



High seed dispersal ability of *Pinus canariensis* in stands of contrasting density inferred from genotypic data

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Abstract

Aim of the study: Models that combine parentage analysis from molecular data with spatial information of seeds and seedlings provide a framework to describe and identify the factors involved in seed dispersal and recruitment of forest species. In the present study we used a spatially explicit method (the gene shadow model) in order to assess primary and effective dispersal in *Pinus canariensis*.

Area of study: *Pinus canariensis* is endemic to the Canary Islands (Spain). Sampling sites were a high density forest in southern slopes of Tenerife and a low density stand in South Gran Canaria.

Materials and methods: We fitted models based on parentage analysis from seeds and seedlings collected in two sites with contrasting stand density, and then compared the resulting dispersal distributions.

Main results: The results showed that: 1) *P. canariensis* has a remarkable dispersal ability compared to other pine species; 2) there is no discordance between primary and effective dispersals, suggesting limited secondary dispersal by animals and lack of Janzen-Connell effect; and 3) low stand densities enhance the extent of seed dispersal, which was higher in the low density stand.

Research highlights: The efficient dispersal mechanism of *P. canariensis* by wind inferred by the gene shadow model is congruent with indirect measures of gene flow, and has utility in reconstructing past demographic events and in predicting future distribution ranges for the species.

Keywords: Bayesian inference; Canary Islands; gene shadow model; microsatellites; parentage analysis.

Citation: López de Heredia, U., Nanos, N., García-del-Rey, E., Guzmán, P., López, R., Venturas, M., Gil Muñoz, P., Gil, L. (2015). High seed dispersal ability of *Pinus canariensis* in stands of contrasting density inferred from genotypic data. *Forest Systems*, Volume 24, Issue 1, e-015, 13 pages. <http://dx.doi.org/10.5424/fs/2015241-06351>.

Received: 03 Jan 2014. **Accepted:** 04 Feb 2015

<http://dx.doi.org/10.5424/fs/2015241-06351>

This work has one supplementary file.

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Funding: Canary Islands Government, the Cabildos of Tenerife and Gran Canaria for long-standing financial and logistical support in the study of Canary Island pine. This study was partially funded by the Spanish Ministry of Science in the Project AGL2009-10606 (VULCAN).

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Seed dispersal strongly influences the demographic cycle of plants (Harper, 1997), and can be split into three categories: primary dispersal, secondary dispersal, and effective dispersal. Primary dispersal occurs when seeds detach from branches and reach the ground. In anemochorous species, wind has a strong incidence in primary dispersal and seeds show morphological adaptations to increase its magnitude (Nathan *et al.*, 2002). Secondary dispersal occurs after primary dis-

persal, until the seeds germinate and settle. The effect of small mammals, birds or ants on secondary seed dispersal has been addressed for many forest species included Pinaceae (Briggs *et al.*, 2009) and Fagaceae (Herrera *et al.*, 1994). Both primary and secondary dispersal constitute the 'basic' dispersal (Bontemps *et al.*, 2013), which is the template for recruitment. Effective dispersal combines primary and secondary dispersal with establishment (Nathan & Muller-Landau, 2000), and ultimately determines population dynamics and colonization patterns.

The integration of methods based on seed or seedling counts in either seed traps or regeneration quadrants, and genetic methods, based on parentage analysis, has been used to model the different components of dispersal (Bontemps *et al.*, 2013). The ‘basic’ and effective dispersal processes can be examined using the gene shadow model (GSM) (Jones & Müller-Landau, 2008) on seeds collected in traps and on established seedlings (Millerón *et al.*, 2013), respectively. This model is a modification of the classical inverse modelling approach (Ribbens *et al.*, 1994). The GSM integrates genetic and non-genetic information by using the numbers of seeds/seedlings of each genotype in a sample from each seed-trap/seedling-quadrant, when the source of each seed/seedling is identifiable by parentage analysis. Likewise inverse modelling, the GSM estimates two elements: 1) a dispersal kernel, or the probability density function, describing the spread of the seedlings around the mother tree (Clark *et al.*, 1999); 2) a fecundity parameter, which is usually estimated as a function of the basal area of the mother tree (Ribbens *et al.*, 1994).

Other genetic methods that utilize frequencies of offspring genotypes, and that rely on a spatially explicit model for the mating event resulting in that offspring can be used to model the ‘basic’ dispersal: 1) the ‘neighbourhood seedling model’ –NSM– (Burczyk *et al.*, 2006) that simultaneously estimates the seed and pollen dispersal kernels; 2) the ‘competing sources model’ –CSM– (Robledo-Arnuncio & García, 2007), which is a simplified version of the NSM. CSM estimates a dispersal kernel based on the frequencies of individual offspring genotypes in each seed-trap/seedling-quadrant using a multinomial error distribution; and 3) the ‘Moran and Clark’s model’ –MCM– (Moran & Clark, 2011), that differs from the NSM in how the genotyping error and the immigrant seeds/pollen are treated. A common way to apply these techniques is by genotyping seeds collected in seed-traps or seedlings occurring in seed-quadrants. All the methods above perform reasonably well for ‘basic’ seed dispersal inference providing a good sampling design on seedlings (for a comparison of the performance of different methods on seeds and seedlings see Millerón *et al.*, 2013). When these methods are applied on seeds collected in seed-traps, they would be estimating the primary component of dispersal.

Disentangling the different components of seed dispersal, by comparing the mean primary and effective dispersal distances (δ_p vs. δ_e), assists in evaluating the relative contributions to the whole dispersal process of the main factors affecting the spatial patterns of recruitment: 1) density dependent mortality (i.e.

the Janzen-Connell effect); and 2) secondary dispersal vectors. The Janzen-Connell hypothesis predicts a lower survival rate in the proximity of the mother plant owing to higher frequency of pathogens, seed predators or herbivorous animals (Janzen, 1970; Connell, 1971). Secondary dispersal may be produced by either biotic (birds, small mammals or insects) and/or abiotic agents (wind or water streams). Both secondary dispersal and the Janzen-Connell effect lead to discordance between the primary seed rain and the spatial patterns of recruits relative to their mothers (Nathan & Muller-Landau, 2000); therefore, if any of both factors operate in the regeneration process, a shift in the mode of the effective dispersal kernel relative to the basic dispersal kernel is expected; hence, ($\delta_e \neq \delta_p$). If there is discordance between primary and effective dispersal, some methodologies that compare the ‘basic’ and effective dispersals have been recently developed to disentangle the effects of secondary dispersal and the Janzen-Connell effect (Steinitz *et al.*, 2011; Bontemps *et al.*, 2013). Otherwise, the primary seed rain can be considered the template for recruitment.

