South African Journal of Botany 89 (2013) 273–280

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

A snapshot of extinction in action: The decline and imminent demise of the endemic *Eligmocarpus* Capuron (Caesalpinioideae, Leguminosae) serves as an example of the fragility of Madagascan ecosystems

Dion S. Devey [a], Félix Forest [a], Frank Rakotonasolo [b], Persephone Ma [a], Bryn T.M. Dentinger [a,c], Sven Buerki [a,*]

[a] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, United Kingdom
[b] Kew Madagascar Conservation Centre, Lot II J 131 B Ambodivoanjo, Ivandry, Antananarivo 101, Madagascar
[c] Institute of Biology, Environment and Rural Sciences, University of Aberystwyth, Penglais, Aberystwyth, Wales, United Kingdom

ARTICLE INFO

Available online 21 July 2013

Edited by JS Boatwright

Keywords:
Biomes
Conservation
Dispersal
Legumes
Madagascar
Restoration ecology
River network

ABSTRACT

The southeastern Madagascan endemic and monotypic genus *Eligmocarpus* is highly threatened due to a combination of factors. Firstly, general human-induced habitat destruction and fragmentation has degraded the environment in which it occurs, leading to an increased threat of extinction for itself and other co-occurring species. Secondly, and more specifically to *Eligmocarpus*, the desirable properties of its timber, which is an excellent construction material, has led to over-collection beyond levels of sustainability. Thirdly, and with the highest relevance for this project, it is a combination of mode of dispersal, germination and seedling establishment. For all these reasons, its range has contracted and the only remaining population (21 trees) is located in Petriky, a future mining site. In this study we investigate the phylogeography and population dynamics of *Eligmocarpus* based on molecular tools (not only conducted on extant individuals but also using herbaria preserved DNA from individuals from neighbouring populations which are no longer alive, to give a glimpse of the past). Prior to human colonisation, the species was successful in using the river network to invade several biomes (most likely from the humid to subarid, where it is now constrained). Hence, due to its location, Petriky is a mosaic of the genetic variability from populations higher up in the river network, therefore, despite the low number of remaining individuals, all hope of restoration is not lost. Within this project we hope that a more complete understanding of the evolution of the flora will allow conservation, not only of current patterns of variation, but also the processes that gave rise to these patterns.

1. Introduction

Madagascar is located in the south-western Indian Ocean and has been recognized as a biodiversity hotspot by Myers et al. (2000) (Fig. 1A). The island is home to 11220 species of vascular plants, >80% of which are endemic (Callmander et al., 2011). Although Madagascar is facing unprecedented rates of deforestation due to human activities (<10% of primary vegetation remains; Moat and Smith, 2007), botanical expeditions conducted in remote areas still result in the discovery and description of new species (e.g. the Galoka and Kalabenono massifs in North-West Madagascar; Callmander et al., 2009). This is especially true for Leguminosae since the genus studied here, *Eligmocarpus* Capuron, was discovered only 62 years ago (but formally published by Capuron, 1968) and is now facing extinction.

Leguminosae is the third most species-rich family of the Madagascan flora (after the Orchidaceae, ca. 850 spp.; Rubiaceae, ca. 650 spp.; Callmander et al., 2011) with more than 600 species, 70% of which are endemic (Du Puy et al., 2002). In addition, 23 of the 113 genera occurring in Madagascar are restricted to the island (Buerki et al., 2013; Du Puy et al., 2002). Although the family occurs mainly in the dry and subarid biomes, representatives are also found in the humid biome (Du Puy et al., 2002; see Buerki et al., 2013 for more information on the biome distributions and their establishment through time). In this study we focus on the threatened endemic and monotypic genus *Eligmocarpus* (Caesalpinioideae), which is restricted to the south-east of Madagascar (Fig. 1), and has been demonstrated by Bruneau et al. (2008) to be sister to another Madagascan endemic genus, *Baudouinia* Baill. *Eligmocarpus cynometroides* Capuron occurs in the transition zone between the humid and subarid biomes, more specifically between the Andohahela national park (NP) and Petriky (representing a distribution area of <50 km^2; Fig. 1A). Population sizes are always very small, with the biggest population found in Petriky (a future mining site) with <30 trees (Randriatafika et al., 2007). As an example of the decline of this species, 27 trees were recorded in Petriky in 2001 and only 23 trees remained in 2004 (Randriatafika et al., 2007). Moreover, when we visited this site in 2012 two more trees had been felled and several trees are now

* Corresponding author. Tel.: +44 2083325397.
E-mail address: s.buerki@kew.org (S. Buerki).

