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Genetic Variation and Evolutionary History of the Threatened *Dipterocarpus turbinatus* C.F.Gaertn. Detected Using Microsatellites

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Abstract: *Dipterocarpus turbinatus* C.F.Gaertn. is a valuable timber tree indigenous to the lowland tropical forests of southern and central Vietnam. It is in danger of extinction due to fragmented habitats and overexploitation. Therefore, assessing the genetic diversity and demographic history of this species is essential to providing a platform for conservation activities. Herein, we analyzed 281 *D. turbinatus* trees from 10 populations representing their natural distribution range in Vietnam using nine polymorphic microsatellites to provide valuable information for conservation activities. We detected genetic diversity within these populations (0.285 and 0.328 for observed and expected heterozygosity, respectively), as well as population genetic differentiation (a Wei and Cockerham value of 0.202 and a Hedrick value of 0.32). Clustering analysis based on different approaches revealed three genetic clusters which were significantly correlated to gene flow across the geographic distribution range of *D. turbinatus* in tropical evergreen forests. Approximate Bayesian computation suggested that the *D. turbinatus* populations had an evolutionary history consistent with divergence during the last glacial maximum. Based on our results, we recommend the in situ conservation of several populations with high genetic diversity, high allelic richness, or private alleles, and the gathering of the seeds of the remaining populations for ex situ conservation.

Keywords: demography; gene exchange; genetic structure; habitat disturbance; species conservation

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1. Introduction

Dipterocarps (Dipterocarpaceae) play a significant role in Asia, both from an ecological and an economic perspective. They can be used in a diverse range of applications, e.g., their wood can be used for plywoods, illumination, and waterproof baskets, boats, decking, and outdoor furniture [1–3], and their resin can be used for paints, lithographic inks, and an anti-corrosive coating compositions for iron [4,5]. More than 45 dipterocarp species from six different genera (*Hopea*, *Anisoptera*, *Vatica*, *Shorea*, *Parashorea*, and *Dipterocarpus*) have been identified in the tropical forests of Vietnam [5]. These dipterocarps have been subjected to overexploitation due to their commercial value. Furthermore, in recent years, the increased human pressure has resulted in a sharp reduction in forest areas and an increase in the

degree of fragmentation in the surviving forests, seriously altering dipterocarp habitats. As a consequence, 33 dipterocarp species are threatened at the global and national levels [6,7].

Dipterocarpus turbinatus C.F.Gaertn., a large evergreen tree that grows up to 40–50 m in height and 160 cm in diameter at breast height (Figure S1), is found in lowland tropical forests in southern and central Vietnam, including the provinces of Gia Lai, Kon Tum, Dak Lak, Tay Ninh, Dong Nai, Da Nang, and Thua Thien-Hue. It has bisexual flowers that are pollinated by insects. *D. turbinatus* trees flower between March and April, and their fruits mature and fall between May and June. The fruit consists of a single-seeded nut with a wing-like calyx. Their seeds are dispersed by wind and disseminated by water. *D. turbinatus* is an important species of tree known to be suitable for the construction of boats, bridges, houses, and furniture. The resin it produces is used in paints and varnishes. As a result, the adult trees of this dipterocarp are overexploited, and this has led to a decrease in their population size over the past few decades. Moreover, the habitat of *D. turbinatus* has been lost as a consequence of agricultural expansion. The remaining populations are severely fragmented and isolated in the surviving forests. These populations are often small in size and isolated by large geographical distances. Such populations consistently face an increased risk of inbreeding depression and decreased genetic variability, which can lead to increased vulnerability to environmental stochasticity [8,9]. *D. turbinatus* is classified as Vulnerable according to the IUCN Red List Categories [10] and the Vietnam Red Data Book [7].

The loss of genetic diversity within populations reduces evolutionary potential and is a consequence of small population sizes and isolated populations [11]. The evolutionary potential of a species in nature depends on its genetic diversity within and between populations. Gene flow within species may influence genetic differences and the evolution of population adaptation [12]. Identifying patterns of genetic variation within and between populations of threatened species can improve our understanding of the spatial distribution of genetic diversity and help establish effective strategies for species conservation, management, and restoration, and possibly lead to genetic improvement in the future.

DNA molecular markers have been used to study genetic diversity in plants in recent decades [13–16]. Due to their distribution across all genomes, their polymorphism, and their reproducibility [17], microsatellite markers, i.e., simple sequence repeats (SSRs), have been widely used to investigate genetic diversity, population structure, gene flow, and mating systems in plants [18,19]. For the purpose of species conservation and management [20–24], nuclear microsatellites have been used in studies of genetic diversity focusing on certain dipterocarp species, such as *Dryobalanops lanceolata* in Sarawak, Malaysia [25], *Shorea curtisii* in Sarangor, Malaysia [26], and *Dipterocarpus tempehes* in Sarawak, Malaysia [27]. The exploration of genetic variability within and among populations of *D. turbinatus* is of crucial importance for species conservation, management, and restoration. To date, the genetic variability of this species is still unknown. Therefore, the objective of the present study is to evaluate genetic diversity within and among *D. turbinatus* populations using a set of nuclear SSRs as genetic markers, and to provide guidelines for the conservation of this endangered species in Vietnam.

2. Materials and Methods

2.1. Sample Collection

The inner bark of adult trees was collected from 10 natural populations of *D. turbinatus* representing the range of its geographical distribution in Vietnam. Two of the populations are situated in both the province of Dong Nai and the province of Dak Lak, and the remaining populations are each located within a single province (Gia Lai, Kon Tum, Tay Ninh, Binh Phuoc, Da Nang, and Thua Thien-Hue) (Table S1 and Figure 1). A total of 281 trees were randomly sampled and preserved in the field in labeled plastic bags that contained silica gel before being transferred to the Genome Laboratory at the Institute of Genome Research, where they were stored at −30 °C until DNA extraction. To avoid sampling clonal material, the samples were collected from transects of about 2.5–3 km, and

the distance between two consecutively collected trees within a population was always at least 50 m. The collection sites were recorded using a global position system (GPS) receiver.

