Great Genetic Diversity but High Selfing Rates and Short-Distance Gene Flow Characterize Populations of a Tree (Foetidia; Lecythidaceae) in the Fragmented Tropical Dry Forest of the Mascarene Islands

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From the CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France (Cuénin, Rivière, Lebreton, Reynaud, and Martos); the Université de La Réunion, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France (Cuénin, Flores, Reynaud); and Institut de Systématique, Evolution, Biodiversité (ISYEB), MNHN, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France (Martos).

Address correspondence to Nicolas Cuénin: CIRAD, UMR PVBMT, 7 chemin de l'IRAT Ligne Paradis, 97410 Saint-Pierre, La Réunion, France, or e-mail: nicolas.cuenin@cirad.fr.

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Abstract

Following the global trend of deforestation and degradation, tropical dry forests in the Mascarenes archipelago on Reunion has undergone harsh reduction and fragmentation within 3 centuries of human occupation. We investigated the genetic diversity, mating system, and gene flow in fragmented populations of the native tree *Foetidia mauritiana* (Lecythidaceae) on Reunion, using microsatellite genotyping of adults (in- and ex situ) and seed progenies (in situ only). To test genetic isolation between the Mascarene islands, we also genotyped conspecific adults on Mauritius, and trees of *Foetidia rodriguesiana* on Rodrigues. We found a high genetic diversity among the trees on Reunion, but no population structure (G'_{ST}: 0.039–0.090), and an increase of the fixation index (F_{IS}) from adults to progenies. A subsequent analysis of mating systems from progeny arrays revealed selfing rates >50% in fragmented populations and close to 100% in lone trees. A paternity analysis revealed pollen flow ranging from 15.6 to 296.1 m within fragments. At broader scale, the populations of *F. mauritiana* on Reunion and Mauritius are genetically differentiated. The morphologically allied taxa *F. rodriguesiana* and *F. mauritiana* are clearly isolated. Therefore, this case study shows that genetic diversity may persist after deforestation, especially in long-lived tree species, but the reproductive features may be deeply altered during this process. This would explain the low seed production and the absence of recruitment in *F. mauritiana*. Restoration programs should take into account these features, as well as the importance that trees ex situ represent in restoring and conserving diversity.

Keywords: genetic erosion, habitat fragmentation, island biota, mating systems, pollen dispersal, tropical dry forests

Erosion of natural habitats is a major threat to the conservation of biodiversity worldwide (Lira et al. 2003; Lindenmayer and Fischer 2013). The ongoing process by which large continuous habitats are converted to smaller, more isolated fragments generally have strong negative impacts on the population sizes and genetic pools of species inhabiting them (Saunders et al. 1991; Young et al. 1996; Lowe et al. 2005; Aguilar et al. 2008). In particular, tropical tree species should be highly vulnerable to habitat fragmentation, due to their demographic and reproductive characteristics, including complex self-incompatibility, high rates of outcrossing, and their mutualistic (often specific) interactions with pollinators and seed dispersers (Dick et al. 2003; Lowe et al. 2005; Ward et al. 2005). Besides population bottlenecks, gene flow between distant forest patches may be disrupted, and then outcrossing may be constrained by the lower availability of compatible genotypes, hence reducing the chance for species to restore some genetic diversity (Eckert et al. 2010). These genetic consequences of habitat degradation may play a considerable role in the survival and long-term adaptability of tree populations (Young et al. 1996; Lowe et al. 2005), particularly in changing environmental conditions. Studying those consequences on diversity and gene flow can offer practical guidelines for prioritizing conservation efforts, and thus may significantly increase chances of success in species conservation (Aguilar et al. 2008; Thomas et al. 2014) and by extension, in habitat restoration.

Empirical studies have shown that while genetic diversity may decrease with reduced population size, not all fragmentation events lead to genetic isolation (Young et al. 1996; Bacles and Jump 2011). For instance, Guidugli et al. (2016) recently showed that geographic isolation in a forest patch was not preventing gene flow in a tropical tree species, *Cariniana estrellensis* (Lecythidaceae), in a semideciduous forest in Brazil. In this study, all adult trees in the forest patch and a fraction of their seed progenies were genotyped at 9 microsatellite loci; gene immigration rates (mainly attributable to pollen) could then be estimated using parentage analyses (Guidugli et al. 2016). From this example as from others (e.g., White et al. 2002; Seltmann et al. 2009), we learn that gene dispersal can be observed over long distances. This should be particularly true in plants having wind-pollinated flowers or wind-dispersed seeds; dispersal events may compensate for the spatial isolation of fragmented populations (see in Bacles and Jump 2011; Guidugli et al. 2016; Moracho et al. 2016; Noreen et al. 2016; Peng et al. 2016).

It appears that in certain cases, increased geographic distance between remnant populations can even enhance outcrossing, leading to an increase of genetic diversity and lack of genetic differentiation, for example, in *Ficus* spp. (Nason and Hamrick 1997) and *Swietenia humilis* (White et al. 2002).

Although these 2 studies showed a decrease in genetic diversity across generations, they did not show inbreeding nor spatial genetic structure (SGS), which suggests the possibility of long-range gene flow, even in highly fragmented habitats. This effect could exist in long-lived species, which are characterized by a long generation time, but not be observed because the time scale considered is too short (Young et al. 1996; White and Boshier 2000; Aguilar et al. 2008; Bacles and Jump 2011).

Tropical and subtropical dry forests represent a major part of tropical forests (42% vs. 33% and 25% of tropical wet forests and rain forests, respectively) (Holdridge 1967; Murphy and Lugo 1986). They are characterized by an average temperature generally over 17 °C, and rainfall ranging from 250 to 2000 mm annually (Holdridge 1967; Dirzo et al. 2011). Species inhabiting these forests generally have to cope with low water availability and fire disturbance (Dirzo et al. 2011). These forest ecosystems are globally threatened by anthropogenic activities (e.g., exploitation, agriculture, fire) (Janzen 1988; Cascante et al. 2002), such that tropical dry forests have lost most of their historic cover (Murphy and Lugo 1986). In sub-Sahara Africa, tropical dry forests are primarily found in the biodiversity hotspot of Madagascar and the Indian Ocean Islands (e.g., the Mascarene archipelago). This hotspot is not only known for its high degree of endemism among plant and animal taxa, but also for ongoing pressure on natural resources and habitats (Olson and Dinerstein 2002; Strasberg et al. 2005). Tropical dry forests have totally disappeared from 2 islands on the Mascarene archipelago, Mauritius and Rodrigues, but patches remain on Reunion, representing ca. 1 % of the original surface cover (56 800 ha) (Strasberg et al. 2005; Sarrailh et al. 2008). These patches are functionally highly degraded (Sarrailh et al. 2008) and remain highly exposed to anthropogenic activities. Conservation actions have been undertaken to restore and preserve the unique biodiversity found in these habitats. In this context, it is crucial to assess the levels of genetic diversity and to understand the genetic consequences of habitat fragmentation in plants that are native to the tropical dry forest of the Mascarene archipelago. The present study focuses on a tropical dry forest tree, *Foetidia mauritiana* (Lecythidaceae), which is endemic to Reunion and Mauritius.

