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

Ang, Cheng Choon ; O'Brien, Michael John ; Ng, Kevin Kit Siong ; Lee, Ping Chin ; Hector, Andy ; Schmid, Bernhard ; Shimizu, Kentaro K

Abstract: 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.

DOI: https://doi.org/10.1080/17550874.2016.1270363

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

Cheng Choon Ang[1], Michael J. O'Brien[1,2], Kevin Kit Siong Ng[3], Ping Chin Lee[4], Andy Hector[5], Bernhard Schmid[1] and Kentaro K. Shimizu[1*]

[1]Department of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich, Switzerland; [2]Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, La Cañada, Almería, España;[3]Genetics Laboratory, Forest Research Institute Malaysia, Kepong, Selangor, Malaysia;[4]Biotechnology Program, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia;[5]Department of Plant Sciences, University of Oxford, Oxford, England (UK);

*Corresponding author: kentaro.shimizu@ieu.uzh.ch

Abstract

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; O'Brien et al. 2014, 2015; Reynolds et al. 2011; Wilcove et al. 2013). Restoration efforts have focused on restoring species diversity and forest structure post-logging, but there has been limited

50 emphasis on genetic diversity within species, which is an important factor for
51 understanding species adaptation and persistence under novel climates and biological
52 interactions (Fitzpatrick et al. 2015; Ratnam et al. 2014; Thomas et al. 2014).
53 Therefore, understanding the impact of both logging and forest restoration on genetic
54 diversity within species is useful for predicting forest recovery.

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

71 To restore logged forests, an enrichment-planting strategy was adopted in
72 Sabah (a state of Malaysia on the island of Borneo). The process normally entails
73 gathering seeds after flowering events, growing them in a nursery and subsequently
74 planting them along lines through the pre-existing logged forests. The strategy has

75 focused mostly on the dominant canopy tree species (i.e. dipterocarps). Enrichment-
76 planting is frequently used to supplement the insufficient natural regeneration in
77 secondary forests (Ådjers et al. 1995). In Sabah, this strategy has been employed in
78 several projects that explicitly focused on timber production (e.g. Innoprise
79 Corporation), carbon storage (e.g. INFAPRO project) and restoration of biodiversity
80 and forest ecosystem structure (e.g. INIKEA project). Hector et al. (2011) established
81 the Sabah Biodiversity Experiment (SBE) to test the effect of dipterocarp diversity on
82 ecosystem functioning. These approaches have focused mainly on either increasing
83 tree growth or species diversity (e.g. Tuck et al. in press) but rarely considered the
84 genetic diversity of planted seedlings. Thus, little attention has been paid to the source
85 of planting materials used in the establishment of these projects nor to subsequent
86 changes in overall genetic diversity.
87 Genetic diversity reflects the reservoir of a species for short-term ecological
88 adaptation and long-term evolutionary change (Templeton 1994; Thomas et al. 2014).
89 Therefore, genetic diversity has been considered crucial for species adaptation to
90 unforeseen environmental changes and for the maintenance of species resilience to
91 pests and diseases. In plant populations, low genetic diversity increases homozygosity
92 and results in inbreeding depression through selfing and biparental inbreeding
93 (Shimizu and Tsuchimatsu 2015). Previous studies have shown that logging activities
94 affect the outcrossing rate and genetic diversity of naturally regenerated dipterocarp
95 species (Lee 2000; Murawski et al. 1994; Ng et al. 2009). Several studies in the
96 Brazilian Amazon forests have also shown that selective logging reduced the level of
97 genetic diversity in the progenies of e.g. *Bagassa guianensis* (Arruda et al. 2015) and
98 *Hymenaea courbaril* (Carneiro et al. 2011). However, those studies did not
99 investigate the genetic diversity of planted seedling in the logged forests.

100 Research on the interaction between two fundamental levels of biodiversity
101 (i.e. species diversity and genetic diversity) has attracted intense interest from both
102 ecologists and population geneticists since the emergence of 'community genetics'
103 (Antonovics 1992). The relationship between the two types of diversity can be
104 positive, negative or absent (i.e. no significant interaction). Both diversity levels share
105 many similarities and are influenced by four processes: mutation/speciation, random
106 drift, migration and selection (Vellend and Geber 2005). Mutations create new alleles,
107 while speciation creates new species, but they occur on a longer timescale than the
108 three other processes. A positive species and genetic diversity correlation would be
109 expected if drivers, for instance, drift, migration and selection act in parallel on both
110 diversity levels (Vellend 2004). Valen's (1965) 'niche variation' idea was adapted to
111 the inference of negative correlation between species and genetic diversity. His
112 hypothesis states that niche breadth (and, therefore, genetic diversity) is highest in
113 communities with low species diversity because species diversity may act to stabilise
114 selection on traits related to interspecific competition. In addition, in a community
115 with a fixed number of individuals, species diversity may also affect genetic diversity
116 within species via its effects on population size.
117 The aim of the present study was to provide a detailed genetic diversity
118 assessment of regenerating dipterocarp species across the gradient from primary
119 undisturbed forests to selectively logged forests and enrichment-planting restoration
120 efforts in Sabah. For this purpose, the genetic diversity of two dipterocarp species
121 (*Shorea leprosula* Miq. and *Parashorea malaanonan* (Blanco) Merr.) was quantified
122 across this gradient. In addition, given the unique set-up of the enrichment-planting
123 strategy used in the SBE, we also investigated the correlation between species
124 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 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 (DVCA; 05°19′21″ N, 117°26′26″ E) is a protected area of 43800 ha of uninhabited primary forest in Sabah, Malaysia (Marsh and Greer 1992). It has been the main field site for many collaborative research programs 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

