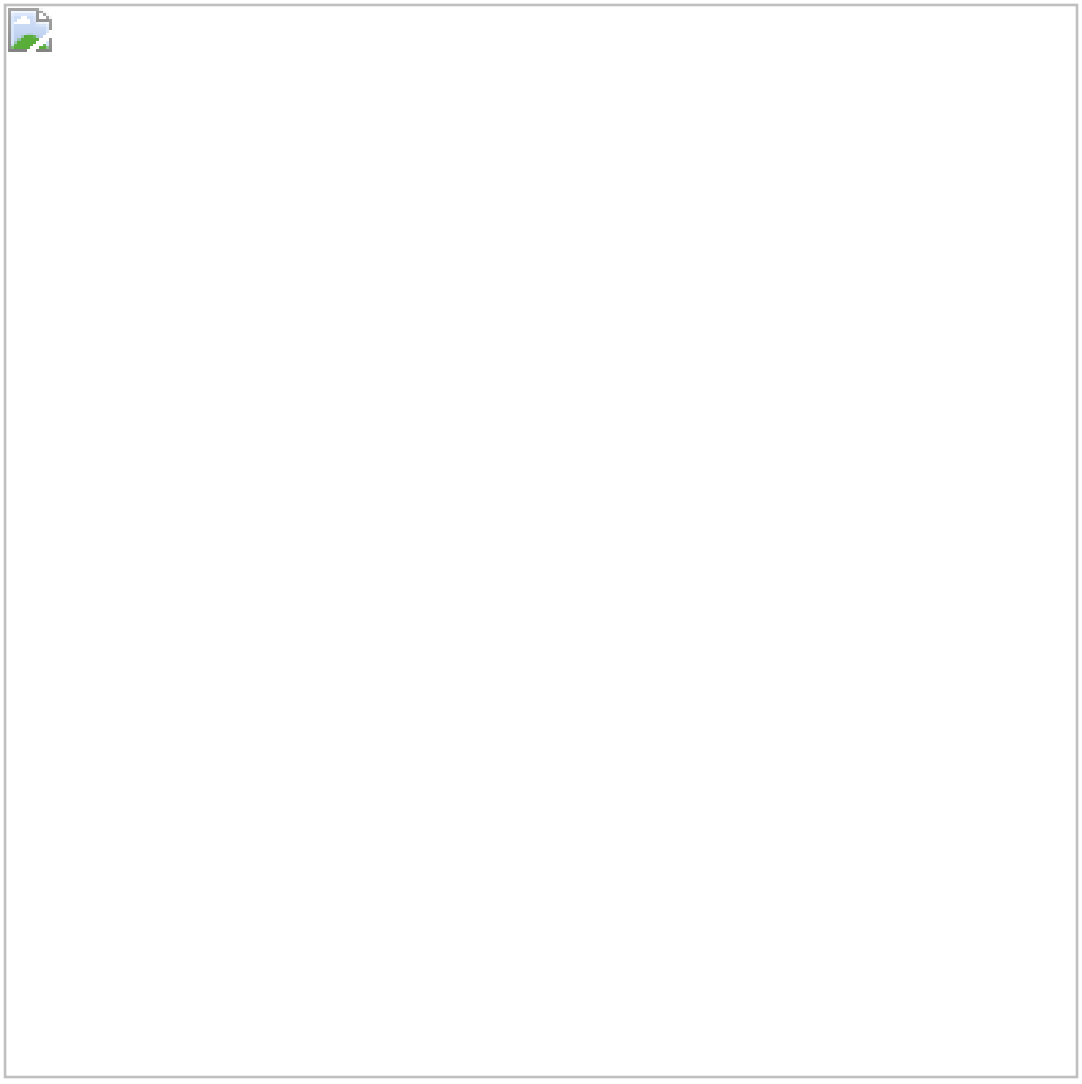

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Seedling Selection Using Molecular Approach for Ex Situ Conservation of Critically Endangered Tree Species (*Vatica bantamensis* (Hassk.) Benth. & Hook. ex Miq.) in Java, Indonesia

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Abstract

Ex situ conservation is an important complementary strategy for in situ to conserve endangered plant species. However, the limited areas designated for ex situ conservation such as in botanic gardens have become a great challenge for conservation practitioners and scientists attempting to optimally conserve the genetic diversity of targeted plant species. Our study aimed to assess genetic diversity and structure of wild seedlings of *Vatica bantamensis*, an endemic and critically endangered dipterocarp from Java (Indonesia). We also estimated genetic differentiation between the wild seedlings and existing ex situ collection and evaluated the genetic diversity preserved in the ex situ collection. Our analysis, using 730 single-nucleotide polymorphisms loci, showed that wild seedlings exhibited higher genetic diversity than the ex situ collection (nucleotide diversity, $\mu = 0.26$ and 0.16, respectively). Significant genetic differentiation was also detected ($F_{ST} = 0.32$) between wild seedlings and ex situ collection. Furthermore, we found high kinship within the ex situ collection suggesting low genetic diversity since the founding collection. We also detected three distinct genetic clusters from all samples combined (analysis of molecular variance, $\phi = 0.48$, $p < .001$), with two clusters present in the wild seedlings that were not represented in the ex situ collection. We recommend that supplementary collections from the two newly identified genetic clusters in the wild seedlings should be incorporated to increase genetic diversity in the ex situ collection. Furthermore, our study demonstrated that understanding the population genetics of targeted endangered species provides better results for ex situ conservation strategies.

Keywords

dipterocarps, endangered tree species, ex situ conservation, MIG-seq, population genetics, single-nucleotide polymorphisms

Introduction

Habitat loss and fragmentation due to land use change is considered as the major driver of plant extinction around the world (Hermy, Honnay, Jacquemyn, & Brys, 2014; Tilman et al., 2017). In Java (Indonesia), the habitat loss is characterized by the conversion of forest into small-scale agriculture (Austin, Schwantes, Gu, & Kasibhatla, 2018). Higginbottom, Collar, Symeonakis, and Marsden (2019) reported that around 40% of montane forest areas have loss due to deforestation for the last 28 years. Conservation of plant species through multiple mechanisms is necessary to prevent

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unprecedented consequences of habitat loss, forest fragmentation, and global climate change (Mantyka-pringle, Martin, & Rhodes, 2012; Pacifici et al., 2015; Volis, 2017). Conservation of plant species in their natural habitats (in situ) is thought to be the preferred method of conservation (Oldfield, 2009); however, conservation outside their natural range (ex situ), including in botanic gardens, arboreta, or seedbanks, can play an unprecedented role in conservation (Potter et al., 2017; D. J. Pritchard, Fa, Oldfield, & Harrop, 2011; H. W. Pritchard et al., 2014).

Currently, there are more than 3,300 botanic gardens around the world (Botanic Gardens Conservation International, 2017a), with a total living plant collections of about 1.3 million individuals, representing more than 526,000 taxa (Botanic Gardens Conservation International, 2017b). Among these living collections, more than 1,300 species are designated critically endangered (CR) or endangered (EN), according to the IUCN Red List (Rivers, Shaw, Beech, & Jones, 2015). The thousands of threatened species conserved in the botanic gardens are irreplaceable resources, not only for conservation but also for research and education. Furthermore, living collections in the botanic gardens are key sources for many elements of conservation programs, including reintroduction, restorations, and population enhancement (Donaldson, 2009; Pennisi, 2010; Sharrock, 2012).

