

Effective gene dispersal and female reproductive success in Mediterranean maritime pine (*Pinus pinaster* Aiton)

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Abstract

Understanding population-scale processes that affect allele frequency changes across generations is a long-standing interest in genetic, ecological and evolutionary research. In particular, individual differences in female reproductive success and the spatial scale of gene flow considerably affect evolutionary change and patterns of local selection. In this study, a recently developed maximum-likelihood (ML) method based on established offspring, the *Seedling Neighbourhood Model*, was applied and exponentially shaped dispersal kernels were fitted to both genetic and ecological data in a widespread Mediterranean pine, *Pinus pinaster* Aiton. The distribution of female reproductive success in *P. pinaster* was very skewed (about 10% of trees mothered 50% of offspring) and significant positive female selection gradients for diameter ($\gamma = 0.7293$) and cone crop ($\gamma = 0.4524$) were found. The selective advantage of offspring mothered by bigger trees could be due to better-quality seeds. These seeds may show more resilience to severe summer droughts and microsite variation related to water and nutrient availability. Both approaches, ecological and of parentage, consistently showed a long-distance dispersal component in saplings that was not found in dispersal kernels based on seed shadows, highlighting the importance of Janzen-Connell effects and microenvironmental variation for survival at early stages of establishment in this Mediterranean key forest tree.

Keywords: dispersal kernels, female reproductive success, gene immigration, inverse modelling, Mediterranean pines, microsatellites, selection gradients

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Introduction

The differential contribution of individual mother plants to future generations and their spatial distribution affect key evolutionary parameters such as the effective population size or the level of within-population gene diversity (Crow & Kimura 1970). An unequal reproductive success and a restricted gene flow both promote the establishment of fine-scale genetic structure and the adaptation to local environments (Doligez *et al.* 1998; Epperson 2003; Rousset 2004). The occurrence of long-distance gene flow can change

the unit at which evolution operates: whereas isolated populations can be considered evolutionary units in themselves, in the case of long-distance gene flow the dynamics of neighbouring populations have also to be taken into account (Ouborg *et al.* 1999).

Fertility variation in plants has often been studied through the analysis of selection gradients (e.g. Burczyk *et al.* 1996; Smouse *et al.* 1999; Morgan & Conner 2001; Wright & Meagher 2004). Selection gradients are defined as the slope of the regression of relative fertility on trait values measured in single plants. Linear slopes identify directional selection and quadratic components are associated with stabilizing (negative values) or disruptive (positive values) selection. Evidence of selection gradients resulting in

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differences of male or female reproductive success among individuals has been found for some traits and plant species (e.g. flower size in *Raphanus raphanistrum*, Morgan & Conner 2001; flower phenology and intensity in *Pseudotsuga menziesii*, Burczyk & Prat 1997). However, selection gradient estimates, even those based on differences in female reproductive success, have not usually considered postdispersal processes, which can affect recruitment greatly.

In plant populations, recruitment occurs only if both the propagules' dispersal and the subsequent establishment of the seedlings are successful. Seed and seedling mortality affects population structure profoundly. At early life-history stages, high density-dependent mortality (Janzen 1970; Nathan & Casagrandi 2004) and/or habitat depletion by mothers (Howe & Smallwood 1982) can promote higher average distances between mothers and successfully established offspring than those expected from seed dispersal alone (the so-called Janzen-Connell effects; see review in Nathan & Casagrandi 2004). Studies based on seed and sapling shadows from multiple seed sources under closed canopies, carried out in different forest systems (e.g. temperate deciduous forest, Hille Ris Lambers & Clark 2003; Mediterranean conifer forest, Nathan *et al.* 2000) suggest the existence of Janzen-Connell (J-C) effects in forest trees, highlighting the importance of postdispersal mortality in shaping population structure and dynamics. In addition, microsite heterogeneity and/or genotype-microsite interactions, especially in spatially variable environments (Hille Ris Lambers & Clark 2003), can limit the recruitment of offspring from particular individuals, despite high offspring production, depending on the spatial location of the mother plants and the patches adequate for establishment (Álvarez-Buylla *et al.* 1996; Nathan & Muller-Landau 2000). Consequently, selection gradients and dispersal estimates based on established offspring are more relevant than the mere description of mating success and seed movements.

While mating system and dispersal estimates that are based on established offspring have been studied in a number of plant species with molecular markers, these studies have generally addressed gene flow (e.g. *Magnolia obovata*, Isagi *et al.* 2000; *Pinus pinaster*, González-Martínez *et al.* 2002; oaks, Dow & Ashley 1996; Valbuena-Carabaña *et al.* 2005; *Cercidiphyllum japonicum*, Sato *et al.* 2006) but only rarely female reproductive success or other mating system features (Meagher & Thompson 1987; Schnabel *et al.* 1998; Kameyama *et al.* 2001). In their pioneering study on *Chamaelirium luteum*, a dioecious long-lived perennial plant, Meagher & Thompson (1987) found a negative correlation for both sexes between plant size (as estimated by leaf count) and number of established progeny. In this species, the number of successfully established progeny showed also a strong correlation with the number of mates (Meagher & Thompson 1987). Female stem diameter and distance to open land explained about 45% of the variation

in female fertility in *Gleditsia triacanthos*, a mostly dioecious tree (Schnabel *et al.* 1998). In this species, female fertility was also quite constant for individual females across offspring age classes. Finally, Kameyama *et al.* (2001) showed that the effects of distance and plant size on adult fertility in *Rhododendron metternichii* var. *hondoense*, a hermaphroditic shrub from western Japan, can vary with the spatial configuration of the population.

