

Long pollen dispersal prevents biparental inbreeding depression in seeds in a natural population of the tropical tree *Shorea laxa*

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ABSTRACT

The aim of this study was to detect biparental inbreeding depression in seeds in a natural population of the tropical tree *Shorea laxa*. Using microsatellite markers, we conducted parentage analysis and directly estimated outcrossing rate and contemporary pollen dispersal across a natural population (100 ha). We found a high outcrossing rate and frequent long pollen dispersal in *S. laxa*, even though the population-level flowering during the study was not intensive. We also found a significant but weak negative correlation between pairwise relatedness and spatial distance between adult trees. Structural equation modeling revealed significant decrease in seed size with increasing pairwise relatedness of seed parents, indicating biparental inbreeding depression and also indicating a positive contribution of maternal internal relatedness and a negative contribution of pollen dispersal distance to the relationships among seed size and pairwise relatedness of seed parents. These results suggest that long-distance pollen dispersal is essential to prevent biparental inbreeding depression in *S. laxa*. When developing conservation strategies for *S. laxa*, attention must be paid to the breeding structure and phenology to ensure long-distance pollen dispersal and prevent reproductive isolation and inbreeding.

1. Introduction

Pollen dispersal in plant populations influences reproductive success and subsequent recruitment success, as it directly determines the inbreeding level of offspring. Offspring fate is therefore determined by both the spatial breeding structure, i.e., pollen dispersal range, and the parental genetic load, which lowers fitness through inbreeding depression. These spatial and genetic factors combine to drive evolution of the mating system (Holsinger, 1986; Charlesworth, 2006). In outcrossing plants, especially for populations that exhibit spatial genetic structure, i.e., spatial clumping of genetically related individuals, long-distance pollen dispersal is needed to prevent the mating of related neighboring individuals and thus biparental inbreeding depression (Ouborg et al., 1999; Ismail et al., 2012; Castilla et al., 2017) and maintain genetic diversity in the offspring population (O'Connell et al., 2007; Jha and Dick, 2010), which is essential for population persistence and “evolutionary potential” (Frankham et al., 2002). A better understanding of spatial breeding structure, including the genetic background of a species and the effect of inbreeding depression, is necessary for evaluating population viability and developing a conservation strategy.

Many studies have shown that tree species in tropical forests are

predominantly outcrossing (Murawski et al., 1994; Obayashi et al., 2002; Ward et al., 2005), and long-distance pollen dispersal is common (Kenta et al., 2004; Fukue et al., 2007; Ismail et al., 2012; Masuda et al., 2014; Tani et al., 2015; Castilla et al., 2017). The high outcrossing rate results from processes of the mating systems of a species to avoid selfing or inbreeding (Ward et al., 2005), such as self-incompatibility (Kenta et al., 2002) and inbreeding depression (Charlesworth and Willis, 2009). Inbreeding depression for selfed individuals has been observed in early life stages in natural populations, and includes phenotypic traits such as smaller seed mass (Naito et al., 2005), low germination rate (Naito et al., 2005; Ismail et al., 2014), and lower plant height (Rymer et al., 2015; Takeuchi et al., 2020), which are disadvantageous traits for survival. Biparental inbreeding (mating of inbred individuals) further reduces fitness (Nason and Ellstrand, 1995). There are indirect estimates of the effect of biparental inbreeding depression, including high outcrossing rate of offspring (Ward et al., 2005) and reduction of inbreeding coefficient in aged cohorts relative to younger cohorts (Stacy, 2001; Kenta et al., 2004; Tambarussi et al., 2017; Castilla et al., 2019). Relatively few studies have directly measured offspring fitness reduction with increasing parents relatedness in the population (Chaves et al., 2011; Jolivet et al., 2013; Pupin et al., 2019; Aguiar et al., 2020; Pereira et al.,

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2020). However, these studies showed which traits might be useful to measure biparental inbreeding depression, for example, seed size, seed mass, and seedling height.