Nevertheless, ex situ conservation in the botanic gardens is facing a huge challenge. The average size of botanic gardens around the world is about 45 ha (Pautasso & Parmentier, 2007), enough to contain only a limited number of living plant collections (Lauterbach, Burkart, & Gemeinholzer, 2012). As a result, the genetic diversity represented in the ex situ collections may not always be adequate for conservation programs (Ensslin, Sandner, & Matthies, 2011). Generally, low number of individuals in a population makes such a population vulnerable to genetic drift and inbreeding depression, possibly causing lower fitness and other deleterious effects that jeopardize future generations (Ellstrand & Elam, 1993; Frankham, 2003). Therefore, limited size of living collections for each endangered plant species in the botanic gardens could have negative effects on conservation. This threat has now become a major concern for many conservation scientists and botanic gardens managers. We were therefore motivated to assess the representation of genetic diversity and intraspecies genetic differentiation of an endemic and threatened tree species from Java (Indonesia) by taking the advantage of a population genetic study, as suggested by Hoban and Schlarbaum (2014).

Vatica bantamensis (Hassk.) Benth. & Hook. ex Miq. was first described by Hasskarl in 1859 under the genus of *Anisoptera* before being classified under the genus of *Vatica* in 1862 and accepted under its current name (The International Plant Names Index, 2012; The Plant List, 2013). The genus of *Vatica* itself was described by Carl Linnaeus in 1771 (The International Plant Names Index, 2012). *V. bantamensis* is the only species in the genus of *Vatica* that is endemic to the island of Java, Indonesia (Ashton, 1982), although two other subspecies (*V. vanulosa* ssp. *venulosa* and *V. javanica* ssp. *javanica*) that are also distributed on the island (Ashton, 1982; Backer & Bakhuizen van den Brink Jr, 1963). *V. bantamensis* is highly regarded for its timber quality, hence it is used in construction (Soerianegara & Lemmens, 1994). This tree species is endemic only to Mount Payung, Ujung Kulon National Park (UKNP; West Java, Indonesia) and is currently classified as Critically Endangered on the IUCN Red List (Robiansyah, 2018). Mature tree of *V. bantamensis* can reach up to 30 m height, with diameter of about 40 cm (Ashton, 1982; Wihermanto, Dodo, Kusuma, & Muhiban, 2015). The fruit is composed of hard shell covering one seed called nut, with two shorter lobes or wings which then dispersed by wind (gyration-dispersed; Ashton, 1982; Suzuki & Ashton, 1996). Several species of *Vatica* (i.e., *V. sumatrana* and *S. sarawakensis*) exhibit hermaphrodite reproductive system with mass flowering event every 3 to 4 years (Brearley, Proctor, Nagy, Dalrymple, & Voysey, 2007). *V. bantamensis* is likely to follow the same pattern.

In this study, we assess the genetic diversity and structure of wild seedlings of *V. bantamensis*, an endemic and critically endangered dipterocarp from Java (Indonesia). We also estimated genetic differentiation between the wild seedlings and existing ex situ collection from the botanic gardens. We further explored the genetic diversity preserved in the ex situ collection and evaluated the possibility of additional collections from the wild and suggested an effective measure for an improved seedling selection for ex situ conservation strategy.

Materials and Methods

Collecting Sites

The UKNP is located on the westernmost part of the island of Java, Indonesia (Figure 1). Protected since 1921 (Whitten, Soeriaatmadja, & Afiff, 1996), the national park is known as the last remaining habitat of the Javan rhinoceros in the world (Fernando et al., 2006). In the national park, tropical lowland forest is commonly found, consisting mainly of primary and secondary forests. The forests are predominantly composed of palms, bamboos, thorny shrubs, and rattan (Hommel, 1990). The elevation ranges from sea level up to ca. 490 m above sea level (m asl; Whitten et al., 1996).

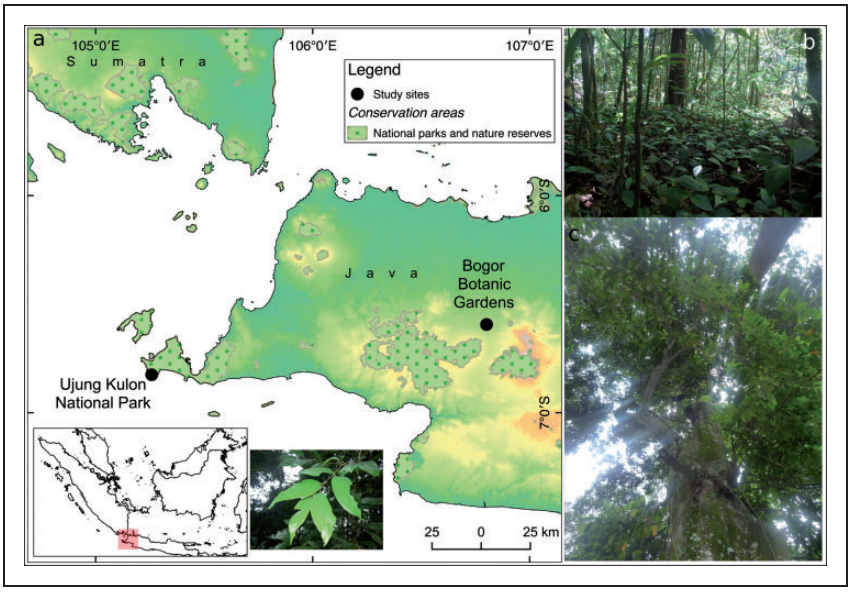

Figure 1. (a) Collection sites of *Vatica bantamensis* on island of Java, Indonesia (map layers were downloaded from https://www.globalforestwatch.org/, https://www.naturalearthdata.com/, and http://www.diva-gis.org/gdata); (b) wild seedlings of *V. bantamensis* in Ujung Kulon National Park; and (c) living collections in the Bogor Botanic Gardens; inset: leaves of *V. bantamensis*.

Sample Materials

In 2014, we sampled leaf tissue of *V. bantamensis* from 65 randomly selected wild seedlings originally collected from UKNP (Mount Payung, altitude ca. 500 m asl) West Java, Indonesia. These wild seedlings were distributed on the northeast side of the mountain within approximately 5 ha area, where four mature trees of *V. bantamensis* were found (Wihermanto et al., 2015). They were collected for the purpose of population reintroduction. The leaf tissue was then preserved in plastic bags containing silica gels. We also collected leaf tissue from all three living individuals of *V. bantamensis* in the only available ex situ collection at the Bogor Botanic Gardens (BBG; tree numbers VII.B.39a, I.K.63, and I.K.63a). Tree number VII.B.39a had been growing in the BBG since 1843, together with three other individuals (numbers VII.B.30a, I.K.40, and I.K.40a) that had already died (Wihermanto et al., 2015). These collections were originally collected from Banten (Java), presumably from the UKNP. The other two individuals, tree numbers I.K.63 and I.K.63a, were planted in February 1990, and they are progeny of the deceased individual (tree number I.K.40; BBG Database, unpublished).

