Keeping it in the family: strong fine-scale genetic structure and inbreeding in *Lodoicea maldivica*, the largest-seeded plant in the world

E. J. Morgan[1]*, C. N. Kaiser-Bunbury[2], P. J. Edwards[3], F. Fleischer-Dogley[4], C. J. Kettle[1]

[1]ITES–Ecosystem Management, ETH Zürich, Universitätstrasse 16, 8092 Zürich, Switzerland
[2]Ecological Networks, Department of Biology, TU Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany
[3]Singapore-ETH Centre, 1 CREATE Way, 06-01 CREATE Tower, Singapore 138602
[4]Seychelles Islands Foundation, La Ciotat Building, Mont Fleuri, PO Box 853, Victoria, Mahé, Seychelles

*Corresponding author
Tel.: +41 44 632 89 45
e-mail: emma.morgan@usys.ethz.ch
ORCID: 0000-0001-8093-8987

Abstract

The fine-scale spatial genetic structure (FSGS) of plant populations is strongly influenced by patterns of seed dispersal. An extreme case of limited dispersal is found in the charismatic yet endangered palm *Lodoicea maldivica*, which produces large fruits (up to 20 kg) dispersed only by gravity. To investigate patterns of seed dispersal and FSGS in natural populations we sampled 1252 individual adults and regenerating offspring across the species' natural range in the Seychelles archipelago, and characterised their genotypes at 12 microsatellite loci. The average dispersal distance was 8.7 ± 0.7 m. Topography had a significant effect on seed dispersal, with plants on steep slopes exhibiting the longest distances. FSGS was intense, especially in younger cohorts. Contrary to what might be expected in a dioecious species, we found high levels of inbreeding, with most neighbouring pairs of male and female trees (≤ 10 m) being closely related. Nonetheless, levels of genetic diversity were relatively high and similar in the various sampling areas, although these differed in disturbance and habitat fragmentation. We discuss potential trade-offs associated with maternal resource provisioning of progeny, seed dispersal and inbreeding, and consider the implications of our findings for managing this globally significant flagship species.

Introduction

Dispersal allows offspring to move away from their parents, thereby promoting gene flow, reducing inbreeding and sibling competition, and enabling new sites to be colonised. In plants, most long-distance gene dispersal, especially in wind-pollinated species, occurs through the transport of haploid pollen (Petit *et al.* 2005), which moves only paternal genes. In contrast, seed dispersal - which is the principal means by which progeny establish away from related individuals - moves both maternal and paternal genes. Both processes influence the degree to which related individuals are aggregated, and therefore the fine-scale spatial genetic structure (FSGS) within a population (Hardy *et al.* 2006). Limited dispersal of either seeds or pollen may result in an increased intensity of FSGS (Ennos 1994; Hardy *et al.* 2006; Seidler & Plotkin 2006) and greater genetic 'isolation-by-distance' (Wright 1943). Conversely, any aspect of a plant's breeding system that promotes outbreeding tends to reduce the intensity of FSGS. An extreme case is dioecy, which is usually regarded as a strategy to avoid inbreeding, especially in small populations (Darwin 1877; Baker & Cox 1984), and which occurs more frequently in the floras of tropical islands than amongst flowering plants as a whole (12−27% versus 4%; Yampolsky & Yampolsky 1922; Bawa 1980).

In general, FSGS is more affected by seed dispersal than by pollen dispersal, especially in large, long-lived plant species (Vekemans & Hardy 2004; Torimaru *et al.* 2007; Grivet *et al.* 2009). However, disruption of gene flow either by seed or pollen dispersal is expected to intensify the FSGS. Thus, disruption of seed dispersal due to habitat fragmentation can prevent the recolonisation of areas from which a species has been extirpated (Ismail *et al.* 2017), while disruption of pollen flow can cause elevated inbreeding and reduced genetic diversity (Ismail *et al.* 2012). In some systems, however, long-distance pollen flow remains sufficient to counteract these negative effects (Dick *et al.* 2007; Ismail *et al.* 2014). In addition, FSGS may be affected by several other factors, including local adaptation in heterogeneous habitats (Galen *et al.* 1991; Parisod and Christin 2008), recent founder events in pioneer tree species (Silvestrini *et al.* 2015), distance and density-dependent mortality (Choo *et al.* 2012), and the spread of sterility-causing mutations (De Cauwer *et al.* 2010).

