



# Morphological and genetic differentiation in populations of the dispersal-limited coco de mer (*Lodoicea maldivica*): implications for management and conservation

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## ABSTRACT

**Aims** Developing plant conservation strategies requires knowledge of ecological and genetic processes underlying population dynamics. We aimed to quantify morphological and genetic differentiation among remnant populations of the iconic coco-de-mer palm *Lodoicea maldivica*. We hypothesized that limited gene flow among widely spaced populations would result in high genetic variation and large phenotypic differences among populations.

**Location** Islands of Praslin and Curieuse (CU), Seychelles, Indian Ocean.

**Methods** We conducted an extensive population survey and recorded morphological parameters for 447 *Lodoicea* in the main populations at Vallée de Mai (VM) and Fond Ferdinand (FF) on Praslin, and on CU. We collected leaf material from 180 trees in these populations for DNA genotyping using amplified fragment length polymorphisms.

**Results** A total of 16,766 *Lodoicea* trees were recorded in the three populations (72.6% of *Lodoicea* on both islands). *Lodoicea* trees at VM and FF showed similar morphology, but differed in most parameters from those at CU, which were shorter, grew more slowly and produced fewer seeds. Mean overall genetic diversity was 0.337, and percentage of polymorphic loci was 91.1. Genetic diversity of the CU population was lower than that at VM and FF. There was weak genetic differentiation between CU and Praslin populations, but 99% of all genetic diversity was within populations.

**Main conclusions** Trees on CU differed in growth and morphology from those of the two Praslin populations. These phenotypic differences, however, were not mirrored in the genetic structure of the populations. All populations were relatively genetically diverse with remarkably little differentiation among populations. This suggests that the capacity of *Lodoicea* to dominate across a range of habitats may be because of high phenotypic plasticity. High genetic connectivity may be maintained through long-distance wind pollination. Given the uncertainty about the extent of underlying adaptive variation, we recommend that restoration projects avoid transferring seeds between island populations.

## Keywords

AFLP, genetic diversity, Indian Ocean, limited seed dispersal, palm conservation, Seychelles Islands.

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## INTRODUCTION

The iconic coco de mer palm, *Lodoicea maldivica* Gmelin, produces the largest seed in the plant kingdom. It is well known as a national emblem of the Seychelles and a flagship species for conservation, as well as being an economically important tourist attraction and commodity. Prior to human colonization in the 18th century, *Lodoicea* was the dominant tree on two neighbouring islands in the Seychelles group, Praslin and Curieuse, occurring across a broad range of habitats from the coast to the uplands (Malavois 1787 quoted in Fauvel, 1909; Quéau de Quincy 1801 quoted in Fauvel, 1915; Bailey, 1942). Human activities over the subsequent 250 years, however, have drastically reduced the extent of *Lodoicea* forest on both islands, and most of the remaining trees are restricted to three disconnected, semi-natural populations (Fischer & Fleischer-Dogley, 2008).

The largest populations occur on Praslin, where males can grow as tall as 30 m and form a high, closed forest, together with four endemic palms (*Deckenia nobilis*, *Phoenixophorium borsigianum*, *Nephrosperma vanhoutteanum* and *Verschaffeltia splendida*) and endemic dicotyledonous trees such as *Parargenipa wrightii*, *Canthium bibracteatum*, *Syzygium wrightii* and *Erythroxylum sechellarum* (Fleischer-Dogley, 2006). In contrast, *Lodoicea* trees on Curieuse are mainly of rather low stature (< 10 m tall) and do not form closed forest. Palm forests on Praslin support a remarkable number of other endemic species, including the Seychelles black parrot (*Coracopsis nigra barklyi*), five geckos (*Phelsuma* and *Ailuronyx* sp.), two chameleons (*Calumma tigris*, *C. sechellensis*), the snail *Pachnodus praslinus*, several bryophyte species, lichens and ferns (Beaver & Chong-Seng, 1992). The fact that so many endemic species are restricted to forests with *Lodoicea* raises interesting questions about mechanisms of co-evolution and adds greatly to the conservation importance of these palm-dominated forests.