DNA Extraction and MIG-seq Analysis

Genomic DNA were extracted from dried leaf tissues using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Multiplexed inter simple sequence repeats (ISSR) genotyping-by-sequencing (MIG-seq) was conducted following the protocol described by Suyama and Matsuki (2015). MIG-seq has an advantage on its applicability to a wide range of DNA quality with a quick, simple, and economical approach compared with other next-generation sequencing (NGS) technologies (Suyama & Matsuki, 2015). In brief, the MIG-seq library was constructed in two polymerase chain reaction (PCR) steps; in the first step, multiple nonrepetitive regions from various ISSRs were amplified by multiplexing with tailed ISSR primers. For the second step, the diluted products of the first PCR were added with the necessary sequences used as described in the original protocol, and the PCR was performed in a GeneAmp PCR System 9700 (Thermo Fisher Scientific, Applied Biosystem, CA, USA). The second PCR products were then pooled in equimolar concentrations and purified. Fragments of a size range 300 to 800 bp were isolated, and library concentrations were measured. Approximately 10 pM of the libraries were used for sequencing on an Illumina MiSeq Sequencer platform (Illumina, San Diego, CA, USA).

After obtaining the sequences, preanalysis steps were applied by removing primer regions and performing quality filtering using FASTX-Toolkit v. 0.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/) and Cutadapt v. 1.16 (Martin, 2011). The filtered reads were used for single-nucleotide polymorphisms (SNPs) detection as implemented in Stacks v. 2.2 (J. Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013; J. M. Catchen et al., 2011). The processed reads were compiled using the "ustack" module to create "stacks" with settings of a minimum depth of coverage ($m = 3$) and a maximum

distance between stacks ($M = 2$). Deleveraging (d) and removal (r) algorithms were also enabled during stacks assembly. Stacks catalogues were created using the "cstacks" module. We allowed mismatches between sample loci ($n = 2$) and matched the stacks produced by "ustacks" against the catalogue created with "cstacks" by using the "sstacks" module. SNPs were called using the "populations" module. We considered all 68 samples (65 wild plus three in cultivation) to be a single population ($p = 1$) and retained only those loci that were present in >50% of individuals ($r = 0.5$). Furthermore, we specified a minimum minor allele frequency (*min_maf* = 0.05) and a maximum observed heterozygosity (*max_obs_het* = 0.95) during the SNPs calling. In addition, for comparison, we also applied *min_maf* = 0.01 (1%). To reduce genotyping error, loci that deviated from Hardy–Weinberg Equilibrium and that showed linkage disequilibrium ($r = 0.8$) were removed with VCFtools v0.1.14 (Danecek et al., 2011).

Population Genetic Analysis

We compared the genetic characteristics between the wild seedlings (UKNP) and ex situ collection (BBG) of *V. bantamensis*. In the case of bi-allelic SNPs markers, nucleotide diversity (π) is also a measure of expected heterozygosity (H_E) and a useful tool with which to measure overall genetic diversity in a population (J. Catchen, Bassham, Wilson, Currey, & Brien, 2013). Therefore, we calculated the mean of nucleotide diversity (π), and Tajima's D for each population using Stacks v2.2 (J. Catchen, Hohenlohe, et al., 2013; J. M. Catchen et al., 2011) and VCFtools v0.1.14 (Danecek et al., 2011), respectively. Tajima's D was calculated with sliding window of 1000 bp. We also computed values for observed heterozygosity (H_O) and inbreeding coefficient (F_{IS}) in Stacks v2.2 (J. Catchen, Hohenlohe, et al., 2013; J. M. Catchen et al., 2011) with 100 bootstrap resampling. We tested for population genetic differentiation using Weir and Cockerham (1984) F_{ST} to accommodate unequal sample size, calculated with hierfstat v0.4.30 (Goudet & Note, 2005). In addition, we computed the values for individual genetic diversity using homozygosity-by-loci (HL) that weighs the contribution of loci depending on their allelic variability (Aparicio, Ortego, & Cordero, 2006). The HL value varies between 0 and 1, with 0 being when all loci are heterozygous, and 1 when all loci are homozygous (Aparicio et al., 2006). In addition, internal relatedness (IR) was also calculated to measure of parental relatedness (Amos et al., 2001). When the IR value is 1, it means that all loci are homozygous, and conversely when the IR value is –1 (Coulon, 2010). HL and IR index computation was undertaken using the Genhet function (Coulon, 2010). Statistical significance of the mean differences of both values was determined using a two-tailed Student's t test.

We used discriminant analysis of principal components (DAPC) implemented in in adegenet v2.0.1 (Jombart, 2008) to assess the genetic structure of *V. bantamensis*. DAPC uses allele frequencies to minimize within-group variability and maximize between-group variability for the same purpose (Jombart, Devillard, & Balloux, 2010). First, we used the *find.clusters* function to estimate K (number of populations) and to retain all the principal components (PCs). Calculation of K was iterated several times to obtain the most representative K, as indicated by an elbow in the Bayesian Information Criterion curve. Second, we used the *dapc* function to discriminate the K. When applying the *dapc* function, retaining excessive PCs with respect to the number of samples can lead to overfitting and instability in the membership probability (Jombart & Collins, 2017). Therefore, we cross-validated the *dapc* by implementing the *xvaldapc* function to determine the optimal number of PCs. The inferred groups from DAPC were further analyzed with analysis of molecular variance (AMOVA; Meirmans, 2012) in poppr v2.6.1 (Kamvar, Tabima, & Grünwald, 2014).

In addition, we also infer genetic cluster using STRUCTURE software (Pritchard et al. 2000) to evaluate potential clusters (K) from 1 to 7 with 10 individual runs for each cluster. We applied admixture model without any prior geographic information, with burn-in value and Markov Chain Monte Carlo replications of 100k times (Porras-Hurtado et al., 2013). The optimal clusters (K) was determined based on ΔK value (Evanno et al., 2005), which was calculated and visualized using pophelper package v1.0.10 (Francis, 2017). All R packages were performed under the R 3.5.1 environment (R Core Team, 2018).

Results

Population Genetic Analysis

A total of 25,949,778 reads were obtained from the 68 samples (online Appendix S1), using the MIG-seq method. After filtering, trimming, and removing short-reads, 18,096,097 reads were retained for SNP calling in stacks software, generating a final catalog of 34,898 loci. From this catalog, a final set of 1,513 SNP loci was successfully called and genotyped. We filtered out loci with minimum minor allele frequency *(min_maf)* $< 5\%$ and removed loci exhibiting high pairwise linkage disequilibrium (threshold $r > 0.8$) and that deviated from Hardy–Weinberg Equilibrium. A final set of diploid 730 SNP loci were obtained from these procedures. A different parameter setting of minimum minor allele frequency (*min_maf* = 0.01) gave higher number of SNP

Table 1. Descriptive Statistics of Genetic Diversity of *Vatica bantamensis* for Each Population, Generated From 730 SNPs.

Population	No. of samples	No. of polymorphic sites	Missing data (%)	μ	Tajima's *D*	H_O	F_{IS}
Wild (Ujung Kulon National Park)	65	719	19.22	0.26 ± 0.005	0.5 ± 0.04	0.73 ± 0.005	–0.04 ± 0.4
Ex situ (Bogor Botanic Gardens)	3	203	7.17	0.16 ± 0.009	1.02 ± 0.05	0.76 ± 0.002	–0.12 ± 0.01

Note. Population genetic statistics are as follows: π = nucleotide diversity, H_O = observed heterozygosity, and F_{IS} = inbreeding coefficient. Mean values are shown with standard error.

