TITLE: Assessing rarity: genomic insights for population assessments and conservation of the most poorly known Neotropical trees

TÍTULO: Evaluación de la rareza: Perspectivas genómicas para la evaluación y conservación de las poblaciones de los árboles neotropicales pobremente conocidos.

Ellen J. Quinlan[a] (quinej18@wfu.edu), David A. Neill[b] (davidneill53@gmail.com), Gonzalo Rivas-Torres[c] (grivast@usfu.edu.ec), and Miles R. Silman[a, d] (silmanmr@wfu.edu)

a) Department of Biology, Wake Forest University, Winston-Salem, North Carolina, USA
b) Universidad Estatal Amazónica, Puyo, Pastaza, Ecuador
c) Estación de Biodiversidad Tiputini, Universidad San Francisco de Quito, Quito, Ecuador
d) The Sabin Center for Environment and Sustainability, Wake Forest University, Winston-Salem, North Carolina, USA

Corresponding Author: Ellen J. Quinlan, Department of Biology, 1834 Wake Forest Rd., Winston-Salem, North Carolina, USA; quinej18@wfu.edu

Acknowledgements: The authors would like to thank Dr. Paul VA Fine and Dr. Craig A. Layman for their helpful comments on earlier versions of this manuscript. Dr. William Farfan-Rios translated the abstract into Spanish. The material used in this study was collected under the research permit MAAE-ARSFC-2021-1250 and permit MAE-DNB-CM-2018-0106 which allows for access to the genetic information of these same species, granted by the Ecuadorian Ministry of the Environment, Water, and Ecological Transition. The mining company Lundin Gold, Inc. provided access and logistical support for collection of samples from tagged trees in the Fruta del Norte mining concession.

Funding: This work was supported by Wake Forest University's Pilot Research Grant [DM1050] awarded to MRS and the Andrew Sabin Center for Environment and Sustainability.

Declaration of interests: The authors declare they have no known financial or personal conflicts of interest.

Declaration of Generative AI in Scientific Writing: The authors confirm that no generative AI or AI-assisted technologies were used in the preparation of this work.

CRediT Authorship Contribution Statement

Ellen Quinlan: conceptualization, methodology, formal analysis, investigation, writing – original draft, writing – review & editing, visualization, funding acquisition; **David Neill:** conceptualization, formal analysis, investigation, writing – review & editing; **Gonzalo Rivas-Torres:** conceptualization, writing – review & editing; **Miles Silman:** conceptualization, writing – review & editing, supervision, funding acquisition

ABSTRACT: Tropical forests comprise a few hyperdominant and many rare tree species, but distinguishing the truly rare from those under-sampled remains a challenge for ecology and conservation. Given the vastness of Amazonia (~6 million km^2, ~$3.9x10^{11}$ individual trees), increasing sampling cannot solve this problem. Still, half of all species are known from three or fewer collections, making predicting their abundances and distributions impossible with census data alone. Here, we integrate census data with genomics to assess the rarity of one of the most poorly known and highly threatened Neotropical trees, *Magnolia yantzazana*. Genetic analyses indicate that while there is relatively high nucleotide diversity among sequences ($\pi > 0.5$), there is also evidence of a loss of heterozygosity ($H_e > H_o$) and inbreeding ($F_{IS} \geq 0.5$), consistent with a small, isolated population. Demographic reconstructions show population decline since the late Pleistocene, with a predicted effective population size (N_e) of ~10^3 in recent millennia. Together, the low heterozygosity, potential inbreeding, demographic trajectory, and census data suggest *M. yantzazana* is in fact a truly rare species, highly vulnerable to ongoing environmental change and anthropogenic threats in the region, notably mining, and support updating its conservation status to Critically Endangered (CR). Here, we offer a framework for using genomic tools to advance our understanding of the rarest tropical trees and establish conservation priorities, despite the limited field collections available for most species.

Keywords: Amazon, biodiversity, conservation, conservation genetics, demographic history, rare species, tropical forests

RESUMEN: Los bosques tropicales están compuestos por pocas especies hiperdominantes y muchas raras, pero distinguir las verdaderamente raras de aquellas poco muestreadas sigue siendo un desafío para la ecología y la conservación. Dada la inmensidad de la Amazonia (~6 millones de km^2, ~3,9 x 10^1 árboles individuales), incrementar el muestreo no puede resolver este problema. Aun así, la mitad de todas las especies se conocen a partir de tres o menos colecciones, lo que hace imposible predecir su abundancia y distribución únicamente con datos de censos. En este estudio, integramos datos censales con la genómica para evaluar la rareza de uno de los árboles neotropicales más desconocidos y altamente amenazados, *Magnolia yantzazana*. Los análisis genéticos indican que, si bien existe una diversidad de nucleótidos relativamente alta entre las secuencias ($\pi > 0,5$), también existe evidencia de una pérdida de heterocigosidad (He > Ho) y endogamia (FIS ≥ 0,5), lo cual es consistente con una población pequeña y aislada. Las reconstrucciones demográficas muestran un declive poblacional desde finales del Pleistoceno, con un tamaño poblacional efectivo (Ne) previsto de ~103 en los últimos milenios. En conjunto, la baja heterocigosidad, la endogamia potencial, la trayectoria demográfica y los datos de censos sugieren que *M. yantzazana* es en realidad una especie verdaderamente rara, altamente vulnerable al cambio ambiental actual y a las amenazas antropogénicas en la región, en particular la minería, y respaldan la actualización de su estado de conservación a En Peligro Crítico (CR). Aquí, ofrecemos un marco para el uso de herramientas genómicas con el fin de avanzar nuestra comprensión sobre los árboles tropicales más raros y establecer prioridades de conservación, a pesar de las limitadas colecciones de campo disponibles para la mayoría de las especies.

Palabras clave: Amazonía, biodiversidad, conservación, genética de la conservación, historia demográfica, especies raras, bosques tropicales

1. INTRODUCTION

The predominant community pattern described for tropical forests from field collections is one of hyperdominance and extreme rarity (e.g., Gentry 1982, Hubbell 2013). Ter Steege et al. (2013) estimated there are approximately 16,000 tree species (≥10 cm dbh) in the Amazon Forest based on forest plot inventory data, finding 227 species (1.4% of the total) so common they account for half of all individual trees ("hyperdominants"). The rarest 11,000 species (~70% of the total) represent just 0.12% of individuals. Though field collections are the best available data on species abundance and distribution, the conclusions that can be drawn from those data have limitations due to spatial autocorrelation and the limited forest area they represent. Moreover, given the large number of species and the exceptionally low predicted abundances of most, increased sampling cannot overcome these limitations. Sampling efforts across the Andes-Amazon system have more than doubled over the last two decades, yet, less than half of all species are known from more than a handful of collections and at least a quarter remain unknown to science (Feeley and Silman 2011a; ter Steege et al. 2013; ter Steege et al. 2016; Guevara Andino et al. 2019).

