Continuous Degradation of Forest Structure and Composition Causes a Shift in Phorophytes and Population Genetics in Aphyllous *Vanilla* Species

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Abstract

Background and Research Aims: The natural habitats of aphyllous vanillas in Madagascar have been extensively degraded by human activities. The implications of this degradation are complex given their intricate interactions with other organisms. This study highlights possible approaches to the conservation of aphyllous vanillas after examining the impact of forest degradation on phorophyte diversity, reproductive modes, and genetic diversity.

Methods: We conducted comparative studies of conserved and degraded forests in two contrasting bioclimatic zones. We characterized the changes in horizontal and vertical forest structures and assessed the ecological importance of the tree species. We identified the preferred phorophytes of two aphyllous *Vanilla* species, *Vanilla madagascariensis* and *Vanilla decaryana*, and investigated their population genetics.

Results and Conclusions: Our findings confirmed a declining trend in tree diversity, number of trees, and cover rate in degraded forests. Canopy openness is associated with an increase in the number of phorophytes and the asexual reproductive mode of *Vanilla* species. Dominant tree species were more likely to become phorophytes, and there was a positive correlation between phorophyte diameter and number of hosted vanilla individuals. Significant deviations from the Hardy-Weinberg equilibrium were observed in each population, particularly in the dry forest and degraded sites, compared with the conserved sites. Additionally, slight differentiation was observed between vanilla populations in conserved and degraded forests, with an alarming effective population size (Ne) that did not exceed 13 individuals.

Conservation Implications: We recommend the introduction of new genotypes to promote genetic enrichment, the ban on logging potential phorophytes, and the inclusion of some degraded forests comprising *Vanilla* species in conservation.

Keywords
climbers, deforestation, genetics, orchids, reproduction, vanilla

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Introduction

Plant taxonomic groups respond differently to anthropogenic disturbances across ecoregions (Irwin et al., 2010; Sfair et al., 2018), causing notable changes in the forest structure and composition (Haq et al., 2019). These alterations significantly affect plant pollination by modifying the microenvironment and pollinator availability and behavior (Torres-Vanegas et al., 2021; Xiao et al., 2016). Consequently, deforestation, which is the conversion of a forest into different land-use types or permanent reduction of tree canopy cover below the 10% threshold (Carter et al., 2017; FAO, 2015), can decrease plant reproductive success (Torres-Vanegas et al., 2021). Numerous studies have indicated that forest fragmentation into smaller, isolated patches surrounded by non-forest land, significantly affects the genetic diversity of plant and animal populations (González et al., 2020; Schlaepfer et al., 2018). Fragmentation leads to reduced population sizes and changes in species richness and composition, and creates residual habitat patches of varying shapes and sizes that disrupt population dynamics (Schlaepfer et al., 2018; Torres-Vanegas et al., 2021). The spatial isolation of populations theoretically erodes genetic variability and increases genetic divergence due to enhanced random genetic drift, high inbreeding, and reduced gene flow between populations (Cheptou et al., 2017; Schlaepfer et al., 2018).

Over the past 60 years, Madagascar's forest cover has declined drastically, leaving only 15% of its land forested (Bond et al., 2008; Harper et al., 2007; Vieilledent et al., 2018). Ecological degradation is primarily driven by economic activities, poverty, and population growth. Poverty forces many people to rely heavily on natural resources for survival, which, in turn, weakens livelihoods and exacerbates poverty (Barbier & Hochard, 2018; Miyamoto, 2020). Key activities contributing to forest degradation, which is a progressive decline of forest structure and composition resulting in a loss of functions (Vásquez-Grandón et al., 2018), include selective logging, targeting of commercially valuable tree species, and slash-and-burn agriculture, which involves clearing unmarketable woody vegetation (Kaneko et al., 2014; Ralimanana et al., 2022; Zaehringer et al., 2015).

Madagascar harbors a significant diversity of orchids, with 1002 species, including several in the genus *Vanilla* (Cribb & Hermans, 2009; Goodman, 2022). Aphyllous *Vanilla* species, wild relatives of cultivated vanillas, exhibit drought adaptation (Botomanga et al., 2024). Conserving wild vanilla resources and recognizing their genetic value are crucial for addressing the current agronomic challenges (Flanagan et al., 2018; Watteyn et al., 2023). Some species are used in traditional medicine as aphrodisiacs (Rakotoarivelo et al., 2019; Randriamiharisoa et al., 2015), raising concerns regarding resource depletion due to overharvesting. Aphyllous *Vanilla* species are semi-epiphytes that initially germinate in the soil before growing on phorophytes, which are host trees that serve as supports for epiphytic plants (Derzhavina, 2019).

The flowers attract bees for potential pollination (Gigant et al., 2014, 2016; Petersson, 2015). Similar to many lianas, they can act as bridges for primates and arboreal animals (Gerolamo et al., 2022; Rowe, 2018). Although epiphytic orchids use trees as phorophytes, not all trees serve effectively as supports (Clemente-Arenas et al., 2023; González-Orellana et al., 2024; Shen et al., 2022). Such selective behaviors can significantly affect the distribution and abundance of epiphytic populations (Cortés-Anzúres et al., 2020; Gowland et al., 2011; McCormick & Jacquemyn, 2014; Rubio-Salcedo et al., 2015; Wagner et al., 2015). The rough bark of phorophytes offers better support and moisture retention, thereby enhancing the germination rates (González-Orellana et al., 2024; Timsina et al., 2016; Zarate-García et al., 2020). Preferences may also be linked to specific fungi involved in orchid mycorrhizal associations (Pecoraro et al., 2021; Rasmussen et al., 2015; Wang et al., 2017).

Vanilla species typically reproduce through a mixed mating system involving vegetative propagation and pollinator-mediated sexual reproduction (de Oliveira et al., 2022; Grisoni & Nany, 2021). In Puerto Rico, the clonality rates for the three *Vanilla* species ranged from 6 to 38% (Nielsen & Siegismund, 1999). For *Vanilla humblotii* in Mayotte, the rate was 12.5%, while seven aphyllous *Vanilla* species in Madagascar averaged 9% (Andriamihaja et al., 2022; Gigant, De Bruyn, et al., 2016a). Forest degradation may enhance the vegetative propagation of vanilla vines due to increased light access. Canopy openness significantly affects liana density (Gianoli et al., 2012; Miranda et al., 2022; Schnitzer et al., 2021), and co-occurrence of liana species (Silva et al., 2016). Consequently, forest degradation could influence liana reproductive modes, justifying the investigation of the impact of canopy openings on the development and reproduction of aphyllous *Vanilla* species.

Highly polymorphic markers have revolutionized population genetics research (Garrido-Cardenas et al., 2018; Nadeem et al., 2018). Vegetative reproduction, often linked to repeated multilocus genotypes (MLG) (Stoeckel et al., 2021a), enhances clonality estimation accuracy. F-statistics (Wright, 1949), widely used in population genetics (Cui et al., 2022; Stoeckel & Masson, 2014; Zimmerman et al., 2020), describe gene flow and breeding structures, especially deviations from random mating. The fixation index of individuals in subpopulations (FIS) assesses genetic divergence within a subpopulation relative to the total population, ranging from -1 to +1. Negative values signify a surplus of heterozygotes, positive values indicate a heterozygote deficit, and zero denotes the Hardy-Weinberg equilibrium, where the genetic distribution is considered stable (Arnaud-Haond et al., 2007; Mishra et al., 2021). Essentially, FIS reflects intra-population differentiation. FST, which ranges from 0 to 1, measures the proportion of the total genetic diversity attributable to differences between subpopulations. Higher FST values indicate greater genetic

divergence among the subpopulations (Arnaud-Haond et al., 2007; Mishra et al., 2021).

The effects of habitat degradation on population genetic diversity have been studied in both conserved and degraded environments (Aguilar et al., 2019; Chung et al., 2014; Depecker et al., 2023). However, these studies are often difficult because of the limited availability of the pristine sites. Traditional societies protect sacred natural sites from various threats (Frascaroli et al., 2019; Ormsby, 2021; Zannini et al., 2021). These areas are typically remnant forests linked to spirits, temples, or burial sites, providing significant protection against logging, agriculture, and other forms of habitat destruction (Frascaroli et al., 2019; Ormsby, 2021; Zannini et al., 2021). To assess the impact of forest degradation on the population of aphyllous *Vanilla* species, we used undisturbed sacred forests as reference sites.

