Late Miocene origin and recent population collapse of the Malagasy savanna olive tree (*Noronhia lowryi*)

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ABSTRACT

Debates regarding the origin of tropical savannas attempt to disentangle the role of people, biotic, and abiotic factors. Understanding savanna origins remains essential to identifying processes that gave rise to habitat mosaics, particularly those found in the Central Plateau of Madagascar. Documenting the evolutionary history and demography of native trees occurring in open habitats may unravel footprints left by past and recent environmental changes. We conducted a population genetic analysis of an endangered Malagasy shrub (*Noronhia lowryi*, Oleaceae) of the Central Plateau. Seventy-seven individuals were sampled from three sites and genotyped at 14 nuclear and 24 chloroplast microsatellites. We found a highly contrasting nuclear and plastid genetic structure suggesting that pollen-mediated gene flow allows panmixia, while seed-based dispersal may rarely exceed tens of meters. From a phylogeny based on full plastomes, we dated the surprisingly old crown age of maternal lineages back to ~6.7 Mya, perhaps co-occurring with the global savanna expansion. In contrast, recent demographic history inferred from nuclear data shows a bottleneck signature ca. 350 generations ago, likely reflecting an environmental shift during the Late Pleistocene or the Holocene. Ancient *in situ* adaptation and recent demographic collapse of an endangered woody plant highlight the unique value and vulnerability of the Malagasy savannas.

ADDITIONAL KEYWORDS: clonal growth – gene flow – human impact – Madagascar – Malagasy olive tree – *Noronhia lowryi* – Oleaceae – savanna.

INTRODUCTION

Tropical savannas can be defined as open canopy environments, with variable tree cover and a continuous grassy ground layer (mostly C₄) maintained at a climax state by fire regimes and/or herbivores (Bond, 2008; Lehmann *et al.*, 2009; Archibald *et al.*, 2020). The contribution of anthropic versus natural factors to the origin and distribution of this habitat remains unclear across the world, and especially so in Madagascar. While the dominant narrative long assumed a major role for human-driven deforestation as a driver of the rise of open canopy environments dominated by grasses (Perrier de la Bâthie, 1921; Koechlin *et al.*, 1974; Burns *et al.*, 2016), there is accumulating evidence for an older expansion of Malagasy savannas and the associated fires since the Miocene (Bond *et al.*, 2008; Vorontsova *et al.*, 2016; Hackel *et al.*, 2018; Solofondranohatra *et al.*, 2018). Furthermore, Quaternary palynological records support the existence of open habitats long before early human arrival (Matsumoto and Burney, 1994; Gasse and Van Campo, 1998, 2001). Fossil records also suggest that a few extinct endemic grazers – giant tortoises, pygmy hippopotamuses, and giant lemurs – may have played a role in the dynamic of Malagasy savannas (e.g. Burney *et al.*, 2004; Virah-Sawmy *et al.*, 2010; Pedrono *et al.*, 2013; Goodman and Jungers, 2014; Godfrey and Crowley, 2016; Godfrey *et al.*, 2016; Samonds *et al.*, 2019). Their demise in the last millennia and the rise of pastoralism ca. ~1000 y BP may have deeply impacted ecosystem function (Johnson, 2009; Crowley, 2010; Veldman, 2016; Vorontsova *et al.*, 2016; Hansford *et al.*, 2018; Godfrey *et al.*, 2019). Together with long-term pastoralism, recent dramatic impacts on the Malagasy landscapes due to human driven deforestation are now well documented and undoubtedly played a major role in the more recent expansion of open habitats (Harper *et al.*, 2007; Dewar, 2014; Waeber *et al.*, 2016; Vieilledent *et al.*, 2018).

A deeper understanding of savanna origins remains essential to our understanding of habitat mosaics (e.g. savannas, gallery and riparian forests and larger forest blocks) in Madagascar. Such a landscape structure may have favored population isolation and divergence in numerous forest organisms, such as lemurs, chameleons, geckos or plants (e.g. Quéméré *et al.*, 2010; Grbic *et al.*, 2015; Yoder *et al.*, 2016; Salmona *et al.*, 2017; Aleixo-Pais *et al.*, 2018; Hackel *et al.*, 2018). In addition, land conservation programs in Madagascar focus on forests in particular [but see references in next section], frequently overlooking open habitats (Bond, 2016) often considered of anthropogenic origin, of low ecological interest and highly impacted by fires and cattle grazing (but see Kull, 2004).

Perennial savanna plants have evolved dedicated adaptations to survive, reproduce, and compete (Simon *et al.*, 2009; Bond, 2016; Pausas *et al.*, 2018; Buisson *et al.*, 2019). Perennial tussock grasses have evolved to be highly flammable, encouraging fires that kill seedlings of forest plants (Simpson *et al.*, 2016; Cardoso *et al.*, 2018). The frequently observed geophyte strategy allows plants to survive below ground and resprout after fire or grazing, such as some pachypods and numerous grasses in Malagasy savannas (Rapanarivo and Leeuwenberg, 1999; Solofondranohatra *et al.*, 2018). Tree species have also developed strategies involving protective tissues (e.g. cork) to survive fire. In Madagascar, such a strategy has been reported for tapia (*Uapaca bojeri* Baill.) and some Sarcolenaceae (e.g. *Sarcolaena oblongifolia* F. Gérard) (Rakotoarisetra, 1997; Solofondranohatra *et al.*, 2018). Furthermore, recent evidence suggests that many non-grass savanna lineages were recruited from nearby ecosystems and evolved *in situ* adaptations to fire and grazing. This is the case for numerous plant lineages of the Cerrado flora (Brazil), that were likely recruited during the Miocene and Pleistocene (Simon *et al.*, 2009). These pieces of evidence again support the local, long persistence of open habitats in different tropical and subtropical regions since at least the Late Miocene (Edwards *et al.*, 2010; Strömberg, 2011).

The Oleaceae genus *Noronhia* encompasses numerous trees and shrubs endemic to Madagascar (i.e. 87 species are presently recognized in the Malagasy flora), but includes only one savanna species – *N. lowryi* Hong-Wa – a small shrub that occurs in patches in the savannas of Itremo and surrounding massifs of the Central Plateau (Hong-Wa, 2016; Fig. 1). *Noronhia lowryi* individuals are often observed in close proximity to one another (Fig. 1) suggesting the occurrence of clonal growth (as already reported for Oleaceae elsewhere in the world; Baali-Cherif and Besnard, 2005), a possible geophyte adaptation to fire (Bond, 2016; Pausas *et al.*, 2018). Despite being considered endangered (Hong-Wa, 2016), *N. lowryi*'s ecology is still poorly documented. Better understanding of its ecology, phylogeography, and demography will, therefore, help conservation stakeholders develop adequate management strategies.

To evaluate the presence of clonal growth, assess the intra-specific genetic diversity, and the connectivity between distant sites, we investigated nuclear and chloroplast genetic diversity of *N. lowryi*. We developed 14 nuclear microsatellite loci and used 24 chloroplast microsatellite markers to assess the relative contributions of pollen and seed dispersal. We then inferred the recent demographic history of *N. lowryi* to evaluate the impact of human colonization on savanna habitat. Finally, from full chloroplast genomes of 11 *Noronhia*

accessions, including the three *N. lowryi* maternal lineages presented here, we investigated the early origin of this species.

MATERIAL AND METHODS

THE MODEL SPECIES AND HABITAT

Noronhia lowryi Hong-Wa, formerly referred to as “spl” in Hong-Wa and Besnard (2014), is known to occur at five localities, on marble-quartzite and basement rocks in the Itremo and surrounding massifs of the Central Plateau of Madagascar, at an elevation of approximately 1200-1800 m (Figs 1 and 2; Supplementary Data Fig. S1; Hong-Wa, 2016). Most of its known habitat across the Itremo and Ibity massifs is dominated by C₄ grasses with sparse *Tapia* (*Uapaca bojeri*) and other woody plants (including *N. lowryi*), and is typical of one of the Malagasy savanna biomes, punctuated with a few humid forest relics (gallery forests; Alvarado *et al.*, 2014, 2015; Nanjarisoa *et al.*, 2017; Goodman *et al.*, 2018). *Noronhia lowryi* is considered endangered due to frequent human-induced fires in these areas and its limited distribution (Hong-Wa, 2016). *Noronhia lowryi* is generally a small shrub (Hong-Wa, 2016), but when protected from fire on cliffs, it can become a small tree (ca. 3 m; G. Besnard, pers. obs.). Populations generally constitute patches of a few small individuals (Fig. 2). Its flower morphology (especially the long tubular corolla) is suggestive of insect pollination (possibly by moths), while its dry nut-like fruits, 1-2 cm drupes with a very thin fleshy layer and a large seed, are likely to be (or have been) dispersed by animals (e.g. by rodents or lemurs). The Itremo and the Ibity Massifs (Fig. 1) harbour several protected areas, respectively managed by KMCC (Kew Madagascar Conservation Center, 2012) and MBG (Missouri Botanical Garden; Alvarado *et al.*, 2014, 2015; Birkinshaw *et al.*, 2018), where the species is presently propagated by seed in restoration programs (Ibity, MBG).