0254-6299/$ – see front matter © 2013 SAAB. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.sajb.2013.06.013

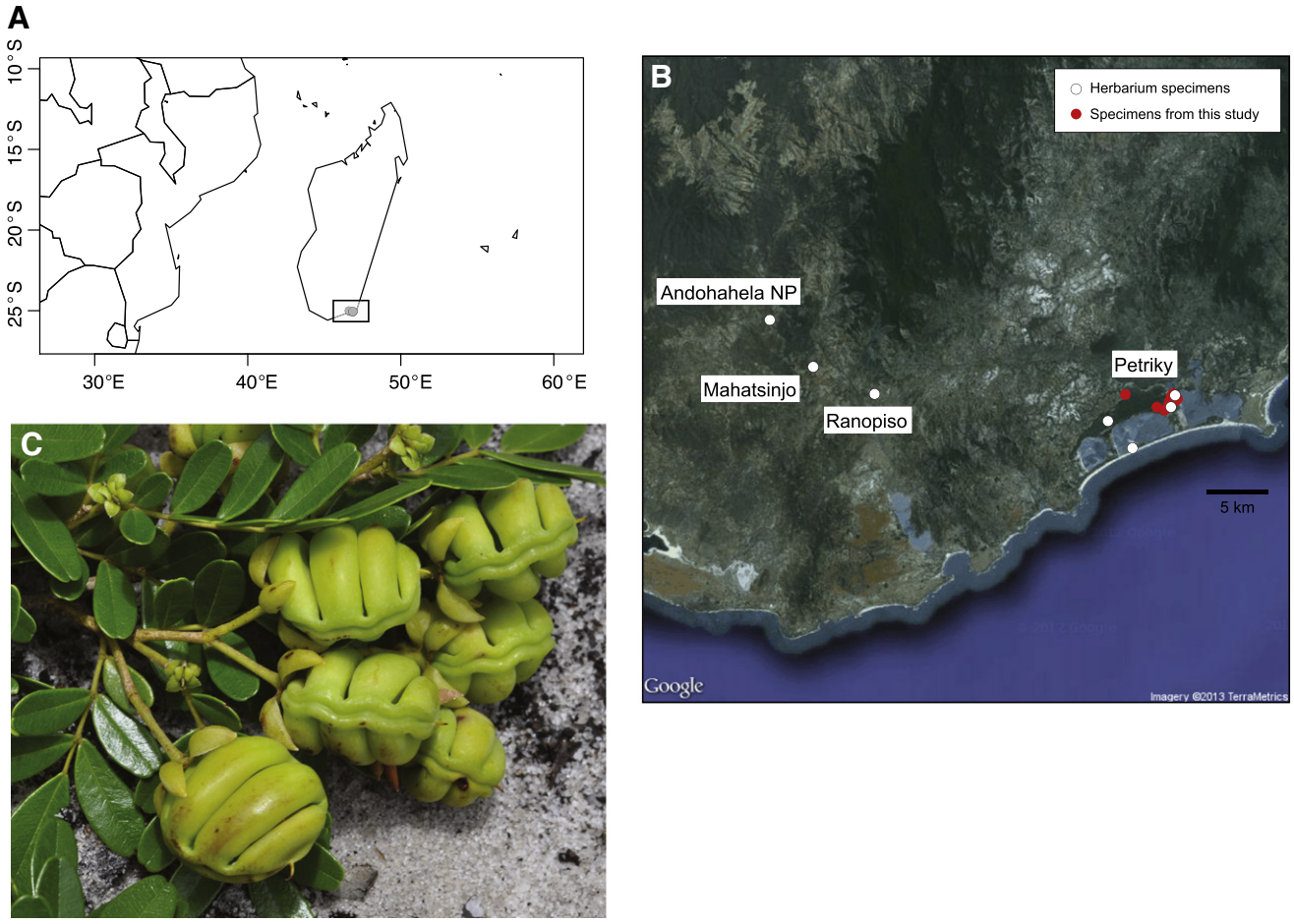

Fig. 1. A. Distribution of *Eligmocarpus cynometroides* in Madagascar. B. Close-up of the distribution of individuals in the Southeast of Madagascar. C. Picture of the fruits of *E. cynometroides* (Buerki et al. 272). [Photograph taken by F. Forest].

surrounded by degraded forest and in the vicinity of villages (Fig. 1). Collection trips, undertaken by the Service Forestier in the 1950s and 1960s, in the forests west of Ranopiso, identified further individuals, but subsequent trips, undertaken by Ratovoson in 1999 were unable to find any trees. This is further evidence that the only viable population today is most likely to be found in Petriky (Ratovoson, pers. comm. 2013). In addition to being threatened by anthropic factors (burning for charcoal, fire, agriculture and construction; Moat and Smith, 2007), this species is widely used by local communities as a timber since its wood is similar to rosewood (*Dalbergia* spp.; Randriatafika et al., 2007). In addition, with the exception of the population found in the Andohahela NP (that might be reduced to only one tree; Ratovoson, pers. comm. 2013), the other individuals occur outside of the national park network and are therefore highly threatened. Outside of Petriky, no individuals of *E. cynometroides* have been collected since 1999 (Table 1). An ecological study conducted on the individuals in Petriky showed that this species has a very low rate of seed production (ca. 1 seed/kg of fruit) and, moreover, its seed germination was also shown to be limited (<5%) (Randriatafika et al., 2007).

In this study we use molecular techniques [DNA sequencing of the Internal Transcribed Spacer (ITS) region and Amplified Fragment Length Polymorphism (AFLP)] to investigate the phylogeography of the species and to assess the genetic variability of the Petriky population (presumably the only remaining population of this species). Results are discussed in light of morphological characters, past climate change and landscape uses. We also extrapolate from our findings to propose a rigorous conservation programme for this species and hopefully to ensure its long-term survival. Finally, we would like to use *E. cynometroides* as a flagship species in order to seek additional funding to protect the unique vegetation found in this area of Madagascar (a rare environment where the humid, dry and subarid biomes meet; see Buerki et al., 2013).

