# Interspecific comparisons with chloroplast SSR loci reveal limited genetic variation in Nigerian montane forests: A study on Cordia Millenii (West African Cordia), Entandrophragma angolense (tiama mahogany), and Lovoa trichilioides (African Walnut)

Authors: Thia, Joshua A. , Hale, Marie L. , and Chapman, Hazel M.

Source: Tropical Conservation Science, 9(1) : 321-337

Published By: SAGE Publishing

URL: https://doi.org/10.1177/194008291600900117

**Research Article**

# Interspecific comparisons with chloroplast SSR loci reveal limited genetic variation in Nigerian montane forests: A study on *Cordia millenii* (West African Cordia), *Entandrophragma angolense* (tiama mahogany), and *Lovoa trichilioides* (African walnut).

**Joshua A. Thia[1, 4], Marie L. Hale[2, 5] and Hazel M. Chapman[2, 3, 6]**

[1] School of Biological Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia
[2] School of Biological Sciences, The University of Canterbury, PB 4800, Christchurch, New Zealand
[3] Nigerian Montane Forest Project, Yelwa Village, Taraba State, Nigeria
[4] Corresponding author; email: josh.thia@live.com
[5] Contributing author; email: marie.hale@canterbury.ac.nz
[6] Contributing author; email: hazel.chapman@canterbury.ac.nz

**Abstract**

The montane forests of south-eastern Nigeria are of immense conservation value due to their high levels of biodiversity and endemism. Yet despite increasing anthropogenic disturbance and forest fragmentation, little is known about the genetics of resident tree populations. We used a set of conserved chloroplast simple sequence repeat (SSR) primers to quantify and directly compare genetic diversity in three tree species: *Cordia millenii*, West African Cordia; *Entandrophragma angolense*, tiama mahogany; and *Lovoa trichilioides*, African walnut, within a single montane forest. Additionally, we assessed the diversity of West African Cordia between forests at a local and regional scale. Results indicate that for our focal loci, in all three species, there is a general lack of chloroplast genetic diversity. Our study is particularly relevant because it considers genetic diversity among multiple tree species simultaneously. This work represents the first study of its kind in the region, and will pioneer the way for future conservation genetic studies in montane Nigeria.

Keywords: Afromontane, chloroplast microsatellites, interspecific population genetic comparisons, Mambilla Plateau, Nigeria.

Les forêts de montagne du sud-est Nigerien sont d'une grande valeur pour la conservation immense en raison de leurs niveaux élevés de biodiversité et d'endémisme. Pourtant, malgré l'augmentation de perturbations et de fragmentations (d'origine) anthropique de la forêt, peu est connu sur la génétique des populations d'arbres résidents. Nous avons utilisé un ensemble de répétition de séquences chloroplaste simples conservées (SSR) amorces pour quantifier et comparer directement la diversité génétique de trois espèces d'arbres (*Cordia millenii*, Cordia Afrique de l'Ouest; *Entandrophragma angolense*, tiama acajou; *Lovoa trichilioides*; noyer africaine) au sein d'une seule forêt de montagne. En outre, nous avons évalué la diversité de Cordia Afrique de l'Ouest entre les forêts à l'échelle locale et régionale. Les résultats indiquent que pour notre loci focal, pour les trois espèces, il y a un manque général de diversité génétique des chloroplastes pour les trois espèces. Notre étude est particulièrement pertinente car elle estime que la diversité génétique à travers les espèces d'arbres multiples simultanément. Ce travail représente la première étude de ce genre dans la région, et pionnier façonne la voie à de futurs travaux de conservation dans montagnarde Nigeria.

**Received**: 8 October 2016; **Accepted** 19 January 2016; **Published**: 28 March 2016



**Cite this paper as:** Thia, J. A., Hale, M. L. and Chapman, H. M. 2016**.** Interspecific comparisons with chloroplast SSR loci reveal limited genetic variation in Nigerian montane forests: A study on *Cordia millenii* (West African Cordia), *Entandrophragma angolense* (tiama mahogany), and *Lovoa trichilioides* (African walnut). *Tropical Conservation Science* Vol. 9 (1): 321-337. Available online: www.tropicalconservationscience.org



## Introduction

The consequences of deforestation on tree population genetics are diverse [1–6] and are intimately related to how different tree species respond to fragmentation [7, 8]. Unlike other organisms, trees are sessile, so long distance dispersal generally occurs during the reproductive cycle as pollen flow or seed dispersal [8, 9]. Fragmentation influences plant population evolution by impacting the interplay among ecological traits, ecological interactions, gene-flow, and the underlying population genetic variation [10].

Studies that investigate population genetic patterns in multiple co-occurring tree species are rare [11–14], and there are issues with finding molecular markers that provide directly comparable measurements across species [15]. While the use of simple sequence repeats (SSRs, *i.e.* microsatellites) from the nuclear genome is a popular method of assessing population genetic parameters, primer sets often have limited transferability amongst taxa, making it difficult to directly compare species [15]. Chloroplast (cp) SSRs, therefore, are an attractive alternative, because primers can be anchored in conserved regions of the chloroplast genome and be used to compare interspecific genetic patterns [16, 17].

The montane forests of Africa have experienced fluctuations in size and distribution due to historic cycles of global warming and cooling [18–22]. However, growing anthropogenic pressure in West Africa is creating unprecedented forest loss, and relative lack of knowledge of the region's biodiversity and ecology demands a greater effort from conservation biologists [23, 24]. In particular, the Mambilla Plateau on the Nigeria-Cameroon border (and part of the Cameroon Highlands) is of high conservation priority due to its high levels of biodiversity in flora and fauna [25, 26] (Fig. 1a).

Three tree species were selected for this study based on their differences in ecology and conservation status: West African Cordia (*Cordia millenii*), tiama mahogany (*Entandrophragma angolense*), and African walnut (*Lovoa trichilioides*). Tiama Mahogany (Welw.) and African walnut (Harms.) are both shade-tolerant, wind-dispersed Meliaceae species [27] that are listed as Vulnerable on the IUCN Red List [28, 29]. West African Cordia (Bak.) is a shade-intolerant, animal-dispersed Boraginaceae species [27, 30], considered to be of Least Concern on the IUCN Red List [31]. All three tree species are under pressure from logging in Nigeria [32–34]. These three species

share a similar geographic range (from West to East Africa), though the distribution of African walnut is not as extensive as that of West African Cordia or tiama mahogany [28, 29, 31].

