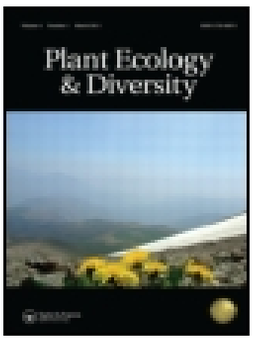

Plant Ecology & Diversity

ISSN: 1755-0874 (Print) 1755-1668 (Online) Journal homepage: http://www.tandfonline.com/loi/tped20

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To cite this article: Cheng Choon Ang, Michael J. O'Brien, Kevin Kit Siong Ng, Ping Chin Lee, Andy Hector, Bernhard Schmid & Kentaro K. Shimizu (2017): Genetic diversity of two tropical tree species of the Dipterocarpaceae following logging and restoration in Borneo: high genetic diversity in plots with high species diversity, Plant Ecology & Diversity, DOI: 10.1080/17550874.2016.1270363

To link to this article: http://dx.doi.org/10.1080/17550874.2016.1270363

Plant Ecology & Diversity, 2017
http://dx.doi.org/10.1080/17550874.2016.1270363

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Genetic diversity of two tropical tree species of the Dipterocarpaceae following logging and restoration in Borneo: high genetic diversity in plots with high species diversity

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(*Received 4 November 2015; accepted 30 September 2016*)

Background: The impact of logging and restoration on species diversity has been well studied in tropical forests. However, little is known about their effects on genetic diversity within species.
Aims: We assess the degree of genetic diversity among dipterocarp seedlings used for enrichment planting of selectively logged forests in Sabah, Malaysia, and compare it with diversity in naturally regenerating seedlings.
Methods: We sampled young leaf tissues from seedlings of *Shorea leprosula* and *Parashorea malaanonan* for DNA genotyping, using microsatellite markers.
Results: The levels of genetic diversity (expected heterozygosity and rarefied allelic richness) of naturally regenerating seedlings were statistically indistinguishable among unlogged, once logged and repeatedly logged forest areas. Enrichment-planted seedlings of *P. malaanonan* exhibited similar levels of genetic diversity to naturally regenerating seedlings whereas those of *S. leprosula* had significantly lower genetic diversity than natural seedlings. Interestingly, reduction of genetic variation was consistently observed in single-species plots relative to mixed-species plots among enrichment-planted seedlings.
Conclusions: There was no reduction of genetic variation in naturally regenerating dipterocarp seedlings in areas of selective logging. However, genetic variation of enrichment-planted seedlings was lower in single-species plots relative to mixed-species plots. This suggests that enrichment-planting strategies should adopt diverse mixtures that should promote levels of both species richness and genetic diversity within species.

Keywords: allelic richness; enrichment planting; forest regeneration; genetic diversity; heterozygosity; microsatellites; *Parashorea malaanonan*; *Shorea leprosula*; species diversity

Introduction

Tropical rain forests are well known for being the most species rich of all the terrestrial ecosystems on Earth (Myers et al. 2000). However, biodiversity in these forests is under threat because of global change drivers – e.g. logging, land-use change and increased severity of droughts (Lewis 2006; Reynolds et al. 2011; Wilcove et al. 2013; O'Brien et al. 2014, 2015). Restoration efforts have focused on restoring species diversity and forest structure post-logging, but there has been limited emphasis on genetic diversity within species, which is an important factor for understanding species adaptation and persistence under novel climates and biological interactions (Ratnam et al. 2014; Thomas et al. 2014; Fitzpatrick et al. 2015). Therefore, understanding the impact of both logging and forest restoration on genetic diversity within species is useful for predicting forest recovery.

In Borneo, several genera of the family Dipterocarpaceae dominate the canopy of rain forests and more than 250 species can be found in the region (Ashton 1988). The family has a unique feature of synchronised mass flowering and fruiting with non-dipterocarp families at irregular intervals in Southeast Asia (Appanah 1985; Sakai 2002), which is triggered by drought (Sakai et al. 2006; Kobayashi et al. 2013). The availability of dipterocarp seeds is limited because of this intermittent flowering and the difficulties of preserving the recalcitrant seeds, which also makes forest restoration practices difficult (Kettle et al. 2010). Multiple cycles of logging have drastically changed the forest structure and the species composition of dipterocarps since the 1950s (Ancrenaz et al. 2010), which has led to a reduction in the diversity of species of both flora and fauna, changes in understorey microclimates, reduced regeneration and altered hydrological functions and biogeochemical cycles in the forest (Turner 1996; McGrath et al. 2001; Achard et al. 2002; Murty et al. 2002; Bruijnzeel 2004; Wilcove et al. 2013). Differing degrees of logging intensity affected most of the forest at least once and many areas twice or thrice (Wilcove et al. 2013) and only few regions remain undisturbed.