2. Material and methods

2.1. Sampling and DNA extraction

A list of specimens of *E. cynometroides* was established based on specimens deposited at G, K, MO and P (Table 1). To infer the spatial distribution of these specimens a map was constructed using the R packages 'RgoogleMaps' and 'rworldmap' (R Development Core Team, 2010). We have evidence suggesting that populations outside of Petriky are limited to very few individuals or even totally extinct due to anthropogenic habitat destruction and wood exploitation (see Section 2.2). In this study, we first investigate the phylogeography of *E. cynometroides* by sequencing the nuclear ITS region from herbarium specimens taken from individuals that are now extinct as well as recent collections, both extant and extinct (see introduction), obtained by the authors in Petriky. Subsequently, a DNA fingerprinting method (AFLP) is applied to assess the genetic variability of individuals in Petriky and to propose a conservation strategy. Herbarium specimens were not included in this latter analysis because their DNAs were too degraded for the AFLP technique to be suitable. Field-collected specimens were dried in silica-gel prior to DNA extraction, following the recommendations of Chase and Hills (1991), though some accessions were extracted from herbarium collections of varying ages. Extraction of genomic DNA followed the 2 × CTAB protocol (Doyle and Doyle, 1987), with the following

Table 1
List of specimens of *Eligmocarpus cynometroides* included in this study and GenBank accession numbers.

Voucher	Year	Location	Longitude	Latitude	Altitude (m)	Herbarium	ITS EMBL no.	ITS haplotype
Herbarium specimens								
Dumetz 1102	1989	Petriky	46.867	−25.083	0–10	MO		
Dumetz 652	1989	Petriky	46.850	−25.067	0–10	K	HF937274	H1
Rabenantoandro 1610	2004	Petriky	46.896	−25.051	6	K	HF937275	H1
Rajoharison 18	–	Petriky	46.893	−25.058	10	MO		
Rajoharison 19	–	Petriky	46.893	−25.058	10	MO		
Randriatafika 325	2001	Petriky	46.850	−25.067	0–10	MO		
Ratovoson 102	1999	Andohahela NP (parcel 3)	46.621	−25.004	180	MO		
Service Forestier 20501	1961	Ranopiso	46.650	−25.033	–	K	HF937276	H2
Service Forestier 28325	1968	Mahatsinjo	46.650	−25.033	–	K	HF937277	H1
Service Forestier 3498	1951	Ranopiso	46.692	−25.050	0–100	P		
Service Forestier 8213	1953	Mahatsinjo	46.650	−25.033	–	P		
Service Forestier 8496	1953	Between Bevilany and Ranopiso	46.650	−25.033	–	P		
Service Forestier 9918	1952	Mahatsinjo	46.650	−25.033	–	P		
Collections done for this study								
Buerki et al. 272.1	2012	Petriky	46.89529	−25.05223	17	K	HF937260	H3
Buerki et al. 272.2	2012	Petriky	46.8932	−25.05162	16	–		
Buerki et al. 272.3	2012	Petriky	46.89324	−25.05136	13	–	HF937261	H3
Buerki et al. 272.4	2012	Petriky	46.89338	−25.05113	13	–	HF937262	H5
Buerki et al. 272.5	2012	Petriky	46.89427	−25.04975	10	–	HF937263	H3
Buerki et al. 272.6	2012	Petriky	46.89434	−25.0522	20	–	HF937264	H3
Buerki et al. 272.7	2012	Petriky	46.89562	−25.05263	20	–	HF937265	H3
Buerki et al. 272.8	2012	Petriky	46.89669	−25.05265	20	–	HF937266	H3
Buerki et al. 272.9	2012	Petriky	46.89728	−25.05309	11	–	HF937267	H3
Buerki et al. 272.10	2012	Petriky	46.89639	−25.05366	16	–	HF937268	H3
Buerki et al. 272.11	2012	Petriky	46.89639	−25.05349	17	–	HF937269	H3
Buerki et al. 272.12	2012	Petriky	46.89585	−25.05295	18	–	HF937270	H4
Buerki et al. 272.13	2012	Petriky	46.8947	−25.05392	15	–		
Buerki et al. 272.14	2012	Petriky	46.89478	−25.05393	16	–	HF937271	H3
Buerki et al. 272.15	2012	Petriky	46.89494	−25.0542	16	–	HF937272	H3
Buerki et al. 272.16	2012	Petriky	46.89197	−25.05402	30	–	HF937273	H3
Buerki et al. 272.17	2012	Petriky	46.89313	−25.05614	15	–		
Buerki et al. 272.18	2012	Petriky	46.89079	−25.05705	16	–		
Buerki et al. 272.19	2012	Petriky	46.88844	−25.05997	12	–		
Buerki et al. 272.20	2012	Petriky	46.88324	−25.05805	20	–	HF937278	H3
Buerki et al. 272.21	2012	Petriky	46.86184	−25.05034	20	–		

modifications: after precipitation with isopropanol and subsequent centrifugation, the DNA pellet was washed with 70% ethanol, dried at 37 °C, then re-suspended in TE buffer (20 mm Tris–HCl, 0.1 mm EDTA).