Much of the scientific interest of *Lodoicea* derives from the unique evolutionary history of the Seychelles archipelago. During the late Cretaceous period, from around 85–65 mya, the granitic islands formed through the break-up of Gondwana, thus making them among the oldest islands in the world (Fischer & Fleischer-Dogley, 2008). The size of the *Lodoicea* seed (on average about 10 kg; Edwards *et al.*, 2003) is a feature of particular ecological and evolutionary interest, providing an extreme example of island gigantism (Whittaker, 1998). Edwards *et al.* (2003) argued that the ancestral palm in its island habitat must have lacked any vector to disperse its fruits, and the resulting sibling competition favoured female trees that produced few but very large seeds. Another unusual feature of the *Lodoicea* life cycle is the small number of ovules produced, which may also be linked to the evolution of enormous seeds.

From a population genetics perspective *Lodoicea* is also of considerable interest. First, several aspects of its life history – very long generation time, low fecundity, negligible seed dispersal and dioecy – should limit the potential for mutations and rapid selection, prerequisites for a species to occur across a

broad range of habitats. Indeed, these characteristics are unusual among tree species, most of which show extensive gene flow and high fecundity (Petit & Hampe, 2006). Second, many of the older trees established before humans settled on the Seychelles, providing a rare opportunity to investigate the phenotypic and genetic composition of an endemic plant species that pre-dates human-induced habitat degradation. Third, small isolated populations are especially vulnerable to a combination of ecological, genetic and demographic processes and understanding how genetic variation is partitioned among extant populations is crucial for scientifically informed conservation management (Lande, 1998; Oostermeijer *et al.*, 2003). This is especially important in rare species, or species with very limited dispersal, due the potentially deleterious effects of genetic erosion and elevated inbreeding (Amos & Balmford, 2001; Frankham, 2002).

The aim of this study was to quantify morphological variation in *Lodoicea* in its three remaining large populations and investigate whether phenotypic differences between populations are reflected in their genetic structure. We hypothesized that gene flow between the remaining and widely spaced populations is limited, resulting in a high genetic structure among the populations. We discuss the ecological significance of the results obtained in this study and provide management recommendations. *Lodoicea*'s unique set of ecological characteristics could compound its vulnerability to habitat degradation, partly through invasive plant species and overexploitation of nuts (Rist *et al.*, 2010).

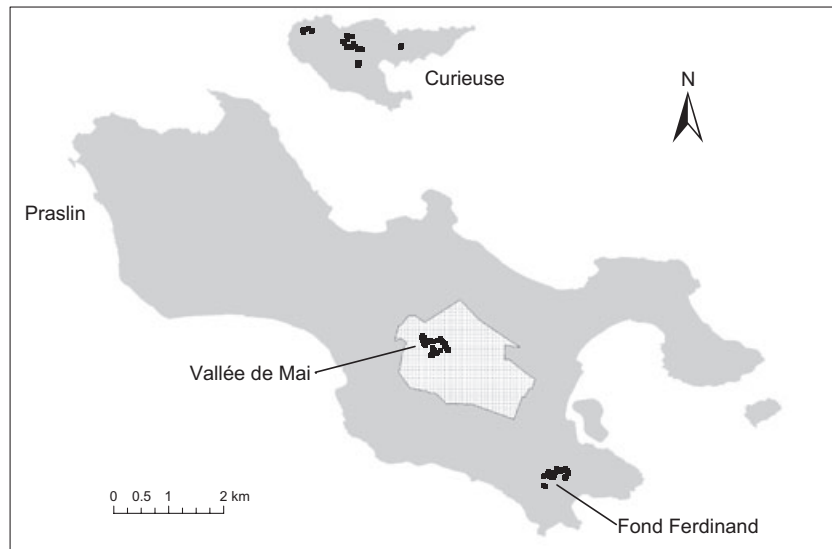
## METHODS

### Population survey and morphology

*Lodoicea maldivica* (Arecaceae) belongs to a monotypic genus within the tribe Borasseae (Uhl & Dransfield, 1987). A review of the life history characteristics of *Lodoicea* is given by Edwards *et al.* (2003). The species is endemic to the Seychelles in the Western Indian Ocean, where it is native on two islands, Praslin (37 km<sup>2</sup>) and Curieuse (3.6 km<sup>2</sup>), although fossil remains have been found on a few neighbouring islets. Praslin and Curieuse are separated by a 1.2- to 1.8-km-wide channel, but previously formed a larger land mass during the glacial periods. Nearly three-quarters of the extant *Lodoicea* trees occur in three natural populations – at Vallée de Mai (VM; 55°44' E, 4°19' S) and Fond Ferdinand (FF; 55°45' E, 4°21' S) on Praslin and on Curieuse (CU; 55°41' E, 4°16' S), which forms part of a Marine Protected Area (Fig. 1). The populations on Praslin are approximately 3.2 km apart, and the distances between CU and VM, and CU and FF are 5.6 km and 8.7 km, respectively.

