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Full Length Research Paper

Economic importance of the endemic Sumatran lowland dipterocarp tree species (*Shorea javanica*)

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Shorea javanica **is an economically important dammar-producing tree, endemic to tropical lowland forest in Sumatra and Java, Indonesia. Total population size, however, is very limited and endangered. We sequenced three non-coding regions of chloroplast DNA and three nuclear genes, and genotyped seven microsatellite loci in six populations representing the current species distribution. There was no variation in the chloroplast DNA regions, except for one unique indel polymorphism. Mean silent site nucleotide diversity across three nuclear regions was 0.0011, which is much smaller than those of common dipterocarp species. Expected mean population heterozygosity in the microsatellite loci was 0.423, and the total heterozygosity was 0.477. These results show genetic variation in *S. javanica* is extremely low at both population and species levels. Genetic differentiation among populations was moderate (F_{ST} = 0.064 for nuclear genes and $F_{ST}^{\{ENA\}}$ = 0.076 for microsatellite loci), but this result is essentially caused by one isolated population in Oku. We suggest that conservation efforts should focus on promoting connectivity and augmenting population size across the species range. *Ex-situ* conservation should also be considered to provide insurance against catastrophic events and to facilitate for reintroduction.**

Key words: *Shorea javanica*, repong dammar, chloroplast DNA, nuclear genes, microsatellite markers.

INTRODUCTION

Rarity is often a precursor to extinction and can be classified as a condition in which a species occurs at low abundance and occupies a narrow environmental range (Rabinowitz, 1981). Under this simple classification scheme, species that are most vulnerable are those with a low density and low population size and that utilize a narrow range of habitats. This is not only a scheme for defining rarity (Manne and Pimm, 2001), but is also one of the most frequently applied criteria (Ladle and Whittaker, 2011). Dissecting the causes and consequences of rarity is often difficult. For example, many rare plants are endangered largely because of their limited population size and restricted habitats. Isolated plant species with a small population size are often vulnerable to demographic, environmental and stochastic genetic degradation (Ellstrand and Elam, 1993; Frankham et al., 2002), and therefore, face a higher risk of local extinction (Lande, 1998; Holsinger, 2000; Keller and Weller, 2002). The loss of genetic diversity limits evolutionary potential (Johansson et al., 2007), and as a result, populations are more likely to experience inbreeding depression (Willi et al., 2006; Wright et al., 2008). Plants with narrow habitat specificity and limited dispersal potential are at particular risk of global extinction, as landscapes become mosaic due to both anthropogenic activities and catastrophic events such as flood, landslide, volcanic eruption, earthquake, and tsunami.

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Southeast Asia is the center for species diversity of the large tree family Dipterocarpaceae (dipterocarps), which consists of 10 genera with 380 species. Dipterocarps show a high rate of endemism; that is, more than half of the species have a narrow distribution range (Ashton, 1982). In the past, conservation of dipterocarps was not an important issue as the family was common and abundant, and therefore, no species were presumed threatened (Lee et al., 2006). However, due to a decline in natural populations as a result of extensive fragmentation in tropical forests, conservation of dipterocarps has started to gain more concern. Analysis of genetic variation and structure is therefore essential in developing an effective conservation plan.

One species that is threatened with extinction as a result of habitat loss and fragmentation is the dammar tree, *Shorea javanica*. *S. javanica* Koord and Valeton is endemic to Indonesia, and has been put forward as a candidate for the IUCN red list of endangered plant species (Ministry of Indonesian Forestry; https://www.forestry.go.id). The dammar of this species, locally known as *damar mata kucing* (cat eye resin) due to its clear resin product, was an important commodity more than a century ago. Indonesia is currently the only dammar producing country in the world, with most of the resin produced from *S. javanica* trees harvested in a single region, Krui, in Western Lampung-Sumatra. Lampung swidden farmers began domesticating dammar from nearby natural forest sometime in the nineteenth century. Damar agroforest, locally knowns as *repong damma*r, are established at the expense of the primary forest. During forest transformation, farmers preserved the dammar trees and intercropped dammar seedlings with upland rice and vegetables in the first two years. Coffee and/or pepper vine take over from the third to tenth year. The relay is then taken by various fruit trees, and the first dammar harvest does not occur until around 20 years after planting. It is a rare case for successful cultivation of an indigenous species in Indonesia (Michon et al., 2000).