The dioecious coco de mer palm *Lodoicea maldivica* (J.F.Gmel.) Pers. (Arecaceae) is endemic to two small islands of the Seychelles. Female trees bear the largest flowers of any palm and the heaviest seeds in the plant kingdom. Geckos are thought to be the main pollination agents, though wind dispersal may also be important (Edwards *et al.* 2002;

Blackmore *et al.* 2012). In contrast, the seeds, which are enclosed in a massive fruit weighing frequently up to 20 kg (Edwards *et al.* 2002, maximum of 45 kg; Tomlinson 2006), lack any mechanism for dispersal apart from gravity, so that most seedlings establish close to the mother tree. The overlapping leaves of the adult tree form a huge funnel that channels water and organic material to the base of the trunk, thereby improving nutrient and moisture conditions for the dispersal-limited young plants (Edwards *et al.* 2015).

Lodoicea provides a unique opportunity to study the genetic consequences of extremely limited seed dispersal in a species that now occurs in both continuous (Praslin Island) and fragmented habitats (Curieuse Island). In previous work, dominant AFLP markers have been used to explore large-scale patterns of genetic differentiation in adult *Lodoicea* across the species' native range (Fleischer-Dogley *et al.* 2011). Here, we use microsatellites to investigate patterns of FSGS and genetic variation in adult trees that probably established before major human impact upon the forest habitat, and in immature plants that established under more or less degraded conditions. Such fragmentation of habitats is predicted to lead to restricted gene flow and consequently elevated inbreeding and reduced genetic diversity within populations of tree species (Lowe *et al.* 2005).

Because of limited seed dispersal, we expected FSGS to be highly developed in natural populations of *Lodoicea*. However, we also expected there to be differences among cohorts, with FSGS being stronger in younger individuals that established under disturbed conditions, and among sub-populations, due to differences in seed dispersal distances in plants growing in different terrain. Specifically, we addressed the following questions: (1) what is the distance between mother plants and their offspring (i.e. the realised seed dispersal)? (2) how does topography influence realised seed dispersal? (3) how does variation in seed dispersal influence FSGS in different sub-populations, cohorts and sexes? (4) how has recent habitat fragmentation affected patterns of inbreeding and genetic diversity? We discuss the genetic implications of limited seed dispersal observed in *Lodoicea* in the broader ecological and evolutionary context of inbreeding avoidance, and consider the genetic consequences of habitat fragmentation for this endemic palm.

Materials and Methods

Study area and study species

The Seychelles archipelago is exceptional amongst tropical islands in that it was uninhabited and apparently undisturbed by humans until the 18th century, when it was colonised by

Europeans (Lionnet 1976). Historical records suggest that the natural vegetation on two of the islands, Praslin and Curieuse, was a dense forest dominated by the coco de mer palm *Lodoicea maldivica* (Malavois 1787, quoted in Fauvel 1909), in the tribe Borasseae (Dransfield *et al.* 2008). Following settlement, and over the past ~150 years especially (Procter 1984), these trees were progressively exploited for their timber, leaves and nuts (Fischer & Fleischer-Dogley 2008), and the forest habitat was cleared for cultivation and development (Bailey 1942). The only semi-continuous stands now remaining are in the southern part of Praslin, and especially in Vallée de Mai World Heritage Site. On Curieuse the species occurs mainly as isolated individuals or in small clusters. More recent pressures include the unsustainable harvesting of nuts, to the extent that there is now almost no natural regeneration except in protected areas, and the spread of invasive alien species (Fischer & Fleischer-Dogley 2008). Nonetheless, because the trees are long-lived - Savage & Ashton (1983) suggest that they may live for 350 years - many of the larger adults probably established before major human disturbance and have a genetic composition reflecting processes operating under natural conditions.