Information about the demographic structure of the populations at VM, FF and CU was obtained from three sources: a comprehensive survey of all trees growing in the Seychelles and from permanent plots and trail transects. The comprehensive survey was conducted between 2001 and 2004 by staff of the management authorities, who collected information on the

**Figure 1** Location of *Lodoicea maldivica* on the islands Praslin and Curieuse (CU), Seychelles. Two populations Vallée de Mai (VM) and Fond Ferdinand (FF) are located on Praslin, and the third population occurs on CU. Sampled *Lodoicea* plants are shown by black squares. The hatched area indicates the Praslin National Park.



status (female, male or juvenile) and the state of flowering or fruiting of all trees (summarized in Fleischer-Dogley, 2006).

The first permanent plots were established in 1985 by a research group from Oxford University, who set-up six 20 × 20 m plots at Vallée de Mai, representing a total area of 0.24 ha or just over 1% of the World Heritage Site (Fleischmann *et al.*, 2005). The plots, which were selected to be typical of the surrounding vegetation, were surveyed in 1985 and resurveyed in 1998 and 2009. In 2004, we established additional plots of the same size but subdivided into 10 × 10 m subplots at FF (three plots at 80 m, 100 m and 170 m a.s.l.) and CU (two plots at 30 m and 50 m a.s.l.). In each plot, all *Lodoicea* plants were marked and included in the survey.

Data from trail transects supplemented the data from the permanent plots and were intended to check whether these were representative of the forest as a whole. Transects ran along established paths and through overgrown areas. At random intervals along the trail, any *Lodoicea* plants present within a radius of 5 m were recorded. We surveyed forest along a total trail length of 11.03 km (VM: 3.48 km, FF: 1.86 km, CU: 5.69 km) in September 2005, increasing the total sample to > 2% of *Lodoicea* individuals (of the largest population) in each population. The length of the trail transects differed between the populations because of varying tree densities. The morphological data from the permanent plots and the trail transects were pooled, and the following parameters were recorded: total height, trunk height and mean length of leaves, diameter at breast height (DBH), number of green and dead leaves, number of inflorescences or catkins (if present), length of male catkins (if present), number of active catkins (if present), and number of nuts (if present). In addition, we counted the distance between successive leaf scars per metre of trunk as a measure of trunk growth per produced leaf. For juvenile plants, the number of leaves (alive and dead) was counted, and total height and mean leaf length (m) were estimated by one surveyor following regular standardization exercises with a measuring pole.

### Population genetics

To quantify genetic diversity within and among the three extant *Lodoicea* populations, in December 2008, we sampled leaf material from 40 adults (20 males and 20 females) and 20 juveniles in each population (i.e. 180 trees in total, representing 1–2% of the total population; see Fig. 1). The trees were selected to cover the spatial distribution of each population (VM and FF: 600 m; CU: 900 m radius), and the location of all trees was recorded using a handheld GPS unit (Garmin 60CSx Map). Leaf samples were collected and immediately stored in silica gel for subsequent DNA extraction and genotyping using amplified fragment length polymorphisms amplified fragment length polymorphism (AFLPs) (for detailed methods see Appendix S1 in Supporting Information).

### Statistical analysis

Because of non-homogeneity of variances, we used nonparametric Kruskal–Wallis tests to compare morphological differences between sites. Multiple pairwise comparisons were conducted with Nemenyi post hoc tests, which are comparable to using the Tukey test for parametric analyses (Zar, 1996).

Genetic diversity within the three *Lodoicea* populations was estimated using allele frequencies of scored AFLP peaks (loci). Nei's genetic diversity ( $H_j$ ) and percentage of polymorphic loci (PPL) were calculated for species-level genetic diversity (adults and juveniles) over all sites, adult populations and juvenile populations using the programs AFLP-SURV (Vekemans, 2002).

