ORIGINAL ARTICLE

High biogeographical and evolutionary value of Canary Island pine populations out of the elevational pine belt: the case of a relict coastal population

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ABSTRACT

Aim Marginal populations are frequently neglected in static views of vegetation types, particularly when defining conservation reserves. The biogeographical and evolutionary importance of a marginal and endangered population of *Pinus canariensis* is addressed in this study to ascertain the need for conservation action. Diversity loss between adults and offspring and patterns of seed dispersal and recruitment were examined to provide evidence of recent degradation of marginal *P. canariensis* pinewoods. The scientific basis for the provision of sound conservation policies was investigated by elucidating the factors responsible for significant population structure.

Location An isolated low-density pinewood community confined to the Arguineguin ravine, in south Gran Canaria, Canary Islands.

Methods Two cohorts, of centenary trees (those older than 100 years) and young recruits, respectively, were found in a detailed inventory of the pine population in the Arguienguin ravine. Chloroplast and nuclear microsatellites were compared to assess the levels of genetic diversity between adults and recruits. Spatial genetic structure and parentage analysis based on highly polymorphic nuclear and chloroplast microsatellites were examined to test limitations in seed dispersal. The underlying environmental factors that led to a clustering effect in the population were tested using point pattern methodologies.

Results Centenary trees retain high levels of genetic diversity and effective population size, suggesting a wider extension of the pinewood forests in the past. A significant loss of genetic diversity was detected between adults and recruits. *Pinus canariensis* dispersal distances were among the longest ever reported for anemochorous species, suggesting that environmental factors account for recruit clustering. Cluster models showed that recruits tend to aggregate in dry streambeds, where soil and water accumulation favours establishment.

Main conclusions Boundary populations of *P. canariensis* are subjected to fragmentation and reduction in effective population size as a result of human impact. Marginal populations were denser in the past and currently require specific conservation efforts. A severe reduction in genetic diversity compromises the future of present populations. Streambeds appear to play a major role in recruit establishment, but data suggest the absence of limitations to seed dispersal.

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INTRODUCTION

The Canary Islands are an oceanic archipelago of volcanic origin with high species endemism. The naturalist Alexander von Humboldt visited the Canary Islands and wrote an excellent synthesis of the geological, botanical and climatological characteristics of the region in his *Personal narrative of a journey to the equinoctial regions of the new continent* (von Humboldt, 1814). von Humboldt described five types of vegetation belts on Tenerife that remain accepted, in the absence of human intervention, by some today: '…the whole island may be considered as a forest of laurels, arbutus and pines, of which the border has scarcely been cleared…' (von Humboldt, 1814, p. 270). However, it must be noted that this description was completed in a short 6-day stay and restricted to the north side of Tenerife, which differs ecologically from the southern slopes. Among other factors, persistent cloud cover supports laurisilva belts in north Tenerife, and pines occur in poor soil environments.

The influential legacy of von Humboldt indicated that the Canary Island pine (*Pinus canariensis* Chr. Sm. ex DC.) woods were confined to precise vegetation belts (Santana *et al.*, 2006), which differed depending on island and slope: 850–1800/800–2000 m in Tenerife (north/south) (del Arco *et al.*, 1992); and 1025–2000/900–2000 m in Gran Canaria (north/south) (Pérez de Paz *et al.*, 1994). However, a detailed analysis of the distribution of *P. canariensis* found that the species occupies a wide range of elevations and climates in sharply different plant communities (Climent *et al.*, 2002), from the slopes of Teide Mountain at 2400 m in Tenerife to arid locations close to sea level in south-western Gran Canaria, where it inhabits mainly salic outcrops with thermo-sclerophyllous shrubs (Salas Pascual *et al.*, 1998; del Arco *et al.*, 2002).

Currently, the Canary Island pine distribution (about 55,000 ha) is the result of successive colonization–extinction processes derived from volcanic activity (Navascués *et al.*, 2006) and human action: much of it has been planted (Glas, 1764; von Buch, 1825; Parsons, 1981; de Nascimento *et al.*, 2009). Despite significant differences among provenances in some adaptive traits, such as ontogeny (Climent *et al.*, 2006) or survival (López *et al.*, 2007) at the seedling stage, the species performance is fairly homogeneous upon reaching maturity (López *et al.*, 2007). According to molecular studies based on neutral variation, gene flow is significant along elevational transects (Navascués *et al.*, 2008), but some level of genetic differentiation is maintained among valleys (Schiller *et al.*, 1999; Gómez *et al.*, 2003). Consistent with other pine species, *P. canariensis* undergoes an outcrossing mating system, and wind-dispersed pollen can potentially travel long distances. Seeds have an adnate wing and are wind-dispersed. *Pinus canariensis* is 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) and where competition is very low. To date, however, studies directly quantifying seed and pollen dispersal in *P. canariensis* or patterns of tree and recruit aggregation have not been conducted.

Canary Island pinewoods are the main habitat for highly endangered bird communities, such as blue chaffinches (*Fringilla teydea*). The Gran Canarian endemic subspecies of blue chaffinch (*Fringilla teydea* ssp. *polatzeki*) is particularly endangered and is very sensitive to habitat disturbance (García-del-Rey *et al.*, 2009). Although the Canary Island pine forests are included in Annex I of the EU Habitat Directive, there have been few efforts to conserve their genetic resources, particularly in populations distributed outside the standard vegetation belts. Recent Canary Island pinewood inventories have described some populations in extreme or marginal environments (Voggenreiter, 1976; Pérez de Paz *et al.*, 1994), which could have favoured singular genes or gene combinations with adaptive value. These populations might have had larger distributions in the past, as there is historical evidence of Canary Island pinewoods in coastal locations on north Tenerife (Barker-Webb & Berthelot, 1840, p. 153) and southern Gran Canaria slopes (von Buch, 1825, p. 24) exclusive of salic outcrops. Coastal pinewoods were excessively exploited for pitch extraction and subsequent ship caulking right after the Castilian settlement (Hakluyt, 1599; Parsons, 1981), these activities being the main income for settlers (Rosa Olivera & Marrero Rodríguez, 1986) until the end of the 17th century. This led to the extermination of the pine populations closest to coastal areas.