Foetidia Comm. ex Lam. is a tree genus of the pantropical Lecythidaceae family, which occurs mainly in Madagascar and the Indian Ocean Islands (Prance and Mori 2004; Prance 2008). Out of 18 species in this genus (Prance 2008; Labat et al. 2011), 2 are endemic to the Mascarene Islands: *Foetidia rodriguesiana* F. Friedmann that is confined to forest remnants on Rodrigues (population size ca. 50 individuals), and *F. mauritiana* Lam. that is found in semi-deciduous dry forests on Reunion and Mauritius (overall population size < 1000 individuals; Debize 2007). These species are morphologically similar and differ in relatively minor phenotypic characters (e.g., leaf margins, tepal morphology) (Prance 2008). However, no molecular analysis has ever been conducted on these species to confirm that they are genetically dissimilar.

Foetidia mauritiana was formerly common on Reunion, but it was already rare in the late 19th century (Cordemoy 1895). Populations of this tree species have declined not only with the reduction of primary lowlands forest habitats where human activities were concentrated, but also because *F. mauritiana* was considered to be one of the best timbers during the period of human settlement on the islands (ca. 1660). Nowadays, forest remnants rarely contain more than 10 individuals of *F. mauritiana*, with the exception of 4 localities in the north-west of the island (Figure 1; Supplementary Table S1). Following the IUCN, *F. mauritiana* is critically endangered in Reunion (UICN France et al. 2012), and vulnerable in Mauritius (Page and d'Argent 1997; Walter and Gillett 1998), and *F. rodriguesiana* is endangered in Rodrigues (Walter and Gillett 1998).

Foetidia mauritiana trees are up to 15–20 m tall and thereby occur in the forest canopy. These plants are evergreen and heterophyllous, meaning that they have distinct types of juvenile and adult foliage. The flowering peak is usually observed during the rainy season around February. The flowers are hermaphroditic with numerous stamens and a style of almost equal length (Prance 2008). They present a large amount of pollen and produce nectar and are thus visited by a wide suite of insects including numbers of honey bee, *Apis mellifera unicolor* particularly (Cuénin N, personal observation).

The first aim of our study was to test whether the populations of *Foetidia* spp. found on the 3 Mascarene islands are genetically different, as this would have strong implications for management

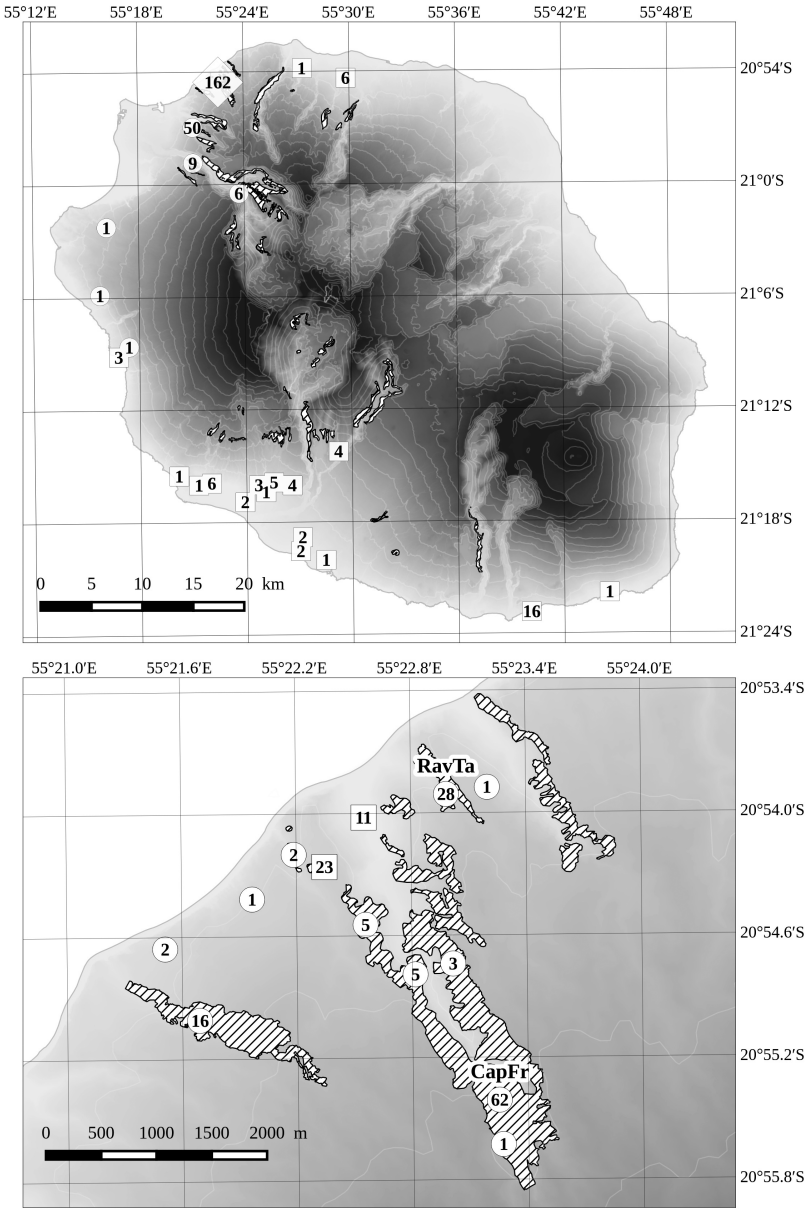

Figure 1. Distribution of *Foetidia mauritiana* trees on Reunion at island scale (top), with a focus on La Montagne massif (down). Wild trees are symbolized by circles, and planted trees by squares. For each locality, numbers within symbols indicate the number of sampled trees. La Montagne sector is symbolized by a diamond on island scale (top). Shading indicates elevation from 0 (sea level) to 3070 m (Piton des Neiges summit) with contour lines indicated every 200 m. The two native populations where fruits were sampled are indicated RavTa (Ravine Tamarins) and CapFr (Cap Francis). *F. mauritiana* on Reunion is only present on the west coast of the island, below an elevation on 800 m. The majority of wild "populations" are nowadays found in the last and highly degraded fragments of native semi-deciduous dry tropical forests (shown as white hashed polygones) (top and down)

strategies. *Foetidia rodriguesiana* is morphologically similar to *F. mauritiana*; however it grows on Rodrigues where *F. mauritiana* is not found (Prance 2008). In a recent study, Martos et al. (2016) showed that most microsatellite loci isolated from *F. mauritiana* on Reunion amplified in individuals of *F. mauritiana* and *F. rodriguesiana* from Mauritius and Rodrigues, respectively. We hypothesized that populations of *F. mauritiana* from different islands should be genetically dissimilar because of limited gene flow across them, with *F. rodriguesiana* as an out-group. We then assessed the levels of genetic diversity and population structure among populations of *F. mauritiana* on Reunion. We hypothesized high genetic differentiation between populations from different islands, but relatively low genetic differentiation among populations on Reunion. Finally, we investigated the genetic characteristics of seed progenies in 2 remnants on Reunion, to understand current patterns of mating and gene flow (mostly via pollen dispersal) in this highly fragmented habitat. Although outcrossing pollination may occur at some frequency, it has been hypothesized that, since there is no spatial isolation between male and female organs at flower scale, the high fruiting rates observed in *F. mauritiana* trees are achieved mainly through selfing (Debize 2007). However, data on plant mating systems in the Mascarene archipelago are limited to certain families (e.g., Sterculiaceae, Humeau et al. 1999; Loganiaceae, Humeau et al. 2003; Rubiaceae, Litrico et al. 2005) and no information has been

provided for *Foetidia* spp. or Lecythidaceae. We hypothesize that the species is self-compatible and that it is mainly reproducing through selfing under such low tree densities.

