Genetic diversity in seedling populations of *Dipterocarpus gracilis* in Kecubung Ulolangan Nature Conservation Reserve, Indonesia

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Abstract. Romadin NI, Indrioko S, Widyatno, Faridah E, Ratnaningrum YWN. 2021. Genetic diversity in seedling populations of Dipterocarpus gracilis in Kecubung Ulolangan Nature Conservation Reserve, Indonesia. Biodiversitas 22: 1138-1145. *Dipterocarpus gracilis* Blume is one of the native *Dipterocarpus* species in Java Island, Indonesia. It has commercial value as timber (wood) and non-timber forest products (oleoressin). This species has been considered vulnerable following the Red List of Threatened Species criteria by The International Union for Conservation of Nature (IUCN). We aimed to study the genetic diversity and genetic structure of seedlings as the natural regenerating population of *Dipterocarpus gracilis* Blume in Kecubung Ulolangan Nature Conservation Reserve, Batang District, Central Java Province, Indonesia. The *D. gracilis* population in this area is distributed in four zones. We observed a total of 137 juvenile seedlings representing all the zones. Isozyme markers detected the genetic diversity of *D. gracilis* by peroxidase (PRX), esterase (EST), and acid phosphatase (ACP) enzymes. The mean observed heterozygosity of all the zones was lower (H_o=0.078) than expected heterozygosity (H_e=0.203). Genetic depletion occurred because of genetic drifts and founder effects due to low parental diversity. The genetic structure of seedlings is similar to the consequence of inbreeding. We conclude that the genetic diversity of *D. gracilis* decreases when mature trees are reduced. This population has essential values in Java Island and should be a priority evaluation in the in-situ and ex-situ conservation of genetic resources.

Keywords: *Dipterocarpus gracilis*, genetic diversity, vulnerable, drift, allozyme

INTRODUCTION

The Dipterocarpaceae are the emblematic family of tropical rain forests in Southeast Asia and much of the continental South and South-East Asia's seasonally dry forests (Brearley et al. 2016). The number of dipterocarps species in Asia is the largest among the regions and has 13 genera and 470 species. In Java Island, only five genera and 11 species are found, although the location is very close to Sumatra and Borneo Islands (Sasaki 2006). Dipterocarpus, one of the genera found in Java Island, is an important component of Dipterocarp forest ecosystems, of which 34 species are listed as critically endangered species (Deb et al. 2017). *Dipterocarpus gracilis* Blume is one of the native *Dipterocarpus* species on Java Island. As a type with a commercial value as carpentry timber, *D. gracilis* produces oleoresin products due to non-timber forests product. The potential chemical content of oleoresin is used as a medicinal ingredient that could be considered a good alternative for human remedy (Aslam et al. 2015; Fernandes and Maharani 2019).

Generally, Southeast Asian rainforests have been facing pervasive threats and challenges of forest fragmentation by agricultural development, the impacts of a changing climate on forests and the biodiversity and ecosystem services, and the restoration of forests degraded by logging (Reynolds et al. 2011). In Java Island, *D. gracilis* exists in the nature conservation reserve and regenerates naturally. In global assessment, this species has been considered vulnerable following the Red List criteria of vulnerable species by The International Union for Conservation of Nature (IUCN) (Ly et al. 2017). But this species is reported as critically endangered in India, and the factor leading to depletion of habitat is degradation/loss (Barik et al. 2018). As a species with a decreasing population, *D. gracilis* should be explored in terms of climate change that affects natural resources and ecosystem services (Hansen and Phillips 2015).

An important concern for understanding species adaptation and survival in new climates and biological interactions is genetic variation among species (Ratnam et al. 2014; Ng et al. 2019). Genetic diversity and genetic differentiation patterns in spatial scales reflect stochastic and environmental impacts linked to viability on key demographic and evolutionary processes e.g. population size, gene flow, adaptive potential (Pavlova et al. 2017). Besides, inbreeding depression can occur at all development stages in dipterocarp species, including seed formation, seed germination, seedling development, and sapling establishment (Tsumura 2011). In the small population, *D. gracilis* probably losing genetic diversity is associated with inbreeding and reducing residual stand.
reproduction. Genetic conservation efforts could be focused on an assessment of genetic diversity.