150 once in the 1980s. The experiment consisted of planting in plots seedlings of 16
151 dipterocarp species using three levels of species diversity (single species, mixture of
152 four species and mixture of 16 species) according to a randomised block design.
153 Thirty-two plots, each 200 m x 200 m, were planted at each diversity level with at
154 least 1000 seedlings per plot in 2002 and 2003. The details of the experimental plots
155 can be found in Hector et al. (2011) and Tuck et al. (in press). The survival and
156 growth of the enrichment-planted seedlings were recorded at regular intervals. Tuck
157 et al. (in press) reported an overall high rate of mortality observed among all 16 tree
158 species with only 36 % of seedlings remaining after 2 years after planting. No
159 significant difference in species growth and survival was observed between plots
160 planted with a single species and mixtures after 10 years (Tuck et al. in press).
161 *Ulu Segama Malua (USM)*.
162 In Ulu Segama Malau forest structure and integrity have been altered by the logging
163 activities since the 1950s. The first phase of logging (1957–1999) used conventional
164 methods with cutting trees with a dbh of ≥ 60 cm. This regime produced ca. 87.5 m^3
165 ha^{-1} of timber from Ulu Segama and 65.5 m^3 ha^{-1} from Malua. In the second phase of
166 logging (1999–2007), conventional logging was used in most places except for some
167 areas that adopted reduced-impact logging (RIL) regimes. A lower yield of wood
168 (46.5 m^3 ha^{-1} in Ulu Segama and 33.0 m^3 ha^{-1} in Malua) was harvested in the second
169 logging although the cutting diameter limit was reduced to 40 cm dbh (Anon, 2008).
170 Only a few protected areas, including the SBE, were not included in the second round
171 of logging.
172 The Sabah Forestry Department classified USM in 2008 as 'very poor forest' with an
173 average density of less than 10 trees (dbh > 40 cm) per ha (Anon 2008). In 2007, an
174 agreement was made between the Sabah Forestry Department, Yayasan Sabah and

175 WWF-Malaysia to protect the area under forest cover through sustainable forest
176 management. Subsequently, it was converted to forest conservation and restoration
177 area, which has involved projects focused on ecosystem services (Reynolds et al.
178 2011).
179
180 *Study species*
181 Two tree species from the family Dipterocarpaceae were used as model
182 species in this study: *Shorea leprosula* and *Parashorea malaanonan*. Both tree
183 species are predominantly outcrossing species (Gamboa-Lapitan and Hyun 2005;
184 Kenta et al. 2002; Lee et al. 2000). They are light-demanding and fast-growing in the
185 early stages of development (Bebber et al. 2002; Massey et al. 2005). They are also
186 regarded as valuable commercial timber species. *Shorea leprosula* is well known as a
187 light red meranti wood while *P. malaanonan* is recognised as white seraya wood
188 (Ashton 1998a, b). Both *S. leprosula* and *P. malaanonan* were listed as endangered
189 species and critically endangered species, respectively, in the IUCN Red List of
190 threatened plants (1998) because of logging and overharvesting. Therefore, these two
191 tree species may be representative of dipterocarp trees under threat of over-
192 exploitation.
193
194 *Sample collection and DNA extraction*
195 At the enrichment-planting site, an intensive sampling of the two selected
196 species was conducted to test the effects of species richness (1-, 4- or 16-species) on
197 the genetic diversity of the planted seedlings after the establishment of SBE in 2000.
198 The ages of planted seedlings varied but were predominantly from a single fruiting
199 event occurred across the USM areas. In June 2014, we randomly sampled 90