Under-sampling and the immense numbers of unidentified collections make it difficult to distinguish species that are truly "rare" from those that maintain low local abundances but may be common at broad geographic scales (i.e., Rabinowitz 1981). For example, Pitman et al. (1999) surveyed 19,252 individuals (≥10 cm dbh) from 21 plots (36 ha) in Manu National Park, Peru, and found 31% of the 829 species (or morphospecies) identified represented singletons (1 individual / 36 ha). When extrapolated to the full department of Madre de Dios where Manu National Park is located (78,415 km^2), the estimated abundance for these rare trees is 200,000 stems, with possibilities ranging anywhere from <1000 to $>10^6$ when smaller stems are included (Pitman et al. 1999). Increasing field sampling efforts alone cannot solve this problem, e.g., ter Steege et al. (2020) showed a 10-fold increase in forest plots would only represent 0.0035% of the Amazonian forest area, capturing <50% of the species richness.

Such uncertainty in our knowledge of Andes-Amazonian biodiversity affects how we understand species richness, ecosystem function, biogeography, the evolution of Neotropical forests and the population biology of the species that comprise them. Further, estimates of abundance and distribution are essential components of any conservation effort, especially those identifying extinction risks and species' responses to anthropogenic land conversion and climate change (e.g., Hoban et al. 2020). However, we will never sample enough individuals through forest inventories alone to have an accurate account of population sizes and ranges, much less demographic history. Instead, the genomes of individuals already collected can offer information for refining these estimates, as the genome of even a single individual represents a population-level sample of genes and their unique histories. Thus, the integration of modern genomics with field collections can improve population inferences from only a small number of sampled individuals (Nazareno et al. 2017; Lemopoulos et al. 2019) and help identify those that are truly rare and threatened from those simply under-sampled.

Advances in sequencing technologies and particularly the development of restriction-site associated DNA sequencing (RAD-seq), have made genome-wide study of non-model organisms such as Neotropical trees possible and cost-effective (Andrews et al. 2016, Parchman et al. 2018). When combined with common metrics of population genomics, such as nucleotide diversity (π), heterozygosity (i.e., H_e, H_o), and inbreeding depression (i.e., F_{IS}, ID), these data offer important insight into the extent, diversity, and interconnectedness of populations (e.g., Linan et al. 2020; Kebaïli et al. 2021, Gautschi et al. 2024), even with low sample sizes (Nazareno et al. 2017; Lemopoulos et al. 2019). For example, low H_o compared to H_e can be a signal of inbreeding depression, indicating an increase in homozygosity and loss of genetic diversity. Similarly, positive F_{IS} values indicate an excess of homozygosity compared to what is expected under Hardy-Weinberg equilibrium, a signal of inbreeding and potentially inbreeding depression if deleterious alleles become more frequent. Measures of genomic diversity can also be used to estimate the effective population size (N_e), a value representing how large a population would need to be to

maintain the observed level of diversity under genetic drift alone, calculated from heterozygosity, generation time, and the neutral mutation rate (Hahn 2018). Though N_e can both underestimate and overestimate census population size (N_C), it can be used to set the bounds of possible census sizes, particularly in orders of magnitude, a goal impossible for most Neotropical tree species given inventory data alone. The demographic history of populations can also be reconstructed by estimating changes in N_e through time, calculating changes in the coalescent rate as N_e is inversely proportional to coalescent time. This is possible due to recombination, which results in different regions of the genome having different gene trees, each of which contains information on population growth, contraction, and divergence in the variants they carry (Excoffier et al. 2013, Hahn 2018).

The genus *Magnolia* is an ancient clade flowering plants (Angiosperm Phylogeny Group et al. 2016), comprising some 390 species of evergreen or deciduous trees and shrubs distributed in temperate and tropical regions across Southeast and East Asia, the Antilles, and the Americas (Cires et al. 2013; Núnez et al. 2024). Nearly half of all *Magnolia* species are globally threatened, and the status of at least another third remains unassessed (Rivers et al. 2016). Many *Magnolia* species are valued for their timber, medicinal, and ornamental use. Overexploitation and human disturbance combined with life history traits such as long generation times and slow recruitment have contributed to population declines (Cires et al. 2013). For these reasons, studies have begun to assess the population structure and genetic diversity of a few rare *Magnolia* species (e.g., Isagi et al. 2007 – *M. obovata*, Yang et al. 2022 – M. fistulosa, Budd et al. 2015 – *M. acuminata*, Tamaki et al. 2019 – *M. kobus*, Hernández et al. 2020 – *M. cubensis subsp. acunae*), but to date none of these studies have included species from Central and South America, an important center of diversity for the genus (~170 species in the Neotropics).

Here, we use *Magnolia yantzazana* F. Arroyo as a case study to explore the application of population genomic and demographic reconstruction methods to understand the population biology and

conservation status of one of the most poorly known and highly threatened Neotropical trees. Specifically, we: (1) test the assumption of extreme rarity in *M. yantzazana* from field collections and (2) assess how its effective population size (N_e) has changed through time, with the overarching goal of setting bounds on potential population size estimates. Here, we offer a framework for advancing understanding of the rarest Neotropical trees and establishing better informed conservation priorities.

2. METHODS

2.1 **Study species and sampling**

Magnolia yantzazana F. Arroyo is an evergreen canopy tree reaching up to 25 m in height (Figure 1b), described from the premontane humid forests on the western slopes of the Cordillera del Cóndor in Zamora-Chinchipe Province, Yantzaza canton, in southeastern Ecuador (Arroyo and Pérez 2013). *M. yantzazana* has been morphologically assigned to *Magnolia sect. Talauma subsect. Dugandiodendron*, one of two clades of *Talauma* that are separated geographically between Mexico and Central America (*subsect Cubenses*, Clade I) and South America and the Caribbean (*subsect. Dugandiodendron*, Clade II; Núnez et al. 2024). All South American *Magnolia* species studied to date belong to *Magnolia sect. Talauma*, *Magnolia sect. Macrophylla*, or *Magnolia sect. Magnolia*, resulting from two hypothesized colonization events. In the first event, the ancestor of *Magnolia sect. Talauma* is thought to have arrived in Venezuela ca. 36 Mya, preceding the most significant Andean mountain-building events in the central and eastern cordilleras (Pérez-Escobar et al. 2022; Núnez et al. 2024). The ancestor of both *Magnolia sect. Macrophylla* and *Magnolia sect. Magnolia*, which both have temperate affinities, is hypothesized to have arrived later, ca. 20 Mya via the Trans-Mexican volcanic belt (Núnez et al. 2024).