This research was designed to study the genetic diversity of the seedling population of *D. gracilis*. The genetic evaluation of the natural population-based on a seedling study was conducted in the Dipterocarpaceae population in the tropical rainforest of Southeast Asia, e.g., *Shorea leprosula* (Widiyatno et al. 2016; Ang et al. 2016) and *Parashorea malaanonan* (Ang et al. 2016). A preliminary study (Romadini, unpublished data) showed the highest density of seedlings (2.67 ind/m²). The sapling density was lower (0.045 ind/m²) than the seedling density but was higher than the pole density (0.0012 ind/m²). The sapling density was lower (0.045 ind/m²) than the seedling density but was higher than the pole density (0.0012 ind/m²). The pole existence was scarce, indicating that accelerated traits were needed to provide a better forest configuration. However, the study to address the genetic diversity of a vulnerable species population in a nature conservation reserve is not yet known. This study aimed to evaluate the genetic diversity of the seedling of *D. gracilis* in a small population. We hypothesized that the genetic diversity of the seedling among the population was low as a result of random genetic drift.

**MATERIALS AND METHODS**

**Study area**

Kecubung Ulolanang Nature Conservation Reserve is a 69.7 ha conservation area located in Subah, Batang District, Central Java Province, Indonesia. The preservation was based on Ministerial Decree of Forestry and Plantation no. 435 / Kpts-II / 1999 dated June 15, 1999 (Ervina and Wasiq 2018). It is situated within the geographical limits of 06°51’46’’–007°11’43’’ S and 109°40’19’’–110°03’06’’ E (Figure 1). The location is classified into B of Schmidt and Ferguson climate types. The *D. gracilis* population in the Kecubung Ulolanang Nature Conservation Reserve is distributed in four zones (Figure 1). The distance between zones exceeds 200–1,300 m and is located at different altitudes (Table 1).

**Procedures**

**Sample collection**

In July 2018, fresh juvenile leaves from 137 *D. gracilis* seedlings were collected from randomly chosen individuals. Leaf samples were placed in a tube and frozen in ice packs prior to allozyme extraction and electrophoresis in the laboratory. Distance between the sampled individuals ranged from 2 m to 30 m, and it was mapped with a Garmin 64S handheld GPS.

**Table 1.** The location used for sampling *Dipterocarpus gracilis* from the four zones

<table>
<thead>
<tr>
<th>Zone</th>
<th>Elevation (m asl)</th>
<th>Number of samples</th>
<th>Number of mature trees*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>137–200</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>144–178</td>
<td>49</td>
<td>94</td>
</tr>
<tr>
<td>C</td>
<td>154–168</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>D</td>
<td>153–156</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

Note: *mature tree is indicated by diameter at breast height (DBH) of >20cm*

**Figure 1.** Location of Kecubung Ulolanang Nature Conservation Reserve in Subah, Batang District, Central Java Province, Indonesia
Isozyme electrophoresis

Isozyme electrophoresis was conducted with vertical polyacrylamide gel electrophoresis followed by the David–Ornstein method (Seido 1993). The leaves were homogenized in a modified extraction buffer and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was loaded onto polyacrylamide vertical slab gels (Sigma Inc., USA) and electrophoresed in a water bath at 4 °C, 220 V, and 200 mA for about 3 hours. After electrophoresis, the gels were stained using a staining solution of each enzyme system. The preliminary analysis screened six enzyme systems, i.e., peroxidase (PRX), shikimate dehydrogenase (SHD), esterase (EST), acid phosphatase (ACP), diaphorase (DIA), and glutamate dehydrogenase (GDH) (unpublished data). Only three enzyme systems, i.e., PRX (E.C. 1.11.1.17.), EST (E.C. 3.1.1.1.), and ACP (E.C. 3.1.3.2.), produced consistent polymorphic bands, and therefore, were applied to genetic analysis for all samples.

Data analysis

Genetic diversity

Genetic parameters of the number of alleles per locus (N_a), the number of effective alleles (N_e) (Kimura and Crow 1964), Shannon’s index (I), observed heterozygosity (H_o), and expected heterozygosity (H_e) were estimated for each zone using GenAlEx 6.5 (Peakall and Smouse 2012). Rare alleles and private alleles were estimated using MS Excel 2010. A bottleneck test was conducted for each population to understand the past population size changes. This analysis was performed under the infinite allele model and the two-phase model using two different tests: the sign test and the Wilcoxon signed-rank test; each test had 1,000 runs in BOTTLENECK 1.2.02 (Cornuet and Luikart 1996).