There is increasing awareness that a species' genetic background must be taken into account for conservation in order to avoid genetic erosion and maintain viable populations of tropical trees (Tito de Moraes et al., 2015; Smith et al., 2018; de Oliveira et al., 2020; Hoban et al., 2020). The reduction of population fitness occurs not only through habitat change but also through genetic processes such as inbreeding depression (Nason and Hamrick, 1997), which are caused by the physical isolation of individuals due to rapid deforestation and forest fragmentation in the tropical area (Curtis et al., 2018). Thus, an understanding of a species' genetic diversity and structure within the population, as well as of the genetic loads that cause inbreeding depression, is essential to effective conservation strategies for tree species.

Shorea laxa (Dipterocarpaceae) is a timber tree found in a natural primary forest in northern Borneo. This species participates in general flowering (GF) events, the mass flowering phenomena that occur only in lowland tropical forests in SE Asia; for GF-participant species, reproductive success is influenced by both population-level and community-level flowering intensity, which determine the abundance and types of pollinators (Momose et al., 1998a,b). In previous studies, we showed a high outcrossing rate for this species in fragmented forests with limited pollen dispersal (Takeuchi et al., 2013), and a higher selfing rate, likely due to the species' reproductive isolation (Takeuchi et al., 2020). Moreover, selfed seedlings have a smaller average initial height, which negatively impacts survival (Takeuchi et al., 2020). We concluded that inbreeding depression in this species is caused by genetic load, and the species likely suffers from biparental inbreeding depression as well.

The goals of this study were to detect biparental inbreeding depression in a natural population of *S. laxa* and determine whether it could be prevented with long-distance pollen dispersal. We examined the multivariate relationships among seed size, relatedness of parent trees and pollen dispersal distance using structural equation modeling (SEM). We focused on seed size because this phenotypic trait is essential for subsequent individual performance, and because smaller seed mass has previously been reported in selfed seeds in other dipterocarp species (Naito et al., 2005). Given the known maternal effect on seed size (Roach and Wulff, 1987; Domic et al., 2020), we also considered the maternal effect in the statistical analysis.

We began by conducting a field survey of *S. laxa* in a natural population within a national park in Malaysia and collecting samples of adult trees and seeds from six mother trees during the GF event in 2005. We genotyped samples using microsatellite markers to identify the pollen parent tree, and calculated pairwise relatedness and spatial distance between parents. SEM was used to determine whether seed size was affected by seed parents' pairwise relatedness, and via this effect, by maternal internal relatedness and pollen dispersal distance. We also used SEM to analyze data from a fragmented forest (5 ha) where pollen dispersal distance was limited to < 60 m (Takeuchi et al., 2013) to identify biparental inbreeding depression in seed size.

2. Materials & methods

2.1. Study site

The field study was conducted in Lambir Hills National Park, Sarawak, Malaysia (4°2'N, 113°50'E, 150 m asl). Consistently high temperatures, with no distinct dry season, characterize the climate at this site. The mean annual temperature and annual precipitation are ca. 26 °C and 2700 mm, with the average monthly precipitation ranging from 167.5 mm in July to 328.4 mm in November (mean of measurements from 1985 to 1998 at a station 1.6 km northwest of the study site, 200 m asl; Sakai et al., 1999; Nakagawa et al., 2000). The study forest is an intact, mature lowland mixed dipterocarp forest (Ashton, 1991;

Ashton and Hall, 1992). The family Dipterocarpaceae dominates the canopy (35–40 m) and over canopy layers (which exceed ~70 m), and the Euphorbiaceae, Burseraceae, and Myristicaceae dominate the lower layers (Nakagawa et al., 2000). In terms of tree species diversity, this is thought to be one of the richest forests in the world (Whitmore, 1984).