S. javanica occurs in primary and secondary forests on dry or periodically inundated areas of flat land or slopes up to 300 to 500 m elevation (Orwa et al., 2009). Soil study in *repong dammar* garden (Torquebiau, 1984) has shown that layer 1 and 2 are composed of a recent deposit of volcanic ash materials, while layer 3 and 4 are characterized by hallosite and methalloysite. These two fractions are the most frequent clay minerals formed by weathering of volcanic materials under the tropical wet climate. This study clearly showed that *S. javanica* required habitat specificity as the species only grown on particularly good fertile volcanic soils. In Sumatra, the Bukit Barisan mountain belt runs for 1700 km down the western side of the island from the top to the bottom, with a width of 50 to 100 km. *S. javanica* is assumed to have been predominant in a narrow coastal strip of lowland forest adjacent to the higher-elevation and more mountainous Bukit Barisan mountain belt. Dried her-barium specimens in Edinburgh Royal Botanic Gardens (www.rbge.org.uk) recorded the presence of the species in the upper part (Singkil, Aceh) and the southwestern coast of Sumatra (Teluk Betung, Lampung). However, its distribution might become smaller, more fragmented and patchily due to extensive deforestation in this region. Indeed, our field surveys showed that at present, most of the populations is maintained in the *repong dammar* and grows naturally only in Bukit Barisan Selatan National Park. Although the literature refers to Central Java as being within the natural distribution (Newman et al., 1996, 1998), only a single mature tree can now be found in Sancang Nature Reserve on the southern coast of West Java Province, along with tree collections in the Bogor Botanical Garden.

After five years of extensive monitoring, Sumatran tropical rainforest was listed as a site of World Heritage in Danger in 2011. Consequently, an emergency conservation plan is a priority. Road construction and agricultural encroachment are among the major threats (whc.unesco.org/list/en/list/1167), and in addition, *repong dammar* farmers are under pressure by commercial operations aimed at converting their gardens into oil palm plantations (Yanagisawa, 1997). Due to its improved infrastructure, and proximity to Jakarta and Java Island, Krui is a prime target for migration and oil palm plantation development (Wollenberg et al., 2004).

In addition to anthropogenic threats, catastrophic events such as frequent earthquakes and tsunamis have also played a role in habitat loss for this species. In the last 10 years, earthquake with the moment magnitude of >7.5 to 9.2 hit Sumatra more than five times. Furthermore, the rupture area for the 2005 Sumatra earthquake is similar to that estimated for the 1861 event and caused a 1-m-deep subsidence at Singkil Baru (New Singkil). The regions affected by the earthquake and tsunami along the southwest coast of Sumatra Islands is known as natural distribution for *S. javanica*, and among them Singkil, Bengkulu, and Liwa are important sites for *repong dammar* cultivation.

Molecular marker-based genetic studies are crucial for elucidating appropriate management units for both rare endemic species and their wide congeners. The chloroplast genome is considered a single, non-recombining unit of inheritance and structurally stable. It is primarily maternally inherited in angiosperms (Olmstead and Palmer, 1994; Birky, 1995). Generally in plants, the mitochondrial genome evolves slowest (0.2 to 1.0 × 10^{-9}), the chloroplast at a slightly faster (1.0 to 3.0 × 10^{-9}) and the nuclear genome at the fastest rate (5 to 30 × 10^{-9}) (Wolfe et al., 1987; Gaut, 1998). Microsatellite markers generally have much high-mutation rates of approximately 10^{-3} at the fastest (Jarne and Lagoda, 1996), resulting in high standing allelic diversity. In species with small or recently bottlenecked populations, markers with

Figure 1. *Shorea javanica* trees managed at repong dammar garden in Krui region (A), its leaf morphology (B) and its fruits (C).

a relatively low mutation rate are likely to be invariant and only those loci with the highest mutation rates are likely to be informative (Hedrick, 1999). Microsatellites have been used to study present day demography and connectivity patterns, changes in the recent past (10 to 100 generations), paternity (Queller et al., 1993), and population structure and gene flow (Kalinowski, 2002; Wilson and Rannala, 2003). Including both uniparetally and biparentally inherited genetic markers with different evolutionary rates, we are expecting to obtain clearer species history from the last glacial period to the recent past. In this study, we hypothesize that since *S. javanica* is an endemic species with limited geographical distribution and a highly specific habitat preference, it harbors low genetic diversity; and as a result of severe habitat loss, range contraction, and population decline, *S. javanica* may be at risk of a deleterious population effect such as significant inbreeding depression. To test these hypotheses, we estimate the level of genetic variation and differentiation among populations based on chloroplast and nuclear DNA sequences and microsatellite markers, and discuss about the historical and demographic causes that shape the population genetic structure *S. javanica*.