PLANT SAMPLING AND DNA EXTRACTION

Seventy-seven samples of *N. lowryi* were collected at three sites (Fig. 1): Only five individuals were observed and collected in Ibity, as well as in Itremo-West, while a larger population was sampled (n = 67) in the eastern part of the Itremo massif (Supplementary Data Table S1). All observed trees were sampled in the southern part of Itremo-East, while three pairs of individuals were collected every 500 meters in the northern part. Sampling was

conducted in 2013 and 2016 by GB, MSV and RJQ. Identifications were further confirmed by CHW. Unfortunately, we were not able to visit the remote sites of Andoharano and Ambohijanaka (Fig. 1). For each sample, we recorded GPS coordinates, collected one leaf dried in silica gel, and a herbarium specimen (deposited at TAN). At Itremo-East, in eight cases, trees were sampled at less than 1.5 m, and suspected of clonal origin (resprout from underground organs; i.e. clonal growth). The same collector's number was assigned to these samples (no. RJQ689-4, 6, 14, 15, 17, 19, 22, 30), but neighboring samples were distinguished with an additional letter (e.g. RJQ689-4A; Supplementary Data Table S1). One sample from a herbarium specimen collected in 1996 at Itremo-East was also included in the study.

Total genomic DNA was extracted from ca. 5 mg of dried leaves. Each sample was ground in a 2-ml tube containing three tungsten beads with a TissueLyser (Qiagen Inc., Texas). The BioSprint 15 DNA Plant Kit (Qiagen Inc.) was then used to extract DNA, which was eluted in 200 µl AE buffer.

MARKER DEVELOPMENT

We used shotgun genome sequencing data from two *N. lowryi*, one *N. brevituba*, one *N. clarinerva*, and one *N. intermedia* (Van de Paer *et al.*, 2018; Olofsson *et al.*, 2019) to assemble 20 nuclear regions with microsatellite motifs (poly GA or GAA) common to the five species. Primers were defined in conserved regions flanking the microsatellite motif to increase their transferability among *Noronhia* species. After PCR tests and preliminary genotyping analyses, we selected 14 loci (Supplementary Data Table S2). Twelve loci are located in a non-coding part of gene (either intron or 3'/5' untranscribed regions), while one tri-nucleotide microsatellite (Nor-13) corresponds to a coding sequence.

GENOTYPING

Nuclear microsatellites were amplified either in multiplex (pair of loci) or separately (Supplementary Data Table S2) using the method of Schuelke (2000). For each locus, an 18-bp tail of M13 was added to the forward or reverse primer. Each PCR reaction (25 µl) contained 10 ng DNA template, 1× reaction buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.15 µmol of one universal fluorescent-labeled M13(-21) primer (5'TGTAAAACGACGGCCAGT-3'; labeled with one of the three following fluorochromes: 6-FAM, AT550 or AT565; Table S3), 0.2 µmol of the reverse primer(s), 0.5 µmol of the

forward primer(s), and 0.5 U of *Taq* DNA polymerase (Promega). We conducted PCR in a Mastercycler pro PCR System (Eppendorf) for 2 min at 94°C, followed by 25 cycles of 30 s at 94°C, 45 s at 56 or 58°C, and 1 min at 72°C, and then by 10 cycles of 30 s at 94°C, 45 s at 51.5°C, and 45 s at 72°C. The last cycle was followed by a 20-min extension at 72°C. In addition, we investigated the chloroplast genetic (cpDNA) diversity with 24 microsatellites (Supplementary Data Table S3) originally developed for the olive tree by Besnard *et al.* (2011). Three to four loci were simultaneously amplified (except locus 19, that was individually amplified) following the protocol described in Besnard *et al.* (2011) and using the universal M13 primer labeled with the YAK fluorochrome. Four nuclear loci and twelve plastid loci PCR products were then multiplexed together with GenScan-600 Liz (Applied Biosystems) and separated on an ABI Prism 3730 DNA Analyzer (Applied Biosystems). Allele size was determined with Geneious v.9.0.5 (Kearse *et al.*, 2012). Multistate cpDNA microsatellites were coded by the number of repeated motifs for each allele (e.g. number of T or A), as described by Besnard *et al.* (2011). Genotyping of three individuals (RJQ689-30A, 32 and 46) was repeated to ensure reproducibility. All individuals with ambiguous genotypes were also systematically repeated. Nuclear and chloroplast microsatellite genotypes are available in Supplementary Data Tables S1 and S3.

ASSESSING THE OCCURRENCE OF CLONAL GROWTH

To investigate the occurrence of clones, we first estimated pairwise allelic distance using the R package *ape* (Paradis *et al.*, 2004; R Development Core Team, 2014) and considered individuals to be potentially genetically identical when harboring a genetic distance of zero (all shared alleles). We then estimated the probability that identical genotypes could result from independent formation of zygotes (P_{gen}) following Parks and Werth (1993) using the R package *poppr* (Kamvar *et al.*, 2014). We further confirmed that identical ramets also share plastid genotypes and excluded clones from subsequent analyses.

GENETIC DIVERSITY

To assess nuclear loci departure from Hardy Weinberg proportions, we estimated Nei F_{IS} statistics (Nei, 1977) using the R package *demerelate* (Kraemer and Gerlach, 2017) and a χ^2 exact test based on 1,000 Monte Carlo permutations of alleles using the R package *pegas* (Paradis, 2010). To assess the overall, per sampling site and per locus genetic diversity, we estimated allelic richness (A_R ; Hurlbert, 1971) using the smallest sampling site size for

rarefaction with the R package *hierfstat*, the number of alleles (A), the observed (H_O) and expected heterozygosities (H_E) with the R package *adeigenet* (Jombart, 2008). To further assess the effect of drift in the potentially small remaining patches of *N. lowryi*, we estimated the average individual inbreeding coefficient F with *adeigenet*, from 100 iterations.

GENETIC STRUCTURE

To assess the nuclear genetic structure, we first examined the pairwise site differentiation using a range of population statistics, such as G_{ST} (Nei and Chesser, 1983), G'_{ST} (Hedrick, 2005), theta (Weir and Cockerham, 1984), D (Jost, 2008), and Weir & Cockerham and Nei's F_{ST} (Nei, 1973) with the R package *diveRsity* (Guevara *et al.*, 2016). Additionally, we investigated the patterns of genetic variance with a Principal Component Analysis (PCA) of allele frequencies. We further assessed the potential presence of substructure using the SnapClust clustering approach (Tonkin-Hill *et al.*, 2019) with default values, for number of clusters K ranging from one to five and selected the most appropriate K value using the goodness of fit AIC and BIC statistics (Akaike, 1974; Schwarz, 1978). To assess the potential effect of distance on gene flow, we then investigated patterns of isolation by distance (IBD) in nuclear and chloroplast DNA. To that respect, we conducted a Mantel test (Mantel, 1967) with 999 permutations, using the R package *ade4* (Chessel and Dufour, 2004), between individual geographic distances and Bruvo's genetic distances (Bruvo *et al.*, 2004) estimated using *poppr*. We investigated patterns of IBD among all samples including all sampling sites. To circumvent possible confounding effects of site substructure on IBD (van Strien *et al.*, 2015), we also investigated within-site IBD using the largest sampling site Itremo-East. To overcome the possible effects of sampling related individuals and oversampled sites, we repeated IBD, PCA, and clustering analyses with a data subset comprising 37 individuals (Supplementary Data Table. S1). Finally, we investigated cpDNA haplotype relationships using a reduced-median network constructed with Network v.5 (Bandelt *et al.*, 1999).

