

# Forest fragmentation genetics in a formerly widespread island endemic tree: *Vateriopsis seychellarum* (Dipterocarpaceae)

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## Abstract

Habitat fragmentation and changed land use have seriously reduced population size in many tropical forest tree species. Formerly widespread species with limited gene flow may be particularly vulnerable to the negative genetic effects of forest fragmentation and small population size. *Vateriopsis seychellarum* (Dipterocarpaceae) is a formerly widespread canopy tree of the Seychelles, but is now reduced to 132 adult individuals distributed in eleven sites. Using ten microsatellite loci, a genetic inventory of all adult trees and a sample of 317 progeny, we demonstrate that despite its restricted range, overall genetic diversity was relatively high ( $H_E$ : 0.56). The juvenile cohort, however, had significantly lower allelic richness (adults  $R_S$ : 3.91; juveniles  $R_S$ : 2.83) and observed heterozygosity than adult trees (adults  $H_O$ : 0.62; juveniles  $H_O$ : 0.48). Rare alleles were fewer and kinship between individuals was stronger in juveniles. Significant fine-scale spatial genetic structure was observed in remnant adults, and parentage analysis indicated that more than 90% of sampled progeny disperse <25 m and pollen dispersed <50 m. The molecular data confirmed that two populations were derived entirely from self-fertilized offspring from a single surviving mother tree. These populations produce viable offspring. Despite this extreme genetic bottleneck, self-compatibility may provide *V. seychellarum* with some resistance to the genetic consequences of habitat fragmentation, at least in the short term. We discuss our findings in the context of other rare and threatened dipterocarp species which are vulnerable to miss-management of genetic resources and population fragmentation.

**Keywords:** conservation genetics, Dipterocarpaceae, Islands, microsatellites, Seychelles, *Vateriopsis seychellarum*

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## Introduction

Fragmentation of formerly continuous forest landscapes reduces population size and increases isolation, potentially altering demographic structure, mating systems, genetic diversity and long-term viability of forest tree species (Bacles *et al.* 2005; Ghazoul 2005; Lowe *et al.*

2005). Following fragmentation, trees may become increasingly reproductively isolated, with more frequent inbreeding and loss of rare alleles, negatively affecting seed production and progeny viability (Ellstrand & Elam 1993; Stacy *et al.* 1996; Cascante *et al.* 2002). Such changes in the breeding system reduce effective population size (Loveless & Hamrick 1984; Rossetto *et al.* 2004). Extreme genetic bottlenecks (survival of only few individuals) will result in considerable loss of genetic diversity, requiring several generations for diversity to

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recover to a new equilibrium (Nei *et al.* 1975; Luikart *et al.* 1998).

Long-distance gene flow can be important in maintaining genetic diversity in fragmented populations by preventing inbreeding and limiting genetic drift (Hartl & Clark 1997). These effects might ensure the viability of populations in the long term (Lande 1988, 1995; Petit & Hampe 2006; Kramer *et al.* 2008). Additionally, breeding system characteristics such as self-compatibility may contribute to the persistence of populations in isolation (Levin 2010). The absence of long-distance gene flow has already been demonstrated to have potentially negative effects on many tropical forest tree species (Finger *et al.* 2011; Sebbenn *et al.* 2011 and refs there in).

Tropical forests have suffered from habitat fragmentation over the last century, mainly owing to forest clearance for agriculture and extensive logging. The Dipterocarpaceae, which are extraordinary in their dominance of lowland forest canopies in Southeast Asia, have been extensively logged and cleared (Sodhi *et al.* 2010). Despite the economic and ecological importance of this timber family, few studies have examined the population genetic and reproductive implications of fragmentation of dipterocarp species (but see Lee *et al.* 2006; Fukue *et al.* 2007). One difficulty has been the challenge of comprehensively mapping the distributions of dipterocarp populations over the large areas necessary to study gene exchange. In this context, *Vateriopsis seychellarum*, the sole representative of the Dipterocarpaceae in the Seychelles, provides an ideal opportunity to study the impacts of fragmentation on a dipterocarp species that has been reduced to eleven discrete populations from a formerly far more widespread distribution.

The rain forest of the Seychelles was extensively exploited for timber and replaced by cinnamon and fruit tree plantations in the 19th century until just a few forest fragments remained at higher altitudes in 1870 (Baker 1877; Diels 1922). As a result, many formerly widespread and abundant species, such as *Campanosperma seychellarum*, *Northea hornei* and *V. seychellarum*, now survive only in small populations scattered across the island (Carlström 1996; Fleischmann 1997). *Vateriopsis seychellarum* was believed to be widespread over the forested lowlands of Mahé (the main island of the Seychelles) up to about 400 m a.s.l. (Diels 1922). As a valuable timber tree it was heavily exploited, and only 132 known adults now survive at 11 sites, two of which are occupied by just single mature individuals. *Vateriopsis seychellarum* is listed as critically endangered by the IUCN Red List (IUCN 2011).

The aim of this study was to determine the extent to which deleterious genetic consequences of small population size, following habitat fragmentation, threaten

*V. seychellarum*. Its insect-pollinated flowers and gravity-dispersed fruit are similar to many dipterocarps. The study of this species may therefore serve as analogue for a wide range of dipterocarp species elsewhere in South and Southeast Asia. Given the shortage of documented evidence for past population history in the remaining forest patches occupied by *V. seychellarum*, we use molecular techniques to evaluate contemporary gene flow, to determine the degree of isolation of current remnant populations and to make inferences on past population history.

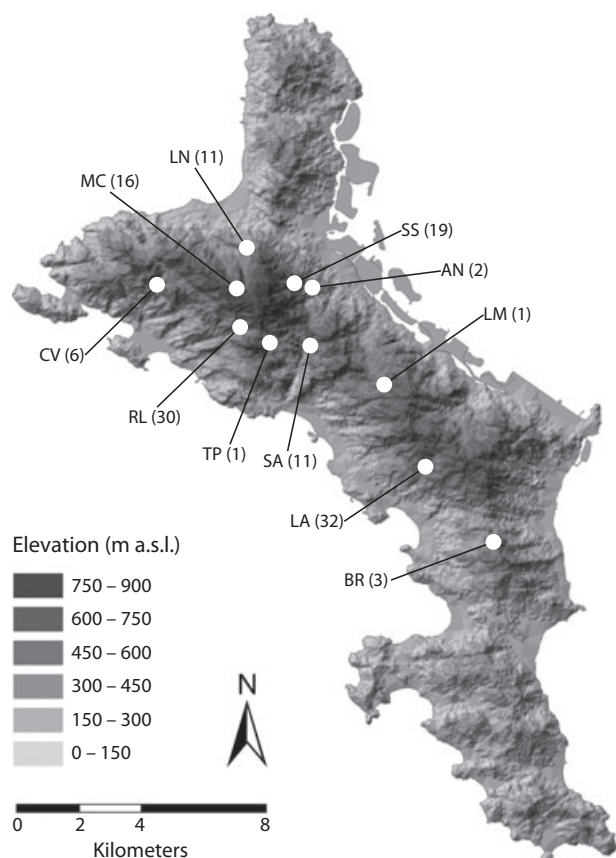
We use a genetic inventory of all known adult trees across all populations and a subset of juveniles to test the hypothesis that this formerly widespread species has experienced extreme genetic bottlenecks and that limited gene flow is insufficient to connect the remnant populations. We ask the questions: (i) is there evidence of extreme population bottlenecks in *V. seychellarum*, indicated by a loss of genetic diversity; (ii) are remnant populations genetically structured; and (iii) is realized contemporary gene flow by both pollen and seed restricted. We address these questions by using ten microsatellite loci to evaluate genetic diversity, inbreeding coefficients and fine-scale spatial genetic structure (FSGS) in adult trees and a parentage assignment of wild juveniles.