Maritime pine (*Pinus pinaster* Aiton) is widely distributed in the western Mediterranean basin, growing in environments ranging from coastal dunes to calcareous, medium-altitude mountains. The current-day distribution of maritime pine in the Iberian Peninsula is explained by the location of Holocene glacial refugia, environmental restrictions and fire-mediated replacement dynamics with other Mediterranean conifers, such as Aleppo pine (Salvador *et al.* 2000; Carrión *et al.* 2001; Gómez *et al.* 2005). To increase our understanding of this key Mediterranean pine, an Intensive Study Plot (ISP) was established in a typical Mediterranean location of the species (Coca, central Spain) in 1999. Previous work in the Coca ISP has shown a significant albeit weak fine-scale spatial structure at short distances, both with neutral molecular markers and individual tree attributes such as total height, and a low level of family substructure within the plot (González-Martínez *et al.* 2003; Nanos *et al.* 2004). A parentage analysis for successfully established saplings showed restricted gene flow and isotropic dispersal within the plot (González-Martínez *et al.* 2002).

In this work, seed trapping data, individual tree phenotypic measurements (size, growth, intraspecific competition and 1-year female reproductive effort) and molecular markers are combined to: (i) produce accurate estimates of seed and pollen immigration among established offspring (ii) identify selection gradients related to female reproductive success, using a novel maximum-likelihood (ML) approach and (iii) study patterns of recruitment within the stand by comparison of dispersal kernels at different life stages (seeds and successfully established saplings). Dispersal kernels for saplings were fitted using both ecological, i.e. inverse modelling (IM) techniques, based on the observed density of offspring, and genetic, i.e. parent-offspring dispersal distributions obtained directly from genetic parentage analyses (PA), methods allowing the comparison for this life-stage of two widespread but rarely combined approaches.

Materials and methods

Study plot and data collection

The Intensive Study Plot (ISP) of Coca is located within a native low-density (~120 stems/ha) stand of *Pinus pinaster* in central Spain (latitude 41°16'N and longitude 4°29'W). The climate is typically Mediterranean, with a mean annual rainfall of 432 mm (72 mm in summer) and a pronounced

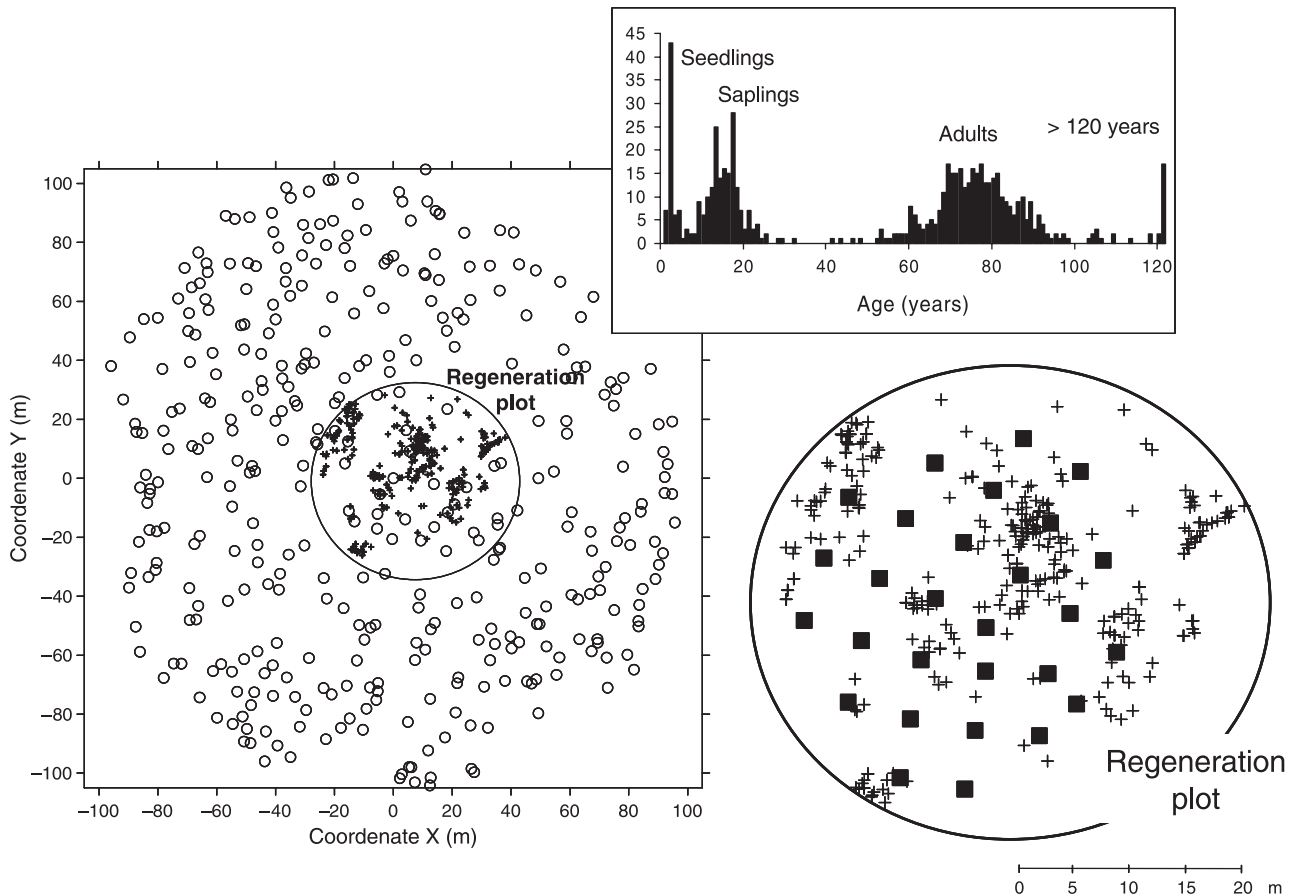


Fig. 1 Intensive Study Plot (ISP) of Mediterranean maritime pine at Coca, central Spain. Crosses and open circles represent seedlings and saplings, and adult trees, respectively. Seed traps are represented by filled squares. An age distribution histogram, showing the different cohorts, is also given.

summer drought. The mean annual temperature is 12.3 °C. The pine forest in this region grows on fossil dunes (from the Tertiary) of different depth and deposited over an impermeable clay layer. This particular geological setting has consequences for the distribution of tree species, as the phreatic level varies from a few centimetres to several metres in depth. Sites with superficial phreatic levels present small relict stands of temperate species such as *Pinus sylvestris*, whereas *P. pinaster* inhabits the sites with ground water at greater depth. The different depth of the ground water also promotes a high microsite variation and the clustering of natural regeneration on more humid sites. The understorey within the plot is scattered and dominated by scrubland species and herbs such as *Retama sphaerocarpa* (L.) Boiss., *Stipa* spp., *Thymus mastichina* (L.) L. and *Helichrysum stoechas* (L.) DC. Some isolated *Pinus pinea* L. trees are also present.