DEMOGRAPHIC HISTORY

The demographic history of *N. lowryi* was investigated using the R package *VarEff* (Nikolic and Chevalet, 2014) that uses an approximate likelihood of the distribution of distance frequencies between alleles in a Monte Carlo Markov Chain framework. This approach offers several advantages: (i) it models several demographic changes, (ii) it implements the three most common microsatellite mutation models, and (iii) it is computationally efficient. This

enabled us to test several combinations of parameters such as the number of population size changes, mutation models, the variance of the prior log-distribution of effective sizes, and the maximal distance between alleles. The analyses were performed using each of the three mutation models (single step, two-phase and geometric), allowing three population size changes. Additional parameters are detailed in Supplementary Methods (Supplementary Data). Although this approach makes the assumption that population structure is negligible (Chikhi *et al.*, 2010; Heller *et al.*, 2012), this was not a major issue for *N. lowryi* according to our results (see below). Since very little available data can help estimating the generation time, we assumed, from field observations, that *N. lowryi* individuals are likely to be mature after a couple of years (≥ 3 years), and may last more than a few decades (~ 30 -50 years). However, individuals protected from fire and grazing in cliffs of rock cavities may reproduce over long periods of time. We therefore considered values of 10, 30 and 50 years, to encompass the possible turnover times between generations. The estimation of the timing of demographic history events is also affected by the assumed mutation rate. We used the value of 5×10^{-3} close to that used and estimated in olive tree demographic inferences (Besnard *et al.*, 2014). Our results - like most other demographic inferences using genetic data - depend on the assumption that the true mutation rate and generation time do not deviate dramatically from the applied values.

EMPIRICAL EVIDENCE FOR DISPERSAL

To investigate effective gene dispersal events, we estimated potential parentage relationships among individuals. All pairs of individuals with a relatedness S_{xy} (Lynch, 1988), estimated in the R package *demerelate* (Kraemer and Gerlach, 2017), superior or equal to 0.5 (that is expected when an individual is parent of another) were first selected. For each putatively affiliated pairs with one shared allele at each locus, we estimated the probability to observe it by chance, $P = \prod \{1-(1-p_{Axy})^2\}_i$, where p_A is the frequency of allele *A* shared by individuals *x* and *y* at locus *i* in the population (Hardy, 1908). To keep the estimate conservative, when the two alleles at a locus were shared by individuals *x* and *y*, we considered the most frequent one.

PLASTOME ASSEMBLY AND PHYLOGENY DATING

We finally estimated the divergence time of *N. lowryi* maternal lineages revealed in our study (see below). The Oleaceae phylogeny based on full plastomes recently reconstructed by

Olofsson *et al.* (2019) was re-used and complemented with five new *Noronhia* accessions: *Noronhia lowryi* (RJQ686-1; Itremo-West, Madagascar), *Noronhia brevituba* (MSV1929-7; Ankafoke, Madagascar), *Noronhia candicans* (GB385-2017; Binara, Madagascar), *Noronhia spinifolia* (GB124-2017; Binara, Madagascar), and *Noronhia mannii* (White 886; Gabon). This sampling thus allowed us to consider the three maternal lineages of *N. lowryi* revealed in our study (see below), as well as another *Noronhia* species (*N. brevituba*) that occurs in the northern Central Plateau (gallery forests), plus five Malagasy species from low elevation forests. In addition, we analysed one representative species of the two Continental African lineages of *Noronhia* (i.e. *N. peglerae* and *N. mannii*; Hong-Wa and Besnard, 2013). DNA was extracted from the five additional accessions, quality checked, and sequenced at the Genopole platform of Toulouse as detailed in Olofsson *et al.* (2019). For each accession, paired-end reads were used to reconstruct the whole plastome, following the method described in Zedane *et al.* (2016). Alignments were produced using MUSCLE in MEGA v.7 (Kumar *et al.*, 2016) followed by manual refinement (particularly in regions with inversions). One inverted repeat region was removed to avoid considering the same sequence twice. A time-calibrated phylogeny was then obtained from the plastome dataset using Bayesian inference as implemented in BEAST v.2.4.3 (Bouckaert *et al.*, 2014) using a GTR + G + I substitution model, which was identified as the best-fit model with Smart Model Selection v.1.8.1 (Lefort *et al.*, 2017) in PhyML v.20120412 (Guindon *et al.*, 2010). A Yule speciation prior was used, which will adequately describe the plastome tree that is composed mainly of different species. Indeed, 90% of the species are represented by a single individual, while *N. lowryi* is represented by three very divergent samples that might be considered as independently evolving chloroplast lineages (See Results). We used a relaxed molecular clock with an uncorrelated log-normal distribution of rates (Drummond *et al.*, 2006). Node calibration was realized as reported in Olofsson *et al.* (2019).

RESULTS

IDENTIFICATION OF CLONES

We detected identical genotypes on two triplets of ramets (RJQ689-4[A-C] and RJQ689-6[A-C]; Supplementary Data Fig. S2). The probability of encountering such genotypes more than once by chance (according to Parks and Werth, 1993) was less than 10^{-8} , and was thus due to

clonal growth. These two triplets of ramets represent 25% of the eight groups of samples labeled as potentially clonal when collected.

GENETIC DIVERSITY

We found a relatively high overall nuclear genetic diversity in *N. lowryi* with a mean of 10.2 alleles per locus (N_a) and a mean heterozygosity of 0.59 (Table 1). Furthermore, this diversity is similar among sites with allelic richness A_r , expected H_E and observed heterozygosities H_O comprised within narrow ranges ($A_r = [3.9-4.2]$, $H_E = [0.53-0.59]$, $H_O = [0.52-0.63]$; Table 1), despite strong discrepancies in sampling size (63 vs 5; Table 1). Furthermore, all individuals showed relatively low estimates of inbreeding (mean < 0.4) from nuclear microsatellite data, with none of the sampling sites showing particularly high inbreeding levels (Fig. 3). Two microsatellites loci, Nor-8 and Nor-14, show a significant excess of homozygosity in the largest sampled population (Itremo-East; Table 1), suggesting the possible presence of null alleles for these loci.

Similarly to nuclear data, *N. lowryi* comprises a relatively high chloroplast genetic diversity with 15 polymorphic loci among 24. Five loci (loci #15, 19, 25, 33, 41) showed at least three alleles, including one locus (#19) with within-site polymorphisms. All together, these polymorphisms allowed us to distinguish six chloroplast haplotypes (Supplementary Data Tables S3).

GENETIC STRUCTURE

At the nuclear level, none of the site pairwise differentiation indices (G_{ST} , G'_{ST} , theta, D , F_{ST}) showed significant signal of differentiation among sites (Fig. 4A). Similarly, the reduced representation of the genetic variance in the PCA exhibits no particular clustering pattern of the sampled individuals, neither within nor among sites (Fig. 4B). In addition, the estimation of cluster number (K) best fitting the data showed no consistency at values > 1 (Supplementary Data Fig. S3), and the clustering approach *snaphust* exhibited no comprehensive signal for $K \geq 2$ (Supplementary Data Fig. S4). Finally, we did not find evidence of continuous structure of lower intensity driven by distance (IBD), neither at the regional level (Fig. 4C), nor at the Itremo-East level (Supplementary Data Fig. S5A). Using the “subset” data (Supplementary Data Tables S1), we found very similar PCA, IBD and clustering results (data not shown), suggesting that the results were not influenced by the high number of Itremo-East individuals and the presence of related individuals.

In contrast, we established a clear cpDNA-based differentiation of the three sites, none of which shared a chloroplast haplotype with any other (Fig. 5A). We found a strong (but based only on six haplotypes) signal of cpDNA IBD (Supplementary Data Fig. S5B). However, we retrieved no signal of IBD when considering Itremo-East data alone (Supplementary Data Fig. S5C). These contradictory signals of cpDNA IBD are likely a consequence of the low number of haplotypes (6 overall, 3 in Itremo-East) and of their peculiar but likely auto-correlated distribution in Itremo-East (Supplementary Data Fig. S6).

DEMOGRAPHIC HISTORY

Using *Vareff*, all three mutation models exhibit a clear and drastic population size decline within the last 350 generations (Fig. 6; Supplementary Data Fig. S7). Altogether, the three models provide mean estimates of a relatively low current effective population size, with $N_e < 900$ (357-854), and larger ancient population sizes with $N_e > 4,800$ (Supplementary Data Table S5).