This study aimed to assess the impact of forest degradation on aphyllous vanilla populations. Specifically, we evaluated the effects of degradation on (i) the diversity of phorophytes and tree flora, (ii) the mode of reproduction, and (iii) the genetic diversity of aphyllous *Vanilla* species. We compared vanilla populations in sacred forests with those in the surrounding degraded forests to test our hypothesis that aphyllous vanilla populations in sacred sites would have greater genetic diversity owing to a less disturbed environment. Conversely, we expected reduced floristic diversity and higher clonality in the degraded forests. We also hypothesized that forest degradation would significantly affect vanilla genetics, leading to differentiation between populations within a locality over time. Ultimately, this study provides insights into the optimal strategies for replanting programs with aphyllous *Vanilla* species, depending on whether the forest is degraded or well-conserved.

Methods

Study Sites

The study was conducted in April and May 2018 at two sites in northern and southern Madagascar, including (i) a sacred forest with relatively intact vegetation and (ii) a degraded forest of secondary formations or 'savoka'. The Ambatofaly sacred forest and Ampitolova degraded forest are in the north (Figure 1, Table 1), whereas the Ampotaka sacred forest and Vohitsara degraded forest are in the south (Figure 1, Table 1). The northern sites are in a sub-humid bioclimatic zone, whereas the southern sites have sub-arid conditions (Table 1).

The Sacred Forests of Ambatofaly and Ampotaka

The Ambatofaly sacred forest (ABF), a relictual forest on the northwestern coast, spans five hectares and is classified as lowland humid tropical forest (Moat & Smith, 2007) (Table 1). In 1825, the forest was consecrated through a ritual that sought blessings from ancestors via the largest tree, an *Andansonia madagascariensis* Baill. This ceremony initiated the sacredness of the Ambatofaly forest, governed by taboos and rules.

The Ampotaka (APTK) sacred forest (Table 1) is situated in the Androy region of southern Madagascar (Figure 1) on the Mahafaly Plateau. The vegetation is classified as a dry, spiny thicket (Moat & Smith, 2007), covering an area of ca. 157 ha. The forest is home to numerous ancestral tombs called 'kibory', and is managed by local communities. Although our study could not determine the exact date, most southern sacred forests predated the colonial era (Tengö et al., 2007). Reports on turtle reintroduction into the APTK forest suggest that it remains largely undisturbed, except for minor traces from goat herds (Randrianjafizanaka, 2014).

The Degraded Forests of Ampitolova and Vohitsara

The Ampitolova (APL) 'savoka' (Figure 1, Figure 2b) is situated approximately 4.5 km away from the ABF forest and is surrounded by small villages. The APL undergoes extensive logging of timber and firewood. The 6–7-year-old successional vegetation primarily consists of young shrubs 1–3 m tall.

The degraded Vohitsara forest (VHT) (Table 1) is situated 30 km east of the sacred APTK forest (Figure 1, Figure 2d). Located in one of the most deprived regions of Madagascar, the VHT is frequently affected by the devastating effects of drought, which limits land-use options (Andriamparany et al., 2014). The local population relies heavily on forest products for subsistence, exacerbating resource overexploitation and further worsening their situation (Andriamparany et al., 2014). The lack of government protection has left VHT forests accessible to the local communities for years.

Study Species

Aphyllous *Vanilla* species (Orchidaceae) are characterized by their succulent stems, which are typically green in color (Figure 2e-f) but may turn brown (Figure 2h) or yellow when exposed to excessive direct sunlight. There are seven known aphyllous *Vanilla* species in Madagascar: *Vanilla bosseri* L. Allorge, *V. decaryana* H. Perrier, *V. humblotii* Rchb.f., *V. madagascariensis* Rolfe, *V. perrieri* Schltr, *V. allorgeae* Andriamihaja & Pailler, and *V. atsinananensis* Andriamihaja & Pailler (Allorge-Boiteau 2005, 2013; Andriamihaja et al. 2022; Cribb & Hermans 2009; Portères 1954). White and yellow flowers bloom only once a year and are ephemeral. Based on the research of Andriamihaja et al. (2021, 2022), the two northern sites, ABF and APL, likely had *V. madagascariensis*, while APTK and VHT were likely home to *V. decaryana*.

Floristic Samplings

In an area with uniform vegetation and aphyllous vanilla vines, a 50-by-20-meter plot was established using the Braun-Blanquet method (Westhoff & Van Der Maarel, 1978). Three comparable plots were established at each site. Trees and
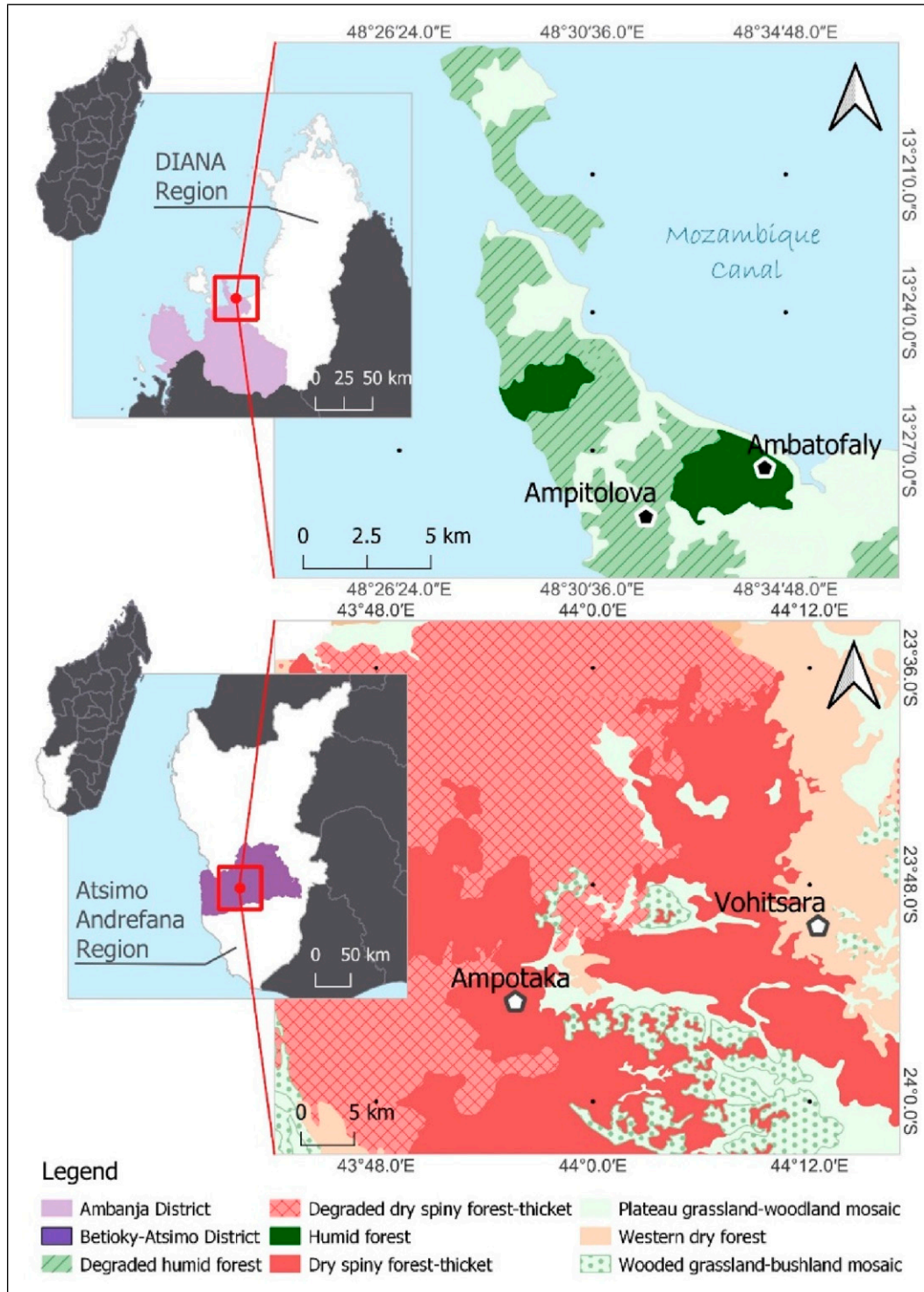
Figure 1. Study areas and locations of *Vanilla* species populations in Madagascar. Map displaying the Ambatofaly sacred forest and Ampitolova degraded forest in the north (up). Map displaying the Ampotaka sacred forest and the Vohitsara degraded forest in the south of Madagascar (Down). The pentagon indicates the geographical positions of the four localities where *Vanilla* population was sampled.

shrubs with a diameter at breast height (DBH at 1.3 meters) of over 5 cm were sampled. The DBH, maximum height, and local name of each specimen were recorded. The total number of trees and phorophytes per plot and the number of vanilla

plants per phorophyte were documented. A vanilla plant was defined as a vine with all its branches, and phorophytes were specifically trees hosting aphyllous vanilla vines. A total of 501 herbarium specimens were collected from every tree or

Table 1. Area, location, geographic information, ecological status, vegetation, and climatic conditions of four study sites in northern (ABF and APL) and southern (APTK and VHT) Madagascar.