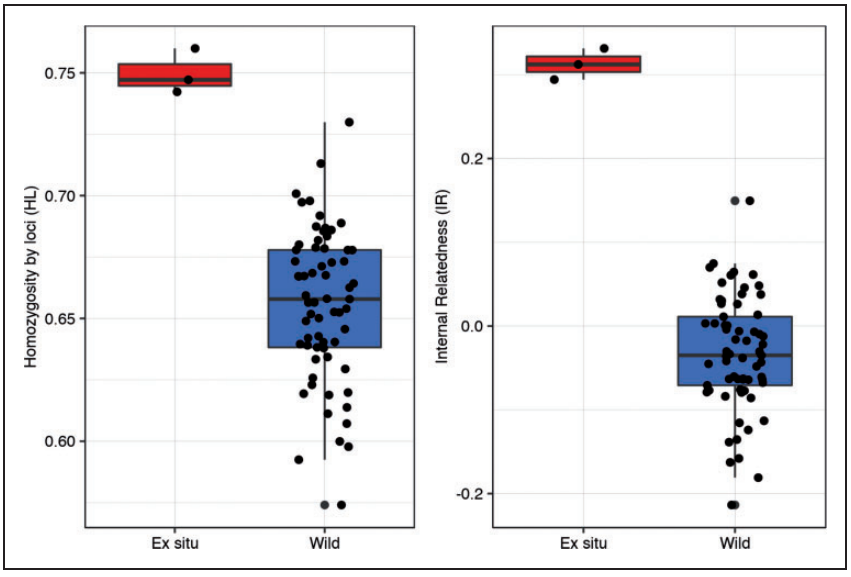

Figure 2. Comparison of the homozygosity-by-loci (HL) and internal relatedness (IR) of the ex situ collection and the wild seedlings of *Vatica bantamensis*.

loci (1,497). However, further downstream analysis (i.e., DAPC) shows similar result (online Appendix S2).

The proportion of missing data was 19.22% in the wild seedlings and 7.17% in ex situ collection. Nucleotide diversity (π) was higher in the wild seedlings (0.26) than in ex situ collection (0.16). Tajima's *D* was lower in the wild seedlings (0.5) than in ex situ collection (1.02). Observed heterozygosity (H_O) values in the wild seedlings and ex situ collection were almost identical (0.73 and 0.76, respectively). Furthermore, a negative inbreeding coefficient (F_{IS}) was detected in the wild seedlings and ex situ collection (–0.4 and –0.12, respectively), but with relatively high standard error (0.4 and 0.01, respectively; Table 1). On average, HL was lower in the wild seedlings than in ex situ (0.66 and 0.75, respectively; $t = 14.48$, $p < .001$; Figure 2). Similarly, IR in the wild is more negative (less related) than in the ex situ (–0.03 and 0.75, respectively; $t = 25.21$, $p < .001$). Moreover, the wild seedlings and ex situ collection of *V. bantamensis* showed strong genetic differentiation ($F_{ST} = 0.32$).

Genetic Structure of V. bantamensis

The genetic structure of *V. bantamensis* in the wild seedlings and ex situ collection was assessed using the DAPC method. The Bayesian Information Criterion value indicated that $K = 3$ was the best representation of the genetic data of *V. bantamensis* (Figure 3, online Appendix S3). DAPC used two discriminant functions from 21 PCs that accounted for 62.3% of the total variance of the genetic data. The results showed that all individuals from the ex situ collection were grouped into a single cluster, whereas the individuals from the wild seedlings were separated into two distinct clusters. On the other hand, in the STRUCTURE analysis, Evanno's ΔK indicated an increased peak from $K = 3$ to $K = 4$ and started to decrease at $K = 5$

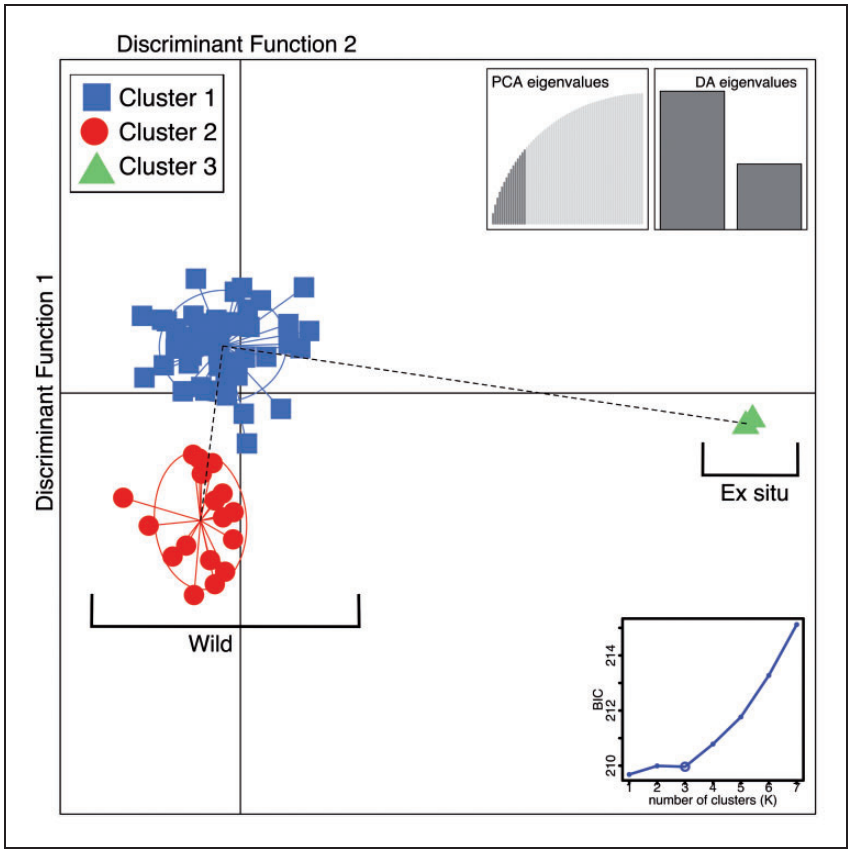

Figure 3. DAPC graph based on SNPs markers depicts three genetic clusters of *Vatica bantamensis*. Individuals belonging to the ex situ collection are separated from all the individuals from the wild seedlings. Clusters are represented by different color with red color coded for ex situ collection and other colors for wild seedlings.
DA = discriminant analysis; PCA = principal component analysis.

(Figure 4, online Appendix S3), suggesting three, four, or five clusters were inferred from the data set. AMOVA of the groups inferred from the DAPC supported the separation of all samples into three genetic clusters ($\phi = 0.48$, $p < .001$, Table 2), with the subdivision of the population explained 47.48% of the genetic variation between the populations and 52.52% of the genetic variation within the populations.

Discussion

Conserving the maximum genetic diversity of threatened species in living collections, including botanic gardens, is very challenging (Wee, Surget-groba, & Corlett, 2015), yet very important. Therefore, understanding the current genetic diversity and the genetic structure of known populations is necessary for developing appropriate conservation plans. Several metrics are used to assess genetic diversity in a population. In the current study, we focused on nucleotide diversity (π), Tajima's D, and the inbreeding coefficient (F_{IS}), because of their ability to measure overall genetic diversity, reduction in observed heterozygosity in a population (J. Catchen, Bassham, et al., 2013; Holsinger & Weir, 2009), and detection population change based on neutral theory (Nielsen, 2005; Simonsen, Churchill, & Aquadro, 1995; Tajima, 1989a, 1989b).