Sample collection

We investigated the FSGS of *Lodoicea* at four locations (hereafter referred to as sub-populations), three on the island of Praslin - Vallée de Mai (VdM), Fond Peper (FP), and Fond Ferdinand (FF) - and one on the island of Curieuse (CU; Fig. 1). Each sub-population on Praslin was characterised by continuous semi-natural vegetation, although the forest on Curieuse was more fragmented. On Praslin, male trees were more abundant than females (57% were males in VdM and FP, and 51% in FF), while on Curieuse female trees were in the majority (46% male; Fleischer-Dogley *et al*. 2011).

In each sub-population, we selected and mapped four 'clusters' of plants. These were selected as groups of individuals that appeared to be the product of natural regeneration and showed minimal signs of human disturbance. These clusters contained between 34 and 193 plants, including individuals of all ages, and had a mean area ± SD: 904.4 ± 800.9 m^2. Within each of the 16 clusters, we geo-referenced (Garmin 60CSx) the locations of all adult trees, and mapped the relative positions of younger plants using a tape measure and compass. Distances between adjacent clusters ranged from 13−360 m. GPS locations of all males within a radius of 80−120 m of each cluster were also recorded. We classified individuals into age classes using the following criteria: *seedlings* had one or two leaves; *juveniles* were trunkless plants with more than two leaves; *immatures* were young individuals with a

developing trunk but no flowers; and *adults* were plants that had reached sexual maturity, as indicated by the presence of male or female flowers. '*Offspring*' refers generally to all non-mature plants. Sampling of leaves for genetic analysis was carried out on all individuals in clusters, and extended uphill to include all potential mothers, and downhill to include all potential offspring (allowing for the possibility that some seeds may have dispersed a long distance). The potential offspring and mothers marked the cluster boundaries. Samples were also collected from male trees within a radius of 80−120 m (many of which were outside of the clusters), and dried and stored in silica gel. This resulted in a total of 1252 samples (sub-population mean $N \pm$ SE: 313 ± 58.8; see Table 1 for sample sizes of each group).

Genotyping

Total genomic DNA was extracted from the leaf tissue using the DNeasy® 96 Plant Kit (Qiagen). Twelve nuclear microsatellite markers were designed for *Lodoicea* at ecogenics GmbH (Balgach, Switzerland), using Roche 454 sequencing. For details of the polymorphic microsatellite loci and PCR conditions see Morgan *et al.* (2016). Fragment length was analysed using the internal size marker LIZ 500 HD in an ABI3730 capillary sequencer (Applied Biosystems), and scored with GeneMarker 2.6.0 (Holland & Parson 2011).

Genetic analysis

Genetic diversity, inbreeding and differentiation

GenAlEx 6.5 (Peakall & Smouse 2006) was used to calculate deviations from Hardy-Weinberg equilibrium (HWE), the mean number of alleles (N_a), the number of private alleles (P_A) and the observed (H_O) and unbiased expected (uH_E) heterozygosities. To control for differences in sample size among groups, we also calculated mean private rarefied allelic richness over loci (Π_s) using HP-RARE 1.1 (Kalinowski 2005). The presence of null alleles and allelic dropout were tested in Micro-Checker 2.2.3 (van Oosterhout *et al*. 2004). Inbreeding coefficients (F_{Is}), allelic richness (A_R; i.e. the number of alleles per locus, corrected for differences in sample size) and pairwise levels of differentiation (F_{ST}) were estimated in FSTAT 2.9.3.2 (Goudet 1995) using 1,000 permutations. Linkage disequilibrium was tested using the log likelihood ratio statistic (G-test) in GENEPOP 4.2 (Raymond & Rousset 1995) using 10,000 iterations. We performed these analyses for each sub-population using data for all adults and offspring combined, and for each age cohort (including males surrounding clusters). In addition, we analysed each cohort separately, but

considering only plants within the clusters so as to have the same sample area for each cohort. To test whether slope affected the patterns of genetic diversity, we selected and pooled the uppermost and lowermost ten individuals from each slope considered to be steep enough (slope > 25%) to permit occasional long-distance seed dispersal (number of clusters: FP $N = 3$; FF $N = 4$; CU $N = 2$). Analyses were also carried out on adult trees separated into male and females, using only individuals from within the clusters, excluding the surrounding males.