Site	Code	Area (ha)	Longitude	Latitude	Altitude (m)	Status	Vegetation	Bioclimate	T (°C)	R (mm)
Ambatofaly	ABF	5	48°34'40.10"E	13°27'9.18"S	20	Conserved	Humid forest	Sub-humid	26	2095
Ampitolova	APL	125	48°32'34.86"E	13°27'53.37"S	26	Degraded	Savoka	Sub-humid	26	2095
Ampotaka	APTK	157	43°56'41.38"E	23°54'13.65"S	108	Conserved	Thicket	Sub-arid	24	547
Vohitsara	VHT	112	44°14'29.63"E	23°53'22.95"S	258	Degraded	Thicket	Sub-arid	24	551

Climatic data were obtained from https://www.worldclim.org, a widely used source of global climate data (data for 1970 – 2000). T: Annual Mean Temperature, R: Annual precipitation.

shrub species to match the reference specimens and provide precise scientific names. This method offers a systematic, comprehensive, and quantitative approach to studying vegetation structure, composition, and ecological interactions.

Forest Vertical and Horizontal Structures

Gautier's method (Gautier et al., 1994) was used to analyze the vertical structure of the forest (Figure S1A), profile the foliage distribution, and estimate the coverage of key species. This involved recording vegetation contact points with a vertical graduated pole placed at one-meter intervals along a transect through the plot center, noting the height of these contacts. These points were projected onto an Excel spreadsheet to create a structural vegetation profile (Figure S1B). Analyzing contact points by height class, quantified coverage, expressed as a percentage of the continuity of forest cover. The coverage scale developed by Godron et al. (1983) was used to interpret the results (Figure S2). The horizontal forest structure, assessed by density, dominance, and species frequency, was determined by evaluating the ecological weight and diameter distribution of all individuals of each species, following Lamprecht (1989). The importance value index (IVI) was calculated based on the collected data for each species as described by Melo and Vargas (2003). IVI was derived as follows: **IVI = RA + RD + RF**, where **RA** is the relative density, **RD** is the relative dominance, and **RF** is the relative frequency. The relative density was calculated as **RA = (n/N) × 100**, where **n** is the number of individuals of each species and **N** is the total number of individuals. Relative dominance was calculated as **RD = (Ga/GT) × 100**, where **Ga** is the basal area of each species, and **GT** is the total basal area. The basal area was calculated using **Ga = (π/4) × (DBH)^2**. The relative frequency was obtained by considering **RF = (aF/TF) × 100**, where **aF** is the absolute frequency, and **TF** is the total frequency. This index aids in identifying the species with the highest ecological value and disturbance indicators in forests (Catalá, 2011).

Flora Diversity

This study used online databases, such as the Madagascar Catalogue of Vascular Plants by Tropicos (2023) and Plants of the World (Royal Botanic Gardens Kew 2023), to determine plant species endemism. Tree species were classified as exotic, indigenous, or endemic. EstimateS software (Colwell & Elsensohn, 2014) was used to calculate the expected number of species at each site, considering rarefaction and extrapolation. We conducted 100 randomization iterations to estimate the number of nodes in each sample by combining the data collected from each site. Classical calculations for Chao1 and Chao2 were used to determine estimators and indices. The extrapolated 'S' values and associated standard deviations were used to construct comparable plot species curves for the different sites. The means of the Chao1 species richness estimator indices were derived using EstimateS, which considers the bias stemming from undetected species and serves as a minimum estimate of the total species richness at each site (Chao, 1984, 1987). To quantify the specific diversity at each site, the Shannon diversity index (H') was computed using EstimateS and the Pielou evenness index using the formula J = H'/ln(S), where S is the number of species found in one locality.

Data Analysis

For statistical analysis, we conducted site-by-site tests using R version 4.3.1 (R Core Team, 2023). To evaluate the number of families, genera, and species present at each site, we combined the data from three separate plots and analyzed their distribution frequency. The percentage of families was calculated considering all individuals. The biodiversityR package (Kindt & Kindt, 2019) was used to calculate the ecological importance value index (IVI, RA, RD, and RF). Two main criteria were assessed to evaluate vanilla preference for phorophytes. First, the most frequently observed phorophyte species among all tree species were identified using the chi2 test to compare the tree species frequencies. Second, the tree species hosting most of the vanilla individuals were determined by excluding phorophytes that hosted vanilla only once. The potential effects of phorophyte species on vanilla abundance were tested using one-factor ANOVA. Mean number of vanilla individuals per phorophyte was then compared using Tukey's post hoc test with the "glht"
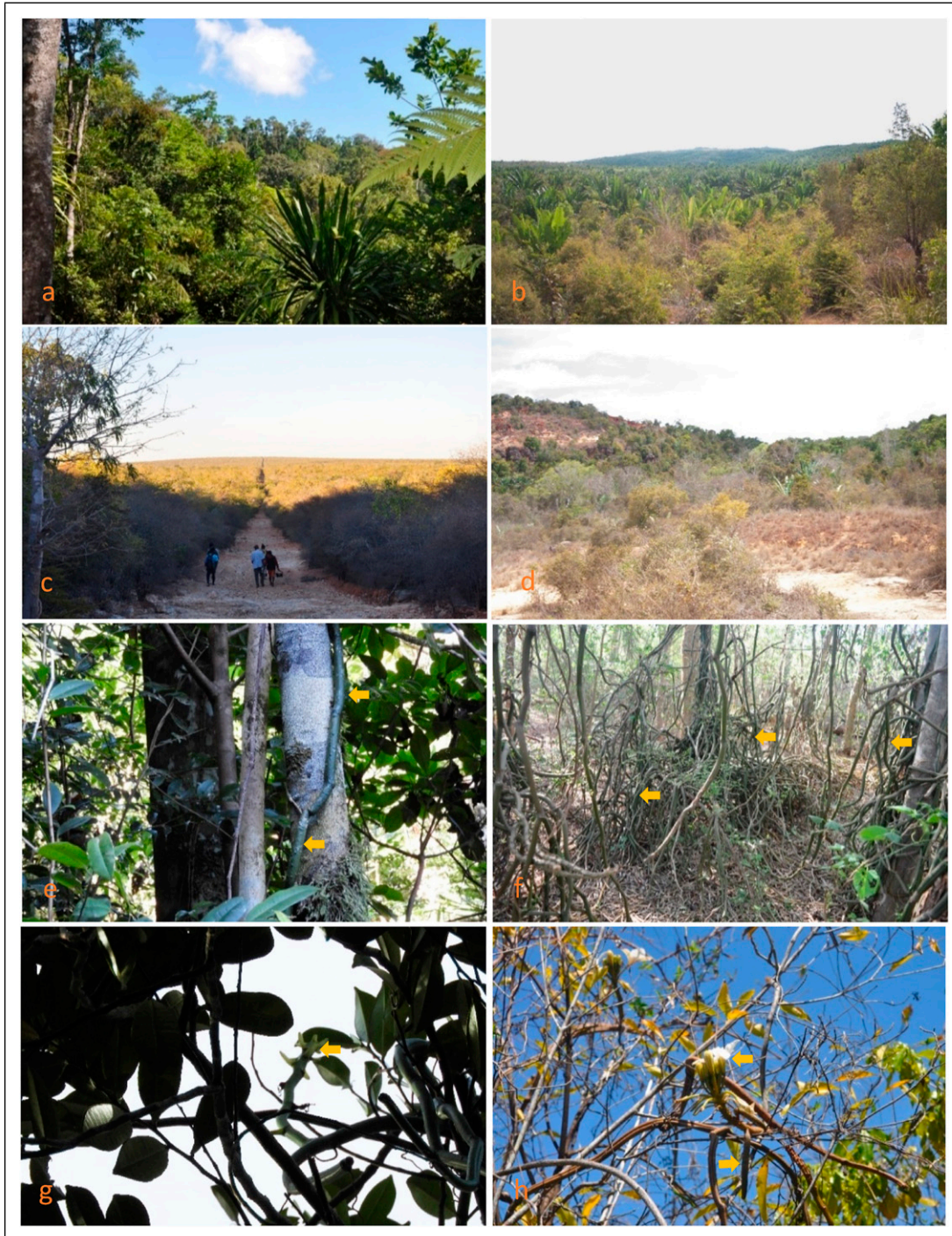
Figure 2. Overview of conserved (left) and degraded (right) forests. (a) Ambatofaly sacred forest; (b) Ampitolova degraded forest; (c) Ampotaka sacred forest; (d) Vohitsara degraded forest; (e) vanilla stem (arrow) at Ambatofaly (semi-closed canopy); (f) vanilla vines (arrow) at Vohitsara (open canopy); (g) vanilla inflorescence (arrow) at Ambatofaly; and (h) vanilla inflorescence and capsules (arrows) at Ampitolova (open canopy).

function from the R package "multcomp" (Hothorn et al., 2015) at a significance level of $p < 0.05$. Pearson's correlation tests were performed to examine the relationship between species density and their identification frequency as phorophytes, as well as the correlation between the number of vanilla individuals and phorophyte diameter. These analyses offer insights into the phorophyte preference of vanillas. Additionally, a split-plot factorial ANOVA was used to assess the average tree height across localities in both the conserved and degraded forests.