If peripheral populations maintain substantial genetic variation, these populations may diverge from more central populations under different selective pressures and reduced gene flow (Lenormand, 2002) and therefore play a role in the maintenance and generation of biological diversity (Mayr, 1970; Channell & Lomolino, 2000). Marginal populations from south Gran Canaria have high chloroplast DNA (cpDNA) diversity, despite small population sizes (Gómez *et al.*, 2003; Vaxevanidou *et al.*, 2006). One population along an eastern hillside of the Arguineguin ravine has the highest number of unique haplotypes (45.8%; 11 of 24 haplotypes detected) of all the Canary Island pine range (average per population of 10.3%), presumably related to the old age of the substrate (Vaxevanidou *et al.*, 2006). In fact, the Arguineguin pines have been suggested as a conservation reserve as they form a relict population under extreme environmental conditions (Voggenreiter, 1976). Voggenreiter (1976) examined *P. canariensis* only on the western hillside of the ravine of Arguineguin and did not indicate tree recruitment. However, the exhaustive inventory of the present study showed that centenary pines (i.e. those older than 100 years) occupy both hillsides of the Arguineguin ravine with an understorey of young recruits.

We hypothesize that the Arguineguin *P. canariensis* population was denser in the past and that it suffered a severe demographic reduction as a result of human activity during the last two centuries. Human-induced fires and goat grazing prevented recruitment, but the absence of human impact in recent decades has led to a cohort of young recruits. Neutral molecular markers will serve to assess any loss of genetic diversity between adults and offspring. Given the extreme environmental conditions in the ravine, spatial structuring of

recruits is expected according to small-scale habitat variation now that human impacts are negligible. First, we modelled seed and pollen dispersal using parentage analysis. Second, we examined aggregation patterns of both cohorts with spatial point pattern analyses. If clusters were detected, we determined if the groups were the result of local seed dispersal or habitat heterogeneity, or both (Law *et al.*, 2009). This served to identify the most suitable niches for *P. canariensis* and provide a scientific foundation for long-term conservation policies.

MATERIALS AND METHODS

Study site

The study plot is an isolated Canary Island pine population located in Arguineguin ravine, 2.5 km inshore from the south coast of Gran Canaria island (27°47′ N; 15°40′ W). The nearest *P. canariensis* population to the study plot is 7 km away in the same ravine, and population density increases up the Roque Nublo volcanic massif slopes, in the centre of the island, where the species grows in continuous stands. The plot occurs in an arid ombrotype with < 200 mm of annual precipitation (del Arco *et al.*, 2002), which restricts arboreal vegetation to a few pines and palm trees at very low density, *Kleinia neriifolia* and *Euphorbia* spp., and the highly endangered tree *Dracaena tamaranae*.

During the inventory phase, two well-defined cohorts were identified, occupying 272 ha: 125 centenary trees (mean diameter = 50 ± 20.8 cm) and 152 young recruits (53 with a mean diameter = 9.5 ± 5.3 cm and 99 with a diameter < 2 cm) (Fig. 1). Density was extremely low: 1.02 trees ha^{-1} (0.46 trees ha^{-1} for the adult cohort and 0.56 trees ha^{-1} for the recruits). The population is distributed on two steep hillsides divided by a road. Dry streambeds transverse the hillsides (mean width = 45 m), where thin soil deposition is possible. The western hillside supports 80.5% of the population (68.8% of adults and 90.2% of recruits). The ravine bottom is 85 m a.s.l., and the first pines occur at 180 m a.s.l. The maximum distance between adult pines spanning the entire population is 2015 m, and the minimum distance is 5.6 m. Maximum and minimum distances
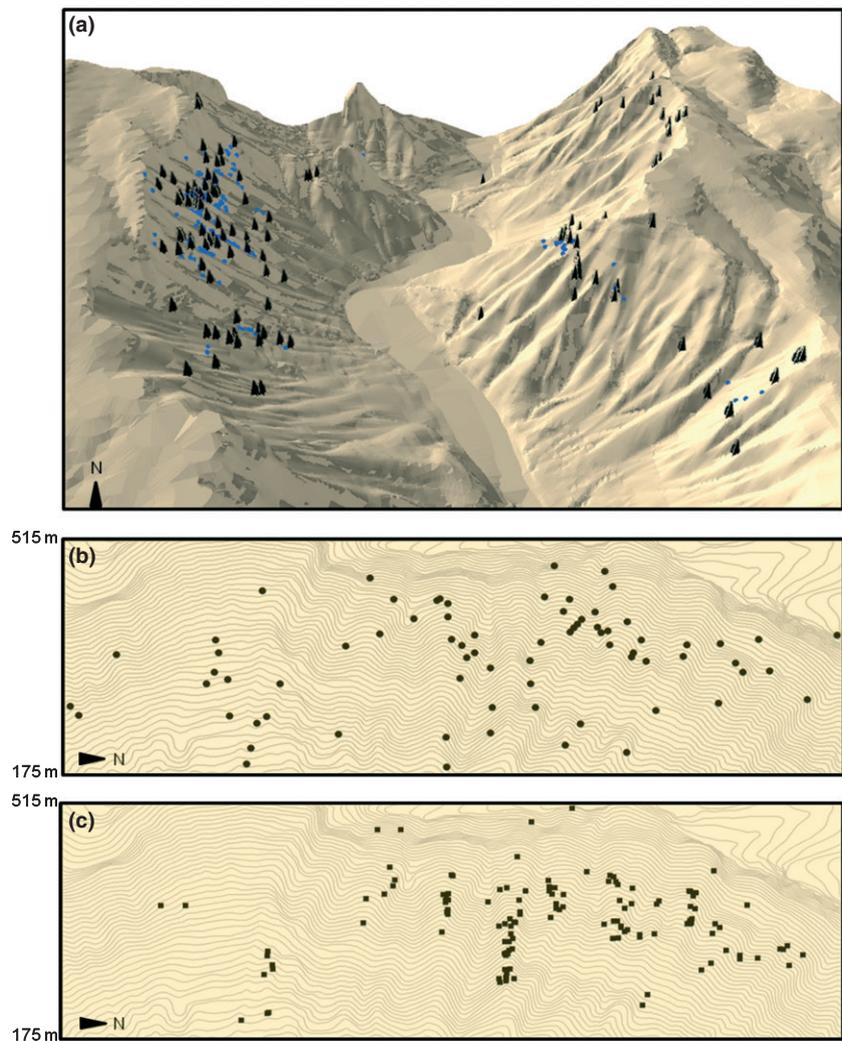