Genetic differentiation among populations was investigated in detail using the following approaches. We calculated the overall genetic differentiation ( $F_{ST}$ ) among populations using a Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999) in the software AFLP-SURV (Vekemans, 2002). Separate analyses were also performed using: (1) all individuals in each population, (2) all adults and

(3) all juveniles. The population pairwise  $F_{ST}$  analogue  $\Phi_{PT}$  was computed with the software GenALex 6.2 (Peakall & Smouse, 2006), and significance was tested using 999 permutations. To explore the hierarchical partitioning of genetic variation between the two islands, we performed an Analysis of Molecular Variance (AMOVA, GenALex 6.2) using only adult populations. Here, we treated the islands as the regional scale, and CU, FF and VM as populations, and used 999 permutations to derive the estimator  $\Phi_{PT}$ .

## RESULTS

### Population survey

In the comprehensive survey from 2001 to 2004, a total of 7065 *Lodoicea* plants were recorded in the Vallée de Mai, 5908 at Fond Ferdinand and 3793 on Curieuse (Table 1). Together, these three populations represented 72.6% of *Lodoicea* trees on Praslin and Curieuse, and 68.3% of those in the Seychelles (Fleischer-Dogley, 2006). While most trees on Praslin and Curieuse probably established naturally, all those on other islands have been planted. Over three-quarters of the trees in the two Praslin populations were juvenile, while the proportion of juvenile trees on CU was much lower (53%). Mature female trees (i.e. those producing nuts) made up 8.8% and 11.4% of the population at VM and FF, respectively, but 25% on CU.

### Morphological differences between populations

There was little evidence for any differences in growth and morphology between adult trees at VM and FF. Juvenile *Lodoicea* were significantly taller at VM than at FF, but average height of male trees at both sites was similar and significantly greater than that of females (Table 2). Furthermore, while the tallest male tree was about 30 m in height, the tallest female trees only reached about 20 m. DBHs of male and female trees were similar at both sites, contradicting some suggestions that the trunks of female trees are broader to support the weight of several mature nuts. The mean number of nuts on female trees was not significantly different between VM and FF (Table 2).

**Table 1** Number and gender of *Lodoicea maldivica* trees in the three main populations. Number in parentheses show proportions of females, males and juvenile to the total number of trees at each site (female, male, juvenile) and the proportion of trees at each site to the total number of known trees (total).

Location	Numbers of trees (% total number of trees)			
	Female	Male	Juvenile	Total
Curieuse	948 (25)	802 (21.1)	2043 (53.6)	3793 (15.4)
Fond Ferdinand	675 (11.4)	705 (11.9)	4528 (76.6)	5908 (24.1)
Vallée de Mai	623 (8.8)	818 (11.5)	5624 (79.6)	7065 (28.8)
Total	2246 (13.4)	2325 (13.9)	12195 (72.7)	16766 (68.3)

Trees on Curieuse were considerably smaller than those in the two Praslin populations, and most parameters except DBH varied significantly between CU and both VM and FF (Table 2). Male and female trees on Curieuse were similar in height (Table 2), and the tallest tree recorded was a female plant of 12.0 m. The mean height increment per leaf, measured as the distance between successive leaf scars, was also much smaller on Curieuse than on Praslin. For a sample of 50 VM trees, there was an average of 8.6 ( $\pm 1.84$  SD) leaf scars per metre of trunk, which corresponds to a height increment of 12.2 cm per leaf. In contrast, for 31 trees on Curieuse, there were 50.6 ( $\pm 8.9$  SD) leaf scars per metre of trunk, equivalent to 1.98 cm per leaf. On average, female trees on CU had fewer than one-third as many nuts as at VM and FF (Table 2). Assuming that a nut takes 6 years to mature (Wise, 1998; Fleischer-Dogley, 2006), the annual number of seeds produced per female tree was only 0.32 on CU, compared with 1.07 and 1.48 at VM and FF, respectively.

### Genetic diversity in *Lodoicea*

Scorable AFLP profiles were obtained from 163 of the 180 individuals investigated. The four primer pairs yielded a total of 336 bands, of which 101 were retained following genotyping error rate estimation of reproducible polymorphic loci (Whitlock *et al.*, 2008). Of these polymorphic loci, a 'private' allele was only recorded in one locus at the FF population.