Understanding the genetic and ecological consequences of habitat degradation and extreme population bottlenecks has broad relevance to the viability of many tropical tree species. This study is especially relevant for the Dipterocarpaceae as some species are known to have limited pollen and seed dispersal (Osada *et al.* 2001; Seidler & Plotkin 2006; Kettle *et al.* 2011a). FSGS is also common in the family (Lee *et al.* 2006; Kettle *et al.* 2011a; Harata *et al.* in press). Consequently, a reduction in population size is predicted to create isolated patches of highly related individuals. Habitat restoration is urgently required, not only for *V. seychellarum* but for many native forest tree species of the Seychelles, and more broadly across tropical Asia. We expect our results to be directly applicable to restoration planning, seed sourcing and conservation management of genetic resources in *V. seychellarum*, as well as other important timber species (particularly dipterocarps) with limited gene dispersal mechanisms and similar functional traits (such as self-compatible mating system).

## Material and methods

### *Study species and populations*

*Vateriopsis seychellarum* is a unique and basal evolutionary lineage of the Dipterocarpaceae (Friedmann 1994) and the sole representative of the Dipterocarpaceae in



**Fig. 1** Map of the Seychelles main Island Mahé, with the 11 known *Vateriaopsis seychellarum* populations. Numbers of individuals for the different populations are shown in brackets. LN = Le Niol, SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, MC = Mt. Cotton, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, AN = André.

the Seychelles. It is endemic to the main island of the Seychelles, Mahé, where only 132 known adults remain spread across eleven sites: Le Niol, Sans Souci, Cap Vert, Tea Plantation, La Misère, Mt. Cotton, Rivière L'Islette, L'Abondance, Brulée, Salazie and André (see Fig. 1). Two populations (Le Niol and André) are purported to be plantations derived from seeds collected from the single isolated Tea Plantation tree (Huber & Ismail 2006; Willy André personal communication). This species was heavily exploited for its timber in the 19th century as the inland forests of the Seychelles were cleared for timber, agriculture and copra production (Baker 1877) and was nearly extinct by the end of the 19th century (Huber & Ismail 2006). The age of the remaining adults is not known and it is difficult to ascertain whether the current adult tree populations pre-date fragmentation. Nevertheless, planted populations with a mean DBH of 7.9–14.8 cm are reported to be 30 years old (Willy André personal communication). A DBH

increment of 1.0–1.5 mm per year has been reported in 10-year-old dipterocarp species plots in Thailand (Bunyavejchewin 1999). Thus, assuming the same DBH increment in *V. seychellarum* trees the remaining natural populations (except Rivière L'Islette) are likely to be 100–200 years old (mean DBH 20.8–29.4 cm, Tea Plantation tree: 63.7 cm), and at least the largest trees probably pre-date fragmentation in the mid 19th century (Table 1). As 30-year-old trees produce juveniles, a generation time of at least 30 years seems plausible for this species.

We mapped all known adult individuals using a GPS (Garmin 60CSx). Two of the sites include only a single tree, while three sites each have fewer than 10 individuals. While most populations are reproductive, the trees from Mt. Cotton produced no flowers or fruits for the duration of the study (2008–2010), and no juveniles could be found (Table 1). Based on our observations, *V. seychellarum* flowers irregularly and nonsynchronously among and within populations. Flowers have a corolla diameter of 3–4 cm and are cream coloured. The pollinator community is not well known but small beetles were observed on the flowers, and generally beetles are important pollinators for many Seychelles endemics on Mahé (Kaiser-Bunbury *et al.* 2011) and other Seychelles islands (Woodell 1979).

Flowers develop into a (mostly) single-seeded fruit that is spheroid (up to 6 cm in diameter) and wingless. Fruits are gravity dispersed and mostly fall directly below the mother tree. Four *V. seychellarum* populations are located in the vicinity of rivers and ravines, so secondary dispersal by water cannot be excluded, although fruits sink. The seeds are recalcitrant and germinate immediately after fruits are shed.

#### Sampling and genetic analysis

We made a complete genetic inventory of all known remaining adults (132) and a sample of 317 juveniles of *V. seychellarum* across the eleven sites on Mahé (Table 1, Fig. 1). Three of the 132 trees could not be genotyped (two at Sans Souci and one at L'Abondance) because of low DNA quality. We collected a subset of juveniles at all available age and height classes in each population. It is difficult to estimate the age of the collected juveniles as these may be retained for relatively long time periods in the seedling stage until the canopy is opened, allowing them to grow into sapling or pole stage trees. We consider that the combined sample of seedlings, saplings and pole stage trees include a range of age classes from a minimum of 1 year up to at least 10 years. Usually, juveniles were aggregated under the mother tree, often with more than 50 juveniles occurring within 10 m of the stem. In such cases, we collected leaf samples of a maximum of ten randomly

**Table 1** Population information for *Vateriaopsis seychellarum*

Pop ID	No. of adults	No. of non mature (estimate)	Max dist adults (m)	Mean tree height (m)	Mean tree DBH (cm)	Lat	Long
LN	11	160	44.13	9.05 ( $\pm 1.01$ )	7.87 ( $\pm 1.36$ )	9488104	326160
SS	19	50	109.59	14.31 ( $\pm 0.94$ )	25.92 ( $\pm 2.64$ )	9486904	327572
CV	6	200	589.71	15.67 ( $\pm 0.33$ )	20.89 ( $\pm 2.82$ )	9486992	323253
TP	1	40	–	18.00	63.69	9485312	326855
LM	1	28	–	15.00	26.59	9483648	330446
MC	16	0	150.01	16.27 ( $\pm 0.55$ )	29.38 ( $\pm 2.45$ )	9486943	325856
RL	30	27	69.55	9.29 ( $\pm 0.70$ )	11.11 ( $\pm 1.65$ )	9485600	325831
LA	32	400	120.75	11.16 ( $\pm 0.78$ )	22.84 ( $\pm 2.09$ )	9481194	331907
BR	3	50	47.63	No data	No data	9478672	334169
SA	11	600	136.49	12.18 ( $\pm 1.43$ )	21.57 ( $\pm 3.25$ )	9485079	328149
AN	2	8	4.67	10.50 ( $\pm 0.50$ )	14.81 ( $\pm 0.48$ )	9486814	327920

Mean tree height and total number of juveniles are estimated values; information on mean DBH, elevation and location are measured values,  $\pm$  Standard Error.