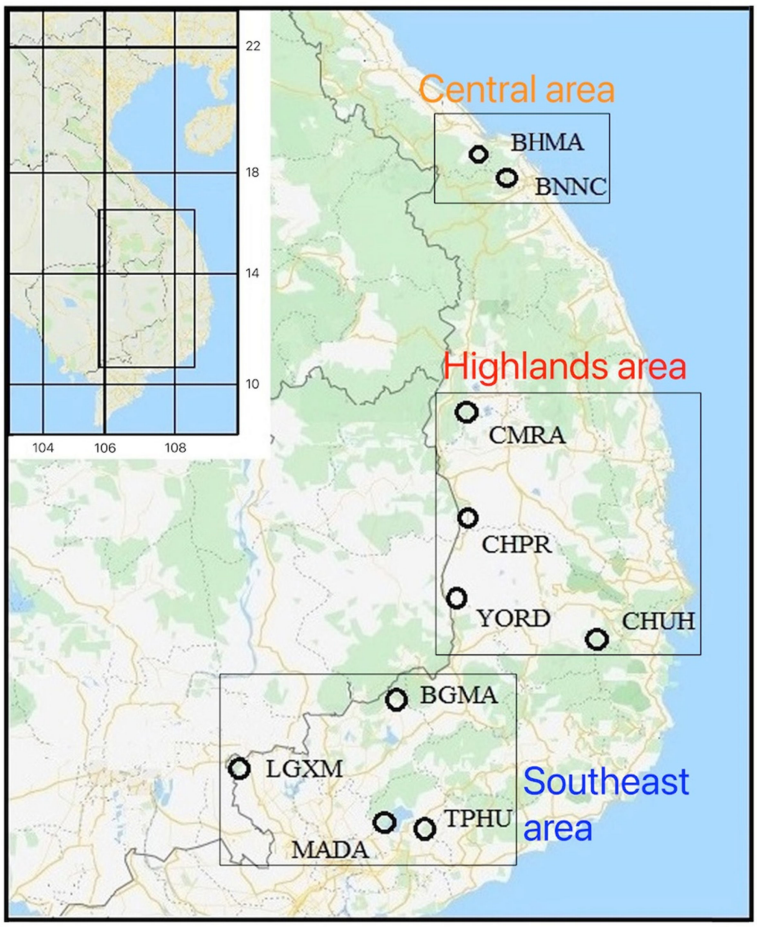

Figure 1. The *Dipterocarpus turbinatus* study sites. BHMA, BNNC, CMRA, CHPR, CHUH, YORD, BGMA, LGXM, MADA, and TPHU are the population names.

2.2. DNA Extraction and Polymerase Chain Reaction

Genomic DNA was isolated from the samples using the CTAB method described by Doyle and Doyle (1990) [28]. About 100 mg of each sample was ground in liquid nitrogen using a Mixer Mill MM 400 (Retsch, Haan, Germany). The total amount of DNA was checked via electrophoresis on 1.2% agarose gel, as well as via spectrophotometer using a NanoDrop 2000C (Thermo Scientific, Waltham, MA, USA), and it was then diluted to a concentration of 10 ng/µL and stored at −20 °C for microsatellite (SSR) analysis.

In order to investigate the genetic diversity and population structures of the *D. turbinatus* specimens, we tested 10 SSRs developed from *Dipterocarpus tempehes* [27] and 9 SSRs developed from *Shorea curtisii* [26]. Nine of these SSRs (Table S2) were selected and used for polymerase chain reaction (PCR) amplification based on their PCR consistency and polymorphism. The reaction mixtures (25 µL volume) contained 10 ng template DNA, 1 × PCR buffer (10 mM Tris HCl, 50 mM KCl, pH 8.4), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1.25 U Taq DNA polymerase, and 10 pmol of each primer. PCR amplification was implemented in the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) as follows: an initial denaturing step at 94 °C for 5 min, 35 cycles at 94 °C for 1 min, an annealing step for each primer pair at 50–60 °C for 30 s with a 1 min extension at 72 °C, and a final extension at 72 °C for 10 min. The amplification products were determined using the Sequi-Gen®GT DNA electrophoresis system (BIO-RAD Co., Ltd., Hercules, CA, USA) of 7% polyacrylamide gels in 1 × TAE buffer and then visualized using GelRed™

Nucleic Acid Gel Stain. The PCR fragment sizes were detected using the GenoSens1850 Gel-Analyzer software (Clinx Science Instruments Co., Ltd., Shanghai, China) with a 25 bp DNA ladder (Invitrogen, Caelsbad, CA, USA).

2.3. Genetic Diversity and Population Structure Analysis

The presence of null alleles in the microsatellite data was determined using the program Micro-Checker v.2.2.3 [29]. The PIC (polymorphism information content) value for each locus was evaluated using Cervus [30]. We estimated the genetic diversity of the samples, including the average number of effective alleles (A_E) per locus, the number of private alleles (A_P), the percentage of polymorphic loci (*PPL*), the observed (H_O) and expected (H_E) heterozygosity, and the fixation index (F_{IS}—inbreeding coefficient) using the program GenAlEx v.6.5 [31]. This program was also used to evaluate the coefficient of total inbreeding (F_{IT}), gene flow (N_m), the Wei and Cockerham genetic differentiation index (F_{ST}) (1984) [32], and that of Hedrick (G'_{ST}) (2005) [33]. The average number of alleles (N_A) per locus and the average allelic richness (A_R) were evaluated using Fstat v.2.9.4 [34]. The inbreeding coefficients were corrected for null allele frequency based on the individual inbreeding model (IIM) using INEst [35]. The Hardy–Weinberg (HW) equilibrium for the heterozygote deficit and the linkage disequilibrium for each pairwise locus combination in each population were tested using Genepop v.4.6 [36]. Bottleneck events for each population were established by comparing the H_E related to the excess H_E relative to the expected equilibrium heterozygosity (H_{EQ}) using Bottleneck v.1.2 [37]. Three mutational/drift models—the infinite allele model (IAM), the stepwise mutation model (SMM), and the two-phased model of mutation (TPM)—were considered, with 70% single-step mutation and 10,000 replicates used for the TPM. Significance was evaluated using the one-tailed Wilcoxon signed rank test. An analysis of molecular variance (AMOVA) was performed to detect the genetic differentiation within and among the populations using Arlequin v.3.5 [38]. A Neighbor-joining tree based on the matrix of F_{ST} values was constructed using Poptree2 to display the genetic relationships among the populations [39]. A principal coordinate analysis (PCoA) based on the G'_{ST} values was also implemented using GenAlEx v.6.5. Finally, the adegenet package [40] was also applied using R 4.0.2 software to perform a discriminant analysis of principal components (DAPC). The number of clusters (K) was run from 1 to 10 to determine the optimal value of K.

