

Effects of Local Density and Forest Fragmentation on Reproductive and Regeneration Success of *Shorea laxa* (Dipterocarpaceae)

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Introduction

Plant–animal interactions such as pollination, seed dispersal, and herbivory play pivotal roles in plant reproductive and regeneration processes (Ghazoul and McLeish 2001). The scale and intensity of these interactions are affected by the local density of host plants and their habitat area (Garcia and Chacoff 2007). Aggregation of host plants may enhance their pollination success, resulting in enhanced seed set, outcrossing, or escape from seed predation as a result of predator saturation. However, recent rapid deforestation, especially in the tropics, has severely reduced pollinator populations and species richness, decreasing the reproductive output of plants in remnant habitats and increasing inbreeding depression (Aizen and Feinsinger 1994; Young et al. 1996). Similarly, environmental change in the remnant forest also results in recruitment losses and rapid changes in species composition (Laurance et al. 2006).

In this study, we focused on the reproduction of *Shorea laxa* Slooten, one of the ecologically and commercially key dipterocarp species in the Southeast Asian tropical forest. We investigated whether reproductive success and seedling recruitment were affected by forest type (primary continuous forest versus fragmented forest, known locally as *pulau*) and local adult tree density in terms of (1) seed set, (2) seed predation by insects, (3) seed predation by vertebrates, (4) the selfing rate, and (5) seedling survival.

Materials and methods

Study site and species

We conducted our field study in and around Lambir Hills National Park (LHNP), Sarawak, Malaysia (4°2'N, 113°50'E; 150 to 200 m a.s.l.). We established a 80-ha study plots in LHNP, which contains continuous stands of primary forest. The fragmented forests resulted from traditional land use by local peoples and were located near LHNP (typically less than 1 km). These areas were originally primary forest, although some selective cutting of trees for house construction has occurred historically. One *pulau* was used for our study plot; it covered 5 ha, was located 200 m from the edge of the primary forest, was surrounded by secondary forests, and was located mainly in hilly terrain.

Shorea laxa (section Richetioides, Dipterocarpaceae) is commonly found in northeastern Borneo and becomes a canopy tree that reaches heights of 30 to 40 m. The mean density of mature stems in primary forest (diameter at breast height \geq 30 cm) of *S. laxa* was 1.8 stems ha⁻¹ (Takeuchi et al. 2005). The species flowers and produces seeds at irregular, supra-annual intervals. The fresh seed mass \pm SD of *S. laxa*

averages 18.3 ± 12.3 g (Takeuchi and Nakashizuka 2007). In LHNP, flowering occurred in early March 2005.

Census of flower and seed fate before seed dispersal, and seedling survival

Two seed traps (0.5 m² surface area) were placed beneath the crowns of 20 trees per plot in each type forest in early March 2005, just before flowering, and were monitored until late October 2005 to describe the patterns of seed fate and predation. Every week or two, we collected all seeds present in the seed traps. Seed traps were made of nylon cloth with a mesh size of 1 mm and were set 1 m above the ground. All collected seeds were counted, measured, and classified into four categories: sound, immature, attacked by insects, or attacked by vertebrates. Seeds that had holes on their surface or larvae or frass inside were categorized as having been attacked by insects. Seeds attacked by arboreal vertebrates were either broken into pieces or exhibited clear tooth marks. Seeds with insect damage rarely germinated; those that did were classified as sound (also see Nakagawa et al. 2005).

Seedling plots containing over 50 seedling individuals (ranging in size from 1×1 m to 1×5 m) were established beneath the canopy after seed dispersal in September 2005. Seedling survival was measured in 7 months later (i.e., in April 2006).

Environmental data

To characterize the light conditions at the forest floor, we measured the percent canopy openness using a digital camera with a fisheye lens (CoolPix 910, Nikon). Two or three images were taken beneath the canopy at each sampling site at ground level and at a 0.2-m height, and were analyzed using Gap Light Analyzer v2.0 (Frazer et al. 1999).