Max dist adults, maximum distance measured between the two most distant trees; LN, Le Niol; SS, Sans Souci; CV, Cap Vert; TP, Tea Plantation; LM, La Misère; MC, Mt. Cotton; RL, Rivière L'Islette; LA, L'Abondance; BR, Brulée; SA, Salazie; AN, André.

selected juveniles underneath individual mother trees. We repeated this sampling strategy for all trees. In the case of single isolated mother trees, we collected 40 juveniles per tree. This sampling strategy was applied over the entire range of each population to ensure a representative sample of the genetic variation in juveniles. Leaf samples were collected at all sites for adults and juveniles from 2008 to 2010 and stored in silica gel. DNA was extracted using the QIAGEN DNeasy Plant Maxi Kit, following the manufacturer's protocol. PCR reactions were carried out in a thermal cycler (Bio-Rad Dyad Cycler). Microsatellite loci were amplified from *c.* 10 ng diluted DNA in a Mastermix containing 1 $\times$  PCR buffer (Promega colorless Flexi GoTaq PCR buffer), MgCl<sub>2</sub>, dNTPs, specific primer pairs and *Taq* polymerase (Promega), for details see Finger *et al.* (2010). We used an ABI3730 for genotyping and GENEMAPPER 3.5 software (Applied Biosystems) for fragment analysis. Ten microsatellite loci were analysed (01, 10, 11, 12, 14, 15, 20, 21, 22 and 23) and the forward primer of each pair was labelled with an M13-tag at its 5'-end as described by Schuelke (2000).

### Statistics

**Assessment of genetic diversity.** Deviation from Hardy–Weinberg Equilibrium (HWE) at each locus was calculated in CERVUS 3.0 (Kalinowski *et al.* 2007). Linkage disequilibrium and inbreeding coefficients ( $F_{IS}$ ) using 10 000 permutations were calculated in ARLEQUIN 3.5 (Excoffier *et al.* 2005). Population genetic parameters including observed and expected heterozygosities ( $H_O$ ,  $H_E$ ), number of alleles ( $N_A$ ) and the number of private alleles ( $P_A$ ) were generated in GENALEX (Peakall &

Smouse 2006). In addition, allelic richness ( $R_S$ ) was calculated in FSTAT 2.9.3.2 (Goudet 1995). Because this study includes a complete sample of all known individuals, there is no risk of sampling bias (Leberg 2002).

**Assessment of genetic structure.** Differentiation between populations was assessed using pairwise  $F_{ST}$  values calculated using ARLEQUIN 3.5. To test for the presence of geographical groupings of related samples, we applied a Bayesian cluster analysis to all individuals using the software STRUCTURE (Pritchard *et al.* 2000). The batch run function was used to carry out a total of 150 runs, 10 each for one to 15 clusters (K1 to K15). For each run, the burn-in and simulation length was 30 000 and 100 000, respectively. We used the refined ad hoc statistic  $\Delta K$  based on the rate of change in the log probability of data between successive  $K$  values (Evanno *et al.* 2005). It was calculated as  $\Delta K = (|mL(K+1) - 2mL(K) + mL(K-1)|) / \text{SDL}(K)$ , where  $L(K)$  is the logarithm of the probability that  $K$  is the correct number of clusters,  $m$  is the mean and SD is the standard deviation.

**Assessment of within-population spatial genetic structure and relatedness among individuals.** Within-population spatial genetic structure was assessed using 10 000 permutations in the program SPAGEDI 1.3 (Hardy & Vekemans 2002). This tests the null hypothesis of no correlation between spatial distance and multilocus kinship ( $F$ ) of individuals. The analysis was conducted over the whole data set of adults and juveniles. The kinship coefficients were estimated using the allele frequencies over all natural populations (excluding Le Niol and André). Based on the maximum within-population distance of 160 m between individuals, the

distance classes were chosen as follows: 0–10 m to 120–160 m in total with 10 m intervals from 0 to 100 m, 20 m intervals from 100 to 120 m and a 40 m interval from 120 to 160. A minimum of 35 pairs per distance class was maintained for this analysis.

The  $Sp$ -statistic as a measure of intensity of fine-scale genetic structure (FSGS) was calculated as  $Sp = -b_F / (1 - F_{(1)})$  where  $b_F$  is the mean regression slope with natural log of distance ( $\ln$ ) and  $F_{(1)}$  is the mean pairwise kinship coefficient for the smallest distance class (Veke-mans & Hardy 2004). The standard deviation is calculated as  $(SE\ b_F) / (1 - F_{(1)})$  where SE is the standard error (Olivier Hardy personal communication).

We also compared the mean within-population individual kinship coefficients  $F$  (Loiselle *et al.* 1995) among all populations and between adults and juveniles in each population.

*Estimating realized gene flow using parentage analysis.* Using multilocus genotypes (ten loci) of 275 juveniles (excluding the juveniles from known plantations Le Niol and André), we applied a maximum likelihood exclusion analysis in CERVUS 3.0, to assign the two most likely candidate parents (Marshall *et al.* 1998; Kalinowski *et al.* 2007) in all natural populations. Pollen dispersal distance was deduced for each juvenile based on the position of the two most likely parent trees assigned by CERVUS. Simulations of paternity were run using the allele frequencies of all adult reproductive trees and the following settings: 10 000 cycles; minimum number of loci typed 5, unknown parents; all surviving adults across the species range were set as candidate parents for juveniles; 1% for proportion of loci mistyped and 97% for proportion of loci genotyped. The proportion of candidate parents sampled was set at 90%, as we could not be absolutely sure to have found all remaining potential parents and know three trees which we could not genotype and are thus missing in the analysis. Assignment was based upon the 96%, 90% and 80% confidence level of the critical LOD score. Seed dispersal was calculated as the distance between the juvenile and its closest assigned parent trees, as we know that seeds are primarily dispersed by gravity we assume that pollen dispersal is greater than seed dispersal.

## Results

### Population genetics

*Genetic diversity and inbreeding. Species level:* The ten loci each yielded between six and 19 alleles, with a total number of 120 alleles over all populations and loci. No linkage disequilibrium was observed for any pair of loci

after Bonferroni correction. Further analyses were performed on multi-locus data from all ten microsatellites. After Bonferroni correction, and exclusion of populations having deviations from HWE in this study (Le Niol, L'Abondance, Sans Souci and Salazie), only one locus (23) of our 10 showed a significant ( $P < 0.05$ ) but low homozygous excess indicating low frequency of null alleles.