The nucleotide diversities detected in the wild seedlings and ex situ collection of *V. bantamensis* were 0.26 and 0.16, respectively. These values were comparable to those reported for *Panax* spp. (Pan, Wang, Sun, Li, & Gong, 2016) and for *Amorphophallus paeoniifolius* (Araceae) studied with restriction site-associated DNA sequencing (RAD-seq) in China (Y. Gao et al., 2017). Other studies on tree species, such as *Dalbergia cochinchinensis* (Fabaceae) in Cambodia (Moritsuka et al., 2017), *Populus balsamifera* (Salicaceae) in Canada (Olson et al., 2010), *Pinus sylvestris* (Pinaceae; Wachowiak, Wójkiewicz, Cavers, & Lewandowski, 2014), and *Fagus sylvatica* (Fagaceae; Lalagüe et al., 2014) in Europe, reported even lower nucleotide diversity levels than those reported in the current study. The earlier studies used various gene sequences, including chloroplast gene, nuclear gene, and expressed sequence

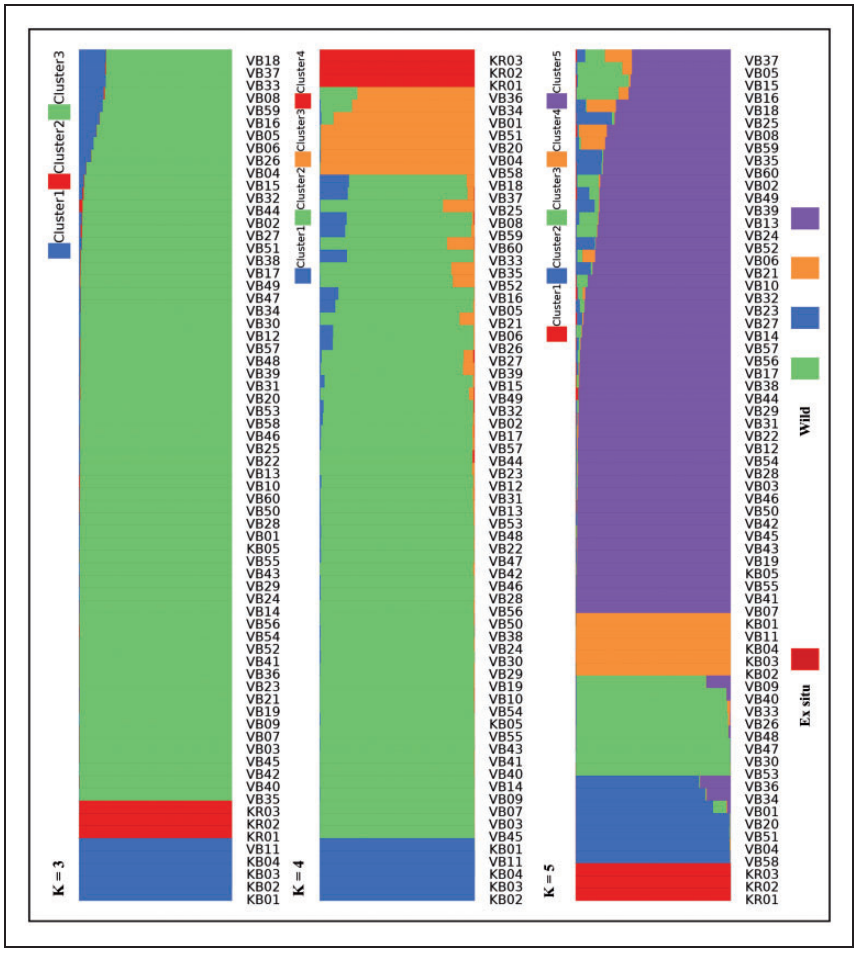

Figure 4. Genetic structure of *Vatica bantamensis* inferred using Bayesian clustering implemented in STRUCTURE. Three different clustering results ($K = 3$, $K = 4$, and $K = 5$) are shown. Clusters are represented by different color with red color coded for ex situ collection and other colors for wild seedlings.

Table 2. Analysis of Molecular Variance for Inferred Genetic Clusters of *Vatica bantamensis* From DAPC.

			Components of variance			
Source of variation	*df*	Sums of squares	Sigma	%	ϕ	*p*[a]
Between populations	2	341.16	20.34	47.48	0.48	.001
Within populations	65	1,462.84	22.50	52.52		
Total	67	1,804.00	42.84	100		

[a]*p*-value was obtained from a randomization test with 999 permutations.

tags to estimate the nucleotide diversity. Various genes or markers used in the studies may result in varying estimates of nucleotide diversity estimation due to variation in evolutionary rates (Nybom, 2004).

In both wild seedlings and ex situ collection of *V. bantamensis*, our analysis detected positive value for Tajima's *D* and negative value for F_{IS}. A positive Tajima's *D* indicates a population differentiation or balancing selection (Charlesworth, 2003). A negative F_{IS} represents an excess of heterozygosity (J. Catchen, Bassham, et al., 2013) caused by a small reproductive population size, over-dominance, negative assortative mating, or asexual reproduction (Stoeckel et al., 2006). Excess of heterozygosity (negative F_{IS}) in particular in the wild seedlings might be attributed to a bottleneck event in the past, resulting in a relatively small population size today. *V. bantamensis* is very rare and narrowly endemic species from the lowland forests in the

Mount Payung area, UKNP (Ashton, 1982). Repeated volcanic eruptions of Krakatoa in 1883, 1680, and hundreds of years before (Lamb, 1970) possibly devastated the population of many plant and animal species during these periods, as has been suggested for the Javan rhinoceros (Fernando et al., 2006). These events might have also reduced the size of the *V. bantamensis* population in UKNP that is close to the volcanic island of Krakatoa, causing a genetic bottleneck. Nevertheless, more thorough historical and demographic analysis is required to test this hypothesis. However, negative F_{IS} in ex situ collection was more likely due to small reproductive population size.

Our comparison of the total genetic diversity of *V. bantamensis* between wild seedlings and ex situ collection indicates that the ex situ collection had lower diversity than did the wild one. There have been many cases where ex situ populations exhibit lower levels of genetic diversity than the remnant wild populations (Ensslin et al., 2011; Y. Gao et al., 2017; Lauterbach et al., 2012; Miao et al., 2015; Rucinska & Puchalski, 2011). Nevertheless, there was a study reporting the opposite results, where the ex situ populations included comparable or greater genetic diversity than did the wild populations (J. Gao, He, & Li, 2012; LaBonte, Tonos, Hartel, & Woeste, 2017). Lower genetic diversity in the ex situ collection of *V. bantamensis* could be attributed to small population size ($n = 3$) and kinship among individuals.

Kinship was indicated by the higher HL value showed in the ex situ collection (mean HL = 0.75) compared with the wild seedlings (mean HL = 0.66; Figure 2). The HL value has been reported to be associated with the level of correlated paternity (Nora, Aparicio, & Albaladejo, 2016). Hence, a high HL in the ex situ collection of *V. bantamensis* suggested strong kinship between the three individuals. Correspondingly, parental relatedness analysis using IR index indicated that the ex situ collection is significantly higher than wild seedlings (mean IR = 0.75 compared with –0.03, $t = 25.31$, $p < .001$). It shows that ex situ collections have more closely related parent than wild seedlings. Generally, ex situ populations suffer most from the two stages of sampling effect that occurs during the establishment (founder effect) and decay of genetic diversity because of small population size (genetic drift; Yang & Yeh, 1992). In addition, genetically related samples (siblings in the case of the ex situ collection of *V. bantamensis*) may also have a strong influence on the reduction in genetic diversity, because on average, full siblings share approximately 50% of their genome (Stadele & Vigilant, 2016; Visscher et al., 2006).