Figure 1 Population of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria. (a) 3-D representation. The steep slopes of the western and eastern hillsides are crossed by perpendicular dry streams. The size of the pine symbols is proportional to the age of the pines and is overrepresented to facilitate the view of the two pine cohorts: black pine symbols, adults; blue dots, recruits. (b) 2-D representation of the adult pines in the observation window employed for point pattern analysis (western hillside). (c) 2-D representation of the recruits in the observation window employed for point pattern analysis (western hillside). Recruits are clumped in the dry streams. Contour lines are displayed each 10 m.

between adults and recruits are 1937.5 and 7 m, respectively (mean = 351 m).

Sampling and laboratory protocols

The spatial coordinates of all trees in the study plot were obtained with a GPS. Fifteen adult trees were dead at the time of sampling. The trunks had fallen and had advanced signs of rot. Therefore, it is unlikely that these trees contributed to the cohort of recruits. One of these pines was *c.* 259 years old, as determined by counting rings from a basal slice, and showed evidence of at least three severe fires *c.* 127, 84 and 26 years before death. These were probably human-induced soil fires to remove tall shrubs for cattle grazing.

Fresh leaves from all living trees were collected and stored at −80 °C. DNA extraction was performed using the protocol of Dellaporta *et al.* (1983) with modifications. Five polymorphic nuclear microsatellites, SPAG 7.14, SPAC 11.5, SPAC 11.8 (Soranzo *et al.*, 1998), Pttx3116, Pttx4001 (Auckland *et al.*, 2002), and five chloroplast microsatellites, Pt15169, Pt26081, Pt30204, Pt87268, Pt71936 (Vendramin *et al.*, 1996), were amplified under standard polymerase chain reaction (PCR) conditions. Fluorescence-labelled PCR products were analysed 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).

Data analysis

Genetic diversity estimates and spatial genetic structure

Standard diversity statistics (number of alleles, effective number of alleles and heterozygosity) were computed for co-dominant nuclear microsatellites to estimate the loss in diversity between adults and recruits. Chloroplast microsatellites were collapsed into haplotypes, and haplotypic diversity was scored for both cohorts (number of haplotypes, effective number of haplotypes, number of private haplotypes and expected heterozygosity). Effective population size (N_e) was computed for adults and recruits and compared with the N_e of a dense *P. canariensis* population from Tenerife. The latter population covers 3 ha and is located in the ravine of Chirigel, Tenerife (28°25′ N, 16°23′ W, elevation 1100 m), and is representative of the high-density pine populations from the south of Tenerife. Three hundred and twenty-eight adults (older than 100 years) and 540 young recruits were sampled in this population and were analysed with the same set of microsatellites. We applied the linkage–disequilibrium method (LD) to nuclear microsatellites derived from single temporal samples (Hill, 1981), using the program LDNE (Waples & Do, 2010). The method estimates the effective number of parents contributing to a sample, assuming no immigration, which is a reasonable assumption for the isolated population of Arrueguin. Reductions in N_e will increase the rates at which genetic diversity is lost.

Spatial genetic structure was investigated using the method of Smouse & Peakall (1999) for both diploid nuclear and haploid chloroplast data with the software GenAlEx 6.1 (Peakall & Smouse, 2006). The autocorrelation coefficient *r* (Smouse & Peakall, 1999) measures the correlation between spatial and genetic data. The analysis is based on distance classes between pairs of individuals. The number of distance classes was chosen so that all classes had approximately the same number of pairs. Tests for statistical significance in GenAlEx were performed by random permutation, that is, by shuffling individual genotypes among spatial locations and re-computing the autocorrelation coefficient (*r*). This generates a distribution of *r* based on the null hypothesis of no spatial genetic structure so that the probability of the observed *r* can be determined.

Parentage analysis with chloroplast and nuclear SSR markers

Distances from seed donors to recruits and from pollen donors to seed donors were directly estimated using the methodology of Lian *et al.* (2008), with modifications. First, 'pollen parents' were assigned by direct exclusion among those trees having the same chloroplast microsatellite (cpSSR) haplotype in a single adult and in one or several recruits. The accuracy of this paternal assignment was evaluated by verifying the presence of one nuclear allele per locus shared by the pollen parents and their progeny. A maternity analysis with known fathers was subsequently conducted on these recruits to obtain their 'seed parent'. Following this preliminary assignment, parentage analysis was used to generate the most likely parent pairs of the recruits. We compared cpSSR haplotypes of possible parent pairs with those of the recruit assigned by nuclear microsatellites (nSSR). If the pollen parent had the same cpSSR haplotype as the recruit, and the seed parent had a different cpSSR haplotype, these two adults were the presumed parent pair. If both adults had different cpSSR haplotypes from the recruit, none of them was considered the pollen parent. If both adults had the same cpSSR haplotype as the recruit, the gender of each parent could not be identified. Second, we compared cpSSR haplotypes of the recruits when nSSR markers had identified a single possible parent. If the cpSSR haplotype of the candidate was not identical to that of the recruit, the candidate was considered the 'seed parent' of the recruit. If the cpSSR haplotype was identical, pollen and seed donors could not be identified.

For each type of parentage analysis (maternal, parent pairs and single parent), five co-dominant nuclear markers were used to simulate the most likely maternal, single-parent and parent-pair assignments based on population allele frequencies for the entire data set. The proportion of sampled parents was set at a conservative 80%; this was because 15 adult trees were dead at the time of sampling and, in addition, long-distance pollen dispersal was possible. The critical log-likelihood ratios, or LOD scores, were obtained by conducting 100,000 simulations. Only those assignations with LOD scores higher than the critical LOD were considered for further analysis. All analyses were performed with CERVUS 3.0.3 (Kalinowski *et al.*, 2007).

