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Gene flow through pollination of dipterocarp tree species in a Bornean rainforest

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Abstract We used microsatellite DNA analysis to assess the gene flow through pollination and the fine-scale spatial genetic structure (FSGS) of dipterocarp tree species in a tropical rain forest in Borneo. The results suggest that the intensity of FSGS of dipterocarp populations is dependent on tree density and pollination system. We found notable FSGS in beetle-pollinated tree populations but not in bee-pollinated tree populations. Both high and low density beetle-pollinated species had clear FSGS, in which genotypes of nearby trees were similar but those of distant trees were genetically different from each other. FSGS was especially strong for beetle-pollinated species with low tree density. These results are particularly important because they suggest that the effects of forest fragmentation by logging might be largely dependent on individual tree species. Logging is likely to form fragmented populations consisting of patches of genetically related trees. Thus, forest fragmentation may intensify FSGS, especially for tree species that have a pollination system with low mobility, such as beetle-pollinated trees. Increasing FSGS of tree populations may lead to loss of genetic diversity, high frequency of inbreeding and reduction of regeneration by inbreeding depression. Even for species pollinated by highly mobile bees, such negative effects on regeneration might be unavoidable under logging fragmentation.

Keywords Dipterocarpaceae, Genetic diversity, Inbreeding, Pollinator, Tree density

Introduction

The world's tropical rain forests are important plant communities due to their high biological diversity, including plant diversity (Whitmore 1998; Myers et al. 2000). To better understand plant community dynamics in tropical rain forest ecosystems, the forces that control the regeneration dynamics of the major component tree species must be evaluated. In the tropical rain forests of Southeast Asia, the plant family Dipterocarpaceae dominates in both biomass and tree species

richness (Condit et al. 2000), and includes 475 species in 13 genera (Ashton 1982). As most of the dipterocarp species grow very large and have great longevity, they regulate the forest environments by occupying the forest canopy layer for long time periods. Thus, the regeneration dynamics of this tree family have important implications for the dynamics of the entire forest community and its biodiversity.

Recently, studies on genetic variation have indicated that genetic diversity and the inbreeding of tree populations can influence the regeneration dynamics of trees. To date, several molecular biology techniques have been utilised (e.g. Lee et al. 2000; Tani et al. 2009) to study the effects of genetic diversity in wild plant species. Studies have revealed that even in large populations, populations with low genetic diversity are considered to be unstable when faced with environmental change (Bawa 1998). The harmful effects of inbreeding on regeneration are even more obvious because inbreeding increases the homozygosity of recessive deleterious alleles. The frequency of inbreeding is considered to be influenced by an interaction between fine-scale spatial genetic structure (FSGS) and pollen dispersal. FSGS, or the non-random spatial distribution of alleles and/or genotypes at fine spatial scales (less than 10 km) (Vekemans and Hardy 2004), often occurs as a result of limited seed and/or pollen dispersal (Hamrick et al. 1993) and is generally characterised by spatial aggregation of genetically similar individuals.

In this study, we selected four dipterocarp species from a tropical rain forest in Borneo and assessed the FSGS together with tree density and different pollinator types. The tree species pollinated by pollinators with long flight distances were expected to have lower FSGS than tree species pollinated by pollinators with low mobility, because longer pollen dispersal will increase the influx of gene flow and break the spatial aggregation of relatives via random gene movement. Tree density was expected to influence pollinator movement, because low tree densities will limit pollinator movement and hence will enhance the intensity of FSGS. In contrast, high tree density may accelerate pollinator movement and result in lower FSGS for these tree species.

Study site

This study was conducted in a 52-ha permanent plot (500 m × 1040 m) established in a tropical rain forest at Lambir Hills National Park, Sarawak, Malaysia by research teams from Sarawak, U.S.A. and Japan. The park is located 24 km south of Miri City (4°12'N, 114°00'E). The park and plot consist mainly of lowlands covered in mixed dipterocarp forest (Lee et al. 2002). The average annual rainfall at Miri Airport, about 20 km north of the study site, is 2725 mm (1967−1998). The park topography is hilly and the plot altitude range is 108.6−240.3 m. A total of 358, 905 trees ≥ 1 cm in diameter at breast height (1.3 m above ground level) were identified, measured, labelled and mapped (Yamakura et al. 1995).