2.2. Determination of the environmental niche of *E. cynometroides*

To characterize the niche of *E. cynometroides*, worldclim data (here the bioclimatic variables BIO1, 5, and 12 and the elevation; available at http://www.worldclim.org/) were downloaded. In addition, information on geology and vegetation types was retrieved from Moat and Smith (2007). These data were extracted for each record using the R package 'raster' (R Development Core Team, 2010). A principal component analysis (PCA) was built based on these data (information on the geology and vegetation types were not included in the analysis since they are not quantitative, but will be discussed) using the R package 'vegan' (R Development Core Team, 2010).

2.3. DNA sequencing and network analysis

The amplification of the ITS region was carried out in two parts, using the primer pairs ITS5 and ITS2, and ITS3 and ITS4 (White et al., 1990). Both amplifications were performed in 25 μL reactions, containing 22.5 μL PCR Abgene mastermix (1.5 mM mg), 1 μL bovine serum albumin (0.04%), 33 ng of each ITS primer and 40 ng of DNA template. The PCR profile was as follows: initial denaturation of 94 °C for 2 min, followed by 28 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 3 min, followed by a final extension of 7 min at 72 °C. All PCR products were purified using Nucleospin DNA purification columns according to the manufacturers' protocols (QIAquick; Qiagen Ltd, Crawley, UK). Dideoxy cycle sequencing was then performed using the chain termination method and ABI Prism Big Dye version 3.1 reaction kit, following the manufacturers' protocols (Applied Biosystems Inc., Warrington, UK). The products were run on an ABI 3730 Genetic Analyser, also according to the manufacturers' protocols. The programme Sequencher v. 4.1 (Gene Codes Corp., Ann Arbor, Michigan, USA) was used to assemble complementary strands and verify software base-calling. Sequences were then aligned by eye, following the guidelines of Kelchner (2000). A maximum parsimony network was inferred based on the ITS dataset using TCS v.1.21 (Clement et al., 2000). The haplotypes defined by TCS were finally plotted on a map using the R package 'RgoogleMaps' (R Development Core Team, 2010).

2.4. AFLP amplification, scoring and analyses

A primer trial was conducted using 12 primer combinations to identify pairs of selective primers that would be most appropriate for this study. The 2C genome size was measured at 0.98 pg (following the guidelines of Pellicer et al., 2010), which is a larger genome than those for which the AFLP kit is optimized, therefore *Eco*R1 primers with four-base tails, rather than the usual three have been used to reduce the number of peaks produced to manageable levels. The rationale behind this is described in Devey et al. (2008). Primer combinations *Mse*1–AGG + *Eco*RI–CTAC and *Mse*1–ACA + *Eco*RI–CTAT (both 5 μm) were used following the manufacturers' instructions to produce AFLP profiles across all analysed accessions, since these combinations yielded suitable numbers of clearly identifiable bands and levels of variation among loci. Generated fragments were

mixed with a 500 ROX size ladder and analyzed with an ABI 3730 Genetic Analyser. In order to detect and calculate the size of AFLP bands, raw electropherograms were analysed using PeakScanner (ABI) with default parameters except a light peak smoothing. A binary matrix of AFLP band presence (1) and absence (0) was built using the automated scoring implemented in the R package 'RawGeno' (Arrigo et al., 2009) with the following parameters: scoring range, 50–500 bp; minimum intensity, 100 rfu; minimum bin width, 0 bp; and maximum bin width, 2 bp. Closely sized and overlapping bins were eliminated. Individuals were randomly distributed in the plates to produce a reliable AFLP dataset. Blank controls and duplicates were also included to ensure the reproducibility of the profiles (see Arrigo et al., 2010). Bands that were clearly not reproducible were discarded from the final dataset.

A principal coordinate analysis (PCoA) was inferred from the AFLP binary matrix using the R package 'vegan' (R Development Core Team, 2010) based on a Jaccard distance matrix. To confirm the grouping obtained by the PCoA analysis the Jaccard distance matrix was also investigated using a clustering approach by building a ward tree and defining four groups. These groups were displayed on the PCoA as well as the ward topology using the 'vegan' function ordicluster. A Mantel test was calculated between genetic and geographic distances among individuals using R packages 'stats' and 'vegan' as done in Arrigo et al. (2010). Finally, the groups were plotted on a map using the R package 'RgoogleMaps' (R Development Core Team, 2010).

3. Results

3.1. Environmental niche of E. cynometroides

Based on the available herbarium specimens (13 collections) and our collections (21 collections from Petriky), we can recognize four populations of *E. cynometroides* (from North-West to South-East): Andohahela NP (parcel 3), Mahatsinjo, Ranopiso and Petriky (Fig. 1). The only remaining population of *Eligmocarpus* (defined following IUCN conventions) occurs in the eastern part of the Petriky site and is composed of 21 adult trees that are concentrated on the edge of the forest in the vicinity of villages (Fig. 1). The PCA shows that i) Petriky receives more precipitation than the other sites, ii) Mahatsinjo is at higher elevation than the other sites (180 m vs. 0–20 m) and iii) Andohahela NP and Ranopiso have higher annual temperatures than the rest of the sites (Fig. 2). However, although Petriky receives more rainfall, its sandy soil (vs. limestone at the other sites) makes its ecological characteristics very similar to the other sites (due to the rapid drainage of water from the soil). Finally, Andohahela NP (parcel 3 of Andohahela is covered by dry forest, but it is at the edge, with the humid forest found at higher elevation) and Mahatsinjo harbour a dry to spiny forest, whereas Ranopiso is covered by spiny forest. The vegetation found in Petriky is unique for Madagascar, because it sits at the boundary of the humid and subarid biomes (Figs. 1–2).