Histograms of 'seed parent-to-offspring' and pollination distances were obtained. The dispersal kernels were fitted directly to the 'seed parent-to-offspring' and pollination distance distributions, respectively, by a maximum likelihood approach. We fitted the parameters for log-normal, gamma and exponential distributions, as they are fat-tailed kernels optimal for data with long-distance dispersal events. We used the *fitdistr* command in R using the Nelder–Mead optimization algorithm of the MASS package (Nelder & Mead, 1965). This maximizes the negative log-likelihood distribution, hence minimizing the log-likelihood. As start parameters, we employed those values provided for other species of *Pinus* (Clark *et al.*, 1999; González-Martínez *et al.*, 2006).

Point pattern analysis

Following the use of molecular data to test for limited seed dispersal, we applied spatial point pattern analysis based on spatial coordinates to examine recruit and adult spatial structure and the effects of environmental covariates, including elevation, slope, aspect and terrain curvature. If there is a strong relationship between adults and recruits or evidence of clustering in the absence of limited seed dispersal, environmental factors must be governing the tree spatial distribution. Point pattern analysis aids in describing and quantifying the spatial patterns of clustering and/or segregation of trees.

Ripley's $K(r)$, the transformed function $L(r) = \sqrt{\frac{K(r)}{\pi}}$ and its derivative, the pair correlation function $g(r) = \frac{K'(r)}{2\pi r}$, are based on the distribution of distances r of pairs of points. These functions are used to describe the small-scale spatial correlation structure of point patterns (Diggle, 2003; Illian *et al.*, 2008). The simplest null model assumes complete spatial randomness (CSR). By comparing the observed $K(r)$, $L(r)$ or $g(r)$ functions with those produced by CSR, we can examine whether the observed patterns show regularity or aggregation, and if this is related to any associated covariate. $L(r)$ is positive for an aggregated pattern and negative for a regular spatial distribution of trees. The pair correlation function $g(r)$ is the probability of observing a pair of points separated by a distance r, divided by the corresponding probability of a Poisson process. Values of $g(r) = 1$ correspond to CSR; values of $g(r) > 1$ suggest clustering at a distance r; and values $g(r) < 1$ suggest inhibition or regularity.

A point pattern analysis with an observation window of 37 ha on the Arguineguin ravine western hillside (within the plot area) was performed (Fig. 1). Trees on the eastern hillside were not included owing to the presence of an artificial barrier, i.e. the road, which generates non-random spatial patterns if the whole plot is considered. ArcGIS 9.2 (ESRI Inc., Redlands, CA, USA) was used to obtain elevation (m), slope and aspect (°) in a grid of 1×1 m. This is a small-scale analysis relative to the study plot size, but it was necessary to address the small clustering observed in the recruits and was also required given the irregularity in the terrain (Fig. 1). In addition, we constructed a 50×50 m grid with values of the horizontal

curvature to depict the dry streambeds. Positive values of this variable indicate convexity, and negative values, concavity. Convex areas are regions with less soil deposition because water flow tends to disperse soil; concave areas are associated with soil deposition, that is, with dry streambeds that may be suitable for *P. canariensis*.

First, point patterns for adults and recruits were tested for CSR by estimating corresponding L-functions. Global upper and lower envelope simulations for theoretical distributions were performed for 999 simulations. We used the R *step* procedure of the *spatstat* package to evaluate the best-fit models for adult and recruit patterns. The *step* procedure adds and drops terms from the function until the model with the lowest Akaike information criterion (AIC) value is found. The best-fit models for intensity were obtained for the inhomogeneous pair correlation functions. Then, an inhomogeneous Thomas process was fitted for the recruits (Baddeley & Turner, 2000).

A homogeneous Thomas process assumes that single clusters are randomly distributed in the observation window, that each cluster contains a random number of points, and that the point locations, relative to the cluster centre, have a two-dimensional normal distribution $h(r, \sigma)$ with variance σ^2. The Thomas process K-function with parameters $\theta = (\kappa, \mu, \sigma)$ is

$$K_\theta(r) = \pi r^2 + \frac{1}{\kappa}\left(1 - \exp\left(-\frac{r^2}{4\sigma^2}\right)\right). \tag{1}$$

The parameter values $\theta = (\kappa, \mu, \sigma)$ are determined by the minimum contrast method through achieving the best match between equation 1 and the estimated data K-function (Diggle, 2003).

The Thomas process assumes a random distribution of clusters in the study area, but this may be incorrect, owing to environmental heterogeneity and habitat association. If habitat association is quantified by any environmental covariate, a Thomas process could be applied in combination with inhomogeneous K-functions (Baddeley & Turner, 2000). A Thomas process is fitted with inhomogeneous clusters as follows: (1) estimate the inhomogeneous intensity $\lambda(u)$ of the process; (2) derive an estimate of the inhomogeneous K-function (and of its derivative, the pair correlation function); and (3) use the method of minimum contrast to estimate the parent intensity k and the cluster scale parameter σ (Gaussian standard deviation). All calculations were conducted with the R *spatstat* package (Baddeley & Turner, 2005).

RESULTS

Genetic diversity and spatial genetic structure

All genetic diversity statistics had higher values for adult trees than for recruits, revealing a drop in genetic diversity between cohorts (Table 1). The loss in the effective number of haplotypes (35.174/10.333) and number of private haplotypes (28/13) is substantial. LD analysis indicated a marked contrast in effective population size (N_e) between adults and recruits

Table 1 Diversity statistics for nuclear microsatellites and chloroplast DNA (cpDNA) haplotypes of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria. N_a, number of alleles; N_{ea}, effective number of alleles; N_{pa}, number of private alleles; N_{ah}, number of haplotypes; N_{eh}, effective number of haplotypes; N_{ph}, number of private haplotypes; H_e, expected heterozygosity. The standard error is given in parentheses.