Materials and Methods

Tree Sampling

We sampled a total of 289 *F. mauritiana* trees in Reunion between 2010 and 2016 (sampling authorized by the local authorities: Parc National de La Reunion, Office National des Forêts, Département de La Reunion, and Conservatoire du Littoral). We then compiled the samples with those collected by Martos et al. (2016) on adult trees of *F. rodriguesiana* on Rodrigues island (n = 30 in 10 localities) and adult trees of *F. mauritiana* on Mauritius (n = 28 in 5 localities).

Unlike in Rodrigues and Mauritius where there remains only a few sparsely distributed trees, *F. mauritiana* in Reunion can be observed in larger forest remnants, smaller stands, and in planted restoration areas. Thus, the number of sampled trees (n = 289) included all wild trees occurring in dry forest remnants on Reunion (n = 196) (67.8%), plus a large representation of the trees grown ex situ on the same island (ex situ conservation; n = 70, 24.2%) (Figure 1). A small proportion of the sampled trees (n = 23, 8%) were trees planted in situ in restoration work. As that class of trees were still saplings at study time, and thus do not participate in gene flow, they have been integrated in the rest of the study in the "ex situ conservation" class for practical reasons (Table 1).

Out of 196 trees sampled in situ, 96.9% were found in fragments where more than 1 individual occurred. Five large patches with more than 6 trees each were found across the entire island and include most of the in situ wild trees (85.7%; Figure 1, Table 1, Supplementary Table S1). They will be designated as "Forest Fragment" in the rest of the study (Table 1, Supplementary Table S1). All lone trees (3.1%) and all trees found in small patches (with less than 6 trees) (11.2%) were computed in the category "Others." Natural establishment is sporadic in *F. mauritiana*, so that we were able to sample only a limited number of saplings (n = 26 plants with a stem diameter at breast height ≤ 3 cm) at one location, Cap Francis (Table 1, Supplementary Table S1). No ambiguity dwells between wild saplings and planted ones due to the fact that all saplings planted during restoration have been tagged.

Seed Sampling and Production of Progenies

We harvested mature fruits on 14 wild *F. mauritiana* trees. Those trees were selected according to the density of conspecific potential pollen donors within the fragment: trees either belonged to 2 fragments where the number of putative pollen donors was high (n = 6 seed trees at Ravine Tamarins; n = 4 seed trees at Cap Francis), or were selected among those that were spatially isolated from any conspecific individual on the island (n = 4 lone trees in 4 distinct localities) (Supplementary Table S1). To avoid immature seeds, fruits of the last fruiting season (March 2015) were selected and collected on the ground at the base of trees, based on their global state of degradation. A total of 1660 fruits (100–200 per seed tree; see details in Supplementary Table S1) were harvested on Reunion between 2015 and 2016.

The fruit of *F. mauritiana* is turbinate (i.e., shaped like a spinning top), indehiscent (remaining closed at maturity), and has 4 ovary locules each containing 0–2 seeds (Friedmann and Scott 1990; Prance 2008). A physical dormancy is caused by an impermeable seed coat (Friedmann and Scott 1990), large seed banks can thus be harvested under adult trees. Each fruit was split with a hammer, and the seeds it contained were extracted from the impermeable matrix with cutting pliers without damaging them. Seed production was estimated by counting the numbers of full seeds per fruit, seed tree, or locality.

We compared seed production using generalized linear model (GLM) with a quasi-Poisson distribution and log link functions. These analysis were performed with the R package *glm2* (Marschner 2011).

Following protocols in Rivière and Schmitt (2004), each full seed was sterilized using a 10% NaCl solution (bleach), sowed on sterilized sand in a room at 25 °C, and watered every 2 days. The germinated seeds were transferred after 1–3 weeks in growing pots and kept in a nursery.

Genotyping of Trees and Seed Progenies

For the 347 adult trees sampled from islands in the archipelago, 1–2 leaves were harvested from each plant and dried in silica gel at 50 °C. For 169 seed progenies grown in the nursery, ca. 100 mg fresh leaf tissue was harvested on each seedling and oven dried at 50 °C. Genomic DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN, Hilden, North Rhine-Westphalia, Germany). We

Table 1. Estimated genetic diversity among *Foetidia mauritiana* trees on Reunion

	N	A_L	A_R	A_P	H_O	H_E	F_{IS}
Cap Francis	62	7.38 ± 1.63	4.36 ± 1.73	3	0.628 ± 0.056	0.649 ± 0.052	−0.040*
Ravine Lataniers	49	7.5 ± 1.66	4.83 ± 1.73	2	0.702 ± 0.059	0.689 ± 0.038	-0.168^{NS}
Ravine Tamarins	28	6.13 ± 1.34	4.30 ± 1.76	0	0.638 ± 0.033	0.644 ± 0.045	0.002^{NS}
Ravine Malheur	16	5.63 ± 0.96	4.14 ± 1.73	0	0.596 ± 0.071	0.643 ± 0.058	−0.010**
Sans-Souci	8	4.88 ± 0.48	4.48 ± 1.13	0	0.672 ± 0.091	0.676 ± 0.044	-0.089^{NS}
Others	31	6.63 ± 1.54	4.47 ± 2.07	2	—	—	—
Ex situ conservation	93	8.38 ± 2.63	4.65 ± 1.86	4	—	—	—
Total/mean	287[a]	6.64 ± 0.53	4.50 ± 1.72	—	0.647 ± 0.028	0.660 ± 0.020	−0.04*

Allelic and genetic diversities were estimated in 5 wild large fragments (n > 6). The category "Others" comprises all wild trees outside these that do not belong to the 5 remnants, and the category "Ex situ conservation" gathers all planted trees. The number of individuals (N) and the number of private alleles (A_P) are presented here, associated with the mean and standard error of the average number of allele per locus (A_L), of the allelic richness estimates by the R package *PopGenReport* (A_R), of the observed (H_O) and expected (H_E) heterozygosity. The values of the fixation index (F_{IS}) are given, with their associated P value: $^{NS}P > 0.05$, $*0.01 < P < 0.05$, $**0.001 < P < 0.01$.

[a]Genetic and allelic diversities were only estimated for 287 trees on 289 sampled, for 2 trees in situ did not amplified.

amplified 9 out of 13 nuclear microsatellite loci that were previously isolated from *F. mauritiana* (Martos et al. 2016): FmCIR29, FmCIR31, FmCIR32, FmCIR43, FmCIR52, FmCIR16, FmCIR57, FmCIR61, and FmCIR11. Although FmCIR31 appeared not to amplify in *F. rodriguesiana*, we selected these 9 loci since they revealed a high degree of polymorphism in the tested tree populations (Martos et al. 2016).

Two mixes of primers were used, based on primer characteristics (Martos et al. 2016), with the first one (Mix1) containing FmCIR29, FmCIR31, FmCIR32, FmCIR43 and FmCIR52, and the second mix (Mix2) containing FmCIR16, FmCIR57, FmCIR61, and FmCIR11. Multiplex PCR was performed in a total volume of 15 µL containing 7.5 µL 2X Type-it Multiplex PCR Master Mix (QIAGEN), 1.5 µL 5× Q-Solution, 0.2 µM each primer, and 20–50 ng template DNA. The thermal cycling protocol was as follows: initial denaturation at 95 °C for 5 min; 28 cycles of denaturation at 95 °C for 30 s, primer annealing at 57 °C for 90 s, extension at 72 °C for 30 s; and final extension at 60 °C for 30 min. PCR products were diluted in HPLC-grade water (1:10), denatured in formamide, and separated on a 16-capillary ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). GeneScan 500 LIZ (Applied Biosystems) was used for sizing alleles in the expected range of 80–300 bp. Allele sizes were estimated using the Microsatellite Plugin version 1.4 implemented in Geneious version 10.0.7 (Biomatters, Auckland, New Zealand).