The parent tree density is determinant to seed dispersal, affecting the shape and scale of the dispersal kernels, and fecundity. Trees in dense forests produce and disseminate fewer seeds per year and unit of basal area than trees in sparser locations (Schurr *et al.*, 2008). With high tree densities the dispersal kernel has a shorter median and a fatter tail. In anemochorous species, wind turbulence is affected by tree and sub-canopy vegetation density (Bohrer *et al.*, 2008). Moreover, sub-canopy vegetation may be a physical barrier to wind dispersed seeds (Pounden *et al.*, 2008) and pollen (Millerón *et al.*, 2012). The tree density may also have an effect on the density and behaviour of predators/dispersers (Carlo & Morales, 2008), which is relevant for animal dispersed species.

In the present study we applied a model that integrates genetic and spatial information (GSM) to estimate the primary and the effective dispersal kernels in two stands of high (site 1) and low (site 2) stand density, respectively, of the endemic insular tree *Pinus canariensis* C.Sm. ex DC. The experimental design consisted in seedling quadrants (in both sites) and seed traps (only in site 1, dense pinewood). We examined the dispersal intensity of *P. canariensis*’ seeds in both dense and sparse stands and tested the following hypotheses: 1) there are no differences between the primary and the effective dispersal kernels in site 1 ($\delta_{p1} \approx \delta_{e1}$), therefore, primary dispersal can be considered the template for recruitment; 2) the mean effective dispersal distances are significantly higher in the sparse than in the dense pinewood ($\delta_{e1} < \delta_{e2}$).

Material and Methods

Species of study and sampling design

Pinus canariensis is an endemic pine to the western Canary Islands (i.e. Tenerife, Gran Canaria, La Palma, La Gomera and El Hierro). Currently, it occupies about 55,000 ha, being much of it planted. In terms of conservation priorities, the natural Canary Island pine formations are Habitats of Community Interest (Habitat Directive 92/43/EEC, 2006/105/EC). It is considered a colonizing species that invades poor soils, that is, salic volcanic substrates with very dry environments that arboreal angiosperms are unable to inhabit (Aboal *et al.*, 2000). As a consequence, *P. canariensis* occupies a wide range of habitats at varying densities, from semi-desert areas in southern Gran Canaria to humid areas in the slopes of Tenerife or La Palma, usually forming pure stands. Out of its natural range, it is considered an invasive species (Rouget *et al.*, 2004).

Sampling was performed in two sites with contrasting densities. Site 1 (Figure 1) is located in the ravine of Chirigel (28° 25' N, 16° 23' W, elevation = 1,100 m a.s.l.) and it is representative of the high density forests from the slopes in the south of Tenerife. Diameter at breast height (DBH) was measured for 328 adults and needles were collected from the same trees. In 2007 seeds were collected from 103 seed traps. The seed traps consisted of a cylindrical metallic oil drum transversally cut. The traps had a diameter of 0.55 m and a height of 0.30 m. The open end of the top of the traps was covered with a metallic net that allowed the entrance of seeds, but prevented predation by frugivores. A portion of the collected seeds (324/1354) were germinated to obtain a final sample size of 315

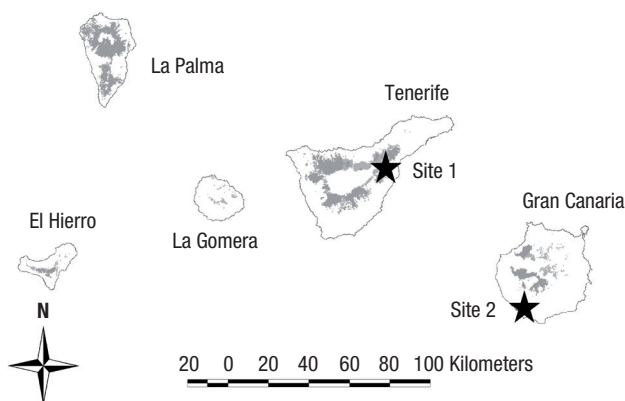


Figure 1. Map of the Canary Islands and location of the study sites. Shaded grey areas represent the distribution range of *P. canariensis*.

seeds, and for further germination studies (data not shown). Collection of seedlings was done in 120 subplots of 1 m² close to the seed traps (Figure 2). The subplots were located in the nodes of a rectangular grid of 12 x 6 m, with E-W orientation, so the smallest size of the grid was set in line with the direction of the maximum slope. All the seedlings contained within the subplots (540 seedlings) were collected and leaves were stored at -80 °C for molecular analysis. Seedlings corresponded to at least two regeneration events.

Site 2 is an isolated stand located in the ravine of Arguineguín, 2.5 km inshore from the south coast of Gran Canaria island (27° 47' N; 15° 40' W, elevation = 350 m a.s.l.; Figure 1). The nearest *P. canariensis* population to the study plot is 7 km away in the same ravine. A hundred and twenty five adult pines grow scattered at extremely low density (0.46 trees ha⁻¹) on two steep hillsides divided by a road. The western hillside supports 80.5% of the population (68.8% of adults and 90.2% of recruits). In the western hillside,