Nuclear microsatellites

Cohort	N_a	N_{ea}	N_{pa}	H_e
Adults	22.800	9.629	5.600	0.771
	(8.935)	(4.910)	(2.926)	(0.082)
Seedlings	21.400	8.170	4.200	0.760
	(7.878)	(3.506)	(2.396)	(0.088)

cpDNA haplotypes

Cohort	N_{ah}	N_{eh}	N_{ph}	H_e
Adults	55.000	35.174	28.000	0.972
Seedlings	40.000	10.333	13.000	0.903

Table 2 Effective population size (N_e) and confidence intervals (CI) for the adults and seedlings of *Pinus canariensis* from the ravine of Arguineguin, Gran Canaria, and of the dense population in Chirigel, south Tenerife.

Population	n	Min. allele freq. used	N_e (CI 95%)
Arguineguin adults	110	0.05	69.0 (39.2–151.4)
		0.02	156.6 (94.6–350.7)
		0.01	176.8 (118.3–315.2)
Arguineguin seedlings	152	0.05	29.0 (20.4–41.4)
		0.02	56.3 (44.4–72.7)
		0.01	89.4 (71.7–114.5)
Tenerife adults (dense)	328	0.05	66.3 (48.8–91.4)
		0.02	100.7 (85.4–119.9)
		0.01	128.2 (109.5–151.6)
Tenerife seedlings (dense)	540	0.05	125.0 (82.3–204.9)
		0.02	221.1 (168.2–305.9)
		0.01	327.4 (251.0–453.1)

(Table 2). Relatively narrow 95% confidence intervals, particularly for Arguineguin recruits, showed that the LD method (Waples, 2006) was a reliable estimator of N_e where demographic information, including sex ratios and number of offspring per male and/or female, is limited. N_e for adults at Arguineguin was similar to that for trees densely distributed in the plot on Tenerife. Because the Arguineguin population maintains such high N_e, the population was probably denser in the past. A comparison of recruits between localities showed a four-fold higher N_e in Tenerife than in Arguineguin, suggesting a marked genetic bottleneck affecting the Arguineguin population at some point in the past.

The r autocorrelograms for chloroplast microsatellites (i.e. for male gametes) did not detect significant genetic structure at any distance class from 0 to 500 m for adults and recruits (Fig. 2a, b). For nuclear microsatellites (i.e. male and female gametes) there was weak but significant genetic structure observed for both adults and recruits (Fig. 2c, d). Although significant, correlation values were < 0.15 in both cases, suggesting weak family structure. For adults, significant values of the r-statistic were scored below 150 m, and for recruits significant values occurred up to 100 m.

Seed and pollen dispersal

Microsatellites showed exclusion probabilities of 99.9% for maternity, single-parent and parent-pair assignments. Simulation results showed a critical LOD score of 2.52 for maternity analysis, when the father was known, at a relaxed confidence level of 80%. The LOD scores for parent-pair and single-pair assignments were respectively 9.94 and 3.82 at the same confidence level. The mother was unambiguously assigned to 59 recruits (38.8% of the data) when only the highly reliable parent–offspring matches were analysed; in 29 recruits, both mother and father were identified (19% of the data). Non-reliable assignments were discarded to fit seed dispersal and pollen distribution. The 59 recruits were the offspring of 38 mothers, suggesting a normal distribution of female reproductive success.

The histogram of 'parent seed-to-offspring' distances showed a leptokurtic (kurtosis = 0.35) and right-skewed (skewness = 1.30) distribution (Fig. 3a). The mean seed-dispersal distance was 368.43 m and the median was 160.55. This indicated that 50% of recruits dispersed at distances below 160.55 m. Long-distance dispersal events were also detected at more than 1.6 km. These results strongly support the hypothesis that limitations to seed dispersal are not in effect. The vector corresponding to this univariate distribution was fitted to log-normal, gamma and exponential dispersal kernels (Table 3). The best fit corresponded to a log-normal dispersal kernel (AIC = 806, μlog = 5.05, sdlog = 1.38) (Fig. 3a).

The pollination-distance histogram was fitted to the exponential and log-normal dispersal kernels (Table 4). The best fit corresponded to an exponential pollen-dispersal kernel (AIC = 432.4, rate = 0.00163). The mean pollination dispersal distance was 614.89 m and the median was 494.69 m; that is, 50% of the pollination distances occurred at distances < 494.69 m. The resulting kernel was flatter than the seed-dispersal kernel estimated with the log-normal model (Fig. 3b).