Genetic structure

Wright’s fixation indices (F IS, F RT, and F ST) were also calculated for four zones using Arlequin 3.5.2.2. POPGENE 1.32 (Yeh et al. 1999) was used to calculate the estimation of Nei’s (Nei 1987) to measure population genetic differentiation between the zones and evaluate the genetic relationships among the four zones. A UPGMA dendrogram was created based on Nei’s allele frequency and standard genetic distance by using POPGENE 1.32. Data from the four zones were used to test whether the genetic variance was partitioned among the zones using Arlequin 3.5.2.2 (Excoffier and Lischer 2010) for AMOVA.

RESULTS AND DISCUSSION

Polymorphic loci

Enzyme systems that showed consistent banding patterns and polymorphisms were PRX, EST, and ACP based on a preliminary study conducted on six enzyme systems (Romadini, unpublished data). The banding patterns were interpreted as the active zone of the enzyme system and interpreted as a locus. Three enzyme systems that produced the genetic diversity and the distribution of allele frequencies are shown in Table 2. The PRX enzyme system could be observed with one locus, i.e., Prx-1 controlled by three alleles. The EST enzyme system could be detected in three loci, namely, Est-1, Est-2, and Est-3 (Table 2). Est-1 was controlled by three alleles, and Est-3 and Est-2 were controlled by two alleles. Although the ACP enzyme system could be observed with only one locus, i.e., Acp-1, which was controlled by three alleles, Acp-1 showed the highest polymorphism in terms of the number of alleles (2.75). Est-2 and Est-3 exhibited the lowest number of alleles (1.25; Table 2). Based on the allele distribution (Table 2), two unique alleles are present in zone A (Est-2-b and Est-3-b) and zone C (Prx-1-c). The presence of unique alleles also indicated that the same alleles were lost in other zones. Prx-1-c alleles presented in zone C were also rare alleles. Besides, rare alleles were found in other zones, namely, Prx-1-a (Zone A) and Acp-1-a (Zone B). Expected heterozygosity (H_e) ranged from 0.017 to 0.359, and the average H_e was 0.205. The alleles that were fixed in this population were Prx-1-c, Est-2-b, and Est-3-b. The expected heterozygosity (H_e) and observed heterozygosity (H_o) values at each locus are shown in Table 2. The mean H_e value based on the locus was 0.205 (0.031-0.359) and the average H_o was 0.0745 (0.0-0.214).

Genetic diversity of Dipterocarpus gracilis

As a key species and one of the Dipterocarps that still occurred naturally in semi-evergreen dry forests on Java Island (Hamilton et al. 2019), the existing D. gracilis population should be maintained. To support forest ecosystem function, the genetic diversity of keystone species is crucial (Ratnam et al. 2014). However, our results showed that genetic drift occurred in this population indicated by the allele fixation and fluctuation of the allele frequency in each locus (Table 2). Oakley and Winn (2012) said that genetic drift in small populations increases both homozygosity within populations and the chance of differentiation among them. These substantiate previous findings in the literature that in small populations, two important genetic mechanisms acting on short to intermediate timescales and threatening are inbreeding depression and increased genetic load due to genetic drift (Pekkala et al. 2014). It was probably caused by a systematic random genetic drift that induced random changes in allele frequencies across zones.

The most striking results (Table 2) were those rare alleles, private alleles, and missing alleles were randomly distributed based on the allele frequencies. The presence of rare and missing alleles suggests that some alleles were not inherited in the next generation (Indrioko and Ratnaingrum 2015). Rare alleles and private alleles were important in this population because these alleles would support the genetic variation. With the loss of rare alleles, genetic variation within a small population can be decreased by random genetic drift and increased homozygosity for common alleles (Gijbels et al. 2015). To maintain its existence, a species requires the ability to survive and adapt, which depends on each individual's genetics.
In all zones, the mean of the effective alleles per locus (N_e) was lower than the number of the allele per locus (N_a; Table 3). The mean N_e was 1.40 (1.26–1.47), with the percentage of polymorphic loci was 40%–80% in each zone. Shannon index in all the zones ranged from 0.26 to 0.43. The number of mature trees would have affected N_a, N_e, and I. Na, Ne, and I in zones B and C were higher than those in zones A and D. A high value was probably related to the number of mature trees with the possibility of having a high diversity, which can be passed on to the offspring.