2.4. Demographic History

Based on the results from the different clustering analyses (NJ tree, PCoA, and DAPC), all the studied *D. turbinatus* populations were assigned to one of three genetic groups corresponding to their geographical distribution area. Thus, three populations were defined. The CET population consisted of two populations (BNNC and BHMA) in the Central area, the HIL population consisted of four populations (CHPR, CMRA, CHUH, and YORD) in the Highlands area, and the SOE population included the remaining four populations (LGXM, MADA, BGMA, and TPHU), located in the Southeast area. To determine the population divergence history, an approximate Bayesian computation (ABC) method was applied using Diyabc v 2.1.0 [41]. Four demographic scenarios were considered for the three populations HIL, CET, and SOE (Figure 2). In scenario 1, the SOE population was derived from the HIL population at time $t1$, which was itself derived from the CET population at time $t2$. In scenario 2, the two populations CET and SOE were merged at time $t1$, and the HIL population at time $t2$. In scenario 3, the HIL population was assumed to originate from an admixture of the two populations SOE and CET at time $t1$. The rate of admixture of the CET and HIL populations was set as "ra", and that of the SOE and HIL populations was set as "1-ra". The CET population merged with the SOE population at time $t2$. Finally, in scenario 4, the three populations CET, HIL, and SOE merged simultaneously at time $t2$. The ancestral population (*NA*) was assumed to exist at time $t3$ in each scenario. No migration between populations was assumed in any of the scenarios. The prior values were used with a uniform distribution for all parameters (Table S3). The generalized stepwise

mutation model [42] and single nucleotide indels (SNI) were also implemented from a set of 10^4 simulations generated for each putative scenario. The posterior probabilities were calculated using a logistic regression approach with 10^6 simulations for each scenario. The goodness-of-fit of each scenario was assessed via a principal component analysis (PCA) performed using the "model checking" option in Diyabc.