Point pattern analysis

The L-function showed that the adult and recruit univariate patterns (Fig. 4a, b) were not under CSR. The L-function suggested some clustering of adults, with an inter-point distance of $r > 40$ m. However, strong clustering was evident for recruits with inter-point distances $r > 3$ m. Because molecular data did not show evidence of restricted dispersal, deviation from CSR suggests that one or several environmental covariates may be generating clustered point patterns, particularly for recruits.
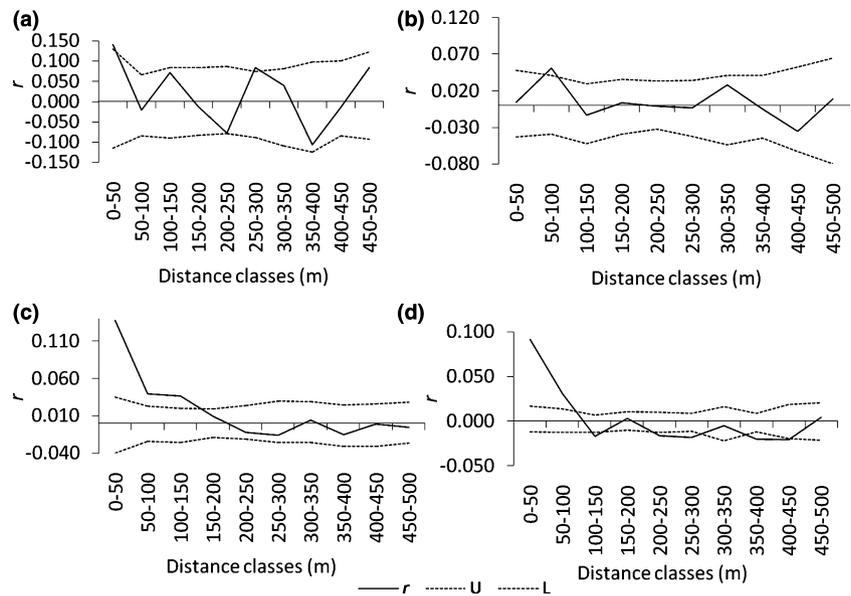
Figure 2 Autocorrelograms of the *r*-statistic for chloroplast DNA for (a) adults and (b) recruits; and for nuclear DNA for (c) adults and (d) recruits of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria. Dashed lines represent the upper (U) and lower (L) confidence intervals based on 20,000 permutations.
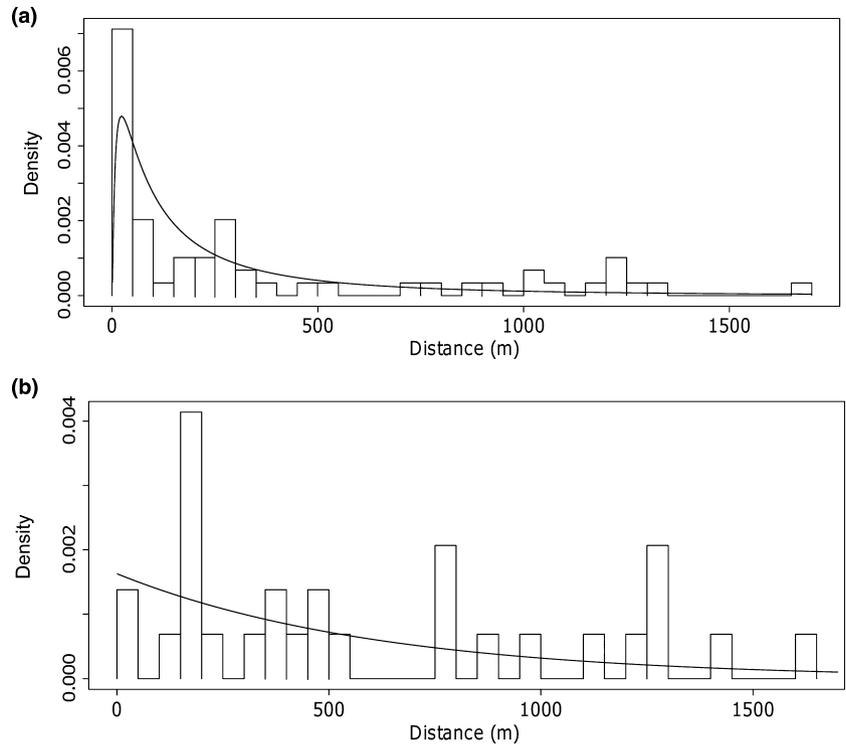

Figure 3 Histogram of (a) 'parent seed-to-offspring' distances and the probability density function of the fitted log-normal model for *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria; and (b) pollination distances and the probability density function of the fitted exponential model for *P. canariensis* in the ravine of Arguineguin, Gran Canaria.

The best models derived from the *step* procedure were as follows. For adults, intensity was exponentially proportional to elevation (*Z*):

$$\lambda(Z) = \exp(\beta_0 + \beta_1 Z). \qquad (Model\,I)$$

For recruits, intensity was an exponential function of horizontal terrain curvature (*C*):

$$\lambda(C) = \exp(\gamma_0 + \gamma_1 C). \qquad (Model\,II)$$

Parameters for both models are shown in Table 5. For adults, intensity decreased with elevation, suggesting that

adults of *P. canariensis* are less frequent in the steep, higher regions of the ravine. For recruits, intensity decreased with positive convex parameters, suggesting that clusters were maintained in concave areas (i.e. streambeds).

We fitted an inhomogeneous Thomas model for recruits, which exhibited stronger clustering in recruits than in adults. Thomas analysis with inhomogeneous clusters for recruits exhibited parameters $\kappa = 0.000036$ and $\sigma = 15.26$. The Gaussian standard deviation σ was the scale parameter that was used to estimate cluster size. Cluster sizes were consequently estimated as circles with mean diameter

Table 3 Summary of fitted parameters for log-normal, gamma and exponential dispersal kernels for seed dispersal of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria.

Distribution	Kernel parameters	AIC
Log-normal	μlog = 5.0540	806.0
	sdlog = 1.3827	
Gamma	Shape = 0.7176	814.1
	Rate = 0.0021	
Exponential	Rate = 0.0004	817.3

AIC, Akaike information criterion.

Table 4 Summary of fitted parameters for log-normal and exponential dispersal kernels for pollen dispersal of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria.

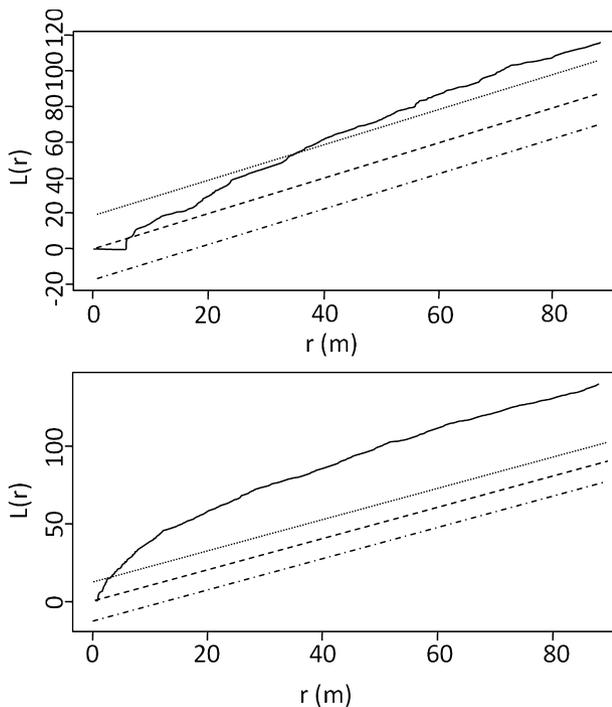| Distribution	Kernel parameters	AIC
Log-normal	μlog = 6.015	437.7
	sdlog = 1.0447	
Exponential	Rate = 0.00163	432.4

AIC, Akaike information criterion.