Estimates of genetic diversity across populations and life stages (adults and juveniles) are summarized in Table 3. The percentage of polymorphic loci (PPL) per population was 76.2% at CU, 98% at VM and 99% at FF. A more detailed analysis showed that low genetic diversity at CU was because of the adults, which had a PPL of 58.4%; in contrast, PPL of juvenile trees was 97%. Mean Nei's genetic diversity ( $H_j$ ) over all individuals across populations (species level) was 0.337 (SE = 0.010).

Overall, the genetic diversity of the CU population was significantly lower than that of FF and VM populations (Fig. 2), but the difference was much less marked for juveniles ( $P = 0.05$ ). Furthermore, genetic diversity between cohorts (juveniles versus adults) within populations was significantly greater in juveniles at CU than adults ( $P = 0.05$ ), while there was no such difference between cohorts for FF or VM (Table 3).

### Genetic differentiation between *Lodoicea* populations

The overall  $F_{ST}$  value was 0.019 ( $P < 0.05$ ), indicating that genetic differentiation among populations was significant but very weak. A slightly higher value was obtained when only adult individuals were considered ( $F_{ST} = 0.027$ ,  $P < 0.01$ ; Table 4), while for juveniles, there was no significant differentiation ( $F_{ST} = 0.007$ ,  $P > 0.05$ ). The results of the AMOVA indicated that 99% of the genetic diversity within *Lodoicea* was within populations (Table S1). Pairwise  $\Phi_{ST}$ , a measure of genetic differentiation between pairs of *Lodoicea* populations, showed significant differentiation between adults at CU and

**Table 2** Means  $\pm$  SD of tree growth parameters of *Lodoicea maldivica* sampled along trail transects on Praslin [Vallée de Mai (VM) and Fond Ferdinand (FF)] and on Curieuse (CU). Significance of variation among sites ( $P$ -value of Kruskal–Wallis test) is shown. Also multiple pairwise comparisons indicate difference between population pairs (Nemenyi test;  $*P < 0.05$ ).

Parameters	VM	FF	CU	Kruskal–Wallis ( $P$ )	VM-FF	VM-CU	FF-CU
Female trees ( $N$ )	21	21	21				
Total height (m)	14.4 $\pm$ 3.5	14.6 $\pm$ 6.6	6.8 $\pm$ 2.2	0.001	ns	*	*
Trunk height (m)	7.8 $\pm$ 4.1	9.3 $\pm$ 5.7	3.9 $\pm$ 2.2	0.001	ns	*	*
Diameter at breast height (DBH) (cm)	28.4 $\pm$ 3.7	28.7 $\pm$ 3.9	26.9 $\pm$ 7.1	ns	ns	ns	ns
Mean leaf length (m)	6.6 $\pm$ 1.4	5.4 $\pm$ 1.1	3.8 $\pm$ 0.07	0.001	ns	*	*
Green leaves per tree	11.8 $\pm$ 2.7	12.6 $\pm$ 2.4	12.3 $\pm$ 2.6	ns	ns	ns	ns
Dead leaves per tree	2.1 $\pm$ 1.1	1.8 $\pm$ 1.3	5.2 $\pm$ 2.3	0.001	ns	*	*
Nuts per tree	6.4 $\pm$ 3.6	8.9 $\pm$ 5.4	1.9 $\pm$ 2.5	0.001	ns	*	*
Inflorescences per tree	0.9 $\pm$ 1.2	0.62 $\pm$ 1.1	5.5 $\pm$ 3.4	0.001	ns	*	*
Male trees ( $N$ )	30	30	30				
Total height (m)	18.7 $\pm$ 6.4	16.9 $\pm$ 6.3	6.5 $\pm$ 2.8	0.001	ns	*	*
Trunk height (m)	14.1 $\pm$ 11.3	10.9 $\pm$ 5.8	3.4 $\pm$ 2.6	0.001	ns	*	*
DBH (cm)	28.3 $\pm$ 4.2	28.3 $\pm$ 3.5	24.3 $\pm$ 8.9	ns	ns	ns	ns
Mean leaf length (m)	6.2 $\pm$ 1.2	6.1 $\pm$ 1.7	3.4 $\pm$ 0.79	0.001	ns	*	*
Green leaves per tree	12.0 $\pm$ 1.8	13.3 $\pm$ 2.0	11.4 $\pm$ 1.8	0.01	ns	ns	*
Dead leaves per tree	1.5 $\pm$ 0.94	1.8 $\pm$ 1.4	4.1 $\pm$ 1.8	0.001	ns	*	*
Active inflorescence per tree	0.83 $\pm$ 0.53	0.63 $\pm$ 0.67	1.1 $\pm$ 0.66	0.05	ns	ns	*
Length inflorescence (cm)	81.7 $\pm$ 23.7	94.6 $\pm$ 22.9	64.1 $\pm$ 17.0	0.001	ns	*	*
Juvenile plants ( $N$ )	112	98	84				
Total height (m)	11.5 $\pm$ 7.3	8.9 $\pm$ 4.4	3.2 $\pm$ 1.2	0.001	*	*	*
Mean leaf length (m)	9.8 $\pm$ 2.7	8.3 $\pm$ 3.9	3.2 $\pm$ 0.95	0.001	ns	*	*
Green leaves per tree	7.7 $\pm$ 3.1	7.4 $\pm$ 3.4	6.5 $\pm$ 2.7	ns	ns	ns	ns
Dead leaves per tree	1.6 $\pm$ 1.6	1.4 $\pm$ 1.3	3.2 $\pm$ 2.1	0.001	ns	*	*