*Comparison between populations:* Genetic diversity indicated by  $R_S$  (based on five diploid individual) and  $H_E$  was highest in population Cap Vert ( $R_S = 5.13$ ;  $H_E = 0.73$ ), lowest values were observed for Le Niol ( $R_S = 1.90$ ;  $H_E = 0.44$ ) and André ( $H_E = 0.38$ ), see Table 1 for more details. The highest number of private alleles was found in Rivière L'Islette (6), Mt. Cotton (8) and L'Abondance (10). Significant  $F_{IS}$  values were observed in the adult and juvenile population of Le Niol (0.30,  $P < 0.01$ ; 0.33,  $P < 0.001$ , respectively) and L'Abondance (0.12,  $P < 0.01$ ; 0.14,  $P < 0.001$ , respectively). No significant  $F_{IS}$  values were detected in any of the other adult populations in which  $F_{IS}$  could be calculated (i.e. sites with more than one individual).

*Comparison between cohorts:* When comparing the mean over populations and loci, a 95% confidence interval showed significantly lower values of genetic diversity in juveniles compared to adults for  $R_S$  and  $H_O$ . Values for  $F_{IS}$ ,  $H_E$  and  $N_A$  were not significantly different between adults and juveniles. In total, 46 rare alleles (with a frequency of 0.05 or lower) were found in adults but of these only 23 were in the juvenile cohort. The overall  $F_{IS}$  for adults was 0.09 ( $P < 0.001$ ) and 0.07 ( $P < 0.01$ ) for juveniles.

We also calculated the overall  $F_{IS}$ ,  $R_S$ ,  $H_O$ ,  $H_E$  and  $N_A$  values excluding the known plantations Le Niol and André. Without these two plantations, comparisons of genetic diversity ( $R_S$ ,  $H_O$ ,  $H_E$ ,  $N_A$ ) did not change substantially (see Table 2). The overall  $F_{IS}$  value was 0.08 ( $P < 0.001$ ) for adults and 0.06 ( $P < 0.001$ ) for juveniles.

A comparison between cohorts among individual populations showed significant differences for  $R_S$  in Cap Vert, Tea Plantation, Brulée and André (all  $P < 0.05$ ). No significant differences were found for  $H_O$  and  $H_E$ . Also, no significant differences were found for  $F_{IS}$  in any population between adults and juveniles after Bonferroni correction (see Table 1).

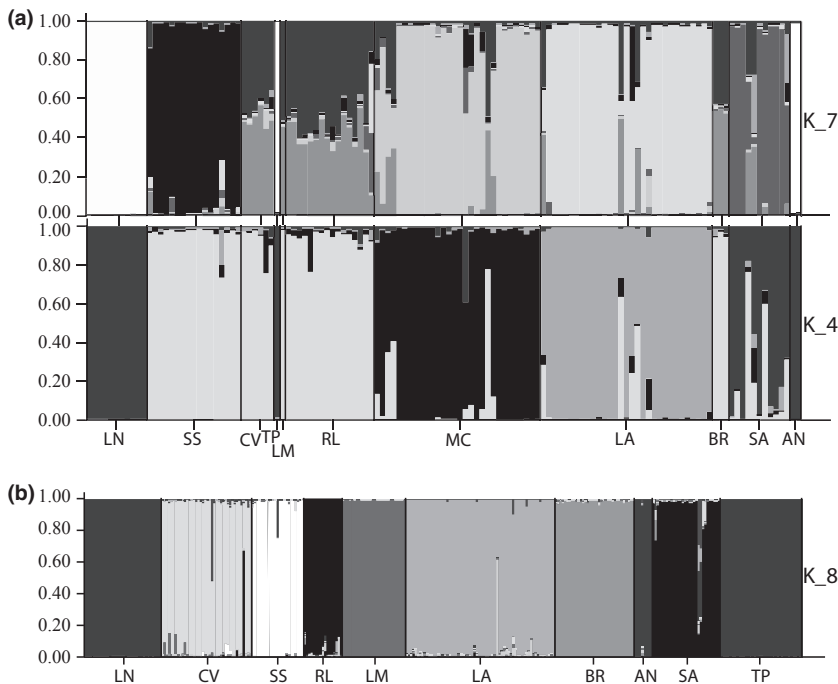
*Genetic differentiation—adults.* The overall  $F_{ST}$  value across all adult populations was 0.30 ( $P < 0.001$ ). Excluding populations Le Niol and André (populations confirmed as planted), overall  $F_{ST}$  value was 0.20 ( $P < 0.001$ ). Pairwise genetic distances (Nei 1972) of populations in which  $F_{ST}$  values could be calculated (with more than one individual) were highest between Le Niol and the rest of the populations. Lowest values

**Table 2** Genetic variability of 10 microsatellite loci estimated for all populations of *Vateriaopsis seychellarum*

POP ID	<i>n</i>		<i>N<sub>A</sub></i>		<i>H<sub>O</sub></i>		<i>H<sub>E</sub></i>		<i>R<sub>S(O)</sub></i>		<i>R<sub>S(S)</sub></i>		<i>P<sub>A</sub></i>		<i>F<sub>IS</sub></i>	
	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg
LN	11	34	1.9 (±0.10)	1.9 (±0.10)	0.35 (±0.07)	0.27 (±0.05)	0.44 (±0.05)	0.33 (±0.05)	1.46 (±0.05)	1.34 (±0.05)	1.90 (±0.10)	1.82 (±0.10)	0	0.30*	0	0.33***
SS	17	21	4.0 (±0.42)	3.6 (±0.50)	0.47 (±0.08)	0.40 (±0.08)	0.55 (±0.06)	0.48 (±0.07)	1.57 (±0.06)	1.49 (±0.06)	3.16 (±0.30)	2.75 (±0.30)	3	0.05 ns	3	0.11 ns
CV	6	40	5.5 (±0.45)	5.6 (±0.58)	0.76 (±0.03)	0.60 (±0.05)	0.73 (±0.04)	0.71 (±0.05)	1.79 (±0.04)	1.72 (±0.04)	5.13 (±0.38)	4.19 (±0.34)	3	0.02 ns	3	0.11**
TP	1	36	2.0 (±0.00)	1.9 (±0.10)	1.00 (±0.00)	0.55 (±0.07)	0.50 (±0.00)	0.45 (±0.05)	2.00 (±0.00)	1.45 (±0.05)	NA	1.90 (±0.10)	0	NA	0	-0.23 ns
LM	1	28	1.5 (±0.17)	1.5 (±0.17)	0.50 (±0.17)	0.23 (±0.08)	0.25 (±0.08)	0.24 (±0.08)	1.50 (±0.17)	1.25 (±0.08)	NA	1.50 (±0.17)	0	NA	0	-0.03 ns
MC	16	0	6.7 (±0.80)	NA	0.65 (±0.05)	NA	0.70 (±0.04)	NA	1.72 (±0.04)	NA	4.48 (±0.41)	NA	8	0.05 ns	NA	NA
RL	30	17	5.6 (±0.69)	4.0 (±0.49)	0.57 (±0.06)	0.58 (±0.07)	0.61 (±0.05)	0.56 (±0.07)	1.62 (±0.05)	1.58 (±0.06)	3.61 (±0.35)	3.11 (±0.34)	6	0.07 ns	6	-0.12 ns
LA	31	66	5.5 (±0.34)	4.9 (±0.48)	0.57 (±0.07)	0.52 (±0.07)	0.65 (±0.06)	0.63 (±0.07)	1.67 (±0.06)	1.64 (±0.06)	3.76 (±0.31)	3.61 (±0.32)	10	0.12**	10	0.14***
BR	3	36	2.7 (±0.26)	3.1 (±0.31)	0.57 (±0.12)	0.49 (±0.09)	0.49 (±0.07)	0.49 (±0.07)	1.59 (±0.08)	1.50 (±0.07)	NA	2.57 (±0.22)	3	-0.08n	3	-0.04 ns
SA	11	30	4.3 (±0.50)	4.0 (±0.39)	0.50 (±0.05)	0.46 (±0.03)	0.58 (±0.04)	0.59 (±0.03)	1.60 (±0.04)	1.60 (±0.03)	3.32 (±0.27)	2.96 (±0.18)	2	0.13 ns	2	0.21***
AN	2	8	1.9 (±0.10)	1.9 (±0.10)	0.40 (±0.10)	0.44 (±0.08)	0.38 (±0.05)	0.34 (±0.05)	1.50 (±0.06)	1.37 (±0.06)	NA	1.85 (±0.10)	0	0.13 ns	0	-0.29 ns
Mean	129	317	3.76 (±0.21)	3.24 (±0.17)	0.58 (±0.03)	0.45 (±0.02)	0.53 (±0.02)	0.48 (±0.02)	1.64 (±0.03)	1.49 (±0.02)	3.62 (±0.16)	2.61 (±0.11)				
Mean No. plantations	116	275	4.17 (±0.23)	3.58 (±0.20)	0.62 (±0.03)	0.48 (±0.03)	0.56 (±0.02)	0.52 (±0.03)	1.67 (±0.03)	1.53 (±0.03)	3.91 (±0.16)	2.83 (±0.13)				