2.2. Study species

Shorea laxa Slooten (Section Richetioides), which belongs to the family Dipterocarpaceae, is a canopy tree found in northeastern Borneo and western Malaysia. This species reaches 30–40 m in height and often grows on ridges. Pollinators include small beetles and thrips, which are common visitors to other *Shorea* spp. (Appanah and Chan, 1981; Sakai et al., 1999; Takeuchi et al., 2010), and these insects would also contribute to the long-distance pollen dispersal (Tani et al., 2009; Masuda et al., 2014). *S. laxa* produces wingless seeds that are dispersed by gravity, so most seedlings show a clumped distribution around the mother tree (Takeuchi et al., 2005). The seeds are consumed and can be, rarely, dispersed secondarily by rodents on the forest floor (Takeuchi and Nakashizuka, 2007). As mentioned above, *S. laxa* has been observed to produce flowers at every GF event in these locations since 2000 (Nakagawa et al., 2019; Ushio et al., 2020). In March 2005, about 33% of adult trees (defined as diameter at breast height (DBH) > 30 cm; 28 out of 84) in the population flowered (Takeuchi, personal observation).

2.3. Sample collection

All adult trees were mapped within the approximately 110 ha plot in Lambir Hills National Park, as reported in a previous study (Fig. 1) (Takeuchi et al., 2010). We randomly selected six seed-bearing trees that were evenly distributed within the plot and at least 80 m from each other. The DBH were 103.2, 61.1, 73.1, 79.3, 111.3, and 98.6 cm in trees ID SL02, SL18, SL37, SL43, SLv230, and SLv237, respectively. Seeds were collected from six seed-bearing trees in 2005 using two seed traps (each 0.5 m² in surface area) placed beneath the crown of each tree from early September to late October 2005 (the number of samples is given in Table 1). The diameter of each seed was measured two times with calipers, and the mean diameter (mm) was used as seed size (the size frequency is shown in Fig. S1). For genetic analysis, we collected a leaf or inner bark sample from all 109 trees in the plot.

2.4. DNA analysis

2.4.1. DNA extraction and microsatellite analysis

Total DNA was extracted from radicles (seed) or cambium (adults) of each individual using a modified CTAB method (Tsumura et al. 1996) or DNeasy Plant Mini Kit (Qiagen). After crude DNA extraction, the samples were purified using a FastDNA Kit (Bio 101). Genotypes of DNA samples were scored using six pairs of microsatellite PCR primers that were developed for relative species: *Shc02* (Ujino et al., 1998), *DT09* (Isagi et al., 2002), *Slc384* and *Slc475* (Lee et al., 2004), *Slu175* (Lee et al., 2006), and *SL39t* (SI text). 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 0.25–0.5 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; Applied Biosystems). The genotypes were determined using an ABI 3100 Genetic Analyzer and GeneScan™ software version 3.7 (PE Applied Biosystems).

2.4.2. Genetic diversity and paternity analysis

Basic measures of genetic diversity consisting of the number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e),