Figure 4 *L*-function plots of point patterns for (a) adults and (b) recruits of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria. Solid line, observed estimated *L*-function; dashed line, theoretical value under complete spatial randomness (CSR). Dotted line, upper envelope for 999 simulations; dotted–dashed line, lower envelope for 999 simulations. Values above the theoretical curve and outside the global envelopes suggest clustering for distance *r*.

$2\sigma = 30.52$ m, with the mean area covered by a cluster equal to $4\pi\sigma^2 = 4843.9$ m^2.

The associated expression of the inhomogeneous pair correlation functions for Model II is shown in Fig. 5. The plot shows strong aggregation up to pair distances of $r < 15$ and stabilizes in values around $g(r) = 1$, congruent with the σ scale parameter used to estimate cluster size.

DISCUSSION

The conservation status of some Canary Island pine populations is not as it was described in the nearly 200-year-old narrative of von Humboldt (1814). Static views that rely on the concept that species tend to associate in natural groups should be avoided (Blumler, 1996, and references therein; Carrión & Fernández, 2009). Vegetation associations are the result of a spatio-temporal dynamic determined by probability, contingency and environmental heterogeneity. Their complexity increases according to evolutionary history and anthropic or natural perturbations. Unfortunately, local extinctions resulting from human impact were not considered when defining conservation priorities in the Canary Island pinewoods. Furthermore, marginal populations, such as that of Arguineguin, were not included in the Natura 2000 network (Santana *et al.*, 2006) because they do not belong to the standard elevational pine belt.

The exhaustive inventory of Arguineguin demonstrated that pine populations outside the pine belt retain high levels of diversity and that recruitment is possible. Therefore, these populations possess high biogeographical and evolutionary importance. In our opinion, the pinewoods of Arguineguin should have been included as priority habitat in the Natura 2000 network, to counteract threats to pinewoods that are the habitat of highly endangered endemic species such as the blue chaffinch (*Fringilla teydea* ssp. *polatzeki*). Indeed, the effective population size of adults in Arguineguin is comparable to that of dense pinewood forests on Tenerife, but with much lower demographics. The maintenance of this level of N_e is possible only if the population was more widespread in the recent past. These results are congruent with the notably high cpDNA diversity levels for the pines from Arguineguin detected by Vaxevanidou *et al.* (2006) compared with the entire range of *P. canariensis*.

One cause of the drastic reduction in demographics is the extensive human impact that occurred in Arguineguin following its passage from public to private property at the end of the 19th century (Ojeda, 1977). Cattle grazing and forest fires severely reduced population size in Arguineguin as well as in other coastal populations. The pinewoods of El Cucaracho, Maspalomas, Lomos de Pedro Afonso, Llanos de Cortadores and also Arguineguin are cited in a document of the 1870s as pinewood communities that disappeared owing to a lack of control of public property (Ojeda, 1977, p. 167). Indeed, Llanos de Cortadores is the adjacent territory to the north of Arguineguin. The property was sold in 1855 (Suárez, 1997, p. 125) and the pinewoods are now gone.

Forest fires that aim to open land for grazing have moderate effects on adult *P. canariensis*, which are able to re-sprout.

Table 5 Fitted coefficients and standard deviation (in parentheses) for the fitted inhomogeneous Thomas models for *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria. Model I is for the adult trees and uses elevation as a covariate. Model II is for the recruits and uses the curvature of the terrain as a covariate.

Model I		Model II	
β_0 (intercept)	β_1 (elevation)	γ_0 (intercept)	γ_1 (curvature)
7.359703895 (0.478336067)	−0.004199641 (0.001569598)	−8.0495534 (0.08807606)	−0.6613013 (0.274821869)

Figure 5 Inhomogeneous pair correlation function of the fitted Thomas Model II for recruits of *Pinus canariensis* in the ravine of Arguineguin, Gran Canaria, which uses the curvature of the terrain as a covariate. The horizontal axis represents the inter-point distance *r*.
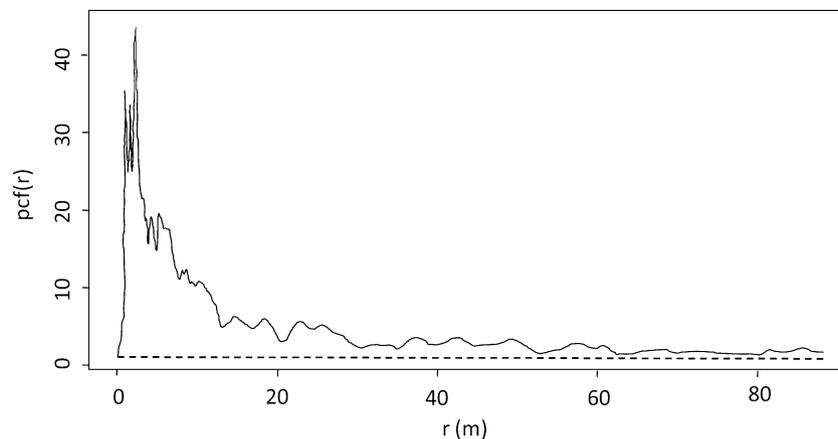
However, recruits suffer complete mortality. In Arguineguin, the pines that are most accessible are those close to the road and they were probably cut for wood and pitch extraction. The lifting of cattle grazing pressures in the last decade has allowed pine recruitment in Arguineguin. However, a loss of genetic diversity from adults to recruits has occurred in both nuclear and chloroplast DNA, particularly in private cpDNA haplotypes. This loss of genetic variation compromises the future of the population. Genetic diversity measurements based on allelic richness are important for conservation genetics. Studies have demonstrated that marker-assisted methods are effective in maximizing the number of conserved alleles (Schoen & Brown, 1993; Bataillon *et al.*, 1996). It is also vital in the long term, as selection limits are determined by initial allelic composition of populations more than by heterozygosity (Petit *et al.*, 1998).