200 individuals of *S. leprosula* and 92 individuals of *P. malaanonan* from six plots where
201 enrichment planting was made with a single species, from four-species mixtures (two
202 plots) and 16-species mixtures (two plots).
203 We randomly sampled leaf tissues from 23–40 naturally established seedlings
204 of the study species from DVCA, USM and SBE, to compare the genetic diversity of
205 both natural regeneration and artificial regeneration (enrichment-planted seedlings) in
206 dipterocarp species. In DVCA, sampling was carried out in a 50 ha permanent plot. In
207 USM, sampling was made in an area encompassing 56.8 km^2 between DVCA and
208 SBE because of the paucity of naturally regenerated seedlings found in the intensively
209 logged forests (Figure S1). In SBE, sampling of the naturally regenerated seedlings
210 was made near the remnant adult trees found within the 500 ha experimental area. To
211 ensure the sampling of natural seedlings from different mother trees, leaf samples
212 were collected from seedlings near to adult trees that were located at least 50 m apart
213 from each other. Total genomic DNA was extracted from young leaf tissues using a
214 modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson
215 1980). We also estimated the adult tree densities of *S. leprosula* according to the GPS
216 coordinates of trees with dbh >30 cm. However, this estimation was not performed
217 for *P. malaanonan* because of its high abundance across the sites. The tree density of
218 *S. leprosula* was inversely correlated with logging intensity, i.e. forests that were
219 unlogged (DVCA = 52.5 tree km^{-2}), logged once (SBE = 44.9 tree km^{-2}) and logged
220 more than once (USM = 19.8 tree km^{-2}).
221
222 *Microsatellite genotyping*
223 We genotyped 183 seedlings of *S. leprosula* and 193 seedlings of *P.*
224 *malaanonan* using 14 and eight nuclear microsatellite loci, respectively (Tables S1-

225 S2). PCR amplifications were carried out on a T100™ thermal cycler (Bio-Rad,
226 Hemel Hempstead, UK) according to the protocol by Ang et al. (2011). Fragment
227 analysis was made using an ABI 3730xl Genetic Analyzer with GeneScan™ 500 LIZ
228 (Applied Biosystems) as the internal size standard in assigning allele sizes, and was
229 further scored by using GeneMapper v 5.0 (Applied Biosystems).
230
231 *Data analysis*
232 Data analysis was carried out separately for the two species (*S. leprosula* and
233 *P. malaanonan*) in two sets: (1) all naturally established seedlings from the three
234 study sites and (2) only enrichment-planted seedlings across the diversity gradient.
235 To measure genetic diversityexpected heterozygosity (*He*) and allelic richness
236 (A_R), have been commonly used. In general, *He* is more frequently used than A_R as *He*
237 counts only the number and relative frequencies of alleles, hence reflecting the
238 'evenness' of allele frequencies (Hale et al. 2012). Conversely, A_R is largely
239 influenced by the sample size of the populations (i.e. large samples are expected to
240 have more alleles than small samples). Nevertheless, a statistical method of
241 rarefaction can be used to compensate for this sampling disparity (Kalinowski 2005).
242 Genetic diversity parameters were calculated using CERVUS (Kalinowski et
243 al. 2007). Because of the unequal sample sizes from the sites, the HP-RARE program
244 was used to undertake rarefaction on the estimation of allelic richness (Kalinowski
245 2005). GENEPOP was used to calculate the fixation index (F_{IS}) – an estimate of
246 inbreeding per population and per locus (Rousset 2008a). The significance value of
247 F_{IS} was determined by FSTAT (Goudet 1995). Linear mixed-effect models were used
248 to analyse the effects of species (fixed factor with two levels: *S. leprosula* and *P.*
249 *malaanonan*), regeneration (fixed factor with two levels: natural and planted),

250 location (fixed factor with three levels: DVCA, USM and SBE) and species richness
251 of the enrichment-planting (continuous explanatory variable) on the *He* and rarefied
252 A_R of the two species. A random effect for microsatellite loci nested within species (a
253 random factor with 22 levels) was incorporated in the model. Furthermore, an *a priori*
254 linear contrast was carried out to test whether richness as a factor explained additional
255 variation in the analysis.

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

262 The PhiPT measure suppresses intra-individual variation to facilitate
263 comparison between codominant data. The significance of PhiPT values was tested
264 with 999 permutations in GENALEX. Furthermore, the F_{ST} estimate was calculated to
265 validate the genetic distance between the locations using the GENEPOP method. This
266 method was adopted because it provides a better estimation of F_{ST} under weak
267 differentiation (Rousset 2008b).

268 In the Bayesian analysis, we specifically chose an admixture model without
269 prior population information and accounted for correlated allele frequencies between
270 populations. This configuration is considered as the best fit in populations with subtle
271 differentiation (Falush et al. 2003). We assumed a number of genetic clusters (K = 1 –
272 8) with repetition for each K occurring 10 times, a burn-in of 10^5 iterations and a run
273 length of 10^6 iterations after the burn-in. The best K value was considered when ΔK
274 reached the highest peak, as described in Evanno et al. (2005) using the

STRUCTURE HARVESTER programme (Earl and Vonholdt 2012). After the best *K* was inferred, the CLUMPP programme (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 (PCA). 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 dataset was comprised of microsatellite genotypes that were scored using 14 microsatellite loci (Table S1) and eight 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

300 species, such that 16-species plots greater *He* and A_R than four-species plots or plots
301 planted with a single species. The F_{IS} values were all significantly positive within
302 each subpopulation and were generally higher in planted seedlings than natural
303 seedlings (Table 1).

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

317

318 *Genetic structure*

319 In general, for *S. leprosula*, a very low genetic distance among the natural
320 seedlings was measured across the three sites. A relatively high genetic differentiation
321 was observed between natural and planted seedlings with PhiPT < 0.11 (Table 3) and
322 F_{ST} < 0.07 (Table S3), with the exception of seedlings in 16-species plots. The planted
323 seedlings in 16-species plots were more genetically related to natural seedlings than to
324 planted seedlings in four-species and single-species plots (Tables 3; Table S3).