Ad, adults; Sg, Juveniles; *N<sub>A</sub>*, mean number of alleles; *H<sub>O</sub>*, observed heterozygosity; *H<sub>E</sub>*, expected heterozygosity; *R<sub>S(O)</sub>*, allelic richness, based on one or five diploid individuals; *P<sub>A</sub>*, number of private alleles; *F<sub>IS</sub>*, inbreeding coefficient; ± SE. LN, Le Nioi; SS, Sans Souci; CV, Cap Vert; TP, Tea Plantation; LM, La Misère; MC, Mt. Cotton; RL, Rivière L'Islette; LA, L'Abondance; BR, Brulée; SA, Salazie; AN, André.

\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.



**Fig. 2** Bayesian structure analysis of *Vateriaopsis seychellarum* with the STRUCTURE software (Pritchard *et al.* 2000). Bars represent individual *V. seychellarum* individuals with their assignment proportions (*y* axis) to the different clusters. Performing the analysis for (a) adults for *K* 4 and *K* 7 (four and seven different grey shades), representing four and seven genetic clusters and (b) *K* 8 (eight different grey shades), for a clustering solution of eight genetic clusters for juveniles. For coloured STRUCTURE bar plots, see Fig. S2a,b (Supporting information).

were found between Cap Vert and Mt. Cotton and all other adult populations (see Table S1, Supporting information). Bayesian structure analysis of *V. seychellarum* individuals discriminated the adult individuals into four and seven clusters. The probability values (mean  $\ln(\text{Pr})$  and  $\Delta K$ ) reached their highest peak at  $K = 4$ . Additional but smaller peaks were observed at  $K = 2$  and  $K = 7$  (Table S1, Supporting information). Following the protocol of Pritchard *et al.* (2010) we chose the number of clusters that firstly had high probability values and secondly made biological sense, based upon this  $K = 4$  and  $K = 7$  are the most plausible clustering solutions. This analysis places Tea Plantation and the populations Le Niol and André constantly into a single cluster (Fig. 2a,b).

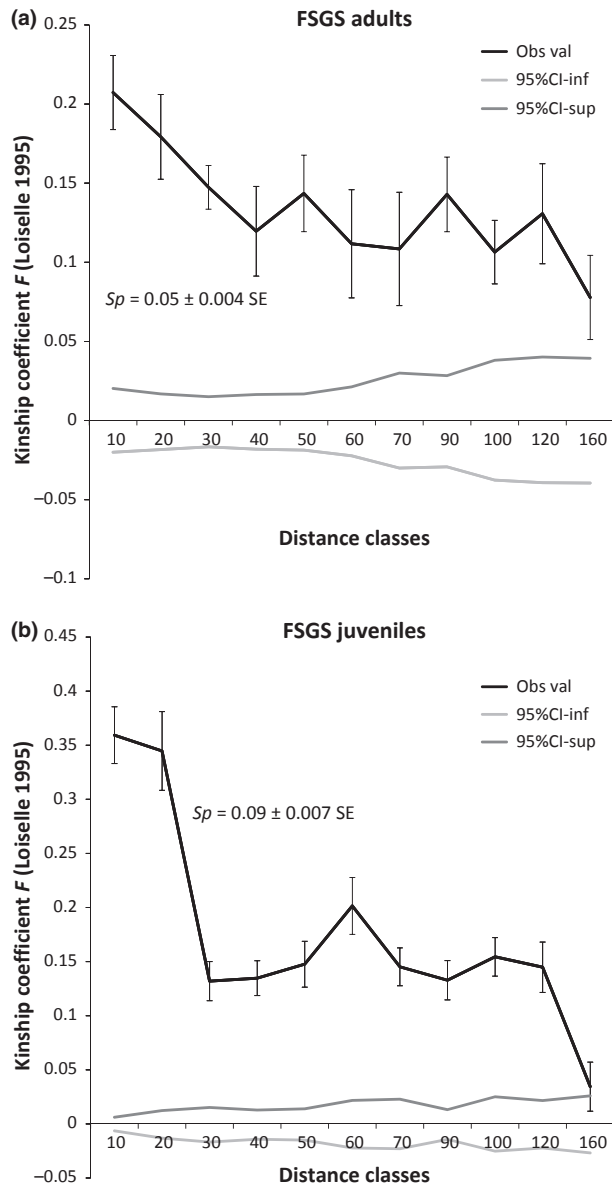
**Genetic differentiation—juveniles.** The overall  $F_{ST}$  value for juveniles was 0.42 ( $P < 0.001$ ), excluding populations Le Niol and André  $F_{ST} = 0.38$  ( $P < 0.001$ ). Pairwise genetic distances (Nei 1972) in all populations were greater in juveniles than in adults (Table S1, Supporting information). The Bayesian structure analysis of *V. seychellarum* juveniles discriminated the juvenile individuals into eight clusters. The probability values [mean  $\ln(\text{Pr})$  and  $\Delta K$ ] reached their highest peak at  $K = 2$ . Nevertheless, the biologically more relevant solution was the second highest peak at  $K = 8$  (Table S1, Supporting information and Fig. 2b).