Sampling and DNA Extraction

We employed a site-specific sampling approach, in which we collected 25 samples from each study site. We excised 15 cm of stems or young buds and preserved them in silica gel, resulting in 100 individuals across the four populations. DNA collection from young buds allowed us to obtain high quality, uncontaminated, and undegraded DNA. To prevent collection from the same clonal shoot, we ensured a minimum distance of 2 m between collected specimens. DNA was extracted from 100 specimens using the DNEASY® plant kit (https://www.qiagen.com).

Microsatellite Genotyping

Seven microsatellite markers developed by Gigant et al. (2011) (Table S1) were used to amplify and genotype each sample. Polymerase chain reaction (PCR) was carried out using fluorescently labeled primers in a total volume of 25 μl using a Promega kit (Promega, 2007). The mixture contained 12.5 μl of GoTaq Colorless Master Mix 2X, 2 μl of DNA solution (5 < DNA < 250 ng), 0.4 μM of each primer, and 8.5 μl of nuclease-free water. Amplifications were carried out under the following conditions: an initial denaturation step at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 45 s, and extension at 72°C for 1 min, and a final extension step at 72°C for 7 min. Capillary electrophoresis with an automated 3130XL ABI Genetic Analyzer (Applied Biosystems) was used to genotype PCR fragments. GENEIOUS PRIME 2019.1 (https://www.geneious.com) was used for allele size coding.

Genetic Diversity

Of the 25 samples collected per site, 23 were considered for ABF, APL, and VHT, and 21 for APTK due to missing data. *Vanilla* species at each site were identified through Bayesian genotypic clustering analysis using STRUCTURE software (Pritchard et al., 2000) on all sampled individuals, and six aphyllous *Vanilla* species (Table S2) from Andriamihaja et al. (2021, 2022). Ten independent replicates were performed for each K value (1 to 10), without admixture and independent allelic frequency models. The analysis was conducted with a burn-in length of 100 000 iterations and 1 000 000 Markov chain Monte Carlo (MCMC) replications. We determined the optimal number of clusters based on the posterior probabilities of K "Ln P (X/K)," and the summary likelihood statistic ΔK (Evanno et al., 2005). Two datasets were generated: one with all sampled individuals ("ramets") and another excluding identical multilocus genotypes ("genets"), identified using multilocus clonality analysis in GenAlex 6.51 (Peakall & Smouse, 2006). The probability of encountering a specific multilocus genotype through sexual reproduction under random mating was calculated using GenAlex 6.51 to identify clones from vegetative reproduction. Genotypic diversity was assessed using the ratio of "genets" (G) to "ramets" (N) (Ellstrand & Roose, 1987). Genetic variability parameters and Hardy-Weinberg equilibrium (HWE) deviations were analyzed using the genet dataset. The average number of alleles per locus, total alleles per population, and private alleles was estimated using GenAlex for each polymorphic locus across populations. Observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation indices (F_{IS}) were calculated using the Genepop 4.7.5 (Rousset, 2008). Exact *p*-value estimates (Guo and Thompson, 1992) were determined at the population level to test for HWE deviations using default parameters. Linkage disequilibrium between loci was tested using Fisher's exact test in Genepop, comparing the results with and without repeated multilocus genotypes. To estimate the effective population size, we used the "genet" data, excluding any missing values. We applied the linkage disequilibrium (LD) method, assuming random mating, using NeEstimator v2.1 software (Do et al., 2014). NeEstimator calculated the 95% confidence interval (CI) by using the JackKnife procedure for each Ne estimate. The LD method requires setting a critical value (PCrit) at a sufficiently high level to exclude alleles present in a single copy (heterozygotes) within the sample. We used PCrit > 1/(2S), where S is the number of individuals with available data for both loci. With our population of 23 to 11 genets, this required PCrit values greater than 1/46=0.02 to 1/22=0.045. We report the results obtained using a PCrit value of 0.05.

Differentiation of Populations

Population differentiation was analyzed by calculating the pairwise F_{ST} index using Genepop, which considered only the 'genet' dataset. Bayesian clustering analysis using STRUCTURE software was performed on the dataset to assign multilocus genotypes to different groups. Bayesian analysis uses probabilistic models to estimate the number of genetic groups (K) present in the data, and assigns each individual to these groups based on their multilocus genotypes. This approach is particularly effective in detecting genetic differentiation patterns, even when the differences between populations are subtle. Ten independent replicates were conducted for each K value, ranging from 1 to 10, to detect any sub-structuring within the samples. The no admixture and correlated allelic frequency models were used, with a burn-in length of 100,000 iterations and 1,000,000 MCMC replications. The optimal number of clusters was determined using the online software Structure Analyses (https://pophelper.com/), which calculates the summary likelihood statistics ΔK.

Results

Changes in Forest Structure Following Degradation and Impacts on Vanilla Species Colonization

A total area of 1.2 hectares was surveyed, including 4 412 vanilla individuals, 1 404 trees, and shrubs, of which

627 were phorophytes. Table 2 compares the forest structure parameters of the four sites located in northern and southern Madagascar. The sacred forest of ABF stood out because of its average tree height of 9.2 ± 3.1m (Table 2). There is a clear trend of a decreasing number of trees in degraded forests compared to conserved forests (Table 2). In addition, a reduction in forest cover was observed in degraded forests, although cover loss was less pronounced in the southern forest of the VHT (7.7%). In contrast, forest degradation caused a significant increase in the number of phorophytes hosting vanilla vines (Table 2). Specifically, there was an 80% increase in phorophytes in the degraded APL forest compared to the sacred ABF forest, and a 48% increase in the degraded VHT forest compared to the sacred APTK forest (Table 2). The number of vanilla individuals was 10 to 16 times higher in open sites, such as the APTK and VHT forests in the south and the degraded APL forest in the north, than in the ABF site where the canopy was semi-closed (Table 2).

Changes in Tree Species Composition Following Degradation and Impacts on Vanilla Species Colonization

In total, 104 tree species belonging to 47 families and 83 genera were identified in the study area. Table 3 presents a summary of the flora diversity parameters at the four sites located in northern and southern Madagascar. From these data, it can be seen that the number of recorded species (S) is always lower in degraded forests. Additionally, the extrapolated accumulation curves (Figure S4) indicated that there were more expected species (Chao 1) in conserved forests (Table 3). The Shannon diversity index (H') observed between conserved and degraded forests revealed significant differences (Table 3), with higher diversity index values for conserved forests. However, Piélou's evenness was statistically similar across all study sites, with values ranging from 0.76 to 0.85, indicating relatively high uniformity of species across the study sites (Table 3). A comprehensive floristic list including all tree species, phorophytes, and non-phorophytes is provided in Table S3. Of these 104 species, 72 were confirmed to be endemic to Madagascar, 14 were indigenous

and seven were introduced. The remaining 11 species were not identified in the present study. Among the 627 phorophyte trees (Table 2), 73 species were identified, representing 70% of all the recorded species. Interestingly, 4% of these phorophyte trees hosted only one individual of vanilla.