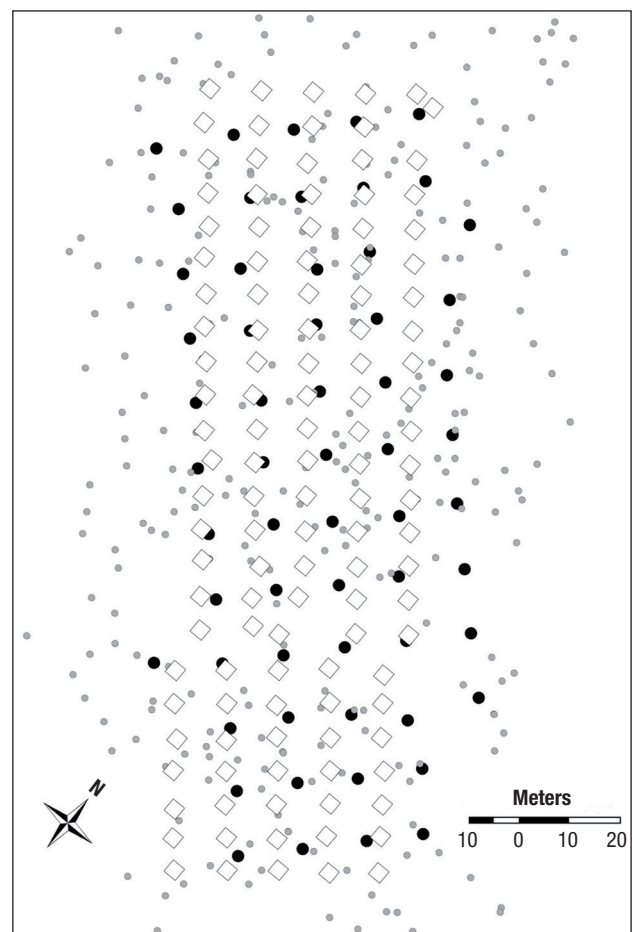


Figure 2. Experimental design in site 1, Chirigel ravine, Tenerife. Grey dots: genotyped adult trees; Black dots: seed traps; Empty diamonds: Seedling quadrants.

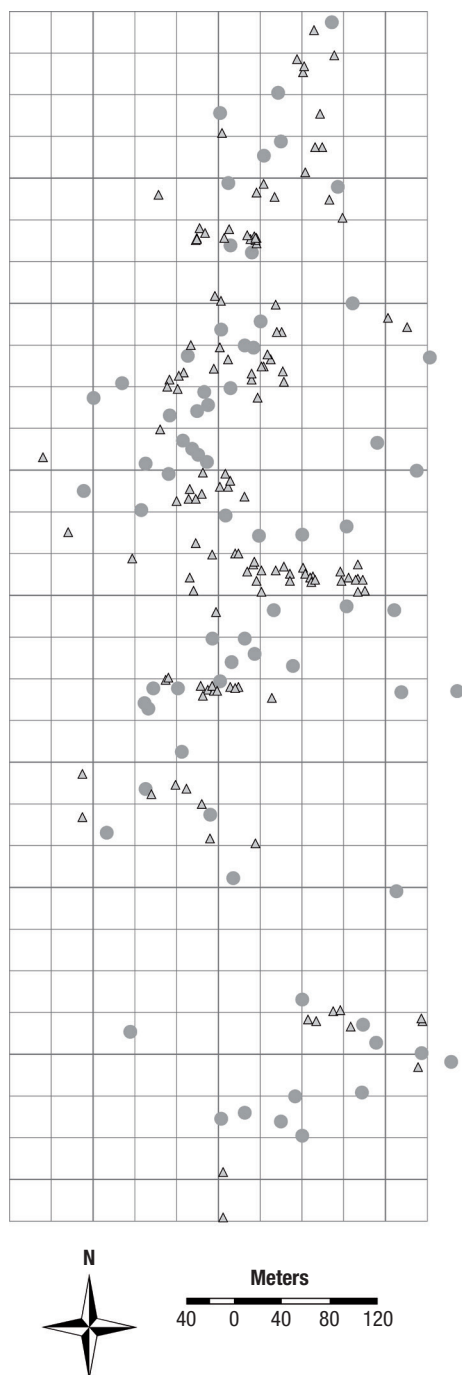


Figure 3. Experimental design in site 2, Arguineguín ravine, Gran Canaria. Grey dots: genotyped adult trees; Grey triangles: seedlings. The reticule represents the seedlings quadrants.

a surface of 350 x 1015 m was divided in 290 quadrants of 35 x 35 m containing 125 seedlings (Figure 3). All the 125 adults were used as potential mothers in the parentage analysis (see below). The coordinates of the central points of the quadrants were used to compute the matrix of distances between the mother trees and the seedling-quadrants. Seed-traps were not used in this site because seed production was very low.

Microsatellite analysis and parentage analysis

DNA extraction was performed with a modification of the protocol by Dellaporta *et al.* (1983). Five polymorphic nuclear microsatellites (nSSRs): SPAG 7.14, SPAC 11.5, SPAC 11.8 (Soranzo *et al.*, 1998), PttX3116, PttX4001 (Auckland *et al.*, 2002); and five chloroplast microsatellites (cpSSRs): Pt15169, Pt26081, Pt30204, Pt87268, and Pt71936 (Vendramin *et al.*, 1996) were amplified under standard PCR conditions. Fluorescence labelled PCR products were analyzed in a 4300 LI-COR automated sequencer (LI-COR biosciences, Lincoln, NE, USA). Allele sizes were determined with SAGA Microsatellite Analysis Software (LI-COR biosciences, Lincoln, NE, USA). Genotyping errors were minimized by genotype double-blind reading, positive and negative controls, and blind duplicate samples. The software MICRO-CHECKER v 2.2.3 (van Oosterhout *et al.*, 2004) was used to test for the presence of null alleles that could bias parentage assignment. Standard diversity statistics were computed with GenAlEx 6.5 (Peakall & Smouse, 2012).