At a population level, the genetic diversity also could be calculated as observed heterozygosity (H_o) and expected heterozygosity (H_e). Zone C showed the highest genetic diversity among the four zones. Zone A had the lowest observed heterozygosity value, which implied that the genetic variation in this zone was smaller than the other zones. Generally, the mean H_o of all the zones was lower (0.078) than H_e (0.203). Our result is similar to that of another Dipterocarpus study that population size had an affect on the low heterozygosity of D. littoralis (Dwijanti et al. 2014). Interestingly, our results were much smaller than the mean H_e obtained from natural populations with various silvicultural systems (Widiyantno et al. 2016). In this forest management practices study, these parameters were not sensitive because several generations were necessary to determine the effect of reduction in H_e. Ratnam et al. (2014) suggest that expected heterozygosity (H_e) is not very sensitive to bottlenecks and perturbations in populations. Therefore, our finding was of significant information because the results showed that the heterozygosity of D. gracilis in the Nature Conservation Reserve indicated a decreasing trend. Low genetic variation in a small population is particularly challenging for conservation management. These findings contrast with a goal of conservation management which is to enhance the population’s adaptive ability, i.e. the capacity of the population to adapt and survive in the face of environmental changes (Sgrò et al. 2011).

Extreme drift leads the genetic bottleneck effects and can be detected by changes in allele frequencies. The loss of this allele reduces the ratio, which results in a population bottleneck. In this case, the bottleneck effect analysis

### Table 2. Distribution of the allele frequencies and genetic diversities of five polymorphic loci from Dipterocarpus gracilis from four zones

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Zone A</th>
<th>Zone B</th>
<th>Zone C</th>
<th>Zone D</th>
<th>N_a</th>
<th>N_e</th>
<th>H_o</th>
<th>H_e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prx-1</td>
<td>a</td>
<td>0.050</td>
<td>0.398</td>
<td>0.288</td>
<td>0.000</td>
<td>2.000 ± 0.408</td>
<td>1.626 ± 0.351</td>
<td>0.085 ± 0.043</td>
<td>0.293 ± 0.145</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.950</td>
<td>0.602</td>
<td>0.538</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.000</td>
<td>0.000</td>
<td>0.173</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est-1</td>
<td>a</td>
<td>0.000</td>
<td>0.066</td>
<td>0.093</td>
<td>0.143</td>
<td>2.250 ± 0.479</td>
<td>1.700 ± 0.351</td>
<td>0.077 ± 0.041</td>
<td>0.324 ± 0.143</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.000</td>
<td>0.382</td>
<td>0.090</td>
<td>0.310</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>1.000</td>
<td>0.553</td>
<td>0.000</td>
<td>0.548</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est-2</td>
<td>a</td>
<td>0.966</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.250 ± 0.250</td>
<td>1.018 ± 0.018</td>
<td>0.000 ± 0.000</td>
<td>0.017 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.034</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Est-3</td>
<td>a</td>
<td>0.933</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.250 ± 0.25</td>
<td>1.036 ± 0.036</td>
<td>0.000 ± 0.000</td>
<td>0.031 ± 0.031</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.034</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acp-1</td>
<td>a</td>
<td>0.017</td>
<td>0.024</td>
<td>0.104</td>
<td>0.000</td>
<td>2.750 ± 0.250</td>
<td>1.608 ± 0.160</td>
<td>0.214 ± 0.071</td>
<td>0.359 ± 0.066</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.586</td>
<td>0.786</td>
<td>0.750</td>
<td>0.897</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.397</td>
<td>0.190</td>
<td>0.146</td>
<td>0.103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>1.900</td>
<td>1.398</td>
<td>1.106</td>
<td>0.745</td>
<td>0.205 ± 0.052</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The standard error of each parameter is shown in parentheses. * = private allele; bold letters = rare allele; the null number in gray background represents the missing allele; N_a = no. of different alleles; N_e = no. of effective alleles; H_o = observed heterozygosity; H_e = expected heterozygosity.