To restore logged forests, an enrichment-planting strategy was adopted in Sabah (a state of Malaysia on

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the island of Borneo). The process normally entails gathering seeds after flowering events, growing them in a nursery and subsequently planting them along lines through the pre-existing logged forests. The strategy has focused mostly on the dominant canopy tree species (i.e. dipterocarps). Enrichment planting is frequently used to supplement the insufficient natural regeneration in secondary forests (Ådjers et al. 1995). In Sabah, this strategy has been employed in several projects that explicitly focused on timber production (e.g. Innoprise Corporation), carbon storage (e.g. Innoprise-FACE Foundation Rainforest Rehabilitation Project (INFAPRO)) and restoration of biodiversity and forest ecosystem structure (e.g. INIKEA project). Hector et al. (2011) established the Sabah Biodiversity Experiment (SBE) to test the effect of dipterocarp diversity on ecosystem functioning. These approaches have focused mainly on either increasing tree growth or species diversity (e.g. Tuck et al. 2016) but rarely considered the genetic diversity of planted seedlings. Thus, little attention has been paid to the source of planting materials used in the establishment of these projects and to subsequent changes in overall genetic diversity.

Genetic diversity reflects the reservoir of a species for short-term ecological adaptation and long-term evolutionary change (Templeton 1994; Thomas et al. 2014). Therefore, genetic diversity has been considered crucial for species adaptation to unforeseen environmental changes and for the maintenance of species resilience to pests and diseases. In plant populations, low genetic diversity increases homozygosity and results in inbreeding depression through selfing and biparental inbreeding (Shimizu and Tsuchimatsu 2015). Previous studies have shown that logging activities affect the outcrossing rate and genetic diversity of naturally regenerated dipterocarp species (Murawski et al. 1994; Lee 2000; Ng et al. 2009). Several studies in the Brazilian Amazon forests have also shown that selective logging reduced the level of genetic diversity in the progenies of, e.g. *Bagassa guianensis* (Arruda et al. 2015) and *Hymenaea courbaril* (Carneiro et al. 2011). However, those studies did not investigate the genetic diversity of planted seedling in the logged forests.

Research on the interaction between two fundamental levels of biodiversity (i.e. species diversity and genetic diversity) has attracted intense interest from both ecologists and population geneticists since the emergence of 'community genetics' (Antonovics 1992). The relationship between the two types of diversity can be positive, negative or absent (i.e. no significant interaction). Both diversity levels share many similarities and are influenced by four processes: mutation/speciation, random drift, migration and selection (Vellend and Geber 2005). Mutations create new alleles, while speciation creates new species, but they occur on a longer timescale than the three other processes. A positive species and genetic diversity correlation would be expected if drivers, for instance, drift, migration and selection act in parallel on both diversity levels (Vellend 2004). Valen's (1965) 'niche variation' idea was adapted to the inference of negative correlation between species and genetic diversity. His hypothesis states that niche breadth (and, therefore, genetic diversity) is highest in communities with low species diversity because species diversity may act to stabilise selection on traits related to interspecific competition. In addition, in a community with a fixed number of individuals, species diversity may also affect genetic diversity within species via its effects on population size.

The aim of the present study was to provide a detailed genetic diversity assessment of regenerating dipterocarp species across the gradient from primary undisturbed forests to selectively logged forests and enrichment-planting restoration efforts in Sabah. For this purpose, the genetic diversity of two dipterocarp species (*Shorea leprosula* Miq. and *Parashorea malaanonan* (Blanco) Merr.) was quantified across this gradient. In addition, given the unique set-up of the enrichment-planting strategy used in the SBE, we also investigated the correlation between species diversity and genetic diversity within species. We hypothesised that the genetic diversity of natural seedlings would be reduced in logged forests compared with unlogged forests because of the loss of adult trees. Moreover, we hypothesised that genetic diversity of planted seedlings would be affected by the diversity of species planted in the experimental plots of the SBE.

Materials and methods

Study site

Three study sites in the lowland dipterocarp rain forests in Sabah, Malaysia, were selected for sampling, to encompass a spectrum of logging intensity and forest management (Figure S1). A 50-ha permanent plot in the primary forest at the Danum Valley Conservation Area (DVCA) provided an unlogged forest control, SBE served as a site once logged and now under regeneration by the use of enrichment planting and Ulu Segama Malua (USM), represented a intensively logged forest, selectively logged with multiple cycles of logging since the 1950s (Ancrenaz et al. 2010).

Danum Valley Conservation Area (DVCA). The DCVA (05°19′21″ N, 117°26′26″ E) is a protected area of 43,800 ha of primary forest in Sabah, Malaysia (Marsh and Greer 1992). It has been the main field site for many collaborative research programmes, in particular the comparative study between primary forest and selectively logged forests since 1980s. Our sampling was carried out in a 50-ha permanent plot from DVCA managed by the Smithsonian Tropical Research Institute's global network (Reynolds et al. 2011).