Quantifying realised seed dispersal distances

To determine the most likely mother tree of each individual within a cluster we used the delta maximum-likelihood exclusion analysis in CERVUS 3.0 (Marshall *et al*. 1998; Kalinowski *et al*. 2007). Females from within the cluster were included as candidate mothers. This analysis simulated 10,000 offspring, with the minimum number of typed loci set at 9, the proportion of mistyped loci set at 1%, with 96.1% of the loci typed, and the significance threshold for maternity assignments set at 95%. We calculated mean dispersal distances for (i) all offspring, (ii) for offspring in each sub-population, and (iii) for offspring in clusters on relatively flat and sloped terrain (slopes < 25% and >25%, respectively). Although our data records the distance between mother plants and surviving offspring, any resulting bias due to differential survival of offspring probably had only a limited effect upon estimated dispersal distances. Moreover, realised estimates are more ecologically relevant.

Estimating FSGS across populations and life stages

To investigate FSGS, we used the software SPAGeDi 1.4c (Hardy & Vekemans 2002) and GeneAlex (Peakall & Smouse 2006). We calculated correlations of pairwise relatedness (*r*) (Peakall *et al*. 2003) using the pairwise spatial distances of the plants. We also calculated multilocus kinship coefficients (*F*) as described in Loiselle *et al*. (1995), due to its robust statistical properties (Vekemans & Hardy 2004). Separate analyses were performed for: (i) all plants in each sub-population, (ii) for offspring and adults separately, and (iii) for male and female plants (adults from within clusters) separately (the reference sample was the whole dataset). The distance classes varied among but not within analyses (see Table 4, Online Resources 1, 2), and were specified to ensure there were sufficient pairs in each class (minimum numbers: sub-population = 341; adults vs. offspring = 172; sexes = 104). To compare the intensity of FSGS - among populations, between adults and offspring, and

between sexes - we used the *Sp*-statistic $Sp = -b_F/(1 - F_1)$, where F_1 is the mean kinship coefficient between the pairs of individuals within the first distance class, and b_F is the mean regression slope of the regression of kinship over the natural log of the distance (Vekemans & Hardy 2004).

An auto-correlational heterogeneity test (as described by Smouse *et al.* 2008) was carried out in GeneAlex (Peakall & Smouse 2006) to test for differences in FSGS among sub-populations, age cohorts and sexes in adult trees. We calculated the squared paired-sample *t*-test statistic (t^2) to test for differences in relatedness within each of the distance classes among the sub-populations. We also carried out tests of correlogram-level heterogeneity (Ω-test) - among sub-populations, between adults and offspring, and between males and females - taking 1% as the level for a significant effect (Banks & Peakall 2012). Sequential Bonferroni corrections (Holm 1979) were applied to all multiple tests. To evaluate the relatedness of potential father and mother adult trees we plotted kinship (*F*) between male and female pairs from all samples, at several distance classes.

Results

Genetic diversity, inbreeding and differentiation

For the complete sample of 1252 *Lodoicea maldivica* plants, we recorded a total of 158 alleles at 12 microsatellite loci, with a mean of 13 alleles per locus (range 5–21). Over all loci, the inbreeding coefficient (F_{IS}) was 0.272. All loci deviated significantly from Hardy-Weinberg Equilibrium (HWE), reflecting an excess of homozygotes. After sequential Bonferroni correction, no significant linkage was detected between loci pairs.

Sub-populations, sexes, age cohorts and topographical position

No significant variation was detected across groups for any measure of genetic diversity (N_a, A_R, H_o or uH_e; Table 1). However, mean private rarefied allelic richness over loci was significantly higher at CU ($\Pi_s = 1.47 \pm 0.47$) than at VdM ($\Pi_s = 0.29 \pm 0.09$, $U = 26.0$, $P < 0.01$) and FP ($\Pi_s = 0.24 \pm 0.12$, $U = 32.0$, $P < 0.05$). Allelic richness increased with cohort age from seedlings to adults, although the differences were not significant (Table 1).