A 30-m radius (0.28 ha) regeneration plot was defined at the centre of the 100-m radius (3.14 ha) ISP (Fig. 1). In 2000, there were 380 adult trees in the ISP and 267 offspring in the regeneration plot. Seed dispersal was investigated

during this year by placing 28 square (1 × 1 m) traps in the regeneration plot in February 2000. Traps were visited weekly until October 2000 and all seeds were collected and counted. The spatial locations of all trees (polar coordinates) were recorded, using a total station. A core was taken from each adult tree at 1.30 m height and kept refrigerated (4 °C) until analysis. The age of trees was estimated by counting growth whorls (seedlings, saplings) or growth rings from the cores (adults). We differentiated three life stages: seedlings ($n = 66$), defined as offspring less than 4 years old and with a low probability of survival; saplings ($n = 201$), defined as offspring successfully established, with an average age of 15 (SD: 4) and maximum of 25 years old, considered to be prereproductive or to have produced so few seeds as to be essentially inactive reproductively, in comparison with older and larger trees and adult trees (i.e. fully reproductive individuals; $n = 380$), with an average age of 79 (SD: 17) years old. Given the low probability of seedling survival (mortality of first-year seedlings during 2000's summer was ~65%), they were not considered in the genetic analyses.

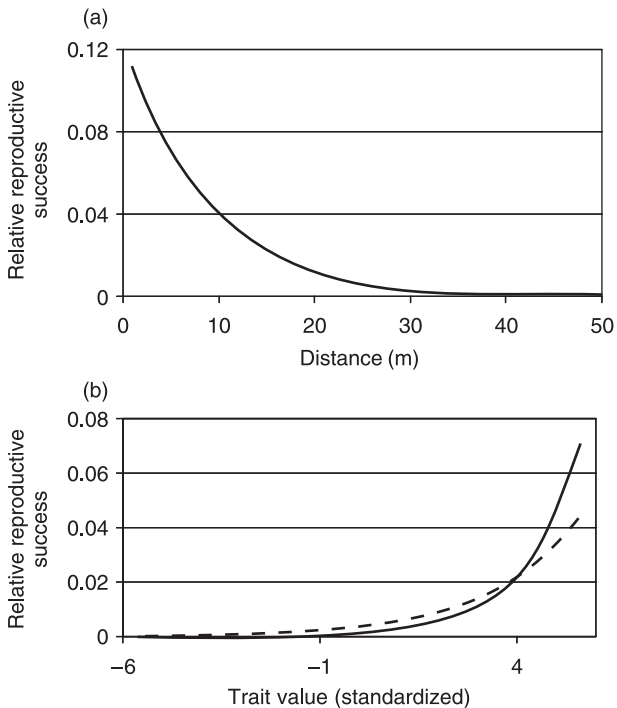


Fig. 2 The relationship between female reproductive success and (a) distance between mother trees and offspring and (b) diameter at 1.30 m (continuous lines) and number of cones (broken lines), based on the interplant distance effects and the linear selection gradients that gave the lowest negative log-likelihood (NLL) in mating models. Trait values are standardized, thus making selection gradients directly comparable.

Seedlings and saplings were measured for total height and age. In adult trees, the following variables were recorded: age, total height, diameter at 1.30 m, diameter increment in the last 10 years, number of female strobili in the spring of 2000 and number of cones in the winter of 2000–2001 (flowering of 1999). Diameter increment was measured from cores using WINDENDRO version 6.5 C software. Numbers of female strobili and cones were estimated by visual counts (see details in Miguel *et al.* 2002). Finally, two types of individual competition indexes were computed: basal area in trees with diameter at 1.30 m larger than the subject tree (*BAL*) and basal area of the six trees closest to the object tree (*BA6*). *BAL* is a 'one-sided' competition index and represents the level of competition for light imposed by larger trees and *BA6* is a 'two-sided' competition index, reflecting competition for below-ground resources, such as nutrients and moisture (Vanclay 1994).

Needle tissue was collected from all adult trees and saplings ($n = 581$) and kept refrigerated for 1–3 days until DNA extraction. Total genomic DNA was extracted following a slightly modified Dellaporta *et al.* (1983) protocol. Three highly polymorphic nuclear microsatellites (ITPH4516, FRPP91 and FRPP94) yielding high exclusion probabilities (EP of

92.22 and 99.87% for single parents and parent pairs, respectively) were analysed, following protocols described by Mariette *et al.* (2001) using a LI-COR 4000 automatic sequencer (LI-COR Inc.).

Gene flow and reproductive success

We used a novel maximum-likelihood model, the *Seedling Neighbourhood Model*, to estimate jointly selfing rates, immigration by seed and pollen and the effect of interplant distance on female reproductive success and selection gradients based on established offspring (see details in Burczyk *et al.* 2006). This model is based on earlier neighbourhood models developed for pollen dispersal (Adams & Birkes 1989, 1991; Burczyk *et al.* 2002). Selection gradients, both linear and quadratic, are computed in a fashion similar to Smouse *et al.* (1999) and Morgan & Conner (2001). Major advantages of the *Seedling Neighbourhood Model* are (i) it includes both selfing and immigration rate (from outside the neighbourhood) as parameters within the likelihood model, thus rendering it suitable for nonisolated populations, a common case in temperate forest trees; and (ii) analyses are based on naturally established offspring populations (rather than on pollen or seed stages). Provided that we have high exclusion probabilities (EP=0.95), a small number of markers (a few microsatellites) is sufficient to produce accurate parameter estimates (Burczyk *et al.* 2006).