325 Furthermore, Bayesian clustering in STRUCTURE identified two distinct clusters in
326 *S. leprosula* based on ΔK (Figure S2a) with natural and planted seedlings in the 16-
327 species plots in one cluster, and those in single-species and four-species plots in
328 another cluster (Figure 1a). Two genetic clusters were observed in *S. leprosula* in the
329 DAPC analysis (Figure S3c), which provided partial support for the STRUCTURE
330 results. However, this analysis clustered all natural seedlings while all planted
331 seedlings were grouped in another cluster. The first discriminant function also
332 supported $K = 2$, indicating divergence among the seedlings into two clusters (Figure
333 S5a). Nevertheless, a small overlap of DVCA with the 16-species plots was observed
334 (Figures S3c & S5a).
335 For *P. malaanonan*, a very low genetic distance was observed among both
336 natural and planted seedlings with PhiPT < 0.05 (Table 3) and $F_{ST} < 0.03$ (Table S3).
337 Although ΔK displayed a distinct peak when $K = 2$ (Figure S2b), the membership
338 assignment of all seedlings across the three sites including the enrichment-planted
339 seedlings, showed population admixture distribution among the two clusters (Figure
340 1b). Therefore, we can infer that the natural and planted seedlings of *P. malaanonan*
341 exhibit weak genetic differentiation. DAPC analysis in *P. malaanonan* did not
342 indicate any significant genetic divergence among the seedlings; all seedlings
343 seemingly shared similar genotypes. No discrete genetic clusters for natural and
344 planted seedlings could be determined (Figures S4c and S5b). Thus, the DAPC
345 analysis was concordant with STRUCTURE, in which weak genetic differentiation
346 was observed in all seedlings of *P. malaanonan.*
347
348 ***Discussion***
349 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 *Shorea leprosula* showed a significant reduction in genetic diversity while *Parashorea 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 four-species and 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. 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

375 diversity in USM was overestimated compared with DCVA and SBE, because the
376 sampling in USM was conducted in a larger area due to the low density of remnant
377 adult trees in the intensively logged forests.

378 The density of remnant adult trees is a key contributing factor to the genetic
379 diversity of regenerated species in logged forests (Ratnam et al. 2014). The genetic
380 diversity parameters for *S. leprosula* were similar at DVCA and SBE, most likely due
381 to the lack of difference in adult tree density (DVCA = 52.5 trees km^{-2}; SBE = 44.9
382 trees km^{-2}) between the two forests. There is a good regeneration in SBE following
383 logging (1957–1999). In USM, although logging reduced the density of large adult
384 trees of *S. leprosula* (19.8 trees km^{-2}), the genetic diversity of the seedlings was
385 maintained at the same level as that observed in unlogged forests. Potentially, the
386 outcrossing of remnant adult trees in the logged forests (USM and SBE) might not be
387 affected by the logging activities because of the comparable species richness of
388 pollinators observed in logged and unlogged forest (Berry et al. 2010). The low F_{IS}
389 values observed in natural seedlings from USM and SBE suggests no increase of
390 inbreeding due to mating among relatives or selfing occurred. Berry et al. (2010)
391 demonstrated that >90% of the species, including insects, documented in DVCA were
392 also present in logged forests near USM. Hence, the genetic diversity in USM may be
393 maintained by the presence of a high diversity of pollinators. Furthermore, pollen
394 flow between flowering trees might not be restricted in the logged forests, as Fukue et
395 al. (2007) reported long-distance gene flow (1000 m) in *S. leprosula*, particularly in
396 populations with a low tree density. This long-distance gene flow might be attributed
397 to the presence of larger pollinators, such as bees, stingless bees, beetles and moths,
398 which can fly over long distances (Appanah and Chan 1981; Corlett and Primack
399 2005; Dayanandan et al. 1990; Momose et al. 1994). Studies have reported that thrips

400 are the main pollinator for *S. leprosula* (Appanah and Chan 1981). However, during
401 mast flowering, other pollinators from the Chrysomelidae and Curculionidae families
402 might also contribute to the pollination event (Sakai et al. 1999). This hypothesis
403 warrants verification based on additional genotype data from flowering trees and
404 records of pollinator density found in the logged forests.
405
406 *Reduced genetic diversity in planted seedlings*
407 In most restoration projects, nursery seedlings are commonly recruited as
408 planting materials, partly because this promotes successful establishment (Godefroid
409 et al. 2011). To establish SBE, seedlings of the 16 species of dipterocarps were
410 bought from the Innoprise-FACE Foundation Rainforest Rehabilitation Project
411 (INFAPRO) nursery. Because the locations of fruiting trees were not well
412 documented by seed collectors during mast fruiting, we were not able to determine
413 the exact mother trees of the planted seedlings. Nevertheless, we are certain that the
414 seedlings originated from the surrounding forest reserves across the USM areas.
415 Seeds of *S. leprosula* could only be collected from a limited number of mother trees,
416 as the adult trees were far less common than *P. malaanonan* in the study areas. This
417 would explain the significant reduction of genetic diversity compared with naturally
418 regenerated seedlings observed among the planted seedlings of *S. leprosula* but not in
419 *P. malaanonan*. The elevated F_{IS} value indicated an excess of homozygous
420 individuals among the planted seedlings of each species. This mirrored the findings of
421 Lee (2000), who demonstrated a high level of correlated mating of *Dryobalanops*
422 *aromatica* Gaertn. in a seed orchard because of the use of related seed sources during
423 the early establishment of the orchard. Conversely, in *P. malaanonan*, the level of
424 genetic diversity did not differ significantly between planted and natural seedlings.