FF, and CU and VM, but no differentiation between FF and VM (Table 4).

## DISCUSSION

### Morphological differences between populations

Our data confirm that *Lodoicea* trees on Curieuse differ considerably in their growth and morphology from those of on Praslin. Not only do trees on Curieuse reach a smaller maximum size, but they grow much more slowly and produce fewer seeds. Trees at FF and VM, in contrast, are very similar in size and productivity, justifying a simple comparison between these combined populations with the population on Curieuse. Unexpectedly, the phenotypic differences are not mirrored in the genetic structure of the populations. All three populations are relatively diverse genetically – although less so on Curieuse – but there is almost no between-population differentiation. This mismatch suggests that the capacity of *Lodoicea* to occur in high numbers across a range of habitats may be because of high phenotypic plasticity. The most favourable habitats are mid-altitude forests and ravines on Praslin, where trees well over 30 m tall were recorded in the past (Ward, 1866). In contrast, in places where the soil is thin and water is a more limiting resource, *Lodoicea* is much smaller, although it is still an abundant species. Such conditions prevail not only on

Curieuse but also in parts of Praslin, and similarly stunted trees occur in these areas.

### Genetic diversity in *Lodoicea*

We found that 30% of the 336 AFLP bands obtained from four primer pairs were polymorphic. This relatively high level of polymorphism is comparable to that recorded in *Cocos nucifera* sampled over its entire geographic range (Teulat *et al.*, 2000) and demonstrates that *Lodoicea* is far from being genetically depauperate. Indeed, the level of gene diversity (0.337) and PPL (91.1) recorded are substantially higher than equivalent values for other rare and threatened palms (Vanuatu: Dowe *et al.*, 1997; Madagascar: Shapcott *et al.*, 2007; NE Africa: Shapcott *et al.*, 2009). These results are consistent with the documented evidence that the populations of *Lodoicea* were formerly much larger, occupying most of the land area of Curieuse and Praslin (Fauvel, 1915).

Within populations, adult *Lodoicea* on Curieuse have significantly lower Nei's genetic diversity (0.263;  $P < 0.01$ ) and substantially fewer polymorphic loci (56%) than the populations sampled from Praslin. These levels of diversity are comparable to those reported for the Madagascar endemic palm *Beccariophoenix madagascariensis* (Shapcott *et al.*, 2007), but substantially greater than for other endemic palms with similarly narrow ranges, albeit with smaller

**Table 3** Summary of genetic diversity estimates based upon amplified fragment length polymorphism (AFLP) analysis in *Lodoicea maldivica* sampled from the species natural range in the Seychelles. Number of scored AFLP loci;  $H_j$  Nei's gene diversity and SE, standard error; No. P, number of polymorphic loci; PPL, percentage of polymorphic loci (with 95% criteria). Bold figures indicate significantly different gene diversity between populations within life stages.

