) Paternity analysis of pollen-mediated gene flow for *Fraxinus excelsior* L. in a chronically fragmented landscape. *Heredity*, **101**, 368–380.
- Bittencourt JVM, Sebbenn AM (2007) Patterns of pollen and seed dispersal in a small, fragmented population of the wind-pollinated tree *Araucaria angustifolia* in southern Brazil. *Heredity*, **99**, 580–591.
- Burczyk J, Adams WT, Shimizu JY (1996) Mating patterns and pollen dispersal in a natural knobcone pine (*Pinus attenuata* Lemmon.) stand. *Heredity*, **77**, 251–260.
- Clark JS (1998) Why trees migrate so fast: confronting theory with dispersal biology and the paleorecord. *American Naturalist*, **152**, 204–224.
- Correia OA, Martins AC, Catarino FM (1992) Comparative phenology and seasonal foliar nitrogen variation in Mediterranean species of Portugal. *Ecologia Mediterranea*, **18**, 7–18.
- Devlin B, Clegg J, Ellstrand N (1992) The effect of flower production on male reproductive success in wild radish populations. *Evolution*, **46**, 1030–1042.
- Dick CW, Hardy OJ, Jones FA, Petit RJ (2008) Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology*, **1**, 20–33.
- Dow BD, Ashley MV (1998) High levels of gene flow in bur oak revealed by paternity analysis using microsatellites. *Journal of Heredity*, **89**, 62–70.
- Franceschinelli EV, Bawa KS (2000) The effect of ecological factors on the mating system of a South American shrub species (*Helicteres brevispira*). *Heredity*, **84**, 116–123.
- Friedman J, Barrett SCH (2009) Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany*, **103**, 1515–1527.
- González-Martínez SC, Gerber S, Cervera MT, Martínez-Zapater JM, Gil L, Alía R (2002) Seed gene flow and fine-scale structure in a Mediterranean pine (*Pinus pinaster* Ait.) using nuclear microsatellite markers. *Theoretical and Applied Genetics*, **104**, 1290–1297.
- González-Varo JP, Albaladejo RG, Aparicio A (2009) Mating patterns and spatial distribution of conspecific neighbours in the Mediterranean shrub *Myrtus communis* (Myrtaceae). *Plant Ecology*, **203**, 207–215.
- Goudet J (2002) FSTAT 2.9.3.2: a computer program to calculate F-statistics, estimate and test gene diversities and fixation indices. Available at <http://www.unil.ch/izea/software/fstat.html>.
- Hall P, Walker S, Bawa K (1996) Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. *Conservation Biology*, **10**, 757–768.
- Hardy OJ, Vekemans X (2002) Spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.
- Hardy OJ, González-Martínez SC, Colas B, Fréville H, Mignot A, Olivieri I (2004) Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics*, **148**, 1601–1614.
- Hardy OJ, Maggia L, Bandou E *et al.* (2006) Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology*, **15**, 559–571.
- Herrera CM (1984) A study of avian frugivores, bird-dispersed plants and their interactions in Mediterranean scrublands. *Ecological Monographs*, **54**, 1–23.
- Irwin AJ, Hamrick JL, Godt MJW, Smouse PE (2003) A multiyear estimate of the effective pollen donor pool for *Albizia julibrissin*. *Heredity*, **90**, 187–194.
- Ivey CT, Wyatt R (1999) Family outcrossing rates and neighborhood floral density in natural populations of swamp milkweed (*Asclepias incarnata*): potential statistical artifacts. *Theoretical and Applied Genetics*, **98**, 1063–1071.
- Jordano P (1988) Polinización y variabilidad de la producción de semilla en *Pistacia lentiscus* L. (Anacardiaceae). *Anales Jardín Botánico de Madrid*, **45**, 213–231.
- Jordano P (1989) Pre-dispersal biology of *Pistacia lentiscus* (Anacardiaceae): cumulative effects on seed removal by birds. *Oikos*, **55**, 375–386.
- Kalisz S, Nason JD, Hanzawa FM, Tonsor SJ (2001) Spatial population genetic structure in *Trillium grandiflorum*: the roles of dispersal, mating, history, and selection. *Evolution*, **55**, 1560–1568.
- Karron JD, Marshall DL (1990) Fitness consequences of multiple paternity in wild radish, *Raphanus sativus*. *Evolution*, **44**, 260–268.
- Knapp EE, Goedde MA, Rice KJ (2001) Pollen-limited reproduction in blue oak: implications for wind pollination in fragmented populations. *Oecologia*, **128**, 48–55.
- Koenig WD, Ashley MV (2003) Is pollen limited? The answer is blowin' in the wind. *Trends in Ecology and Evolution*, **18**, 157–159.
- Levin DA, Kerster HW (1974) Gene flow in seed plants. *Evolutionary Biology*, **7**, 139–220.
- Lloret F, Peñuelas J, Estiarte M (2005) Effects of vegetation canopy and climate on seedling establishment in Mediterranean shrubland. *Journal of Vegetation Science*, **16**, 67–76.
- Loiselle BA, Sork VL, Nason JD, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, **82**, 1420–1425.
- Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, **15**, 65–95.
- de-Lucas AI, Robledo-Arnuncio JJ, Hidalgo E, González-Martínez SC (2008) Mating system and pollen gene flow in Mediterranean maritime pine. *Heredity*, **100**, 390–399.
- Martínez-Pallé E, Aronne G (2000) Reproductive cycle of *Pistacia lentiscus* (Anacardiaceae) in southern Italy. *Plant Biosystems*, **134**, 365–371.
- Nakanishi A, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2005) Interannual genetic heterogeneity of pollen pools accepted by *Quercus salicina* individuals. *Molecular Ecology*, **14**, 4469–4478.
- Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, **13**, 1143–1155.
- O'Connell LM, Mosseler A, Rajora OP (2006) Impacts of forest fragmentation on the mating system and genetic diversity of white spruce (*Picea glauca*) at the landscape level. *Heredity*, **97**, 418–426.
- Pakkad G, Ueno S, Yoshimaru H (2008) Gene flow pattern and mating system in a small population of *Quercus semiserrata* Roxb. (Fagaceae). *Forest Ecology and Management*, **255**, 3819–3826.