Parentage analysis based on nSSRs was used to generate the most likely parent pairs. Parentage analysis is based on exclusion probabilities for single parent and parent pair assignment as implemented in software FAMOZ (Gerber *et al.*, 2003). Statistical confidence in parentage assignment was based on offspring simulations from allele frequencies and genotyped parents. To account for possible genotyping errors, we considered a relaxed error level of 0.05 as input in FAMOZ. Then, we compared cpSSR haplotypes of possible parent pairs with those of the offspring assigned by nSSRs. If the pollen donor had the same cpSSR haplotype as the offspring, and the seed donor had a different cpSSR haplotype, these two adults were the presumed parent pair. If both adults had different cpSSR haplotypes from the offspring, none of them was considered the pollen donor. If both adults had the same cpSSR haplotype as offspring, the gender of each parent could not be identified. Second, we compared cpSSR haplotypes of the offspring when nSSR markers had identified a single possible parent. If the cpSSR haplotype of the candidate was not identical to that of the offspring, the candidate was considered the seed donor. If the cpSSR haplotype was identical, seed donors could not be identified and were considered as coming from outside the plot.

The Gene Shadow Model (GSM)

Both basic and effective seed dispersals were assessed using the 'gene shadow model' (GSM) (Jones

& Müller-Landau, 2008). While the primary seed dispersal was estimated from seeds collected in traps from site 1, effective seed dispersal was modeled using data from the seedlings genotyped in site 1 and site 2. Unlike the classical inverse modeling approach that is applied to the numbers of offspring in each seed-trap/seedling-quadrant (Ribbens *et al.*, 1994), the GSM extends the model to the numbers of offspring of each genotype in a sample from each trap/seedling-quadrant.

The fecundity of the tree i as the seeds produced per year (Q_i) is estimated as:

$$Q_i = \exp(\beta_i) b_i \quad [1]$$

where β_i is the fecundity parameter of tree i in seeds per m^2 and b_i the basal area of tree i in m^2 .

The expected number of offspring of genotype g to be identified among the seedlings sampled from seed quadrant j , is calculated by summing the expected contributions of all parent trees k having genotype $G_k = g$

$$\hat{s}_{ij} = c_j a \sum_i^{G_k=g} Q_i F(r_{ij}) \quad [2]$$

where c_j is the proportion of all offspring in trap/quadrant j that are genotyped, assuming that none of these offspring could have come from non-genotyped parents, a is the area of the traps/quadrants, Q_i is the fecundity of tree i estimated as in equation [1] and $F(r_{ij})$ is the dispersal kernel component.

In equation [2] it is assumed that none of these offspring could have come from non-genotyped parent trees. The total likelihood of all the genotyped data for seeds matching known trees alone is

$$\prod_j \prod_i Pr(s_{ij} | \hat{s}_{ij}) \quad [3]$$

where s_{ij} is the observed number of offspring of genotype i in quadrant j . We assumed a Poisson error distribution.

The expected number of offspring whose genotype does not match that of any parent on the plot, i.e. immigrant seeds, can be calculated as (Jones & Müller-Landau, 2008):

$$\hat{s}_j = c_j a \frac{\sum_i Q_i}{m} \iint_{\text{areaffplot}} \left(\sqrt{(x_j - x)^2 + (y_j - y)^2} \right) dx dy \quad [4]$$

where m is the total mapped area, x_j and y_j are the coordinates of the trap j , x and y are the coordinates of points off the plot. It is assumed that the genotypes of the offspring coming from parents off the plot do not

match any parent on the plot. The total likelihood of all the genotyped data is

$$\prod_j \prod_i Pr(s_{ij} | \hat{s}_{ij}) \prod_j Pr(s_j | \hat{s}_j) \quad [5]$$

where \hat{s}_j is the observed number of offspring whose genotype does not match that of any parent.

Bayesian implementation of the GSM

Model fitting was performed under a Bayesian framework using modified programs run in R (R Development Core Team 2010), kindly provided by Drs. H. Müller-Landau and A. Jones (Jones & Müller-Landau, 2008). We fitted the parameters for lognormal, Weibull, Clark's 2Dt and generalized exponential distributions (see Suppl. File S1 [pdf on line] for an explanation of the different dispersal kernels).

We ran 10 independent Markov chains of 100,000 iterations to estimate the posterior probabilities. We used non-informative priors for all fitted parameter estimates, because priors have a strong influence in dispersal parameter estimation (Kass & Wasserman, 1996), and we had no previous information about seed dispersal in *P. canariensis*. A re-parameterization of the models was used by which the fitted parameters were log-transforms of the original parameters. We used the Poisson error distribution as it performs better for wind-dispersed species with medium to large dispersal means (Greene *et al.*, 2004). We used the median of the posterior distributions of each parameter as our best estimates, and computed 95% symmetric credible intervals. Convergence of the models was evaluated using the potential scale reduction factor – PSRF– (Brooks & Gelman, 1998). A model was assumed to have converged when the PSRF was close to 1 (PSRF < 1.1). Mean and median dispersal distances were estimated for each model's parameters when convergence was achieved. Due to the high proportion of offspring with no parents assigned within the plots (see below), we used the version of the model that considers immigrant seed flow (equations [4] and [5]). After several preliminary runs, and due to the distance at which other adult trees are found in site 1, we considered a radius of 1 km around the plot.

Hypothesis testing

The Deviance Information Criterion (DIC) (Spiegelhalter *et al.*, 2002) was computed to perform model comparisons and choose the optimal distribution fitting to our data. We compared the ranges of the 95 % credible

intervals between site 1 and site 2, and between dispersal estimated from seeds and seedlings, in order to test for discordance between primary and effective dispersals. In Bayesian inference, a probability interval is a probabilistic region around a posterior moment, and is similar in use to a frequentist confidence interval. We specifically tested the following hypotheses: 1) Hypothesis I: patterns are not discordant between the primary and the effective dispersal kernels in site 1 ($H_0: \delta_{p1} = \delta_{e1}$; $H_a: \delta_{p1} \neq \delta_{e1}$); 2) Hypothesis II: the mean effective dispersal distances were significantly higher in site 2 (low density) than in site 1 (high density) ($H_0: \delta_{e1} = \delta_{e2}$; $H_a: \delta_{e1} < \delta_{e2}$).