Under the canopy of each tree, we quantified the soil-water potential using the method of Deka et al. (1995). Soil samples were taken on the morning of 25 October 2006, after a relatively dry spell with no rain for 6 consecutive days. We extracted two 3-cm cores to a depth of 10cm beneath the canopy after removing the surface litter. Each soil sample was sealed in a plastic box for 8 days with filter paper (Whatman No. 42) placed in the soil. On 31 October 2006, the filter paper was weighed to within 1 mg after quickly removing soil with a small paintbrush, and the soil-water potential was calculated (Deka et al. 1995).

DNA analysis and estimation of selfing rate of seeds

Seed samples for estimating selfing rate were collected beneath the 14 focal trees in late August. Total DNA was extracted from the cotyledon (seeds) or cambium (adult trees) of each individual using the DNeasy Plant Mini Kit (Qiagen). Genotypes of DNA samples were scored using five to six pairs of microsatellite PCR primers that had been developed for *Dipterocarpus tempehes* Slooten (Isagi et al. 2002), *Shorea leprosula* Miq. (Lee et al. 2004), and *Shorea lumutensis* Sym. (Lee et al. 2006). For PCR, we used 10-μL reaction mixtures containing 1× PCR Buffer (Promega), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 200 nM of each primer (one of each pair was fluorescently labeled), 1 ng of template DNA, and 0.25 to 0.50 units of *Taq* polymerase (Promega). PCR amplification was carried out for 5 min at 94°C, followed by 30 cycles of 30 s denaturing at 94°C, 30 s annealing at the optimized temperature, and 30 s extension at 72°C, with a

final 3-min incubation at 72°C, using a GeneAmp™ PCR System (Model 9700 and Model 2700; PE Applied Biosystems). The genotypes were determined using an ABI 3100 Genetic Analyzer and version 3.7 of the GeneScan™ software (PE Applied Biosystems). To estimate whether the analyzed seed had been self-fertilized, we examined the genotype similarity between the seed and the presumed mother tree using Cervus 2.0 software (Marshall et al. 1998).

Nearest-neighbor index (PULAU)

We calculated a nearest-neighbor index (*NNI*) for each focal tree based on the proximity of conspecific trees:

$$NNI = \sum \left(\frac{1}{n_{ij}} \right)$$

where n_{ij} is the distance (m) between the focal tree i and the other conspecific tree j located within 200 m of the focal tree (Ghazoul and McLeish 2001). By summing the reciprocal of the distances, this index considers both the number of surrounding flowering trees and their distance, weighted more heavily for the nearest neighbors. Thus, a high index value indicates low isolation and the existence of many nearby neighbors.

Statistical analysis

Data were analyzed using the stepwise generalized linear mixed model (GLMM) techniques implemented in the R statistical software (<http://www.r-project.org/>) to test correlations between seed fate and *NNI*. For seedlings, we tested the effects of *NNI* and environmental variability across habitats (canopy openness and soil-water potential) on the seedling survival. The *NNI* values were ln-transformed, canopy openness values were arcsine-transformed, and water potential values (negative values, converted into positive values for analysis) were ln-transformed before the analysis.

Results

Effect of forest type

Large numbers of seeds fell before they had matured (97% or more, Table 1). The proportion of mature seeds was significantly higher in *pulau* than in primary forest (Table 1). The proportion of seeds >1 cm in diameter that were attacked by insects exceeded the proportion attacked by vertebrates in both forest types, but the difference was greater in *pulau*. Predation by vertebrates was significantly higher in the primary forest, but predation by insects did not differ significantly between forest types (Table 1). The selfing rate of mature seeds was significantly higher in primary forest than in *pulau* (Table 2).

Seedling survival did not differ significantly between primary forest and *pulau* (Table 3). *Pulau* had significantly more light (a greater canopy openness) and significantly less water (lower water potential) than the primary forest (Table 4). We found no interaction between light intensity and water potential (data not shown).