The inbreeding coefficients (F_{IS}) for each sub-population, age cohort and sex were significantly greater than zero. F_{IS} values were similar among sub-populations and age cohorts, and did not differ according to sex (adults only) or topographic location (Table 1).

There were also no differences in genetic diversity or inbreeding among age cohorts when considering only individuals from within cluster boundaries (Online Resource 3).

At the sub-population level, all pairwise F_{ST} values differed significantly from zero at the adjusted 5% level (which was 0.83%; Table 2). Overall, genetic differentiation across sub-populations was low (F_{ST} = 0.018 ± 0.004), with the biggest differences being between populations on different islands. The sub-populations FP and FF - although not the closest geographically (Fig. 1) - were the most similar. Genetic differentiation among sub-populations was greater for offspring than for adults (F_{ST} = 0.027 ± 0.006 vs. 0.015 ± 0.003).

Realised seed dispersal

Seed dispersal distances were estimated using the maternity analyses for offspring within clusters (Fig. 2). Across all clusters 56.5 ± 5.3% (mean ± SE; N = 267) of offspring could be assigned to a probable mother tree within the site. However, this calculation included two sites with anomalously low assignment rates (5.6% at FF1 and 20.0% at FF3). We later discovered that these sites had been burnt, which would account for these lower assignment rates, if many of the mother trees had been killed. The calculation also included plants from extreme locations downhill of the female trees, most of which must have originated from female trees outside our sample areas and were only sampled to be sure of finding all offspring. With these outlier individuals and anomalous sites excluded, the average assignment rate increased to 78.0 ± 3.1%, with one site (VdM2) having an assignment rate of 93.3% (Online Resource 4).

Overall, the mean dispersal distance across all sub-populations was 8.7 ± 0.7 m, with 50.6% of offspring growing within 5 m from the mother tree. The mean dispersal distance was greater in FF (17.0 ± 2.6 m) than in the other sub-populations (CU: 5.6 ± 1.2 m; FP: 7.5 ± 1.0 m; VdM: 7.8 ± 0.8 m; χ^2 = 32.241, df = 3, pairwise tests all P < 0.001). Mean dispersal was also greater on sloping sites, where clusters formed elongated ellipses downslope of the mother trees, than on level sites (11.3 ± 1.2 m vs. 6.5 ± 0.6 m; U = 7,499.0, df = 1, P < 0.05). The longest individual distance of 77.4 m was recorded on a steeply sloping site at FF1.

Evaluating patterns of FSGS

Sub-populations

FSGS was most intense at FP ($Sp = 0.017 \pm 0.002$), and least so at FF ($Sp = 0.010 \pm 0.002$; Fig. 3, Table 3). Both Loiselle's F in SPAGeDI and the spatial autocorrelation r in GenAlEx indicated similar relatedness levels between tree pairs in all sub-populations (Table 3). At the shortest distance class (≤ 10 m), F was highest in FP ($F_1 = 0.098 \pm 0.010$) and lowest in FF ($F_1 = 0.041 \pm 0.006$), a pattern supported by r (Table 3). Using the multi-class test criterion (Ω) to test for heterogeneity of the multilag correlograms, FSGS differed significantly between all pairs of sub-populations, with the strongest contrast being between FP and CU ($\Omega = 100.796$, $P < 0.001$). The largest single difference was between FP and FF at the 10 m class ($t^2 = 92.028$, $P < 0.001$; Table 4), a pattern consistent with the Sp-statistic.