M. yantzazana has large ovate leaves and ellipsoid fruits (Figure 1c), and has been found growing on sandstone plateaus from 1400 – 1650 m elevation. While beetles are the predominant pollinator of *Magnolia* flowers, *Diptera* (flies) and *Hymenoptera* (bees, etc.) have also been observed, and birds are

the principal disperser (Thien et al. 1996). *M. yantzazana* is one of the rarest known magnolias, a narrow endemic confined to a small geographic area (~20 km^2; Vázquez-García et al. 2015). It's known range is exclusively within the watershed of the Machinaza River, a tributary of the Zamora River, and is entirely within the "Fruta del Norte" mining concession operated by the Canadian-based company Lundin Gold, Inc. under license from the government of Ecuador (see Supporting Information for more information and an extinction risk assessment; Figure S1).

Originally described from a single collection, there have been 15 individuals identified since 2008, with 31 total records in the Global Biodiversity Information Facility (GBIF.org) reflecting the original collections and their duplicates deposited in herbaria (Figure S1). For this study, *M. yantzazana* trees that were previously identified to species and tagged in survey plots within the intact forest for the Lundin Gold mining concession were located, and individuals selected to represent the known geographic distribution and elevation range of the species; a total of five trees were sampled (N=5). Voucher collections were obtained for individuals, in some cases with flowers or fruit as well as leaves, and deposited in the Ecuadorian herbarium ECUAMZ and LOJA (Table 1, Figure 2).

2.2 DNA extraction and sequencing

Leaf tissue was field-collected and stored in silica. High molecular weight DNA was extracted from ~50 mg dried tissue using Aboul-Maaty and Oraby's (2019) modified CTAB protocol for non-model plants, with the following modifications: samples were ground with a bead-beater, incubated at -20˚C overnight (and up to 24 hrs), and the DNA pellet was washed 2x with 500 µl 70% ethanol. DNA was checked for quality and quantity on a 0.8% agarose gel and Qubit 2.0 fluorometer dsDNA HS assay kit (Invitrogen) before being sent to Floragenex (Beaverton, OR) for double-digest RAD (ddRAD) library prep and sequencing. ddRAD is a variation of RAD-sequencing that uses two restriction enzymes to create more evenly distributed and predictable cut sites, allowing for better genome coverage, less data loss, and

fewer sequencing errors, ideal for non-model species. Samples were ligated with 6 bp barcodes and digested with the PstI/MseI +2 enzyme pair.

2.3 Sequence filtering and assembly

Genomic data were demultiplexed using iPYRAD v. 0.9.95. (Eaton & Overcast 2020) with an average 3.13 million ± 1.03 million reads recovered per sample. Reads were assembled both de novo and mapped to the reference genome *Magnolia sinica* (PRJNA774088) in iPYPRAD and the two assemblies were compared. Both assemblies used the default parameters in iPYRAD except the minimum number of samples required per locus was set to 2 due to the small sample size. The final de novo dataset retained 3129 SNPs across 2828 loci, with a final concatenated sequence length of 358,502 total sites and 51.34% missing data. The reference assembly retained 1419 SNPs across 2214 loci, with a final concatenated sequence of 269,528 bp and 42.6% missing data.

SNPs were filtered in VCFtools v. 0.1.16 (Danecek et al. 2011) for minor allele frequency (-maf) and missing data (-max-missing). The minor allele frequency (MAF) filter was set to 0.05 and the missing data filter was run at 0.4 and 0.6 for both assemblies. The *de novo* assembly retained 3114 sites with the 0.4 filter and 1311 sites with the 0.6 filter. The reference-mapped assembly retained 1339 sites with the 0.4 filter and 645 sites with the 0.6 filter.

2.4 Genomic diversity

The nucleotide diversity (π), expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{IS}) were calculated using VCFtools for each assembly. Additionally, we used COLONY v.2.0.7.0 (Wang 2004) to assess kinship and inbreeding. The COLONY parameters were set to allow for inbreeding, polygamy, and monoecism. All other parameters were set to the default, and the analyses were performed for three replicate runs per dataset. Marker type was set to codominant for all sites, and the allelic dropout rate and genotyping error were conservatively set to 0.005 and 0.01 respectively.

2.5 Inference of demographic history

We used Stairway plot 2 (Liu and Fu 2020) to infer the demographic history of *M. yantzazana*, using a mutation rate of 4e -9 (calculated for the reference species *M. sincia*, Yang et al. 2022). Generation time for *M. yantzazana* is unknown, but as generation time for *M. sinica* is estimated to be 10 years from cultivation records (Yang et al. 2022), and other magnolias have known generation times of up to 25 years, we ran the model with three different generation times: 10, 25, and 50 years. The folded (*de novo*assembly) and unfolded (reference assembly) site-frequency-spectrum was generated in easySFS (https://github.com/isaacovercast/easySFS; Gutenkunst et al. 2009) from the 0.4 filtered SNP matrices and thinned to one SNP per locus, selecting the projection that retained the highest number of segregating sites for each.

3. RESULTS

3.1 Genomic diversity

The genetic diversity statistics calculated for individuals and the population were similar across all datasets (Table 2). Observed heterozygosity (H_o) was low compared to expected heterozygosity (H_e) within individuals and the population. Among individuals, H_o values ranged from 0.091–0.232 (de novo) and 0.152–0.254 (reference), and H_e ranged from 0.433–0.509 (de novo) and 0.414–0.494 (reference). The estimated population H_os were 0.197 ± 0.03 and 0.123 ± 0.03 (de novo), and 0.213 ± 0.03 and 0.161 ± 0.004 (reference; Table 2). The estimated population H_es were 0.497 ± 0.009 and 0.445 ± 0.006 (de novo), and 0.480 ± 0.01 and 0.423 ± 0.004 (reference) (Table 2). Nucleotide diversity (π) was relatively high among sequences at 0.452 - 0.523 and the Weir and Cockerham (1984) inbreeding coefficient (F_{IS}) was also high at 0.555 – 0.723 across assemblies, indicative of a highly inbred population (Table 2).