MATERIALS AND METHODS

Plant materials

Sampling sites in Sumatra were selected based on references of *S. javanica* distribution (Newman et al., 1996, 1998) to cover the present geographical distribution. Sampling was also performed in an additional two sites in Java. In Sumatra, 15 to 16 mature trees were sampled from each site. Here, mature trees refer to trees, currently being tapped for dammar resin, generally aged 15 years or more with a minimum diameter of 25 cm at breast height. The morphology of the species is shown in Figure 1. A total of 80 mature trees were sampled in seven different geographic locations (Table 1, Figure 2); four from *repong damar* in Krui and one within the only remaining natural population in Kubu Perahu (Kp), Bukit Barisan Selatan National Park. In Java, since only one remaining tree was found in Sancang Nature Reserve, samples were also taken from the trees in Bogor Botanical Garden, which were collected from Java (exact sampling site is not known; personal communication), these samples were pooled together as the Java population. Sampled leaves were dried with silica gel and used for DNA extraction. Total genomic DNA was extracted from the leaf samples using a modified CTAB method (Murray and Thompson, 1980).

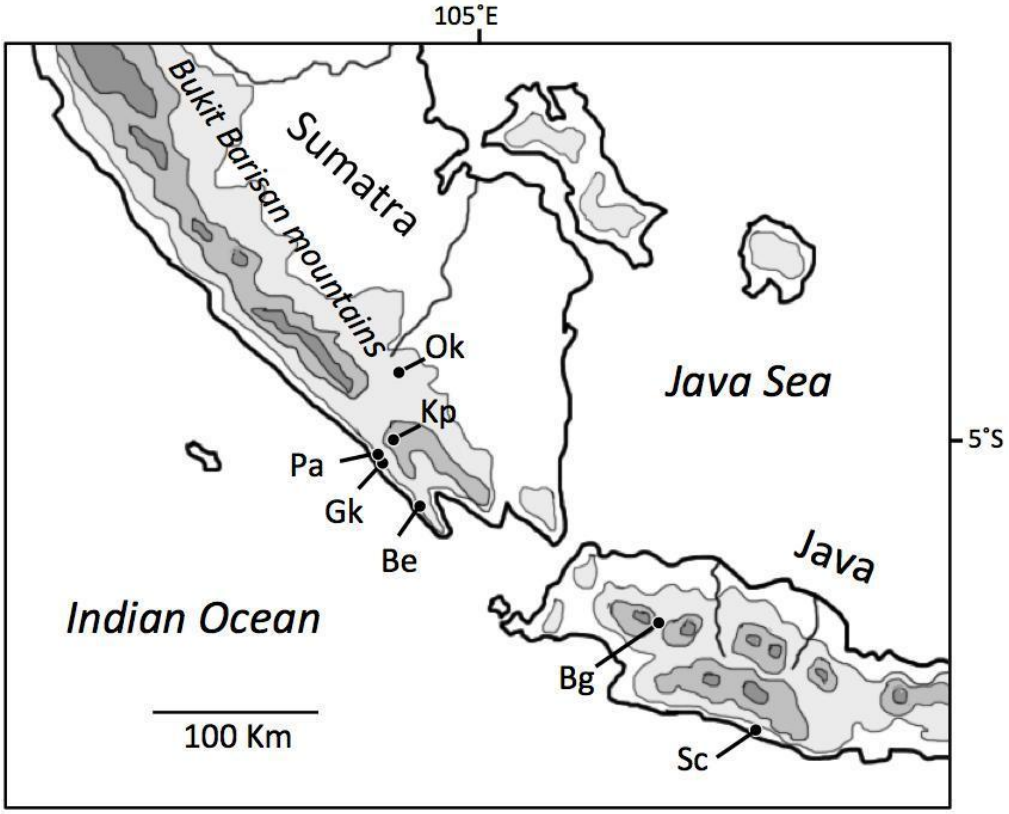

Figure 2. Sampling sites of *Shorea javanica* in Sumatra and Java.

Table 1. Sampling sites and sizes of the *Shorea javanica* populations examined.