Our objective was to provide a preliminary investigation into interspecific patterns of maternally-inherited genetic diversity of trees on the Mambilla Plateau. Our hypotheses pertain to the distribution of diversity within and amongst patchily distributed forests. Considering all three species, we expected that: (1) at the within-forest scale, our three focal species would exhibit different genetic patterns, related to their different dispersal ecologies. Focusing solely on West African Cordia, we also expected that: (2) between forests at a local scale (<10km apart) some small genetic differences might exist between forests; and (3) at a broader regional scale (>40km apart) significant genetic differences would exist between forests.

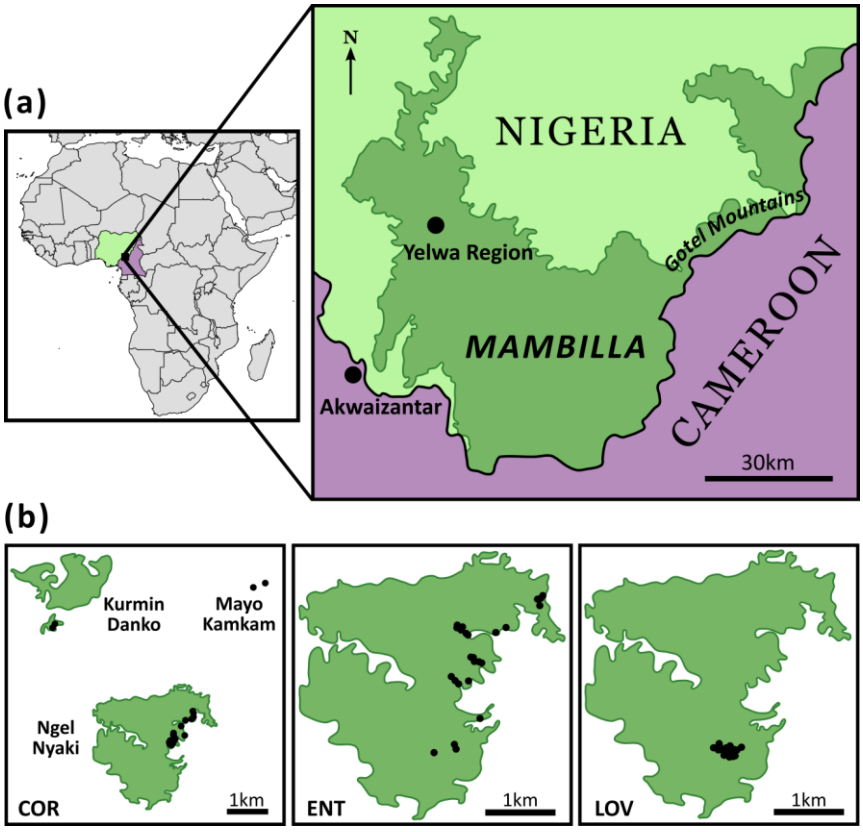


**Fig. 1. (a) Geographic context of our study (modified from [25]). The Mambilla Plateau (dark green) is situated on the highlands that straddle Nigeria (light green) and Cameroon (purple). Trees were sampled from the Yelwa area (Ngel Nyaki Forest, near Kurmin Danko Forest, and Mayo Kamkam) and Akwaizantar Forest (black circles). (b) Locations of individual trees sampled in the Yelwa area (black dots) for each focal species, abbreviated as follows: *C. millenii* (West African Cordia) = COR; *E. angolense* (tiama mahogany) = ENT; and *L. trichilioides* (African walnut) = LOV.**

## Methods

Our main site for interspecific comparisons at the 'within forest' scale was Ngel Nyaki Forest Reserve (07°05'N and 011°05'E), a forest fragment of approximately 5.5km$^2$ on the western escarpment of the Mambilla Plateau. Elevation ranges from 1,400–1,600m. The mean annual rainfall is 1,800mm, occurring mainly between mid-April and mid-October. Mean maximum/minimum monthly temperatures for the wet/dry season are 26.1/13.1°C, and 23.1/16.1°C (respectively) based on Nigerian Montane Forest Project (NMFP) weather records. Sampling occurred during the years 2012 and 2013. Where possible leaf tissue was collected, otherwise a cambium core was taken [35].

We were able to sample Ngel Nyaki Forest relatively extensively. The NMFP has established a network of transects throughout the east side of the forest. We sampled all adult individuals, from each tree species, known to occur on those transects. A tree was classed as an adult when its DBH (diameter at breast height) was >10cm and its height was >4m. The distribution of trees in all sites is illustrated in Fig. 1b. West African Cordia are found in small stands throughout the forest. Tiama

mahogany is dispersed throughout the forest. All known African walnut occur in the southern part of Ngel Nyaki Forest and form a single large stand. Based on DBH measurements, we sampled a broad age range for each species—our collection was thus not biased towards older or younger trees.

Additional samples of West African Cordia were also obtained from a small forest fragment bordering Kurmin Danko Forest (~07°06'N 01°01'E), and from degraded riparian forest bordering the Mayo Kamkam (~07°07'N 011°04'E). These three sites are proximal to Yelwa Village and were also classed as being sampled from the Yelwa area. In addition we obtained a sample from the more distant Akwaizantar Forest (~06°52'N 10°55'E), ~43km away (Fig. 1a), as a herbarium specimen (31795) from the Royal Botanic Gardens, Kew. These additional sample sites allowed us to assess the distribution of genetic variation in populations of West African Cordia at the 'between forests' scale at both the local and regional level. Total samples for each species and each site are detailed in Table 1.