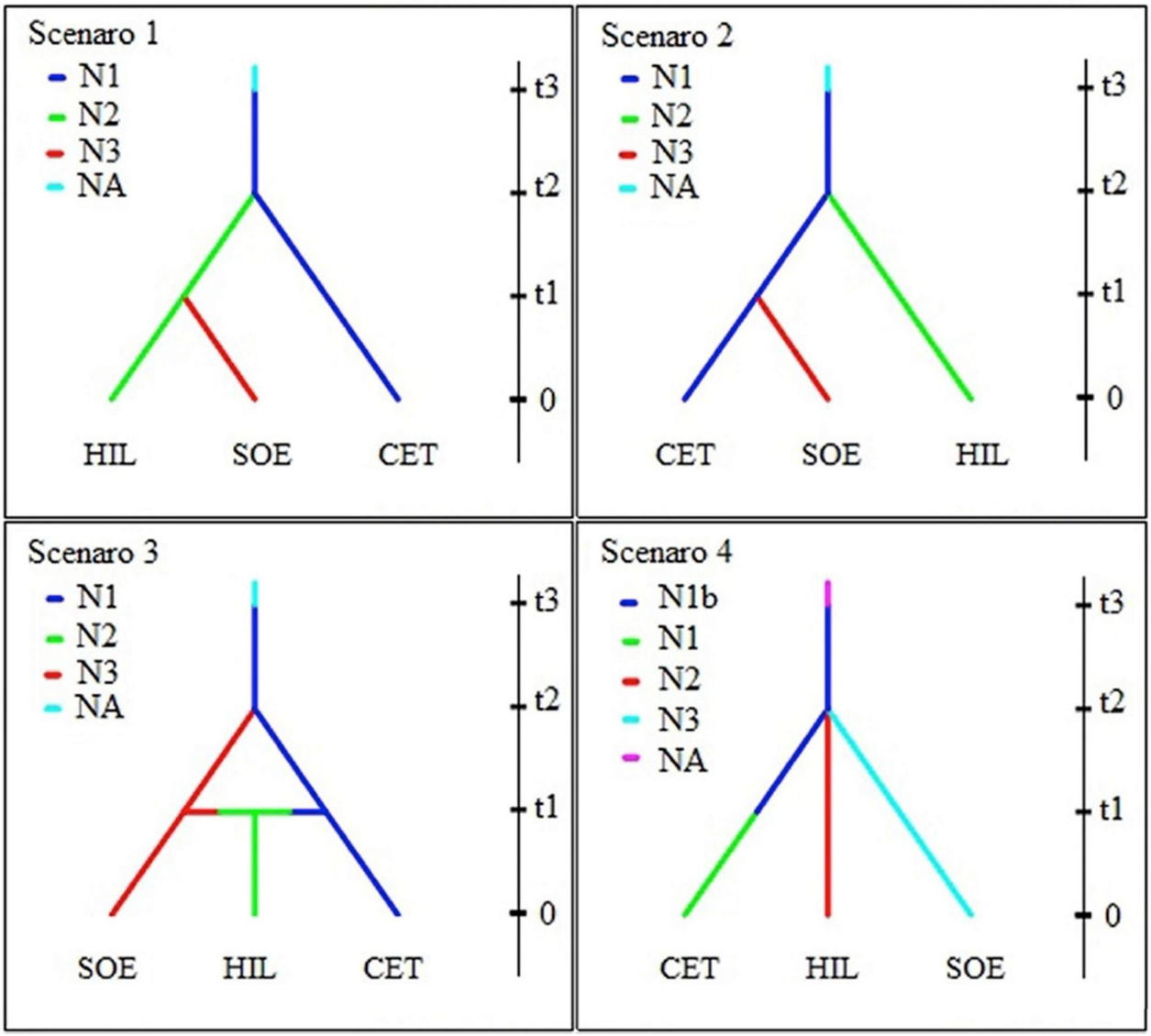

Figure 2. Four scenarios of population demography history examined in a Diyabc analysis of *D. turbinatus*. *t#* is the time scale measured in generations; N1–3 and NA are the effective population sizes of the populations CET, HIL, SOE, and the ancestral population, respectively.

3. Results

3.1. Genetic Diversity

A total of 29 different alleles were produced across 9 SSR loci from all 281 trees in the 10 natural populations. The BHMA population had the lowest number of alleles (18), and two populations CMRA and LGXM had the highest number of alleles (23). One allele was recorded at two loci (Shc01 and DT18P) in the BHMA population. Similarly, the highest number of alleles (4) was recorded at the Shc11 locus in the LGXM population and at the DT38 locus in the CHPR population. The most common alleles (allelic frequencies > 0.8) were found at two loci in three populations (CHUH, CMRA, and YORD) and in seven loci in the TPHU population. Further, all nine of the SSRs were polymorphic (Table 1). The lowest

number of alleles (N_A) was 2 (in the BHMA population), while the highest number of alleles was 2.6 (in the two populations CMRA and LGXM), making an average of 2.31. The effective alleles (A_E) averaged 1.5 (1.3–1.8). The allelic richness (A_R) averaged 2.29 (1.97–2.54). One private allele was found at Shc01 in the CMRA population and at DT38 in the YORD population. The mean observed heterozygosity (H_O) and the expected heterozygosity (H_E) were 0.285 and 0.328, respectively. The lowest heterozygosity was found in the BHMA population (H_O = 0.193 and H_E = 0.227), while the highest heterozygosity was observed in the CHUH population (H_O = 0.37 and H_E = 0.397). The fixation index (F_{IS}) varied from 0.043 in the BGMA population to 0.178 in the CMRA population (an average of 0.112), suggesting a mixed breeding system involving both inbreeding and outbreeding. However, the deficiency of the heterozygosity was significant ($p < 0.001$). The inbreeding coefficients were corrected for null alleles based on the individual inbreeding model (F_{IS}IIM) and ranged from 0.018 in the BNNC population to 0.298 in the CHPR population (an average of 0.075), which also showed the heterozygote deficits. However, the F_{IS}IIM value was lower than the inbreeding coefficient (F_{IS}). The inbreeding index calculated for the total populations (F_{IT}) varied from 0.025 at Shc11 to 0.492 at DT20, an average of 0.306 (Table S4), indicating an excess of homozygosity in the populations. Significant excess heterozygosity was detected in four populations (BNNC, CHUH, YORD, and MADA) in the two IAM and TPM models ($p < 0.5$ and $p < 0.01$), whereas the SMM model did not show significant excess heterozygosity in any of the studied populations (Table 1). These results do not indicate any signs of a recent bottleneck in any of the *D. turbinatus* populations.

Table 1. Genetic diversity values and results of bottleneck tests for 10 *D. turbinatus* populations.

Population	N	PPL (%)	N_A	A_E	A_R	H_O (SE)	H_E (SE)	F_{IS} (SE)	F_{IS} IIM	*p* Value of Bottleneck		
										IAM	TPM	SMM
BNNC	21	100	2.1	1.6	2.1	0.317	0.355	0.096 *	0.018	0.007 *	0.01 *	0.125
BHMA	27	77.8	2.0	1.3	1.97	0.193	0.227	0.126 **	0.022	0.234	0.469	0.766
CHUH	30	100	2.4	1.7	2.4	0.370	0.397	0.073 *	0.031	0.002 **	0.018 *	0.150
CHPR	29	100	2.4	1.5	2.4	0.280	0.318	0.102 ***	0.298	0.285	0.41	0.674
CMRA	28	100	2.6	1.7	2.52	0.313	0.392	0.178 ***	0.048	0.010 *	0.125	0.367
YORD	30	100	2.3	1.7	2.3	0.330	0.383	0.122 *	0.049	0.007 **	0.018	0.125
BGMA	32	100	2.4	1.4	2.4	0.240	0.260	0.043	0.136	0.545	0.820	0.898
LGXM	24	100	2.6	1.8	2.54	0.306	0.386	0.144 ***	0.049	0.01 *	0.125	0.213
MADA	29	100	2.1	1.5	2.1	0.303	0.320	0.108 **	0.035	0.018 *	0.082	0.150
TPHU	31	100	2.2	1.3	2.14	0.197	0.246	0.128 *	0.068	0.326	0.787	0.898
Mean		97.8	2.31	1.5	2.29	0.285 (0.012)	0.328 (0.014)	0.112 *** (0.021)	0.075			

Notes: N: sample size; PPL: percentage of polymorphic loci per population; N_A: mean number of alleles per locus; A_E: mean effective alleles; A_R: mean allelic richness; H_O and H_E: observed and expected heterozygosity; F_{IS}: fixation index (Wright's inbreeding coefficient); F_{IS}IIM: corrected inbreeding coefficient for null alleles; IAM: heterozygosity excess (one-tailed test); TPM: heterozygosity excess (one-tailed test); SMM: heterozygosity excess (one-tailed test); SE: standard error. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