3.2. DNA sequencing and network analysis

Sadly, the amplification of the ITS regions failed for most of the herbarium specimens, probably due to severe degradation caused by the use of an ethanol-based preservation methodology; however it was successful for two individuals from the Mahatsinjo population and collections from the Petriky population. From Petriky, we have also been able to sequence individuals that were felled by local communities in the last 5–10 years (Fig. 3). Based on the ITS matrix, five different haplotypes (based on single substitutions, rather than insertions/deletions) have been recognized: H1–5 (Fig. 3). The haplotype H1 is shared between Mahatsinjo and Petriky, whereas the other haplotypes are restricted either to Mahatsinjo (H2), or Petriky (H3–5) (Fig. 3). It is worth mentioning that the haplotype H1 is currently not found in Petriky, since these trees were recently

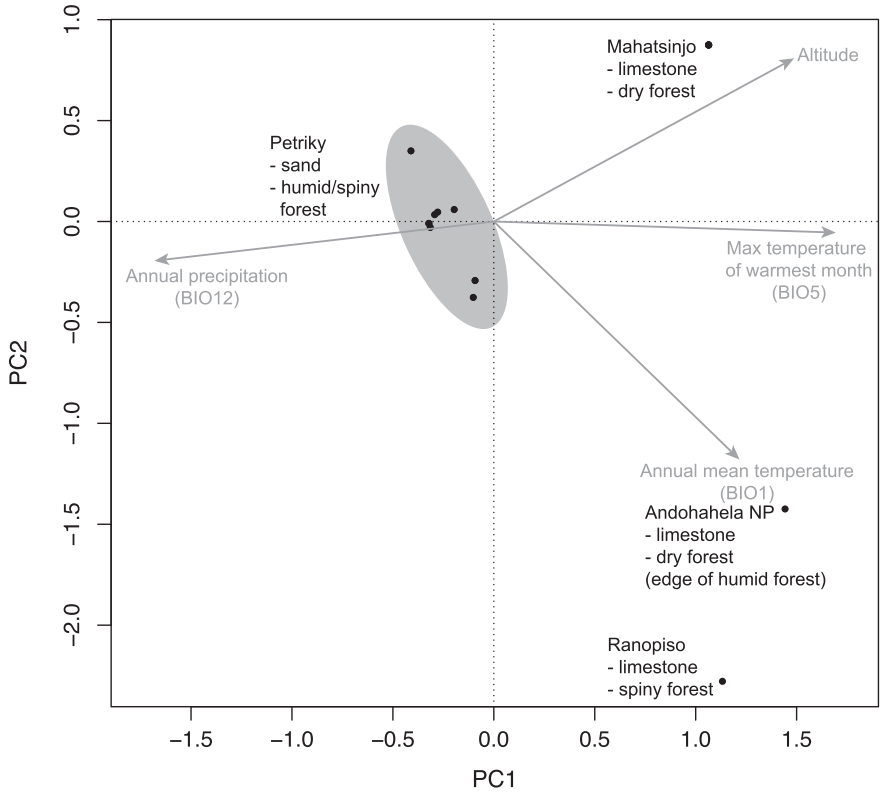

Fig. 2. Principal component analysis of the environmental variables for the four sites where *Eligmocarpus cynometroides* occurs.

felled (Fig. 3; Table 1). The tree was unrooted for several reasons; the lack of available ITS sequences for a suitable outgroup, the focus of the study being on population genetics rather than phylogenetics and the prior establishment of *Baudouinia* as a sister group to *Eligmocarpus* (Bruneau et al., 2008).

3.3. AFLP analyses

The AFLP data produced a total of 113 bands with an average of 66.7 bands per individual. The measured error rate was 3.54%. The approach combining the PCoA and clustering analysis allowed the definition of four groups of individuals within the Petriky population (Fig. 4). This showed that although there are very few trees remaining, the genetic diversity within the population is still relatively high. The Mantel test was not significant and therefore suggested that there is no correlation between the genetic and geographic distances. Finally, the AFLP groups are displayed on the map of the Petriky area and show that almost all the remaining trees are found on the edge of the forest on a dune (Fig. 5).

4. Discussion

4.1. Why is Eligmocarpus doing so badly?

A survey performed on *Eligmocarpus* in 2001 counted 27 trees and noted that seedlings were present near only three trees (<10 seedlings were still alive one year later; Randriatafika et al., 2007). In February 2012, only 21 trees remained and there was almost no sign of regeneration. Local communities felled the seven missing trees (decreasing the population by >22% in eleven years and also reducing the number of haplotypes, i.e. haplotype H1 is not represented in the population anymore; Fig. 3), despite the fact that this species has been established as critically endangered and protection/conservation planning was proposed by the mining company in 2007 (Randriatafika et al., 2007). In addition to being threatened by the activities of local communities, the remaining trees are located on the proposed mining pathway (Randriatafika et al., 2007). At this rate of felling, the whole population might be extinct even before the mining activity takes place at Petriky. This statement is especially true since most of the remaining trees occur in the vicinity of the villages and are found in open, degraded habitats (Fig. 5). This species is also of high concern because of poor seed production (ca. 1 seed/kg of fruits) and displays very low germination rates (<5% if not treated with an increase to 43% when soaked in cold water for 48 h) (Randriatafika et al., 2007). Interestingly, the individual belonging to the group 4 is the most successful in producing seeds (Landry pers. comm. 2012; Figs. 4–5). More attention should therefore be focused on this individual in order to understand the breeding system and pollination syndrome of this species, and to try to improve seed production.