Population	Sample size ( <i>n</i> )	No. loci	$H_j$	SE	No. P	PPL (95%)
CU	58	101	<b>0.281</b>	0.018	59	76.20
FF	47	101	0.361	0.016	100	99.00
VM	58	101	0.369	0.015	98	98.00
Mean (over all)			0.337	0.010		91.07
Adults						
CU	38	101	<b>0.263</b>	0.017	59	58.4
FF*	32	101	0.386	0.013	100	99
VM	38	101	0.369	0.014	98	97
Mean (adults)	36		0.305	0.011		76.6
Juveniles						
CU	20	101	<b>0.341</b>	0.015	98	97
FF	15	101	0.391	0.012	101	100
VM	20	101	0.417	0.011	101	100
Mean (juveniles)	18		0.383	0.013		99

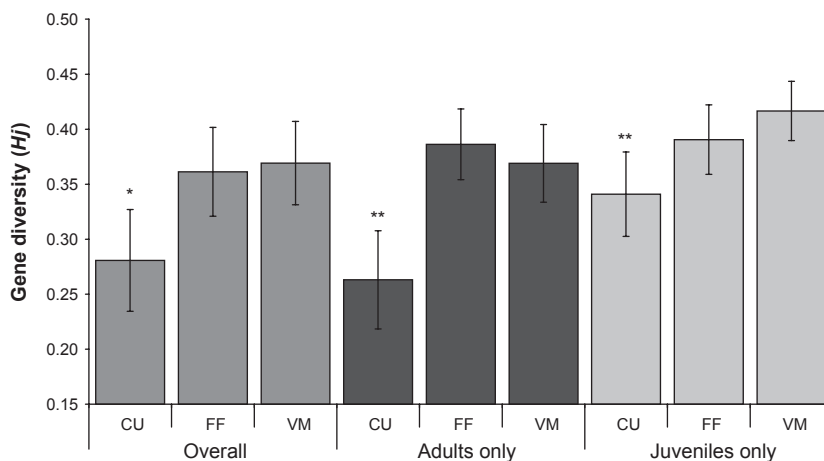
\*Private alleles = 1.

populations (Dowe *et al.*, 1997; Shapcott, 1999; Shapcott *et al.*, 2009). The low differentiation between juvenile cohorts in *Lodoicea* suggests that contemporary gene flow is sufficient to prevent erosion of genetic diversity within populations by random genetic drift or inbreeding, processes known to threaten the conservation status of plant species surviving in small fragmented populations (Ouborg *et al.*, 2006). The comparatively low genetic diversity on CU may reflect a founder effect, as the population on Curieuse became isolated from that on Praslin by rising sea levels after the last ice age, some 8000 years ago.

### Genetic differentiation between *Lodoicea* populations

The destruction of the formerly continuous *Lodoicea* forest on Praslin by the first settlers may have created new barriers to its already limited dispersal potential. Nevertheless, we found no evidence of genetic differentiation between the VM and FF populations, and very little differentiation between the islands of Curieuse and Praslin. Our findings are comparable with those for some other palm species. For example, Shapcott *et al.* (2009) found no genetic differentiation among populations of *Livistona carinensis* across several river catchments. Similarly, low levels of differentiation were observed over comparable distances in the endangered palm *Beccariophoenix madagascariensis* (Shapcott *et al.*, 2007). Seeds of most palm species, however, are far smaller than those of *Lodoicea*, and many are known to be dispersed by animals (Zona & Henderson, 1989; Zona, 2006).

Our results indicate that historic gene flow was sufficient to prevent genetic differentiation in *Lodoicea*, even across the extremes of its natural range. It is unlikely that this genetic connectivity could be achieved through seed dispersal; assuming a dispersal rate of 10 m per generation (say 100 years in females), it would take *c.* 800 generations or 80,000 years for a gene to disperse via seeds across the 8 km from Fond Ferdinand to Curieuse. A more likely explanation is that genetic connectivity is maintained through pollen-mediated gene flow. Although little is known about the pollination of *Lodoicea*, both male and female flowers produce a strong sweet smell and female flowers produce nectar (Kaiser-Bunbury *et al.* unpublished data), suggesting that biotic pollination is important. Indeed, geckos and small flies are commonly observed feeding on pollen on the male catkins (Noble *et al.*, in press), and geckos also visit female flowers. Wind transport may also play a role in pollination, especially over longer distances. Anecdotal evidence for this is provided by relatively isolated female trees – for example in the botanic gardens on Mahé – which sometimes produce fruits. Whatever the mechanism for gene dispersal, it seems that the 1- to 2-km-wide channel separating Praslin from Curieuse is a barrier to



**Figure 2** Histogram of within populations genetic diversity Nei's gene diversity ( $H_j$ ) of *Lodoicea* at the species (overall), adults and juvenile life stages, across two islands Curieuse (CU) and Praslin and three populations (CU, FF and VM). Error bars are 99% confidence intervals. \*Different between populations at  $P = 0.05$  or \*\* $P = 0.01$ .