**Within-population fine-scale spatial genetic structure and kinship.** Significant FSGS was detected across all popu-

lations, in distance classes up to 160 m for adult (Fig. 3a) and juvenile cohorts (Fig. 3b), as indicated by significant regression of kinship against spatial distance, adults  $Sp = 0.05 \pm 0.004$  SE and juveniles  $Sp = 0.09 \pm 0.007$  SE.

The lowest mean individual kinship coefficient ( $F$ ) was observed in Cap Vert adults:  $F = 0.06$  ( $\pm 0.03$  SE), highest values in La Misère juveniles:  $F = 0.70$  ( $\pm 0.004$  SE). The highest kinship coefficients between individuals of different populations were recorded for individuals of the Tea Plantation, Le Niol and André (Tea Plantation—Le Niol:  $0.43 \pm 0.01$  SE; Tea Plantation—André:  $0.38 \pm 0.01$  SE; André—Le Niol:  $0.44 \pm 0.04$  SE). The species-level mean individual kinship coefficient was greater in juveniles compared to adults (Juveniles  $F = 0.29 \pm 0.002$  SE; Adults  $F = 0.19 \pm 0.004$  SE, respectively), see Fig. S1 (Supporting information).

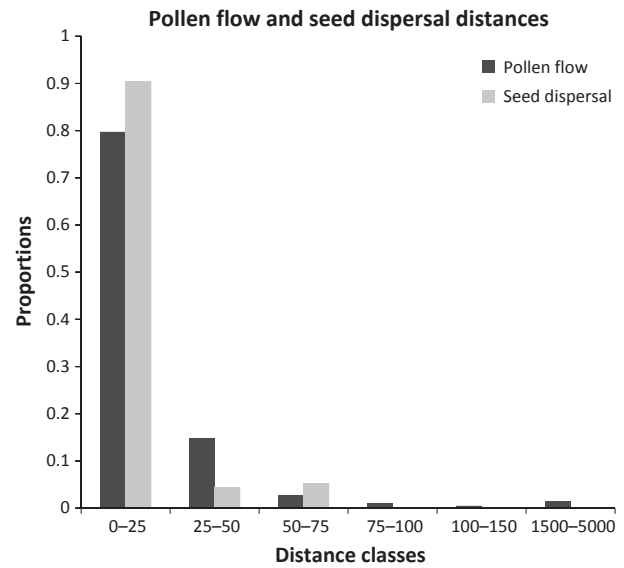
**Contemporary pollen and seed dispersal and mating system.** Using parentage assignment we were able to assign 79% (216) of our juveniles ( $n = 275$ ) with 90% confidence, and 60% of all juveniles with 96% confidence. On a more relaxed 80% confidence level we were able to assign 99% of the juveniles, we present here the more conservative 90% confidence level. Realized pollen dispersal ranged between 0 m (selfed) to 4.7 km in one case, with 94% of all juveniles resulting from mating events of less than 50 metres. Assuming that the nearest parent was the maternal tree, 90% of assigned realized seed dispersal events were  $< 25$  m, only 10% of seed dispersal was found between 25 and



**Fig. 3** Fine-scale spatial genetic structure of *Vateriopsis sechellarum* adults (a) and juveniles (b). Correlogram of average kinship coefficient (*F*) of adult individuals over all populations for 11 distance classes. The solid line represents the average *F* values. The dotted lines represent the 95% (two-tailed) CI of the average *F* distribution calculated from 10 000 permutations of spatial distance among pairs of adults.

100 m, with a maximum seed dispersal distance of 67.3 m (Fig. 4).

Selfing rates (number of cases where parentage analysis determined the father and mother as the same tree) are relatively high in most populations but considerably higher in Salazie (80%), Cap Vert (39%) and Sans Souci (38%). Lower values are observed in L'Abondance (18%) and Brulée (17%) and in Rivière L'Islette (10%). Individual isolated trees (La Misère, Tea Plantation)



**Fig. 4** Frequency distributions of realized pollen and seed dispersal distances in *Vateriopsis sechellarum* populations, calculated from *n* = 275 juveniles and parent pairs.

**Table 3** Results from the parentage analysis for 74% juveniles (*n* = 217) based on 90% assignments confidence showing the total numbers of contributing adults to juvenile production and selfing rate in the different populations

Population	No. of contributing adults	No. of assigned juveniles	Freq > 20%	Prop. selfed juveniles
CV	7	36	3	0.39
SS	7	13	2	0.38
RL	9	10	1	0.10
LA	27	40	0	0.18
BR	3	28	2	0.17
SA	6	26	1	0.80
TP	1	36	1	1.00
LM	1	28	1	1.00

The numbers of trees that contributed to more than 20% to juvenile production are given in Freq > 20%.

SS, Sans Souci; CV, Cap Vert; TP, Tea Plantation; LM, La Misère; RL, Rivière L'Islette; LA, L'Abondance; BR, Brulée; SA, Salazie; MC, Mt. Cotton.

had exclusively selfed juveniles (Table 3). The overall selfing rate was 53%.

In the populations Cap Vert and Sans Souci, three assigned fathers are found to be from outside the populations (1.4% events in a total of 216 assigned juveniles). In Sans Souci, the father from two juveniles was identified as one tree at Mt. Cotton (Distance Sans Souci to Mt. Cotton = 1.7 km), and in Cap Vert, one juvenile was assigned to a father from the population Salazie (Distance Cap Vert to Salazie = 4.8 km).



## Discussion

*Vateriopsis seychellarum* is a critically endangered species, found in small numbers in only a few isolated populations in the Seychelles. Its landscape context is, on a small scale, typical of many dipterocarp species in Southeast Asia, the main centre of dipterocarp species diversity, where logging and agricultural expansion has fragmented lowland forest habitats. Our study of *V. seychellarum* provides insights into opportunities for ecological and genetic restoration efforts, and explores how the patterns of contemporary gene flow and mating system characteristics influence the viability of *V. seychellarum* in particular and other dipterocarps more generally.

### *Evidence for extreme genetic bottlenecks*

The genetic diversity in *V. seychellarum* adults is comparable among populations with the exception of two plantation populations (Le Niol and André) and the single tree at La Misère where genetic diversity was lower. A parentage analysis of the adult trees from Le Niol and André, using all other adult trees as potential parents, demonstrated that these trees are selfed progeny derived from the single isolated tree at Tea Plantation. This tree is the largest known remaining *V. seychellarum* individual (DBH: 63.7 cm, Height: 18 m) and is easily accessible for collecting seed.