Results

Parentage analysis

The standard diversity statistics for nuclear (Table 1) and chloroplast microsatellites (Table 2) showed poly-

morphism enough to perform parentage analysis. The theoretical multilocus exclusion probabilities (Jamieson & Taylor, 1997) exceeded 0.999 for the combination of nuclear and chloroplast microsatellite loci for both seeds and seedlings in the two sites (Table 3). Under this approach, the chloroplast DNA is considered as a single independent locus and each haplotype as an allele. In site 1, seeds showed a critical LOD-score of 3.62 for a single parent, and 9.01 for a parent pair, at a confidence level of 95 %. For seedlings, the critical LOD-scores were 3.62 for a single parent, and 8.92 for a parent pair, at the same confidence level than for seeds. The mother was assigned to 90 seeds (28.6 % of genotyped seeds) and to 151 seedlings (28.0 % of genotyped seedlings). Interestingly, the same percentage of immigrants was scored for seeds (71.4 %) and seedlings (72.0 %).

In site 2, the LOD-scores for single parent and parent-pair assignments were, respectively, 3.82 and 9.94 (Table 3). The mother was unambiguously assigned to 59 seedlings (38.8% of the data) when only

Table 1. Standard diversity statistics for each nuclear microsatellite locus and cohort.

	Locus	N ^a	Na ^b	Ne ^c	Ho ^d	He ^e
Adults (site 1)	SPAG 7.14	324	22	8.374	0.846	0.881
	Pttx4001	283	10	3.983	0.749	0.749
	Ptx3116	318	9	2.177	0.538	0.541
	SPAC 11.5	273	33	17.379	0.821	0.942
	SPAC 11.8	262	49	17.265	0.836	0.942
Seeds (site 1)	SPAG 7.14	314	31	8.111	0.860	0.877
	Pttx4001	312	9	4.241	0.737	0.764
	Ptx3116	314	9	2.206	0.519	0.547
	SPAC 11.5	271	31	13.145	0.657	0.924
	SPAC 11.8	279	45	17.727	0.781	0.944
Seedlings (site 1)	SPAG 7.14	524	22	7.602	0.794	0.868
	Pttx4001	534	8	4.101	0.706	0.756
	Ptx3116	528	9	2.246	0.568	0.555
	SPAC 11.5	481	38	17.056	0.692	0.941
	SPAC 11.8	483	54	20.156	0.818	0.950
Adults (site 2)	SPAG 7.14	105	16	7.792	0.752	0.872
	Pttx4001	107	9	2.943	0.542	0.660
	Ptx3116	107	9	2.032	0.486	0.508
	SPAC 11.5	110	23	6.589	0.791	0.848
	SPAC 11.8	103	57	28.790	0.699	0.965
Seedlings (site2)	SPAG 7.14	143	15	8.211	0.741	0.878
	Pttx4001	148	9	2.842	0.581	0.648
	Ptx3116	146	7	1.897	0.445	0.473
	SPAC 11.5	150	26	6.488	0.773	0.846
	SPAC 11.8	131	50	21.411	0.573	0.953

^aN: Sample Size

^bNa: Number of Alleles

^cNe: Number of Effective Alleles

^dHo: Observed Heterozygosity

^eHe: Expected Heterozygosity

Table 2. Standard diversity statistics for chloroplast microsatellite haplotypes and for each cohort.

	N	Na	Ne	h	uh
Adults (site 1)	291	56	12.879	0.922	0.926
Seeds (site 1)	314	68	10.849	0.908	0.911
Seedlings (site 1)	398	68	14.018	0.929	0.931
Adults (site 2)	110	55	35.174	0.972	0.980
Seedlings (site 2)	152	40	10.333	0.903	0.909

^aN: Sample Size^bNa: Number of Alleles^cNe: Number of Effective Alleles^dh: Haplotypic diversity^euh: Unbiased haplotypic diversity

the highly reliable parent-offspring matches were analysed.

Primary and effective dispersal estimates and hypothesis testing

Convergence was achieved for fecundity and scale parameters of the lognormal, Weibull and Clark's 2Dt distribution in all three models (PSRF < 1.001). The model for the generalized exponential dispersal kernel did not converge in site 2. The models using the lognormal kernel showed the smallest DIC values and largest estimates for mean and median dispersal distances (Table 4). The DIC values scored for the Weibull, Clark's 2Dt and generalized exponential kernels were higher than for the lognormal, but the mean and median dispersal distance estimates were more consistent across these models.

Both primary and effective dispersal estimates showed a remarkable dispersal ability for *P. canariensis* in sites 1 and 2 (Table 4; Figure 4). Using the Weibull dispersal kernel, the average mean and median dispersal distances were *c.* 60 m in site 1, but over 800 m in site 2 (Table 4). The fecundity parameters were consistent with '*de visu*' predictions about seed production made at the sampling time showing large differences between sites: site 1 had much higher fecundity parameter for both seeds (2242 seeds m⁻²) and seedlings (448 seedlings m⁻²) than site 2 (2.3 seedlings m⁻²).

For Hypothesis I, there was no apparent discordance between primary and effective dispersals, as the 95 % credible intervals for seed and seedling dispersal distance overlapped irrespective of the kernel of choice (Table 4). That is, the GSM for seeds and seedlings converged to very similar dispersal parameters and we cannot reject the null hypothesis of equal primary and effective dispersals. For Hypothesis II, the 95% credible intervals for seedling dispersal parameters showed remarkable differences between sites. The sparse stand (site 2) showed higher mean effective dispersal distance than the dense stand (site 1). Therefore, we rejected the null hypothesis of equal effective dispersal distances in dense and sparse stands.

Discussion

Model selection

The hypotheses tested in the present study were validated for any dispersal kernel we used. However, there were differences in the intensity of the dispersal process depending on the kernel of choice. According to DIC, the best models were the ones with lognormal

Table 3. Results of the maternity parentage analyses in sites 1 and 2.

Site	Offspring	EP ^a	LOD-s (SP) ^b	LOD-s (PP)	N _a ^c	N _s ^d	N _m ^e	m _s ^f
Site 1	Seeds	>0.999	3.62	9.01	328	315	90	0.714
Site 1	Seedlings	>0.999	3.62	8.92	328	540	151	0.720
Site 2	Seedlings	>0.999	3.82	9.94	125	125	59	0.582

^aEP: exclusion power of the microsatellites for the data.^bLOD-s: critical LOD-scores for single-parent inference (SP) and parent-pair inference (PP).^cN_a: number of potential parents genotyped within the plots.^dN_s: number of offspring genotyped within the plots.^eN_m: number of offspring with assigned mothers.^fm_s: ratio of immigrant seeds coming off the plot.