### Table 3. Genetic diversity of Dipterocarpus gracilis in the four zones

<table>
<thead>
<tr>
<th>Zone</th>
<th>Number</th>
<th>N_a</th>
<th>N_e</th>
<th>P (%)</th>
<th>I</th>
<th>H_o</th>
<th>H_e</th>
<th>No. of rare alleles*</th>
<th>No. of private alleles**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>2 (0.32)</td>
<td>1.26</td>
<td>80</td>
<td>0.27</td>
<td>0.05</td>
<td>0.16</td>
<td>0.31</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.18)</td>
<td></td>
<td>0.13</td>
<td>0.04</td>
<td>0.09</td>
<td></td>
<td>(0.21)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>2 (0.45)</td>
<td>1.53</td>
<td>60</td>
<td>0.43</td>
<td>0.06</td>
<td>0.27</td>
<td>0.21</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.24)</td>
<td></td>
<td>0.18</td>
<td>0.02</td>
<td>0.12</td>
<td></td>
<td>(0.21)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>C</td>
<td>47</td>
<td>2 (0.45)</td>
<td>1.47</td>
<td>60</td>
<td>0.41</td>
<td>0.13</td>
<td>0.23</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.28)</td>
<td></td>
<td>0.20</td>
<td>0.08</td>
<td>0.12</td>
<td></td>
<td>(0.00)</td>
<td>(0.20)</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>1.6 (0.4)</td>
<td>1.33</td>
<td>40</td>
<td>0.26</td>
<td>0.07</td>
<td>0.15</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.27)</td>
<td></td>
<td>0.19</td>
<td>0.04</td>
<td>0.11</td>
<td></td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>Average</td>
<td>1.90</td>
<td>1.40</td>
<td>60</td>
<td>0.34</td>
<td>0.078</td>
<td>0.203</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The standard error of each parameter is shown in parentheses. N_a = allele numbers per locus/observed number of alleles; N_e = effective number of alleles (Kimura and Crow, 1964); I = Shannon’s information index; H_e = expected heterozygosity; *) allele frequency 0.05; **) alleles that are found only in a single population among a collection of populations.
results revealed that the results were not significantly different in all the zones and possibly did not reflect the founder effect since this plant existed. This finding indicated that the genetic alterations were relatively unchanged from the start. However, the minimal number of individuals and the relatively incompatible flower’s pollination and fertilization likely led to the extinction of this species if no effort was devoted to conserving it, especially if a drastic environmental change would trigger a bottleneck effect (Table 4).

**Differentiation and genetic structure**

Synchronized flowering effectively promotes successful pollination and mass general flowering events of Dipterocarpaceae species are important for maintaining genetic diversity (Tani et al. 2012). Generally, the outcrossing rate of Dipterocarpaceae species is high. However, the outcrossing rate appears to be poor in forests with a lower density of mature trees, depending on the key pollinators (Tsumura 2011). The calculated fixation index ($F_{IS}$) showed a positive value of 0.6778 which indicates that all populations have a trend to reduce heterozygosity which leads to inbreeding. A high $F_{IS}$ value indicates a high inbreeding as a result of a small and isolated population that can increase deleterious allele fixation (Lynch et al. 1995). $F_{IT}$ showed positive value (0.758). This is also possibly occurred because dipterocarps are not synchronous in the flowering phase, and they pollinate nearby trees that tend to have a high genetic relationship (Widiyatno et al. 2017). Besides, where mature trees are at low density, it will result in lower outcrossing caused by poorly dispersing pollinators.

The genetic differentiation between individuals was higher (50.76%) than that of between zones (25.1%) (Table 5), in accordance with the overall $F_{ST}$ value (0.251). The proportion of variation in individuals was 24.1%. $F_{ST}$ was calculated to evaluate the genetic differentiation between zones and had a positive value (0.251), which indicated a very high genetic differentiation within the zone (Hartl and Clark 1997). Strong genetic differentiation in Dipterocarpaceae species (such as *S. macrophylla*), probably associated with short seed dispersal distances and limited habitats (Utomo et al. 2018). Gene flow due to the random movement of living things such as pollinators can reduce genetic differences between populations and increase population variation. Connectivity between zones can still be maintained if the presence of pollinators and seed dispersal processes can still be present. The presence of pollen donors from outside the zone can help to reduce $F_{IS}$. But, our result showed that the $N_{m}$ value obtained was 0.746, resulting from $F_{ST}$ value. $N_{m}$ < 1 values indicate genetic drift as a cause of population differentiation (Slatkin 1987). Similar results were reported in *D. alatus* (Tam et al. 2014) and *D. costassus* (Duc et al. 2016), wherein $N_{m}$ value was under 1 ($N_{m} < 1$) with low genetic diversity which is a consequence of inbreeding within the small and isolated population. This lends support to previous findings in the literature that genetic drift usually decreases the total amount of genetic variation and when spatial structure becomes more pronounced, this effect is comparably stronger for the lower levels of gene flow (Star and Spencer 2013).