425 The seeds for *P. malaanonan* were sourced from various fruiting trees, as a high
426 density of adult trees exists in the vicinity of INFAPRO.
427
428 *Positive species and genetic diversity correlations*
429 The significant positive relationship found between species richness and
430 genetic diversity metrics may be due to selective mortality of certain genotypes in
431 monocultures with more stochastic mortality in mixtures. Although we do not know
432 the exact mother trees of the planted seedlings, the random plot design and systematic
433 planting strategy used in the SBE should have ensured that the initial level of genetic
434 diversity across plots was similar for each species (Hector et al. 2011). Furthermore,
435 the genetic diversity of naturally regenerated seedlings from USM, SBE and DVCA
436 are statistically indistinguishable. Because all of the seeds for planting stock in the
437 SBE were sourced from these three areas, the initial level of genetic diversity across
438 plots for each species was likely to have been similar. Therefore, the current genetic
439 diversity observed in the plots is likely due to the loss of genotypes from post-
440 planting mortality over the last decade. Selective loss of genotypes in plot with a
441 single species may be the result of increased density-dependent mortality. For
442 example, species-specific insects or pathogens may spread more easily in
443 monocultures (Zhu et al. 2000; Zuppinger-Dingley et al. 2014). If mortality from
444 these mechanisms preferentially affected genotypes with poor defensive strategies,
445 then the genotypic diversity of the surviving seedling population would be lower. In
446 contrast, species mixtures disrupt the spread of these mortality mechanisms that
447 preferentially limit specific genotypes (Zhu et al. 2000). Nevertheless, it is also
448 possible that the genetic differentiation observed in planted seedlings was caused by
449 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 encouraged 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 numbers 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 Claraz Foundation.

Notes on contributors

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 analyzed 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. CCA is interested in the underlying genetic architecture and diversity of adaptive traits observed in dipterocarps. MOB is interested in the effect of climate change on community composition and intra- versus inter-specific competition of seedlings in Malaysian, Borneo. KKSN is interested in the population genetics and genomics of tropical tree species. PCL specializes in the field of microbiology, molecular biology and cellular biology. AH is interested in biodiversity loss and its consequences for the stability and functioning of ecosystems and the provision of ecological services. BS is the expert in biology of species interactions focusing in variation among individuals, populations and species of plants and animals, and how they interact in nature and under controlled experimental

500 conditions. KKS is interested evolutionary and ecological genomic studies by
501 integrating novel genomics tools and systems biology to predict evolutionary and
502 plastic responses in changing environments.
503
504 **References**

505 Achard F, Eva HD, Stibig H-J, Mayaux P, Gallego J, Richards T, Malingreau J-P.
506 2002. Determination of deforestation rates of the world's humid tropical
507 forests. Science 297(5583):999–1002.
508 Ådjers G, Hadengganan S, Kuusipalo J, Nuryanto K, Vesa L. 1995. Enrichment
509 planting of dipterocarps in logged-over secondary forests: effect of width,
510 direction and maintenance method of planting line on selected *Shorea* species.
511 Forest Ecology and Management 73(1):259–270.
512 Ancrenaz M, Ambu L, Sunjoto I, Ahmad E, Manokaran K, Meijaard E, Lackman I.
513 2010. Recent surveys in the forests of Ulu Segama Malua, Sabah, Malaysia,
514 show that orangutans (*P. p. morio*) can be maintained in slightly logged
515 forests. PLoS One 5(7):e11510.
516 Ang CC, Lee SL, Lee CT, Tnah LH, Zakaria RM, Ng CC. 2011. Isolation and
517 characterization of microsatellite loci in an endangered palm,
518 *Johannesteijsmannia lanceolata* (Arecaceae). American Journal of Botany
519 98(5):e117–e119.
520 Anon 2008. Forest Management Plan for the Ulu Segama Malua Forest Reserve.
521 Sandakan, Sabah: Sabah Forestry Department.
522 Antonovics J. 1992. Toward community genetics. In: Fritz RS, Simms EL (eds) Plant
523 resistance to herbivores and pathogens: ecology, evolution, and genetics.
524 University of Chicago Press. p. 426–449.