Table 4. Posterior mean estimates for seed dispersal fitted for one parameter’s Clark’s 2Dt distribution ($p = 1$), lognormal, Weibull and generalized exponential distributions using the gene shadow model.

Dispersal Component ^a	Dispersal kernel	Fitted parameters (CI) ^b		Distance (CI) ^c		DIC ^d	
		β	p	Log(u)	Mean		Median
PD (site 1)	Clark’s 2Dt	-6.09 (-6.31, -5.89)	1	9,23 (8.98, 9.49)	63.5 (56.1, 72.4)	114.7 (101.2, 130.6)	1337.8
ED (site 1)	Clark’s 2Dt	-7.69 (-7.86, -7.54)	1	9,16 (8.96, 9.37)	61.40 (55.6, 68.1)	110.77 (100.2, 123.0)	2364.7
ED (site 2)	Clark’s 2Dt	-12.63 (-12.83, -12.45)	1	14,86 (14.48, 15.25)	1060.77 (877.2, 1289.2)	1915.0 (1583.6, 2327.3)	1487.3
		β	σ	μ	Mean	Median	DIC
PD (site 1)	Lognormal	-3.83 (-4.57, -3.09)	1.01 (0.83, 1.17)	5.60 (4.99, 6.18)	450.4 (207.4, 957.6)	270.8 (147.4, 484.2)	1186.1
ED (site 1)	Lognormal	-5.61 (-6.16, -5.05)	1.02 (0.87, 1.16)	5.47 (4.98, 5.94)	399.5 (212.4, 744.6)	236.9 (145.5, 380.7)	2109.1
ED (site 2)	Lognormal	-8.69 (-9.12, -8.29)	1.76 (1.62, 19.94)	9.64 (9.40, 9.89)	72315.9 (44900.3, 129547.1)	15419.5 (12116.9, 19839.9)	1369.8
		β	λw	k	Mean	Median	DIC
PD (site 1)	Weibull	-6.10 (-7.32, -5.89)	1.95 (1.65, 2.28)	69.81 (62.62, 77.45)	61.9 (56.0, 68.6)	57.8 (50.1, 65.9)	1335.5
ED (site 1)	Weibull	-7.71 (-7.87, -7.55)	1.79 (1.57, 2.03)	67.50 (61.63, 73.69)	60.0 (55.3, 65.3)	55.0 (48.8, 61.5)	2365.5
ED (site 2)	Weibull	-12.99 (-13.20, -12.78)	1.84 (1.47, 2.23)	919.76 (756.57, 1142.58)	817.2 (684.7, 1011.9)	753.6 (589.6, 969.4)	1480.1
		β	α	λeg	Mean	Median	DIC
PD (site 1)	Generalized exponential	-6.09 (-6.29, -5.89)	1.80 (1.15, 2.52)	1877.84 (71.13, 67050.48)	56.3 (53.8, 88.9)	7.9 (4.6, 11.5)	1332.0
ED (site 1)	Generalized exponential	-7.68 (-7.84, -7.52)	1.38 (0.97, 1.81)	217.76 (26.42, 1926.52)	48.4 (42.2, 56.1)	5.8 (3.7, 7.9)	2355.0

^a PD: Primary dispersal; ED: Effective dispersal.

^b Fitted parameters and 95% upper and lower credible intervals (CI) for the fitted parameters: β : fecundity parameter; p, u : shape and scale parameters for Clark’s 2Dt distribution; μ, σ : mean and standard deviation of the variable logarithm for lognormal distribution; λ_w, k : shape and scale parameters for Weibull distribution; λ_{eg}, α : shape and scale parameters for generalized exponential distribution

^c Mean and median distances of the fitted kernels and 95% upper and lower credible intervals (CI) for the mean and median distances estimated from the fitted parameters.

^d Deviance Information Criterion.

dispersal kernel. The median dispersal distance characterizes better the dispersal process than the mean for estimations based on the lognormal kernel. However, median dispersal distances for these models were excessively large: four times the median using the Weibull kernel for site 1, and more than 20 times the median obtained with the Weibull kernel for site 2. When using Clark’s 2Dt, Weibull, and generalized exponential kernels the mean dispersal values were similar for both sites. When using Clark’s 2Dt, Weibull, and generalized exponential kernels the mean dispersal values were very similar for both sites and cohorts. Therefore, we

will discuss the results in terms of the models fitted with the Weibull dispersal kernel.

High dispersal ability of *P. canariensis*

Both basic and effective mean dispersal estimates suggest high dispersal ability for *P. canariensis*, even in dense stands. The mean primary dispersal distance estimated for other species using inverse modeling on seeds collected in traps was lower than the mean primary dispersal distance for *P. canariensis* ($\delta_{p1} = 61.9$ m):