The ABF inventory identified 35 tree species across 31 genera and 22 families, with Loganiaceae being the most prevalent, accounting for 22% of trees. Among these species, 22 were endemic, five indigenous, four introduced, and four unidentified (Figure 3). The most dominant species were *Strychnos decussata* (Pappe) Gilg (IVI = 48.11), *Diospyros gracilipes* Hiern (IVI = 40.88), and *Trilepisium madagascariensis* DC. (IVI = 27.77) (Table 4). Additionally, the inventory allowed the recording of 118 individuals of *V. madagascariensis* hosted by 11 different phorophyte species (Table 2 and Table 3), six of which were endemic, two autochthonous, two introduced, and one unidentified (Table S3). On average, 4.9 ± 2.6 vanillas were observed on each phorophyte. Among the 24 surveyed phorophyte trees, *Diospyros gracilipes* was the most commonly encountered, with seven trees observed, followed by *Trilepisium madagascariensis*, which was observed four times as phorophytes. However, none of the phorophyte species showed significant affinity for vanillas ($\chi^2 = 16.33$; df = 10; $p = 0.09$). It is noteworthy that *Trilepisium madagascariensis* had the highest number of

Table 3. Comparison of parameters describing flora diversity and ecology between conserved and degraded forests in the northern (ABF and APL) and southern (APTK and VHT) regions of Madagascar.

| Site	F	G	S	H'	J	Chao1	Php
ABF	22	31	35	2.48 ± 0.1[a]	0.76 ± 0.3[a]	56.4[a]	11
APL	17	24	24	2.19 ± 0.2[b]	0.84 ± 0.6[a]	25.12[b]	23
APTK	28	39	46	2.68 ± 0.4[a]	0.77 ± 0.1[a]	53.3[a]	35
VHT	10	15	17	2.12 ± 0.7[b]	0.85 ± 0.1[a]	17.2[b]	17

F: number of families, G: number of genera, S: species richness, H': Shannon's diversity index, J: Pielou's evenness index, Chao1: Species richness estimates, Php: Number of species identified as phorophytes. ABF: Ambatofaly, APL: Ampitolova, APTK: Ampotaka, VHT: Vohitsara. Means followed by the same letter within the same column are not significantly different ($p > 0.05$).

Table 2. Comparison of forest structure parameters, with abundance of *Vanilla* species between conserved and degraded forests located in the northern (ABF and APL) and southern regions (APTK and VHT) of Madagascar.

Site	H_{max}	C_{co} (%)	C_{co} scale	N_t	N_p (%)	V
ABF	9.2 ± 3.1[b]	64.6	Semi-open stratum	363	24 (6.6)	118
APL	4.4 ± 1.7[a]	28.6	Open stratum	290	253 (87.2)	1958
APTK	4.7 ± 1.7[a]	37.5	Open stratum	532	174 (32.7)	1160
VHT	4.7 ± 2.4[a]	29.8	Open stratum	219	176 (80.3)	1176

H_{max}: Tree maximal height, C_{co}: Canopy cover rate, C_{co} scale: Canopy cover scale, N_t: Total number of trees, N_p: Total number of phorophyte trees, with the percentage in parentheses indicating the proportion of the total number of trees present on the site, V: number of vanilla individuals. ABF: Ambatofaly, APL: Ampitolova, APTK: Ampotaka, VHT: Vohitsara. Means followed by the same letter within the same column are not significantly different ($p > 0.05$).
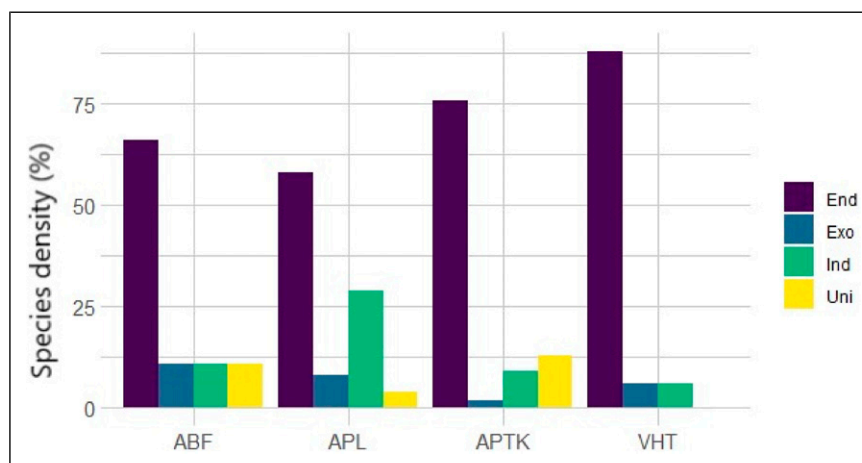
Figure 3. Number of tree species per locality and according to endemism. Endemism: End (endemic), Exo (exotic) et Ind (indigenous), Uni (Unidentified), Locality: ABF (Ambatofaly), APL (Ampitolova), APTK (Ampotaka), VHT (Vohitsara).

Table 4. List of the five species with the highest ecological importance value index (IVI) at the studied sites.

Locality	Species	Dis	Den	RF (%)	RA (%)	RD (%)	IVI (%)	Php	PhpS	AvV
ABF	*Strychnos decussata* (Pappe) Gilg	Nat	80	4.05	22.04	22.02	48.11	Yes	3	3.66
ABF	*Diospyros gracilipes* Hiern	End	67	4.05	18.46	18.37	40.88	Yes	7	4.42
ABF	*Trilepisium madagascariense* DC.	Nat	53	4.05	14.60	9.11	27.77	No		
ABF	*Albizia lebbeck* Benth.	Exo	30	4.05	8.26	14.10	26.41	Yes	1	8
ABF	*Schizolaena parviflora* (F. Gérard) H. Perrier	End	24	4.05	6.61	4.22	14.88	Yes	2	5
APL	*Ravenala agathea* Haev. & Razanats.	End	51	8.11	17.59	58.65	84.35	Yes	32	17,56
APL	*Schizolaena parviflora* (F. Gérard) H. Perrier	End	45	8.11	15.52	7.12	30.75	Yes	40	5,27
APL	*Gaertnera arenaria* Boulanger	End	40	8.11	13.79	4.43	26.33	Yes	37	6,27
APL	*Mascarenhasia arborescens* A. DC.	Nat	33	8.11	11.38	6.20	25.69	Yes	33	6,3
APL	*Dypsis ambanjae* Beentje	End	12	5.41	4.14	6.01	15.55	Yes	13	7,2
APTK	*Gyrocarpus americanus* Jacq.	Nat	91	3.23	17.11	13.74	34.07	Yes	23	6,5
APTK	*Commiphora lamii* H. Perrier	End	57	3.23	10.71	14.27	28.21	Yes	14	11,9
APTK	*Euphorbia fiherenensis* Poiss.	End	62	3.23	11.65	10.87	25.75	Yes	22	6,63
APTK	*Croton geayi* Leandri	End	51	3.23	9.59	12.02	24.83	Yes	3	
APTK	*Cedrelopsis gracilis* J.-F. Leroy	End	41	3.23	7.71	10.92	21.85	Yes	12	8
VHT	*Euphorbia antso* Denis	End	72	9.09	32.88	38.04	80.01	Yes	55	7,2
VHT	*Croton geayi* Leandri	End	21	6.06	9.59	9.01	24.66	Yes	19	6,68
VHT	*Croton salviformis* Baill.	End	18	9.09	8.22	6.31	23.62	Yes	14	5.2
VHT	*Terminalia ulexoides* H. Perrier	End	16	9.09	7.31	6.48	22.88	Yes	15	5,9
VHT	*Ehretia cymosa* Thonn.	Exo	13	3.03	5.94	5.44	14.40	Yes	12	5

Ambatofaly (ABF), Ampitolova (APL), Ampotaka (APTK), and Vohitsara (VHT). Within each site, the list was arranged in decreasing order of IVI. Den: Density of each species; Dis: Distribution, End: Endemic, Exo: Exotic, Ind: Indigenous, Uni: Unidentified, IVI: Importance Value Index, RA: Relative density; RD: Relative dominance; RF: Relative frequency; Php: Phorophyte status; PhpS: Number of individual phorophytes; AvV: Average number of aphyllous vanilla individuals per phorophyte.

hosted vanilla plants, with an average of 7.2 ± 3.5 individuals per tree, but did not differ significantly from the other phorophytes. No significant correlation was observed between the number of aphyllous vanilla individuals and phorophyte diameter ($r = 0.2$; $p > 0.05$). However, a significant positive correlation was observed between the tree species abundance and their role as phorophytes ($r = 0.7$, $p < 0.01$).