**Table 1.** Sample sizes for each of our focal species. The proximally close sites, Ngel Nyaki Forest, Kurmin Danko Forest, and Mayo Kamkam, were grouped as the Yelwa area to be compared to a sample sourced from Akwaizantar Forest at the regional scale. Species abbreviations: *C. millenii* (West African Cordia) = COR; *E. angolense* (tiama mahogany) = ENT; and *L. trichilioides* (African walnut) = LOV.

| Species | Ngel Nyaki | Kurmin Danko | Mayo Kamkam | Akwaizantar | TOTAL |
|---|---|---|---|---|---|
| COR | 37 | 2 | 2 | 1 | 41 |
| ENT | 25 | | | | 25 |
| LOV | 26 | | | | 26 |
| | Sampled from the Yelwa area | | | | |

DNA extractions were prepared from 5–6mg ground cambial or leaf tissue using a modified CTAB method sourced from Brunner *et al.* (2001) or a PowerPlant® DNA Isolation Kit (MoBio Laboratories). KAPA3G Plant PCR Kit (Kapa Biosystems) was used for PCR reactions. A 20µL reaction volume was used with the reagent mix: 10µL KAPA Plant PCR Buffer (2×), 0.3µM each primer, 5mM additional $MgCl_2$, ~20ng DNA, 0.2µL KAPA3G Plant DNA Polymerase, and PCR water as required. Some reactions required addition of KAPA3G Enhancer solution at 1× final concentration. Primers implemented in this study were sourced from a conserved set of chloroplast SSR primers for dicotyledonous angiosperms [16]. After screening all 10 loci it was determined ccmp2, ccmp3, ccmp4, ccmp5, ccmp6, ccmp7, and ccmp10 would amplify in our study species (Appendix 1).

The PCR conditions were: 3 minutes at 95°C (initial denaturing); 40 cycles of 20 seconds at 95°C (denaturing), 15 seconds at 50°C (annealing), and 45 seconds at 72°C (extension); completed with 30 seconds at 72°C (final extension). For some particularly difficult samples, increasing the annealing time to 20 seconds and the extension time to 50 seconds, assisted successful amplification. Genotyping was carried out using an ABI Prism 3130xl Genetic Analyzer. For all species, loci could be pooled into two groups for genotyping: (i) ccmp2, ccmp3, ccmp4, and ccmp5; and (ii) ccmp6, ccmp7, and ccmp10. Each genotyping reaction contained 0.6µL PCR product per locus, 12uL HiDi™ (Applied Biosystems) and 0.3µL 500 LIZ™ (Gene Scan™; an internal size standard). The resulting chromatrograms were aligned with the internal size standard and analyzed with Gene Marker v1.97 (SoftGenetics LLC, CA, USA). As in Weising & Gardner [16], alleles for each ccmp locus were scored as the second to largest peak. We were able to resolve alleles to 1bp and any ambiguous alleles were re-run on the sequencer. Because the genotypes produced by cpSSRs are haploid, there was no need to assess for null alleles as should be done for nSSRs [37].

The effective number of haplotypes ($n_e$) and the haplotypic diversity ($H_E$) were calculated for each population of tree species, following Nei [38]. Average genetic distances amongst individuals within each tree species' respective population were measured using the metric $\overline{D}^2_{SH}$ [39, 40]. $\overline{D}^2_{SH}$ is based on a step-wise mutational model [41] and measures the mean squared pair-wise haplotype differences amongst all possible pairs of individuals in the population using absolute size differences in their alleles. If $n$ is the number of individuals, $L$ is the number of loci, $a_{ik}$ and $a_{jk}$ are the allele sizes for individuals $i$ and $j$ at locus $k$, $\overline{D}^2_{SH}$ is calculated as follows:

$$\overline{D}^2_{SH} = \frac{1}{[n(n-1)]/2} \times \frac{1}{L} \times \sum_{i=1}^{n} \sum_{j=i+1}^{n} \left[ \sum_{k=1}^{L} | a_{ik} - a_{jk} | \right]^2$$

$\overline{D}^2_{SH}$ is a useful and commonly used statistic because it provides a simple way to interpret measures of genetic dissimilarity among individuals at cpSSR loci [39, 40, 42, 43].

In order to compare our results to previous investigations into genetic diversity within adult tropical/subtropical tree populations, we reviewed 16 papers that measured cpDNA diversity in 20 species. Diversity measures included cpSSRs, restriction sites, indels, and substitutions, and covered a range of spatial scales (Appendix 2). It was not always possible to extract the same genetic diversity statistics from these studies, so we focused on the mean number of haplotypes per population for each species in each study. To understand how the number of cpDNA loci and number of populations sampled might impact the mean number of haplotypes observed, we conducted linear regressions on these data; models were fit using R (R Core Team 2014).

## Results

The results from the ccmp SSR analysis showed incredibly low cpDNA genetic variation in each focal tree species, within Ngel Nyaki Forest. Of the seven loci examined, all were monomorphic in West African Cordia and tiama mahogany; African walnut was only polymorphic at one locus, ccmp6 (Table 2). One haplotype was therefore observed in West African Cordia and tiama mahogany, and two in African walnut. The lack of genetic diversity at the ccmp cpSSRs used in this study is consequently reflected in the three genetic diversity indices used. Both West African Cordia and tiama mahogany had values of $n_e$ = 1.00, $H_E$ = 0.00 and $\overline{D}^2_{SH}$ = 0.00. African walnut exhibited slightly different values due to the small amount of variation present at ccmp6. In this population: $n_e$ = 1.17, $H_E$ = 0.15, and $\overline{D}^2_{SH}$ = 0.02, which are very small and suggest almost no variation.

We also observed complete genetic homogeneity of West African Cordia samples between forests at both the local and regional scale. From all the sampled sites, only a single haplotype was recovered (Table 2). This was surprising, because we expected at least some variation to exist between forests, particularly when comparing trees from Akwaizantar Forest and those in the Yelwa area.

Our review of other tropical tree cpDNA diversity studies provided some interesting insights into studying population genetic patterns in the chloroplast genome. We considered how both number of loci and number of populations sampled might impact the mean number of haplotypes observed. However, neither the number of loci ($P$ = 0.807), nor the number of populations ($P$ = 0.984), were a statistically significant predictor of the mean number of haplotypes observed within populations of the 20 tree species investigated (Appendix 3). One species, *Dysoxylum malabaricum* (white cedar)[45] was an outlier in our analysis with an average of 7.667 haplotypes per sampled population, notably more than in all the other species studied (Appendix 3). However, removing *D. malabaricum* from the analysis did not alter the statistical non-significance of the relationship

between the number of loci, and the number of populations sampled, with the mean number of haplotypes.