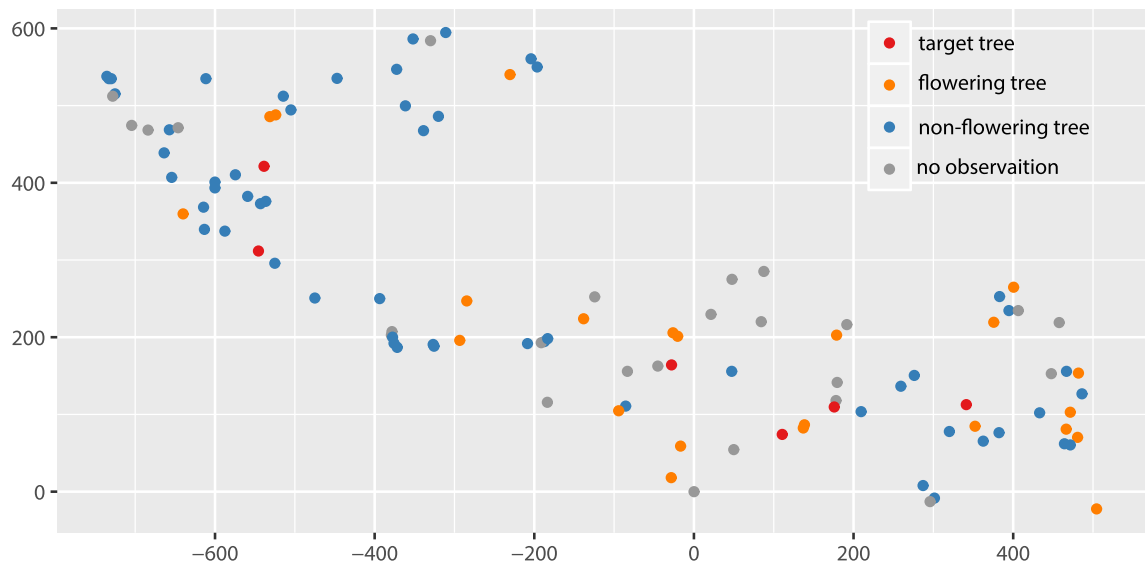


Fig. 1. The spatial distribution of adult trees within a plot (approximately, 1400 m (x axis) × 600 m (y axis) area). Flowering status is indicated with colors: red, mother trees ($N = 6$), orange, flowering trees ($N = 22$), blue, non-flowering tree ($N = 56$), gray, unobserved trees ($N = 25$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Genetic diversity of Adult population and seed arrays of six mother trees.

No. of samples		N		N_a		H_o		H_e		F		
Adults		109	108.43	(0.43)	10.57	(2.64)	0.62	(0.05)	0.72	(0.05)	0.14	(0.03)
Seeds	SL02	40	39.83	(0.17)	3.67	(0.42)	0.46	(0.09)	0.41	(0.09)	−0.14	(0.06)
	SL18	39	38.17	(0.48)	4.50	(0.67)	0.42	(0.09)	0.43	(0.08)	0.08	(0.08)
	SL37	46	45.17	(0.54)	4.83	(0.95)	0.34	(0.10)	0.35	(0.09)	0.02	(0.06)
	SL43	40	39.67	(0.21)	5.17	(0.83)	0.59	(0.10)	0.53	(0.07)	−0.11	(0.10)
	SLv230	41	40.83	(0.17)	3.33	(0.71)	0.37	(0.12)	0.36	(0.10)	−0.01	(0.08)
	SLv237	31	30.50	(0.34)	4.50	(0.76)	0.42	(0.08)	0.42	(0.07)	0.00	(0.09)
	Mean	39.5	39.03	(0.75)	4.33	(0.30)	0.43	(0.04)	0.42	(0.03)	−0.03	(0.03)

Fixation index (F), and allele frequencies were calculated using GenAEx 6.4.1 (Peakall and Smouse, 2006). To estimate the pollen parent of each seed, we used the software program Cervus 3.0 for maximum-likelihood-based paternity assignment (Kalinowski et al., 2007), commonly used to analyze parentage in natural plant populations (Ismail et al., 2012; Jolivet et al., 2013; Aguiar et al., 2020). Trees that had flowered or were not observed to have flowered were treated as the candidate pollen parents ($N = 53$, Fig. 1). The paternity exclusion rates among the candidate pollen parents were 0.94 and 0.99 for the first and second parent, respectively. The allele frequency of the candidate trees was used for the simulation rather than the frequency for the whole sample. For the simulation, we selected 10,000 cycles with the parameter of 100% of proportion of candidate parents sampled, 5% of the rate of typing error, and 95% strict confidence levels and 80% relaxed confidence levels. We used the results of 80% for paternity analysis.

2.4.3. The metrics of inbreeding

To estimate genetic relatedness of each seed and mother trees, we used two different metrics: pairwise relatedness between the mother tree and the estimated pollen tree, and internal relatedness of each mother tree within the population. The first metric is a direct metric on the inbreeding level of each seed, and our primary method to detect “biparental inbreeding depression.” The other metric indicates the relative inbreeding level of each mother tree in the population. The metric was used to detect how the maternal genetic background or maternal effect affected seed size. For pairwise relatedness, we used method-of-moments estimators of pairwise relatedness of adult trees in the population using three different algorithms—RI (Ritland, 1996), LRM (Lynch and Ritland, 1999) and QGM (Queller and Goodnight,