Methods

We selected four dipterocarp species that differed in tree density in the plot and by pollinator type. *Dryobalanops aromatica* and *Shorea acuta* had high tree density and were distributed continuously along a ridge (Fig. 1a and b, respectively). In contrast to these two species, *Dipterocarpus crinitus* and *S. ovata* had low tree density and formed clumps containing a small number of trees (Fig. 1c and d, respectively). *D. aromatica* is mainly pollinated by the giant honeybee (*Apis dorsata*) (Momose et al. 1998). Based on field observations and flower morphologies, *D. crinitus* would be mainly pollinated by the giant honeybee, whereas *S. acuta* and *S. ovata* seem to be mainly

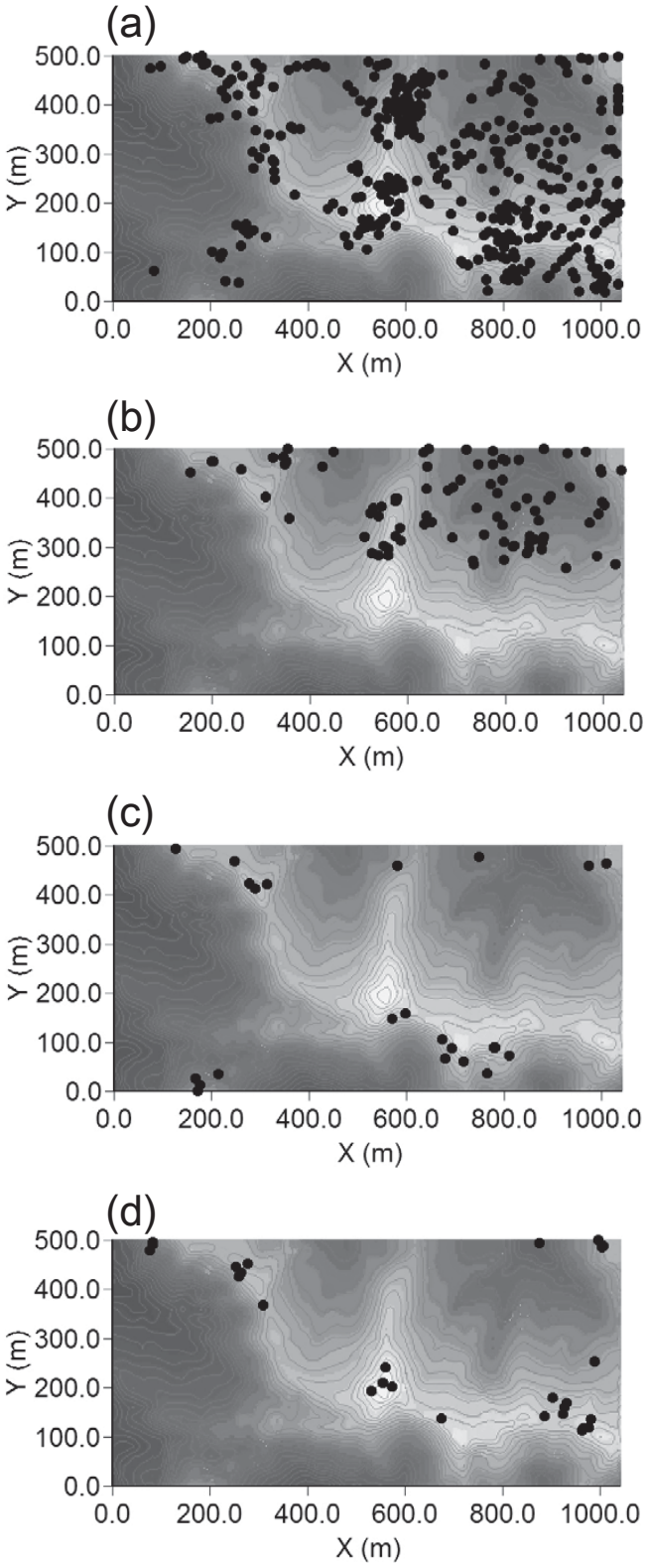

Fig. 1 The spatial distributions of adult dipterocarp tree species in the 52-ha plot, Lambir Hills National Park, Sarawak, Malaysia: (a) *Dryobalanops aromatica* (*n* = 397), (b) *Shorea acuta* (*n* = 89), (c) *Dipterocarpus crinitus* (*n* = 25) and (d) *Shorea ovata* (*n* = 40). Note that only trees included in the analysis are mapped in the plot.