Population	Location	Sample size (*N*)
Pahmungan (Pa)	05°09'40.32" S; 103°56'27.37" E	16
Gunung Kemala (Gk)	05°11'30.04" S; 103°55'49.43" E	16
Kubu Perahu (Kp)	05°02'07.29" S; 104°04'31.30" E	15
Bengkunat (Be)	05°36'12.83" S; 104°24'10.61" E	15
Ogan Komering Ulu (Oku)	04°16'51.33" S; 104°06'29.54" E	15
Sancang Natural Reserve (Sc)	06°36'03.06" S; 106°47'50.36" E	1
Bogor Botanical Garden (Bg)	07°43'03.04" S; 107°51'03.08" E	2

DNA sequencing of cpDNA noncoding regions and nuclear genes

Three cpDNA regions, *trn*L-*trn*F, *psb*C-*trn*S, and *trn*S-*trn*fM, were amplified by PCR using the universal primers described in Taberlet et al. (1991) for *trn*L-*trn*F and Demesure et al. (1995) for *psb*C-*trn*S and *trn*S-*trn*fM. Three nuclear genes, *GapC*, *PgiC* and *GBSSI*, were partially sequenced. The *GapC* was amplified and sequenced using primers designed by Ishiyama et al. (2003). We newly designed primers for *PgiC* and *GBSSI* based on DNA sequences of *Shorea* species deposited in GenBank (Kamiya et al., 2005; Kamiya et al., unpublished). Information on these primer sequences is shown in Table 2. PCR was performed in 20 μl of solution containing 10 ng of genomic DNA, 5 pmol of each forward and backward primer, and 10 μl of Go Taq® Hot Start Colourless Master Mix (Promega, Wisconsin, USA) according to the manufacturer's instructions. Initial denaturation was performed at 95°C for 2 min, followed by 30 to 35 cycles of denaturation at 95°C for 1 min, annealing at 52 to 62°C and polymerization at 72°C for 2 min, and final extension at 72°C for 7 min. Prior to sequencing, the PCR products were purified using rAPid Alkaline Phosphatase™ (Roche, Germany) and exonuclease I (New England Biolabs, Massachusetts, USA). The purified PCR products were sent for automated sequencing by Hokkaido System Science (Sapporo, Japan). DNA sequencing was performed for both strands with the primers used for the PCR amplifications.

Table 2. Primers used in this study.

Locus	Primer	Sequences (5'–3')	Length (bp)	Annealing temp (°C)	Reference
*trn*L-*trn*F	trnL-c trnL-f	CGAAATCGGTAGACGCTACG ATTTGAACTGGTGACACGAG	953	52	Taberlet et al. (1991)
*psb*C-*trn*S	psbC-f trnS-r	GGTCGTGACCAAGAAACCAC GGTCCGAATCCCTCTCTCTC	1510	57	Demesure et al. (1995)
*trn*S-*trn*fM	trnSfM-f trnSfM-r	GAGAGAGAGGGATTCGAACC CATAACCTTGAGGTCACGGG	1475	62	Demesure et al. (1995)
GapC	GapC dipF GapC dipR	AATGAAGGATTGGAGAGGTG CAATCTTTACCTGCTATCAC	751	53	Ishiyama et al. (2003)
PgiC	PgiC 14F PgiC 18R	TGGAATGTTTCGTTTCTTGG TCATGGTTGCTAACCACCTC	674	52	Kamiya unpublished
GBSSI	GBSSI dipF GBSSI dip 1F GBSSI dip 1R	GTGGTCTGCCACCTGCAAT TCAGTTTGATGTTGGAATTGG ACCTGCAGAGTAGGATTAGGG	778	58	Kamiya unpublished

Microsatellite genotyping

We initially screened 36 microsatellite loci derived from *Shorea leprosula* (Ng et al., 2009), *Shorea curtisii* (Ujino et al., 1998), and *Dipterocarpus tempehes* (Isagi et al., 2002). Seven loci, *Shc01, Shc04, Shc07, SleE08, SleE09, SleE10*, and *SleE14*, showed good amplification and were therefore used in this study. PCR premixture (10 μl) contained 10 ng genomic DNA, 1 × Type-it Multiplex PCR Master Mix (Qiagen, Duesseldorf, Germany), and 5 pmol each of forward primer labeled with either FAM, VIC, NED, or PET and backward tailed-primer (Applied Biosystems). PCR was performed with initial denaturation at 95°C for 5 min, followed by 31 cycles of 95°C for 0.30 s, annealing at 48 to 52°C for 1 min 30 s, and 72°C for 30 s. A final cycle at 60°C for 30 min was used to complete extension before holding the samples at 4°C. PCR products were diluted 200-fold with water, and 1 μl of diluted PCR product was mixed with 0.1 μl of Gene Scan^{TM} 500 LIZ size standard and 12μl Hi-Di^{TM} Formamide (Applied Biosystems). This was followed by denaturing at 95°C for 5 min and immediate chilling on ice. PCR products were resolved by capillary electrophoresis with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Allele sizes were assigned with GeneMapper software (Applied Biosystems).