1989)—which are calculated in GenAEx (Peakall and Smouse 2006). The RI estimator reflected the number of shared alleles relative to population allele frequencies. The LRM estimator uses a regression calculation to determine relatedness coefficients for any pair of individuals based on shared identity by descent (IBD) alleles. The QGM is a coefficient based only on the estimated IBD (Grafen, 1985). For all three estimators, a higher value indicates higher relatedness, and a lower indicates lower relatedness among the pairs in the population. These estimators are considered to be sensitive to allele frequencies (Bink et al., 2008). For example, comparing to another common kinship parameter by Loiselle et al. (1995), those weight to rare alleles in the population relatively more.

For mother tree relatedness, we used internal relatedness (IR), which is an indirect measure of individual inbreeding level within the population. The IR among frequencies of homozygous alleles was used to estimate parental relatedness (Amos et al. 2001). This method is based on the relatedness measure described by Queller and Goodnight (1989), which is usually applied to comparisons between pairs of individuals. This method does not adjust allele frequency estimates for alleles carried by the individual concerned (Queller and Goodnight, 1989; Amos et al., 2001). When calculated over several loci, the resulting values are approximately normally distributed and centered on zero for individuals of unrelated parents, with negative values suggesting relatively outbred individuals and high positive values suggesting inbreeding (Amos et al., 2001).

2.5. Statistical methods

Fine-scale genetic structure within the adult population was

analyzed by examining the correlation between the pairwise relatedness metrics of RI, LRM, and QGM and spatial distance among the trees. The Mantel test was used to find the correlation between genetic relatedness and spatial distances among the adult trees, using the function “mantel” of the library “ecodist” in R (Goslee and Urban, 2007), with 999-times permutation in the Mantel test.

SEM was used to determine if seed size is influenced by relatedness of each seed, and via this effect by pollen dispersal distance and maternal relatedness (Fig. 4). The mother tree ID was treated as a random intercept. The model was fit using the R package brms (Bürkner, 2017) with 2 chains, 100,000 iterations and a warmup of 25,000 runs with 20 thin. Using the standard priors, the model converged with R values close to 1 (Gelman and Rubin’s diagnostic) and effective sample sizes > 5,000 for all estimated effects. Population-level effects were assumed to be significant if the 95% credible interval of an effect did not include zero. Model validation was performed using approximate leave-one-out cross-validation (LOOIC) using the loo package (Vehtari et al., 2020). We also estimated Bayesian R^2 values for each model to examine goodness of fit.

2.5.1. The data analysis of the fragmented forest

To examine whether biparental inbreeding depression was also found in a fragmented population where pollen dispersal was limited, we also conducted the SEM analysis using the genotypic datasets from our former study (Takeuchi et al., 2013). The target fragmented forest was a remnant of primary forest about 5 ha. This forest has probably been gradually fragmented since LHNP was established in 1975. The forest was located at least 90 m from the edge of LHNP and was surrounded by secondary forest (or fallows) and a rubber tree plantation, and thus physically disconnected from the primary forest. The seed samples were collected from five mother trees within the fragment forest in 2005 and genotyped, with the pollen parent tree also estimated. This study used all but one of the six microsatellite markers from the former study (Takeuchi et al., 2013). This study used the marker *SL39t* instead of *SL475* (Lee et al., 2004) because we detected a high probability of null alleles in the *SL475* locus in the population of primary forest. However, the mean H_e and paternity exclusion rates for the second parent in the fragmented forest were 0.70 and 0.99, respectively, which were equivalent to those in this study. Therefore, these two studies are comparable. Again using SEM, we examined the influence on seed size of relatedness via the mother tree’s relatedness and pollen dispersal distance in the dispersal-limited fragment forest.