Genetic Diversity

All pairs of loci were tested for genotypic disequilibrium using a Bonferroni-corrected probability test in Genepop v. 4.5.1 (Raymond and Rousset 1995; Rousset 2008). The frequencies of null alleles for each locus were estimated using Micro-Checker v 2.2.3 (Van Oosterhout et al. 2004). The mean number of alleles per locus (A_L), the number of private alleles (A_P), and observed and expected heterozygosity over all loci (H_O and H_E) were calculated using GenAlEx v. 6.5 (Peakall and Smouse 2006). The fixation index of Wright (F_{IS}) was calculated according to Weir and Cockerham (1984) and statistically tested based on heterozygosity deficiency hypothesis (Hardy–Weinberg exact tests) using Genepop v. 4.5.1 (Raymond and Rousset 1995; Rousset 2008), with 100 batches of 1000 iterations sampled along Markov chains. Allelic richness was estimated using the rarefaction method described by El Mousadik and Petit (1996) and implemented by the R package *PopGenReport* (Adamack and Gruber 2014) to make direct comparisons between populations regardless of their size. These estimates were calculated for each large forest fragment independently (Table 1), then for each life stage (i.e., adults, saplings and progenies) in 2 fragments (Table 2). Moreover, the allelic diversity preserved ex situ was estimated from 70 trees in all ex situ localities and 23 planted in restoration work. Similarly, allele diversity was estimated for all small fragments clustered with isolated wild trees (Table 1).

Genetic Structure

Genetic differentiation was first investigated between islands; that is, *F. mauritiana* on Reunion versus on Mauritius, and both populations versus *F. rodriguesiana* on Rodrigues. Second, genetic differentiation was investigated among *F. mauritiana* populations on Reunion. For this analysis, we only considered 5 large forest fragments (Table 1). The fixation index among sampling locations (F_{ST}) was estimated with Weir and Cockerham's (1984) theta (θ) for all populations and for each pair of locations using Genepop5.1 (Raymond and Rousset 1995; Rousset 2008). A permutation test was used to assess statistical significance using FSTAT v. 2.9.3 (n = 999; Goudet 2001). Theta estimates were then compared with the standardized version of Nei's G_{ST}, G'_{ST} (Hedrick 2005) computed by dividing G_{ST} by the maximum value it can obtain given the observed within-population diversity to ease interpretation (Nei 1973; Hedrick 2005, Meirmans and Hedrick 2011).

This index was estimated between each pair of the 5 wild fragments studied with the *mmod* R package (Winter 2012).

Genetic variation in the 5 main forest fragments in Reunion trees was first investigated using the Bayesian clustering based software Structure 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). Spatial differentiation was assessed based on Monte Carlo Markov chains of length 10 000, sampled after burn-in (n = 10 000), following Evanno et al. (2005). We tested a range of K values from 1 to the number of sampled localities (5) plus 5. The amount of variation of the likelihood for each K (ΔK) was quantified using 100 runs for each data set. We selected the K value that best describes the data using the maximum value of ΔK as proposed by Evanno et al. (2005).

Discriminant analysis of principal components was then performed (DAPC; Jombart 2008; Jombart et al. 2010). DAPC optimizes the genetic variance between groups while minimizing the variance within groups (Jombart 2008). Here, the main advantage of DAPC over the previous approach is that it does not make any hypothesis based on the populations being at Hardy–Weinberg equilibrium. DAPC analysis was performed using the *adegenet* package of R (Jombart 2008; Jombart and Ahmed 2011).

Mating Systems

Mating systems were inferred from genotyping 168 progenies of *F. mauritiana* (see *Seed sampling and production of progenies* subsection), produced by 14 mother plants from 2 forest remnants on Reunion (Ravine Tamarins, n = 89 for 6 trees, and Cap Francis, n = 70 for 4 trees), or in lone trees (n = 9 progenies for 4 trees). These

Table 2. Pairwise genetic differentiation of *Foetidia mauritiana* between 5 forest fragments on Reunion

	Cap Francis	Ravine Lataniers	Ravine Tamarins	Ravine Malheur	Sans-Souci
Cap Francis	—	0.090	0.041	0.043	0.080
Ravine Lataniers	0.042**	—	0.060	0.084	0.072
Ravine Tamarins	0.024**	0.022**	—	0.039	0.053
Ravine Malheur	0.024**	0.039**	0.022**	—	0.088
Sans-Souci	0.029**	0.025**	0.010*	0.032*	—

Values below the diagonal depict θ, or Wright's index of genetic differentiation F_{ST} estimate (Weir and Cockerham 1984). Values above the diagonal in gray correspond to G'_{ST}, Hedrick's standardized G_{ST} (2005).

*$P < 0.05$, **$P < 0.01$.

limited numbers of progenies were obtained after examination of several hundreds of fruits in each locality (Ravine Tamarins n = 756, Cap Francis n = 503, and lone trees = 401; total = 1660), thus revealing the low reproductive success of this tree species on Reunion (see *Results* section).

The analysis of mating systems was performed using MLTR v. 3.2 (Ritland 1990; Ritland 2002). This program allows inferences of mating systems from progeny arrays (dominant or co-dominant genetic markers), using maximum likelihood estimation of several inbreeding parameters: multilocus population outcrossing rate (tm), single-locus population outcrossing rate (ts), and correlation of paternity, that is, fraction of siblings that share the same father ($rp(m)$) (Ritland 1990; Ritland 2002). Standard errors of estimates were calculated using bootstrapping (n = 999 replicates). From these parameters, we could estimate selfing rate ($1 - tm$), biparental inbreeding, that is, mating among relatives ($tm - ts$), and the number of pollen donors ($n = 1 / rp(m)$) (Ritland 1990; Ritland 2002).

Paternity Analysis

To estimate pollen dispersal in fragmented populations of *F. mauritiana* on Reunion, 2 paternity analyses were conducted. At first, a maximum likelihood method of parentage analysis using all loci was applied using Colony v. 2.0.6.4 (Jones and Wang 2010). This program is based on a full-likelihood method, at "family" or pedigree scale (Jones and Wang 2010; Harrison et al. 2013).

Firstly the data set is divided in 3 categories: OFS (Offspring Sample, with full/half siblings or not related), CFS (Candidate Father Sample) and CMS (Candidate Mother Sample). Then, a maximum-likelihood method is used to discriminate family clusters that group together siblings and potential parents. As this program allows the simultaneous inference of parentage and sibship, it accommodates genotyping errors (Jones and Wang 2010; Jones et al. 2010; Harrison et al. 2013).

Secondly, we conducted an exclusion method, by inferring the paternal haplotype knowing the mother tree genotype for each progeny. We eliminated all candidate fathers that could not produced a gamete with that inferred parental haplotype. In the case of more than one possible pollen donor, we considered all candidates as equally probable. We calculated exclusion probabilities based on observed allelic frequencies following Hamilton (2009). These approaches could be carried out on 165 genotyped progeny arrays in total at Cap Francis (n = 70), Ravine Tamarins (n = 86), or in lone trees (n = 9). Whenever more than one pollen donor were inferred for a progeny, we only retained paternal assignments showing more than 60% probability. When no known pollen donor could be inferred or when the associated assignment probability was too low, the corresponding pollination event was labeled "cryptic."