Adult and offspring cohorts

FSGS was more strongly developed in offspring cohorts ($Sp = 0.018 \pm 0.002$) than in adults ($Sp = 0.009 \pm 0.001$) (Online Resources 1, 5). The degree of kinship differed significantly between adult and offspring cohorts for nine of 21 distance classes (Online Resource 6), with both F and r being higher for the ≤ 10 m distance class in the offspring than in the adults (offspring: $F_1 = 0.093 \pm 0.009$, $r_1 = 0.134$; adults: $F_1 = 0.066 \pm 0.006$, $r_1 = 0.098$). We also investigated the intensity of FSGS within adult and offspring cohorts separately in the various sub-populations. Overall, intensity was greatest for FP offspring ($Sp = 0.027 \pm 0.003$), and least for FP adults ($Sp = 0.006 \pm 0.001$) (Online Resource 7).

Male and female adults

Male and female *Lodoicea* within the clusters showed similar FSGS ($Sp = 0.012 \pm 0.003$ vs. $Sp = 0.012 \pm 0.002$) (Online Resources 2, 8), though the distance over which individuals were significantly related was greater for females ($Dist_F = 30$ m vs. 15 m, respectively).

The relatedness (F) between male and female adult pairs varied according to distance class ($\chi^2 = 375.90$, df $= 15$, $P < 0.0001$; Fig. 4). Mean kinship values between male/female pairs were highest at distances of less than 10 m ($F = 0.066 \pm 0.009$), and remained significantly higher than expected from spatially random pairs up to a distance of 500 m.

Discussion

With its huge seeds, lacking any mechanism for dispersal apart from gravity, *Lodoicea maldivica* provides an extraordinary example of island evolution. In a previous molecular genetic study using dominant AFLP markers, Fleischer-Dogley *et al.* (2011) found surprisingly little genetic differentiation across the natural range of *Lodoicea maldivica.* Our aim in this study was to investigate in more detail patterns of genetic differentiation, focusing especially upon fine-scale genetic pattern. We discuss the significance of our results for gene flow in natural populations of *Lodoicea,* and explore the potential conservation implications for this keystone palm species in the face of increased fragmentation.

Limited seed dispersal and intense fine-scale spatial genetic structure

Our sampling scheme was successful in locating the progeny of the sampled female trees, with an overall assignment rate of nearly 80% (excluding outlier plants and anomalous sites). Of the unassigned seedlings, 11% had genotypes that differed from a female tree in the study area by a single allele, and some of these were probably not assigned because the pair possessed several particularly common alleles. Many of the remainder may have been unassigned because mother trees had died; this was almost certainly the case for trees on two sites affected by fire, and was probably the case for all sites, given that the offspring develop very slowly and many were several decades old when they were sampled.

The spatial distribution of the offspring confirms what we had initially suspected, that realised seed dispersal distances are mainly very short. As a consequence, offspring of *Lodoicea* usually establish either in dense clusters around the mother tree or, if they are growing on a steep slope, in elongated ellipses downhill of the mother tree (e.g. at FF). This clustered pattern of regeneration probably results in small-scale founder effects with a few individuals contributing to the colonisation of a cluster, thus causing intense FSGS; if so, this effect would be less pronounced on steep slopes. Our results also show that FSGS is less intense among adult plants than among offspring. This could occur if selection favours progeny that are less inbred or less related to the nearest adult tree, so that FSGS declines as the plants mature, as has been documented for other palms (Choo *et al.* 2012). Alternatively, the observed decline might reflect a disruption in pollen-mediated gene flow due to habitat fragmentation, which would exacerbate FSGS in cohorts that established after fragmentation. Our data do not enable us to distinguish between these two possibilities. The *Sp*-statistic observed for adult trees in this study (0.009) is comparable to, although slightly lower than those calculated for other tropical arboreal palms - for example *Astrocaryum aculeatum* (*Sp* =

0.0139; Ramos *et al.* 2016) and *Acrocomia aculeata* (*Sp* = 0.014; Araújo *et al.* 2017) - in which seed dispersal is also limited.

Within all sub-populations, pairs of individuals within 10 m from each other had an average kinship coefficient value of F = 0.07. To put this into perspective, an F value between 0.125 and 0.031 suggests that the most recent common ancestor of these individuals is between one or two generations (equivalent to first or second cousins). We also found that relatedness between male and female adult trees increased with proximity, and that pairs separated by less than 10 m also had a mean F value of 0.07. The high relatedness over short geographical distances, coupled with the very high inbreeding coefficients at all life stages (see the 'Patterns of genetic diversity and inbreeding' section below), is consistent with the idea that mating between spatially proximate pairs is common in *Lodoicea,* with little evidence for selection for outbred pollen during fertilisation. Studying contemporary pollen dispersal in *Lodoicea* will be important in resolving this.