Sabah Biodiversity Experiment (SBE). The SBE (05°05′ 20″ N, 117°38′32″ E, 102 m a.s.l.), which is a large-scale enrichment-planting project, is located in the southern

part of the Malua Forest Reserve. The SBE was established in 2000 on a 500-ha area that had been logged once in the 1980s. The experiment consisted of planting in plots seedlings of 16 dipterocarp species using three levels of species diversity (single species, mixture of four species and mixture of 16 species) according to a randomised block design. Thirty-two plots, each 200 m × 200 m, were planted at each diversity level with at least 1000 seedlings per plot in 2002 and 2003. The details of the experimental plots can be found in Hector et al. (2011) and Tuck et al. (2016). The survival and growth of the enrichment-planted seedlings were recorded at regular intervals. Tuck et al. (2016) reported an overall high rate of mortality observed among all 16 tree species with only 36% of seedlings remaining after 2 years after planting. No significant difference in species growth and survival was observed between plots planted with a single species and mixtures after 10 years (Tuck et al. 2016).

Ulu Segama Malua (USM). In USM forest structure and integrity have been altered by the logging activities since the 1950s. The first phase of logging (1957–1999) used conventional methods with cutting trees with a dbh of ≥60 cm. This regime produced ca. 87.5 m^3 ha^{-1} of timber from Ulu Segama and 65.5 m^3 ha^{-1} from Malua. In the second phase of logging (1999–2007), conventional logging was used in most places except for some areas that adopted reduced-impact logging (RIL) regimes. A lower yield of wood (46.5 m^3 ha^{-1} in Ulu Segama and 33.0 m^3 ha^{-1} in Malua) was harvested in the second logging although the cutting diameter limit was reduced to 40 cm dbh (Anon 2008). Only a few protected areas, including the SBE, were not included in the second round of logging.

The Sabah Forestry Department classified USM in 2008 as 'very poor forest' with an average density of less than 10 trees (dbh >40 cm) per ha (Anon 2008). In 2007, an agreement was made between the Sabah Forestry Department, Yayasan Sabah and WWF-Malaysia to protect the area under forest cover through sustainable forest management. Subsequently, it was converted to forest conservation and restoration area, which has involved projects focused on ecosystem services (Reynolds et al. 2011).

Study species

Two tree species from the family Dipterocarpaceae were used as model species in this study: *S. leprosula* and *P. malaanonan*. Both tree species are predominantly outcrossing species (Lee et al. 2000; Kenta et al. 2002; Gamboa-Lapitan and Hyun 2005). They are light-demanding and fast-growing in the early stages of development (Bebber et al. 2002; Massey et al. 2005). They are also regarded as valuable commercial timber species. *S. leprosula* is well known as a light red meranti wood while *P. malaanonan* is recognised as white seraya wood (Ashton 1998a, 1998b). Both *S. leprosula* and *P. malaanonan* were listed as endangered species and critically endangered species, respectively, in the IUCN Red List of threatened plants (Ashton 1998a, 1998b) because of logging and overharvesting. Therefore, these two tree species may be representative of dipterocarp trees under threat of over-exploitation.

Sample collection and DNA extraction

At the enrichment-planting site, an intensive sampling of the two selected species was conducted to test the effects of species richness (1, 4 or 16 species) on the genetic diversity of the planted seedlings after the establishment of SBE in 2000. The ages of planted seedlings varied but were predominantly from a single-fruiting event occurred across the USM areas. In June 2014, we randomly sampled 90 individuals of *S. leprosula* and 92 individuals of *P. malaanonan* from 6 plots where enrichment planting was made with a single species, from 4-species mixtures (2 plots) and 16-species mixtures (2 plots).

We randomly sampled leaf tissues from 23–40 naturally established seedlings of the study species from DVCA, USM and SBE, to compare the genetic diversity of both natural regeneration and artificial regeneration (enrichment-planted seedlings) in dipterocarp species. In DVCA, sampling was carried out in a 50-ha permanent plot. In USM, sampling was made in an area encompassing 56.8 km^2 between DVCA and SBE because of the paucity of naturally regenerated seedlings found in the intensively logged forests (Figure S1). In SBE, sampling of the naturally regenerated seedlings was made near the remnant adult trees found within the 500-ha experimental area. To ensure the sampling of natural seedlings from different mother trees, leaf samples were collected from seedlings near to adult trees that were located at least 50 m apart from each other. Total genomic DNA was extracted from young leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). We also estimated the adult tree densities of *S. leprosula* according to the GPS coordinates of trees with dbh >30 cm. However, this estimation was not made for *P. malaanonan* because of its high abundance across the sites. The density of *S. leprosula* was inversely correlated with logging intensity, i.e. forests that were unlogged (DVCA = 52.5 tree km^{-2}), logged once (SBE = 44.9 tree km^{-2}) and logged more than once (USM = 19.8 tree km^{-2}).