COLONY did not detect any evidence of close kinship among the individuals, with each sample inferred to be unrelated in the best configuration, with unique parents. The Full-Likelihood Pairwise Likelihood Scoring (FL_PLS) estimated both inbreeding and selfing to be 1, extreme values likely inflated by the small sample size and lack of sibling structure. However, the David, Hartl, and Wenzel (1997)

inbreeding coefficient (Fis) was ≥0.7 (de novo) and ≥0.5 (reference; Table 3), similar values to those calculated by VCFtools (Table 2). Identity disequilibrium (ID) was also high, ≥0.9 (all assemblies; Table 3). These values do not rely on family structure and are less sensitive to small sample sizes. Taken together, the diversity statistics across assemblies support a signal of high inbreeding and/or selfing in the population.

3.2 Demographic history

The models show a general pattern of population collapse over the last several hundred thousand years in both the folded (unmapped) and unfolded (reference mapped) datasets and all generation time scenarios (t_g = 10, 25, 50 years; Figure 4). The demographic history inferred from the folded SFS suggests *M. yantzazana* reached a maximum N_e around 200,000 and began to decline 100,000 years ago (t_g = 10), and 400,000 years ago (t_g = 25, 50), stabilizing with a N_e of approximately 1,000–2,000 between 1,000 (t_g = 10) and 10,000 (t_g = 50) years ago. The demographic histories inferred from the unfolded (reference mapped) SFS showed similar population trends, though they were generally associated with narrower confidence intervals, and the magnitude of the maximum inferred N_e was larger, reaching approximately 500,000. N_e began to decline between 100,000 years ago (t_g = 10) and 400,000 years ago (t_g = 50), again stabilizing with a N_e of 4,000–20,000 between 2,000 and 10,000 years ago. Under all scenarios, the models infer at least one prolonged period of stability lasting at least 50,000 years, following the initial population crash. However, the models disagree on the inferred N_e during this stabilizing period, with the folded model predicting a N_e ~5,000 and the unfolded ~10,000.

4. DISCUSSION

Even in the largest forest inventories, most Andean and Amazonian tree species appear as singletons or are completely undetected, hyper-rare, and for all practical purposes, invisible to conservation efforts. As of 2024, the Amazonian Tree Diversity Network (ATDN) maintains forest inventory plots covering >2000 ha and 5,122 species, less than half of the named Amazonian trees. Thousands more remain completely

unknown to science. *M. yantzazana* is one such hyper-rare species, originally described as a singleton from the premontane tropical forests of southeastern Ecuador, and after thorough field investigation, is still known only from a single locality and a handful of individuals. However, using the genomic information stored in these field collections, we can infer genomic diversity and demographic trends to provide a first conservation assessment.

Population genomic statistics suggest that while there is relatively high nucleotide diversity within *M. yantzazana* among sequences ($\pi \approx 0.5$), there is also evidence of a loss of heterozygosity ($H_e > H_o$) and inbreeding ($F_{IS} \geq 0.5$, ID ≥ 0.9). This may reflect a historically larger population with diverse ancestry, that due to a population bottleneck now experiences restricted mating with limited gene flow. The values calculated here for *M. yantzazana* are comparable to those obtained for other rare *Magnolias*. For example, Yang et al. (2022) calculated an F_{IS} value of 0.316 for *M. fistulosa*, and Hernandez et al. (2020) found similar values for π (0.504), H_o (0.434), and H_e (0.469) in *M. cubensis subsp. acunae*. Such a high F_{IS} is particularly concerning for conservation, as inbreeding may reduce the fitness of the population and reduce the genetic variation available for natural selection to act upon, leading to inbreeding depression and greater vulnerability to changing conditions (Lewontin 1974; Hoban et al. 2020).

COLONY did not detect any kinship among the individuals in our sample, despite the high signal of inbreeding. This is possibly due in part to selfing, as magnolias are known to exhibit varying capacities for self-pollination though not preferentially. For example, *M. stellata* (Hirayama et al. 2005), *M. sinica* (Chen et al. 2016), and *M. grandiflora* (Sukumaran et al. 2020), have been shown to undergo self-pollination in response to pollen shortage and at a significant cost to fruit and seed set. In a small, geographically isolated population, self-pollination can be a double-edged sword that in the short term provides reproductive assurance but over time exacerbates the effects of inbreeding depression.

290 The inferred demographic history from both datasets (folded and unfolded) and all generation
291 time scenarios (t_g = 10, 25, 50) suggest that *M. yantzazana* experienced a population collapse in the late
292 Pleistocene. Looking at a global sample of 15 economically important plants including herbs, shrubs, and
293 trees, Patil et al. (2021) found a similar late Pleistocene bottleneck trend across tropical species. Patil et
294 al. (2021) proposed this joint decline in N_e for tropical species globally corresponds to changing
295 environmental conditions, such as prolonged drought and a decrease in CO_2 concentration. However,
296 conditions in the western Amazon became wetter over the same period (Cheng et al. 2013). More
297 studies on non-model tropical forest trees are required to disentangle the potential drivers and
298 regionality of these perceived late-Pleistocene population bottlenecks.

299 Perhaps more significant is the dynamism of commonness and rarity through time. *M.*
300 *yantzazana* was once at least 1–2 orders of magnitude more common than it is today, now seemingly
301 isolated within a single watershed and mining concession (Figure S1; GBIF.org). While some species may
302 recover their populations (as Patil et al. 2021 proposed for *Faidherbia albida*) other once rarer species
303 surely capitalized on these community shifts and are now more common on the landscape. One such
304 example is the "hyperdominant" species complex *Protium heptaphyllum*, which experienced an increase
305 in population size after diverging from its ancestors (ca. 5 mya), followed by several diversification and
306 population expansion events throughout the Pleistocene (Damasco et al. 2021). Understanding how
307 patterns of commonness and rarity change over time and the causes are fundamental questions in
308 ecology, particularly in hyper-diverse tropical forests, that have traditionally been unanswerable given
309 the coarseness of paleo reconstructions. Whether the species that are common today have always been
310 common underpins our most basic understanding of community structure and function, and is critical for
311 both biodiversity conservation and community ecology theory testing.