The genetic diversity between the zones compared with the genetic diversity between individuals was supported by cluster analysis results. Based on cluster analysis, the four zones of *D. gracilis* in this nature conservation reserve are divided into two main groups (Figure 2). The first group consisted of Zone A, B, and D. Two sub-group were identified within the first group. Zone C was grouped into the second sub-group. In this case, genetic and geographical distances did not have a linear relationship. Zones B and D, which were geographically relatively far apart, tend to have proximity compared with zone C (Table 6).

**Table 4. Results of bottleneck tests on *Dipterocarpus gracilis* in the four zones under two models of mutation: the infinite allele model (IAM), the three-phase model (TPM), and SMM (with variance = 30 and probability = 70%)**

<table>
<thead>
<tr>
<th>Zone</th>
<th>IAM</th>
<th>TPM</th>
<th>SMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.388 (0.27)</td>
<td>0.335 (0.17)</td>
<td>0.322 (0.10)</td>
</tr>
<tr>
<td>B</td>
<td>0.103 (0.04)</td>
<td>0.523 (0.09)</td>
<td>0.572 (0.20)</td>
</tr>
<tr>
<td>C</td>
<td>0.485 (0.22)</td>
<td>0.460 (0.35)</td>
<td>0.408 (0.47)</td>
</tr>
<tr>
<td>D</td>
<td>0.733 (0.21)</td>
<td>0.747 (0.30)</td>
<td>0.742 (0.37)</td>
</tr>
</tbody>
</table>

Note: The standard error of each parameter is shown in parentheses.

**Table 5. Analysis of molecular variance (AMOVA) partitioning of the total genetic diversity within and among the zones**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of square</th>
<th>Variance components</th>
<th>Percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among zone</td>
<td>34.096</td>
<td>0.19447</td>
<td>25.104</td>
</tr>
<tr>
<td>Among individual</td>
<td>105.207</td>
<td>0.39324</td>
<td>50.763</td>
</tr>
<tr>
<td>Within individual</td>
<td>21.500</td>
<td>0.18694</td>
<td>24.1%</td>
</tr>
<tr>
<td>Total</td>
<td>160.803</td>
<td>0.77465</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6. Nei’s unbiased measure of genetic identity and genetic distance**

<table>
<thead>
<tr>
<th>Zone</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>0.9203</td>
<td>0.7340</td>
<td>0.9432</td>
</tr>
<tr>
<td>B</td>
<td>0.0830</td>
<td></td>
<td>0.9172</td>
<td>0.9606</td>
</tr>
<tr>
<td>C</td>
<td>0.3093</td>
<td>0.0864</td>
<td></td>
<td>0.8767</td>
</tr>
<tr>
<td>D</td>
<td>0.0584</td>
<td>0.0402</td>
<td>0.1315</td>
<td></td>
</tr>
</tbody>
</table>

Note: Nei’s genetic identity (above diagonal) and genetic distance (below diagonal)

**Figure 2. Dendrogram based on Nei’s (1978) genetic distance with UPGMA**
While *D. gracilis* pollinators have not been documented, the donor pollen from outside the zone is possible because pollination on Dipterocarpaceae is generally assisted by insects (Tito de Morais et al. 2015). The characteristics of *D. gracilis* fruits are winged and easily carried by the wind or gravity and it can be distributed to other zones, although the distribution is not optimal in the process because of disturbances such as the presence of understorey plants. A previous study (Smith et al. 2015) revealed that seed dispersal distances of *Dipterocarpus* species such as *D. crinitus* and *D. cornutus* is predicted to be 7-11 m. In this study, the large geographical distances between the zones could be the limitation of seed dispersal. Natural boundaries could also be the causes of the emergence of discontinuous populations and often occur in Dipterocarpaceae in natural forests.