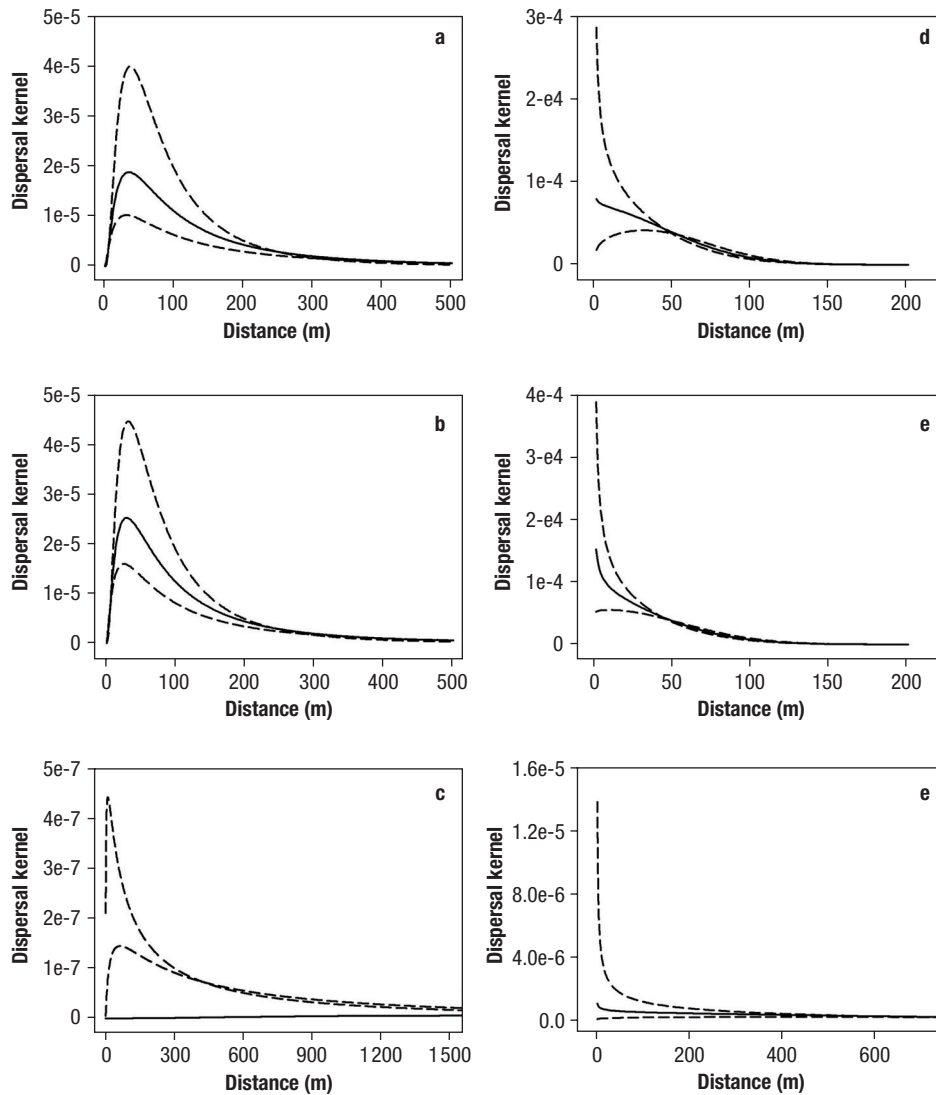


Figure 4. Comparison of primary and effective dispersal kernels for the lognormal (a, b) and Weibull (d, e) distributions in site 1. Effective dispersal kernel in site 2 for the lognormal (c) and Weibull (f) distributions. Broken lines represent upper and lower 95% credible intervals. Notice the different scale of dispersal distance between sites 1 and 2.

P. rigida ($\delta_p = 15.1$ m) (Clark, 1998), *P. strobus* ($\delta_p = 13$ -30 m) (Greene *et al.*, 2004) or *P. pinaster* ($\delta_p = 8.8$ -21.24 m) (González-Martínez *et al.*, 2006). Considering only studies that used molecular data and parentage analysis on seedlings and saplings, *P. canariensis* showed higher effective dispersal ability than other pine species ($\delta_{e1} = 60$ m; $\delta_{e2} = 817.2$ m), such as *P. pinaster* ($\delta_e = 26.5$ m) (González-Martínez *et al.*, 2006), *P. halepensis* ($\delta_e = 41.6$ m) (Steinitz *et al.*, 2011) or *P. sylvestris* ($\delta_e = 53$ m) (Burczyk *et al.*, 2006). These studies used different sampling schemes, and therefore may not serve as adequate comparisons to *P. canariensis*. We cannot rule out that models based on genetic information may overestimate the dispersal distances when sampling is biased in areas with high seed density, because they only use a small proportion of the total amount of seeds

collected in traps (Jones & Muller-Landau, 2008). There is also a source of error associated to microsatellite mistyping and mother assignment that could inflate the percentage of immigrant seeds, thus increasing the mean dispersal estimates for *P. canariensis*. Furthermore, the miss-specification of the fecundity term in the dispersal model may also lead to overestimation of the dispersal distances (Nanos *et al.*, 2010), because very often fecundity does not have a straightforward relation with the basal area of the parent trees.

Despite the limitations of the genetic models, the high dispersal ability of *P. canariensis* is not surprising in a > 35 m tall species that inhabits steep slopes subjected to strong winds. In fact, one of the factors that may explain the efficiency in seed dispersal is that the wing of the Canary Island pine seeds is adnate, which is a rare

trait in the species of subgenus *Pinus* (*Diploxylon*). Pines with adnate seed-wings disperse by wind after seeds are released from the cone. The persistence of this trait, which is also present in some island pine species (Eck-enwalder, 2009), could be related to the ability for long distance dispersal that allows the colonization of bare soils. Together with *Pinus caribaea*, *P. canariensis* is acknowledged as an “island-jumper” (Klaus, 1989).

The high percentage of immigrant seeds scored for *P. canariensis* in both sampling sites is consistent with the invasive character of the species. A previous study conducted in site 1, evaluating the seed rain along one year (García-del-Rey *et al.*, 2011) showed a remarkable variation in seed deposition between the upper and the lower parts of the plot. However, all seed-traps received more than 25 seeds m⁻². Our results based on parentage analysis suggest that a large part of this seed rain came from outside the plot. Moreover, even in the apparently isolated site 2, the percentage of immigrant seeds was very high. Such as other pine species, *P. canariensis* undergoes an outcrossing mating system. Seeds are wind-dispersed and they can potentially travel long distances providing the species with a very efficient mechanism of gene flow. Consistently with this, molecular studies have shown effective gene flow among islands (Vaxevanidou *et al.*, 2006) and evidence of recent range expansions (López de Heredia *et al.*, 2014).