312 Demographic reconstructions have inherent uncertainties. N_e is often an underestimate of N_c
313 and has a lag time of several generations before significant changes in N_c may be reflected. Thus, N_e is

best used to set the bounds of possible census sizes and as a proxy for genetic erosion, as N_e has an inverse and non-linear relationship with genetic erosion which accelerates as N_e declines (Hahn 2018; Hoban et al. 2020). The demographic histories inferred here, for all models, suggest the bounds of modern estimates of N_e for *M. yantzazana* are ~10^3 individuals (over the last 1000–2000 years). This is a comparably small population for a premontane Amazonian tree, where populations of the most common species have been estimated to be >10^8 and only the rarest 5800 species have population estimates of <1000 (ter Steege et al. 2013). Generation time is one of the greatest uncertainties in these models (Liu & Fu 2015), yet is ill-defined across the demographic literature. Further, these values are either unknown or difficult to constrain in rare species, much less common ones, with implications for the biogeographic interpretations of such reconstructions (i.e. Caswell 2009; Figure 4). In this paper, we dealt with this uncertainty for *M. yantzazana* by modeling with three different generation times spanning the range of values estimated for other *Magnolia* species (i.e. Yang et al. 2022) and long-lived tropical forest trees generally (Lieberman et al. 2009).

The most significant limitation of this study is sample size, making the inferences coarse, though still informative. Simulations have shown that sampling many SNPs from across the genome (>1000 SNPs) can accurately estimate genomic diversity with small sample sizes, and increasing sample size beyond eight individuals has diminishing returns (Nazareno et al. 2017). Still, with such a small sample size, COLONY results are likely undersampling relatedness in the population because relationships will go undetected. Here, we used COLONY to simply assess relatedness among the individuals in our sample and inform downstream analyses. Similarly, the FL_PLS statistics calculated in COLONY rely on relationships among individuals to estimate inbreeding and selfing. Without any relationships detected, these values will be inflated and uninformative. Other inbreeding metrics such as F_{IS} (DHW) and identity disequilibrium (ID), do not rely on pedigree information. In Stairway Plot 2, sample size controls the resolution in the demographic reconstructions. The number of chromosomes defines the number of

frequency classes or bins in the site-frequency-spectrum (SFS), thus with few chromosomes the resolution becomes coarse, and subtle or recent demographic changes become difficult to detect. The number of SNPs in the SFS determines the accuracy or stability of the frequency estimates in each bin, and too few SNPs can lead to overfitting or unstable demographic trajectories. Here, we did not observe signals of overfitting or instability, as the curves show reasonable confidence intervals with trajectories that are consistent across models. Though the inferences that can be drawn from any of these models or statistics individually are limited, together they reveal a consistent trend of demographic decline and inbreeding that advances our understanding of a previously poorly known species.

While a larger sample size may increase the resolution and informativeness of these models and statistics, such sampling is not always possible, particularly with rare tropical species. At least half of all tree species predicted for the Amazon basin have fewer than three collections, with 90% having < 90 (ter Steege et al. 2016). Efforts should thus focus on leveraging the genomic data stored in samples already in collections, which, when combined with field census data, can help distinguish the truly rare species in need of conservation protection from those simply under-sampled. Alternate sequencing technologies to RADseq such as low-coverage whole genome sequencing may offer greater genomic resolution and reduced missing data, but often require reference genomes which are not available for most tropical species and can be cost-prohibitive. Here, the combined evidence from genomic diversity statistics, demographic inference, and census data from a handful of individuals and a few thousand RAD loci indicate *M. yantzazana* is a truly rare tree rather than an artifact of under-sampling, with an estimated modern population size of ~10^3, though once 1-2 orders of magnitude more common.

Ongoing acute threats from human disturbance, notably mining, in the region suggest *M. yantzazana* is now of significant concern and unlikely to recover without urgent management intervention. Species in the premontane forests of the Andes-Amazonian system (700 m -1500 m) such

as *M. yantzazana* are particularly vulnerable, given that they live at elevations where coffee, coca, other agricultural crops, and high value timber species are grown, and have thus been the target of much deforestation (e.g. Tapia-Armijos et al. 2015). These threats, combined with the data presented here, support an IUCN status assignment of Critically Endangered (CR; IUCN 2012, 2024; S1). Future work may benefit from modeling many species from the same geographic area together to help to shed light on population trends in the region and highlight specific localities of concern.

5. DATA AVAILABILITY STATEMENT

All genetic data generated for this study are deposited in the GenBank online repository under PRJNA1206534 (www.ncbi.nlm.nih.gov/bioproject/PRJNA1206534).

6. REFERENCES

Aboul-Maaty, N. A. F., & Oraby, H. A. S. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, *43*(1), 1-10.

Andrews KR, Good JM, Miller MR, Luikart G, and Hohenlohe PA. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, 17 (2) 81 - 92.

Angiosperm Phylogeny Group, Chase, M. W., Christenhusz, M. J., Fay, M. F., Byng, J. W., Judd, W. S., ... & Stevens, P. F. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, *181*(1), 1-20.

Arroyo, F. & Pérez, A. J. (2013). Three new species of *Magnolia* (Magnoliaceae) from Ecuador. Phytoneuron, 55, 1-6.

Budd, C., Zimmer, E., & Freeland, J. R. (2015). Conservation genetics of Magnolia acuminata, an endangered species in Canada: can genetic diversity be maintained in fragmented, peripheral populations? *Conservation Genetics*, *16*, 1359-1373.

Caswell, H. (2009). Stage, age and individual stochasticity in demography. *Oikos*, *118*(12), 1763-1782.

Cheng, H., Sinha, A., Cruz, F. W., Wang, X., Edwards, R. L., d'Horta, F. M., ... & Auler, A. S. (2013). Climate change patterns in Amazonia and biodiversity. *Nature Communications*, *4*(1), 1411.

Cires, E., De Smet, Y., Cuesta, C., Goetghebeur, P., Sharrock, S., Gibbs, D., ... & Samain, M. S. (2013). Gap analyses to support ex situ conservation of genetic diversity in Magnolia, a flagship group. *Biodiversity and Conservation*, *22*, 567-590.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., ... & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156-2158.

Eaton, D. A., & Overcast, I. (2020). ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*, *36*(8), 2592-2594.

Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V. C., & Foll, M. (2013). Robust demographic inference from genomic and SNP data. *PLoS Genetics*, *9*(10), e1003905.

Feeley, K. J., & Silman, M. R. (2011a). The data void in modeling current and future distributions of tropical species. *Global Change Biology*, 17(1), 626-630.