EMPIRICAL EVIDENCE FOR SHORT-DISTANCE DISPERSAL

At Itremo-East, we identified 17 pairs of samples sharing at least one allele per nuclear locus (Supplementary Data Table S6; Supplementary Data Fig. S8). The Bonferroni corrected probability that these individual pairs share at least one allele at each locus by chance was relatively low (p_{BF} : $9.6 \cdot 10^{-3}$ to $1.3 \cdot 10^{-5}$; Supplementary Data Table S6). These observations likely reflecting parent-progeny affiliations were distributed at distances ranging from one meter to ~5.9 km (median = 7.3 m). All the 17 pairs did share a chloroplast haplotype and we thus cannot determine if the parental affiliation is maternal or paternal, except for related individuals RJQ689-6 and RJQ689-7 that uniquely share the rare chlorotype A-3 suggesting a mother-progeny relationship with seed dispersal at ca. 30 m. Most of the related pairs (15) are found at distances inferior to 32 m indicative of strong dispersal limitation of propagules. As pollen seems efficiently dispersed (see above), short-distance parent-progeny affiliations may mainly reflect limited seed dispersal.

PHYLOGENETIC RECONSTRUCTION BASED ON PLASTOMES

The phylogeny inferred from plastomes (Supplementary Data Fig. S9) is well resolved, and fully congruent with Olofsson *et al.* (2019). The three *N. lowryi* maternal lineages form a clade sister to another species collected on the Central Plateau (*N. brevītuba*). The node dating

of the Oleaceae phylogeny is consistent with Olofsson *et al.* (2019). The crown age of *N. lowryi* appears surprisingly old with a split from the *N. brevifolia* lineage ~9.1 Mya [95% confidence: (12.1–6.6)] and a split between Ibity and Itremo sites at ~6.7 Mya (9.3–4.5). Furthermore, the chloroplast lineages of the two geographically close Itremo sampling sites also show an ancient split [~5.2 Mya (7.7–3.0)]. These old within-species splits do not seem driven by the use of the Yule model, since other within-species datings show, as expected, very recent splits (i.e. *Cartrema americana*, *Chionanthus brassii*, and *Fraxinus ornus*; Supplementary Data Fig. S9).

DISCUSSION

REPRODUCTIVE STRATEGY OF A MALAGASY SAVANNA TREE

Madagascar High Plateau savannas faced increasingly recurrent fires since the advent of agropastoralism ~1,000 y BP (Burney *et al.*, 2003; Dewar, 2014), but also dealt with little predictable climate variations (Dewar and Richard, 2007), natural fires, and grazing since the expansion and diversification of C₄ grasses ~8-3 Mya (Bond *et al.*, 2008; Edwards *et al.*, 2010; Strömberg, 2011; Hackel *et al.*, 2018). In such environmental conditions, many plants developed geophyte strategies to persist over a long period (Bond, 2016), and clonal growth was expected in the Malagasy savanna olive. From new nuclear microsatellite markers, our results confirm only two out of eight suspected cases of clonal growth (25%) on ramets sampled at close proximity. The area covered by these ramets never exceeded 1 m² (Fig. 2C) and was small when compared to that reported in the Saharan olive, whose surface of the stump can reach 80 m² (Baali-Cherif and Besnard, 2005). Whether *N. lowryi* uses clonal growth to respond to harsh environmental conditions remains an open question, but an efficient sexual reproduction seems to be the prominent reproductive strategy.

MALAGASY SAVANNA OLIVE TREE GENETIC DIVERSITY

Despite the threats faced by *N. lowryi*, and the low number of known and sampled localities of occurrence (five and three respectively; Fig. 1), it harbours levels of genetic diversity of a sustainable population ($H_E = 0.63$; Table 1; Supplementary Data Table S5), comparable, for instance, to those measured with microsatellites in two olive subspecies (Baali-Cherif and Besnard, 2005). To our knowledge, the genetic diversity of Malagasy savanna trees has only

been assessed in two endangered palm species of *Dypsis* with amplified fragment-length polymorphisms (AFLPs) (Gardiner *et al.*, 2017). A relatively low genetic diversity ($H_E = 0.233-0.239$) was revealed in both species. However, as microsatellites usually reveal higher within-population diversity than AFLPs (e.g. Mariette *et al.*, 2001; Maguire *et al.*, 2002), comparison of genetic diversity between *Dypsis* and *N. lowryi* is not possible. The estimated genetic diversity of *N. lowryi* further suggests that its distribution could be larger than currently known (Fig. 1), with populations to be discovered further away from the main roads and accessible areas. However, the contrast between the relatively high genetic diversity and the low number of known occurrence localities suggests that the estimated genetic diversity may depart from equilibrium and represent a relic of the diversity of an ancient larger population (see also demographic history reconstruction, below). A di-allelic self-incompatibility system maintained in distantly related Oleaceae genera (*Olea*, *Phillyrea*, and *Fraxinus*; Vernet *et al.*, 2016; Saumitou-Laprade *et al.*, 2017) is likely present in *Noronhia* [which belongs to the *Olea-Phillyrea* lineage (Olofsson *et al.*, 2019)], and may have played a role in the maintenance of substantial levels of genetic diversity in *N. lowryi*, despite its likely recent population size contraction (see below).

CONTRASTING NUCLEAR AND CHLOROPLAST GENETIC MAKEUP MEDIATED BY POLLEN AND SEED MOVEMENTS

The strikingly different patterns of nuclear and chloroplast structure (Figs 4 and 5) across *N. lowryi* sampling sites can be surprising at first but make sense when interpreted in the light of its biology. Contrasting nuclear and chloroplast genetic differentiation has been reported in numerous plants, notably in the *Dalbergia monticola* populations from humid forests in eastern Madagascar (Andrianolelina *et al.*, 2019), and such patterns result from contrasted pollen- and seed-mediated gene flow. For *N. lowryi*, the apparent nuclear panmixia is evidence that pollen movement is not encountering resistance at the scale of this study (~80 km-wide), in contrast to the two *Dypsis* species from Itremo for which a clear IBD pattern has been observed (Gardiner *et al.*, 2017). Our results suggest that *N. lowryi*'s pollen (supposedly dispersed mainly by insects) commonly travels long distances. It should be mentioned, however, that the markers used in this study (14 nuclear SSR loci) may lack the power to reveal putative fine differentiations that could be elicited by more intensive genotyping approaches. In contrast, the strong structure revealed by maternally inherited chloroplast markers suggests that the three sampled sites do not exchange seeds. This pattern

is in agreement with the deep age of the *N. lowryi*'s chloroplast lineage (6.25 My BP) and suggests that fruits may only be dispersed over short distances (Supplementary Data Table S6), through barochory or by small mammals (rodents). It further suggests that the dry nut-like drupes (with thin fleshy mesocarp and thin endocarp), shared by most *Noronhia* species (Hong-Wa, 2016), are not primarily spread by birds. Considering its large kernel (seed) size compared to the thin meso- and endo-carp layers, it seems more likely that *N. lowryi*'s seeds are dispersed via non-destructive storage by rodents rather than through (likely destructive) ingestion by animals.

RECENT DEMOGRAPHIC HISTORY OF *N. LOWRYI*

Our inferences of *N. lowryi*'s demography through time suggest that its effective population size dramatically declined approximately 350 generations ago. To our knowledge this is the first demographic collapse of a Malagasy plant documented from genetic data. The exact timing of such population decrease is, however, difficult to infer with precision since it depends on accurate knowledge of generation time. Additional studies on the average time necessary to replace reproducing individuals of a population will help resolve the reason(s) of *N. lowryi* decline. At this stage, two alternative explanations can be given: (1) Assuming a generation time of 10 years, this major decline would have occurred within the last four millennia, and could originate from a change in fire regime. Indeed, since the human colonization of Madagascar during the Early Holocene if not earlier (Hansford *et al.*, 2018; but see: Anderson, 2019; Douglas *et al.*, 2019), the frequency of fire ignition may have continuously risen as a consequence of population density (Guyette *et al.*, 2002). Major fire peak traces, likely due to the advent of agro-pastoralism, have been dated to the last millennium (Burney *et al.*, 2003; Dewar, 2014; Voarintsoa *et al.*, 2017). In an interconnected open habitat, a long-lasting continuous rise in ignition frequency may have had earlier heavy impacts (Archibald *et al.*, 2012). It's also possible that a drought event occurring at or since the Mid-Holocene transition (Burney, 1993; Kiage and Liu, 2006; Virah-Sawmy *et al.*, 2010) may have exacerbated fire ignition frequency and spread intensity, and acted synergistically with human-driven impact as suggested for other species in various regions of Madagascar (Goodman and Jungers, 2014; Salmona *et al.*, 2017; Godfrey *et al.*, 2019); (2) Alternatively, considering generation times of 30 or 50 years, this decline would coincide with the Last Glacial Maximum (LGM; that ended ~12 kya in the area). On the central highlands, at the onset of the Holocene, the major vegetation shift from Ericaceous bush to grass-dominated

habitat (Gasse and Van Campo, 1998, 2001) likely impacted *N. lowryi* populations. Furthermore, vegetative reproductive strategies may have dominated during unfavourable periods (altering generation time), allowing long-term maintenance of surviving individuals, as already reported, for instance, in the Saharan olive (Baali-Cherif and Besnard, 2005). A bottleneck signal could thus reflect a strong decline of the population during the LGM, with isolated, relic patches of individuals subsequently reconnecting when conditions became favourable at the onset of the Holocene.