Microsatellite genotyping

We genotyped 183 seedlings of *S. leprosula* and 193 seedlings of *P. malaanonan* using 14 and 8 nuclear microsatellite loci, respectively (Tables S1–S2). Polymerase Chain Reaction (PCR) amplifications were carried out on a T100™ thermal cycler (Bio-Rad, Hemel Hempstead, UK) according to the protocol by Ang et al. (2011). Fragment analysis was made using an ABI 3730xl

Genetic Analyzer with GeneScan™ 500 LIZ (Applied Biosystems) as the internal size standard in assigning allele sizes, and was further scored by using GeneMapper v 5.0 (Applied Biosystems).

Data analysis

Data analysis was carried out separately for the two species (*S. leprosula* and *P. malaanonan*) in two sets: (1) all naturally established seedlings from the three study sites and (2) only enrichment-planted seedlings across the diversity gradient.

To measure genetic diversity, expected heterozygosity (*He*) and allelic richness (A_R), have been commonly used. In general, *He* is more frequently used than A_R as *He* counts only the number and relative frequencies of alleles, hence reflecting the 'evenness' of allele frequencies (Hale et al. 2012). Conversely, A_R is largely influenced by the sample size of the populations (i.e. large samples are expected to have more alleles than small samples). Nevertheless, a statistical method of rarefaction can be used to compensate for this sampling disparity (Kalinowski 2005).

Genetic diversity parameters were calculated using CERVUS (Kalinowski et al. 2007). Because of the unequal sample sizes from the sites, the HP-RARE program was used to undertake rarefaction on the estimation of allelic richness (Kalinowski 2005). GENEPOP was used to calculate the fixation index (F_{IS}) – an estimate of inbreeding per population and per locus (Rousset 2008). The significance value of F_{IS} was determined by FSTAT (Goudet 1995). Linear mixed-effect models were used to analyse the effects of species (fixed factor with two levels: *S. leprosula* and *P. malaanonan*), regeneration (fixed factor with two levels: natural and planted), location (fixed factor with three levels: DVCA, USM and SBE) and species richness of the enrichment-planting (continuous explanatory variable) on the *He* and rarefied A_R of the two species. A random effect for microsatellite loci nested within species (a random factor with 22 levels) was incorporated in the model. Furthermore, an *a priori* linear contrast was carried out to test whether richness as a factor explained additional variation in the analysis.

The genetic structure of the seedlings at the study sites was determined using several complementary methods: (1) pairwise PhiPT genetic distance in GENALEX (Peakall and Smouse 2012) and F_{ST} genetic distance in GENEPOP (Rousset 2008); (2) Bayesian model-based clustering in STRUCTURE (Pritchard et al. 2000; Hubisz et al. 2009) and (3) discriminant analysis of principal components (DAPC) (Jombart et al. 2010) in the R package ADEGENET (Jombart 2008).

The PhiPT measure suppresses intra-individual variation to facilitate comparison between codominant data. The significance of PhiPT values was tested with 999 permutations in GENALEX. Furthermore, the F_{ST} estimate was calculated to validate the genetic distance between the locations using the GENEPOP method. This method was adopted because it provides a better estimation of F_{ST} under weak differentiation (Rousset 2007).

In the Bayesian analysis, we specifically chose an admixture model without prior population information and accounted for correlated allele frequencies between populations. This configuration is considered as the best fit in populations with subtle differentiation (Falush et al. 2003). We assumed a number of genetic clusters (K = 1–8) with repetition for each K occurring 10 times, a burn-in of 10^5 iterations and a run length of 10^6 iterations after the burn-in. The best K value was considered when ΔK reached the highest peak, as described in Evanno et al. (2005) using the STRUCTURE HARVESTER program (Earl and Vonholdt 2012). After the best K was inferred, the CLUMPP program (Jakobsson and Rosenberg 2007) was used to match all the replicates from the inferred K. In this case, we used 10^3 permutations for 10 replicates of the chosen K using the FullSearch algorithm. Lastly, the output from CLUMPP was used to generate bar plots of the assigned cluster membership, using DISTRUCT (Rosenberg 2004).

DAPC is a multivariate method that involves a two-step analysis. First, we transformed the genetic data to principal components using a principal component analysis. Next, clusters were identified using a discriminant analysis. This analysis allows discriminant functions that show group differences while minimising variation within clusters. Unlike STRUCTURE, this method does not require any prior population model and provides membership probabilities of each individual to the different groups based on the discriminant functions.

Results

Genetic diversity

The complete data set comprised microsatellite genotypes that were scored using 14 microsatellite loci (Table S1) and 8 microsatellite loci (Table S2) from 183 seedlings of *S. leprosula* and 193 seedlings of *P. malaanonan*, respectively. The sample sizes of the two species ranged from 23 to 40 for each location, and from 30 to 32 for each plot (Table 1).