**Table 2.** Frequency of haplotypes (written in the numeric order of ccmp loci, *i.e.*: ccmp2/ccmp3/ccmp4/ccmp5/ccmp6/ccmp7/ccmp10) for the focal species**.** Alleles are represented by number of base pairs. Frequency and sample size (*n*) for *C. millenii* sampled from within Ngel Nyaki Forest only, the Yelwa area (Ngel Nyaki + Kurmin Danko + Mayo Kamkam), and Akwaizatar Forest, are separated with a "/" (respectively). Species abbreviations: *C. millenii* (West African Cordia) = COR; *E. angolense* (tiama mahogany) = ENT; and *L. trichilioides* (African walnut) = LOV.

| Species | Haplotype | Allelic composition | Frequency | *n* |
|---|---|---|---|---|
| COR | C1 | 185/106/127/102/70/125/109 | 1.00/1.00/1.00 | 37/41/1 |
| ENT | E1 | 213/104/120/92/124/136/107 | 1.00 | 25 |
| LOV | L1 | 206/105/120/90/120/137/107 | 0.08 | 26 |
| | L2 | 206/105/120/90/121/137/107 | 0.92 | |

## Discussion

The primary objective of this study was to assess levels and distribution of cpDNA diversity in three tree species in the montane forest Ngel Nyaki, but also in forests on the Mambilla Plateau. We chose seven conserved ccmp primers for a directly comparable measure of genetic variation among species. Our diversity estimates were very low compared to some other studies that had used cpSSRs [39, 40, 43, 46] as well as the ccmp primers used by us [45, 47, 48]. In those studies, population $H_E$ was found to be substantial in many cases (*i.e.* >0.50).

However, relative to many comparable studies on tropical tree species, our low cpDNA diversity was not unusual. All but three (*Dysoxylum malabricum*, white cedar [45]; *Dicorynia guianensis*, Angelique batard [49]; and *Ficus insipidia* subsp. *insipidia*, swamp fig [50]) of the 20 species, in the 16 studies we reviewed, had populations fixed for a single haplotype. While it is possible that our results reflect our choice of universal cpSSRs, which can produce a lack of observable polymorphisms from ascertainment bias in the original subject species [15, 37], two observations support our conclusion that our cpSSR diversity estimates reflect general cpDNA trends in our focal species. First, ccmp primers have revealed considerable variation—where it exists—in tropical trees [45, 51, 52]. Indeed, using a single ccmp primer, Bodare *et al.* [45] observed an average of 7.667 haplotypes per population of white cedar. Second, our West African Cordia cpSSR dataset corroborates a cpDNA and nDNA sequencing study conducted by Thia [53] on these exact same individuals. Sequencing of various chloroplast intergenic spacers and the nuclear ITS1 showed that all individuals had the same sequences. Given that cpSSRs should be more mutable, the lack of genetic variation observed in this present study is even more poignant.

The chloroplast genome is highly susceptible to genetic drift. Because chloroplasts are almost always uniparentally inherited, they have a much smaller effective population size relative to the nuclear genome, increasing the rate of drift. Fixation of particular haplotypes creates the scenario where most of the genetic diversity occurs among populations [54, 55], and it is common for nDNA diversity to exceed cpDNA diversity [54, 56–58]. However, in cases where seed dispersal exceeds the distance of pollen dispersal, cpDNA diversity may be greater [45] because adequate dispersal helps to buffer the effects of small population size.

The montane forests of the Mambilla Plateau are highly fragmented; patches of forest habitat are nested in a surrounding matrix of grassland. The ability to disperse between forest patches in our three focal species differs. Tiama mahogany and African walnut are both wind-dispersed and are unlikely to disperse between forests in a single generation. In contrast, West African Cordia is primate-dispersed and seeds could theoretically be moved considerable distances. However, the identical patterns of limited cpDNA diversity in all three species imply that dispersal mode is not a major contributor to apparent genetic patterns.

There are three possible scenarios that may explain the low cpDNA diversity in our three focal species: (1) tree populations are only recently colonized (on evolutionary timescales) from a limited number of sources; (2) tree populations have been isolated for a long period of time, perhaps representing relict populations; and (3) natural selection has fixed tree populations at the chloroplast. The first two scenarios propose that genetic patterns have been influenced by fluctuations in forest cover as a result of climatic changes associated with glacial-interglacial cycles [14, 18, 59]. Recent analysis of pollen cores from Lake Bambili in the Cameroon Highlands suggest that forest cover in these mountainous areas might have been very low during the last glacial maximum, which was approximately 20 kya [60]. At the glacial-interglacial transition, some 12–18 kya, an increased presence of arboreal pollen indicates the colonization and expansion of forest at Lake Bambili around this time [60]. Given that the Mambilla Plateau is also part of the Cameroon Highlands, if the pattern of forestation at Lake Bambili is representative of that across this montane region, forests on Mambilla may have been seeded relatively recently in evolutionary time as a result of post-glacial forest expansions. Recent colonization from a limited number of sources could create low contemporary patterns of genetic diversity [50, 59]. However, forests on the Mambilla Plateau might instead be relict populations that have been isolated for considerable time. If this is the case, isolation and drift likely explain low genetic diversity. Unfortunately, to truly tease apart these two scenarios, we would need either fossil pollen data (to determine the floristic composition history of Mambilla) and/or genetic data from populations at the continental scale (to estimate patterns of isolation/migration).

A final possibility that selection has contributed to low cpDNA diversity cannot be ruled out. Regardless of whether tree species are recent colonists, or represent relict populations, strong selection on the chloroplast genome could drive haplotypes to fixation. Testing this scenario would, again, require extensive sampling throughout each species' respective range to correlate haplotype distribution with environmental variables. A rigorous test of selection would also involve reciprocal transplants between populations.