Results

Archipelago Genetic Differentiation

Genetic differentiation was first investigated among populations from different islands using a DAPC (Figure 2). Individuals from each island formed a distinct group, underlining the existence of genetic differentiation between islands. As could be expected, the cluster formed by *F. rodriguesiana* differs more from the 2 others than the cluster of *F. mauritiana* in Reunion does from the one in Mauritius. However, although *F. mauritiana* is present on those 2 islands, 2 genetically isolated groups are highlighted here, 1 for each island.

Genetic Diversity

We estimated the levels of genetic diversity among 287 *F. mauritiana* trees occurring in dry forest remnants on Reunion. The average number of alleles per locus was medium to high over all localities, ranging from 4.88 (Sans-Souci) to 7.38 (Cap Francis) (Table 1). The unbiased allelic richness (A_R) ranged from 4.14 (Ravine Malheur) to 4.83 (Ravine Lataniers), with an average value of 4.50. Only 2 forest fragments revealed private alleles, with their number ranging from 2 (Ravine Lataniers) to 3 (Cap Francis). Nonetheless, when ex situ individuals and lone trees are added to the analysis, it appears

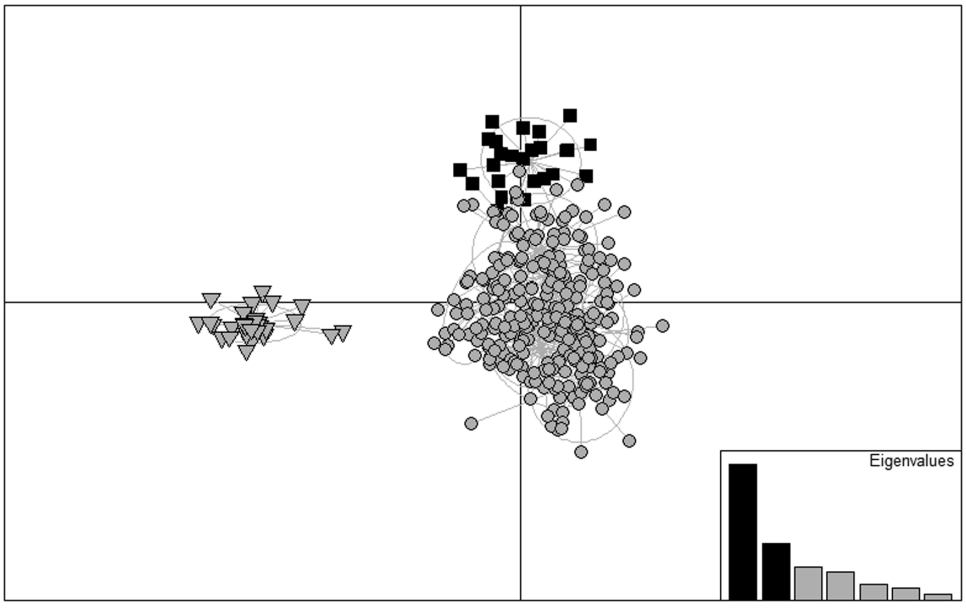

Figure 2. Discriminant analysis of principal components realized on adults of *Foetidia* sp. on three islands of the Mascareignes (Indian Ocean) for a number of optimum clusters given by *adegenet* (K = 10) (Jombart 2008; Jombart and Ahmed 2011). Individuals from Rodrigues (n = 30) are represented by grey inverse triangle, those from Reunion (n = 287) by grey circles, and those from Mauritius (n = 28) by black squares.

that the largest average number of alleles per locus is obtained by ex situ trees (A_R = 8.38). Furthermore, ex situ and lone trees present, respectively, 4 and 2 private alleles.

There was no significant difference between the observed and expected heterozygosities in 3 of the 5 localities. The fixation index (F_{IS}) ranged from –0.168 (Ravine Lataniers) to 0.002 (Ravine Tamarins) and was only significantly different from 0 in Cap Francis and Ravine Malheur (Table 1). We found moderate to high levels of genetic diversity in remnant populations of *F. mauritiana* on Reunion (e.g., Van Etten et al. 2015; Guidugli et al. 2016; Moracho et al. 2016), and unexpectedly in ex situ trees as well. Moreover, 3 locations, Ravine Lataniers, Ravine Tamarins, and Sans-Souci, showed significant no deviation between observed and expected heterozygosity, which was consistent with these populations being at Hardy–Weinberg equilibrium.

Genetic Structure

Population structure was then investigated among the forest fragments on Reunion. According to θ, or Wright's index of genetic differentiation F_{ST} estimate (Weir and Cockerham 1984), the genetic differentiation was low for each population pair (0.010–0.042), but was significantly different from 0 in all cases ($P < 0.05$, Table 2). These results were consistent with those provided by G'_{ST}.

Inferring the genetic structure among 5 large patches using 2 methods (Structure software and DAPC) provided different optimal numbers of clusters (Figure 3a,b). Following the method described in Evanno et al. (2005), we found an optimum of 5 clusters (K = 5, with ΔK_{max} = 0.45), whereas the methods implemented in the R package adegenet (Jombart 2008; Jombart and Ahmed 2011) provided an optimum of 6 clusters. However, in both cases, poor spatial structuring in *F. mauritiana* population on Reunion was detected (Figure 3a,b). No correlation can be inferred between the locality of a tree and its inferred genetic cluster. Looking at those results, it appears that *F. mauritiana* on Reunion does not show genetically different populations, and can be considered as one homogeneous population.

Seed Production

Full seed production for 14 seed trees in different localities (Cap Francis, n = 4, Ravine Tamarins, n = 6, and lone trees, n = 4) highlighted significant variation between lone trees and grouped trees, as lone trees produced fewer full seeds (GLM quasi-Poisson distribution and log link function, df = 2, t = –4.15, $P < 10^{-3***}$). However, this production does not differ between seed trees from Cap Francis (n = 250) and Ravine Tamarins (n = 305) (GLM quasi-Poisson distribution and log link function, df = 2, t = 1.04, $P = 0.30^{NS}$).