Table 1 The genetic diversity and inbreeding coefficient for microsatellite loci of dipterocarp trees in the 52-ha plot, Lambir Hills National Park, Sarawak, Malaysia. The microsatellite loci analysis included: N_a, observed number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity under Hardy-Weinberg equilibrium; F, the inbreeding coefficient estimated by FSTAT (Goudet 2001). Note that n is the number of analysed adult trees, which does not include all mapped and labelled trees in the plot. Asterisks denote significant differences from zero (*P < 0.05; ***P < 0.001).

Dryobalanops aromatrica (n = 397)

Locus	N_a	H_o	H_e	F
Dra266	28	0.868	0.896	0.031*
Dra426	9	0.673	0.676	0.004
Dra428	5	0.103	0.245	0.580***
Dra471	10	0.578	0.734	0.213***
Dra519	16	0.723	0.746	0.031
Mean	13.6	0.589	0.659	0.172

Shorea acuta (n = 89)

Locus	N_a	H_o	H_e	F
Shc03	5	0.517	0.518	0.008
Shc04	18	0.764	0.795	0.045
Shc07	21	0.876	0.874	0.003
Shc09	9	0.854	0.829	−0.025
Sle079	8	0.472	0.674	0.305*
Sle111a	14	0.888	0.882	−0.001
Sle465	15	0.787	0.821	0.047
Sle562	11	0.831	0.856	0.034
Mean	12.6	0.749	0.781	0.052*

Dipterocarpus crinitus (n = 25)

Locus	N_a	H_o	H_e	F
DT07	3	0.522	0.567	0.102
DT09	9	0.609	0.688	0.137
DT20	6	0.870	0.768	−0.110
DT29	10	0.870	0.864	0.016
DT39	11	0.957	0.878	−0.067
Sle118	3	0.348	0.294	−0.162
Sle303a	4	0.652	0.653	0.024
Mean	6.6	0.689	0.673	−0.009

Shorea ovata (n = 40)

Locus	N_a	H_o	H_e	F
Sle111a	11	0.857	0.870	0.033
Sle118	14	0.750	0.872	0.158
Sle280	5	0.750	0.663	−0.113
Sle290	16	0.714	0.895	0.220*
Sle303a	15	0.821	0.804	−0.003
Sle384	10	0.929	0.864	−0.056
Sle392	7	0.821	0.786	−0.026
Sle605	5	0.750	0.663	−0.113
Mean	10.4	0.799	0.802	0.041

pollinated by beetles (Harata et al. 2012).

We collected leaf samples from adult trees ≥ 30 cm diameter at breast height in the plot. The leaves were ground to a powder using a mortar and pestle in liquid nitrogen. DNA was extracted using a modified CTAB method (Murray and Thompson 1980). For each species, 5–8 microsatellite markers (Ujino et al. 1998; Isagi et al. 2002; Lee et al. 2004: Nanami et al. 2007) were used (Table 1). PCR amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, USA) using TaKaRa LA *Taq* (TaKaRa Bio Inc., Japan). The reaction mixture for PCR (10 μl) consisted of 4.1 μl dH_2O, 1.0 μl 10 × buffer, 1.0 μl 25 mM $MgCl_2$, 0.2 μM primer F, 0.2 μM primer R, 0.8 μl dNTP mixture, 0.05 μl *Taq* polymerase and 1.0 ng template DNA. The PCR protocol consisted of initial denaturation at 94°C for 3 min, 38 cycles of denaturation at 94°C for 45 sec, 30 sec at the optimized annealing temperature, extension at 72°C for 45 sec, and a final incubation at 72°C for 3 min. Fragment analysis was performed using an ABI 310 Genetic Analyzer (Applied Biosystems) and genotypes were detected using GeneMapper software 3.0 (Applied Biosystems).

Based on the genotype data, the observed number of alleles (N_a), the observed heterozygosity (H_o), the expected heterozygosity under Hardy-Weinberg equilibrium (H_e) and the inbreeding coefficient (F) were calculated using FSTAT software (Goudet 2001). The relatedness coefficient between tree pairs was calculated using GeneAlEx 6.4 software (Peatkall and Smouse 2006).

Results

The values of N_a, H_o, H_e and F for each of the four dipterocarp species are shown in Table 1. The F values were significantly positive for three loci of *Dryobalanops aromatica* and for one locus each of *Shorea acuta* and *Shorea ovata*. Significant negative values were not detected.