Data analysis

DNA sequences were checked visually, and forward and backward traces were assembled using the ATGC program (Genetyx Corporation, Japan). Because the PCR products of nuclear regions were directly sequenced, it was not possible to determine the genotype if there were more than two heterozygous sites. However, estimates of certain population genetic parameters were obtained, such as the number of segregating sites and heterozygosity without determination of individual haplotype sequences. Haplotype diversity and nucleotide diversity (Nei, 1987) were calculated using DnaSP version 5.10 (Rozas et al., 2003). Tests of neutrality, Tajima *D* (1989), Fu and Li's *D** and *F** (1993), Fu's *Fs* (1997), *rg* (Harpending et al., 1993) and R_2 (Ramos-Onsins and Rozas, 2002), were also performed using DnaSP.

Population structure was examined using ARLEQUIN ver. 3.5 (Excoffier et al., 2005). Genetic differentiation, F_{ST} of nuclear loci was estimated through locus-by-locus analysis of molecular variance (AMOVA). Overall *F*-statistic values were determined for three nuclear genes by summing the variance components.

Based on the microsatellite genotype data, GenAlex (Peakall and Smouse, 2006) was used to calculate the average number of alleles per locus (*A*), number of effective alleles (A_e), and observed (H_o) and expected (H_e) heterozygosities. ARLEQUIN 3.5 (Excoffier et al., 2005) was used for conformance to Hardy-Weinberg expectation using the exact probability test with 100,000 permutations and linkage disequilibrium between every pair of loci in each population. MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004) was used to check the presence of null alleles for each population. The overall frequency of null alleles (*r*) in each population was estimated using the method of Brookfield (1996). We also used the IIM approach (individual inbreeding model) in INEst (Chybicki and Burczyk, 2009) to partition out the influence of null alleles on F_{IS} value. The level of population differentiation was quantified by computing global and pairwise estimator of F_{ST} (Weir, 1996). Following Chapuis and Estoup (2007), we used the ENA (excluding null alleles) correction method to efficiently correct for the positive bias induced by the presence of null alleles on estimation of F_{ST}. F_{ST} $^{\{ENA\}}$ values were computed using the program FreeNA (http://www.montpellier.inra.fr/URLB/).

RESULTS

DNA sequence variation

Sequences of 953, 1510, and 1475bp were determined

excluding alignment gaps for three noncoding regions of cpDNA, *trn*L-*trn*F, *psb*C-*trn*S, and *trn*S-*trn*fM, respectively. There were no segregating sites except for single occurrence of a 6-bp indel (TTTTTA) at *psb*C-*trn*S region in four of the 16 individuals from the Gk population. Sequences of 751, 778, and 674bp were determined excluding alignment gaps for three nuclear genes, *GapC*, *GBSSI*, and *PgiC*, respectively. A total of six segregating sites were found (Table 3). All mutations occurred at silent sites (that is, synonymous sites and introns). The average number of nucleotide differences per silent sites (π_S) in total populations ranged from 1.08 to 1.22 × 10^{-3}. Tajima's *D* showed no significant results (Table 3) and neither did the other four neutrality tests (data not shown).

Microsatellite variation

Three of the seven microsatellite loci (*Shc01*, *SleE09*, *SleE10*) were monomorphic in all of the six populations. Excluding these loci, only four polymorphic loci were used for further analysis. The mean expected heterozygosities (H_e) ranged from 0.352 (Ja) to 0.476 (Be) and inbreeding coefficients (F_{IS}) were significantly positive in four populations (Gk, Kp, Oku, and Be) (Table 4). At locus level, we found no significant deviation from Hardy-Weinberg equilibrium except for two loci in Be (*Shc07* and *SleE08*) and three loci in Oku (*Shc07*, *SleE08*, and *SleE14*), and no significant linkage dis-equilibrium for pairs of loci except for Be (*Shc04/SleE14, Shc07/SleE14*) and Oku populations (*Shc07/SleE14, SleE08/SleE14*) (data not shown). Null alleles were detected by MICRO-CHECKER in at least one locus at populations Kp, Be and Oku. The overall frequency of null alleles (*r*) in each population ranged from -0.012 (Pa) to 0.090 (Be). F_{IS} after excluding the presence of null alleles by individual inbreeding model implemented in INEst ($F_{IS;\ IIM}$) were ranged from 0.021 (Pa) to 0.118 (Be) (Table 4). None of them were significantly deviated from zero.