Our present study also revealed that the founding collections of *V. bantamensis* in BBG already suffered from low genetic diversity, as indicated by the higher HL of all the ex situ individuals compared with the wild individuals (Figure 2). This result shows that understanding individual genetic diversity, that is, from using HL index, is important before selecting the wildlings to take part in living collections for ex situ conservation. Selecting for the greatest genetic diversity among all collected wild seedlings from the wild population will benefit the living collection in the future because it minimizes the risk of random genetic drift occurring in a small population.

Our current study also found that there was strong genetic differentiation between the wild seedlings and ex situ collection of *V. bantamensis* ($F_{ST} = 0.32$). The high F_{ST} value obtained using Weir and Cockerham formula takes into account of unequal sample size; however, a possibility of overestimation is also acknowledged. Genetically distinct populations in the ex situ and wild populations have also been reported in other species, including *Silene otites* (Caryophyllaceae; Lauterbach et al., 2012), *Taxus yunnanensis* (Taxaceae; Miao et al., 2015), and *Amorphophallus paeoniifolius* (Araceae; Y. Gao et al., 2017), indicating distinct genetic differences between cultivated (ex situ) and wild (in situ) populations. The ex situ collection of *V. bantamensis* has a very small population size and comes from two different generations, the original seedling from the wild population and its progeny. Therefore, a small effective population size (founder effect), interacting with genetic drift (Ellstrand & Elam, 1993; Lauterbach et al., 2012), contributed to the strong genetic differentiation between the ex situ collection and the wild population of *V. bantamensis*.

We found that, based on DAPC, the genetic structure of *V. bantamensis* demonstrated clear separation into three genetic clusters (Figure 3). This result was also supported by AMOVA ($\phi = 0.48$, $p < .001$). Based on DAPC analysis, Clusters 1 and 3 consisted of all the individuals from the wild seedlings, whereas Cluster 2 was composed of all the individuals from the ex situ collection. The two genetic clusters (Clusters 1 and 3) were observed in the wild seedlings did not attributed to geographical separation. Although, genetic differentiation between both genetic clusters was relatively low ($F_{ST} = 0.14$), the genetic variation may provide genetic pool that is important for future generation (Paaby & Rockman, 2014). Similar pattern on genetic clustering was also inferred based on STRUCTURE analysis with $K = 3$ (Figure 4). Moreover, group membership in DAPC results was precisely similar to the STRUCTURE analysis for $K = 3$ (Figure 4, online Appendix S4). On the other hand, small genetic similarity between ex situ individuals and the wild seedlings indicates that the original population source of the ex situ individuals is likely to have been extirpated. In addition, STRUCTURE analysis also detects more genetic clusters (K=4 and K=5) from the data set,

indicating possibility of more subdivision within the wild seedlings. However, to avoid possible bias and overrepresentation of clustering (Gilbert, 2016; Puechmaille, 2016), we choose the $K = 3$ which is congruent with the result of DAPC for the optimal genetic structure.

We suggested that the individuals from genetic Clusters 1 and 3 are potential new sources for collection from the wild to augment diversity in the ex situ collection. These new accessions from the new distinct genetic Clusters (1 and 3) would increase genetic diversity if incorporated into the ex situ collection. Ex situ collections benefits plant conservation by providing propagation materials for population improvement and reintroduction (Cochrane, Crawford, & Monks, 2007; Donaldson, 2009; Pennisi, 2010; Sharrock, 2012). Therefore, the preservation and maintenance of high levels of genetic diversity in ex situ collection are indispensable (Cavender et al., 2015; Hoban, Kallow, & Trivedi, 2018).

Conservation Implications

Conservation action of *V. bantamensis* is urgently required because of the low population size and the existence of only a single population in the wild, as far as is currently known. Similarly, the number of individuals in the living *V. bantamensis* collection in BBG is also relatively small. Yet, this is the only available ex situ collection of the species. Low genetic diversity in the ex situ collection indicates that additional collection from the wild is necessary to increase the genetic diversity of the ex situ collection. Therefore, it is important that the two genetic clusters identified from the wild seedlings are also represented in the ex situ collection to increase the genetic diversity.

In addition, we also illustrate in the study how using molecular markers to detect genetic structure in the population of a threatened plant species can help to determine the optimum number of collections for ex situ conservation. We underline the importance of performing such analyses in other living collections in the BBG and other botanic gardens, and to take the approach into account for the future ex situ conservation plans. Finally, our study contributes to the development of more effective and rationalized measure for ex situ conservation strategies in the limited confines of the botanic gardens. In particular, this can be achieved by providing a method to evaluate the genetic representativeness of the ex situ collections and to select the most appropriate candidates for the living collection from the wild to cover most of the genetic variations present in the wild.

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References

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D., & Coulson, T. (2001). The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1480), 2021–2027. doi:10.1098/rspb.2001.1751

Aparicio, J. M., Ortego, J., & Cordero, P. J. (2006). What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology*, *15*(14), 4659–4665. doi:10.1111/j.1365-294X.2006.03111.x

Ashton, P. S. (1982). Dipterocarpaceae. *Flora Malesiana - Series 1, Spermatophyta*, *9*(2), 237–552. Retrieved from http://www.biodiversitylibrary.org/item/90889

Austin, K. G., Schwantes, A., Gu, Y., & Kasibhatla, P. S. (2018). What causes deforestation in Indonesia? *Environmental Research Letters*, *14*(2), 024007. doi:10.1088/1748-9326/aaf6db

Backer, C. A., & Bakhuizen van den Brink, R. C., Jr. (1963). *Flora of Java volume I (Spermatophytes only)*. Groningen, The Netherlands: N.V. P. Noordhoff.

Botanic Gardens Conservation International. (2017a). *GardenSearch online database*. Retrieved from www.bgci.org/garden_search.php

Botanic Gardens Conservation International. (2017b). *PlantSearch online database*. Retrieved from www.bgci.org/plant_search.php

Brearley, F. Q., Proctor, J., Nagy, S. L., Dalrymple, G., & Voysey, B. C. (2007). Reproductive phenology over a 10-year period in a lowland evergreen rain forest of central Borneo. *Journal of Ecology*, *95*(4), 828–839. doi:10.1111/j.1365-2745.2007.01258.x

Catchen, J., Bassham, S., Wilson, T., Currey, M., & Brien, C. O. (2013). The population structure and recent colonization history of Oregon threespine stickleback determined using restriction-site associated DNA-sequencing. *Molecular Biotechnology*, *22*, 2864–2883. doi:10.1111/mec.12330

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. doi:10.1111/mec.12354

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J. H., & De Koning, D. J. (2011). Stacks: Building and genotyping loci de novo from short-read sequences. *G3*, *1*(3), 171–182. doi:10.1534/g3.111.000240

Cavender, N., Westwood, M., Bechtoldt, C., Donnelly, G., Oldfield, S., Gardner, M., … McNamara, W. (2015). Strengthening the conservation value of ex situ tree collections. *Oryx*, *49*(3), 416–424. doi:10.1017/S0030605314000866

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *358*(1434), 1051–1070. doi:10.1098/rstb.2003.1296

Cochrane, J. A., Crawford, A. D., & Monks, L. T. (2007). The significance of ex situ seed conservation to reintroduction of threatened plants. *Australian Journal of Botany*, *55*(3), 356–361. doi:10.1071/BT06173