The model assumes that each offspring is mothered by either (i) a female located outside of an arbitrarily defined local population (i.e. the offspring's neighbourhood) due to seed immigration (with probability m_s) or (ii) a local female growing within the offspring's neighbourhood (with probability $1-m_s$). The neighbourhood is defined as a circular area surrounding an offspring, in our case a 50-m radius. The neighbourhood radius was fixed at 50 m in order to avoid border effects, while providing a fair chance of including a high number of parent trees within the neighbourhood. For each offspring with a local mother, it is assumed that the paternal gamete could have come from one of three sources: (i) self-fertilization (with probability s) (ii) migrant pollen from outside of the mother's neighbourhood (with probability m_p) or (iii) outcross fertilization with males located within the mother's neighbourhood (with probability $1-s-m_p$). The probability of observing a multilocus diploid genotype G_i among the offspring therefore is:

$$P(G_i) = m_s \cdot P(G_i | B_s) + (1 - m_s) \cdot \sum_j \Psi_{ij} \cdot \left[s \cdot P(G_i | M_{ij}, M_{ij}) + m_p \cdot P(G_i | M_{ij}, B_p) + (1 - s - m_p) \cdot \sum_k \phi_{ijk} \cdot P(G_i | M_{ij}, F_{ijk}) \right] \quad (1)$$

where $P(G_i | B_s)$ is the transition probability that an offspring immigrating from mother trees located outside of an

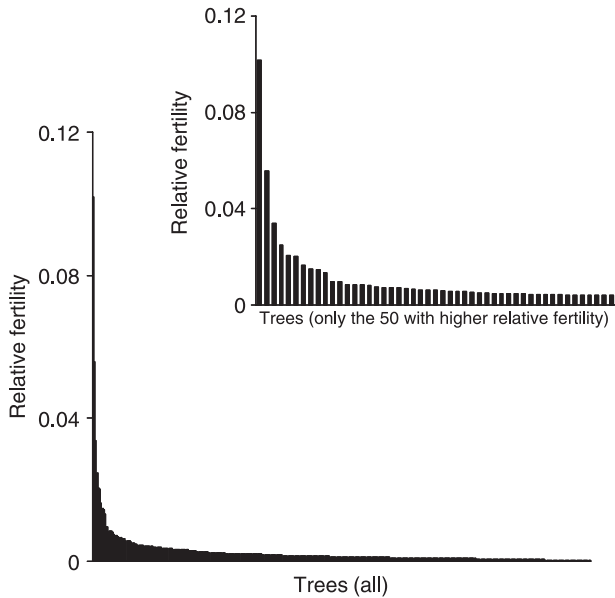


Fig. 3 Estimates of relative female reproductive success for each mother tree obtained by combination of the two selection gradient obtained from the best-fitting mating model. An amplified graph for the 50 trees with the highest individual relative female reproductive success is also shown.

offspring's neighbourhood (background females) has genotype G_i and $P(G_i | M_{ij}, M_{ij})$, $P(G_i | M_{ij}, B_p)$, $P(G_i | M_{ij}, F_{ijk})$ are the transition probabilities that an offspring has diploid genotype G_i when a mother plant of genotype M_{ij} is, respectively, self-pollinated, pollinated by a distant unknown background male or pollinated by a neighbouring plant having genotype F_{ijk} . The parameter ψ_{ij} is the relative reproductive success of the j -th female in the neighbourhood of the i -th offspring and ϕ_{ijk} is the relative reproductive success of the k -th male within the neighbourhood of the ij -th female. The effects of interplant distance and individual-tree attributes on reproductive success are estimated using a log-linear model. For instance, the reproductive success of the j -th female within the i -th seedling neighbourhood, ψ_{ij} , is expressed as:

$$\psi_{ij} = \frac{\exp(\omega_{ij})}{\sum_{h=1}^r \exp(\omega_{ih})} \quad (2)$$

where

$$\omega_{ij} = \gamma_d z_{dj} + \gamma_1 z_{1j} + \gamma_2 z_{2j} \quad (3)$$

is a linear function of the effect γ_d of interplant distance z_{dj} and one or two factors (γ_1, γ_2) influencing the mating success of the j -th female within the offspring's neighbourhood.

The denominator of (2) is the sum of $\exp(\omega_{ij})$ over all the potential local mothers (r) within a given seedling neighbourhood. In (3), the factors γ_1 and γ_2 are the linear selection gradients (equivalent to the β_j for male reproductive success in Smouse *et al.* 1999). The linear function (3) can be extended to include quadratic terms representing the effect of stabilizing or disruptive selection on reproductive success (Morgan & Conner 2001; Wright & Meagher 2004).

Mating system parameters, including linear and quadratic selection gradients, were estimated simultaneously by fitting the neighbourhood model to multilocus genotypic data using ML methods. Previously to model fitting, trait values were standardized in order to compare the relative strength of selection gradients. Neighbourhood models including selfing (s) and other parameters of male reproductive success (ϕ_{ijk}) consistently produced estimates for these parameters that did not differ from zero (data not shown). This was probably an effect of the high rates of pollen immigration within the plot (see Results) which reduced sample sizes to the extreme, so that only a few offspring-male parent pairs were available to estimate male selection gradients. Consequently, these parameters were removed from the models in further analyses. Several models were iterated to find the optimal fit to the data. The first series of models included only one gamma parameter, alternatively the distance between offspring and potential mothers, age, total height, diameter at 1.30 m, diameter increment in the last 10 years, number of female strobili, number of cones and the two (*BAL* and *BA6*) competition indices. The next series of models included the effect of interplant distance as the first gamma parameter and the effects of the rest of the variables were treated individually as second gamma parameters. Finally, a mating model with all three gamma parameters was attempted, selecting those variables that produced better fits (in terms of log-likelihood) in previous models. An individual estimate of relative female reproductive success for each tree was computed from equations (2) and (3) using the estimates of γ_1 and γ_2 (i.e. the two fitted selection gradients) from the final model.