Table 1 Number of focal tree, analyzed seeds and proportion of each seed fate.

Difference between forest type(primary vs. *pulau*) was tested by ANOVA.

Forest	No. of focal tree	Average no of total analyzed seeds \pm SD	Proportion of Mature seeds \pm SD	Proportion of seed fate (> 1cm diameter) \pm SD		
				Insect	Vertebrates	Sound
Total	20	2058.2 \pm 923.8	0.027 \pm 0.012	0.262 \pm 0.094	0.147 \pm 0.092	0.590 \pm 0.137
Primary	15	2152.9 \pm 1011.2	0.026 \pm 0.012	0.276 \pm 0.101	0.183 \pm 0.076	0.540 \pm 0.115
Pulau	5	1774.0 \pm 583.0	0.030 \pm 0.013	0.220 \pm 0.061	0.040 \pm 0.012	0.740 \pm 0.070
<i>p</i>			<0.05	n. s.	<0.01	<0.01

Table 2 Number of focal tree and selfing rate in both forest type.

Difference between forest type (primary vs. *pulau*) was tested by ANOVA. All analyzed seeds were mature.

Forest	No. of focal tree	Average no of total analyzed mature	Selfing rate \pm SD
Total	14	42.93 \pm 17.26	0.080 \pm 0.125
Primary	9	42.33 \pm 5.34	0.094 \pm 0.147
Pulau	5	44.00 \pm 30.14	0.054 \pm 0.080
<i>p</i>			<0.01

Table 3. Number of focal tree and survival rate (after 8 month) of seedlings in both forest types. Difference between forest type (primary vs. *pulau*) was tested by ANOVA.

Forest	No. of focal tree	Average no of total analyzed seedlings \pm	Survival rate \pm SD
Total	11	50.0 \pm 13.05	0.665 \pm 0.173
Primary	6	58.0 \pm 7.78	0.699 \pm 0.184
Pulau	5	40.0 \pm 11.55	0.623 \pm 0.173
<i>p</i>			n. s.

Table 4. Environment difference between primary forest and *pulau*.

	Water Potential(kPa) \pm SD	Canopy Openness (%) \pm SD
Primary	-0.01 \pm 0.01	5.90 \pm 0.54
Pulau	-0.28 \pm 0.29	7.40 \pm 0.40
<i>p</i>	<0.05	<0.05

Effect of local host density

Seed mature rates (the proportion of mature seeds) were negatively correlated with *NNI* in both forest types; the correlation was significant in primary forest and marginally significant in *pulau* (Table 5). The proportion of seeds predated by insects was not significantly correlated with *NNI* in either forest type, but the proportion of seeds predated by vertebrates was marginally positively correlated with *NNI* in the primary forest. We also found a significant negative correlation between the proportion of sound seeds and *NNI* in primary forest. The selfing rate of mature seeds also showed a significant and negative correlation with *NNI* in the primary forest, which suggests that isolation promotes self-fertilization. The correlation between *NNI* and the rates of sound seed production and selfing was not significant in *pulau*.

Seedling survival 8 months after seedling establishment in the primary forest was significantly affected by *NNI* and light intensity. On the other hand, seedling survival in *pulau* was negatively correlated with both light intensity (marginally significant) and water potential (significant), but we found no significant correlation with *NNI* (Table 6).

Table5 Results of stepwise GLMM for seed fate.