Coulon, A. (2010). Genhet: An easy-to-use R function to estimate individual heterozygosity. *Molecular Ecology Resources*, *10*(1), 167–169. doi:10.1111/j.1755-0998.2009.02731.x

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. doi:10.1093/bioinformatics/btr330

Donaldson, J. S. (2009). Botanic gardens science for conservation and global change. *Trends in Plant Science*, *14*(11), 608–613. doi:10.1016/j.tplants.2009.08.008

Ellstrand, N. C., & Elam, D. R. (1993). Population genetic consequences of small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics*, *24*(1993), 217–242. doi:10.1146/annurev.es.24.110193.001245

Ensslin, A., Sandner, T. M., & Matthies, D. (2011). Consequences of ex situ cultivation of plants: Genetic diversity, fitness and adaptation of the monocarpic *Cynoglossum officinale* L. in botanic gardens. *Biological Conservation*, *144*(1), 272–278. doi:10.1016/j.biocon.2010.09.001

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14* (8), 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Fernando, P., Polet, G., Foead, N., Ng, L. S., Pastorini, J., & Melnick, D. J. (2006). Genetic diversity, phylogeny and conservation of the Javan rhinoceros (*Rhinoceros sondaicus*). *Conservation Genetics*, *7*(3), 439–448.

Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendus Biologies*, *326*, 22–29. doi:10.1016/S1631-0691(03)00023-4

Francis, R. M. (2017). pophelper: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources, 17*(1), 27–32. doi: 10.1111/1755-0998.12509

Gao, J., He, T., & Li, Q. M. (2012). Traditional home-garden conserving genetic diversity: A case study of *Acacia pennata* in southwest China. *Conservation Genetics*, *13*(4), 891–898. doi:10.1007/s10592-012-0338-x

Gao, Y., Yin, S., Wu, L., Dai, D., Wang, H., Liu, C., & Tang, L. (2017). Genetic diversity and structure of wild and cultivated *Amorphophallus paeoniifolius* populations in southwestern China as revealed by RAD-seq. *Scientific Reports*, *7*, 14183. doi:10.1038/s41598-017-14738-6

Gilbert, K. J. (2016). Identifying the number of population clusters with structure: Problems and solutions. *Molecular Ecology Resources*, *16*(3), 601–603. doi:10.1111/1755-0998.12521

Goudet, J., & Note, P. (2005). HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, *2*(1), 184–186. doi:10.1111/j.1471-8278

Hermy, M., Honnay, O., Jacquemyn, H., & Brys, R. (2014). Conservation of Plants. In eLS, John Wiley & Sons, Ltd (Ed.). doi: 10.1002/9780470015902.a0003353.pub2'

Higginbottom, T. P., Collar, N. J., Symeonakis, E., & Marsden, S. J. (2019). Deforestation dynamics in an endemic-rich mountain system: Conservation successes and challenges in West Java 1990–2015. *Biological Conservation*, *229*, 152–159. doi:10.1016/j.biocon.2018.11.017

Hoban, S., Kallow, S., & Trivedi, C. (2018). Implementing a new approach to effective conservation of genetic diversity, with ash (*Fraxinus excelsior*) in the UK as a case study. *Biological Conservation*, *225*, 10–21. doi:10.1016/j.biocon.2018.06.017

Hoban, S., & Schlarbaum, S. (2014). Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. *Biological Conservation*, *177*, 90–99. doi:10.1016/j.biocon.2014.06.014

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: Defining, estimating and interpreting F(ST). *Genetics*, *10*, 639–650. doi:10.1038/nrg2611

Hommel, P. W. F. M. (1990). A phytosociological study of a forest area in the humid tropics (Ujung Kulon, West Java, Indonesia). *Vegetatio*, *89*(1), 39–54.

Jombart, T. (2008). Adegenet: An R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. doi:10.1093/bioinformatics/btn129

Jombart, T., & Collins, C. (2017). *A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 2.1.0*. London, England: Imperial College London.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94. doi:10.1186/1471-2156-11-94

Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: An R package for genetic analysis of populations

with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, *2*, e281. doi:10.7717/peerj.281

LaBonte, N., Tonos, J., Hartel, C., & Woeste, K. E. (2017). Genetic diversity and differentiation of yellowwood [*Cladrastis kentukea* (Dum.Cours.) Rudd] growing in the wild and in planted populations outside the natural range. *New Forests*, *48*(2), 1–12. doi:10.1007/s11056-017-9566-8

Lalagüe, H., Csilléry, K., Oddou-Muratorio, S., Safrana, J., de Quattro, C., Fady, B., … Vendramin, G. G. (2014). Nucleotide diversity and linkage disequilibrium at 58 stress response and phenology candidate genes in a European beech (*Fagus sylvatica* L.) population from south-eastern France. *Tree Genetics and Genomes*, *10*(1), 15–26. doi:10.1007/s11295-013-0658-0

Lamb, H. H. (1970). Vulcanic dust in the atmosphere; with a chronology and assessment of its meteorological significance. *Philosophical Transactions of the Royal Society A*, *266*(1178), 425–533.

Lauterbach, D., Burkart, M., & Gemeinholzer, B. (2012). Rapid genetic differentiation between ex situ and their in situ source populations: An example of the endangered *Silene otites* (Caryophyllaceae). *Botanical Journal of the Linnean Society*, *168*(1), 64–75. doi:10.1111/j.1095-8339.2011.01185.x

Mantyka-pringle, C. S., Martin, T. G., & Rhodes, J. R. (2012). Interactions between climate and habitat loss effects on biodiversity: A systematic review and meta-analysis. *Global Change Biology*, *18*(4), 1239–1252. doi:10.1111/j.1365-2486.2011.02593.x

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10–12. doi:10.14806/ej.17.1.200

Meirmans, P. G. (2012). AMOVA-based clustering of population genetic data. *Journal of Heredity*, *103*(5), 744–750. doi:10.1093/jhered/ess047

Miao, Y. C., Su, J. R., Zhang, Z. J., Lang, X. D., Liu, W-. D., & Li, S. F. (2015). Microsatellite markers indicate genetic differences between cultivated and natural populations of endangered *Taxus yunnanensis*. *Botanical Journal of the Linnean Society*, *177*(3), 450–461. doi:10.1111/boj.12249

Moritsuka, E., Chhang, P., Tagane, S., Toyama, H., Sokh, H., Yahara, T., & Tachida, H. (2017). Genetic variation and population structure of a threatened timber tree *Dalbergia cochinchinensis* in Cambodia. *Tree Genetics and Genomes*, *13*(6), 115–126. doi:10.1007/s11295-017-1199-8

Nielsen, R. (2005). Molecular signatures of natural selection. *Annual Review of Genetics*, *39*(1), 197–218. doi:10.1146/annurev.genet.39.073003.112420

Nora, S., Aparicio, A., & Albaladejo, R. G. (2016). High correlated paternity leads to negative effects on progeny performance in two mediterranean shrub species. *PLoS One*, *11*(11), 1–15. doi:10.1371/journal.pone.0166023

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, *13*(5), 1143–1155. doi:10.1111/j.1365-294X.2004.02141.x

Oldfield, S. F. (2009). Botanic gardens and the conservation of tree species. *Trends in Plant Science*, *14*(11), 581–583. doi:10.1016/j.tplants.2009.08.013

Olson, M. S., Robertson, A. L., Takebayashi, N., Silim, S., Schroeder, W. R., & Tiffin, P. (2010). Nucleotide diversity and linkage disequilibrium in balsam poplar (*Populus balsamifera*). *New Phytologist*, *186*(2), 526–536. doi:10.1111/j.1469-8137.2009.03174.x