525 Appanah S. 1985. General flowering in the climax rain forest of South-east Asia.
526 Journal of Tropical Ecology 1:225–240.
527 Appanah S, Chan HT. 1981. Thrips: the pollinators of some dipterocarps. Malaysian
528 Forester 44(2/3):234–252.
529 Arruda CCB, Silva MB, Sebbenn AM, Kanashiro M, Lemes MR, Gribel R. 2015.
530 Mating system and genetic diversity of progenies before and after logging: a
531 case study of *Bagassa guianensis* (Moraceae), a low-density dioecious tree of
532 the Amazonian forest. Tree Genetics and Genomes 11(1):1–9.
533 Ashton PS. 1988. Dipterocarp biology as a window to the understanding of tropical
534 forest structure. Annual Review of Ecology and Systematics 19(1):347–370.
535 Ashton, P. 1998a. *Parashorea malaanonan*. The IUCN Red List of Threatened
536 Species 1998: e.T33097A9751302. Avaialble from
537 http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33097A9751302.en
538 (accessed 10 March 2016).
539 Ashton, P. 1998b. *Shorea leprosula*. The IUCN Red List of Threatened Species 1998:
540 e.T33123A9759177. Available from
541 http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33123A9759177.en
542 (accessed 10 March 2016).
543 Bebber D, Brown N, Speight M. 2002. Drought and root herbivory in understorey
544 *Parashorea* Kurz (Dipterocarpaceae) seedlings in Borneo. Journal of Tropical
545 Ecology 18(05):795–804.
546 Berry NJ, Phillips OL, Lewis SL, Hill JK, Edwards DP, Tawatao NB, Ahmad N,
547 Magintan D, Khen CV, Maryati M, et al. 2010. The high value of logged
548 tropical forests: lessons from northern Borneo. Biodiversity and Conservation
549 19(4):985–997.

550 Bruijnzeel LA. 2004. Hydrological functions of tropical forests: not seeing the soil for
551 the trees? Agriculture, Ecosystems and Environment 104(1):185–228.
552 Carneiro FS, Lacerda AEB, Lemes MR, Gribel R, Kanashiro M, Wadt LHO, Sebbenn
553 AM. 2011. Effects of selective logging on the mating system and pollen
554 dispersal of *Hymenaea courbaril* L. (Leguminosae) in the Eastern Brazilian
555 Amazon as revealed by microsatellite analysis. Forest Ecology and
556 Management 262(9):1758–1765.
557 Corlett R, Primack R. 2005. Dipterocarps: trees that dominate the Asian rain forest.
558 Arnoldia 63(3):3–7.
559 Dayanandan S, Attygalla D, Abeygunasekera A, Gunatilleke I, Gunatilleke C. 1990.
560 Phenology and floral morphology in relation to pollination of some Sri Lankan
561 dipterocarps. In: Bawa KS, Hadley M (eds) Reproductive ecology of tropical
562 forest plants. UNESCO, Paris and Parthenon Publishing Carnforth. p. 103–
563 133.
564 Earl DA, Vonholdt BM. 2012. STRUCTURE HARVESTER: a website and program
565 for visualizing STRUCTURE output and implementing the Evanno method.
566 Conservation Genetics Resources 4(2):359–361.
567 Edwards DP, Woodcock P, Edwards FA, Larsen TH, Hsu WW, Benedick S, Wilcove
568 DS. 2012. Reduced-impact logging and biodiversity conservation: a case study
569 from Borneo. Ecological Applications 22(2):561–571.
570 Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals
571 using the software STRUCTURE: a simulation study. Molecular Ecology
572 14(8):2611–2620.

573 Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using
574 multilocus genotype data: linked loci and correlated allele frequencies.
575 Genetics 164(4):1567–1587.
576 Fitzpatrick CR, Agrawal AA, Basiliko N, Hastings AP, Isaac ME, Preston M,
577 Johnson MTJ. 2015. The importance of plant genotype and contemporary
578 evolution for terrestrial ecosystem processes. Ecology 96(10):2632–2642.
579 Fukue Y, Kado T, Lee SL, Ng KKS, Muhammad N, Tsumura Y. 2007. Effects of
580 flowering tree density on the mating system and gene flow in *Shorea*
581 *leprosula* (Dipterocarpaceae) in Peninsular Malaysia. Journal of Plant
582 Research 120(3):413–420.
583 Gamboa-Lapitan P, Hyun JO. 2005. Mating system of *Parashorea malaanonan* (M.
584 Blanco) Merr. (Bagtikan) in Mt. Makiling, Laguna, Philippines. Philippine
585 Agricultural Scientist 88(1):109–121.
586 Godefroid S, Piazza C, Rossi G, Buord S, Stevens A-D, Aguraiuja R, Cowell C,
587 Weekley CW, Vogg G, Iriondo JM, et al. 2011. How successful are plant
588 species reintroductions? Biological Conservation 144(2):672–682.
589 Goudet J. 1995. FSTAT (Version 1.2): A computer program to calculate *F*-statistics.
590 Journal of Heredity 86(6):485–486.
591 Hale ML, Burg TM, Steeves TE. 2012. Sampling for microsatellite-based population
592 genetic studies: 25 to 30 individuals per population is enough to accurately
593 estimate allele frequencies. PLoS One 7(9):e45170.
594 Hector A, Philipson C, Saner P, Chamagne J, Dzulkifli D, O'Brien M, Snaddon JL,
595 Ulok P, Weilenmann M, Reynolds G, et al. 2011. The Sabah Biodiversity
596 Experiment: a long-term test of the role of tree diversity in restoring tropical

forest structure and functioning. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 366(1582):3303–3315.