At the species level, the alleles (N_A) averaged 3.2, ranging from 2 at three loci to 5 at one locus (Table S4). The averaged allelic richness (A_R) was 2.9 and ranged from 2 at three loci to 4.3 at one locus. The effective allele (A_E) mean was 1.5 and ranged from 1.3 to 1.7. The average observed heterozygosity (H_O) was 0.285 (0.196–0.331), whereas the average expected heterozygosity (H_E) was 0.328 (0.225–0.411). The mean H_E was higher than that of H_O at all the studied loci except DT20. The average fixation index (F_{IS}) was 0.125 across all the populations. Eight of the studied loci had a positive fixation index, indicating heterozygous deficits and inbreeding. Of these, seven loci showed significant inbreeding ($p < 0.05$). The negative value of the remaining locus was not significant. Based on the Micro-Checker analysis, null alleles were found at six of the studied loci (but not at the remaining three, DT20, Shc07, and DT10) at a p value of 0.05 (Table S4). Tests for

genotype linkage disequilibrium between all the loci pairs in each population determined that 89 out of the 360 loci pairs indicated significant deviation ($p < 0.05$).

3.2. Genetic Differentiation and Population Structure

The averaged global genetic differentiation of the loci (F_{ST}) was 0.202 (0.1–0.556), $p = 0.001$, whereas the mean G'_{ST} value was 0.32 (0.118–0.728), $p = 0.001$ (Table S5). Low genetic differentiation was observed for population pairs in the same area, and high genetic differentiation was observed for population pairs in different areas. Our results also detected high gene flow (N_m) for population pairs within each area (an average of 6.427), and low levels of gene flow were found between populations in different areas (an average of 1.257). The lowest genetic differentiation ($F_{ST} = 0.018$ and $G'_{ST} = 0.02$) and the highest gene flow ($N_m = 13.639$) were found for the population pair of YORD/CHUH in the Highlands area, and the highest genetic differentiation ($F_{ST} = 0.275$ and $G'_{ST} = 0.505$) and the lowest gene flow ($N_m = 0.762$) were detected between the YORD (Highlands area) and BHMA (Southeast area) populations. Significant genetic differentiation (F_{ST} values) was found between each of the populations ($p < 0.01$) except the CHPR/CMRA and CHUH/YORD population pairs ($p > 0.05$). The AMOVA analysis revealed that most of the genetic variation was found within individual trees (62.24%). The variance was found between different areas (21.52%). The lowest variance was observed between populations within an area (5.38%). The molecular variance among the *D. turbinatus* populations was significant ($p < 0.0001$, Table 2). The average N_m value between the populations was 1.922. Clustering analyses, NJ, and PCoA were used to analyze the genetic relationships between the 10 populations (Figure 3). Our results determined that the 10 populations were divided into 3 clusters. The first group consisted of two populations in the Central area. The second group included the four populations in the Southeast area. The four populations in the Highlands area formed the third cluster.

Table 2. Analysis of molecular variance from the 10 *D. turbinatus* populations.

	df	Sum of Squares	Variance Components	Total Variation (%)	*p* Value
Between populations	9	226.7	0.422	22.03	<0.001
Within populations	552	824.9	1.494	77.97	
Total	561	1051.6	1.916		

df: degree of freedom; *p* value: significance.

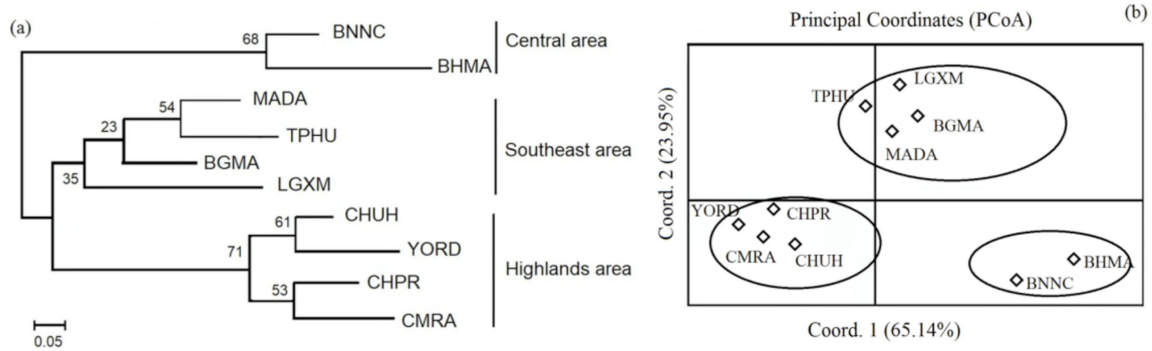

Figure 3. Genetic relationships between the 10 *D. turbinatus* populations based on the NJ tree made using the F_{ST} values received from Poptree2 (**a**) and the PCoA conducted using the G'_{ST} values received from GenAlEx (**b**). BHMA, BNNC, CMRA, CHPR, CHUH, YORD, BGMA, LGXM, MADA, and TPHU are the population names. PCoA: principal coordinate analysis; Coord.: coordinate.

These results indicate a close relationship between the populations. Populations from the same area were grouped together. We also analyzed the genetic structures of the 281 sampled *D. turbinatus* trees using the DAPC method. The DAPC without prior

information identified three genetic groups after retaining 18 principal components using the *xval* DAPC function (Figure 4B). The first discriminant function clearly distinguished the three groups (Figure 4A). Group I was composed of 85 trees, 79 of which were collected in the Southeast area, and 6 of which came from the Highlands area (Table S6, Figure S2). Group II included 123 trees, 110 of which were obtained from the Highlands area, 3 of which came from the Central area, and 10 of which came from the Southeast area. Group III included 45 trees from the Central area, 1 from the CHUH population (Highlands area), and 26 from the Southeast area. The DAPC with prior information detected genetic relationships between the *D. turbinatus* populations (Figure 4C). A high overlap of the populations was related to close geographic distances, indicating low genetic differentiation and high gene flow between these populations.