Population structure

Analysis of Molecular Variance (AMOVA) analysis for population differentiation across three nuclear loci showed 94% total variation within individuals and 6% ($P < 0.05$) among populations. A similar tendency was also observed using microsatellite data. F_{ST} for overall six sampled populations estimated by ENA method indicated that around 8% variation existed among population ($F_{ST\ \{ENA\}}$ = 0.0764; 95% C.I. = 0.0016 to 0.2268). Pairwise $F_{ST\ \{ENA\}}$ based on microsatellite data showed that the Oku population was differentiated from most of the other populations. Similar result was obtained from the nuclear gene data (Table 5). Accordingly, we excluded the Oku population and then re-conducted analysis. No population structure was subsequently detected for both nuclear loci and microsatellites (data not shown).

DISCUSSION

Low genetic variation in *S. javanica*

Endemic species tend to have a low level of genetic diversity due to their small population sizes (Hamrick and Godt, 1996). Therefore, considering the high habitat specificity and very restricted distribution of *S. javanica*, it is expected that populations show lower genetic variation than other dipterocarp species with a wider distribution. This is indeed the case for *S. javanica*, both at the population and species level. There were no variations in three cpDNA regions (*trn*L-*trn*F, *psb*C-*trn*S, and *trn*S-*trn*fM) among the 80 individuals analyzed. The very low cpDNA genetic diversity in this species is unusual compared with other dipterocarp species studied previously. A total of 21 cpDNA haplotypes were detected from 32 populations of *Neobalanocarpus heimii* (*trn*L intron, *trn*G intron, *trn*K intron, and *psb*K-*trn*S spacer regions; Tnah et al., 2009) and 15 haplotypes were found in eight populations of *S. curtisii* (*trn*H-*psb*A-*trn*K and *trn*L-*trn*F regions; Kamiya et al., 2011). These studies also showed that most of the populations contained multiple haplotypes. It could be that the low level of genetic variation is specific in the cpDNA regions examined in this study. However, considerable numbers of substitutions were previously observed within and between *Shorea* species in *trn*L-*trn*F and *psb*C-*trn*S regions (Tsumura et al., 2011). This observation suggests that the extremely low level of genetic variation in the cpDNA regions examined here is specific for *S. javanica* since other *Shorea* species maintain some extent of genetic variation within the populations.

S. javanica also showed a very low level of genetic diversity in nuclear DNA. The silent nucleotide diversity in the *GapC* region in *S. javanica* (π_S = 1.2 × 10^{-3}) was lower than that in *S. acuminata* (3.4 × 10^{-3}), *S. curtisii* (4.5 × 10^{-3}), *S. leprosula* (5.0 × 10^{-3}), and *S. parvifolia* (4.7 × 10^{-3}) (Ishiyama et al., 2003; Ishiyama et al., 2008). In the *PgiC* region, *S. javanica* also revealed much lower diversity (1.1 × 10^{-3}) compared with *S. acuminata* (11.2 × 10^{-3}), *S. curtisii* (5.5 × 10^{-2}), *S. leprosula* (2.6 × 10^{-3}) and *S. parvifolia* (6.4 × 10^{-3}) (Ishiyama et al., 2008).

Mean microsatellite heterozygosity across six popu-lations of *S. javanica* (H_e = 0.423) was not extremely low, but still relatively smaller than the estimates for other common dipterocarp species, including *S. curtisii*, *S. leprosula* and *S. macroptera* (H_e = 0.70 to 0.80; Ng et al., 2006). Comparatively high microsatellite genetic diversity was also observed in a rare endemic dipterocarp species, *S. lumutensis*

Table 3. Nucleotide variation in *Shorea javanica* as inferred from nuclear genes. *N*: number of sequences analyzed. H_d: haplotype diversity. *s*: number of segregating sites. π_s: nucleotide diversity in silent sites. θ_s: theta in silent sites.