Feeley, K. J., & Silman, M. R. (2011b). Keep collecting: accurate species distribution modelling requires more collections than previously thought. *Diversity and Distributions*, *17*(6), 1132-1140.

Gautschi, D., Heinsohn, R., Ortiz-Catedral, L., Stojanovic, D., Wilson, M., Crates, R., ... & Neaves, L. (2024). Genetic diversity and inbreeding in an endangered island-dwelling parrot population following repeated population bottlenecks. *Conservation Genetics*, 1-13.

Gentry, Alwyn H. "Patterns of neotropical plant species diversity." *Evolutionary Biology: Volume 15*. Boston, MA: Springer US, 1982. 1-84.

Guevara Andino, J. E., Pitman, N. C., Ulloa Ulloa, C., Romoleroux, K., Fernández-Fernández, D., Ceron, C., ... & Ter Steege, H. (2019). Trees of Amazonian Ecuador: a taxonomically verified species list with data on abundance and distribution.

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS genetics*, *5*(10), e1000695.

Hahn MW. (2018). Molecular Population Genetics. Oxford University Press.

Hoban, S., Bruford, M., Jackson, J. D. U., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P. A., ... & Laikre, L. (2020). Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. *Biological Conservation*, *248*, 108654.

Hubbell S. (2013). Tropical rain forest conservation and the twin challenges of diversity and rarity. *Ecology and Evolution*, 3 (10) 3263 – 3274.

IUCN. (2012). IUCN Red List Categories and Criteria, Version 3.1. Second edition. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland; Cambridge, United Kingdom.

IUCN. (2024). Guidelines for using the IUCN Red List categories and criteria. Version 16. Prepared by the IUCN Standards and Petitions Committee.

Isagi, Y., Tateno, R., Matsuki, Y., Hirao, A., Watanabe, S., & Shibata, M. (2007). Genetic and reproductive consequences of forest fragmentation for populations of Magnolia obovata. *Sustainability and Diversity of Forest Ecosystems: An Interdisciplinary Approach*, 382-389.

Kebaïli C, Sherpa S, Rioux D, and Després L (2021). Demographic inferences and climatic niche modelling shed light on the evolutionary history of the emblematic cold-adapted Apollo butterfly at regional scale. *Molecular Ecology*, 31, 448 – 466.

Lemopoulos, A., Prokkola, J. M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., ... & Vainikka, A. (2019). Comparing RADseq and microsatellites for estimating genetic diversity and relatedness—Implications for brown trout conservation. *Ecology and Evolution*, *9*(4), 2106-2120.

Lewontin, R. C. (1974). The genetic basis of evolutionary change. Columbia University Press

Lieberman, D., Lieberman, M., Hartshorn, G., & Peralta, R. (1985). Growth rates and age-size relationships of tropical wet forest trees in Costa Rica. *Journal of Tropical Ecology*, 1(2), 97-109.

Linan, A. G., Lowry, P. P., Miller, A. J., Schatz, G. E., Sevathian, J. C., & Edwards, C. E. (2021). RAD-sequencing reveals patterns of diversification and hybridization, and the accumulation of reproductive isolation in a clade of partially sympatric, tropical island trees. *Molecular Ecology*, *30*(18), 4520-4537.

Liu, X., & Fu, Y. X. (2015). Exploring population size changes using SNP frequency spectra. *Nature Genetics*, *47*(5), 555-559.

Liu, X., & Fu, Y. X. (2020). Stairway Plot 2: demographic history inference with folded SNP frequency spectra. *Genome Biology*, *21*(1), 280.

Nazareno, Alison G., et al. "Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species." *Molecular Ecology Resources* 17.6 (2017): 1136-1147.

Núñez, F. A. A., Guzmán-Díaz, S., Veltjen, E., Asselman, P., Jiménez, J. E., Sánchez, J. V., ... & Samain, M. S. (2024). Phylogenomic insights into Neotropical Magnolia relationships. Heliyon, 10(20).

Parchman, T. L., Jahner, J. P., Uckele, K. A., Galland, L. M., & Eckert, A. J. (2018). RADseq approaches and applications for forest tree genetics. *Tree Genetics & Genomes*, *14*, 1-25.

Patil, A. B., Shinde, S. S., Raghavendra, S., Satish, B. N., Kushalappa, C. G., & Vijay, N. (2021). The genome sequence of Mesua ferrea and comparative demographic histories of forest trees. *Gene*, *769*, 145214.

Pérez-Escobar, O. A., Zizka, A., Bermúdez, M. A., Meseguer, A. S., Condamine, F. L., Hoorn, C., ... & Chomicki, G. (2022). The Andes through time: evolution and distribution of Andean floras. *Trends in Plant Science*, *27*(4), 364-378.

Pitman, N. C., Terborgh, J., Silman, M. R., & Nuñez V, P. (1999). Tree species distributions in an upper Amazonian forest. *Ecology*, *80*(8), 2651-2661.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945-959.

Rabinowitz, D. (1981). Seven forms of rarity. Biological aspects of rare plant conservation.

Rivers, M., Beech, E., Murphy, L., & Oldfield, S. (2016). *The Red List of Magnoliaceae: revised and extended*. Richmond, Surrey, UK: Botanic Gardens Conservation International (BGCI).

Tamaki, I., Kawashima, N., Setsuko, S., Lee, J. H., Itaya, A., Yukitoshi, K., & Tomaru, N. (2019). Population genetic structure and demography of Magnolia kobus: variety borealis is not supported genetically. *Journal of Plant Research*, *132*, 741-758.

462 Tapia-Armijos, M. F., Homeier, J., Espinosa, C. I., Leuschner, C., & De La Cruz, M. (2015). Deforestation
463 and forest fragmentation in South Ecuador since the 1970s–losing a hotspot of biodiversity. PloS one,
464 10(9), e0133701.

465 Ter Steege, H., Pitman, N. C., Sabatier, D., Baraloto, C., Salomão, R. P., Guevara, J. E., ... & Silman, M. R.
466 (2013). Hyperdominance in the Amazonian tree flora. *Science*, *342*(6156), 1243092.

467 Ter Steege, H., Vaessen, R. W., Cárdenas-López, D., Sabatier, D., Antonelli, A., De Oliveira, S. M., ... &
468 Salomão, R. P. (2016). The discovery of the Amazonian tree flora with an updated checklist of all known
469 tree taxa. *Scientific Reports*, *6*(1), 29549.

470 Ter Steege H, Prado PI, de Lima RAF, Pos E, et al. (2020). Biased-corrected richness estimates for the
471 Amazonian tree flora. *Scientific Reports,* 10, 10130.