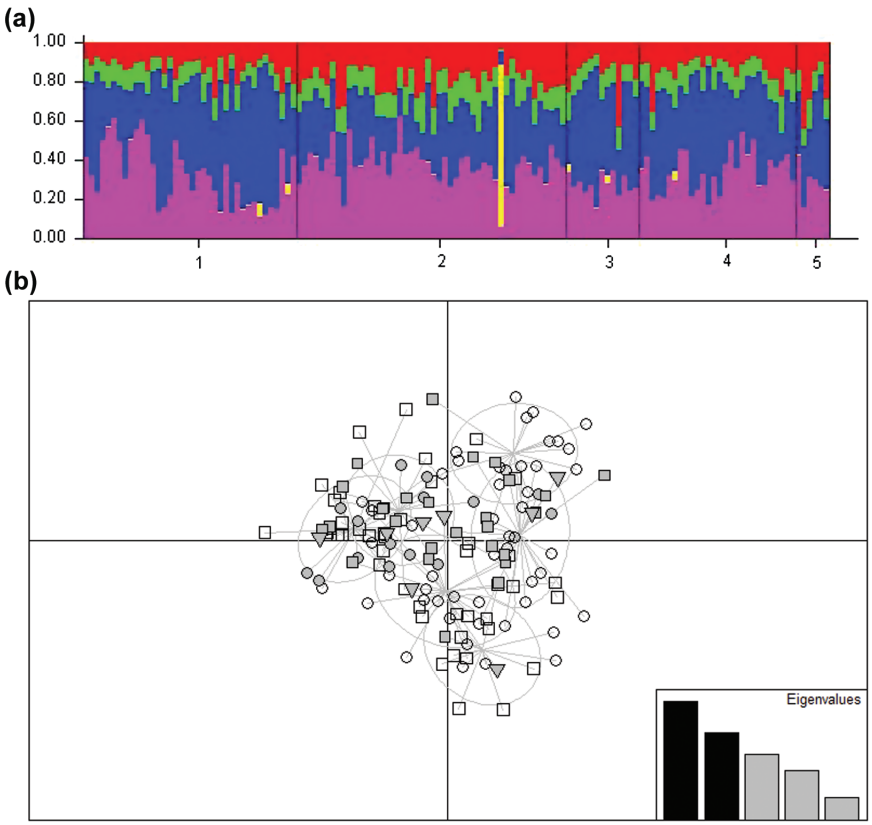

Figure 3. (**a**) Population structure inferred using the Structure software from genotype data in 155 wild trees of *Foetidia mauritiana* on Reunion. Each individual is represented by a single vertical line broken into K colored segments (here, assumed K = 5), with lengths proportional to each of the K inferred clusters. The numbers (1–5) correspond to the different localities, with 1: Cap Francis, 2: Ravine Lataniers, 3: Ravine Malheur, 4: Ravine Tamarins, and 5: Sans-Souci. The individual of locality 2 (Ravine Lataniers) that is mainly composed of yellow segment only amplified for 2 loci on 8. (**b**) Discriminant analysis of principal components (DAPC) from genotype data in 155 wild trees of *Foetidia mauritiana* on Reunion. The number of optimum clusters (gray ellipses) was given by *adegenet* (K = 6) (Jombart 2008; Jombart and Ahmed 2011). Individuals from the same locality are represented by the same symbol.

Empty seeds were the majority in *F. mauritiana* fruits (average of 7 empty seeds/fruit, compared to less than 1 (0.37) full seed/fruit). Their production shows the same pattern as full seeds production. Whereas no significant difference exists between Cap Francis (n_{ES} = 3086) and Ravine Tamarins (n_{ES} = 5591) (2-sample Kolmogorov–Smirnov test, D = 0.08, P = 0.06^{NS}), lone trees show less empty seeds (n_{ES} = 2997) (GLM quasi-Poisson distribution and log link function, df = 2, t = 25.93, $P < 10^{-3}$***). Interestingly, it appears that variation also exists among lone trees. For instance, 2 lone seed trees presented more empty seeds than the 2 others (2-sample Kolmogorov–Smirnov test, D = 0.73, $P < 10^{-3}$***), showing empty seed amounts equivalent to those found in grouped trees.

Genetic Diversity among Progenies

We compared genetic diversity across adult trees, saplings, and their progenies in 2 forest fragments, in Cap Francis and Ravine Tamarins. The average number of alleles (A_L) and the unbiased allelic richness (A_R) slightly decreased from adults to saplings (in Cap Francis), or from adults to their offspring (at both sites) (Table 3). Interestingly, the number of private alleles dropped from 9 in adults to 1 in progenies in Cap Francis, and from 5 to 2 in Ravine Tamarins, suggesting that only a small fraction of the trees contributed to reproduction in these populations. Furthermore, 1 and 2 private alleles were found among progenies in Cap Francis and Ravine Tamarins, respectively, indicated that allopatric pollen flow had occurred in both populations.

The observed heterozygosity was lower in progeny than in adults for both populations (0.492 against 0.651 in Cap Francis; 0.554 against 0.638 in Ravine Tamarins, Table 3). The fixation index (F_{IS}) was much higher among progenies than among adults, and was significantly different from 0 only in progenies, at both sites ($P < 0.001$). The progeny arrays in Cap Francis and Ravine Tamarins thus showed strong deviation from Hardy–Weinberg equilibrium, opposite to adult trees (Tables 1 and 3). Both sites also revealed a highly increasing homozygosity among recent offspring.

Mating Systems and Pollen Dispersal

The analysis of mating systems using MLTR (Ritland 1990; Ritland 2002) revealed selfing rates (1 – *tm*) higher than 0.50 in both populations (0.69 in Cap Francis and 0.53 in Ravine Tamarins), and close to 1.00 in lone trees (Table 4). The overall selfing rate was then 0.63.

These results were similar with those obtained through the analysis of paternity for progenies. The 2 approaches used for paternity analysis, either based on maximum likelihood in Colony (Jones and Wang 2010) or the exclusion paternity assignment method, yielded very similar results (Table 5, and Supplementary Table S3 for markers exclusion probability). Because the maximum likelihood approach uses a powerful probabilistic framework that integrates uncertainy due to genotyping errors, here, we present results for paternity obtained under maximum likelihood inference. Each mother tree was rightly inferred by Colony with a 100% confidence, and we managed to attribute a pollen donor for 133 progenies on 165 (80.6%) with more than 60% confidence. Selfing rates derived from paternity analysis were 0.67 at Cap Francis, 0.56 in Ravine Tamarins, and 0.89 in lone seed trees (Table 5). Biparental inbreeding rates was estimated as close to null by MLTR, ranging from –0.063 (Ravine Tamarins) to 0.037 (Cap Francis) (Table 4). This result indicates that mating between related trees does not, or rarely, occurs in situ.

Outcrossing rate estimates from MLTR (*tm*) were around 0.38 in all trees combined, ranging from 0.32 in Cap Francis to 0.47 in Ravine Tamarins, with a minimum rate of less than 0.01 for lone trees (Table 4). These differences were also found with Colony (Jones and Wang 2010) between the outcrossing rates in Cap Francis and Ravine Tamarins but with weaker values, especially in Cap Francis (respectively, 0.06, 0.27, and 0.00) (Table 5). In fact, MLTR estimates are based and calculated on alleles frequencies in the progenies. In Colony (Jones and Wang 2010), those estimates are performed after inferring progenies paternity. The difference between estimates given by those 2 methods is thus explained by the unassignment rate (or here the cryptic pollen flow rate) by Colony (Jones and Wang 2010) in the offspring, close to 0.27 and 0.17 for Cap Francis and Ravine Tamarins, respectively (Table 5). Therefore, although selfing is high in fragmented populations of *F. mauritiana*, both methods show that outcrossing pollination may represent a significant part of the species reproduction.

Pollination distances calculated from the outcome of paternity analysis showed that pollen dispersal occurs at distances from 15.55 m (Ravine Tamarins) to 296.14 m (Cap Francis), with an average value of 110.57 m in Ravine Tamarins and 245.81 m in Cap Francis (Table 5).

Discussion

Preserved Genetic Diversity but High Selfing in Remnants

A mixed reproductive system, with high level of selfing (>50%), has been detected in fragmented populations of *F. mauritiana* on Reunion Island. This finding is consistent with other Lecythidaceae species in South America that generally present a mixed reproduction system, but with high outcrossing mating levels (Mori et al. 2010; Guidugli

Table 3. Comparison of genetic diversity in 3 life stages of *Foetidia mauritiana* between in 2 forest fragments on Reunion.