Hubisz MJ, Falush D, Stephens M, Pritchard JK. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9(5):1322–1332.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806.

Jombart T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24(11):1403–1405.

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):1.

Kalinowski ST. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5(1):187–189.

Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16(5):1099–1106.

Kenta T, Shimizu KK, Nakagawa M, Okada K, Hamid AA, Nakashizuka T. 2002. Multiple factors contribute to outcrossing in a tropical emergent *Dipterocarpus tempehes*, including a new pollen-tube guidance mechanism for self-incompatibility. American Journal of Botany 89:60–66.

Kettle CJ, Ghazoul J, Ashton PS, Cannon CH, Chong L, Diway B, Faridah E, Harrison R, Hector A, Hollingsworth P, et al. 2010. Mass fruiting in Borneo: a missed opportunity. Science 330(6004):584–584.

622 Kobayashi MJ, Takeuchi Y, Kenta T, Kume T, Diway B, Shimizu KK. 2013. Mass
623 flowering of the tropical tree *Shorea beccariana* was preceded by expression
624 changes in flowering and drought-responsive genes. Molecular Ecology
625 22(18):4767–4782.
626 Lee SL. 2000. Mating system parameters of *Dryobalanops aromatica* Gaertn. f.
627 (Dipterocarpaceae) in three different forest types and a seed orchard. Heredity
628 85(4):338–345.
629 Lee SL, Wickneswari R, Mahani MC, Zakri AH. 2000. Mating system parameters in
630 a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae), from
631 Malaysian lowland dipterocarp forest. Biotropica 32(4):693–702.
632 Lee SL, Tani N, Ng KKS, Tsumura Y. 2004. Isolation and characterization of 20
633 microsatellite loci for an important tropical tree *Shorea leprosula*
634 (Dipterocarpaceae) and their applicability to *S. parvifolia*. Molecular Ecology
635 Notes 4(2):222–225.
636 Lewis SL. 2006. Tropical forests and the changing earth system. Philosophical
637 Transactions of the Royal Society of London B: Biological Sciences.
638 361(1465):195–210.
639 Marsh CW, Greer AG. 1992. Forest land-use in Sabah, Malaysia - an introduction to
640 Danum Valley. Philosophical Transactions of the Royal Society of London B:
641 Biological Sciences. 335(1275):331–339.
642 Massey FP, Press MC, Hartley SE. 2005. Long- and short-term induction of defences
643 in seedlings of *Shorea leprosula* (Dipterocarpaceae): support for the
644 carbon:nutrient balance hypothesis. Journal of Tropical Ecology 21(2):195–
645 201.

McGrath DA, Smith CK, Gholz HL, Oliveira FdA. 2001. Effects of land-use change on soil nutrient dynamics in Amazonia. Ecosystems 4(7):625–645.

Momose K, Nagamitsu T, Inoue T, Inoue T. 1994. Reproductive ecology of an emergent tree, *Dryobalanops lanceolata*, Dipterocarpaceae, in a non-general flowering period in Sarawak. In: Inoue T, Hamid (eds) Plant reproductive systems and animal seasonal dynamics: long term study of dipterocarp forests in Sarawak Canopy Biology Programme in Sarawak. Series I, Kyoto University, Japan. p. 158–172.

Murawski DA, Gunatilleke I, Bawa KS. 1994. The effects of selective logging on inbreeding in *Shorea megistophylla* (Dipterocarpaceae) from Sri Lanka. Conservation Biology 8(4):997–1002.

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Resourses 8(19):4321–4325.

Murty D, Kirschbaum MUF, McMurtrie RE, McGilvray H. 2002. Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. Global Change Biology 8(2):105–123.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403(6772):853–858.

Ng KKS, Lee SL, Ueno S. 2009. Impact of selective logging on genetic diversity of two tropical tree species with contrasting breeding systems using direct comparison and simulation methods. Forest Ecology and Management 257(1):107–116.