**Table 4** Summary table of genetic differentiation in *Lodoicea maldivica* across the species natural range on the Seychelles based upon 101 amplified fragment length polymorphism loci overall  $F_{ST}$  across three populations (CU, Curieuse; FF, Fond Ferdinand; VM, Vallée de Mai), among adults only and among juveniles only. Pairwise  $\Phi_{ST}$  among populations was calculated within adults only and among juveniles only. Upper diagonal are  $P$ -values based upon 999 permutation test. Bold figures indicate significant differentiation, \*Significantly different from zero at  $*P < 0.05$ .

$F_{ST}$	Pairwise $\Phi_{ST}$		
	CU	FF	VM
Overall			
0.0193*	CU	0.001	0.001
	FF	<b>0.013</b>	0.398
	VM	<b>0.015</b>	0.000
Adults			
0.0265*	CU	0.002	0.001
	FF	<b>0.016</b>	0.429
	VM	<b>0.015</b>	0.000
Juveniles			
0.0067	CU	0.165	0.102
	FF	0.012	0.365
	VM	0.018	0.000

gene flow, with the consequence that genetic diversity on Curieuse is lower.

The adult trees in the three populations probably established through natural regeneration, and many of the tallest trees certainly date back to before the islands were colonized (Fischer & Fleischer-Dogley, 2008). The current juvenile cohorts, in contrast, established at times of great anthropogenic disturbance, and their population structures are probably more affected by human intervention than natural processes. For example, the size structure of juveniles in VM coincides with a period of planting between 1951 and 1954 (3283 nuts; Fleischer-Dogley, 2006), after which there was reduced regeneration because nuts were collected and sold (Rist *et al.*, 2010). Similarly, the introduction of *Lodoicea* seed from Praslin to Curieuse may explain why juvenile plants at CU exhibited more genetic variation than adults. Anthropogenic gene dispersal – through the movement of seeds between populations – could be sufficient to obscure any underlying genetic differentiation in the juvenile cohort. Indeed, human trade and cultivation have resulted in both genetic erosion and reduced differentiation in other palm species (Dowe *et al.*, 1997; Shapcott *et al.*, 2009). In the peach palm *Bactris gasipae* (Adin *et al.*, 2004), for example, which is dispersed over relatively short distances by birds (100–200 m), humans appear to have been responsible for extensive long-distance gene flow (> 20 km).

### Implications for management

Our study reveals that there are still large populations of *Lodoicea* on the two islands where the species is native and that the species is not under immediate threat, although the

effective population sizes are certainly much smaller than the census population size. This conclusion is consistent with IUCN's designation of the species as 'Vulnerable'. It is, however, a matter of concern that almost all nuts of *Lodoicea* are harvested for commercial use (Rist *et al.*, 2010). Management of *Lodoicea* in the Seychelles should make a clear distinction between the objectives of (1) managing populations to ensure the sustainable harvest of nuts and (2) management within protected areas aimed at maintaining the most natural conditions possible and therefore minimizing human influences upon the species composition of this ecosystem and upon demographic processes of constituent species.

We conclude, tentatively, that the smaller stature and slower growth of *Lodoicea* on Curieuse reflects less favourable environmental conditions and that there is little genetic differentiation between the populations on the two islands indicated by neutral marker variation. The extent to which these populations differ in adaptive traits, however, can only be determined using common garden and reciprocal transplant experiments, which because of the slow growth of *Lodoicea* would take many decades to yield results. Detailed studies of contemporary gene flow by pollen using paternity analysis would enable us to better understand the potential for gene exchange among islands. Given the present uncertainty, we recommend that restoration projects and *ex situ* collections avoid mixing seed across the extant populations.

The population on Curieuse represents an important part of the ecological range of *Lodoicea*. Understandably, most research has concentrated upon the more spectacular and diverse VM population, but this has led to a biased picture of the ecological role of this remarkable plant in the Seychelles. It is important to ensure that the population on Curieuse is conserved and that research on this island is encouraged.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Methods on DNA extraction.



**Table S1** Summary table of analysis of molecular variance (AMOVA) for *Lodoicea maldivica* populations CU, FF and VM.

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