HIGH PLATEAU SAVANNA ORIGINS AND THE ESTABLISHMENT OF *N. LOWRYI*

Our phylogenetic work, built on previous larger-scale efforts (Olofsson *et al.*, 2019), supports the monophyly of *N. lowryi*, complementing nuclear data (this work) and field evidence (Hong-Wa, 2016) to confirm *N. lowryi* a distinct unique lineage, despite its geographic discontinuity (Fig. 1). Furthermore, the antiquity of the *N. lowryi* clade (~6.7 Mya) does surprisingly co-occur with the documented global and local Miocene grassland expansion (Bond *et al.*, 2008; Stromberg, 2011; Hackel *et al.*, 2018). Although our phylogenetic analyses based on full plastomes are not yet comprehensive, they identified a taxon occurring in humid forests (*N. brevītuba*) as sister to *N. lowryi*. Altogether these results suggest adaptations to grazed and fire-prone savanna habitat evolved *in situ*, from a forest ancestor during the rise of the savanna biome, as also described for the Cerrado (Brazil; Simon *et al.*, 2009). Finally, the deep chloroplast divergence estimated among the three sites (~6.7-5.2 Mya) suggests that seed-mediated dispersal between these has been very limited over the past million years. The environmental factors that prevented seed dispersers from ensuring connectivity at this regional scale remain to be identified. An ancient fragmented distribution (on marble-quartzite soils) combined with a low seed dispersal by small mammals (e.g. rodents) could explain such a strong chloroplast DNA differentiation.

ON THE CONSERVATION OF A WOODY SPECIES IN THE HIGH PLATEAU SAVANNAS

There is little doubt, on the basis of these results, that *N. lowryi* should remain “Endangered” as suggested by Hong-Wa (2016). Although the species has now been recorded in five locations (Fig. 1), the new localities in Itremo-West and Ibity do not drastically affect the distributions estimated by Hong-Wa (2016). It now includes two protected areas (i.e. Itremo and Ibity), a positive contribution to the conservation of *N. lowryi*. However, our estimates of effective population size ($N_e \sim 350-850$) suggest that the IUCN criteria C2a are also

applicable, leading to the following updated set of criteria [EN B1ab(i,ii,iii,iv,v) + 2ab(i,ii,iii,iv,v) + C2a(i,ii)]. The very low estimated seed dispersal distance additionally suggests that *N. lowryi* is unlikely to recolonize areas from which it was recently extirpated. Furthermore, the lack of knowledge on its potential seed disperser(s) still limits our ability to improve seed dispersal via indirect conservation actions (i.e. endemic rodents conservation). In that respect, our analyses suggest that the current seed propagation of *N. lowryi* in a restoration program (Ibity, MBG) is not likely to cause a particularly negative out-breeding effect. Indeed, the distant sites do not show particular structure and are already likely exchanging genes at relatively high rates through pollen dispersal. Ongoing management of fire and grazing activities at Itremo and Ibity (Kew Madagascar Conservation Centre, 2012; Birkinshaw *et al.*, 2018; C. Birkinshaw, pers. comm.) are also likely to be among the most efficient measures to protect current *N. lowryi*'s populations. These actions will likely benefit the entire diverse endemic biota present at both sites (Alvarado *et al.*, 2014, 2018), which hopefully still includes *N. lowryi*'s seed dispersers.

CONCLUSIONS

Ancient *in situ* adaptation and recent demographic collapse of an endangered woody plant highlight the antiquity and uniqueness of Malagasy savannas. Building upon accumulating evidence of a climate-driven expansion of Malagasy savannas since the Late Miocene (Bond *et al.*, 2008; Vorontsova *et al.*, 2016; Hackel *et al.*, 2018), our results show for the first time that the ancient establishment of open habitats also included woody plants and highlight the need for a clear and evidence-based change in narrative concerning the origin of savannas in Madagascar. In addition, our results stress the urgency of conservation of this unique and antique forsaken habitat, likely collapsing at an unprecedented and underestimated pace.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website: <https://academic.oup.com/biolinnean/article/129/1/227/5626515>

Table S1. Nuclear microsatellite data, chlorotypes and GPS coordinates of *Noronhia lowryi* individuals included in the present study. **Table S2.** Characteristics of microsatellite loci developed in the present study. **Table S3.** Data matrix of the six plastid DNA haplotypes (chlorotypes) revealed in *Noronhia lowryi*. **Table S4.** Data matrix used for the reduced-

median network reconstruction with the NETWORK software. **Table S5.** *VarEff* posterior effective population size (N_e) estimates. **Table S6.** Parent affiliation and short distance dispersal in *Noronhia lowryi*. **Figure S1.** Sampling of *Noronhia lowryi* in the western Itremo and Ibity massifs. **Figure S2.** Identification of clones using pairwise allelic genetic distances. **Figure S3.** Most likely number of panmictic clusters in *Noronhia lowryi*. **Figure S4.** *Noronhia lowryi* genetic clustering analysis results. **Figure S5.** Representation of the relation between genetic and geographic distances within the Itremo-East site. **Figure S6.** Spatial distribution of chlorotypes in *Noronhia lowryi* at the Itremo-East site. **Figure S7.** *Noronhia lowryi* demographic history inferred with *VarEff*. **Figure S8.** Geographic representation of *Noronhia lowryi* pairs of samples sharing at least one allele per locus. **Figure S9.** Dated maximum-likelihood phylogenetic tree inferred from the alignment of whole plastomes using BEAST. **Supplementary Methods 1.** Description of the *VarEff* analysis.

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REFERENCES

- Akaike H. 1974.** A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.
- Aleixo-Pais I, Salmons J, Sgarlata GM, et al. 2019.** The genetic structure of a mouse lemur living in a fragmented habitat in Northern Madagascar. *Conservation Genetics* **20**: 229–243.
- Alvarado ST, Buisson E., Rabarison H, Rajeriarison C, Birkinshaw C, Lowry PP II. 2014.** Comparison of plant communities on the Ibity and Itremo massifs, Madagascar, with contrasting conservation histories and current status. *Plant Ecology & Diversity* **7**: 497–508.
- Alvarado ST, Buisson E, Carrière SM, et al. 2015.** Achieving sustainable conservation in Madagascar: the case of the newly established Ibity Mountain Protected Area. *Tropical Conservation Science* **8**: 367–395.
- Alvarado ST, Silva, TSF, Archibald S. 2018.** Management impacts on fire occurrence: A comparison of fire regimes of African and South American tropical savannas in different protected areas. *Journal of Environmental Management* **218**: 79–87.
- Anderson A. 2019.** Was there mid Holocene habitation in Madagascar? A reconsideration of the OSL dates from Lakaton'i Anja. *Antiquity* **93**: 478–487.
- Andrianoelina O, Favreau B, Ramamonjisoa L, Bouvet JM. 2009.** Small effect of fragmentation on the genetic diversity of *Dalbergia monticola*, an endangered tree species of the eastern forest of Madagascar, detected by chloroplast and nuclear microsatellites. *Annals of Botany* **104**: 1231–1242.
- Archibald S, Staver AC, Levin SA. 2012.** Evolution of human-driven fire regimes in Africa. *Proceedings of the National Academy of Sciences, USA* **109**: 847–852.
- Archibald S, Bond WJ, Hoffmann W, Lehmann C, Staver C, Stevens N. 2020.** Distribution and determinants of savannas. In: Scogings PF & Sankaran M (Eds), *Savanna Woody Plants and Large Herbivores*, Chap. 1, pp. 1–24. John Wiley & Sons Ltd.
- Baali-Cherif D, Besnard G. 2005.** High genetic diversity and clonal growth in relict populations of *Olea europaea* subsp. *laperrinei* (Oleaceae) from Hoggar, Algeria. *Annals of Botany* **96**: 823–830.
- Bandelt HJ, Forster P, Röhl A. 1999.** Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37–48.
- Besnard G, Hernández P, Khadari B, Dorado G, Savolainen V. 2011.** Genomic profiling of plastid DNA variation in the Mediterranean olive tree. *BMC Plant Biology* **11**: 80.
- Besnard G, Dupuy J, Larter M, Cuneo P, Cooke D, Chikhi L. 2014.** History of the invasive African olive tree in Australia and Hawaii: evidence for sequential bottlenecks and hybridization with the Mediterranean olive. *Evolutionary Applications* **7**: 195–211.
- Birkinshaw C, Tahirinirainy D, Rakotozafy BFL, Raharimampionona J Ramahefamanana N. 2018.** *Plan de Gestion du Feu pour la Nouvelle Aire Protégée du Massif d'Ibity*. Missouri Botanical Garden, Madagascar Research and Conservation program.
- Bond WJ. 2008.** What limits trees in C₄ grasslands and savannas? *Annual Review of Ecology Evolution and Systematics* **39**: 641–659.
- Bond WJ, Silander Jr JA, Ranaivonasy J, Ratsirarson J. 2008.** The antiquity of Madagascar's grasslands and the rise of C₄ grassy biomes. *Journal of Biogeography* **35**: 1743–1758.
- Bond WJ. 2016.** Ancient grasslands at risk. *Science* **351**: 120–122.
- Bouckaert R, Heled J, Kühnert D, et al. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537.
- Buisson E, Le Stradic S, Silveira FAO, et al. 2019.** Resilience and restoration of tropical and subtropical grasslands, savannas, and grassy woodlands. *Biological Reviews* **94**: 590–609.