For *D. aromatica*, the correlation between spatial distance and relatedness of tree pairs was not significant (Fig. 2a, $r = –0.025$, $P > 0.05$). The fine-scale spatial genetic structures (FSGS) of *S. acuta* (Fig. 2b, $r = –0.122$, $P < 0.001$), *Dipterocarpus crinitus* (Fig. 2c, $r = –0.160$, $P < 0.001$) and *S. ovata* (Fig. 2d, $r = –0.257$, $P < 0.001$) were significantly negative, indicating that the genotypes of nearby trees were more similar to each other than the genotypes of distant trees.

Discussion

We found strong correlations between FSGS and tree density that were dependent on pollinator type. Our findings indicated that the highly genetically structured populations of dipterocarp species had low-mobility pollinator types whose mating was limited to nearby related trees. We also found that the most intensely structured populations were beetle-pollinated species with low tree density, such as *Shorea ovata*. The spatial distance and the genetic relatedness between *Shorea acuta* individuals were also negatively correlated, even though *S. acuta* had a high tree density. In contrast, *Dryobalanops aromatica* is mainly pollinated by bees flying long distances, allowing mating both between nearby related trees, and between distant unrelated trees. We found that *D. aromatica* had low FSGS. Interestingly, the bee-pollinated species *Dipterocarpus crinitus* had significantly negative FSGS. One conceivable reason for these results may be that spatial isolation from other conspecific trees hindered mating over a large area.

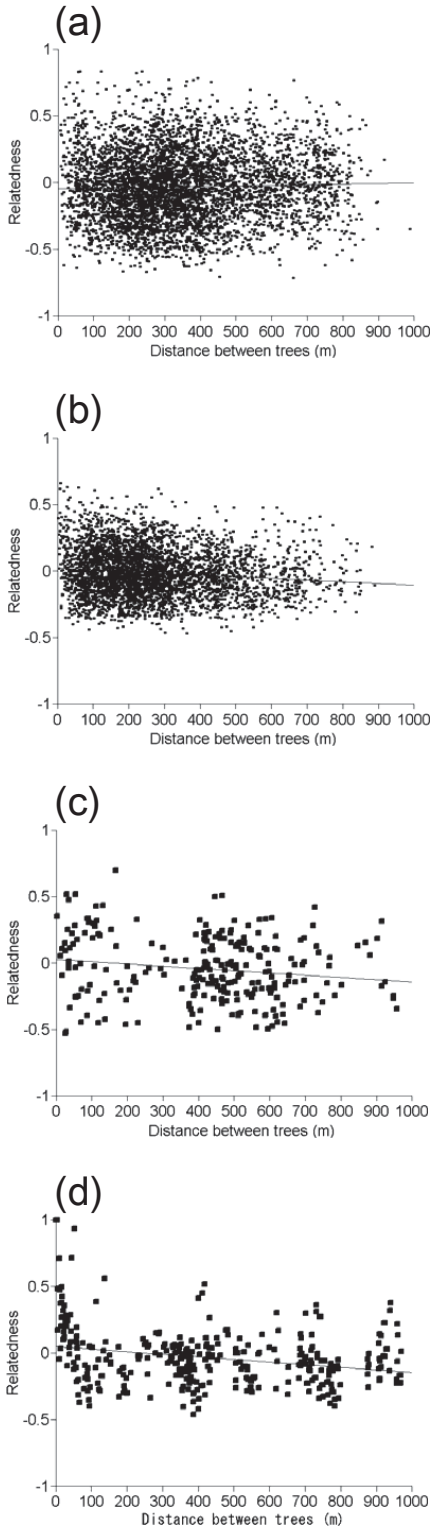

Fig. 2 The relationships between the relatedness coefficients and pairwise spatial distances for dipterocarp species in the 52-ha plot, Lambir Hills National Park, Sarawak, Malaysia: (a) *Dryobalanops aromatica* (r = –0.025, P > 0.05), (b) *Shorea acuta* (r = –0.122, P < 0.001), (c) *Dipterocarpus crinitus* (r = –0.160, P < 0.001) and (d) *Shorea ovata* (r = –0.257, P < 0.001).

The effects of forest fragmentation caused by logging might differ among tree species with distinct pollinators. Logging is likely to increase the patches of fragmented tree populations containing genetically related trees. Especially for the beetle-pollinated tree species, forest fragmentation will accelerate the frequency of inbreeding (Obayashi et al. 2002) and this may lead to loss of genetic diversity, a higher frequency of inbreeding and a reduction in regeneration resulting from inbreeding depression. Even for the bee-pollinated species, such negative effects might be unavoidable. Future work should focus on the effects of fragmentation on the relationship between FSGS, pollinator type and inbreeding depression in forest dynamics.

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