Loci	Length (bp)	Population	*N*	*S*	H_d	($\times 10^{-3}$)		Tajima's *D*
						π_s	θ_s	
		Pa	32	2	0.571	0.94	0.48	1.557
		Gk	32	1	0.514	0.95	0.46	1.634
		Kp	30	2	0.662	1.50	0.93	1.235
GapC	751	Be	30	1	0.480	0.89	0.47	1.408
		Oku	30	2	0.660	1.56	0.93	1.356
		Java	6	1	0.536	1.11	0.81	1.445
		Total	160	2	0.581	1.22	0.65	1.223
		Pa	32	1	0.498	1.02	0.51	1.535
		Gk	32	3	0.508	1.25	1.53	-0.422
		Kp	30	1	0.508	1.04	0.52	1.578
GBSSI	778	Be	30	1	0.257	1.06	0.53	1.623
		Oku	30	1	0.370	0.76	0.52	0.727
		Java	6	1	0.536	1.10	0.79	1.167
		Total	160	3	0.509	1.08	1.09	-0.009
		Pa	32	1	0.508	1.16	0.57	1.578
		Gk	32	1	0.484	1.10	0.56	1.448
		Kp	30	1	0.434	0.99	0.57	1.124
PgiC	674	Be	30	1	0.515	1.17	0.56	1.647
		Oku	30	1	0.370	0.84	0.57	0.727
		Java	6	1	0.536	1.22	0.88	1.167
		Total	160	1	0.499	1.13	0.40	1.915

Table 4. Genetic diversity of microsatellite loci in six populations of *Shorea javanica.*

Population	*N*	*A*	A_e	H_o	H_e	F_{IS}	*r*	$F_{IS;IIM}$
Pa	16	2.50	2.00	0.456	0.418	-0.050	-0.012	0.021 (0.025)
Gk	16	2.25	2.05	0.344	0.432	0.234*	0.032	0.049 (0.056)
Kp	15	2.50	2.01	0.217	0.423	0.513**	0.063†	0.086 (0.093)
Be	15	2.50	2.12	0.179	0.476	0.647**	0.090†	0.118 (0.130)
Oku	15	2.50	2.14	0.183	0.438	0.604**	0.088†	0.061 (0.086)
Ja	3	2.50	1.83	0.313	0.352	0.250	*na*	0.080 (0.107)
Total	80	3.00	2.17	0.281	0.477	0.383**	0.068	0.077 (0.151)

N: sample size. *A*: mean number of alleles per locus. A_e: number of effective alleles. H_e: expected heterozygosity. F_{IS}: fixation index. *: significant deviation from HWE at $P < 0.05$; **: significant at $P < 0.01$ as calculated by GenAlex. *r*: estimated frequency of null alleles using the method of Brookfield, where † denotes null alleles detected in at least one locus using MICRO-CHECKER; *na*: not available. $F_{IS;IIM}$: fixation index by individual inbreeding model implemented by INEst with standard errors calculated by FreeNA in the parentheses.

(H_e = 0.609 to 0.673; Lee et al., 2006). More recently, Harata et al. (2011) studied microsatellite variation in 10 dipterocarps, indicating similar hetero-zygosity among locally common and rare species. These results suggest that rare species are not always characterized by low microsatellite heterozygosity. High microsatellite diversity could be observed in rare species if population size reduction and fragmentation occurred too recently to have resulted in a decrease in genetic variation (Lee et al., 2006). However, if this is the case, we should observe a comparable level of genetic diversity in the chloroplast and nucleotide sequence data. If monomorphic loci are not considered when estimating mean heterozygosity across microsatellite loci, the estimates of genetic diversity

Table 5. Pairwise F_{ST} between six populations of *Shorea javanica.*

Population	Pa	Gk	Kp	Be	Oku	Java
Pa	—	-0.026	0.081*	-0.007	0.140*	-0.018
Gk	0.000	—	0.091*	-0.009	0.158*	-0.034
Kp	0.022	0.010	—	0.043	0.052*	0.050
Be	0.009	0.005	0.005	—	0.077*	-0.064
Oku	0.154*	0.188*	0.210*	0.124	—	0.055
Java	0.067	0.006	0.024	0.010	0.314*	—

Pairwise *FST* values estimated from nuclear loci (upper-right) and microsatellites (*FST {ENA}*, lower-left). *: $P < 0.05$.

genetic diversity will not reflect the actual level of genomic variability. Indeed, mean H_e values within populations of *S. javanica* were about two times lower when monomorphic loci were included (H_e = 0.242). A differential level of nucleotide diversity between populations with a similar level of microsatellite diversity occurs partially as a result of exclusion of monomorphic or less variable loci in population surveys (Vali et al., 2008). In our study, it is noticeable that total heterozygosity in *S. javanica* is also low (H_e = 0.477; Table 4), which is at the similar level of the mean heterozygosity at population level. This means genetic variation in microsatellite loci in *S. javanica* is comparatively low at both population and species level.