## Implications for Conservation

While we cannot rule out that greater diversity might have been observed had we sampled more extensively from forests dispersed throughout Mambilla, or used different loci, our data do support the possibility that chloroplast lineages may be constrained in their diversity on Mambilla. Within a single forest, three separate species showed identical genetic trends of complete (or nearly complete) genetic homogeneity. Furthermore, the fact that all West African Cordia in the Yelwa area had the same haplotype as a single individual found >40km away in Akwaizantar Forest implies that regional genetic homogeneity could be on the scale of tens of kilometers.

Whether or not the low cpDNA diversity observed is due to demographic history or selection has different consequences for management. Low genetic diversity and resulting inbreeding effects can reduce a tree population's fitness: *e.g.* pollen incompatibility [61–63], abortion of seeds [62], or greater juvenile mortality [64, 65]. Plant populations that lose genetic diversity will likely have increased extinction risk, especially if they are self-incompatible [61, 62]. If low cpDNA diversity is a

product of either founder effects or long-term isolation, populations may benefit in the future from the addition of new genetic variants. However, if selection on the chloroplast has been strong, addition of non-locally adapted genetic variants would create outbreeding depression and decrease fitness [66].

Conservation efforts will need to focus on identifying what variation does exist and at what scale; how populations are connected across the landscape; how human presence may disrupt gene dispersal; and how this may impact fitness and recruitment patterns. Whether the analogous patterns of low cpDNA diversity in our focal species are due to the same or different mechanisms also needs to also be tested. Our study provides an initial stepping stone for future tree population genetic studies on the Mambilla Plateau and highlights the need for more such studies in montane Africa.

## Acknowledgements

This project was made possible with generous funding from Chester Zoo, The Explorers Club, and The Royal Society of New Zealand Canterbury. We thank the supporters of the Nigerian Montane Forest Project: Chester Zoo, A. G. Leventis Foundation, Nexen Nigeria, and the Taraba State Government. This work would have not been possible without the assistance of Misa, Hammasumo, Musa, Peter, Alfred and Hammadu. Thank you also to Lucie Malard for helping us with translations into French. Finally we thank our reviewers for their comments.

## References

[1] Fuchs E. J., Lobo J. A., Quesada M. 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropicald dry forest tree *Pachira quinata*. *Conservation Biology* 17:149–157.

[2] Quesada M., Herrerías-Diego Y., Lobo J. A., Sánchez-Montoya G., Rosas F., Aguilar R. 2013. Long-term effects of habitat fragmentation on mating patterns and gene flow of a tropical dry forest tree, *Ceiba aesculifolia* (Malvaceae: Bombacoideae). *American Journal of Botany* 100:1095–1101.

[3] Lobo J., Solís S., Fuchs E. J., Quesada M. 2013. Individual and temporal variation in outcrossing rates and pollen flow patterns in *Ceiba pentandra* (Malvaceae: Bombacoidea). *Biotropica* 45:185–194.

[4] Noreen A. M. E., Webb E. L. 2013. High genetic diversity in a potentially vulnerable tropical tree species despite extreme habitat loss. *PLoS One* 8:e82632.

[5] Jolivet C., Rogge M., Degen B. 2012. Molecular and quantitative signatures of biparental inbreeding depression in the self-incompatible tree species *Prunus avium*. *Heredity* 110:439–448.

[6] Piotti A. 2009. The genetic consequences of habitat fragmentation: the case of forests. *iForest* 2:75–76.

[7] Kramer A. T., Ison J. L., Ashley M. V., Howe H. F. 2008. The paradox of forest fragmentation genetics. *Conservation Biology* 22:878–885.

[8] Bacles C. F. E., Jump A. S. 2011. Taking a tree's perspective on forest fragmentation genetics. *Trends in Plant Science* 16:13–18.

[9] Dick C. W., Hardy O. J., Jones F. A., Petit R. J. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forests trees. *Tropical Plant Biology* 1:20–33.

[10] Jacquemyn H., De Meester L., Jongejans E., Honnay O. 2012. Evolutionary changes in plant reproductive traits following habitat fragmentation and their consequences for population fitness. *Journal of Ecology* 100:76–87.

[11] Stacy E. A., Hamrick J. L., Nason S. P., Hubbell S. P., Foster R. B., Condit R. 1996. Pollen dispersal in low-density populations of three Neotropical tree species. *The American Naturalist* 148:275–298.

[12] Hardy O. J., Maggia L., Bandou E., Breyne P., Caron H., Chevallier M.-H., Doligez A., Dutech C., Kremer A., Latouche-Hallé C., Troispoux V., Veron V., Degen B. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology* 15:559–571.

[13] Duminil J., Heuertz M., Doucet J.-L., Bourland N., Cruaud C., Gavory F., Doumenge C., Navascués M., Hardy O. J. 2010. CpDNA-based species identification and phylogeography: Application to African tropical tree species. *Molecular Ecology* 19:5469–5483.

[14] Jump A. S., Carr M., Ahrends A., Marchant R. 2014. Genetic divergence during long-term isolation in highly diverse populations of tropical trees across the Eastern Arc Mountains of Tanzania. *Biotropica* 46:565–574.

[15] Barbará T., Palma-Silva C., Paggi G. M., Bered F., Fay M. F., Lexer C. 2007. Cross-species transfer of nuclear microsatellite markers: Potential and limitations. *Molecular Ecology* 16:3759–3767.

[16] Weising K., Gardner R. C. 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome* 42:9–19.

[17] Provan J., Powell W., Hollingsworth P. M. 2001. Chloroplast microsatellites: New tools for studies in plant ecology and evolution. *Trends in Ecology & Evolution* 16:142–147.

[18] Maley J. 1996. The African rain forest – main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceedings of the Royal Society of Edinburgh* 104B:31–73.

[19] Dupont L. M., Donner B., Schneider R., Wefer G. 2001. Mid-Pleistocene environmental change in tropical Africa began as early as 1.05 Ma. *Geology* 29:195–198.