The small population at Cap Vert, despite comprising only six individuals, retains one of the highest values of genetic diversity (allelic richness) and underlines the value of some very small populations for conservation of genetic variation (Schneller & Holderegger 1996). Allelic richness, observed heterozygosity and number of alleles decreased from adult to juvenile stages, and the kinship coefficient between individuals increased, possibly caused by limited pollen and seed dispersal and frequent self-fertilization. We interpret the absence of 50% of rare alleles in the *V. seychellarum* juvenile cohort as an early sign of genetic erosion following habitat fragmentation (Kettle *et al.* 2007). This difference in levels of genetic diversity could also be a consequence of differences in effective breeding population sizes, for example, a mass-fruiting event where all remaining adults contribute to juvenile production could greatly increase the observed diversity in a single cohort. A number of Asian dipterocarp species show annual flowering, but there is no evidence of superannual mass flowering for *V. seychellarum* on the Seychelles. The Mt. Cotton population, despite having relatively high genetic diversity, currently lacks any natural regeneration, and during 3 years of field visits no flowers, fruits or juveniles were observed. Numerous ecological con-

straints such as habitat quality, mycorrhiza availability (Tedersoo 2007), herbivory or pollinator limitation might conceivably limit recruitment success in this population, although there is no evidence to implicate any one of these factors specifically.

### *Genetic structure, seed and pollen dispersal*

*Vateriopsis seychellarum* adults have strong genetic differentiation, revealed as high pairwise  $F_{ST}$  values, private alleles and a clear genetic clustering. The FSGS analysis over all adults within populations demonstrates a close relatedness of individuals up to 150 m. This implies that despite a formerly more widespread distribution, gene dispersal has been somewhat restricted. The most plausible explanation for this is limited pollen and seed dispersal. The wingless fruit of *V. seychellarum* provides no obvious means for dispersal beyond the shadow of the mother tree's canopy. Pollen flow is likely to be more extensive, but the pollinators, although poorly known, are likely to be small beetles which are not thought to cover large distances. Restricted gene flow has been noted for several dipterocarp species elsewhere, suggesting that the genetically structured populations found in *V. seychellarum* are not uncommon for this family. In Malaysian Borneo, *Shorea xanthopylla* and *Parashorea tomentella* show significant FSGS partly owing to limited seed and pollen dispersal (Kettle *et al.* 2011b). Dipterocarp species with highly mobile insect pollinators appear to have weaker FSGS compared with species with smaller pollinators (Kettle *et al.* 2011a; Harata *et al.* in press). The mountainous topography of Mahé might further impede gene flow in *V. seychellarum*. The wind-pollinated *Shorea robusta* in hilly regions in Nepal, showed higher levels of genetic differentiation among populations, naturally disjunct along river valleys, compared with larger continuous populations (Pandey & Geburek 2010). These examples indicate that habitat fragmentation may have similar genetic consequences for many dipterocarp species of different geographical regions as gene dispersal modes are often comparable.

Genetic differentiation among populations of *V. seychellarum* is stronger in juvenile cohorts (higher pairwise  $F_{ST}$ ), which could be interpreted as increased genetic isolation in currently fragmented populations. This observation is supported by a stronger clustering solution in the Bayesian analysis for the juveniles (eight genetic clusters compared to four and seven genetic clusters in adults). Furthermore, the intensity of FSGS is stronger in juveniles, as indicated by the higher  $Sp$  value (0.09 in juveniles and 0.05 in adults). These results might also be a consequence of sampling juveniles which were produced over comparatively few (possibly up to 10) years. Nevertheless, as these juveniles repre-

sent the main contribution to future generations of this species, the increased differentiation as indicated by the clustering analysis does suggest greater isolation. The parentage analysis of 275 juveniles demonstrated that contemporary seed dispersal is limited, and 94% of juveniles genotyped were <25 m from their assigned mother, with a maximum observed seed dispersal distance of 67 m. Limited seed dispersal in *V. seychellarum* is consistent with its gravity mode of seed dispersal, a common characteristic of dipterocarps (Suzuki & Ashton 1996; Seidler & Plotkin 2006).

Pollen dispersal distances were low in *V. seychellarum* (90% <50 m), and most assigned fathers were within populations. We observed three (1.4% of all observations) apparent long-distance pollen dispersal events, with pollen donors identified as Mt. Cotton and Salazie individuals, for one juvenile at Cap Vert (Distance Cap Vert to Salazie = 4.8 km) and two at Sans Souci (Distance Sans Souci to Mt. Cotton = 1.7 km). It is possible that small pollinators (possibly small beetles) could have crossed to relatively distant populations but this seems unlikely on account of both distance and topography. Pollen dispersal of over 500 m has been observed in other dipterocarp species (Kenta *et al.* 2004), but an alternative explanation for apparent long-distance pollen dispersal events in *V. seychellarum* could be erroneous assignment resulting from failure to sample the true fathers in Sans Souci and Cap Vert: two adults from Sans Souci could not be genotyped and there is the possibility that we may not have found all adults at Cap Vert. It may also be possible that the potential parents have been logged and may thus no longer exist. No pollen donors from Mt. Cotton or Salazie were observed at closer sites and the Mt. Cotton population showed no sign of recruitment or flowering, further supporting low probability of long-distance pollen flow. When excluding Mt. Cotton and Salazie from the analysis, all juveniles were assigned to parents within the same population and juveniles assigned to the adult at Mt. Cotton had allele mismatches indicating they are probably not the real parents. Although we cannot completely exclude the potential for rare long-distance pollen flow in *V. seychellarum*, such long-distance dispersal has not been sufficient to prevent genetic structuring over relatively narrow spatial scales (<5 km), we conclude that it must be very rare, if it occurs at all.

#### *Mating system and its implications for population viability*

A high degree of selfing was apparent in some populations of *V. seychellarum* (Salazie 80%, Sans Souci 38% and Cap Vert 39%), and all juveniles sampled from the single isolated individuals (Tea Plantation, La

Misère) were selfed. Studies in other dipterocarps have shown a range of mating systems from complete outcrossing to apomixis (Bawa 1998). In *Shorea acuminata* which is predominantly visited by thrips, single pollen grain genotyping collected from insects demonstrated high levels of self-pollination through geitonogamy (Kondo *et al.* 2011). In predominately outcrossing dipterocarps, selfing rates tend to increase with reduced population density and fewer flowering conspecifics (Nagamitsu *et al.* 2001; Fukue *et al.* 2007; Naito *et al.* 2008; Tani *et al.* 2009). Selfing might, however, have benefits in terms of predator avoidance (Ghazoul & Satake 2009), reducing maladapted genes or assuring reproduction (Levin 2010). It is noteworthy that in a comparison of two dipterocarp species with contrasting mating systems, the outcrossing *Shorea leprosula* exhibited a greater loss of genetic diversity owing to logging than the self-compatible *Shorea ovalis* (Ng *et al.* 2009). Nevertheless, selfed progeny tend to have higher rates of abortion or mortality at early ontogenic stages as seen in representatives of three dipterocarp genera: *Dipterocarpus obtusifolius*, *Shorea siamensis* and *Dryobalanops lanceolata* (Momose *et al.* 1996; Ghazoul 1997; Ghazoul *et al.* 1998). Self-fertilization can also lead to genetic problems associated with increased homozygosity and inbreeding depression, as well as reduced effective population sizes and genetic diversity (Schoen & Brown 1991; Hamrick & Godt 1996; Ingvarsson 2002). In other dipterocarp species such as *Neobalanocarpus heimii*, the seed mass and germination success of selfed seeds is lower than that of outcrossed seeds (Naito *et al.* 2005). At this stage, it is difficult to predict whether inbred and selfed populations of *V. seychellarum* will be able to maintain their reproductive output and performance over multiple generations, but a precautionary approach to population restoration efforts avoiding inbreeding through careful selection of seed sources may be advisable.