472 Thien, L. B., Kawano, S., Azuma, H., Latimer, S., Devall, M. S., Rosso, S., ... & Jobes, D. (1996). The floral
473 biology of the Magnoliaceae. In *In: Hunt, David, ed. Magnolias and their allies. Proceedings of an*
474 *International Symposium. Egham, Surrey, UK: Royal Holloway, University of London: 37-58.* (pp. 37-58).

475 Yang, F., Cai, L., Dao, Z., & Sun, W. (2022). Genomic data reveals population genetic and demographic
476 history of Magnolia fistulosa (Magnoliaceae), a plant species with extremely small populations in Yunnan
477 Province, China. *Frontiers in Plant Science*, *13*, 811312.

478 7. TABLES AND FIGURES

479 Table 1. Voucher numbers and sampling locations for the *Magnolia yantzazana* individuals.
480

Voucher #	ID	Herbaria	Altitude (m)	Latitude	Longitude
Neill 18868	Cantera 1	LOJA, ECUAMZ	1625	3°46’41” S	78°30’00” W
Neill 18869	Cantera 2	LOJA, ECUAMZ	1620	3°46’41” S	78°30’00” W
Neill 18870	Portal 1	LOJA, ECUAMZ	1424	3°45’49” S	78°30’30” W
Neill 18871	Portal 2	ECUAMZ	1425	3°45’48” S	78°30’30” W
Neill 18872	NAR	ECUAMZ	1445	3°45’21” S	78°32’46” W

481
482 Table 2. Summary of population genetic diversity statistics calculated in VCFtools for each assembly.
483

Assembly	Num. Loci	π	$H_o \pm SD$	$H_e \pm SD$	F_{IS}
De novo	3114	0.523	0.197 ± 0.03	0.497 ± 0.009	0.604
De novo	1311	0.452	0.123 ± 0.03	0.445 ± 0.006	0.723
Reference	1339	0.504	0.213 ± 0.03	0.480 ± 0.01	0.555
Reference	645	0.504	0.161 ± 0.004	0.423 ± 0.004	0.619

484
485 Table 3. Summary of inbreeding statistics calculated in COLONY v2. for each assembly.

Assembly	Num. Loci	F_{IS} (DHW) Inbreeding	F_{IS} (DHW) Selfing	ID
De novo	3114	0.658	0.794	0.924
De novo	1311	0.655	0.792	0.891
Reference	1339	0.488	0.656	0.923
Reference	645	0.530	0.693	0.879

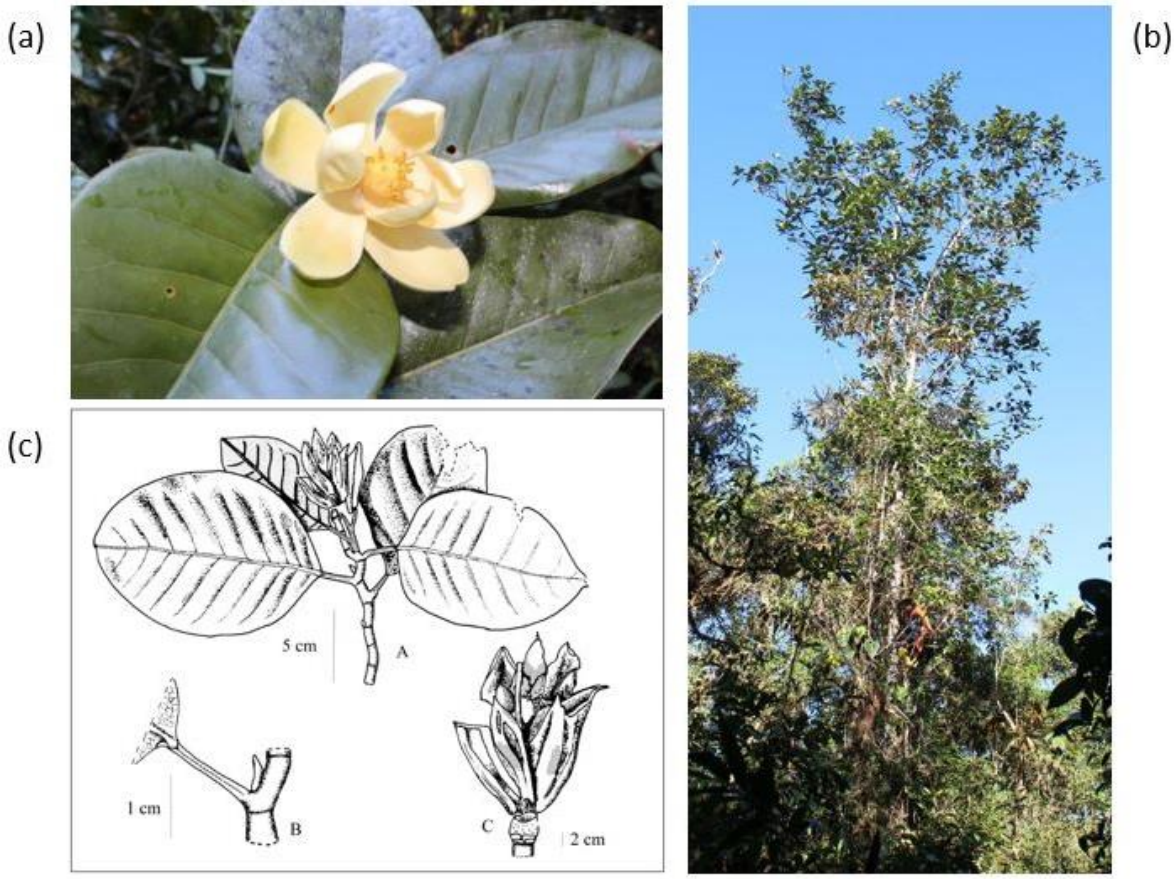
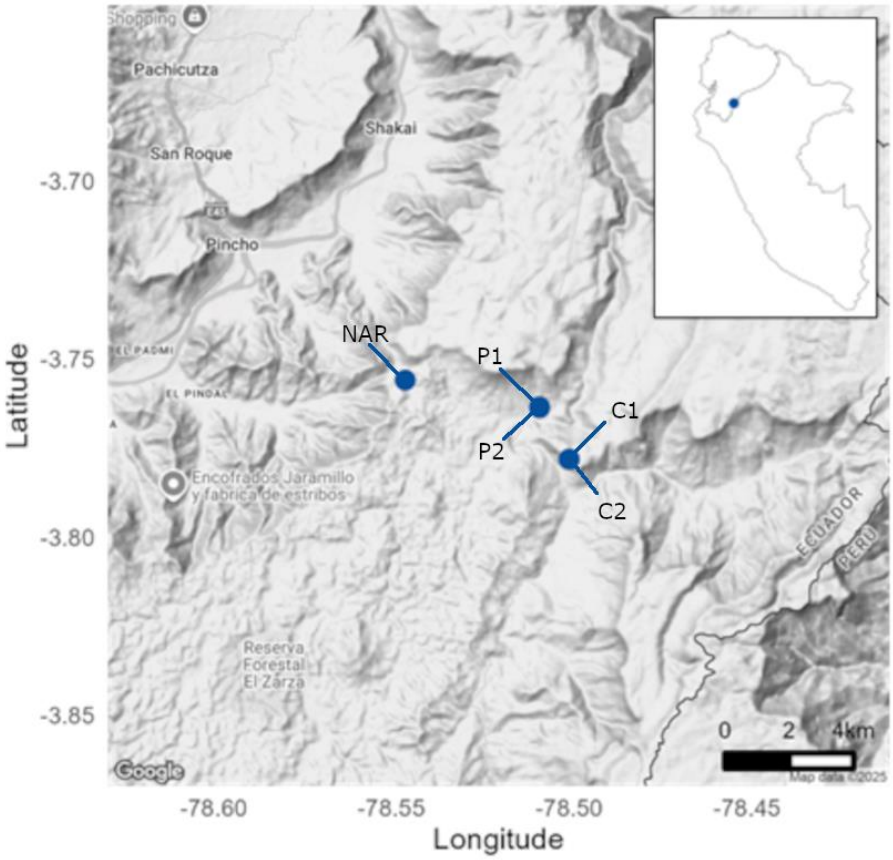