Spatial genetic structure and dispersal model analyses derived from parentage analysis revealed patterns of genetic structure consistent with substantial effective seed dispersal (i.e. dispersal plus establishment) up to 100 m from source trees. A non-skewed distribution in female reproductive success was observed, with 64.4% of adults producing seeds that successfully passed to the sapling stage. In addition, significant long-distance dispersal events were observed. It must be stressed that difficulty in parental assignment increased with the level of relatedness among potential parents (Marshall *et al.*, 1998). Therefore, a potential bias existed in the parent-pair estimates towards parents separated by large distances. This is because the level of relatedness among individuals is expected to vary with distance if spatial genetic structure is present. However, spatial genetic structure was weak for nSSRs and absent for cpSSRs, thus reducing the potential bias.

The weak spatial genetic structure found for adults and recruits was related to the high frequency of effective long-distance seed-dispersal events, and, above all, to the homogenizing effect of pollen dispersal. Autocorrelograms for cpSSR data indicated a lack of spatial structure. Indeed, pollen travelled from distances of at least 7 km, the distance to the nearest population of *P. canariensis*, as shown by private haplotypes found in the recruits but not present in the Arguineguin adults. However, a potential bias in the pollen-dispersal kernel must be considered, given the low number of pollination distances used to construct the model.

It is of considerable interest that, compared with other anemochorous temperate trees, *P. canariensis* is able to effectively disperse its progeny over long distances. For example, through modelling seed-shadow and sapling distributions in a *Pinus pinaster* forest, González-Martínez *et al.* (2006) found average sapling dispersal distances of *c.* 40–60 m, while the average effective dispersal distance for *P. canariensis* in Arguineguin is *c.* 324.9 m. Effective dispersal distances for other temperate tree species are also smaller than for *P. canariensis*; for example, *c.* 60–80 m for *Gleditsia triacanthos* (Schnabel *et al.*, 1998), *c.* 15–40 m for oaks (Dow & Ashley, 1996; Valbuena-Carabaña *et al.*, 2005), *c.* 75 m for *Cercidiphyllum japonicum* (Sato *et al.*, 2006) and *c.* 30 m for *Abies sachalinensis* (Lian *et al.*, 2008). These studies are from species with different dispersal mechanisms and sampling schemes, and therefore may not serve as adequate comparisons to

P. canariensis. Seeds with adnate wings facilitate long-distance gene flow in *P. canariensis*, a rare trait present in some island pine species. Pines with adnate seed wings disperse by wind after seeds are released from the cone.

The 351-m average distance between adults and recruits in Arguineguin is a result of the low population density, resulting from the historical degradation of pinewood communities. In fact, the past reduction in adult density may have influenced effective dispersal distances. The absence of other trees allowed seeds to disperse greater distances without obstacles, and seedlings established in the absence of competition. Indeed, the population configuration in Arguineguin does not allow a generalized model of effective dispersal applicable to dense *P. canariensis* populations. However, it does apply to disturbed pinewoods, or to early stages of colonization in unstable environments, such as parts of the volcanic Canary Islands. After Strombolian-type eruptions (i.e. violent eruptions of incandescent lava flows), *P. canariensis* populations are thought to have been affected by severe bottlenecks (Navascués *et al.*, 2006), resulting in low effective population sizes, similar to the case for the recruits in our study. Our results of effective seed dispersal indicate the potential of *P. canariensis* to initiate recolonization at long distances, which is enhanced by the species capacity to inhabit the salic soils abundant after volcanic perturbations (Ancochea *et al.*, 1990).

Today, in the absence of intensive human impact, erosive processes strongly influence recruitment. The ravines of south Gran Canaria were formed by sequential lava and pyroclast volcanic episodes, as a consequence of the last eruption of Roque Nublo 3.5–4.3 Ma (Pérez-Torrado *et al.*, 1995). Since then, the area has been subjected to intensive erosion, which has resulted in poor salic soils that *P. canariensis* inhabits preferentially. The presence of pines since the Tertiary in the eroded Arguineguin ravine (Navascués, 2006) would explain the reported high levels of N_e and cpDNA diversity (Vaxevanidou *et al.*, 2006). At present, Arguineguin is a semi-desertic environment of rocky soils with little opportunity for soil development owing to the very sparse vegetation cover. The only places where thin soil layers develop are in small dry streambeds. In these niches, the scarce precipitation of the region converges and sediments flow, favouring an edapho-hygrophylous microenvironment optimal for recruit establishment. The estimated diameter of the clusters of recruits (30.52 m) was consistent with the mean width of the dry streams (45 m), suggesting that recruits are located at the bottom of the streambeds.

The patterns for adults indicated less structured clustering than did those for recruits. During forest development, forest composition typically establishes a uniform tree distribution by self-thinning (Hamrick *et al.*, 1993; Wiegand *et al.*, 2007). This may result in a loss of environmental covariate signal and explains the absence of clustering in the bottom of dry streambeds by adult trees. The fitted model for adult point patterns demonstrated that elevation had a low but significant effect on intensity (i.e. density). In fact, structural factors may prevent adult pine trees from merging in the steep higher areas of the plot.

Today's marginal stands in coastal locations were populations with increased genetic diversity in the past and require special conservation efforts. The high levels of diversity and differentiation reported in this study together with the decline in genetic diversity in recruits make these relict populations eligible for special conservation efforts (Eckert *et al.*, 2008; Lorenzo *et al.*, 2009). Limitations to seed dispersal and recruitment were not detected in Arguineguin; however, conservation of the population is desirable owing to a decline in the genetic diversity of recruits. We advocate that rather than extensive reforestation, conservation management should focus on recovering an effective population size using local stock (Mijnsbrugge *et al.*, 2010) to preserve alleles or haplotypes not present in recruits. However, given adult isolation, there is a low probability of pollination. The trees still produce cones but with a low percentage of viable seeds. Vegetative propagation of adults by grafting and the establishment of an *ex situ* conservation clonal bank may be the best solution to achieve preservation of the population. Seeds from the clonal bank should be planted in optimal areas for recruitment that lack natural recruits. Pine population recovery would favour conditions suitable for the recruitment of other thermo-sclerophyllous plants typical of south-west Gran Canaria, including *Juniperus turbinata* ssp. *canariensis* and *Dracaena tamaranae*, which are more ecologically demanding species than pines.

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BIOSKETCH

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