In general, the genetic diversity of *D. gracilis* from the four zones in this nature conservation reserve was not suitable due to a decrease in heterozygosity, which is indicated by drift and the tendency to increase in inbreeding. It was similar to *Santalum album* case in Gunung Sewu (Java Island) with genetic depletion. Heavy-exploitation, natural disturbance, genetic drift, bottleneck effect, and founder effect has reduced its genetic diversity (Ratnaningrum et al. 2015). Therefore, large gene flow and broad genetic base are important for maintaining genetic processes (Ratnaningrum et al. 2017). Besides, on Java Island, a large proportion of deforestation was significantly driven by small-scale agriculture (Austin et al. 2019). Based on these conditions, we must pay attention to the genetic resources of Dipterocarpaceae population under various landscape structures, especially on Java Island.

**Implications for the sustainable management of tropical rainforests**

As a keystone and potentially lesser-known species, *D. gracilis* should be a priority of evaluation in the in-situ and ex-situ conservation of genetic resources. As generally known that poor integration of genetics and evolutionary biology into conservation planning is a major cause of ineffective forest management (Ralls et al. 2018). The population of Kecubung Ulolalang Nature Conservation Reserve has important values in Java Island, and its management must focus on genetic aspects and be based on a landscape scale. Genetic drift that occurs in this population, the existence of these alleles must be preserved. We suggest the improvement of the connectivity between zones to counterforce declining population size, low genetic diversity, and local extinction (Griffiths et al. 2020). This implies that management should be conducted not only in the zone where *D. gracilis* are growing but also in the surrounding area as a buffer zone. Areas between zones that are not covered by *D. gracilis* are growing but also in the surrounding area as a buffer zone. Areas between zones that are not covered by *D. gracilis* individuals should be enriched to maintain gene flow. Genetic diversity, which is the genetic potential in a population, should be maintained. It is important not only in conserving natural species but also in utilizing breeding materials to increase product utilization value. These study results are considered as the basis for determining the conservation actions that should be taken.

Human intervention can help maintain the genetic potential of the population. Enrichment planting in the population of *D. gracilis* in Kecubung Ulolalang Nature Conservation Reserve is needed to increase genetic diversity and improve conservation to support ecological important species (Millet et al. 2013). But we need a selection of species for enrichment planting that involves native species which is important to maintain the diversity of late succession species (Widiyatno et al. 2020). Efforts to minimizing the loss of rare alleles can maintain the variation in the population. Besides, the private allele transfer from one zone to another can be performed to increase the genetic diversity of the population across zones. But we also need much attention for seed collecting processes, Finger et al. (2012) suggest that to minimize the likelihood of disrupting any local adaptation, seeds must be collected from donor sites that are not only diverse and outbred but also from similar site conditions.

Individuals from outside the nature conservation reserve can be introduced to increase population diversity. The steps that may be implemented to keep the genetic diversity of *D. gracilis* include maintaining the existence of the population and encouraging the success of natural regeneration. The population quality should be maintained and improved to keep the existence of pollinating agents. Pollinators may contribute to maintaining the diversity of species (Kettle et al. 2011). The reproductive success and continuity of *D. gracilis* generation are determined in terms of the level of the genetic diversity of parents and the success of the interbreeding pattern of living trees (remnant trees). This approach increases the genetic diversity and adaptability of *D. gracilis* in Kecubung Ulolalang Nature Conservation Reserve and in the natural ecosystem of Java Island, whose sustainability contributes to ecological balance and other living things.

**ACKNOWLEDGEMENTS**

We appreciate the financial support of the PMDSU Scholarship in 2018 (No.148/SP2H/PTN/HRP2018 and 5717/UN1.DITLIT/DIT-LIT/LT/2018) from the Ministry of Research, Technology, and Higher Education in this research, which was part of Nesty Pratini Romadini’s Ph.D. We thank our colleagues from Universitas Gadjah Mada, Yogyakarta, Indonesia and *Balai Konservasi Sumberdaya Alam* of Central Java, Indonesia, for providing insights and offering their expertise.

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