[20] Elenga H., Peyron O., Bonnefille R., Jolly D., Cheddadi R., Guiot J., Andrieu V., Bottema S., Buchet G., de Beaulieu J.-L., Hamilton A. C., Maley J., Marchant R., Perez-Obiol R., Reille M., Riollet G., Scott L., Straka H., Taylor D., Van Campo E., Vincens A., Laarif F., Jonson H. 2000. Pollen-based biome reconstruction for southern Europe and Africa 18,000 yr BP. *Journal of Biogeography* 27:621–634.

[21] Hamilton A. C., Taylor D. 1991. History of climate and forests in tropical Africa during the last 8 million years. *Climate Change* 19:65–78.

[22] Eeley H. A. C., Lawes M. J., Piper S. E. 1999. The influence of climate change on the distribution of indigenous forest in KawZulu-Natal, South Africa. *Journal of Biogeography* 26:595–617.

[23] Bergl R., Oates J., Fotso R. 2007. Distribution and protected area coverage of endemic taxa in West Africa's Biafran forests and highlands. *Biological Conservation* 134:195–208.

[24] Norris K., Asase A., Collen B., Gockowksi J., Mason J., Phalan B., Wade A. 2010. Biodiversity in a forest-agriculture mosaic – The changing face of West African rainforests. *Biological Conservation* 143:2341–2350.

[25] Chapman J. D., Chapman H. M. 2001. *The forests of Taraba and Adamawa States, Nigeria*. Department of Plant and Microbial Sciences, University of Canterbury, Christchurch.

[26] Chapman H. M., Olson S. M., Trumm D. 2004. An assessment of changes in the montane forests of Taraba State, Nigeria, over the past 30 years. *Oryx* 38:282–290.

[27] Keay R. W. J. 1989. *Trees of Nigeria*. Oxford University Press, New York. Revised.

[28] IUCN. 2015. Entandrophragma angolense. *IUCN Red List*. http://www.iucnredlist.org/details/33049/0.

[29] IUCN. 2015. Lovoa trichilioides (African Walnut, Congowood, Tigerwood). *IUCN Red List*. http://www.iucnredlist.org/details/33057/0.

[30] Dutton P. E., Chapman H. M., Moltchanova E. 2014. Secondary removal of seeds dispersed by chimpanzees in a Nigerian montane forest. *African Journal of Ecology* 52:438–447.
[31] IUCN. 2015. Cordia millenii (Drum Tree, West African Cordia). *IUCN Red List*. http://www.iucnredlist.org/details/33042/0.
[32] Adekunle V. A. J., Olagoke A. O., Ogundare L. F. 2010. Rate of timber production in a tropical rainforest ecosystem of Southwestern Nigeria and its implications on sustainable forest management. *Journal of Forestry Research* 21:225–230.
[33] Adekunle V. A. J. 2006. Conservation of tree species diversity in tropical rainforest ecosystem of South-West Nigeria. *Journal of Tropical Forest Science* 18:91–101.
[34] Louppe D., Oteng-Amoako A. A., Brink M. eds. 2008. *Plant Resources of Tropical Africa 7(1): Timbers*. PROTA Foundation / Backhuys Publishers / CTA, Wageningen, Netherlands.
[35] Colpaert N., Cavers S., Bandou E., Caron H., Gheysen G., Lower A. J. 2005. Sampling tissue for DNA analysis of trees: Trunk cambium as an alternative to canopy leaves. *Silvae Genetica* 54:265–269.
[36] Brunner I., Brodbeck S., Büchler U., Sperisen C. 2001. Molecular identification of fine roots of trees from the Alps: reliable and fast DNA extraction and PCR-RFLP analyses of plastid DNA. *Molecular Ecology* 10:2079–2087.
[37] Selkoe K. A., Toonen R. J. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9:615–629.
[38] Nei M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York, USA.
[39] Vendramin G. G., Anzidei M., Madaghiele A., Bucci G. 1998. Distribution of genetic diversity in *Pinus pinaster* Ait. as revealed by chloroplast microsatellites. *Theoretical and Applied Genetics* 97:456–463.
[40] Echt C. S., De Verno L. L., Anzidei M., Vendramin G. G. 1998. Chloroplast microsatellites reveal population genetic diversity in red pine, Pinus resinosa Ait. *Molecular Ecology* 7:307–316.
[41] Goldstein D. B., Inares A. R., Cavalli-Sforzaf L. L., Feldman M. W. 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139:463–471.
[42] Quintela-Sabarís C., Vendramin G. G., Castro-Fernández D., Fraga M. I. 2011. Chloroplast DNA phylogeography of the shrub Cistus ladanifer L. (Cistaceae) in the highly diverse Western Mediterranean region. *Plant Biology* 13:391–400.
[43] Clark C. M., Wentworth T. R., O'Malley D. M. 2000. Genetic discontinuity revealed by chloroplast microsatellites in eastern North American *Abies* (Pinaceae). *American Journal of Botany* 87:774–782.
[44] R Core Team 2014. R: A language and environment for statistical computing. http://www.r-project.org.
[45] Bodare S., Tsuda Y., Ravikanth G., Shaanker R. U., Lascoux M. 2013. Genetic structure and demographic history of the endangered tree species *Dysoxylum malabaricum* (Meliaceae) in Western Ghats, India: implications for conservation in a biodiversity hotspot. *Ecology and Evolution* 3:3233–3248.
[46] Terrab A., Paun O., Talavera S., Tremetsberger K., Arista M., Stuessy T. F. 2006. Diversity and population structure in natural populations of Moroccan Atlas cedar (*Cedrus atlantica*; Pinaceae) determined with cpSSR markers. *American Journal of Botany* 93:1274–1280.
[47] Cubas P., Pardo C., Tahiri H. 2005. Genetic variation and relationships among *Ulex* (Fabaceae) species in southern Spain and northern Morocco assessed by chloroplast microsatellite (cpSSR) markers. *American Journal of Botany* 92:2031–2043.
[48] Rendell S., Ennos R. A. 2003. Chloroplast DNA diversity of the dioecious European tree *Ilex aquifolium* L. (English holly). *Molecular Ecology* 12:2681–2688.
[49] Caron H., Dumas S., Marque G., Messier C., Bandou E., Petit R. J., Kremer A. 2000. Spatial and temporal distribution of chloroplast DNA polymorphism in a tropical tree species. *Molecular Ecology* 9:1089–1098.