669 O'Brien MJ, Leuzinger S, Philipson CD, Tay J, Hector A. 2014. Drought survival of
670 tropical tree seedlings enhanced by non-structural carbohydrate levels. Nature
671 Climate Change 4(8):710–714.
672 O'Brien MJ, Burslem DFRP, Caduff A, Tay J, Hector A. 2015. Contrasting
673 nonstructural carbohydrate dynamics of tropical tree seedlings under water
674 deficit and variability. New Phytologist 205(3):1083–1094.
675 Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population
676 genetic software for teaching and research: an update. Bioinformatics
677 28(19):2537–2539.
678 Pinard MA, Putz FE. 1996. Retaining forest biomass by reducing logging
679 damage. Biotropica 28(3):278–295.
680 Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using
681 multilocus genotype data. Genetics 155(2):945–959.
682 Ratnam W, Rajora OP, Finkeldey R, Aravanopoulos F, Bouvet J-M, Vaillancourt RE,
683 Kanashiro M, Fady B, Tomita M, Vinson C. 2014. Genetic effects of forest
684 management practices: global synthesis and perspectives. Forest Ecology and
685 Management 333:52–65.
686 Reynolds G, Payne J, Sinun W, Mosigil G, Walsh RP. 2011. Changes in forest land
687 use and management in Sabah, Malaysian Borneo, 1990–2010, with a focus
688 on the Danum Valley region. Philosophical Transactions of the Royal Society
689 B: Biological Sciences 366(1582):3168–3176.
690 Rosenberg NA. 2004. DISTRUCT: a program for the graphical display of population
691 structure. Molecular Ecology Notes 4(1):137–138.
692 Rousset F. 2008a. GENEPOP'007: a complete re-implementation of the GENEPOP
693 software for Windows and Linux. Mol Ecol Resour 8(1):103–106.

694 Rousset F. 2008b. Inferences from spatial population genetics. In: Balding DJ, Bishop
695 M, Cannings C (eds) Handbook of statistical genetics. John Wiley and Sons,
696 Ltd. p. 945–979.
697 Sakai S, Momose K, Yumoto T, Kato M, Inoue T. 1999. Beetle pollination of *Shorea*
698 *parvifolia* (Section Mutica, Dipterocarpaceae) in a general flowering period in
699 Sarawak, Malaysia. American Journal of Botany 86(1):62–69.
700 Sakai S. 2002. General flowering in lowland mixed dipterocarp forests of South-east
701 Asia. Biological Journal of the Linnean Society 75(2):233–247.
702 Sakai S, Harrison RD, Momose K, Kuraji K, Nagamasu H, Yasunari T, Chong L,
703 Nakashizuka T. 2006. Irregular droughts trigger mass flowering in aseasonal
704 tropical forests in Asia. American Journal of Botany 93(8):1134–1139.
705 Shimizu KK, Tsuchimatsu T. 2015. Evolution of selfing: recurrent patterns in
706 molecular adaptation. Annual Review of Ecology, Evolution and Systematics
707 46(1):593–622.
708 Templeton AR. 1994. Biodiversity at the molecular-genetic level: experiences from
709 disparate macroorganisms. Philosophical Transactions of the Royal Society of
710 London Series B-Biological Sciences 345(1311):59–64.
711 Thomas E, Jalonen R, Loo J, Boshier D, Gallo L, Cavers S, Bordacs S, Smith P,
712 Bozzano M. 2014. Genetic considerations in ecosystem restoration using
713 native tree species. Forest Ecology and Management 333:66–75.
714 Tuck SL, O'Brien M, Philipson C, Saner P, Tanadini M, Dzulkifli D, Godfray HCJ,
715 Godoong E, Nilus R, Ong RC, et al. 2016. Insurance effects of tree diversity in
716 tropical forest restoration: Survival and growth during the first decade of the
717 Sabah Biodiversity Experiment. Proceedings of the Royal Society B: *in press*

718 Turner I. 1996. Species loss in fragments of tropical rain forest: a review of the
719 evidence. Journal of Applied Ecology 33(2):200–209.
720 Ujino T, Kawahara T, Tsumura Y, Nagamitsu T, Yoshimaru H, Ratnam W. 1998.
721 Development and polymorphism of simple sequence repeat DNA markers for
722 *Shorea curtisii* and other Dipterocarpaceae species. Heredity 81(4):422–428.
723 Valen LV. 1965. Morphological variation and width of ecological niche. The
724 American Naturalist 99(908):377–390.
725 Vellend M. 2004. Parallel effects of land-use history on species diversity and genetic
726 diversity of forest herbs. Ecology 85(11):3043–3055.
727 Vellend M, Geber MA. 2005. Connections between species diversity and genetic
728 diversity. Ecology Letters 8(7):767–781.
729 Wilcove DS, Giam X, Edwards DP, Fisher B, Koh LP. 2013. Navjot's nightmare
730 revisited: logging, agriculture, and biodiversity in Southeast Asia. Trends in
731 Ecology and Evolution 28(9):531–540.
732 Wyatt-Smith, J. 1963. Manual of Malayan Silvicultural for Inland Forests. Vols. 1 and
733 2. Malayan Forest Records No. 23. Forest Research Institute Malaysia, Kuala
734 Lumpur.
735 Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, Fan J, Yang S, Hu L, Leung H, et al.
736 2000. Genetic diversity and disease control in rice. Nature 406(6797):718–
737 722.
738 Zuppinger-Dingley D, Schmid B, Petermann JS, Yadav V, De Deyn GB, Flynn DFB.
739 2014. Selection for niche differentiation in plant communities increases
740 biodiversity effects. Nature 515(7525):108–111.
741

742