The degraded forest in APL exhibited 17 plant families, 24 genera, and 24 species, including one unidentified species. Among the species, two were introduced, seven were indigenous, and 14 were endemic. Four endemic species were common to the neighboring ABF-sacred forest. The Sarcolaenaceae family was the most represented (21.4%), with three species, followed by the Rubiaceae (20.4%) (Table S3). The most dominant species at the APL site was *Ravenala*

agatheae Haevermans & Razanatsoa (IVI = 84.35), followed by *Schizolaena parviflora* (F. Gérard) H. Perrier (IVI = 30.75) and *Gaertnera arenaria* Boulanger (IVI = 26.33) (Table 4). A total of 1958 *V. madagascariensis* and 23 phorophyte species were recorded, representing 95.8% of the potential phorophytes in the APL. Endemic species represented 14 of the 23 phorophyte species, with *Schizolaena parviflora* being the most frequent (15.8%), followed by *Gaertnera arenaria* (14.6%). Analysis of the frequency distribution of these phorophytes showed a significant difference (χ^2 = 308.32; df = 22; $p < 0.001$), highlighting a pronounced preference for these two species for aphyllous vanillas over other phorophytes. APL differed from the other three sites in having a high number of vanillas per host tree, with an average of 7.7 ± 7.2 individuals. This was illustrated by the species *Ravenala agatheae,* which harbored an average of 17.5 ± 11.5 vanilla individuals per tree. A significant positive correlation was found between the number of aphyllous vanilla individuals and the diameter of their phorophytes (r = 0.7; $p < 0.001$), and between the density of a tree species and its role as a phorophyte (r = 0.9; $p < 0.001$).

The APTK forest comprised 46 tree and shrub species from 39 genera and 28 families, including 41 endemic, four indigenous, and one introduced species. The Euphorbiaceae family was the most prevalent, representing 24.8% of the recorded species. *Gyrocarpus americanus* Jacq., *Commiphora lamii* H. Perrier, and *Euphorbia fiherenensis* Poiss. were the most dominant species, with IVI values of 34.07, 28.21, and 25.75, respectively (Table 4). A total of 1160 individuals of *V. decaryana* individuals hosted by 35 different phorophyte species were recorded (Table 2). According to these findings, aphyllous vanilla was hosted by 76% of the tree species found in APTK, with endemic trees dominating the landscape. Of the 35 phorophyte species, 28 were endemic, two were indigenous, and one was introduced. The remaining four species were not identified in this study. The most common phorophyte for vanilla was *Securinega seyrigii* Leandri (14.9%), followed by *Gyrocarpus americanus* (13.2%). These species exhibited significantly higher affinity for vanillas (χ^2 = 310.13; df = 33; $p < 0.001$). Analysis of the number of vanillas hosted by phorophytes indicated an average of 6.6 ± 4.6 vanillas per host tree. *Commiphora lamii* hosted an elevated number of vanillas, with an average of 12.1 ± 9.2 per tree. The analysis revealed significant positive correlations between the number of aphyllous vanilla individuals and phorophyte diameter (r = 0.6; $p < 0.001$) as well as between the abundance of a tree species and its role as a phorophyte (r = 0.8; $p < 0.001$).

The VHT vegetation included ten families, 15 genera, and 17 tree species: 15 endemic, one indigenous, and one introduced. Euphorbiaceae constituted 50.7% of the recorded families. *Euphorbia antso* Denis was the dominant species (IVI = 80) followed by *Croton geayi* Leandri (IVI = 24.66). Of the endemic species, eight were common to the APTK sacred forest. Vanilla recorded at the VHT revealed

1176 individuals of *V. decaryana*, with the majority being associated with *Euphorbia antso*, accounting for 31.3% of the phorophytes, followed by *Croton geayi* at 10.8%. Analysis of the frequency distribution of these phorophytes showed a significant difference (χ^2 = 244.94; df = 16; $p < 0.001$), underscoring the distinct preference of *Euphorbia antso* for aphyllous vanilla over alternative phorophytes. On average, each phorophyte hosted 6.6 ± 4.3 of the vanilla individuals, and no species exhibited a significantly higher number of vanilla individuals compared to others. Significant positive correlations were found between the number of aphyllous vanilla individuals and the diameter of the phorophytes (r = 0.6; $p < 0.001$), and between the abundance of a tree species and its role as a phorophyte (r = 0.9; $p < 0.001$).

Genetic Diversity of Aphyllous Vanillas

The results of STRUCTURE analysis for species determination in studied sites revealed that individuals sampled from ABF and APL sites belong to the species *V. madagascariensis*, while those from APTK and VHT sites belong to the species *V. decaryana* (Figure S3). Clonal individuals were found exclusively in southern populations of *V. decaryana*. In total, 16 clones were identified, with six belonging to the VHT population (G/N = 0.52) and ten to the APTK population (G/N = 0.78) (Table 5), which were distributed across five distinct multilocus genotypes (MLGs). Our Psex analysis (*Psex* < 0.001) confirmed that all MLGs originated from vegetative reproduction and we observed no common MLGs between the different populations. Following the elimination of clones, we identified 80 unique 'genets' out of the 96 examined samples. We identified 97 alleles across seven loci, with the number of alleles per locus ranging from seven to 18 (Table S1). The average number of alleles per locus varied between 4.5 to 7.5, depending on the population (Table 5). Allelic diversity was slightly lower in the conserved forests than in the degraded forests, with an average of 6.8 and 4.5 alleles per locus compared to 7.5 and 5 alleles per locus in the degraded forests, with *V. madagascariensis* and *V. decaryana*, respectively (Table 5). Additionally, the degraded forests in the north and south exhibited a higher number of private alleles (1.7 for APL and 1.8 for VHT) than the conserved forests, with 1.2 for ABF and 1.4 for APTK, respectively (Table 5). The multilocus exact test indicated significant deviations from the Hardy-Weinberg equilibrium ($p < 0.05$) for each population (Table 5). Nei heterozygosity (H_E) values showed relatively high genetic diversity ($H_E >$ 0.6) in all four populations (Table 5). When comparing the two northern sites, the expected heterozygosity (H_E) of the degraded forest (H_E APL = 0.72) was higher than that of the conserved forest (H_E ABF = 0.63). Conversely, for the two southern sites populated by *V. decaryana*, the expected heterozygosity was higher at the conserved forest (APTK), with a value of H_E = 0.65, whereas the degraded forest (VHT) had a slightly lower value of H_E = 0.62. All four populations

Table 5. Summary of genetic diversity at seven microsatellite loci in four populations (Ambatofaly, Ampitolova, Ampotaka, and Vohitsara) of aphyllous *Vanilla* species from Madagascar.

POP	Species	N	G	G/N	Na	Pa	H_O	H_E	F_{IS}	HWE	Ne [95%CI]
ABF	*V. madagascariensis*	23	23	1	6.8 ± 0.8	1.2 ± 0.4	0.73	0.63	- 0.1724	**	6.9 [3 - 13.3]
APL	*V. madagascariensis*	23	23	1	7.5 ± 0.9	1.7 ± 0.4	0.85	0.72	- 0.1721	***	9.3 [5.7 - 15.2]
APTK	*V. decaryana*	21	11	0.52	4.5 ± 0.7	1.4 ± 0.5	0.85	0.65	- 0.3187	***	6.5 [2.1 - 47]
VHT	*V. decaryana*	23	18	0.78	5 ± 0.9	1.8 ± 0.9	0.90	0.63	- 0.4356	***	12.1 [3.2 - 36.6]

N: number of individuals sampled (ramet), G: number of unique genotypes (Genet), Na: mean number of alleles per locus, Pa: mean number of private alleles per locus, H_O: observed heterozygosity for all loci, H_E: expected heterozygosity for all loci, F_{IS}: fixation index, HWE: results of deviation tests from Hardy-Weinberg, Ne: effective population (LD method) with 95% confidence interval (CI) for allelic frequency threshold of 0.05, obtained from JackKnife method.

had H_O values above H_E, resulting in negative F_{IS} values, indicating an excess of heterozygotes. Heterozygote excess was more significant in *V. decaryana* populations but also in degraded versus conserved forests (Table 5). The ABF population exhibited a significant excess of heterozygotes at only one locus, whereas the APL and APTK populations exhibited a significant excess at five of the seven loci (Table S4). In the VHT population, all studied loci showed a significant excess of heterozygotes (Table S4). In this study, we observed effective population size (Ne) values below N, indicating the accuracy of the estimates obtained, as suggested by England et al.(2006). The aphyllous vanilla populations yielded very low Ne values, suggesting an effective population size of < 13 individuals. Moreover, populations in degraded forests exhibited higher Ne values than those in sacred forests (Table 5). When evaluating possible linkage disequilibrium among the 21 loci combinations, significant disequilibrium ($p < 0.05$) was observed for nine loci pairs in the 'genets' file and 11 loci pairs in the 'ramets' file. However, linkage disequilibrium was not due to physical linkage between loci, as previous studies have demonstrated that all loci are independent from each other (Andriamihaja et al., 2021; Gigant, De Bruyn, et al., 2016a; Gigant, Rakotomanga, et al., 2016b).