- Bruvo R, Michiels NK, D'Souza TG, Schulenburg H. 2004.** A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* **13**: 2101–2106.
- Burney DA. 1993.** Late Holocene environmental changes in arid southwestern Madagascar. *Quaternary Research* **40**: 98–106.
- Burney DA, Robinson GS, Burney LP. 2003.** *Sporormiella* and the Late Holocene extinctions in Madagascar. *Proceedings of the National Academy of Sciences, USA* **100**: 10800–10805.
- Burney DA, Burney LP, Godfrey LR, et al. 2004.** A chronology for late prehistoric Madagascar. *Journal of Human Evolution* **47**: 25–63.
- Burns SJ, Godfrey LR, Faina P, et al. 2016.** Rapid human-induced landscape transformation in Madagascar at the end of the first millennium of the Common Era. *Quaternary Science Reviews* **134**: 92–99.
- Cardoso AW, Oliveras I, Abernethy KA, et al. 2018.** Grass species flammability, not biomass, drives changes in fire behaviour at tropical forest-savanna transitions. *Frontiers in Forests and Global Change* **1**: 6.
- Chessel D, Dufour A, Thioulouse J. 2004.** The *ade4* package – I – One-table methods. *R News* **4**: 5–10.
- Chikhi L, Sousa VC, Luisi P, Goossens B, Beaumont MA. 2010.** The confounding effects of population structure, genetic diversity and the sampling scheme on the detection and quantification of population size changes. *Genetics* **186**: 983–995.
- Crowley BE. 2010.** A refined chronology of prehistoric Madagascar and the demise of the megafauna. *Quaternary Science Reviews* **29**: 2591–2603.
- Dewar RE, Richard AF. 2007.** Evolution in the hypervariable environment of Madagascar. *Proceedings of the National Academy of Sciences, USA* **104**: 13723–13727.
- Dewar RE. 2014.** Early human settlers and their impact on Madagascar's landscapes. In: Scales IR (Ed.), *Conservation and Environmental Management in Madagascar*, pp. 44–64, Earthscan, London, UK.
- Douglass K, Hixon S, Wright HT, et al. 2019.** A critical review of radiocarbon dates clarifies the human settlement of Madagascar. *Quaternary Science Reviews* **221**: 105878.
- Drummond AJ, Ho, SY, Phillips, MJ, Rambaut A. 2006.** Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**: e88.
- Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, C₄ Grasses Consortium. 2010.** The origins of C₄ grasslands: integrating evolutionary and ecosystem science. *Science* **328**: 587–591.
- Gardiner LM, Rakotoarinivo M, Rajaovelona LR, Clubbe C. 2017.** Population genetics data help to guide the conservation of palm species with small population sizes and fragmented habitats in Madagascar. *PeerJ* **5**: e3248.
- Gasse F, Van Campo E. 1998.** A 40,000 year pollen and diatom record from Lake Tritrivakely, Madagascar, in southern tropics. *Quaternary Research* **49**: 299–311.
- Gasse F, Van Campo E. 2001.** Late Quaternary environmental changes from a pollen and diatom record in the southern tropics (Lake Tritrivakely, Madagascar). *Palaeogeography, Palaeoclimatology, Palaeoecology* **167**: 287–308.
- Godfrey LR, Crowley BE. 2016.** Madagascar's ephemeral palaeo-grazer guild: who ate the ancient C₄ grasses? *Proceedings of the Royal Society, London, Series B* **283**: 20160360.
- Godfrey LR, Crowley BE, Muldoon KM, Kelley EA, King SJ, Best AW, Berthaume MA. 2016.** What did *Hadropithecus* eat, and why should paleoanthropologists care? *American Journal of Primatology* **78**: 1098–1112.
- Godfrey LR, Scroxton N, Crowley BE, et al. 2019.** A new interpretation of Madagascar's megafaunal decline: the "Subsistence Shift Hypothesis". *Journal of Human Evolution* **130**: 126–140.
- Goodman SM, Jungers WL. 2014.** *Extinct Madagascar: Picturing the Island's Past*. The University of Chicago Press, Chicago, Illinois, 296 pages.

- Goodman SM, Raherilalao J, Wohlhauser S. 2018.** *The Terrestrial Protected Areas of Madagascar*. Association Vahatra, University of Chicago Press, 1716 pages.
- Grbic D, Saenko SV, Randriamoria TM, Debry A, Raselimanana AP, Milinkovitch MC. 2015.** Phylogeography and support vector machine classification of colour variation in panther chameleons. *Molecular Ecology* **24**: 3455–3466.
- Guevara MR, Hartmann D, Mendoza M. 2016.** *diverse*: an R package to measure diversity in complex systems. *The R Journal* **8**: 60–78.
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010.** New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307–321.
- Guyette R, Muzika R, Dey D. 2002.** Dynamics of an anthropogenic fire regime. *Ecosystems* **5**: 472–486.
- Hackel J, Vorontsova MS, Nanjarisoa OP, Malakasi P, Hall RC, Besnard G. 2018.** Grass diversification in Madagascar: in situ radiation of two large C₃ shade clades and support for a Miocene to Pliocene origin of C₄ grassy biomes. *Journal of Biogeography* **45**: 750–761.
- Hansford J, Wright P, Rasoamiamanana A, et al. 2018.** Early Holocene human presence in Madagascar evidenced by exploitation of avian megafauna. *Science Advances* **4**: eaat6925.
- Hardy GH. 1908.** Mendelian proportions in a mixed population. *Science* **28**: 49–50.
- Harper GJ, Steininger MK, Tucker CJ, Juhn D, Hawkins F. 2007.** Fifty years of deforestation and forest fragmentation in Madagascar. *Environmental Conservation* **34**: 325–333.
- Hedrick P. 2005.** A standardized genetic differentiation measure. *Evolution* **59**: 1633–1638.
- Heller R, Chikhi L, Siegmund HR. 2013.** The confounding effect of population structure on Bayesian skyline plot inferences of demographic history. *PLoS One* **8**: e62992.
- Hong-Wa C. 2016.** A taxonomic revision of the genus *Noronhia* (Oleaceae) in Madagascar and the Comoro Islands. In: Callmender MW & Lowry PP II (Eds), *Boissiera* **70**, Conservatoire et Jardin Botanique, Genève, Switzerland, 291 pages.
- Hong-Wa C, Besnard G. 2013.** Intricate patterns of relationships in the olive family as inferred from multi-locus plastid and nuclear DNA sequence analyses: a close-up on *Chionanthus* and *Noronhia* (Oleaceae). *Molecular Phylogenetics and Evolution* **67**: 367–378.
- Hong-Wa C, Besnard G. 2014.** Diversification and species limits in the Madagascar olive. *Botanical Journal of the Linnean Society* **174**: 141–161.
- Hurlbert SH. 1971.** The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**: 577–586.
- Johnson CN. 2009.** Ecological consequences of Late Quaternary extinctions of megafauna. *Proceedings of the Royal Society, London, Series B* **276**: 2509–2519.
- Jombart T. 2008.** *adeigenet*: aR package for the multivariate analysis of genetic markers *Bioinformatics* **24**: 1403–1405.
- Jost L. 2008.** G_{ST} and its relatives do not measure differentiation. *Molecular Ecology* **17**: 4015–4026.
- Kamvar ZN, Tabima JF, Grünwald NJ. 2014.** *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2**: e281.
- Kearse M, Moir R, Wilson A, et al. 2012.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.
- Kew Madagascar Conservation Centre. 2012.** *Plan d'Aménagement et de Gestion de la Nouvelle Aire Protégée du Massif d'Itremo-Ambatofinandrahana Région Amoron'I Mania*. KMCC, Antananarivo, Madagascar.
- Kiage LM, Liu KB. 2006.** Late Quaternary paleoenvironmental changes in East Africa: a review of multiproxy evidence from palynology, lake sediments, and associated records. *Progress in Physical Geography* **30**: 633–658.