In general, the genetic diversity estimates (i.e. *He* and A_R) of natural seedlings were higher than those of planted seedlings. Within both species, naturally regenerated seedlings exhibited a similar level of genetic diversity across the three sites. Genetic diversity had a positive trend with increasing diversity of planted species, such that 16-species plots harboured greater *He* and A_R than 4-species plots or plots planted with a single species. The F_{IS} values were all significantly positive within each subpopulation and were generally higher in planted seedlings than natural seedlings (Table 1).

Table 1. Genetic diversity parameters and fixation index of two tree species in the Dipterocarpaceae family at three sites, Danum Valley Conservation Area (DVCA), Ulu Segama Malua Forest Reserve (USM) or the Sabah Biodiversity Experiment (SBE) under different levels of species richness of the enrichment plantings (i.e. Mono: one species, 4sp: four species or 16sp: 16 species) in Sabah, Malaysia.

Species	Regeneration	Locations/plots	*n*	*A*	*Ho*	*He*	A_R	F_{IS}
Shorea leprosula	Natural	DVCA	40	10.93	0.678	0.743 (0.058)	9.03 (0.928)	0.089*
Natural seedlings		USM	30	10.93	0.697	0.762 (0.048)	9.67 (1.185)	0.086*
		SBE	23	10.07	0.726	0.762 (0.061)	9.70 (1.331)	0.047*
Shorea leprosula	Planted	Mono	30	7.00	0.557	0.635 (0.059)	6.74 (0.677)	0.121*
Enrichment-planted seedlings		4sp	30	7.50	0.533	0.624 (0.062)	7.26 (0.849)	0.146*
		16sp	30	8.86	0.633	0.709 (0.053)	8.53 (1.033)	0.106*
Parashorea malaanonan	Natural	DVCA	34	13.38	0.674	0.803 (0.053)	11.75 (2.271)	0.155*
Natural seedlings		USM	31	13.25	0.687	0.802 (0.061)	12.25 (2.621)	0.139*
		SBE	36	13.25	0.706	0.805 (0.047)	11.40 (2.214)	0.123*
Parashorea malaanonan	Planted	Mono	30	10.38	0.608	0.777 (0.053)	10.20 (2.032)	0.220*
Enrichment-planted seedlings		4sp	32	12.00	0.617	0.810 (0.046)	11.62 (1.942)	0.238*
		16sp	30	11.63	0.669	0.820 (0.033)	11.42 (1.996)	0.187*

Notes: *n*, number of individuals; *A*, mean number of alleles per locus; *Ho*, observed heterozygosity; *He*, expected heterozygosity; A_R, rarefied allelic richness; F_{IS}, fixation index within a subpopulation (significance level: $*P < 0.005$). Figures in the brackets are the estimated standard errors of the mean for genetic diversity parameters.

Table 2. ANOVA tables from the linear mixed-effects model of expected heterozygosity and rarefied allelic richness of two tree species in the Dipterocarpaceae family at three sites under different management or restoration strategies in Sabah, Malaysia.

Genetic diversity estimates	Expected heterozygosity			Rarefied allelic richness		
Source of variation	d.f.	denominator d.f.	*F*	d.f.	denominator d.f.	*F*
Species	1	20	1.44	1	20	1.994
Regeneration	1	100	34.14***	1	100	24.23***
Species x regeneration	1	100	19.25***	1	100	5.394*
Location	2	100	0.28	2	100	0.921
Species x location	2	100	0.15	2	100	0.733
Richness	1	100	13.48***	1	100	12.04***
Species x richness	1	100	1.84	1	100	1.511
Factor (richness)	1	100	0.19	1	100	2.122
Species x factor (richness)	1	100	1.97	1	100	2.254

Note: d.f., degrees of freedom; denominator d.f., denominator degrees of freedom; *F*, conditional *F* statistic; $*P < 0.05$, $***P < 0.001$. Species: *Shorea leprosula* or *Parashorea malaanonan*, regeneration: natural or planted, location: Danum Valley Conservation Area, Ulu Segama Malua Forest Reserve or the Sabah Biodiversity Experiment, richness: species richness of the enrichment planting (a continuous explanatory variable), factor (richness): species richness of the enrichment planting as a factor.

The results of the linear mixed-effects models analysis of *He* and rarefied A_R are shown in Table 2. *He* and A_R varied significantly with regeneration (natural vs. enrichment-planted seedlings) and the interaction between species and regeneration was significant. The enrichment-planted seedlings of *S. leprosula* had a much lower *He* and A_R than did natural seedlings (Table 1). Conversely, *He* and A_R of the natural seedlings of the two species did not differ significantly among sites. A significant difference in *He* and A_R was consistently observed with the species richness of the enrichment planted sites (1, 4 or 16 species) in both species with reduced genetic diversity found in the single-species plots relative to the mixed-species plots. Furthermore, the linear contrast in the model showed that richness as a factor was not significant after accounting for the linear trend. These results further support the linear relationship between genetic diversity and species richness of the enrichment planting.