The dispersal ability of *P. canariensis* has important implications for landscape dynamics. Pines are the first plant species colonizing salic outcrops after volcanic disturbance. In line with this, pines will show a high potential for the colonization of new ecological niches above the tree-line that will become abundant in a global change scenario (VVAA, 2011). High dispersal ability may have aided in the substitution of the thermophilous forests that occurred in the Canary Islands in the last 2,000 years (de Nascimento *et al.*, 2009), promoting the colonization of degraded areas by pines. Out of its natural range, *P. canariensis* is a potential invasive species (Rouget *et al.*, 2004). Invasiveness is enhanced by the high dispersal efficiency and by the resilient character of the species, which is one of the few pines able to re-sprout (Climent *et al.*, 2004). Due to the dispersal characteristics of the species, *P. canariensis* is highly competitive with other species of pines (Arévalo *et al.*, 2005) and angiosperms (Aboal *et al.*, 2000).

Concordance of primary and effective dispersals

Spatial patterns of recruitment largely depend on the initial template established by primary seed dispersal, but are also influenced by successive post-dispersal

factors such as seed predation, germination and seed mortality (Nathan & Muller-Landau, 2000). The spatial concordance of primary and effective dispersals indicates a positive relationship between seed rain and recruitment. Conversely, if there is spatial discordance between primary and effective dispersal, negative relationship between seed rain and recruitment is inferred (Houle, 1995).

The comparison of dispersal estimates in site 1 shows that effective dispersal does not differ from primary dispersal; therefore, primary dispersal can be considered the ‘basic’ seed dispersal (*sensu* Bontemps *et al.*, 2013), i.e. the template for recruitment. Two main conclusions derive from this result. First, there is no evidence for a Janzen-Connell effect for *P. canariensis*. The Janzen-Connell hypothesis predicts that a lower survival rate in the proximity of the mother plant owing to pathogens, seed predators or herbivorous, which can occur directly under it (Janzen, 1970; Connell, 1971). The Janzen-Connell effect was originally described for tropical forests, but recently it was empirically shown for *P. halepensis* (Steinitz *et al.*, 2011). However, at least at the scale of our study, such density dependent mortality effects seem not to be operating in *P. canariensis*.

Second, it seems that animal mediated secondary dispersal of *P. canariensis*’ seeds has no effect on the spatial arrangement of recruits. Animal mediated dispersal of pine-nuts has been addressed for Mediterranean pines (Rost *et al.*, 2010), as well as for other American and European pines (Briggs *et al.*, 2009). The number of potential predators/dispersers on islands is much more reduced than in the mainland, but they can compensate this effect by increasing population densities (MacArthur *et al.*, 1972). Traditionally, only two avian species are considered as pine-wood specialists in the Canary Islands (Volsoe *et al.*, 1955): the blue chaffinch (*Fringilla teidea*) (Bannerman & Bannerman, 1963; García-del-Rey *et al.*, 2009) and the great spotted woodpecker (*Dendrocopos major canariensis*) (García-del-Rey *et al.*, 2007). While the blue chaffinch is mainly a predator of post-dispersed seeds, the great spotted woodpecker is not considered a real pine-nut predator. According to our results, none of both bird species can be considered a true disperser, and therefore, a landscape modeler. Other factors, such as shade tolerance or competition with the understory (Otto *et al.*, 2010) may be more important in dense humid pine-woods in order to explain the patterns of recruitment. In low density forests from arid environments, such as site 2, where evidence of local adaptation to drought was found (López *et al.*, 2013), López de Heredia *et al.* (2010) showed strong evidence for clustering of recruits on hygrophilous stream beds.

Open landscapes facilitate long distance dispersal

The landscapes dominated by *P. canariensis* have a varying stand structure (del Arco *et al.*, 1992; Pérez de Paz, 1994), from low density pine-woods in the south of Gran Canaria to dense forests on the humid slopes of La Palma and Tenerife. In the present study we compared the effective dispersal distributions from two pure stands that lack sub-canopy vegetation. As was hypothesized, the open pine-wood from site 2 showed much higher mean effective dispersal distance than the dense forest from site 1. This result was expected, because standing vegetation probably acts as a physical barrier to wind dispersed seeds across the landscapes (Pounden *et al.*, 2008), and wind speed increases in open canopies (Katul *et al.*, 2005) promoting long distance dispersal. The trunks and canopies of dense stands intercept seeds, which fall to the ground and are rapidly covered by litter that confers protection against predators, and a favourable microclimate for germination. Changes in vegetation density also induce changes in abundance and behaviour of predators and dispersers (Bannerman & Bannerman, 1963; Jordano *et al.*, 2007), but, as we have shown above, it does not seem that birds have a significant incidence in the seed dispersal process of *P. canariensis*.

Despite interception of seeds by the overstorey seems the most plausible scenario to explain the decrease of dispersal distances in dense pine-woods, we cannot discard that other factors associated to density may have an effect on the extent of seed dispersal. For instance, under the canopy of a dense pine-wood the optimal conditions for regeneration are created (Otto *et al.*, 2010). Therefore, a higher percentage of the seeds dispersed at shorter distances are able to establish. Conversely, in open landscapes subjected to high irradiance, the seedlings will install depending on edaphic factors and, mainly, water availability. In line with this, López de Heredia *et al.* (2010) found that establishment in site 2 was strongly related to the presence of hygrophilous bed-streams where seedlings could develop. The spatial arrangement of these optimal niches for seedling occurrence relative to the mother trees (i.e. directional bias due to the anisotropy of the habitat) may strongly bias the effective seed dispersal inference (Schurr *et al.*, 2008). In dense pine-woods, however, the habitat is less heterogeneous and, therefore, we expect less bias of seed dispersal inference.

Conclusions

Models that combine genotypic and spatial information, such as the GSM, have proven valuable to infer

seed dispersal parameters in stands of *P. canariensis* of contrasting density. In this paper we have demonstrated that *P. canariensis* has a remarkable dispersal ability compared to other pine species. This efficient mechanism of seed dispersal by wind is congruent with indirect measures of gene flow, and needs to be considered in the reconstruction of past demographic events and in the prediction of future distribution ranges for the species. Moreover, we have shown that low stand densities enhance the extent of seed dispersal, and that secondary seed dispersal by animals is limited. Therefore, considering that primary seed dispersal is the template for recruitment in the species, the inferred patterns of seed dispersal of *P. canariensis* have utility for forest managers.

Acknowledgments

We wish to thank G. González-Gordaliza for field assistance.

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