4.2. Origin and genetic diversity of the Petriky population

The sequencing of herbarium specimens from populations outside of Petriky allowed the establishment of past genetic connectivity between populations (haplotype H1) as well as haplotypes specific to each of the sites (Fig. 3). This strongly suggests that gene flow was

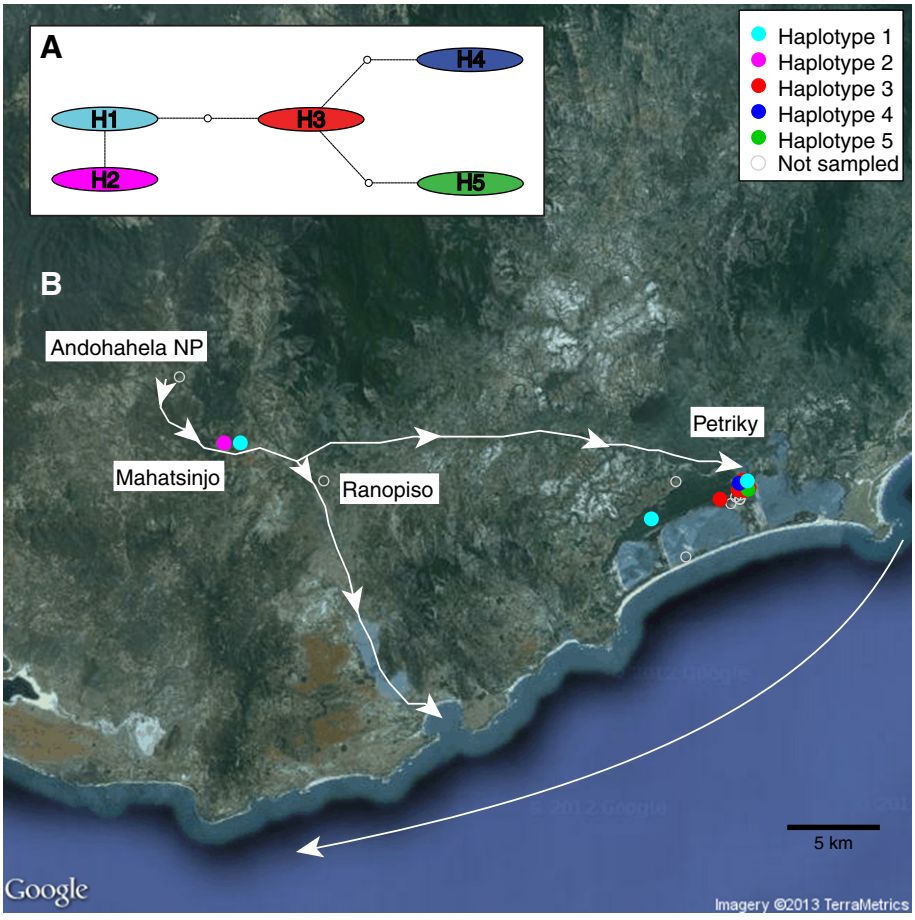

Fig. 3. A. Maximum parsimony network of *Eligmocarpus cynometroides* based on ITS data and inferred using TCS. B. Spatial distribution of the haplotypes. The arrows symbolise the river system and sea current.