Patterns of genetic diversity and inbreeding

Evidence that fragmentation leads to declines in genetic diversity or restricted gene flow

We expected to find greater genetic diversity in adult trees at the bottom of slopes, and a decline in genetic diversity from adults to seedlings, as has been observed in other fragmented species (Vranckx *et al.* 2012). We also expected the more intact sub-populations on Praslin to be genetically more diverse than the sub-population on degraded sites on the island of Curieuse (Pither *et al.* 2003). However, our results demonstrated neither of these effects, though there was the non-significant trend for higher genetic diversity in adults than in offspring. The only significant difference was a greater mean rarefied number of private alleles on Curieuse than at other sub-populations, perhaps as a consequence of restricted gene flow between the two islands, and random genetic drift in the smaller Curieuse population (Slatkin 1985). The very long generation time of *Lodoicea* could mean that the genetic consequences of fragmentation and subsequent population reduction have yet to occur (Kramer *et al.* 2008), or they may only be detectable in pollen (Breed *et al.* 2012) or seeds (Rosas *et al.* 2011). A small proportion of adult trees in our study may have established after the forests were fragmented, although this probably had little effect on our results.

High levels of genetic diversity in endangered species may indicate either historically large populations or continuing gene flow among apparently separate populations (Shapcott *et al*. 2007). The levels of genetic diversity found in *Lodoicea* are similar to, or higher than, those observed in other rare or endangered tropical palm species (Shapcott *et al*. 2007; Shapcott *et al.* 2012; Nazareno & dos Reis 2014), lending support to the idea that populations of *Lodoicea* were formerly large and continuous (as indicated also by historical records; Fauvel 1915), and that their decline is rather recent.

Although outcrossing rates and levels of genetic diversity are typically high in tropical trees, their populations tend to be more differentiated than those of temperate species (reviewed in Dick *et al.* 2008). In *Lodoicea*, low but significant genetic differentiation was observed among all sub-population pairs, with the greatest differences being between Curieuse and Praslin. This suggests that the channel between the islands, albeit a relatively recent result of sea level rise around 8000 years ago, is a barrier to gene dispersal (see also Fleischer-Dogley *et al.* 2011). Another historical factor to consider is that the offspring established under conditions of greater fragmentation and disturbance than did the adults, which could explain the higher genetic differentiation in the offspring than the adults (Browne *et al.* 2015). Historically, the Fond Peper and Fond Ferdinand *Lodoicea* on Praslin formed one continuous tract of forest, and even today the two areas are still partially connected, which presumably accounts for the low genetic differentiation between these sub-populations.

Is inbreeding higher in the more isolated sub-populations and in the younger cohorts?

Inbreeding was unexpectedly high in all sub-populations and, contrary to our initial hypothesis, was not higher in the smaller, fragmented population on Curieuse, nor in the younger age cohorts (as has been found in other studies of long-lived plants; Kettle *et al.* 2007; Finger *et al.* 2012). Several processes, not mutually exclusive, can lead to a homozygote excess, including inbreeding, null alleles and the Wahlund effect (Wahlund 1928). We exclude null alleles because the pattern of homozygotes was consistent across loci (with only one locus, Lm6026, showing a frequency slightly over $P = 0.20$), which would be unlikely for null alleles (Dakin & Avise 2004). We can also exclude the Wahlund effect, since this would require significant barriers to gene flow that evidently do not exist on the two islands.

In general, theory predicts that a decrease in population size will result in an increase in inbreeding in predominately outcrossing plants (Oostermeijer *et al.* 2003), as has been observed in another long-lived dioecious plant in fragmented habitat (Dubreuil *et al.* 2010).