[50] Coronado E. N. H., Dexter K. G., Poelchau M. F., Hollingsworth P. M., Phillips O. L., Pennington R. T. 2014. *Ficus insipida* subsp. insipida (Moraceae) reveals the role of ecology in the phylogeography of widespread Neotropical rain forest tree species. *Journal of Biogeography* 41:1697–1709.

[51] Lemes M. R., Dick C. W., Navarro C., Lowe A. J., Cavers S., Gribel R. 2010. Chloroplast DNA microsatellites reveal contrasting phylogeographic structure in mahogany (*Swietenia macrophylla* King, Meliaceae) from Amazonia and Central America. *Tropical Plant Biology* 3:40–49.

[52] Sun Y., Hu H., Huang H., Vargas-Mendoza C. F. 2014. Chloroplast diversity and population differentiation of *Castanopsis fargesii* (Fagaceae): A dominant tree species in evergreen broad-leaved forest of subtropical China. *Tree Genetics & Genomes* 10:1531–1539.

[53] Thia J. A. Y. W. 2014. The Plight of Trees in Disturbed Forest: Conservation of Montane Trees, Nigeria. Dissertation (University of Canterbury).

[54] Fontaine C., Lovett P. N., Sanou H., Maley J., Bouvet J.-M. 2004. Genetic diversity of the shea tree (*Vitellaria paradoxa* C.F. Gaertn), detected by RAPD and chloroplast microsatellite markers. *Heredity* 93:639–648.

[55] Lira C. F., Cardoso S. R. S., Ferreira P. C. G., Cardoso M. A., Provan J. 2003. Long-term population isolation in the endangered tropical tree species *Caesalpinia echinata* Lam. revealed by chloroplast microsatellites. *Molecular Ecology* 12:3219–3225.

[56] Daïnou K., Bizoux, Jean-Philippen tree with high colonization capacity M. excelsa (Moraceae)., Doucet J.-L., Mahy G., Hardy O. J., Heuertz M. 2010. Forest refugia revisited: nSSRs and cpDNA sequences support historical isolation in a wide-spread African tree with high colonization capacity, *Milicia excelsa* (Moraceae). *Molecular Ecology* 19:4462–4477.

[57] Derero A., Gailing O., Finkeldey R. 2011. Maintenance of genetic diversity in *Cordia africana* Lam., a declining forest tree species in Ethiopia. *Tree Genetics & Genomes* 7:1–9.

[58] Pakkad G., Ueno S., Yoshimaru H. 2008. Genetic diversity and differentiation of *Quercus semiserrata* Roxb. in northern Thailand revealed by nuclear and chloroplast microsatellite markers. *Forest Ecology and Management* 255:1067–1077.

[59] Lowe A., Harris D., Dormontt E., Dawson I. 2010. Testing putative African tropical forest refugia using chloroplast and nuclear DNA phylogeography. *Tropical Plant Biology* 3:50–58.

[60] Lézine A.-M., Assi-Kaudjhis C., Roche E., Vincens A., Achoundong G. 2013. Towards an understanding of West African montane forest response to climate change. *Journal of Biogeography* 40:183–196.

[61] Glémin S., Petit C., Maurice S., Mignot A. 2008. Consequences of low mate availability in the rare self-incompatible species *Brassica insularis*. *Conservation Biology* 22:216–221.

[62] Jones F. A., Comita L. S. 2008. Neighbourhood density and genetic relatedness interact to determine fruit set and abortion rates in a continuous tropical tree population. *Proceedings of the Royal Society B* 275:2759–2767.

[63] Quesada M., Stoner K. E., Rosas-Guerrero V., Palacios-Guevara C., Lobo J. A. 2003. Effects of habitat disruption on the activity of nectarivorous bats (Chiroptera: Phyllostomidae) in a dry tropical forest: Implications for the reproductive success of the Neotropical tree *Ceiba grandiflora*. *Plant Animal Interactions* 135:400–406.

[64] Collevatti R. G., Hay J. D. 2011. Kin structure and genotype-dependent mortality: A study using the Neotropical tree *Caryocar brasiliense*. *Journal of Ecology* 99:757–763.

[65] Ismail S. A., Ghazoul J., Ravikanth G., Shaanker R. U., Kushalappa C. G., Kettle C. J. 2012. Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of *Dysoxylum malabaricum* in an agro-forest landscape. *Molecular Ecology* 21:5484–5496.

[66] Hoffmann A. A., Sgrò C. M. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485.

[67] Dutech C., Maggia L., Joly H. I. 2000. Chloroplast diversity in *Vouacapoua americana*

(Caesalpiniaceae), a neotropical forest tree. *Molecular Ecology* 9:1427–1432.

[68] Islam M. S., Lian C., Kameyama N., Hogetsu T. 2015. Analysis of the mating system, reproductive characteristics, and spatial genetic structure in a natural mangrove tree (*Bruguiera gymnorrhiza*) population at its northern biogeographic limit in the southern Japanese archipelago. *Journal of Forest Research* 20:293–300.

[69] Silvestrini M., McCauley D. E., Zucchi M. I., Santos F. A. M. dos. 2015. How do gap dynamics and colonization of a human disturbed area affect genetic diversity and structure of a pioneer tropical tree species? *Forest Ecology and Management* 344:38–52.

[70] Tnah L. H., Lee S. L., Ng K. K. S., Lee C. T., Bhassu S., Othman R. Y. 2012. Phylogeographical pattern and evolutionary history of an important peninsular malaysian timber species, *Neobalanocarpus heimii* (Dipterocarpaceae). *Journal of Heredity*:ess076.