- Quézel P (1981) Floristic composition and phytosociological structure of sclerophyllous matorral around the Mediterranean. In: *Mediterranean-Type Shrubland* (eds Di Castri F, Goodall DW, Spetch RL). pp. 107–121, Elsevier, Amsterdam, The Netherlands.
- Ritland K (2002) Extensions of models for the estimation of mating systems using n independent loci. *Heredity*, **88**, 221–228.
- Robledo-Arnuncio JJ, Austerlitz F (2006) Pollen dispersal in spatially aggregated populations. *American Naturalist*, **168**, 500–511.
- Robledo-Arnuncio JJ, Gil L (2005) Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. *Heredity*, **94**, 13–22.
- Robledo-Arnuncio JJ, Alía R, Gil L (2004) Increased selfing and correlated paternity in a small population of a predominantly outcrossing conifer, *Pinus sylvestris*. *Molecular Ecology*, **13**, 2567–2577.
- Robledo-Arnuncio JJ, Austerlitz F, Smouse PE (2006) A new method of estimating the pollen dispersal curve independently of effective density. *Genetics*, **173**, 1033–1045.
- Robledo-Arnuncio JJ, Austerlitz F, Smouse PE (2007) POLDISP: a software package for indirect estimation of contemporary pollen dispersal. *Molecular Ecology Notes*, **7**, 763–766.
- Smouse PE, Sork VL (2004) Measuring pollen flow in forest trees: an exposition of alternative approaches. *Forest Ecology and Management*, **197**, 21–38.
- Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landscape Ecology*, **21**, 821–836.
- Sork VL, Davis FW, Smouse PE *et al.* (2002) Pollen movement in declining populations of California Valley oak, *Quercus lobata*: where have all the fathers gone? *Molecular Ecology*, **11**, 1657–1668.
- Streiff R, Labbe T, Bacilieri R, Steinkellner H, Glossl 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.
- Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, **13**, 921–935.
- Verdú M, García-Fayos P (1998) Ecological causes, function, and evolution of abortion and parthenocarpy in *Pistacia lentiscus* (Anacardiaceae). *Canadian Journal of Botany*, **76**, 134–141.
- Verdú M, García-Fayos P (2001) The effect of deceptive fruits in predispersal seed on predation by birds in *Pistacia lentiscus*. *Plant Ecology*, **156**, 245–248.
- Verdú M, García-Fayos P (2002) Ecología reproductiva de *Pistacia lentiscus* L. (Anacardiaceae): un anacronismo evolutivo en el matorral mediterráneo. *Revista Chilena de Historia Natural*, **75**, 57–65.
- Verdú M, González-Martínez SC, Montilla AI, Mateu I, Pannell JR (2006) Ovule discounting in an outcrossing, cryptically dioecious tree. *Evolution*, **60**, 2056–2063.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Young JPW (1981) Sib competition can favour sex in two ways. *Journal of Theoretical Biology*, **88**, 755–756.

R.G.A. is interested in natural hybridization and the consequences of habitat fragmentation and in plant species. S.C.G.-M. has long-term interests in population genetics and genomics of forest trees and the evolution of plant diversity in the Mediterranean region. M.H. is interested in empirical and simulation studies of mating systems, gene flow and genetics structure in plants. G.G.V. is interested in conservation genetics, genetic structure studies and genomics of forest trees. A.A. is interested in population biology and conservation genetics of Mediterranean plants.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Simulation sets performed to assess the effect of pollen dispersal, relative male fecundity and density and spatial aggregation of males on the levels of within- and among-mothers correlated paternity

Table S2 Genetic diversity statistics at the seven resolved loci for *P. lentiscus* in the adult population and the pollen pool in the two study years. A = number of alleles; H_E = Nei's gene diversity; f = Weir & Cockerham's inbreeding coefficient; H = gene diversity based on pollen gametes

Table S3 Individual-plant mating system estimates in *P. lentiscus*. Multilocus (t_m) and single-locus (t_s) outcrossing rates were computed following the method of moments of Ritland (2002), and within-mothers correlated paternity (r_{pWM}) was computed following Hardy *et al.* (2004)

Fig. S1 Averaged values of among-mothers sibships correlated paternity (r_{pAM}) based on simulated progeny arrays as in Fig. 5, but with male reproductive effort fitted to a lineal function of plant size

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.