Dioecy is thought to have evolved as a mechanism to overcome inbreeding depression, though it has clearly been insufficient to prevent high inbreeding in *Lodoicea*. Indeed, the evolution of a very large seed has resulted in a breeding system characterised by high levels of inbreeding. This raises the question of how *Lodoicea* avoids the deleterious effects of inbreeding depression? One possibility is that deleterious recessive alleles have been purged from this long-lived species over generations for thousands of years. This is consistent with the ancient origins of the Seychelles archipelago (Baker & Miller 1963). Infrequent long-distance pollen or seed dispersal (e.g. downhill) may be sufficient to prevent inbreeding depression at the population level. However, without genotype data from pollen and seeds it is difficult to relate pollination success and survival rates of offspring to the genetic relatedness of mother and father pairs.

Ecological and conservation implications of limited seed dispersal in *Lodoicea*

Monodominant tropical forests such as those of *Lodoicea* appear to be restricted to regions that have been stable for very long periods (Hart *et al.* 1989). Indeed, it has been argued that a species such as *Lodoicea*, with its huge seed, could only have evolved under very stable conditions (Edwards *et al.* 2015). If pollen dispersal is frequently over short distances, the intense FSGS and relatively high relatedness among male and female individuals in close proximity would give rise to the high inbreeding coefficients we observe. The consistently high levels of inbreeding and similar levels of genetic diversity among life stages and study sites, regardless of different levels of habitat degradation, suggest that mating between related individuals has long been the strategy of reproduction in *Lodoicea*. One possibility is that the advantages of maternal resource provisioning for progeny via the interception and funnelling of nutritious material to the tree's base (Edwards *et al.* 2015) outweigh the potentially negative costs of inbreeding.

Conservation of rare and threatened species is often undermined by genetic processes, especially inbreeding and inbreeding depression (Frankham 2005). Our results suggest that inbreeding does not greatly reduce reproductive success of *Lodoicea*, though it possibly reduces the chances of some offspring surviving to adulthood. More important may be how contemporary patterns of gene flow vary among sites. The potential consequences of disrupting the natural patterns of genetic structure are also unknown.

Therefore, we conclude that the systematic collection, removal and translocation of seeds should be avoided. Future studies investigating the pollination, germination and establishment consequences of moving seeds to alternative sites will help to inform

management. Understanding how canopy openness and habitat quality influence local pollinator communities and their mobility will be critical to conserving patterns of genetic diversity *per se* rather than preventing inbreeding. These results highlight that for species with extremely limited seed dispersal and intense FSGS, fragmentation genetics needs to be placed in the wider context of the species ecology.

Acknowledgements

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Data accessibility

DNA sequences: GenBank accessions KT897315–KT897326. Microsatellite genotypes and sampling locations: will be uploaded on Dryad.

Figure Captions

Fig. 1 Locations of the sites of sampled *Lodoicea maldivica* on Praslin and Curieuse islands. Centres of the clusters are shown by circles, and labelled. The crossed area indicates Praslin National Park, and within it, Vallée de Mai and Fond Peper boundaries, and the lined area indicates Fond Ferdinand. Inserted map shows the Fond Ferdinand 2 cluster with boundary and some of the surrounding males (black squares = males; diamonds = females; grey stars = offspring)

Fig. 2 Frequency distribution of realised seed dispersal distances in *Lodoicea maldivica*. Flat = seven clusters from relatively flat ground (slope < 25%), sloped = nine clusters from relatively steep slopes (slope > 25%). Obtained using maternity analysis assignments at the 95% confidence level. Candidate mothers were restricted to the same cluster as the offspring

Fig. 3 Fine-scale spatial genetic structure for all individuals of *Lodoicea maldivica* from each of the four sub-populations. **a)** Vallée de Mai, **b)** Fond Peper, **c)** Fond Ferdinand and **d)** Curieuse. Solid lines represent mean F values (Loiselle *et al.* 1995). Dotted lines represent bootstrapped upper and lower 95% confidence intervals. Error bars denote 1SE

Fig. 4 Mean kinship coefficient ($F \pm 1$SE) among male and female *Lodoicea maldivica* pairs at various distance classes