#### *Implications for conservation and management*

We detected extreme genetic bottlenecks in the two planted populations of *V. seychellarum* (Le Niol and André). All other populations (with more than one individual) retain high genetic diversity and low kinship coefficients, consistent with the idea that these are remnants of formerly larger 'natural' populations. Low selfing rates (L'Abondance and Rivière L'Islette) and high numbers of contributing parents to juvenile production in L'Abondance could indicate less disturbed habitats but could also mirror the higher numbers of individuals in these populations. Mt. Cotton, having high genetic diversity and low pairwise  $F_{ST}$  values compared to other populations, supports the view that this is a refu-

gial population. Based on these findings, we make a number of management recommendations.

Like all dipterocarp species, *V. seychellarum* seeds are recalcitrant and cannot easily be stored in conventional seed banks. *Ex-situ* conservation will thus only be achieved through nursery grown seedling banks. Ideally about 20 seeds from each individual tree should be collected from populations where inbreeding is known to be low in adults and juveniles, such as Mt. Cotton, Rivière L'Islette, Sans Souci and Brulée, to ensure sampling the whole range of adult genetic variety. *Ex-situ* collections have been able to capture 94% of the whole species genetic diversity in a rare palm (*Leucothrinax morrisii* Arecaceae) within 58 individuals (Namoff *et al.* 2010) demonstrating that even a relatively modest number of individuals (100–200) can help conserve genetic diversity *ex-situ*. Similarly, in the endangered endemic dipterocarp *Vatica guangxiensis* in China, 28 *ex-situ* individuals conserved 88.31% of the neutral genetic variation of natural populations (Li *et al.* 2002). Larger-scale *ex-situ* seedling banks (several 1000 juveniles) could provide germplasm for habitat restoration and material for transplant experiments into their original populations. Such population augmentation should ensure that seeds are collected from donor sites which are not only diverse and outbred, but share similar site conditions, to minimize the likelihood of disrupting any local adaptation. In addition, common garden experiments using progeny from controlled pollination experiments could be used to test whether inbreeding or outbreeding depression occur in these populations. We expect outbreeding depression to be unlikely due to the similarity of habitats and limited potential for local adaptation.

New populations should also be established at strategic locations. Such locations should consider the ecological context of the relatively short gene dispersal distances (mostly <50 m) and should thus be located close to and between remaining populations to enhance connectivity of existing populations. Clearly, management efforts will only be successful if large natural populations such as L'Abondance, Rivière L'Islette, Sans Souci and Mt. Cotton are protected.

Trees from both plantations (Le Niol and André) produce progeny that were assigned as within-population matings and these juveniles and saplings showed no obvious detrimental inbreeding effects. Even so, self-compatible species often reveal the effects of inbreeding depression at late life stages (Husband & Schemske 1996; Thiele *et al.* 2010) and the long-term growth and survival of *V. seychellarum* progeny warrant further investigation. Repeated bouts of inbreeding within these plantations are likely to further erode the genetic base of these plantations, decreasing their potential to adapt to novel pests and pathogens which

are issues of special concern for island populations (Namkoong 1991; Burdon 2001; Spielman *et al.* 2004). Collecting seeds from these two sites in the future is therefore unlikely to contribute much to preserving the species-wide genetic diversity. Efforts should be invested in collecting seed from a variety of population sources, including relict trees, to ensure the maintenance of species-wide genetic diversity in restoration efforts. Such a strategy has been shown to be potentially successful for restoration in the Australian *Eucalyptus leucoxylon* (Ottewell *et al.* 2009). However, owing to the reduced genetic diversity found in seed collections from some single isolated trees in *V. seychellarum* (Tea Plantation tree), our study indicates that this should be conducted with caution, especially in self-compatible species.

Our results suggest that limited gene flow in *V. seychellarum* may lead to long-term population isolation. Many other dipterocarp species have also been observed to have relatively limited pollen and seed dispersal (Ghazoul *et al.* 1998; Osada *et al.* 2001; Seidler & Plotkin 2006; Kettle *et al.* 2011b). This has implications for a large number of rare and threatened dipterocarp species which continue to experience habitat degradation and fragmentation. Estimates of habitat loss in Sabah (Malaysia) show that 32 of 33 dipterocarp species are threatened, particularly species with narrow distributions (Maycock *et al.* 2012). Ng *et al.* (2009) demonstrate that even a single logging event can cause genetic erosion in an outcrossing dipterocarp (*S. leprosula*) by loss of rare alleles. Another dipterocarp, *Shorea lumutensis*, lost 80% of its population size owing to logging in the Peninsular of Malaysia (Lee *et al.* 2006). Similar to *V. seychellarum*, *S. lumutensis* survives in few populations with few mature individuals (about 500) but retains a large amount of genetic variation in adults that probably pre-date habitat fragmentation. Our study suggests that species like *S. lumutensis* may be particularly vulnerable to population isolation because of significant spatial genetic structure and relatively low pollen flow rates (175 m). Other tropical tree species with limited dispersal potential may show similar trends, for example, the response of *Copaifera langsdorffii* (Fabaceae) in Brazil to habitat fragmentation (Sebbenn *et al.* 2011).

Less than half of the original forests of Southeast Asia remain (Sodhi *et al.* 2004) and Southeast Asian forest restoration is now seen to be an essential tool for dipterocarp (and other forest tree species) conservation and recovery (Kettle 2010). This study advances our understanding of how patterns of gene flow and existing genetic diversity relate to population recruitment and viability and will lead to better informed conservation and restoration.

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- 
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### Data accessibility

DNA sequences: GenBank accessions GU591481–GU591490. Sample locations and microsatellite data deposited in the Dryad repository: doi:10.5061/dryad.pk8997k0.

### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Pairwise  $F_{ST}$  values for *Vateriopsis seychellarum* adults and seedlings after Bonferroni correction.

**Table S2**  $\Delta K$  values calculated from the mean  $\ln(\text{Pr})$  values obtained from the STRUCTURE analysis for *Vateriopsis seychellarum* adults and seedlings.

**Fig. S1** Average kinship coefficient ( $F$ ) of *Vateriopsis seychellarum* adults and juveniles for the eleven individual populations and over all populations.

**Fig. S2** Bayesian structure analysis of *Vateriopsis seychellarum* with the STRUCTURE software (Pritchard *et al.* 2000).

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