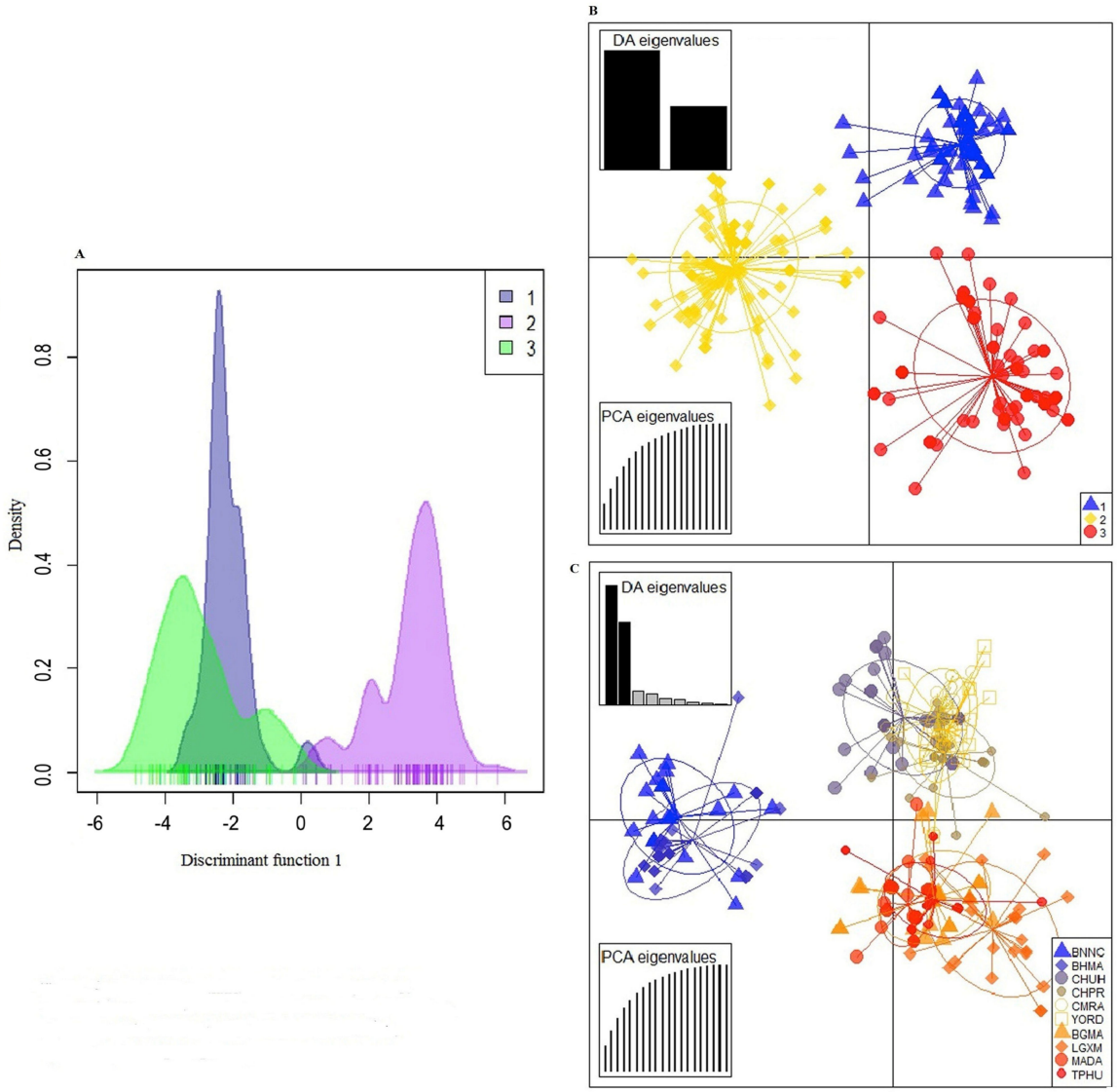

Figure 4. Analysis of population structures using DAPC. (**A**) Densities of individuals on the first discriminant function. (**B**) Scatterplot of DAPC without prior information. (**C**) Scatterplot of DAPC with prior information. PCA: principal component analysis; 1, 2 and 3: genetic clusters; DA: discriminant analysis; BHMA, BNNC, CMRA, CHPR, CHUH, YORD, BGMA, LGXM, MADA, and TPHU: population names.

3.3. Demographic History

Of the four tested scenarios, the best supported scenario in the Diyabc analysis of the *D. turbinatus* populations was scenario 1 (Figure 5A,B), in which the Highlands (HIL) and the Southeast (SOE) populations were merged at time *t*1, and the Central (CET) population merged with them at time *t*2.

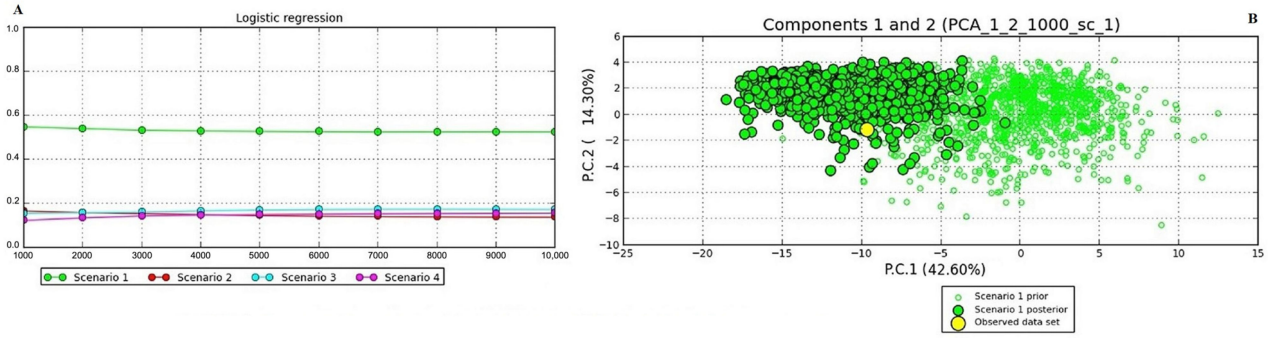

Figure 5. Demographic Diyabc analysis of *Dipterocarpus turbinatus* populations. (**A**) Logistic regression plot for the simulated scenarios. (**B**) Principal component analysis to estimate the scenario with the best goodness-of-fit.