	Primary			Pulau		
		coefficient	p		coefficient	p
Mature rate	AIC:6981			AIC: 2215		
	Intercept	-4.652	<0.001	Intercept	-3.819	<0.001
	<i>NNI</i> _ln	-0.709	0.049	<i>NNI</i> _ln	-0.629	0.087
Predation by insects	AIC: 1551			AIC: 374.5		
	Intercept	-1.003	<0.001	Intercept	-1.204	<0.001
	<i>NNI</i> _ln					
Predation by vertebrates	AIC: 1235			AIC: 120.4		
	Intercept	-0.500	0.380	Intercept	-3.136	<0.001
	<i>NNI</i> _ln	0.817	0.060			
Sound	AIC: 1809			AIC: 403.1		
	Intercept	-0.973	0.025	Intercept	0.997	<0.001
	<i>NNI</i> _ln	-0.878	0.007			
Selfing	AIC: 200			AIC: 59.67		
	Intercept	-9.285	0.001	Intercept	-2.637	0.030
	<i>NNI</i> _ln	-5.310	0.023	<i>NNI</i> _ln	5.700	0.220

Table6 Results of stepwise GLMM for seedling survival.

	Primary			Pulau		
		coefficient	p		coefficient	p
Seedling survival after 8 months	AIC: 317.8			AIC: 195.5		
	Intercept	3.2	0.051	Intercept	2.724	0.114
	<i>NNI</i> _ln	-8.081	<0.001	Openness_arcsin	-41.364	0.071
	Openness_arcsin	-193.243	<0.001	Water potential ln	-0.523	0.002

Discussion

Effect of forest type on reproductive and recruitment success of Shorea laxa

We observed a significantly higher seed maturation rate in *pulau* than in primary forest. This suggests that pollination might function better in *pulau* than in primary forest. However, seed predation by vertebrates was significantly lower in *pulau* whereas predation by insects did not differ significantly between forests. These results can be explained if forest fragmentation has a stronger effect on populations of canopy vertebrates than on populations of insects. This is consistent with the results of Nakagawa et al. (2006), who also reported decreased rodent populations in *pulau* compared with primary forest. These results suggest that vertebrates may be more vulnerable than insects to forest fragmentation.

On the other hand, these differences did not lead to significant differences between forest types in terms of seedling survival 8 months after seedling establishment. However, the higher light intensity and decreased soil moisture in *pulau* compared with primary forest (Table 4) suggests that forest fragmentation may degrade the environment, even if these results are not apparent in the short term (i.e., our results reflect survival after less than 1 year). Seedling survival was affected by environmental conditions (Table 6). The long-term effect of the environmental changes that result from forest fragmentation may thus affect the survival pattern of seedlings.

In this study, we found that the difference between the *pulau* environment and the primary forest environment had no strong negative effects on the reproductive and recruitment success of *S. laxa*, at least in the short term (Tables 1 and 2). In other words, the *pulau* forest type, which represents a traditional form of forest reserve, may offer a similar function to that of primary forest for *S. laxa*.

Effect of local density on reproductive and recruitment success of Shorea laxa

Local host density affected the seed maturation rate and marginally the predation rate by vertebrates (Table 5). This suggests that aggregation of the study species had a negative effect on reproductive success in terms of both seed maturation and predation by vertebrates. It is not clear whether the decreased seed maturation rate results from seed abortion as a result of insect predation, which did not differ significantly among sites (Table 1), and/or inbreeding (selfing), which was significantly higher in primary forest (Table 2). The selfing rate of mature seeds decreased significantly with increasing local tree density, suggesting that tree isolation may increase the rate of pollination failure. The predation rate by vertebrates was marginally positively correlated with tree density in primary forest, suggesting that vertebrates respond to the local density of a food resource. On the other hand, we found that local host density did not affect insect predation in either forest type. This may result from differences in feeding behavior and range between insects and vertebrates.

Seedling survival was significantly correlated with *NNI* in primary forest. This indicates that predators, herbivores, and pathogens, which are a cause of seedling mortality in *S. laxa*, would exhibit density-dependent behavior.

In conclusion, our results show that *S. laxa* obtains advantages from low tree density in terms of seed maturation, avoidance of seed predation by vertebrates, and seedling recruitment. These results provide a possible explanation for why tropical trees tend to be sparsely distributed.

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