Genetic Differentiation Between Aphyllous Vanilla Populations

Bayesian assignment analysis using STRUCTURE showed a distribution of ΔK values, with a maximum for K = 2, indicating the presence of two distinct genetic clusters (Figure 4). Populations of *V. madagascariensis* in the north (ABF and APL) and *V. decaryana* in the south (APTK and VHT) exhibited genetic homogeneity in terms of their structure. However, analysis of genetic differentiation based on F_{ST} revealed significant differences ($p < 0.001$) between the four populations (Table 6). Consistent with the STRUCTURE results, a strong differentiation was observed between the populations of *V. madagascariensis* and *V. decaryana*, with F_{ST} values ranging from 0.24 0.32. However, a slight yet significant differentiation was noted between populations of *V. madagascariensis* growing in conserved

and degraded humid forests (F_{ST} = 0.04 between ABF and APL) as well as between the populations *V. decaryana* growing in conserved and degraded dry forests (F_{ST} = 0.07 between APTK and VHT).

Discussion

Forest Degradation Alters Tree Communities and Dominant Species

Our findings confirmed a decrease in woody species richness due to prolonged forest degradation. This decline in tree numbers significantly affects forest structure, leading to biodiversity loss and altering ecological dynamics by changing habitats, resource competition, and species interactions (Arroyo-Rodríguez et al., 2017; Lohbeck et al., 2015). Endemic species are highly prevalent in floristic studies, which is unsurprising given the high flora endemism rate of Madagascar (Binggeli, 2003; Goodman, 2022; Ralimanana et al., 2022). Despite degradation at the APL and VHT sites, exotic and shrub species remained low. However, habitat degradation significantly alters floristic composition. For instance, *Ravenala agatheae*, common in degraded forests, dominates the APL (Irwin et al., 2010; Randrianasolo et al., 2019). Continuous pressure, such as firewood and timber collection by riparian communities and gemstone mining, likely explains the low number of common species between the conserved and degraded forests.

Larger and Most Abundant Tree Species are the Preferred Phorophytes

We documented the total number of phorophyte species corresponding to 70% of the tree and shrub flora in the study area. Host specificity for epiphytic orchids has been widely discussed (Huang et al., 2018; Otero et al., 2007; Zarate-García et al., 2020), and our study revealed that some phorophyte species show significant affinities with aphyllous vanilla. Specifically, *Securinega seyrigii* and *Commiphora lamii* at the APTK site, *Schizolaena parviflora* and *Ravenala agatheae* at the APL site, and *Euphorbia antso* at the VHT site exhibited strong connections with vanilla. Surprisingly,
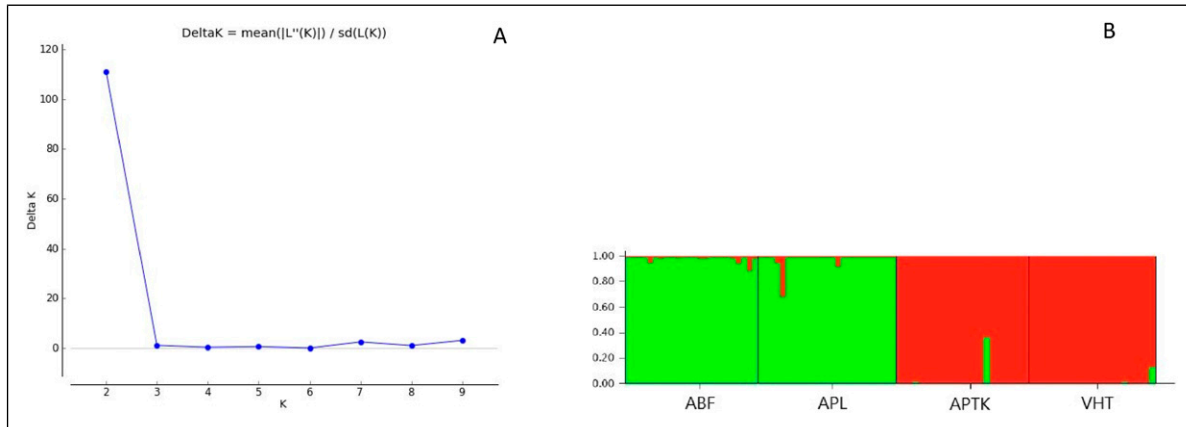
Figure 4. Genetic structuring of the four aphyllous vanilla populations. (A) DeltaK value (max K= 2) obtained by Structure Analyses (https://pophelper.com/). (B) Genetic assignment plot of individuals evaluated by the STRUCTURE program (K = 2). Each individual is represented by a vertical bar partitioned into segments of K colors, which represent the probability of the individual belonging to the cluster of this color.

Table 6. Results of genetic differentiation analyses (F_{ST} values) between four populations (Ambatofaly, Ampitolova, Ampotaka, and Vohitsara) of aphyllous *Vanilla* species from Madagascar.

Pop	ABF	APL	APTK
APL	0.0413 ***		
APTK	0.2525 ***	0.3236 ***	
VHT	0.2436 ***	0.3110 ***	0.0697 ***

p-value for each population pair across all loci (Fisher's method) $p < 0.001$, ***.

these phorophyte species were the most ecologically significant at the study sites. Because of their prevalence, these species play key ecological roles, including carbon storage (Måren & Sharma, 2021; Speak et al., 2020), and influence hydrological regimes, nutrient cycles, and critical ecosystem processes (Lindenmayer & Laurance, 2017). Among the three phorophyte species significantly associated with vanilla in the south, only *S. seyrigii* was commonly used for charcoal production, whereas *C. lamii* and *E. antso* were not (Ranaivoson et al., 2017). Similarly, *R. agathea* is a tree species that remains unutilized for charcoal production, thus safeguarding it from deforestation. Moreover, our observations revealed that, in degraded forests, phorophytes represented 95% of both shrubs and large tree species. These findings suggest that vanillas can colonize a diverse range of available tree species. Except for the ABF site, where the number of vanilla individuals was low and most phorophytes had large diameters, the other three sites displayed significant correlations between the phorophyte diameter and the number of aphyllous vanilla individuals they hosted. Larger phorophytes were able to support a greater number of vanilla individuals, which explains the high density of vanilla observed on *Ravenala agatheae* at APL. These results reflect those of Shen et al. (2022), who suggested that large trees offer more surface than small trees and have been available

for a longer time than younger hosts for epiphyte colonization. This also accords with several studies that reported root climbers' ability to colonize phorophytes of different sizes (Botomanga et al., 2024; Mori et al., 2016; Orihuela & Waechter, 2010). Interestingly, most phorophyte species hosted more than one individual vanilla. However, this outcome is contrary to those of several authors (Orihuela & Waechter, 2010; Visser et al., 2018), who found that isolated occurrence was by far the most common. It is possible that the newly established aphyllous vanilla on phorophytes tended to propagate rapidly via ramification before splitting into different individuals. Another explanation is that degraded remnant forests are less densely populated, which increases the chance of a tree becoming a phorophyte of several vanilla individuals. The phorophyte preference of aphyllous vanilla plants may have evolved with the ongoing degradation of forests in Madagascar. The scarcity of large trees in secondary vegetation limits phorophyte options for smaller trees. However, owing to the high plasticity of their attachment roots, aphyllous vanilla plants can effectively colonize these smaller trees (Botomanga et al., 2024).

Canopy Openness is Associated With Increasing Aphyllous Vanilla Species Colonization

Except for the ABF sacred forest, which had a semi-open canopy, all other forests exhibited a moderately open stratum. These sites had a significantly higher number of vanillas, indicating that canopy openness positively influences the propagation of aphyllous vanillas. These findings align with existing knowledge that forest gaps and low tree density promote the establishment of heliophilous species and the proliferation of lianas (He et al., 2023; Schnitzer et al., 2021). The closed canopy of the ABF forest resulted in a lower *density of V. madagascariensis*, thereby reducing the number of phorophytes at this site. However, considering the "sparse

and deciduous" nature of southern Madagascar's dry forests (Seddon & Tobias, 2007), the increase in light due to gaps in the degraded forests of VHT likely led to only a marginal increase in light intensity compared to the conserved APTK forest. This may explain the similar numbers of *V. decaryana* individuals recorded in both the conserved and degraded forests in the south.