Reproductive systems

Most dipterocarp species are insect pollinated (Bawa et al., 1985) and predominantly outcrossed (Ashton, 1969; Bawa, 1998; Lee et al., 2000; Nagamitsu et al., 2001). Controlled pollination experiments have indicated that many dipterocarps are self-incompatible (Chan and Appanah, 1980). Therefore, it is not surprising that no deviation from the HWE was observed in most dipterocarp species previously studied (Konuma et al., 2000; Kenta et al., 2004; Fukue et al., 2007; Harata et al., 2011). In contrast, we observed significantly positive F_{IS} in four out of the six population of *S. javanica* studied here (GK, Kp, Oku, and Be), indicating excess homozygotes (Table 4). We checked the presence of null alleles by MICRO-CHECKER and recalculated F_{IS} value excluding null alleles. F_{IS} values were reduced to essentially zero in all the six populations when null alleles were excluded ($F_{IS;IIM}$, Table 4). Considering the characteristic of reproductive system in dipterocarp and the values of adjusted F_{IS} ($F_{IS;\ IIM}$), deviation from HWE in most populations was primarily due to the presence on null alleles and not inbreeding. However, it is important to note that although there is indication for the presence of null alleles, several lines of evidence suggest that any potential bias stemming from null alleles is not likely to alter other population genetic parameters. For instance, simulation studies have shown that null alleles lead to an underestimation of allelic diversity and observed and expected heterozigosity, but that bias is particularly low for expected heterozigosity (Chapuis et al., 2008).

Population structure

The F_{ST} value for the total population in nuclear genes was 0.058, while for microsatellite loci the value was 0.076. Based on the qualitative guidelines for interpretation of F_{ST} suggested by Wright (1978), a moderate level of genetic differentiation (F_{ST} = 0.05 - 0.15) was found across all loci. Pairwise F_{ST} among pairs of populations revealed Oku as differentiated from the other populations in both nuclear and microsatellite loci. After excluding the Oku population from the analysis, no significant population differentiation was observed. This implies that the population structure of *S. javanica* is based mainly on the Oku population. During the last glacial epoch (c. 10,000 to 115.000 years ago; Cox and Moore, 2010), western and northern Sumatra together with northern and eastern Borneo, and the Mentawai Islands existed as rainforest refugia (Thomas, 2000; Gathorne-Hardy et al., 2002; Slik et al., 2003). The extant tropical forest in central and eastern Sumatra, therefore, appeared only recently after the last glacial maximum (c. 20,000 years ago). Considering the climatic history together with high habitat specificity and limited geographic distribution, very low genetic variation in *S. javanica* suggests that this species has been isolated and scattered on the west side of the Bukit Barisan mountain belts as relicts of the last glacial epoch. Krui might be one of those regions. The glacial epoch might also have separated Oku population from the others, and the occurrence of Bukit Barisan mountain belts has been acted as a geographic barrier.

Implication for conservation

Repong dammar has been managed over these 100

years by local peoples in Krui region. Nevertheless, the impact of human activities on the pattern of genetic variation in *S. javanica* appears to be scarce. Compare to the natural population in Kp, our study showed that genetic diversity were not reduced and inbreeding coefficients were not significantly different from zero, suggesting that selfing is weak or absent in this tree both in natural and *repong dammar* population. Population differentiation was also weak and only significant between Oku and other populations. Our field observations and interviews revealed that farmers collected seeds preferably from high-dammar-producing trees. This selection process might have reduced genetic diversity and have affected population structure. Considering the species has maintains low genetic variation since its divergence, it seems that the species has been able to avoid excessive inbreeding depression. But, current selection pressure and declining of population size are potent to reduce inter- and intra-population gene flow and may generate systematic genetic erosion.

From the view point of preserving genetic variation, which is one of the basic factors of biological diversity, connectivity within and among populations, including augmentation of population size across the species range should be promoted. Our genetic study also implied that both the natural population and *repong dammar* gardens play an important role equally in conservation of the species. Although *repong dammar* was established primarily for dammar production, certainly it acts as biodiversity refuges in the landscape. Given the rate of deforestation in Sumatra, 2.5 times more than the loss of humid tropical forest worldwide (Gaveau et al. 2009); anthropogenic activity is warranted as a major factor in further declines of this species.

As has been mentioned earlier (Michon et al. 2000), because the traditional agroforestry like *repong damar* has been managed by adapting to the local environmental conditions and includes diversity in the maintaining method itself, the system will be considered as providing biological diversity as well as genetic diversity as a whole. Thus, maintaining various *repong dammar* is a primary concern and conversion to another land use, especially monoculture-based system, should be avoided. Conserving *S. javanica* population in its native habitat is also an important issue; however, *ex-situ* conservation might also be considered to provide insurance against catastrophic loss and to facilitate for reintroduction.

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