Dispersal kernels for different life stages

Dispersal kernels show how the probability of offspring deposition varies with distance from the mother plant. The *Seedling Neighbourhood Model* described above includes an interplant distance term as a covariate but is not adequate to describe the shape of the dispersal curve, because it relies on an arbitrarily defined neighbourhood (in our case 50 m). The distribution of dispersal distances may fit various functional forms (Clark *et al.* 1998, 1999; Turchin 1998; Nathan & Muller-Landau 2000; Greene *et al.* 2004). Distributions of exponential type can be used to describe a multitude of functional forms:

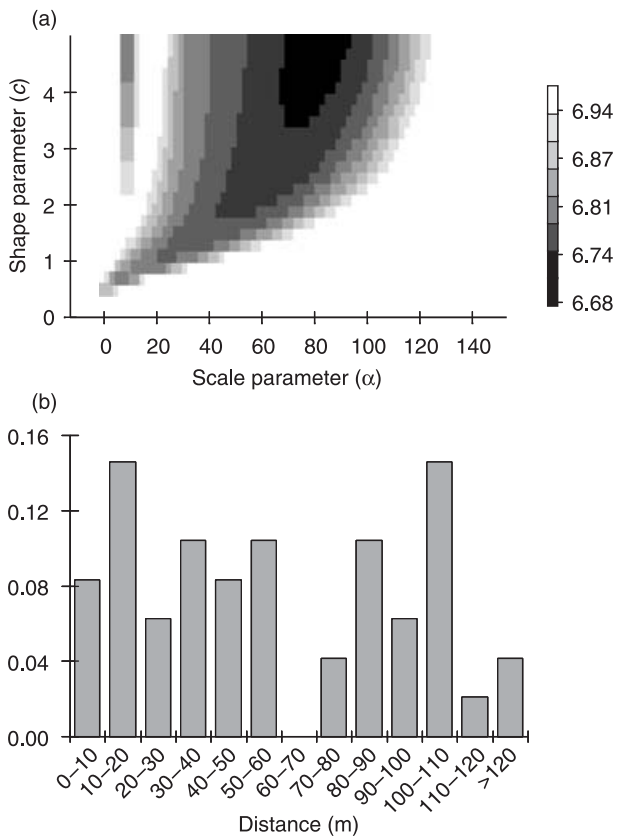


Fig. 4 Short- and long-distance dispersal components in sapling kernels; (a) likelihood surface for sapling dispersal kernels fitted using inverse modelling showing two areas of minimum values; (b) bimodal dispersal distance distribution obtained directly from parentage analysis.

$$f(x) = \frac{c}{2\pi\alpha^2\Gamma\left(\frac{2}{c}\right)} \exp\left[-\left(\frac{x}{\alpha}\right)^c\right] \quad (4)$$

where $f(x)$ is the dispersal kernel estimating the probability of a seed landing per unit area as a function of distance x from the point of release, c and α are shape and scale parameters, respectively, and Γ is the gamma function. We selected this distribution because it accommodates several popular distributions such as the Gaussian ($c = 2$) and the negative exponential ($c = 1$) as special cases (Clark *et al.* 1998). We also examined Clark's $2Dt$ distribution (Clark *et al.* 1999) but our attempts to fit this function resulted in unstable parameter estimates. Dispersal kernels for saplings were fitted using both ecological (inverse modelling based on the density of offspring observed in sampling plots) and genetic (the identified parent-offspring pairs from a molecular parentage analysis) data and were compared to dispersal kernels for seeds obtained by seed trapping and inverse modelling.

To obtain a parent-offspring distance distribution directly, a molecular parentage analysis was conducted. The most-likely parents and parent pairs were detected using log-likelihood ratios or LOD-scores (Meagher & Thompson 1986; Gerber *et al.* 2000) and population allele frequencies estimated from the whole dataset ($n = 581$). Only highly reliable parent-offspring matches (LOD-score > 4.4), as shown by simulation (see details in Gerber *et al.* 2000; González-Martínez *et al.* 2002), were used to fit the dispersal kernel ($n = 48$; see histogram in Fig. 4b). For most cases ($n = 45$), an offspring matched only one parent in the stand and this parent was assumed to be the seed parent. In three cases, a parent pair was found and the closest parent was assumed to be the seed parent. The dispersal kernel (equation 4) was fitted directly to the parent-offspring distance distribution, using a maximum-likelihood approach.

Inverse modelling techniques require knowledge of the spatial arrangement of offspring sampling plots and of all the potential seed sources (adult plants) and their relative fecundity. In our study, seed shadows were estimated from seed trapping, while sapling density was estimated by counting the plants in 2.5-m radius circles centred at the midpoint of each seed trap station. We repeated this procedure for circles of 4-m radius and obtained essentially the same results. Although the spatial location of offspring sampling plots and potential seed sources are known, the actual dispersal distances of observed offspring remain unknown. Inverse modelling techniques estimate dispersal distances only indirectly, by fitting a certain sampling distribution to the observed offspring number in the sampling plots. Previous studies assumed either Poisson (e.g. Clark *et al.* 1999) or negative binomial (e.g. Clark *et al.* 1998) sampling distributions. We used the negative binomial distribution because it constantly exhibited convergence while the Poisson distribution gave unstable results. Fecundity (Q_i), the number of seeds dispersed from the i -th tree, was assumed to be a linear function of the tree basal area (B_i) (Clark *et al.* 1998, 1999). For seed dispersal kernels, the dispersal shape parameter (c) was fixed ($c = 0.5$, $c = 1$, $c = 2$ and $c = 3$), while the dispersal scale parameter (α), the clumping parameter of the negative binomial and the fecundity parameter (b) were fitted. For sapling dispersal kernels, the fecundity parameter was computed based on seed fecundity and field observations on seed-to-seedling (0.001 of annual mortality) and seedling-to-sapling (0.1 of annual mortality) transitions ($b \times 0.001 \times 3 \text{ years} \times 0.1 \times 22 \text{ years} = 32.43$ saplings/m² basal area, where b is the fitted fecundity parameter from the best seed dispersal kernel). The best dispersal kernel was chosen based on negative log-likelihood (NLL) surfaces provided by the nonlinear optimization function 'nlminb' implemented in S-PLUS (S-PLUS 2000, MathSoft).