Figure 1. (a) The flower of *Magnolia yantzazana* F. Arroyo (photo by David A. Neill); (b) 25 m tall *M. yantzazana* tree (photo by David A. Neill); (c) *M. yantzazana* holotype showing the fruiting branchlet, detailed petiole, and mature fruit at dehiscence (from original species description in Arroyo and Pérez 2013).



493
494

495 Figure 2. Map of *Magnolia yantzazana* sampling localities in Zamore Chinchipe, Yantzaza canton,
496 Ecuador, within the Ludin Gold Inc. mining concession.

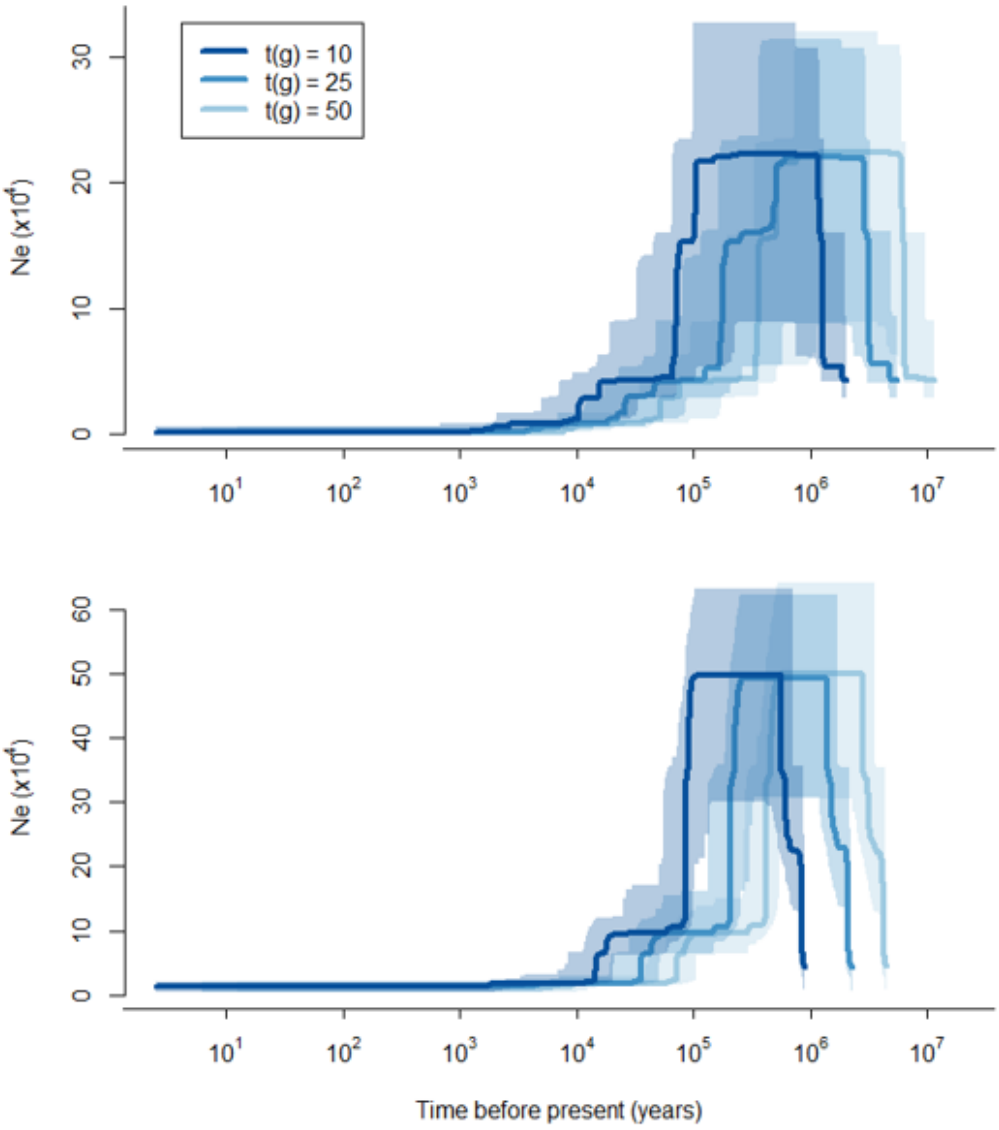


Figure 4. Demographic history of *Magnolia yantzazana* inferred by Stairway plot 2. The three generation time scenarios are represented by different shades of blue with (a) inferenced from the folded SFS and (b) the unfolded (reference mapped) SFS. Lines show predicted change in effective population size N_e over time and shading indicates the 95% confidence interval.