- Koechlin J, Guillaumet JL, Morat P. 1974. *Flore et Végétation de Madagascar*. Vaduz: Gantner.
- Kraemer P, Gerlach G. 2017. *demerelate*: calculating interindividual relatedness for kinship analysis based on codominant diploid genetic markers using R. *Molecular Ecology Resources* **17**: 1371–1377.
- Kull CA. 2004. *Isle of Fire: The Political Ecology of Landscape Burning in Madagascar*. University of Chicago Press, 256 pages.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870–1874.
- Lefort V, Longueville JM, Gascuel O. 2017. SMS: Smart Model Selection in PhyML. *Molecular Biology and Evolution* **34**: 2422–2424.
- Lehmann CER, Archibald SA, Hoffmann WA, Bond WJ. 2009. Deciphering the distribution of the savanna biome. *New Phytologist* **191**: 197–209.
- Lynch M. 1988. Estimation of relatedness by DNA fingerprinting. *Molecular Biology and Evolution* **5**: 584–599.
- Maguire T, Peakall R, Saenger P. 2002. Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae) detected by AFLPs and SSRs. *Theoretical and Applied Genetics* **104**: 388–398.
- Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209–220.
- Mariette S, Chagné D, Lézier C, et al. 2001. Genetic diversity within and among *Pinus pinaster* populations: comparison between AFLP and microsatellite markers. *Heredity* **86**: 469–479.
- Matsumoto K, Burney DA. 1994. Late Holocene environments at Lake Mitsinjo, northwestern Madagascar. *The Holocene* **4**: 16–24.
- Nanjarisoa OP, Besnard G, Ralimanana H, Jeannoda V, Vorontsova MS. 2017. Grass survey of the Itremo Massif records endemic central highland grasses. *Madagascar Conservation & Development* **12**: 34–40.
- Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* **70**: 3321–3323.
- Nei M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* **41**: 225–233.
- Nei M, Chesser R. 1983. Estimation of fixation indices and gene diversities. *Annals of Human Genetics* **47**: 253–259.
- Nikolic N, Chevalet C. 2014. Detecting past changes of effective population size. *Evolutionary Applications* **7**: 663–681.
- Olofsson JK, Cantera I, Van de Paer C, et al. 2019. Phylogenomics using low-depth whole genome sequencing: a case study with the olive tribe. *Molecular Ecology Resources* **19**: 877–892.
- Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**: 289–290.
- Paradis E. 2010. *pegas*: an R package for population genetics with an integrated-modular approach. *Bioinformatics* **26**: 419–420.
- Parks JC, Werth CR. 1993. A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany* **80**: 120–126.
- Pausas JG, Lamont BB, Paula S, Appezzato-da-Glória B, Fidelis A. 2018. Unearthing belowground bud banks in fire-prone ecosystems. *New Phytologist* **217**: 1435–1448.
- Pedrono M, Griffiths OL, Clausen A, et al. 2013. Using a surviving lineage of Madagascar's vanished megafauna for ecological restoration. *Biological Conservation* **159**: 501–506.
- Perrier de la Bâthie HP. 1921. La végétation malgache. *Annales du Musée Colonial de Marseille*, Challamel.

- Quéméré E, Amelot X, Pierson J, Crouau-Roy B, Chikhi L. 2012.** Genetic data suggest a natural pre-human origin of open habitats in northern Madagascar and question the deforestation narrative in this region. *Proceedings of the National Academy of Sciences, USA* **109**: 13028–13033.
- R Development Core Team. 2014.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rakotoarisetra FN. 1997.** *Monographie de Uapaca densifolia dans la Forêt d'Ambohitantely*. Mémoire de fin d'études, Ecole Supérieure des Sciences Agronomiques (ESSA), Département des Eaux et Forêts, Antananarivo, Madagascar.
- Rapanarivo SHJV, Leeuwenberg AJM. 1999.** Taxonomic revision of *Pachypodium*: Series of revisions of Apocynaceae XLVIII. In: Rapanarivo SHJV, Lavranos JJ, Leeuwenberg AJM, & Rösli W (Eds), *Pachypodium (Apocynaceae): Taxonomy, Habitats and Cultivation*, Chap. I, pp. 1–82. A.A. Balkema Publishers, Rotterdam, Netherlands.
- Salmona J, Heller R, Quéméré E, Chikhi L. 2017.** Climate change and human colonization triggered habitat loss and fragmentation in Madagascar. *Molecular Ecology* **26**: 5203–5222.
- Samonds KE, Crowley BE, Rasolofomanana TR, et al. 2019.** A new late Pleistocene subfossil site (Tsaramody, Sambaina basin, central Madagascar) with implications for the chronology of habitat and megafaunal community change on Madagascar's Central Highlands. *Journal of Quaternary Science*, doi.org/10.1002/jqs.3096.
- Saumitou-Laprade P, Vernet P, Vekemans X, et al. 2017.** Elucidation of the genetic architecture of self-incompatibility in olive: Evolutionary consequences and perspectives for orchard management. *Evolutionary Applications* **10**: 867–880.
- Schuelke M. 2000.** An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* **18**: 233–234.
- Schwarz GE. 1978.** Estimating the dimension of a model. *Annals of Statistics* **6**: 461–464.
- Simon MF, Grether R, de Queiroz LP, Skema C, Pennington RT, Hughes CE. 2009.** Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by *in situ* evolution of adaptations to fire. *Proceedings of the National Academy of Sciences, USA* **106**: 20359–20364.
- Simpson KJ, Ripley BS, Christin PA, et al. 2016.** Determinants of flammability in savanna grass species. *Journal of Ecology* **104**: 138–148.
- Solofondranohatra C, Vorontsova MS, Hackel J, et al. 2018.** Grass functional traits differentiate forest and savanna in the Madagascar central highlands. *Frontiers in Ecology and Evolution* **6**: 184.
- Strömberg CAE. 2011.** Evolution of grasses and grassland ecosystems. *Annual Review of Earth and Planetary Sciences* **39**: 517–544.
- Tonkin-Hill G, Lees JA, Bentley SD, Frost SDW, Corander J. 2019.** Fast hierarchical Bayesian analysis of population structure. *Nucleic Acids Research* **47**: doi: 5539–5549.
- Van de Paer C, Bouchez O, Besnard G. 2018.** Prospects on the evolutionary mitogenomics of plants: a case study on the olive family (Oleaceae). *Molecular Ecology Resources* **18**: 407–423.
- van Strien MJ, Holderegger R, van Heck HJ. 2015.** Isolation-by-distance in landscapes: considerations for landscape genetics. *Heredity* **114**: 27–37.
- Veldman JW. 2016.** Clarifying the confusion: old-growth savannahs and tropical ecosystem degradation. *Philosophical Transactions of the Royal Society, London, Series B* **371**: 20150306.
- Vernet P, Lepercq P, Billiard S, Bourceaux A, Lepart J, Dommée B, Saumitou-Laprade P. 2016.** Evidence for the long-term maintenance of a rare self-incompatibility system in Oleaceae. *New Phytologist* **210**: 1408–1417.