Table 1 Simple mating models including seed (m_s) and pollen (m_p) immigration into 50-m neighbourhoods, and either interplant distance (γ_d) or one selection gradient (γ_i) for mother-tree reproductive success. Standard deviations are given between parentheses. *Dist*, distance between mother-trees and offspring; *DBH*, diameter at 1.30 m; *BAL*, basal area in trees with larger diameter at 1.30 m than the subject tree; *BA6*, basal area of the six closest trees to the object tree; *Flower*, number of female strobili; *Cones*, number of cones; *DI10*, diameter increment in the last 10 years; *NLL*, negative log-likelihood

Variables in the model	Parameter estimates					NLL
	m_s	m_p	γ_d	γ_1	γ_2	
$m_s, m_p, Dist, Age$	0.5127 (0.0738)	0.8216 (0.1210)	-0.1356 (0.0195)	0.1625 (0.2829)	—	2240.92
$m_s, m_p, Dist, Height$	0.5181 (0.0733)	0.8119 (0.1230)	-0.1373 (0.0196)	0.2071 (0.1949)	—	2240.55
$m_s, m_p, Dist, DBH$	0.4906 (0.0727)	0.8348 (0.1193)	-0.1228 (0.0181)	0.5842 (0.1776)	—	2236.64
$m_s, m_p, Dist, BAL$	0.4942 (0.0716)	0.8414 (0.1189)	-0.1237 (0.0178)	-0.6153 (0.2063)	—	2236.95
$m_s, m_p, Dist, BA6$	0.5163 (0.0731)	0.8165 (0.1212)	-0.1366 (0.0208)	-0.0067 (0.2156)	—	2241.08
$m_s, m_p, Dist, Flower$	0.4944 (0.0751)	0.8314 (0.1167)	-0.1282 (0.0198)	0.1446 (0.1380)	—	2240.63
$m_s, m_p, Dist, Cones$	0.4712 (0.0764)	0.8340 (0.1115)	-0.1247 (0.0188)	0.2721 (0.1253)	—	2239.12
$m_s, m_p, Dist, DI10$	0.5164 (0.0729)	0.8172 (0.1207)	-0.1375 (0.0199)	-0.0378 (0.1667)	—	2241.05
$m_s, m_p, Dist, DBH, Cones$	0.4323 (0.0710)	0.8502 (0.1015)	-0.1177 (0.0158)	0.7293 (0.1628)	0.4523 (0.1151)	2231.12

Results

Effective gene flow and female reproductive success

Selfing was not significant in any of the models. The immigration rates for both seed and pollen were high, considering a neighbourhood size of 50 m. Bigger neighbourhood sizes produced highly biased estimates due to border effects (data not shown). Simple models, including only one gamma parameter for maternal reproductive success, gave estimates of seed and pollen immigration from outside the 50-m neighbourhood of 51–62% and 82–100%, respectively (see Table S1, Supplementary material). These values were close to the final estimates based on models with more than one gamma parameter, which attributed $43.23 \pm 7.10\%$ and $85.02 \pm 10.15\%$, respectively, to seed and pollen immigration based on established offspring.

Simple models (only one gamma parameter) identified several significant correlations between dispersal distance or tree-attributes and female reproductive success (see Table S1). Distance between mothers and offspring was negatively correlated with female reproductive success ($\gamma_d = -0.1368$), giving the best fitting of this series of models. There was also evidence of positive directional selection (linear selection gradients) for diameter at 1.30 m, number of female strobili and number of cones. In contrast, intraspecific competition for light (*BAL* competition index) had a negative correlation with female reproductive success. Quadratic selection gradients were also significant for the same traits as linear selection gradients, but models including both linear and quadratic terms did not show a better fit than those including only linear terms. Only age, height, *BA6* and diameter increment in the last 10 years did

not show any significant effect on female reproductive success.

More complex significant models included interplant distance (first gamma parameter, included in all models as a covariate) along with diameter at 1.30 m, competition for light (*BAL*) and number of cones (Table 1). The number of female strobili and the number of cones were moderately correlated ($r = 0.44$) but only the number of cones was significant in this second series of models. As in the case of simple mating models, adding quadratic terms to the models did not result in any significant improvements (data not shown). Diameter at 1.30 m (*DBH*) and competition for light (*BAL*) were highly negatively correlated ($r = -0.97$), so only *DBH* was included in the final model. The final mating model included interplant distance and two linear selection gradients, diameter at 1.30 m and number of cones. The relationship between distance from females to offspring and female reproductive success was negative and relatively strong ($\gamma_d = -0.1177$) whereas *DBH* and number of cones showed positive and moderate correlations (selection gradients of 0.7293 and 0.4524, respectively, see also Fig. 2). This model had higher goodness-of-fit than simpler models with a lower number of parameters (-2 log-likelihood ratios of 11.04 and 19.92 relative to the best models with two and one gamma parameters, respectively; both ratios were highly significant, $P < 0.000$, as shown by LLR tests). Using the fitted selection gradients from the final mating model, we computed rough estimates of relative female reproductive success for each mother tree. The distribution of reproductive success was highly skewed. The 10 most successful mother trees (out of 380 sampled) were predicted to produce approximately 32% of the natural regeneration. However, less than 10% of the adult trees had relative female reproductive success below 0.00045, which corresponds,