Paaby, A. B., & Rockman, M. V. (2014). Cryptic genetic variation: Evolution's hidden substrate. *Nature Reviews Genetics*, *15*, 247–258. doi:10.1038/nrg3688

Pacifici, M., Foden, W. B., Visconti, P., Watson, J. E. M., Butchart, S. H. M., Kovacs, K. M., Rondinini, C. (2015). Assessing species vulnerability to climate change. *Nature*, *5*, 215–225. doi:10.1038/nclimate2448

Pan, Y., Wang, X., Sun, G., Li, F., & Gong, X. (2016). Application of RAD sequencing for evaluating the genetic diversity of domesticated *Panax notoginseng* (Araliaceae). *PLoS One*, *11*(11), e0166419. doi:10.1371/journal.pone.0166419

Pautasso, M., & Parmentier, I. (2007). Are the living collections of the world's botanical gardens following species-richness patterns observed in natural ecosystems? *Botanica Helvetica*, *117*(1), 15–28. doi:10.1007/s00035-007-0786-y

Pennisi, E. (2010). Tending the global garden. *Science*, *329*(5997), 1274–1277. doi:10.1126/science.329.5997.1274

Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., & Lareu, M. V. (2013). An overview of STRUCTURE: applications, parameter settings, and supporting software. *Frontiers in Genetics, 4*(98), 1–13. doi:10.3389/fgene.2013.00098

Potter, K. M., Jetton, R. M., Bower, A., Jacobs, D. F., Man, G., Hipkins, V. D., & Westwood, M. (2017). Banking on the future: Progress, challenges and opportunities for the genetic conservation of forest trees. *New Forests*, *48*(2), 153–180. doi:10.1007/s11056-017-9582-8

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945–959. doi: 10.1111/j.1471-8286.2007.01758.x

Pritchard, D. J., Fa, J. E., Oldfield, S., & Harrop, S. R. (2011). Bring the captive closer to the wild: Redefining the role of ex situ conservation. *Oryx*, *46*(1), 18–23. doi:10.1017/S0030605310001766

Pritchard, H. W., Moat, J. F., Ferraz, J. B. S., Marks, T. R., Luís, J., Camargo, C., … Ferraz, I. D. K. (2014). Innovative approaches to the preservation of forest trees. *Forest Ecology and Management*, *333*, 88–98. doi:10.1016/j.foreco.2014.08.012

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3), 608–627. doi:10.1111/1755-0998.12512

R Core Team. (2018). R: *A language and environment for statistical computing*. Vienna, Austria. Retrieved from https://www.r-project.org/

Rivers, B. M., Shaw, K., Beech, E., & Jones, M. (2015). *Conserving the world's most threatened trees a global survey of ex situ collections*. London, England: Botanic Gardens Conservation International.

Robiansyah, I. (2018). *Vatica bantamensis* (errata version published in 2018). *The IUCN Red List of Threatened Species*

2018: e.T31319A135558901. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T31319A125626167.en

Rucinska, A., & Puchalski, J. (2011). Comparative molecular studies on the genetic diversity of an ex situ garden collection and its source population of the critically endangered polish endemic plant. *Biodiversity & Conservation*, *20*, 401–413. doi:10.1007/s10531-010-9965-z

Sharrock, S. (2012). *Global strategy for plant conservation a guide to the GSPC all the targets, objectives and facts*. Richmon, England: Botanic Gardens Conservation International.

Simonsen, K. L., Churchill, G. A., & Aquadro, C. F. (1995). Properties of statistical tests of neutrality for DNA polymorphism data. *Genetics*, *141*, 413–429.

Soerianegara, I., & Lemmens, R. H. M. J. (Eds.). (1994). *Plant resources of South-East Asia No. 5(1): Timber trees: Major commercial timbers*. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation (PUDOC).

Stadele, V., & Vigilant, L. (2016). Strategies for determining kinship in wild populations using genetic data. *Ecology and Evolution*, *6*(17), 6107–6120. doi:10.1002/ece3.2346

Stoeckel, S., Grange, J., Fernández-Manjarres, J. F., Bilger, I., Frascaria-Lacoste, N., & Mariette, S. (2006). Heterozygote excess in a self-incompatible and partially clonal forest tree species - *Prunus avium* L. *Molecular Ecology*, *15*(8), 2109–2118. doi:10.1111/j.1365-294X.2006.02926.x

Suyama, Y., & Matsuki, Y. (2015). MIG-seq: An effective PCR- based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. *Scientific Reports*, *5*, 16963. doi:10.1038/srep16963

Suzuki, E., & Ashton, P. S. (1996). Sepal and nut size ratio of fruits of Asian Dipterocarpaceae and its implications for dispersal. *Journal of Tropical Ecology*, *12*(6), 853–870.

Tajima, F. (1989a). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*(3), 585–595.

Tajima, F. (1989b). The effect of change in population size on DNA polymorphism. *Genetics*, *123*(3), 597–601.

The International Plant Names Index. (2012). *Vatica* L. Retrieved from http://www.ipni.org

The Plant List. (2013). *The Plant List, Version 1.1*. Retrieved from http://www.theplantlist.org/

Tilman, D., Clark, M., Williams, D. R., Kimmel, K., Polasky, S., & Packer, C. (2017). Future threats to biodiversity and pathways to their prevention. *Nature*, *546*(7656), 73–81. doi:10.1038/nature22900

Visscher, P. M., Medland, S. E., Ferreira, M. A. R., Morley, K. I., Zhu, G., Cornes, B. K., … Martin, N. G. (2006). Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. *PLoS Genetics*, *2*(3), e41. doi:10.1371/journal.pgen.0020041

Volis, S. (2017). Complementarities of two existing intermediate conservation approaches. *Plant Diversity*, *39*(6), 379–382. doi:10.1016/j.pld.2017.10.005

Wachowiak, W., Wójkiewicz, B., Cavers, S., & Lewandowski, A. (2014). High genetic similarity between Polish and North European Scots pine (*Pinus sylvestris* L.) populations at nuclear gene loci. *Tree Genetics & Genomes*, *10*(4), 1015–1025. doi:10.1007/s11295-014-0739-8

Wee, A. K. S., Surget-groba, Y., & Corlett, R. (2015). Genetic optimization of trees. *BGjournal*, *12*, 18–20.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370. doi:10.2307/2408641

Whitten, T., Soeriaatmadja, R. E., & Afiff, S. A. (1996). *Ecology of Java & Bali*. Periplus Editions (HK) Limited. Retrieved from https://books.google.co.jp/books?id=_pIcG_aZGjsC

Wihermanto, D., Kusuma, Y. W. C., & Muhiban. (2015). Autekologi Kokoleceren *Vatica bantamensis* (Hassk.) Benth. & Hook. ex Miq. di Taman Nasional Ujung Kulon [Autecological study of Vatica bantamensis (Hassk.) Binn. & Hook. ex Miq. in Ujung Kulon National Park]. *Proceedings of "Ekspose Dan Seminar Pembangunan Kebun Raya Daerah"*, 161–170.

Yang, R., & Yeh, F. (1992). Genetic consequences of in situ and ex situ conservation of forest trees. *The Forestry Chronicle*, *68*(6), 720–729. Retrieved from http://pubs.cif-ifc.org/doi/abs/10.5558/tfc68720-6