Locality	Life stage	N	A_L	A_R	A_P	H_O	H_E	F_{IS}
Cap Francis	Adults	42	7.00 ± 1.46	6.24 ± 3.43	9	0.651 ± 0.061	0.649 ± 0.056	0.016^{NS}
	Seedlings	20	5.13 ± 0.92	5.63 ± 3.02	3	0.606 ± 0.060	0.624 ± 0.044	0.035^{NS}
	Progenies	70	5.00 ± 0.60	4.37 ± 1.27	1	0.492 ± 0.061	0.602 ± 0.044	0.191***
Ravine Tamarins	Adults	28	6.13 ± 1.34	4.40 ± 1.85	5	0.638 ± 0.033	0.656 ± 0.046	0.013^{NS}
	Progenies	84	5.75 ± 1.03	5.02 ± 2.05	2	0.554 ± 0.043	0.642 ± 0.052	0.13***

The number of individuals (N) and of private alleles (A_P) are depicted here, associated with the mean and standard error of the average number of allele per locus (A_L), of the allelic richness estimates by the R package *PopGenReport* (A_R), of the observed (H_O) and expected (H_E) heterozygosity. The values of the fixation index (F_{IS}) are given, with their associated P value: $^{NS}P > 0.05$, ***$P < 0.001$.

Table 4. Maximum likelihood inference of mating systems in 2 population remnants and in lone trees on Reunion

	Total	Cap Francis	Ravine Tamarins	Lone trees
Number of seed trees/progenies	14/165	4/70	6/86	4/9
tm (±SE)	0.375 ± 0.062	0.315 ± 0.087	0.467 ± 0.103	0.001 ± 0.362
ts (±SE)	0.363 ± 0.067	0.279 ± 0.086	0.530 ± 0.129	0.001 ± 0.364
tm – *ts* (±*SE*)	0.012 ± 0.021	0.037 ± 0.013	–0.063 ± 0.044	0.000 ± 0.059
1 – *tm*	0.625	0.685	0.533	0.999

The multilocus and single-locus outcrossing rates (*tm* and *ts*, respectively), the mating rate among relatives (*tm* – *ts*) and the selfing rate (1 – *tm*) are depicted.

Table 5. Paternity analysis in 2 populations remnants and in lone trees on Reunion

	Cap Francis		Ravine Tamarins		Lone tree	
Number of seed trees/progenies	4/70		6/86		4/9	
Selfing	67.1%	62.3%	56.2%	54.8%	88.9%*a*	100.0%
Outcrossing	5.7%	4.3%	26.9%	29.8%	0	0
Cryptic pollen flow	27.2%	33.4%	16.9%	15.4%	11.1%*a*	0
Mean ± SD	245.8 ± 18.0	213.2 ± 110.2	110.6 ± 90.4	85.5 ± 77.5	0	0
Min.	219.1	48.3	15.6	15.6	0	0
Max.	296.1	296.1	293.8	293.8	0	0

The selfing, the outcrossing rate, the rate of cryptic pollen flow and pollination distances resulting of paternity analysis are shown. In each case, first column of values has been calculated using maximum likelihood method implemented by Colony (Jones and Wang 2010) for paternal assignments >0.60 probability. Second column show results obtained with an exclusion method, realized without respect of the expected frequency for the inferred parental haplotypes in the case of more than one possible pollen donor.

[a]These values are not, respectively, equal to 100% and 0% due to one paternal assignment lower than 0.60 probability.

et al. 2016). Those high levels of outbreeding in Neotropical species are likely explained by the spatial structure of the flower that tends to inhibit self-pollination (e.g., *Couroupita* spp.), rather than by an incompatibility system (Mori et al. 2010; Guidugli et al. 2016). In *F. mauritiana*, such spatial separation between the anthers and the stigma does not exist. Although strong presumptions exist about self-compatibility of *F. mauritiana* due to the fact that lone trees usually produce many fruits (Debize 2007), our attempts to experimentally assess its breeding system did not succeed as the flowers did not survive emasculation (data not shown). Experiments like pollen manual supplementation could provide further evidence on that point (e.g., Seltmann et al. 2009).

In spite of high selfing, we found no lack of heterozygosity, associated with an elevated genetic diversity among adult trees in the studied forest remnants. High adult diversity combined with observed selfing both in mating system and paternity analysis seem to support inbreeding depression. Higher mortality of homozygotes during early life stages would leave more trees alive as adults that originated from biparental matings (Charlesworth and Charlesworth 1987). Another possibility is that adults exclusively constitute relicts of populations from before forest fragmentation, with overall very weak recruitment, or even an absence of it.

The persistence of high genetic diversity in highly fragmented populations as documented here is described in many studies as a paradox of habitat fragmentation. It is generally admitted that in cases of long-living species and recent fragmentation, not enough time may have passed for drift to reduce genetic diversity (Lowe et al. 2005; Kramer et al. 2008; Bacles and Jump 2011). Genetic erosion is expected to be less severe than in short-lived species (Young et al. 1996; White and Boshier 2000), as several generations have to pass for a decrease in diversity to be detected (Aguilar et al. 2008; Kramer et al. 2008; Bacles and Jump 2011). In the case of *F. mauritiana*, adult trees are presumed to be mostly contemporary to the fragmentation of their natural habitat, assuming that the largest individuals could be several centuries old. It is thus likely that adult genetic diversity reflects ancient random mating in highly genetically diverse populations, occurring in an untouched habitat.

High Mortality in Early Life Stages

Because early life stages are literally absent in situ and fruits were often infertile, population dynamics of *F. mauritiana* are strongly limited by the lack of recruitment (e.g., Isagi et al. 2007; Van Etten et al. 2015). Indeed, a high proportion of empty seeds was observed in sampled fruits of all individuals. Geographically isolated individuals with 100% inbreeding mating rate showed very low viable seed production rate. Low seed viability has been described for other tree species and can be related to self-incompatibility (Seltmann et al. 2009) or inbreeding depression (Ritland 1996; Cascante et al. 2002; e.g., Lowe et al. 2005; Isagi et al. 2007; Van Etten et al. 2015). In addition, seedlings impacted by biparental inbreeding, and in an extreme case by selfing, may have a lower survival than those produced by outcrossed mating. This process can lead to the over representation of outcrossed seedlings around a given seed tree compared with inbred ones (Isagi et al. 2007).

Populations that have been recently fragmented (e.g., by habitat degradation/destruction) commonly experiment incompatibility and inbreeding depression (Moracho et al. 2016). This pattern is consistent with the recent anthropogenic fragmentation of the natural populations of *F. mauritiana* in Reunion. However, in cases of inbreeding depression, homozygosity should decrease through

generations, and not increase as is observed here. We found that most seed production happens to occur by selfing. One can hypothesize that even with high mortality of inbred individuals, the outcrossing rate is so low that it results in a global increase in inbred individuals frequency. Thus, inbreeding depression stands as a plausible hypothesis, but has to be further explored.

Furthermore, with the lack of clear evidence for self-incompatibility of *F. mauritiana*, it is difficult to discriminate between the effects of these 2 phenomena on early life stages mortality (Porcher and Lande 2005). Moreover, the special case of one isolated tree located in the humid forest of Mare-Longue showing a production of full-seeds equivalent to trees within patches suggests that some individuals may be resilient to inbreeding, and/or that the compatibility system is affected by environmental conditions (all other trees are found in semi-dry areas on the island) (e.g., Balogh and Barrett 2018). Self-fertilization may be possible in the case of a weaker expression of S-alleles. This evidence suggests that *F. mauritiana* could present a variation in self-incompatibility expression, which may occur with a change in the density of individuals (Fuchs et al. 2003; Busch and Schoen 2008) and/or inbreeding depression (Porcher and Lande 2005; Balogh and Barrett 2018).