Table 2 Summary of fixed and fitted parameters for competing seed and sapling dispersal kernels adjusted by inverse modelling. For seeds, the shape parameter (c) was fixed, while the scale parameter (α), the clumping parameter of the negative binomial and the fecundity parameter (b) were fitted. For saplings, the clumping and the fecundity parameters were also fixed to facilitate model convergence. NLL, negative log-likelihood

Fecundity (b)	Clumping parameter	Kernel parameters		Estimated dispersal distance (m)			NLL
		Shape (c)	Scale (α)	Mean	Median	95th percentile	
Seeds							
4893.89	8.42	0.5	1.06	21.24	14.29	63.73	74.05
4913.56	9.91	1	6.09	12.19	10.22	28.90	73.36
4925.78	9.96	2	10.55	9.35	8.78	18.26	73.57
4925.37	9.28	3	12.00	8.86	8.66	18.86	73.98
Saplings							
32.43	140	0.5	1.74	34.94	23.46	104.60	6.76
32.43	140	1	20.26	40.52	34.00	96.11	6.71
32.43	140	2	52.93	46.91	44.06	91.61	6.66
32.43	140	3	68.60	50.66	49.53	90.67	6.64

considering offspring density (0.07 saplings/m²), to less than one successfully established offspring within the plot. Visual analysis did not reveal any anisotropy in the spatial distribution of relative female reproductive success.

Dispersal kernels for different life stages

Distributions of exponential type ($c = 1$) gave the best fit for seed dispersal kernels (NLL = 73.36) with a scale parameter equal to 6.09, resulting in mean and median dispersal distances of 12.19 and 10.22 m, respectively (Table 2). The fecundity parameter was found to be stable during the minimization procedure, giving estimates of 4913.56 seeds/m² of basal area. This estimate is consistent with field observations based on cone counting in 2000 (9549.99 seeds/m² basal area) and 2006 (3230.69 seeds/m² basal area).

Contrary to seed dispersal kernels, the minimization of the negative log-likelihood was difficult to obtain for saplings due to numerical inconsistencies. The clumping parameter of the negative binomial distribution tended to converge for very high parameter values (more than 140) and it was fixed (140) in order to facilitate convergence. It should be noted that both the kernel and the fecundity parameters were insensitive to changes in the clumping parameter (from 10 to > 140). Dispersal kernels with lower NLL were nonleptokurtic ($c > 1$) and showed two areas of minimum values (Fig. 4a). The presence of two areas of minimum values can be attributed to a bimodal distribution of sapling dispersal distances, as shown also by the direct distance distribution obtained from parentage analysis (Fig. 4b). Apart from a short-distance dispersal component, also found for seeds, this last fact indicates a long-distance component, probably related to postdispersal processes. The best dispersal kernel fitted to the distance distribution

obtained from parentage analysis showed mean (median) distances of 58.16 m (49.52 m) (kernel parameters of $c = 1.06$ and $\alpha = 32.44$) similar to those obtained from inverse modelling based on sapling shadows (mean of 40.52–50.66 m and median of 34.00–49.53 m for $c = 1$ –3; see Table 2).

Discussion

Gene flow and female reproductive success

The absence of selfing and the high levels of immigration into the 50-m neighbourhoods by pollen ($85.02 \pm 10.15\%$) and seed ($43.23 \pm 7.10\%$) were in agreement with the life-history traits and ecology of pines (Richardson 1998) and other conifers (see reviews in Lanner 1998; Boshier 2000; Smouse & Sork 2004). Conifers typically lack prezygotic self-incompatibility systems but severe inbreeding depression usually results in negligible self-fertilization at the seed and sapling stages. However, in small isolated populations selfing can be high (Robledo-Arnuncio *et al.* 2004). Using the *Seedling Neighbourhood Model*, we identified statistically significant distance effects ($\gamma_d = -0.1177$). A high probability of offspring being mothered by nearby trees due to restricted dispersal is a common finding in forest trees (e.g. Nathan *et al.* 2000; González-Martínez *et al.* 2002; Greene *et al.* 2004). Using the *Seedling Neighbourhood Model*, we were able to estimate the median sapling dispersal distance (i.e. the neighbourhood radius at which seed immigration based on established offspring was 50%). This estimate was approximately 35.20 m, a value similar to estimates from dispersal kernels based on parentage analysis (median of 49.52 m) and inverse modelling (median of 34.00–49.53 m).

A remarkable result of our study is the highly skewed distribution of female reproductive success (see Fig. 3).

Other studies of female reproductive success in tree species have also shown the reproductive dominance of a very small number of mature individuals. Schnabel *et al.* (1998) found ~50% of honey locust females to be reproductively inactive. Only two trees were responsible for about 50% of the established seedlings in *Symphonia globulifera*, a tropical tree species (Aldrich *et al.* 1998). Valbuena-Carabaña *et al.* (2005) found one third of *Quercus pyrenaica* saplings to be generated by a single adult. Highly skewed distributions of female reproductive success might result in low effective population density at the local scale (Schnabel *et al.* 1998; Hedrick 2005; see also Smouse & Sork 2004 for effective number of males) leading to pronounced fine-scale spatial genetic structure and biparental inbreeding (Rousset 2004; Vekemans & Hardy 2004).