- Vieilledent G, Grinand C, Rakotomalala FA, et al. 2018.** Combining global tree cover loss data with historical national forest cover maps to look at six decades of deforestation and forest fragmentation in Madagascar. *Biological Conservation* **222**: 189–197.
- Virah-Sawmy M, Willis KJ, Gillson L. 2010.** Evidence for drought and forest declines during the recent megafaunal extinctions in Madagascar. *Journal of Biogeography* **37**: 506–519.
- Voarintsoa NRG, Railsback LB, Brook GA, et al. 2017.** Three distinct Holocene intervals of stalagmite deposition and nondeposition revealed in NW Madagascar, and their paleoclimate implications. *Climate of the Past* **13**: 1771–1790.
- Vorontsova MS, Besnard G, Forest F, et al. 2016.** Madagascar's grasses and grasslands: anthropogenic or natural? *Proceedings of the Royal Society, London, Series B* **283**: 20152262.
- Waeber PO, Wilmé L, Mercier JR, Camara C, Lowry PP. 2016.** How effective have thirty years of internationally driven conservation and development efforts been in Madagascar? *PLoS One* **11**: e0161115.
- Weir BS, Cockerham CC. 1984.** Estimating *F*-statistics, for the analysis of population structure. *Evolution* **38**: 1358–1370.
- Yoder AD, Campbell CR, Blanco MB, et al. 2016.** Geogenetic patterns in mouse lemurs (genus *Microcebus*) reveal the ghosts of Madagascar's forests past. *Proceedings of the National Academy of Sciences, USA* **113**: 8049–8056.
- Zedane L, Hong-Wa C, Murienne J, Jeziorski C, Baldwin BG, Besnard G. 2016.** Museomics illuminate the history of an extinct, paleoendemic plant lineage (*Hesperelaea*, Oleaceae) known from an 1875 collection from Guadalupe Island, Mexico. *Biological Journal of the Linnean Society* **117**: 44–57.

Table 1. *Noronhia lowryi* genetic diversity and microsatellite summary statistics by sampling site and on the whole sample. Number of genotypes per sampling site and on the whole sample is given in parenthesis. N_a : number of alleles, A_r : allelic richness, H_E : expected heterozygosity, H_O : observed heterozygosity, F_{IS} : departure from HW proportions index, HW: departure from HW proportions (Chi² p-value).

Site	Itremo-East (63)						Ibity (5)					Itremo-West (5)					Overall				
Locus	N_a	A_r	H_o	H_e	F_{IS}	HW	$N_a=A_r$	H_o	H_e	F_{IS}	HW	$N_a=A_r$	H_o	H_e	F_{IS}	HW	N_a	H_o	H_e	F_{IS}	HW
Nor-01	7	4.3	0.65	0.76	0.15	0.132	7	1.00	0.84	-0.08	1.000	4	0.80	0.70	-0.03	0.587	10	0.68	0.77	0.12	0.108
Nor-02	12	4.9	0.70	0.79	0.12	0.248	5	0.60	0.72	0.27	0.453	5	0.20	0.78	0.79	0.004	12	0.66	0.79	0.17	0.036
Nor-03	19	6.9	0.86	0.90	0.05	0.567	9	1.00	0.88	-0.03	1.000	8	1.00	0.84	-0.08	1.000	21	0.88	0.91	0.04	0.600
Nor-05	10	4.9	0.75	0.76	0.03	0.153	5	0.80	0.74	0.03	0.897	5	0.80	0.76	0.06	0.351	10	0.75	0.77	0.03	0.396
Nor-06	3	2.1	0.27	0.27	0.00	0.503	1	0.00	0.00	1.00	1.000	1	0.00	0.00	1.00	1.000	3	0.23	0.23	0.02	0.432
Nor-07	17	6.6	0.87	0.89	0.02	0.084	4	0.80	0.66	-0.10	1.000	6	1.00	0.78	-0.18	0.748	18	0.88	0.89	0.02	0.138
Nor-08	9	4.7	0.60	0.78	0.23	0.004	4	0.80	0.72	0.00	0.248	5	0.80	0.74	0.03	0.909	11	0.63	0.80	0.22	0.000
Nor-09	19	6.6	0.83	0.88	0.07	0.069	5	0.60	0.68	0.23	0.260	7	0.60	0.84	0.38	0.052	19	0.79	0.87	0.09	0.005
Nor-10	5	2.3	0.30	0.30	-0.01	0.287	3	0.40	0.34	-0.07	1.000	2	0.20	0.18	0.00	1.000	5	0.30	0.29	-0.03	0.316
Nor-11	4	1.5	0.11	0.11	-0.03	1.000	1	0.00	0.00	1.00	1.000	3	0.40	0.34	-0.07	1.000	4	0.12	0.12	-0.04	1.000
Nor-12	3	2.2	0.49	0.45	-0.09	0.794	2	0.20	0.42	0.60	0.323	1	0.00	0.00	1.00	1.000	3	0.44	0.43	-0.02	1.000
Nor-13	3	2.3	0.41	0.37	-0.10	0.894	2	0.20	0.18	0.00	1.000	2	0.60	0.42	-0.33	1.000	3	0.41	0.37	-0.11	0.841
Nor-14	12	5.1	0.68	0.79	0.15	0.034	4	0.40	0.64	0.47	0.149	5	0.80	0.72	0.00	1.000	15	0.67	0.80	0.17	0.010
Nor-15	9	4.7	0.81	0.77	-0.04	0.554	3	0.60	0.46	-0.20	1.000	5	1.00	0.76	-0.21	0.851	9	0.81	0.78	-0.03	0.404
Mean	9.4	4.2	0.59	0.63	0.04	-	3.9	0.53	0.52	0.22	-	4.2	0.59	0.56	0.17	-	10.2	0.59	0.63	0.05	-
Total	132	59.0					55					59					143

Figure 1. Distribution of *Noronhia lowryi* and location of sampled sites. The sampled region is located in Madagascar Central High Plateau (green dot). Samples used in this study are represented with orange dots. Herbarium records of *N. lowryi* are represented by red triangles [from Hong-Wa (2016), the Tropicos database and the Museum national Histoire Naturelle (MNHN) of Paris]. Protected areas are represented in green and national roads in red. Note that the protected area encompassing the site of Andoharano in the center-south of the map is considered since 2008 as a potential site to develop a protected area. We have no particular information suggesting that it is currently managed.

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870 **Figure 2.** Sampling of *Noronhia lowryi* in the eastern part of the Itremo massif. (A) General
 871 view of the landscape; (B) Fruiting individual (RJQ689-10); (C) Three neighbor ramets
 872 (RJQ689-4A, B and C) with the same genotype supporting a case of clonal growth; and (D)
 873 Three neighbor ramets (RJQ689-30A, B and C) with distinct genotypes. (Photo credit: Jan
 874 Hackel).

Figure 3. Inbreeding and genetic diversity in *Noronhia lowryi*. (A) Levels of averaged individual inbreeding. Most individuals have a relatively low inbreeding coefficient (< 0.4) from nuclear microsatellites data, and none of the three sampling sites show particularly high inbreeding levels. (B) Loci diversity expressed by the observed diversity plotted against the expected diversity. The graph shows that loci deviates very little from Hardy-Weinberg expectations for sites with large sample sizes. (C) Boxplot of sampling sites diversity represented by the observed (H_O) and expected diversity (H_E) across loci. The figure shows that the three sampling sites show overall similar diversity levels. IE: Itremo-East; Ib: Ibity; IW: Itremo-West; all: overall estimates.

Figure 4. *Noronhia lowryi*'s spatial nuclear genetic structure. (A) Measures of genetic divergence among sites with their confidence interval (dashed arrows). Abscise labels represent pairs of compared sites, IE: Itremo-East; Ib: Ibity; IW: Itremo-West. None of the divergence statistics differ from the null expectation under Hardy-Weinberg equilibrium (zero, horizontal black line). (B) Principal Component Analysis (PCA) of the genetic variance showing that the sampling origin does not drive nuclear genetic variance. (C) Graphic representation of the relationship between genetic and geographic distances (isolation by distance) suggesting that nuclear genetic differentiation of *N. lowryi* is not influenced by distance.

Figure 5. Spatial distribution of chloroplast genetic diversity in *Noronhia lowryi*. Pie charts indicate the frequency of each haplotype in the three populations. Their size is proportional to the number of genotyped individuals. No haplotypes are shared among sampling sites. Phylogenetic relationships between the six detected haplotypes were analyzed with a reduced-median network (Bandelt *et al.*, 1999) based on the dataset given in Supplementary Data Table S4. This analysis allowed distinguishing three lineages (namely A, B, C). For the distribution of chloroplast haplotypes at a finer scale (for Itremo-East) see Supplementary Data Fig. S6.

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907 **Figure 6.** *Noronhia lowryi*'s demographic history inferred with *VarEff* (Nikolic and Chevalet,
908 2014). Mode (black line), and kernel density (color scale) of the posterior distribution of the
909 effective population size (N_e) over time (in generations), inferred under three distinct mutation
910 models: (A) Single Step Mutation Model (SMM), (B) Geometric Mutation Model (GMM),
911 and (C) Two-Phase Mutation Model (TPM). The results only support a demographic decline
912 between 300 and 400 generations ago. See also Supplementary Data Fig. S7 for a more
913 extensive description of demographic history results.