Current Lack of Gene Flow within/between *F. mauritiana* Patches

When comparing different life stages, a decrease of genetic diversity is observed in terms of decreasing allele richness and increasing homozygosity, due to higher inbreeding rates in progenies compared with adults. However, no spatial differentiation between adult trees has been found. As previously said, adult trees are presumed to result from mating events mostly contemporary to fragmentation of their habitat, and must have been weakly affected by this phenomenon. This absence of spatial genetic structure underlines high levels of gene flow in the past, possibly via both pollen and seed dispersion. First, the lack of biparental inbreeding observed, or the scarcity of sympatric adult trees that are genetically related, suggests that fruit dispersal occurred at long distance, presumably thanks to efficient seed dispersers, such as parrots or tortoises. Historical reports also witness high rates of bat visitation (*Pteropus* sp.) during flowering episodes of *F. mauritiana* (de Lanux 1772; Fleming et al. 2009). Bat pollination may have commonly occurred across the species range. This type of long range pollination can compensate isolation-by-distance effects between populations, and favor outcrossed pollen flow between populations, as often seen in others systems (Young et al. 1996; Nason and Hamrick 1997).

In a fragmented habitat, as distances between patches increase, gene flow generally decreases which further increases differentiation through higher inbreeding. Moreover, after suffering a drastic decrease in population size, differentiation between patches is generally believed to increase rapidly through genetic drift (Barrett and Kohn 1991; Ellstrand and Ellam 1993; Young et al. 1996). Meanwhile, some studies suggest that some species may be resistant to fragmentation and habitat degradation thanks to high levels of relictual diversity within populations, which seems to be the case for *F. mauritiana*, and the existence of long-distance gene flow across them (Hamrick 2004; Kramer et al. 2008; Guidugli et al. 2016).

Generally, pollen dispersal can be under-evaluated when only considering sympatric trees as potential donors (Kramer et al. 2008). Here, however, exhaustive sampling of trees on Reunion showed that gene flow between patches of *F. mauritiana* seems to have been severely disrupted and might be not functional anymore. Scarcity of gene dispersal events between patches could relate to the disappearance of seed dispersal and/or pollen flow. In the first place, the complete loss of potential seed dispersers (parrots, tortoises) soon after human settlement on the island could have decreased or stopped gene flow resulting from seed dispersal between patches (e.g., Wotton and Kelly 2011). However, poor archival clues exist about interactions between *F. mauritiana* and those extinct fruit dispersers. The seeds could have always been gravity-dispersed, and the species' genetic structure mainly shaped by inbreeding depression.

Especially in the case of self-incompatibility, a tree's mating system could be particularly sensitive to changes in pollination altering the ratio of outcrossed to self-pollen on stigmas (Fuchs et al. 2003; Johnson et al. 2004; Petit and Hampe 2006; Eckert et al. 2010). Here, as pollen dispersal across the distances separating patches (min. 255 m; max. 3237 m) scarcely occurs (max. 296.1 m in Cap Francis), pollination shift is another likely cause for the lack of gene flow between *F. mauritiana* patches (e.g., Johnson et al. 2004; Van Etten et al. 2015). Today, *Pteropus* sp. are reported as extinct on Reunion. Most flower visitation is due to Hymenoptera, essentially *Apis melliferra*, and occasionally by beetles and the Reunion gray white-eye (*Zosterops borbonica*) (Cuénin N, personal observation). Honey-bee behavior on flowers results in high levels of self-pollination through geitonogamy (e.g., Fuchs et al. 2003; Van Etten et al. 2015), and could also induce a "pollen pollution" on stigmas due to the simultaneous flowering of other species (Westerkamp 1991). Geitonogamy might have existed before fragmentation, but due to higher densities in *F. mauritiana* populations, honey-bee foraging on *F. mauritiana* might have also allowed stronger pollen flow between individuals, and on a large timescale, contributed to a genetically homogenized population. This pattern is consistent with the historical description of *F. mauritiana* being abundant along the West coast of Reunion under 800 m, described in the late 19th century as "a common tree in the past, although it has become rare" (Cordemoy 1895). In opposition to some studies (e.g., Visscher and Seeley 1982; Dick et al. 2003), increasing distances between patches of *F. mauritiana* have not been compensated by bees, leading pollen flow to be restricted only to small distances. The domestication of *A. melliferra* on the island may also have impacted its population size, and consequently its visitation frequency on flowers as well. Nowadays, pollen cloud is likely composed of self pollen, which may explain the low seed-set recorded (Quesada et al. 2004; Lowe et al. 2005; Seltmann et al. 2009; Lagache et al. 2013).

Implications for Conservation and Restoration, and the Role of Ex Situ Living Collections

Ex situ conservation trees represent a major part of the Reunion population of *F. mauritiana*. Although these trees are generally found in gardens, and so are traditionally seen as minor actors in the survival of the species in situ, our results showed that they can be considered as an actual bank of genetic diversity. Firstly, ex situ trees are representatives of the diversity existing in situ. When ex situ trees and lone trees were not considered in the analysis, the number of private alleles increased in every population (data not shown). Thus, we assume that most of the fruits that were used to produce ex situ trees came from the studied fragments, especially for the recently planted ones. We presume that the last natural fragments of the species remain endangered and extremely sensible to habitat degradation, so that ex situ trees with lower threats could represent a chance of restoring the diversity of genotypes that could be lost in wild fragments such as Ravine Tamarins or Sans-Souci.

Secondly, when considering all sampled trees, the largest number of private alleles was found in ex situ individuals. It remains possible that these trees are progeny from now dead trees or rather unlikely, from unknown living seed trees that were not sampled in this study. Nevertheless, the high number of private alleles involved here clearly underlines their role as the last representatives for these alleles. These trees or their progenies could be also reintroduced in situ to extend the diversity existing in wild patches of *F. mauritiana* on Reunion.

As a dry forest canopy structuring species of Mascarene Islands, *F. mauritiana* is a key species in dry forest restoration, not only on Reunion but also on Mauritius, Rodrigues island (for *F. rodriguesiana*), and even in other parts of the Southwest Indian Ocean region (e.g., *F. comorensis* in the Comoros, *F. delphinensis* in Madagascar) (Labat et al. 2011). This study offers guidelines for restoration managers, and practical tools to be employed in that purpose. A main result is the pollination distances highlighted in this study, which would have to be taken into account when reintroducing individuals in situ to promote outcrossed pollen stepping-stones flux between fragments, as no spatial structuration is found at island scale. However, reintroduction in Reunion of genotypes from Mauritius must be avoided. The population of Reunion and Mauritius are genetically differentiated, which gives evidence for a lack of gene flow between these islands. In case of genetic differentiation, promoting the introduction of nonlocal genotypes can lead to unadapted genotypes, or outcrossing depression, which is reduced fitness of hybrids (Hufford and Mazer 2003; Tallmon et al. 2004; Falk et al. 2006).

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

- Sampling characteristics and sampling size of fruits uploaded as Supplementary Material S1
- Primers sequences uploaded as Supplementary Material S2
- Microsatellite genotypes of adults trees sampled in the Mascarenes archipelago and of studied seedlings: Dataverse https://dataverse.org/

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Author Contributions

F.M., B.R., and E.R. designed the work. E.R., G.L., N.C., and F.M. collected the data. N.C., F.M., and O.F. analyzed and interpreted the data. N.C., F.M., B.R., and O.F. wrote the article. All authors approved this submission.

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Data Accessibility

We have deposited the primary data underlying these analyses as follows: Dataverse doi:10.18167/DVN1/EHWIGR

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