Genetic structure

In general, for *S. leprosula*, a very low genetic distance among the natural seedlings was measured across the three sites. A relatively high genetic differentiation was observed between natural and planted seedlings with PhiPT <0.11 (Table 3) and F_{ST} <0.07 (Table S3), with the exception of seedlings in 16-species plots. The planted seedlings in 16-species plots were more genetically related to natural seedlings than to planted seedlings in 4-species and single-species plots (Tables 3; Table S3). Furthermore, Bayesian clustering in STRUCTURE identified two distinct clusters in *S. leprosula* based on ΔK (Figure S2a) with natural and planted seedlings in the 16-species plots in one cluster, and those in single-species and 4-species plots in another cluster (Figure 1a). Two genetic clusters were observed in *S. leprosula* in the DAPC analysis (Figure S3c), which provided partial support for the STRUCTURE results. However, this analysis

Table 3. Pairwise PhiPT genetic distance of two tree species in the Dipterocarpaceae family at three sites, Danum Valley Conservation Area (DVCA), Ulu Segama Malua Forest Reserve (USM) or the Sabah Biodiversity Experiment (SBE) under different levels of species richness of the enrichment plantings (i.e. Mono: one species, 4sp: four species or 16sp: 16 species) in Sabah, Malaysia.

	Shorea leprosula						*Parashorea malaanonan*					
	DVCA	USM	SBE	Mono	4sp	16sp	DVCA	USM	SBE	Mono	4sp	16sp
DVCA	–	0.043	0.019	0.001	0.001	0.001	–	0.015	0.122	0.001	0.012	0.001
USM	0.010	–	0.206	0.001	0.001	0.001	0.013	–	0.001	0.001	0.001	0.001
SBE	0.015	0.006	–	0.001	0.001	0.001	0.006	0.027	–	0.039	0.013	0.005
Mono	0.083	0.092	0.08	–	0.124	0.001	0.036	0.051	0.015	–	0.003	0.004
4sp	0.103	0.103	0.083	0.012	–	0.001	0.019	0.028	0.017	0.028	–	0.007
16sp	0.039	0.064	0.050	0.088	0.148	–	0.038	0.041	0.025	0.035	0.027	–

Notes: PhiPT values are indicated below the diagonal. Probability based on 999 permutations is shown above the diagonal.

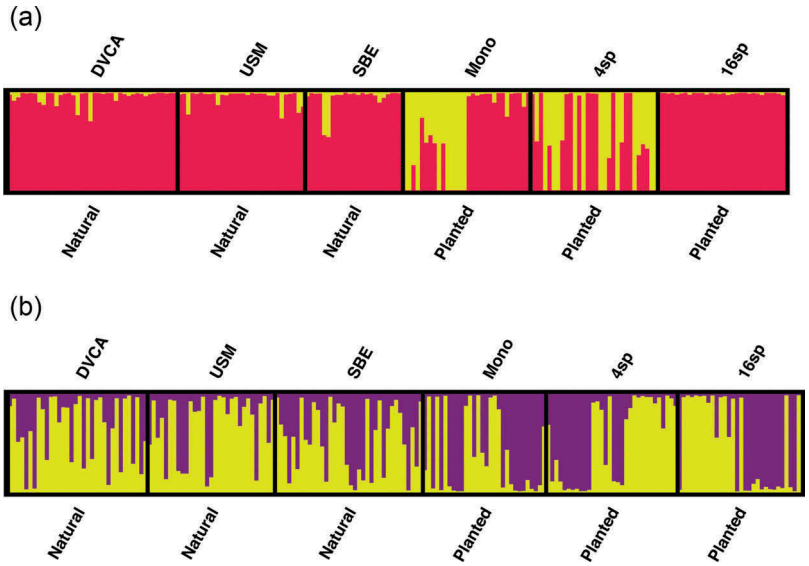

Figure 1. Population structure of natural and planted seedlings from (a) 183 seedlings of *Shorea leprosula* and (b) 193 seedlings of *Parashorea malaanonan* at three sites under different management or restoration strategies in Sabah, Malaysia. Each individual is represented by a single vertical line, with coloured segments indicating individual's estimated membership fraction in each of the *K*-inferred clusters.

clustered all natural seedlings while all planted seedlings were grouped in another cluster. The first discriminant function also supported $K = 2$, indicating divergence among the seedlings into two clusters (Figure S5a). Nevertheless, a small overlap of DVCA with the 16-species plots was observed (Figures S3c and S5a).