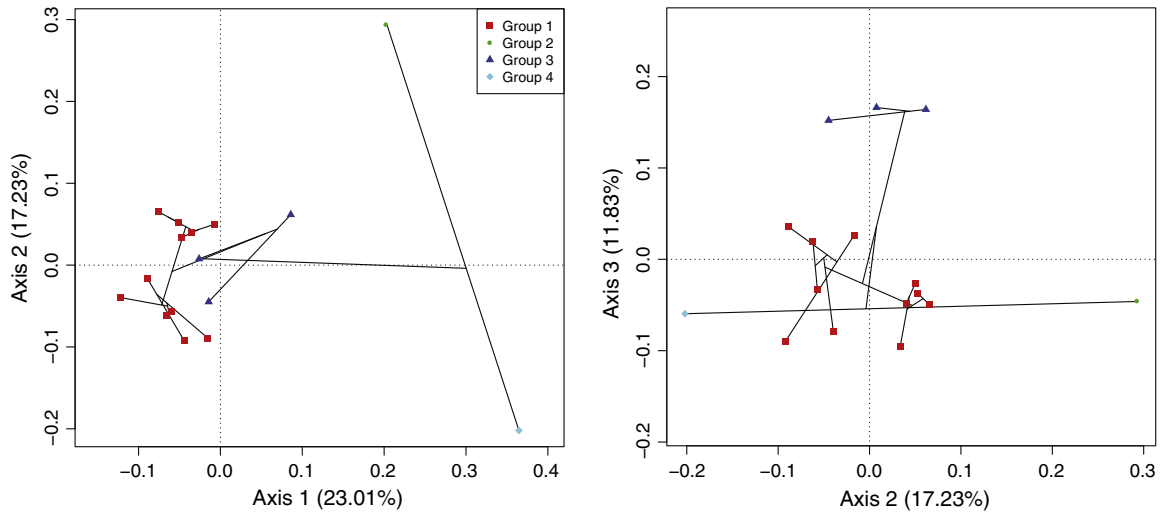

Fig. 4. Principal coordinate analysis based on the AFLP data of *Eligmocarpus cynometroides*. The result of the clustering analysis is also plotted (see groups) as well as the ward topology.

effective prior to human settlements (ca. 1500–500 years ago; see below). The AFLP data showed that although there is a core of individuals in Petriky that are genetically similar (group 1), the population is still genetically diverse, but that this diversity is not correlated to geography (the groups are intermixed, making conservation practices even more challenging; Figs. 4–5). If we take fruit morphology into account (Fig. 1C), we can hypothesize that the river network has played an important role in dispersing *Eligmocarpus* (Fig. 3). In this hypothesis, the origin would have been close to the populations currently located in the Andohahela area and the individuals used the river network to colonize the area and finally reach Petriky (Fig. 3). Interestingly, Ranopiso is at the intersection between two river basins and we

Fig. 5. Spatial distribution of the AFLP groups (see Fig. 4) of *Eligmocarpus cynometroides*.

might expect that *Eligmocarpus* previously occurred in the whole area prior to human deforestation (ca. 1150 years ago; Virah-Sawmy et al., 2009). This hypothesis is even more likely based on a fruit morphology that is indicative of an aquatic mode of dispersal and the evidence of increasing levels of seed germination when seeds were soaked in cold water (Fig. 1C; Randriatafika et al., 2007). Whilst some variation from this model may be possible, such as the occasional dispersal of seed by lemur activity and sporadic times when backwash, initiated by high tides etc., could transport seed back upriver, this hypothesis assumes that seed has travelled predominantly downstream from Andohahela. This hypothesis would also explain the large ecological niche of *E. cynometroides*, which had the ability to shift between biomes by using the river network to establish new populations on riverbanks and seacoasts. As the individuals in Petriky are currently restricted to the dune (10 m) and do not have direct contact with either rivers or the sea (Fig. 5), further investigation into previous climatic conditions was necessary to validate the aquatic-dispersal hypothesis. During the Holocene (more specifically 6500 years ago), the sea level in this region was 10–15 m higher due to a combination of the tilt of the planet and effects due to the last glaciations (Virah-Sawmy et al., 2009; Roberts et al., 2012). At this point in time, the individuals of *Eligmocarpus* would have been in close proximity to water, thereby allowing the fruit to float and act as a diaspore, and thus, seem to have subsequently been able to reach the riverbanks and shore again, but were felled by local communities in the last ten years (Figs. 1, 5). The relatively high genetic richness of the population in Petriky (supported by both ITS sequences and AFLP data) might suggest that it results from several independent dispersal events.

4.3. A brief summary; history, protection and restoration

In this section we will discuss the implications of our study for understanding the dynamics of this species through time and propose some lines of investigations to ensure the future prosperity of this taxon.

At a geographic level, the disruption of gene flow between populations of *Eligmocarpus* is likely to be primarily the result of habitat fragmentation, but compounded by the specialised dispersal mode utilised by *Eligmocarpus*. The habitat fragmentation is positively correlated with the intensity of agriculture, charcoal production and need for wood to build houses in the south-east that took place ca. 500 years ago, as attested by archaeological evidence (Virah-Sawmy et al., 2009 and references therein). Human settlement in this area started ca. 1150 years ago, but populations were small and patchy and their effect on the vegetation was rather limited (Virah-Sawmy et al., 2009 and references therein).

In many respects, the actions of the mining company are admirable, in that an attempt is being made to provide a net positive impact, by providing protected offset areas into which seedlings can be transplanted. However, the results of this study clearly demonstrate that without a thorough understanding of the ecology and evolutionary history of the flora and fauna of an area, this approach, on its own, is insufficient. In this case, protecting an offset area of forest with a different ecotype and no geographical connection to Petriky would do nothing to aid *Eligmocarpus*.

One simple but effective way to improve seedling survival and increase the chance of future successful germination would be to transplant seedlings onto riverbanks or shores in protected areas. In addition, tests will also be conducted at the Millennium Seed Bank at the Royal Botanic Gardens, Kew, in order to determine the optimal conditions for germination. Now that we have a much wider understanding of the biology of this species, further fieldwork is required in the area, to look for individuals along the river network from which seed could be collected (it is unlikely that trees will be found on the shore since most of the villages are located there). Finally, a study should be conducted focusing on the breeding system and pollination syndrome to improve seed production for restoration ecology purposes.

The case of *E. cynometroides* can be viewed as a proxy study for little-understood taxa in complex environments with high levels of endemism. This genus was first discovered in 1951, formally named in 1968 and, at the present rate of destruction, will probably be extinct before it reaches its hundredth birthday. However, we are confident that if the conservation and scientific communities, as well as local communities actively work together we will be still be able to secure the future of this unique lineage of Legume.