The highest posterior probability was detected in this scenario with for which $p = 0.5269$ and which had a 95% confidence interval from 0.4974 to 0.5564 (Table S7). The type I and mean II error values for scenario 1 were 0.397 and 0.123 (0.059–0.159), respectively. The relative median absolute error values were moderate in the parameter estimates (Table S8). The prior and posterior distributions are shown in Figure S3. The posterior distribution of the effective population sizes for the three populations CET, HIL, and SOE had median values of 1080, 3580, and 1360, respectively (Table S8). The ancestral population (*NA*) had a median value of 5360. The median values of the divergence times *t*1 and *t*2 were 349 generations ago and 2080 generations ago, respectively. The population size change time *t*3 was 6440 generations. If the generation time of *D. turbinatus* trees is assumed to be 35 years (our obs. data; [5]), then the divergence times could be approximately 72,800 years ago for the CET population and 12,215 years ago for the two populations SOE and HIL. The time of the ancestral population size change was 225,400 years ago. The median values of the microsatellite mutation rates (μmic) and single nucleotide insertions/deletions (snimic) at the examined loci were estimated to be 1.08×10^{-4} and 1.95×10^{-8}, respectively. The observed mean allelic numbers and mean expected heterozygosity values in each population differed significantly from corresponding values in the simulated datasets, which were based on the parameters drawn from the posterior distributions of the four scenarios ($p < 0.05$). The F_{ST} values of the populations were not significant in any of the scenarios.

4. Discussion

4.1. Genetic Diversity

Genetic diversity reflects the history and ecology of a species. A species with wide distribution, a large population size, a long lifespan, predominant outcrossing, and successional stages will often maintain high genetic diversity compared with other species [43,44]. High levels of genetic diversity provide better environmental adaptability. Dipterocarps are regionally distributed, long-lived, and predominantly outcrossed. In the present study, *D. turbinatus* exhibited lower levels of genetic diversity ($H_O = 0.285$ and $H_E = 0.328$) compared with several other dipterocarp species, such as *Shorea leprosula* [45–47], *S. robusta* [17], *D. tempehes* [48], *Dryobalanops aromatica* [24], and *D. dyeri* [49]. Furthermore, our results show that the observed number of alleles for each locus was lower ($N_A = 3.2$) than that of *S. leprosula* [45], *D. tempehes* [48], and *D. aromatica* [24,50]. *D. turbinatus* also showed a reduction in allelic richness. The allelic richness values in our study were similar to the

values obtained in previous studies for threatened species such as *Hopea chinensis* [51] and *D. alatus* [52]. The genetic diversity results of our study were also similar to those obtained for other threatened species, such as *Parashorea malaanonan* [53], *S. javania* [21], *D. alatus* [54], *D. costatus* [52], *H. odorata* [55], and *H. chinensis* [51]. Genetic diversity is affected by the degree of anthropogenic disturbance and can be decreased via genetic drift and increased homozygosity for common alleles due to the loss of rare alleles [56,57]. Such populations are inherently vulnerable and at high risk of extinction [58]. Of the 10 studied populations, BHMA, BGMA, and TPHU had lower genetic diversity. The low genetic diversity levels in these three populations were strongly associated with anthropogenic disturbance. Deforestation and overexploitation may be the main factors reducing genetic diversity within all the studied populations. Anthropogenic disturbances and population declines may have caused a reduction in effective population size and genetic diversity associated with the effects of inbreeding. Among the seven preserved populations located in protected areas, the four populations CHUH, CMRA, YORD, and LGXM had higher genetic diversity, whereas the three populations MADA, BGMA, and CHPR had lower genetic diversity. Thus, *D. turbinatus* populations can suffer from disturbances, even those populations that are protected in nature reserves and national parks. The significant heterozygosity deficits across the 281 trees from the 10 populations suggest that a relatively high level of heterozygosity deficiency can exist within *D. turbinatus* populations. This shows that inbreeding occurs in small populations, although dipterocarp species are predominantly outcrossed [48,59,60].

4.2. Genetic Structure and Demographic History of Populations

In the present study, the genetic differentiation between the *D. turbinatus* populations was determined by F_{ST} values of 0.202 and G'_{ST} values of 0.32, which were consistent with the results of the AMOVA analysis (21.52% genetic variation between the different areas). The genetic differentiation observed between the *D. turbinatus* populations was similar to that seen in *D. alatus* [54], *H. ordorata* [55], and *D. beccarii* [24] populations. The genetic differentiation of a population is influenced by gene flow and genetic drift [61]. Low population genetic differentiation reflects high gene flow. High gene flow ($N_m > 1$) indicates a high number of migrants in each generation and may prevent genetic differentiation among populations due to genetic drift [62]. In this study, our results showed that the relatively high gene flow ($N_m = 1.922$) was able to counteract the genetic drift effect, decreasing the population genetic differentiation while increasing the genetic diversity within the populations. Gene flow is determined by the dispersal of pollen grains and seeds [63–65], and it contributes to population genetic differentiation and genetic structure. Dipterocarps are long-lived, predominantly outcrossed, insect-pollinated, and late successional [48–60,66]. Their seeds are dispersed by wind and water [1]. *D. turbinatus* trees are pollinated by insects (bees), and their seeds are dispersed via wind and small mammals (bats and rodents). Because pollen dispersal depends primarily on insects, an increase in the distance between trees (i.e., increased isolation) will reduce pollen transmission between the *D. turbinatus* populations. Recently *D. turbinatus* populations have been fragmented into smaller populations consisting of several individual trees. Thus, the fragmenting of habitats, which leads to increasingly isolated populations, might have reduced the gene exchange among the populations both by pollen and seeds, and this may have influenced their genetic structure in recent decades. Our results showed low population genetic differentiation in the same areas, such as $F_{ST} = 0.055$ between two populations (BNNC and BHMA) in the Central area, $F_{ST} = 0.018$–0.041 among four populations (CHPR, CMRA, BGMA, and YORD) in the Highlands area, and $F_{ST} = 0.03$–0.088 among four populations (LGXM, MADA, BGMA, and TPHU) in the Southeast area. These results were also consistent with the AMOVA analysis of the variance between populations within an area (5.38%). High population genetic differentiation was found between populations located in different areas, such as $F_{ST} = 0.227$ between BHMA and CHUH, $F_{ST} = 0.186$ between BNNC and TPHU, and $F_{ST} = 0.275$ between YORD and BHMA, all of which are separated by large geographic dis-