For *P. malaanonan*, a very low genetic distance was observed among both natural and planted seedlings with PhiPT <0.06 (Table 3) and F_{ST} <0.03 (Table S3). Although ΔK displayed a distinct peak when $K = 2$ (Figure S2b), the membership assignment of all seedlings across the three sites including the enrichment-planted seedlings, showed population admixture distribution among the two clusters (Figure 1b). Therefore, we can infer that the natural and planted seedlings of *P. malaanonan* exhibit weak genetic differentiation. DAPC analysis in *P. malaanonan* did not indicate any significant genetic divergence among the seedlings; all seedlings seemingly shared similar genotypes. No discrete genetic clusters for natural and planted seedlings could be determined (Figures S4c and S5b). Thus, the DAPC analysis was concordant with STRUCTURE, in which weak genetic differentiation was observed in all seedlings of *P. malaanonan.*

Discussion

Our results indicated a similar level of genetic diversity (*He* and A_R), with no significant genetic depletion among naturally regenerated seedlings of the two dipterocarp species in the logged sites relative to the unlogged site. The planted seedlings of *S. leprosula* showed a significant reduction in genetic diversity while *P. malaanonan* maintained a level of genetic diversity that was similar to that of natural seedlings. For enrichment-planted seedlings, the

16-species mixture plots had significantly higher genetic diversity than seedlings in 4-species or single-species plots.

Maintenance of genetic diversity in natural seedlings

In our study, we did not find significant differences in genetic diversity among the naturally regenerated seedlings of *S. leprosula* and *P. malaanonan*, regardless of the number of logging cycles, i.e. whether they were logged once (SBE) or more often (USM). Ng et al. (2009) showed a substantial reduction of allelic diversity in *S. leprosula* after 51 years of regeneration in a logged forest. These contrasting findings may be attributed to the differences in the markers used for microsatellite genotyping and in logging severity between the two sampling areas: Ng et al. (2009) conducted their research at a site that had experienced complete removal of all trees greater than 45 cm dbh based on the Malayan Uniform System (Wyatt-Smith 1963). The complete removal of large trees had a detrimental effect on the demographic structure, which subsequently led to the loss of allelic diversity within species Ng et al. 2009). In our study, RIL techniques have been implemented in USM since 1992 (Pinard and Putz 1996), to minimise degradation and residual damage (Wilcove et al. 2013). It is likely that USM and SBE retained a sufficient number of adult trees that enhanced outcrossing and provided adequate pollinator densities for reproductive assurance of both *S. leprosula* and *P. malaanonan*. Nevertheless, it is also possible that the genetic diversity in USM was overestimated compared with DCVA and SBE, because the sampling in USM was conducted in a larger area due to the low density of remnant adult trees in the intensively logged forests.

The density of remnant adult trees is a key contributing factor to the genetic diversity of regenerated species in logged forests (Ratnam et al. 2014). The genetic diversity parameters for *S. leprosula* were similar at DVCA and SBE, most likely due to the lack of difference in adult tree density (DVCA = 52.5 trees km^{-2}; SBE = 44.9 trees km^{-2}) between the two forests. There is a good regeneration in SBE following logging (1957–1999). In USM, although logging reduced the density of large adult trees of *S. leprosula* (19.8 trees km^{-2}), the genetic diversity of the seedlings was maintained at the same level as that observed in unlogged forests. Potentially, the outcrossing of remnant adult trees in the logged forests (USM and SBE) might not be affected by the logging activities because of the comparable species richness of pollinators observed in logged and unlogged forest (Berry et al. 2010). The low F_{IS} values observed in natural seedlings from USM and SBE suggests no increase of inbreeding due to mating among relatives or selfing occurred. Berry et al. (2010) demonstrated that >90% of the species, including insects, documented in DVCA were also present in logged forests near USM. Hence, the genetic diversity in USM may be maintained by the presence of a high diversity of pollinators. Furthermore, pollen flow between flowering trees might not be restricted in the logged forests, as Fukue et al. (2007) reported long-distance gene flow (1000 m) in *S. leprosula*, particularly in populations with a low tree density. This long-distance gene flow might be attributed to the presence of larger pollinators, such as bees, stingless bees, beetles and moths, which can fly over long distances (Appanah and Chan 1981; Dayanandan et al. 1990; Momose et al. 1994; Corlett and Primack 2005). Studies have reported that thrips are the main pollinator for *S. leprosula* (Appanah and Chan 1981). However, during mast flowering, other pollinators from the Chrysomelidae and Curculionidae families might also contribute to the pollination event (Sakai et al. 1999). This hypothesis warrants verification based on additional genotype data from flowering trees and records of pollinator density found in the logged forests.