**Appendix 1.** Location, repeat motif, and primer sequences for each cpSSR. Dye choice for each primer was based on differences in product size (as observed from gel electrophoresis) to facilitate pooling. Repeat motifs mentioned here are those deduced from the original source species, *i.e.* tobacco [16], and are not necessarily the same in our focal species.

| Primer | Location | Repeat motif | Primer sequence | Dye |
|---|---|---|---|---|
| ccmp2 | 5' to *trn*S | $(A)_{11}$ | 5'–GATCCCGGACGTAATCCTG–3'<br>5'–ATCGTACCGAGGGTTCGAAT–3' | 6-FAM |
| ccmp3 | *trn*G intron | $(T)_{11}$ | 5'–CAGACCAAAAGCTGACATAG–3'<br>5'–GTTTCATTCGGCTCCTTTAT–3' | PET |
| ccmp4 | *atp*F intron | $(T)_{13}$ | 5'–AATGCTGAATCGAYGACCTA–3'<br>5'–CCAAAATATTBGGAGGACTCT–3' | 6-FAM |
| ccmp5 | 3' to *rps*2 | $(C)_7(T)$<br>$(T)_5C(A)_{11}$ | 5'–TGTTCCAATATCTTCTTGTCATTT–3'<br>5'–AGGTTCCATCGGAACAATTAT–3' | VIC |
| ccmp6 | ORF77–ORF82 intergenic | $(T)_5C(A)_{17}$ | 5'–CGATGCATATGTAGAAAGCC–3'<br>5'–CATTACGTGCGAACTATCTCC–3' | NED |
| ccmp7 | *atpB*–*rbc*L intergenic | $(A)_{13}$ | 5'–CAACATATACCACTGTCAAG–3'<br>5'–ACATCATTATTGTATACTCTTTC–3' | PET |
| ccmp10 | *rpl*2–*rps*19 intergenic | $(T)_{14}$ | 5'–TTTTTTTTTAGTGAACGTGTCA–3'<br>5'–TTCGTCGDCGTAGAAATAG–3' | VIC |

**Appendix 2.** Review of 16 cpDNA diversity studies in 20 tropical and subtropical tree species across different continents. The mean and total number of haplotypes observed in these studies is listed. The total number of populations sampled is also given. Methods of cpDNA diversity estimates were obtained in different ways: cpSSRs = chloroplast microsatellites; restriction sites = digestion of PCR products; indel sites = sequence insertions and deletions; substitution sites = nucleotide base changes. The number of loci used is also indicated. The spatial scale is with respect to kilometers. Exploratory analyses of this data can be found in Appendix 3. We report measures of adult genetic diversity only from those studies that considered different age classes.

| Species | Common name | Continent | Mean haplotypes | Total haplotypes | Populations sampled | Method | Loci | Scale (km) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Dysoxylum malabaricum* | White cedar | Asia | 7.667 | 24 | 12 | cpSSRs | 1 | 100 | Bodare *et al.* [45] |
| *Dicorynia guianensis* | Angelique batard | South America | 3.6 | 12 | 5 | cpSSRs; restriction sites | 10 | 100 | Caron *et al.* [49] |
| *Vouacapoua americana* | Brownheart | South America | 1.214 | 6 | 14 | Restriction and indel sites | 5 | 100 | Dutech *et al.* [67] |
| *Vitellaria paradoxa* | Shea butter tree | Africa | 1.333 | 7 | 12 | cpSSRs | 4 | 1000 | Fontaine *et al.* [54] |
| *Bruguiera gymnorrhiza* | Burmese mangrove | Asia | 2 | 2 | 1 | cpSSRs | 6 | 0.01 | Islam *et al.* [68] |
| *Ficus insipida* subsp. *insipida* | Swamp fig | South/Central America | 1.648 | 19 | 54 | Restriction, indel, and substitution sites | 1 | 1000 | Coronado *et al.* [50] |
| *Swietenia macrophylla* | Big leaf mahogany | South/Central America | 3.563 | 31 | 16 | cpSSRs | 6 | 1000 | Lemes *et al.* [51] |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Quercus semiserrata* | Wú chǐ Qīnggāng | Asia | 2 | 16 | 10 | cpSSRs | 9 | 100 | Pakkad *et al.* [58] |
| *Croton floribundus* | Velame | South America | 1.375 | 4 | 8 | cpSSRs | 6 | 1 | Silvestrini *et al.* [69] |
| *Castanopsis fargesii* | Kăo | Asia | 2.778 | 47 | 27 | cpSSRs | 8 | 1000 | Sun *et al.* [52] |
| *Neobalanocarpus heimii* | Chengal | Asia | 2.344 | 15 | 32 | Substitution sites | 5 | 100 | Tnah *et al.* [70] |
| *Irvingia gabonensis* | African mango | Africa | 1.182 | 2 | 11 | Restriction sites | 12 | 1000 | Lowe *et al.* [59] |
| *Macaranga capensis* | Wild poplar | Africa | 1.3 | 12 | 9 | Indel and substitution sites | 3 | 100 | Jump *et al.* [14] |
| *Newtonia buchananii* | Forest Newtonia | Africa | 0.9 | 5 | 7 | Indel and substitution sites | 2 | 100 | Jump *et al.* [14] |
| *Ocotea usambarensis* | East African camphor | Africa | 1.7 | 17 | 7 | Indel and substitution sites | 3 | 100 | Jump *et al.* [14] |
| *Xymalos monospora* | Lemon wood | Africa | 2.4 | 23 | 7 | Indel and substitution sites | 2 | 100 | Jump *et al.* [14] |
| *Zanthoxylum gilletii* | Large-leaved knobwood | Africa | 1.4 | 14 | 6 | Indel and substitution sites | 2 | 100 | Jump *et al.* [14] |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Cordia africana* | Large-leaved Cordia | Africa | 1.637 | 22 | 22 | cpSSRs | 2 | 100 | Derero *et al.* [57] |
| *Caesalpinia echinata* | Peachwood | South America | 1.286 | 8 | 7 | cpSSRs | 3 | 1000 | Lira *et al.* [55] |
| *Milicia excelsa* | Mvule | Africa | 1.818 | 14 | 22 | cpSSRs; indel and substitution sites | 3 | 1000 | Daïnou *et al.* [56] |

12

**Appendix 3.** Mean number of haplotypes (sum of within population haplotype count/number of sampled populations) for each species in reviewed studies (Appendix 2) as a function of the number of loci used (a), and the number of populations sampled (b). Trend lines were fit with a simple linear regression in the statistical package, *R*; $R^2_{ajd}$ = adjusted $R^2$ value, *P* = statistical significance.