Aphyllous Vanilla Populations Displayed High Genetic Diversity and an Excess of Heterozygotes

The results of our structural analysis indicated that vanilla populations in the north correspond to *V. madagascariensis*, whereas those in the south belong to *V. decaryana*. These findings corroborate those of the earlier studies conducted by Andriamihaja et al. (2021, 2022). Information on species reproductive patterns and the level of habitat degradation is crucial for understanding the genetic structure of populations and their evolutionary trends. Our results indicate a high genetic diversity of aphyllous *Vanilla* species at all studied sites ($H_E > 0.6$). This high diversity corroborates the results reported by Andriamihaja et al. (2021) for 22 locations where aphyllous *Vanilla* species are present, with an H_E values that can exceed 0.8. However, in contrast to our results for the northern sites, conserved forests, mainly corresponding to national parks, tended to have higher H_E values, which also appears to be the case for the southern sites investigated in our study. Nevertheless, it is important to note that Andriamihaja et al. (2021) did not examine the degree of openness of these sites, and tree density in protected forests can also be highly variable, with some being sparser than others. In addition, the ABF sacred forest has a total area of only 5 ha, much smaller than the national park average. Forest fragment size can have a dramatic bottleneck effect on orchid species diversity (Minasiewicz et al., 2018; Yamashita et al., 2023).

Our study found that all vanilla populations had negative F_{IS} results, suggesting an excess of heterozygotes. One possible explanation for this is the small size of the breeding population (Balloux, 2004; Templeton, 2018). Another explanation is the theory of overdominance, which suggests that selection against recessive alleles increases the frequency of heterozygotes (Hedrick et al., 2016; Mérot et al., 2020; Schöfl et al., 2018). Negative F_{IS} values can also occur due to negative assortative mating between individuals carrying different alleles (Fishman & Sweigart, 2018; Glémin et al., 2001; Schoen & Baldwin, 2023). Another plausible explanation for negative F_{IS} is asexual reproduction, which does not involve mixing of the DNA of the two parents, as in sexual reproduction (Arnaud-Haond et al., 2020; Probowati et al., 2023; Stoeckel & Masson, 2014). However, our results revealed a frequently observed paradox in which high genotypic diversity coexists with a significant excess of heterozygotes (Dia et al., 2014; Orantes et al., 2012). These two observations appear contradictory; high genotypic diversity

suggests active sexual reproduction, whereas an excess of heterozygotes is generally associated with near-strict clonality. Stoeckel et al. (2021a) suggested that this apparent contradiction could be due to the strong prevalence of clonal reproduction in partially clonal organisms, which may explain the observed duality. Lack of genetic recombination can increase mutational heterozygosity over generations, with each new mutation immediately inherited by all descendants (Arnaud-Haond et al., 2020; Stoeckel, Arnaud-Haond, et al., 2021a; Stoeckel, Porro, et al., 2021b). Our results are consistent with those of Stoeckel et al. (2006, 2021b), who observed an excess of heterozygosity, but noted strong variations in F_{IS} values between loci and populations. Given the reproductive modes of aphyllous *Vanilla* species and the presence of genome-wide linkage disequilibria, we strongly suspect that the negative F_{IS} observed in the examined populations indicates the asexually mediated propagation of aphyllous *Vanilla* species.

The vanilla populations in the southern region exhibited a significantly greater proportion of heterozygotes than those in the north, which is supported by the more significant *P* values obtained in the HWE H1 test for excess heterozygosity (Table S5). For the southern populations, the (G/N) index was less than 1, the observation of linkage disequilibrium between the pairs of loci, and the high negative value of F_{IS} suggested a strong clonal structure in the populations (Halkett et al., 2005; Stoeckel, Porro, et al., 2021b; Stoeckel & Masson, 2014). Indeed, ten of the 21 APTK individuals and six of the 23 VHT individuals were identified as clones. Consequently, the significant increase in heterozygosity in the southern populations is due to this unusually high rate of asexuality. In support of this hypothesis, our results regarding the regeneration of aphyllous vanillas revealed the complete absence of new recruitments within populations in southern Madagascar (Botomanga A., unpublished data). Moreover, the HWE H1 test for excess heterozygosity was always more significant in degraded forests than in the corresponding conserved forests (non-significant for ABF) (Table S5), suggesting increased asexual reproduction in degraded forests. We observed significant and non-significant variations in F_{IS} values across loci in the APL and APTK populations (Table S4), which also suggested the occurrence of infrequent sexual reproduction events in these populations (Balloux et al., 2003; Halkett et al., 2005; Stoeckel, Porro, et al., 2021b; Stoeckel & Masson, 2014). Altogether, these results confirm that canopy openness promotes aphyllous *Vanilla* species colonization via asexual reproduction

Forest Degradation Leads to Slight Differentiation of Aphyllous Vanilla Species Populations

The genetic differentiation between populations from the conserved and degraded forests, as measured by a F_{ST} value of 0.04 for *V. madagascariensis*, was comparable to those

reported by Andriamihaja et al. (2021) across four localities (Ambanja, Ankarana, Analamerana, and Maromandia) with F_{ST} values ranging from 0.01 to 0.04. Similarly, the genetic differentiation between populations of *V. decaryana* from conserved and degraded forests (F_{ST} = 0.7) aligned with F_{ST} values from three localities (Anja, Antandroka, and Kirindy Mitea) reported by Andriamihaja et al. (2021). The significant pairwise genetic differentiation detected between the populations of the conserved and degraded forests in our study might be attributed to the loss of natural habitats in Madagascar over the decades, which may have resulted in the erosion of genetic variability linked to changes in the vanilla reproductive system (Fajardo et al., 2017; Jump & Peñuelas, 2006; Li et al., 2020; Pandey et al., 2015). This translates into the increased vegetative propagation of aphyllous vanilla plants and a reduction in gene flow rates between populations, ultimately leading to population differentiation. Previous studies have shown that the effects of habitat degradation on plant genetic diversity are discernible only after 50–100 years of environmental fragmentation (Aguilar et al., 2008). The sacredness of the two conserved forests fell within this period, which could explain the subtle distinctions between the vanilla populations in the sacred and degraded forests. However, it is possible that the number of generations owing to habitat fragmentation and degradation is insufficient to generate high values for genetic differentiation (Ehlers & Pedersen, 2000).

Limitations

The shortcoming of this study lies in the insufficient number of true replicates for each species within a single bioclimatic zone. This might have hindered the statistical power of our findings. Nevertheless, we noticed a similar trend in the responses of the two unrelated *Vanilla* species to forest degradation, although they evolved in distinct bioclimatic zones.

Conclusion

Our results showed that aphyllous *Vanilla* species thrived best in areas with open canopies. Thus, conserved forests with closed canopies are not necessarily appropriate refugia. Forest degradation forces the growing vanillas to climb the remaining uncut trees and is accompanied by a reduction in genetic diversity that may originate from excessive clonality but also from a low recruitment rate. The lessons drawn from this study could be useful for the conservation of orchids that rely on phorophytes for their development.

Implications for Conservation

To prevent the erosion of genetic diversity and inbreeding, a population size (Ne) of over 100 individuals is crucial (Frankham et al., 2014; Hoban et al., 2020). Our study found that the effective population size (Ne) of the four aphyllous vanilla populations did not exceed 13 individuals. Thus, we recommend introducing new genotypes from the surrounding subpopulations to enhance their genetic diversity. Alternatively, replanting seedlings raised *in vitro* is an option to compensate for low *in situ* recruitment rates. Passive restoration, seeding, and mixed sources can significantly increase the genetic diversity of the restored plant populations (Bucharova et al., 2019; Wei et al., 2023). Nevertheless, restocking should be conducted cautiously to reduce the risk of outbreeding depression (Bucharova et al., 2019; Rogers et al., 2024) and prevent the introduction of genotypes that might promote invasiveness (Le Roux et al. 2007; Matesanz & Sultan, 2013). The inclusion of degraded forests in conservation areas could be a suitable option for forest management. In some degraded forest spots, halting wood harvesting may be beneficial for increasing the chance of a tree becoming a phorophyte. Additionally, stricter regulations on the wildcrafting of vanilla stems for trade should be enacted via the establishment of a quota system to ensure the sustainability of isolated populations.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability Statement

Data utilized to derive the findings in this study have been deposited in the Science Data Bank (ScienceDB: https://www.scidb.cn/en) for open access and availability: - Genetic data from four leafless vanilla populations. DOI: 10.57760/sciencedb.09416. - Ecological data from four leafless vanilla populations. DOI: 10.57760/sciencedb.09419

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Supplemental Material

Supplemental material for this article is available online.

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