Reduced genetic diversity in planted seedlings

In most restoration projects, nursery seedlings are commonly recruited as planting materials, partly because this promotes successful establishment (Godefroid et al. 2011). To establish SBE, seedlings of the 16 species of dipterocarps were bought from the INFAPRO nursery. Because the locations of fruiting trees were not well documented by seed collectors during mast fruiting, we were not able to determine the exact mother trees of the planted seedlings. Nevertheless, we are certain that the seedlings originated from the surrounding forest reserves across the USM areas. Seeds of *S. leprosula* could only be collected from a limited number of mother trees, as the adult trees were far less common than *P. malaanonan* in the study areas. This would explain the significant reduction of genetic diversity compared with naturally regenerated seedlings observed among the planted seedlings of *S. leprosula* but not in *P. malaanonan*. The elevated F_{IS} value indicated an excess of homozygous individuals among the planted seedlings of each species. This mirrored the findings of Lee (2000), who demonstrated a high level of correlated mating of *Dryobalanops aromatica* Gaertn. in a seed orchard because of the use of related seed sources during the early establishment of the orchard. Conversely, in *P. malaanonan*, the level of genetic diversity did not differ significantly between planted and natural seedlings. The seeds for *P. malaanonan* must have been sourced from various fruiting trees, as a high density of adult trees exists in the vicinity of INFAPRO.

Positive species and genetic diversity correlations

The significant positive relationship found between species richness and genetic diversity metrics may be due to selective mortality of certain genotypes in monocultures with more stochastic mortality in mixtures. Although we do not know the exact mother trees of the planted

seedlings, the random plot design and systematic planting strategy used in the SBE should have ensured that the initial level of genetic diversity across plots was similar for each species (Hector et al. 2011). Furthermore, the genetic diversity of naturally regenerated seedlings from USM, SBE and DVCA are statistically indistinguishable. Because all of the seeds for planting stock in the SBE were sourced from these three areas, the initial level of genetic diversity across plots for each species was likely to have been similar. Therefore, the current genetic diversity observed in the plots is likely due to the loss of genotypes from post-planting mortality over the last decade. Selective loss of genotypes in plot with a single species may be the result of increased density-dependent mortality. For example, species-specific insects or pathogens may spread more easily in monocultures (Zhu et al. 2000; Zuppinger-Dingley et al. 2014). If mortality from these mechanisms preferentially affected genotypes with poor defensive strategies, then the genotypic diversity of the surviving seedling population would be lower. In contrast, species mixtures disrupt the spread of these mortality mechanisms that preferentially limit specific genotypes (Zhu et al. 2000). Nevertheless, it is also possible that the genetic differentiation observed in planted seedlings was caused by the unevenness of seed sources of these outcrossing tree species during the seed collection. Additional experimental evidence is required to confirm the mechanisms underlying these phenomena, and tests on additional species are needed to understand the breadth of the effect observed. However, these results would encourage us to further investigate and understand the underlying positive interaction between species diversity and genetic diversity within species of dipterocarps.

Conclusions

Our findings suggest that the degree of logging experienced by our study sites did not affect the genetic diversity of the regeneration in two outcrossing dipterocarp species in selectively logged forests. We observed the maintenance of a substantial level of genetic diversity in the seedlings after 10–30 years of forest recovery. Concurrently, we also observed a reduction of genetic diversity in single-species enrichment planting relative to mixed-species plots at least 10 years after establishment of a forest restoration experiment. In the future, restoration of tropical tree species should employ a planting strategy that uses diverse mixtures of species, rather than single species, or planting material collected from a limited number of related mother trees.

Acknowledgements

We would like to thank the staff of the Sabah Biodiversity Experiment for their assistance with the field and shade-house experiments. We appreciate the help from Alexander Karolus for providing the coordinates of dipterocarp species in the 50-ha plot, DVCA. We acknowledge the Sabah Biodiversity Centre and Danum Valley Management Committee for granting us the permission to conduct our research in the areas. This research is manuscript number 14 of the Sabah Biodiversity Experiment and supported by the University Research Priority Program on Global Change and Biodiversity of the University of Zurich, Swiss National Science Foundation, MEXT KAKENHI Grant Number 16H06469. AH is supported by the NERC Human-modified Tropical Forests programme, and CCA by Georges and Antoine Claraz-Donation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research is manuscript number 14 of the Sabah Biodiversity Experiment and supported by the University Research Priority Program on Global Change and Biodiversity of the University of Zurich, Swiss National Science Foundation, MEXT KAKENHI Grant Number 16H06469. AH is supported by the NERC Human-modified Tropical Forests programme, and CCA by Georges and Antoine Claraz-Donation.

Supplemental data

Supplemental data for this article can be accessed here.

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The project was conceived by AH and KKS with input from MOB and BS. CCA and MOB designed the field sampling strategy and wrote the initial draft of the manuscript. CCA performed the field sampling and analysed the genetic data. KKSN and KKS provided technical and analytical advice on the genetic analysis. MOB and BS contributed in the ANOVA analysis. PCL contributed logistical and strategic help to facilitate sampling and laboratory assistance in Sabah. AH setup the SBE experiment, which was the basis for sampling the enrichment-planted seedlings. AH and BS provided sampling and statistical advice. BS and KKS are the PIs on the project that funded this research. KKSN helped with genetic analysis. All authors contributed to manuscript revisions.

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