The reproductive effort of females in 1 year, estimated here by cone counts in the winter of 2000–2001 was correlated with female reproductive success over the whole regeneration period, which, in Mediterranean forests of maritime pine, probably comprises more than 20 years. Similarly, Schnabel *et al.* (1998) showed constancy in female fertility estimates of *Gleditsia triacanthos* across years, based on the mother-tree contributions to different age classes of the offspring cohort. Interannual correlation in strobilus and/or cone crop for single trees is typically reported in pine species (Mutke *et al.* 2005; see Miguel *et al.* 2002 for maritime pine). The diameter and the number of cones per tree were only slightly correlated in our sample ($r = 0.19$, $n = 380$) and were both significant in the final mating model, reflecting different aspects of female reproductive success. The number of cones per mother-tree is a surrogate of female reproductive effort (i.e. fruit set), whereas diameter is related to mother-tree size and vigour. Bigger and more vigorous plants usually have better-quality seed (Harper & White 1974), which might result in selective advantage for their offspring (Reich *et al.* 1994; Castro 1999) and in positive selection gradients for vigour-related traits, in particular in severe environments. Differences in diameter among mother-trees can result from additive (heritable) genetic variation (although narrow-sense heritability, h^2 , for diameter is relatively low in maritime pine from central Spain; ~0.13, M.R. Chambel, personal communication) and/or from microenvironmental variation in soil fertility and humidity.

Surprisingly, mother-tree age and female reproductive success were not correlated. Natural regeneration in maritime pine native populations is the result of accumulative reproductive episodes over several years. In our study plot, older adult trees (more than 120 years old; see Fig. 1) were supposedly fully reproductive much earlier than younger adult trees, which could have resulted in a positive correlation between female reproductive success and age due to the higher chances of the older trees' progeny of finding an empty patch for establishment. Nevertheless,

age and diameter, which showed the strongest selection gradient of all variables, were moderately correlated ($r = 0.49$, $n = 380$). The positive correlation of diameter with relative female reproductive success would reflect, then, not only the vigour of the tree or the differences in soil fertility within the plot but also the habitat availability at early stages of the regeneration process, as it has also been suggested for other long-lived trees (*G. triacanthos*, Schnabel *et al.* 1998; *Quercus petraea* and *Q. pyrenaica*, Valbuena-Carabaña 2006). In herbs, however, surrogates of female plant size, such as leaf count, were negatively correlated with reproductive success as a possible consequence of a trade-off between vegetative vigour, which would result in bigger plants, and reproductive investment (Meagher & Thompson 1987).

Dispersal kernels for different life stages

To our knowledge, this study is the first to compare dispersal kernels fitted for the same population by genetic (direct parentage analysis, PA) and ecological (inverse modelling, IM) methods. Sapling dispersal kernels estimated by the two methods were remarkably similar, showing nonleptokurtic kernels ($c > 1$) and average sapling dispersal distances of ~40–60 m. More leptokurtic dispersal functions, showing relatively high probabilities very close to the source and very far at the distribution tail and lower probabilities at the shoulders (Nathan & Muller-Landau 2000), have often been found for wind-dispersed pollen (e.g. Austerlitz *et al.* 2004; Robledo-Arnuncio & Gil 2005) and, in some cases, also for seeds (e.g. Nathan *et al.* 2000; Greene *et al.* 2004; Jones *et al.* 2005) in other forest trees. Estimates of average dispersal distances based on successfully established offspring using molecular markers are only available for a few temperate tree species: ~60–80 m in honey locust (Schnabel *et al.* 1998), ~15–40 m in oaks (Dow & Ashley 1996; Valbuena-Carabaña *et al.* 2005), and ~75 m in *Cercidiphyllum japonicum* (Sato *et al.* 2006), and are difficult to compare to our case because of different population configuration and dispersal mechanisms. Using inverse modelling techniques, Hille Ris Lambers & Clark (2003) found average dispersal distances for saplings of different forest trees of ~10–45 m in a temperate deciduous forest (Southern Appalachians, western North Carolina, USA).

Average dispersal distances for saplings in maritime pine (~40–60 m) were shorter than expected from average distances among saplings and adult trees (~75 m), and larger than those from dispersal kernels for seeds (mean of 12.19 m and median of 10.22 m). This is in agreement with the existence of a long-distance component in maritime pine sapling dispersal that, as it was not found in seeds, can be related to postdispersal processes. Dispersal kernels based on saplings can exhibit larger dispersal distances than seeds due to J-C effects (see *Introduction*). Such effects

have also been documented for the wind-dispersed Mediterranean pine *Pinus halepensis*, using both seed-trap and sapling survey data (Nathan *et al.* 2000) and nuclear simple sequence repeat (nuSSR)-based PA (Troupin 2005; Troupin *et al.* 2006).

Apart from J-C effects, our results suggest a strong impact of the microenvironmental variation in survival and early growth stages in a Mediterranean pine and, indeed, it has also been found for other Mediterranean conifers (e.g. Nathan & Ne'eman 2004; Troupin 2005). Survival of seedlings and saplings in Mediterranean maritime pine might be determined by environmental factors, patchily distributed, that are not related to the seed shadow produced by dispersal. Such factors could be biotic (e.g. higher densities of mycorrhiza or localized predation) or abiotic (e.g. fertility, shelter by shadow, or humidity). Microsite requirements of juveniles explained, for instance, the greater seedling and sapling survival of *Liriodendron tulipifera* close to mother trees, in a mesic temperate forest (Hille Ris Lambers & Clark 2003).

Natural selection is a major evolutionary force; its effects, although notable, are extremely difficult to evaluate in natural conditions. We have shown that insights about selective constraints on quantitative traits in the wild can be obtained indirectly through correlating reproductive success and phenotypes. These studies are most useful when dealing with successfully established offspring, as only those individuals that reach reproductive maturity can affect next-generation population allele frequencies and promote evolutionary change. However, refined methods, such as the *Seedling Neighbourhood Model*, and the combination of ecological and genetic approaches and techniques, are necessary to accomplish this goal. Genetic diversity and population structure of key forest trees, such as the Mediterranean maritime pine, may well have ecosystem-wide effects, through their 'extended' phenotypes (Whitham *et al.* 2003). Thus, differential selection promoted by microenvironmental variation at the population level and the extent to which selected genes disperse in these species can be relevant for whole-ecosystem dynamics.

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Supplementary material

The supplementary material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3118/MEC3118sm.htm>

Table S1 A. Linear components; S1 B. Quadratic components

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