tances. Such large geographic distances might reduce the likelihood that pollen grains are moved between these populations via insects. Additionally, the dipterocarp fruit consists of a single-seeded nut with wings developed from the calyx. The movement of seeds via the wind can be restricted due to their heavy weight (6.07 g/fruit). Zhirenko and Nguyen (2022) [67] found several young *D. turbinatus* trees located at distances of up to 500 m from one another. In this study, the genetic structure of the *D. turbinatus* trees was determined using different clustering analyses (NJ, PCoA, and DAPC) in which the 281 studied trees were grouped based on their geographic origin. Their particular genetic structures might be a consequence of the gene flow, and they could lead to the formation of different groups of *D. turbinatus*. Large geographic distances can increase the isolation of a population. Pollen dispersal can affect the gene flow and population genetic structure of *D. turbinatus* trees.

Our ABC analysis produced a splitting model in which population divergence took place from the Highlands area (HIL) to the Southeast area (SOE) c. 6.09 kya, and the HIL population diverged from the Central area (CET) population c. 28.59 kya. These results suggest that the oldest divergence event occurred during the last glacial maximum (c. 30–11.7 kya). This period was characterized by conditions that were drier and colder than those of today, with fewer tropical rainforests and extensive savannas. In Southeast Asia, tropical rainforests were also affected, and these would have included a diverse range of taxa from tropical, subtropical, and submontane communities in which the Dipterocarpaceae species would have been conspicuous [68]. The distribution range of tropical rainforests was extended in the early Holocene (c. 9–7 kya) due to the warming and moistening of the climate [69]. However, our estimates of the divergence times showed that the confidence intervals for *t*1 and *t*2 were broad. Moreover, it was assumed in the Diyabc analysis that no gene flow took place between the populations after divergence. The current *D. turbinatus* populations might have been formed from those of the Central and Highlands areas before the SOE population was split from the Highlands population. This model fits the dataset better than the other three models. The ABC analysis in the present study provides demographic information that may be useful for the conservation of relevant *D. turbinatus* populations. In the analysis, no gene flow between populations was assumed. However, our population genetic differentiation result (G'_{ST} = 0.32) indicated gene flow between the *D. turbinatus* populations (N_m = 1.922). Thus, the species distribution range may limit the gene exchange between populations. Clear genetic clusters were shown in the present study. The species should be preserved in three distinct clusters.

5. Conclusions

The results of the present study revealed that *D. turbinatus* has moderate genetic diversity. However, lower genetic diversity was detected in the three populations BHMA, BGMA, and TPHU than in the remaining seven populations. This could be a consequence of highly anthropogenic disturbances in these populations. Low population genetic differentiation was revealed in those populations located in the same area compared with the population genetic differentiation observed between the populations located in different areas. Besides genetic diversity, a higher allelic richness was detected in the six populations CHUH, CHPR, CMRA, YORD, BGMA, and LGXM than in the remaining populations. Private alleles were revealed in the two populations CMRA and YORD. These alleles are an important resource for maintaining populations and adapting to altered selection pressures [70]. They are suitable parameters for conservation. Therefore, populations such as CHUH, CMRA, YORD, and LGXM, which have high genetic diversity or private alleles, should be prioritized for in situ conservation. In the future, seeds from the remaining populations should be gathered for the purpose of ex situ conservation. An increase in population size may prevent a decrease in genetic diversity through genetic drift and homozygosity for common alleles due to the loss of rare alleles, and thus ensure the conservation of *D. turbinatus* trees. The clustering analyses revealed three different gene pools, and these genetic sources may contribute to *D. turbinatus* conservation. The ABC analysis indicated an early divergence in

the Central area that occurred during the last glacial maximum, followed by an increase in the size of the derived populations in the Highlands area during the early Holocene.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15080894/s1, Figure S1: *Dipterocarpus turbinatus* tree in Tan Phu; Figure S2: Number of individuals per population (rows) assigned to each genetic cluster (columns) using DAPC without prior information; Figure S3: Prior and posterior distribution of each parameter of scenario 1 produced via Diyabc analysis of *D. turbinatus.* Table S1: *Dipterocarpus turbinatus* collection locations; Table S2: SSR primer nucleotide sequences and allele size range, and genetic diversities of *D. turbinatus*; Table S3: Prior distributions of parameters for simulated scenarios in Diyabc analysis of *Dipterocarpus turbinatus*; Table S4: Microsatellite diversity values of nine SSR loci for *D. turbinatus*; Table S5: Pairwise genetic differentiation among the 10 *D. turbinatus* populations obtained using GenAlEx; Table S6: Number of individuals per population assigned to a cluster; Table S7: Posterior probability of four putative scenarios, and type I and type II errors for scenario 1 in Diyabc analysis of *Dipterocarpus turbinatus*; Table S8: Demographic parameters obtained via Diyabc analysis of *Dipterocarpus turbinatus*.

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