Acknowledgements

We would like to thank the KMCC team (especially Helene Ralimanana), Stuart Cable, Natasha Ali, Paul Smith and the QMM team in Fort-Dauphin (especially Faly Randriatafika and Johny Rabenantoandro) and the “Service des Eaux et Forets” for the collection permits. We would also like to thank Fidy Ratovoson for useful information on both modern and historical collection locations. The fieldwork of this study was funded by QMM Madagascar. We are grateful to Bruce Pavlik for funding the laboratory and his constant support, and to Jaume Pellicer for genome-size measurements. We are grateful to the curators of the following herbaria G (Laurent Gautier), K (Gwil Lewis and Ruth Clark), MO (Peter B. Phillipson, Porter P. Lowry II, Martin Callmander) and P. We are also grateful to the editor and two anonymous reviewers for their valuable comments that improved our manuscript. Finally, we would like to warmly thank the committee of the VI Legume conference held in Johannesburg (January 2013) for allowing us to submit this contribution.

References

Arrigo, N., Tuszynski, J.W., Ehrich, D., Gerdes, T., Alvarez, N., 2009. Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. BMC Bioinformatics 10.

Arrigo, N., Felber, F., Parisod, C., Buerki, S., Alvarez, N., David, J., Guadagnuolo, R., 2010. Origin and expansion of the allotetraploid *Aegilops geniculata*, a wild relative of wheat. New Phytologist 187 (4), 1170–1180.

Bruneau, A., Mercure, M., Lewis, G.P., Herendeen, P.S., 2008. Phylogenetic patterns and diversification in the caesalpinioid legumes. Botany-Botanique 86 (7), 697–718.

Buerki, S., Devey, D.S., Callmander, M.W., Phillipson, P.B., Forest, F., 2013. Spatio-temporal history of the endemic genera of Madagascar. Botanical Journal of the Linnean Society 171 (2), 304–329.

Callmander, M.W., Rakotovao, C., Razafitsalama, J., Phillipson, P.B., Buerki, S., Hong-Wa, C., Rakotoarivelo, N., Andriambololonera, S., Koopman, M.M., Johnson, D.M., Deroin, T., Ravoahangy, A., Solo, S., Labat, J.-N., Lowry, P.P., 2009. New species from the Galoka and Kalabenono massifs: two unknown and severely threatened mountainous areas in NW Madagascar. Candollea 64 (2), 179–202.

Callmander, M.W., Phillipson, P.B., Schatz, G.E., Andriambololonera, S., Rabarimanarivo, M., Rakotonirina, N., Raharimampionona, J., Chatelain, C., Gautier, L., Lowry, P.P., 2011. The endemic and non-endemic vascular flora of Madagascar updated. Plant Ecology and Evolution 144 (2), 121–125.

Capuron, R., 1968. Contributions à l'étude de la flore forestière de Madagascar. Adansonia 8 (2), 199–222.

Chase, M.W., Hills, H.H., 1991. Silica-gel — an ideal material for field preservation of leaf samples for DNA studies. Taxon 40 (2), 215–220.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9 (10), 1657–1659.

Devey, D.S., Bateman, R.M., Fay, M.F., Hawkins, J.A., 2008. Friends or relatives? Phylogenetics and species delimitation in the controversial European orchid genus Ophrys. Annals of Botany 101 (3), 385–402.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19, 11–15.

Du Puy, D.J., Labat, J.-N., Rabevohitra, R., Villiers, J.-F., Bosser, J., Moat, J., 2002. The Leguminosae of Madagascar. Kew Publishing, UK.

Kelchner, S.A., 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. Annals of the Missouri Botanical Garden 87 (4), 482–498.

Moat, J., Smith, P., 2007. Atlas of the Vegetation of Madagascar. Kew Publishing, UK.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403 (6772), 853–858.

Pellicer, J., Fay, M.F., Leitch, I.J., 2010. The largest eukaryotic genome of them all? Botanical Journal of the Linnean Society 164 (1), 10–15.

R Development Core Team, 2010. R: A Language and Environment for Statistical Computing, Reference Index Version 2.8.1. R Foundation for Statistical Computing, Vienna, Austria.

Randriatafika, F., Rabenantoandro, J., Birkinshaw, C., Vincelette, M., 2007. Biology, ecology, risk of extinction, and conservation strategy for *Eligmocarpus cynometroides* (Fabaceae): a priority species at Petriky. In: Ganzhorn, J.U., Goodman, S.M., Vincelette, M. (Eds.), Biodiversity, Ecology and Conservation of Littoral Ecosystems in Southeastern Madagascar, Tolagnaro (Fort-Dauphin), Washington, pp. 369–377.

Roberts, G.G., Paul, J.D., White, N., Winterbourne, J., 2012. Temporal and spatial evolution of dynamic support from river profiles: a framework for Madagascar. Geochemistry, Geophysics, Geosystems 13. http://dx.doi.org/10.1029/2012gc004040.

Virah-Sawmy, M., Willis, K.J., Gillson, L., 2009. Threshold response of Madagascar's littoral forest to sea-level rise. Global Ecology and Biogeography 18 (1), 98–110.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications, New York, USA, pp. 315–322.