3. Results

3.1. Genetic diversity and pollen dispersal

Among all adult trees, the six loci resolved that the mean N_a (\pm SD) and the mean H_e were 4.58 ± 1.05 and 0.72 ± 0.05 , respectively (Table 1). Among the seed arrays, the diversity varied among mother trees; the seed array of SL43 had lowest genetic diversity (mean N_a 3.33 ± 0.71 and mean H_e 0.36 ± 0.10), while SLv230 had highest genetic diversity (mean N_a 5.17 ± 0.83 and mean H_e 0.53 ± 0.07). The mean genetic diversity in seed arrays was lower than the mean genetic diversity of adults (mean N_a 4.33 ± 0.30 and mean H_e 0.42 ± 0.03), likely due to limited sample collection, e.g., derived from only six mother trees, in the seed array.

Table 2
Summary of the no. of samples and results of paternity analysis.

	SL02	SL18	SL37	SL43	SLv230	SLv237	Total
95% confidence level	12	6	4	10	14	6	52
80% confidence level	23	28	35	25	24	21	156
Total no. of samples assigned the pollen parent	35	34	39	35	38	27	208
No. of assigned pollen parent	4	10	11	12	10	7	
The mean pollen dispersal distance in meter (SD)	193.0 (316.90)	516.7 (128.30)	494.5 (186.39)	714.1 (405.18)	366.7 (252.21)	563.4 (224.82)	

We assigned pollen parents with 95% and 80% confidence levels to 52 (22%) and 156 (66%) seeds, respectively (Table 2). The genotypes of the remaining 29 seeds (12%) were consistent with those of trees within their respective plots, though with < 80% confidence. Among seeds whose paternities were assigned, there were no selfing seeds (0%), which indicates high outcrossing rate.

We also calculated the distance between the mother tree and the assigned pollen parent tree with > 80% confidence. The pollen dispersal distance ranged from 8.6 to 1106 m, with a mean of 193 to 714 m (Table 2, Fig. 2), which was less than the average pairwise distance between trees of each mother tree. The pollen dispersal distance frequency varied among mother trees (Fig. 2). Pollen from the distance class between 400 and 800 m was most abundant in three out of six mother trees.

3.2. Spatial genetic structure of adult trees and biparental inbreeding depression in seed size

Pairwise relatedness of RI, LRM and spatial distance had a significant negative correlation (Fig. 3, $r = -0.05$, $p < 0.01$, Fig. S2(a), $r = -0.03$, $p < 0.05$, Mantel test, respectively); QGM was also negatively correlated with spatial distance, but was not significantly (Fig. S2(b), $r = -0.02$, $p > 0.05$, Mantel test). The SEM analysis for both primary and fragmented forests yielded a good fit to the data in all models (the Pareto shape k parameter was < 0.7 in all models). The pairwise relatedness (RI) of seed parents were negatively correlated with pollen dispersal distance and positively correlated with maternal IR. Biparental relatedness was negatively correlated to seed size; i.e., seeds with higher relatedness were smaller on average in both primary and fragmented forests (Fig. 4). The relatedness metrics LRM and QGM has comparable results although some correlations were not statistically significant in both primary and fragmented forests (Fig. S3).

4. Discussion

4.1. Predominant outbreeding and long pollen dispersal in *S. laxa*

We found the dominant outcrossing and frequent long-distance pollen dispersal of *S. laxa* during the 2005 GF event. The intensity of community-level flowering was high that year (Ushio et al., 2020), although population-level flowering intensity was not; flowering trees were only 33% of the population (vs. > 50% in other flowering events). Lower flowering tree density generally reduces outcrossing rate and pollen dispersal distance (Naito et al., 2008b; Tani et al., 2009), but that result was not seen in the present study. We believe there are two reasons for the difference. First, the movement and abundance of generalist pollinators (small beetles and thrips) of the tree are less affected by population-level flowering intensity. Since community-level flowering intensity was strong, flower resources in the forest were abundant, which kept the pollinator population stable. A previous study reported that in another *Shorea* species with the same types of generalist pollinators, pollen dispersal distance did not differ between sporadic and intensive GF years (Masuda et al., 2014), indicating the stability of generalist pollinators’ function regardless of flowering intensity in a population/community e.g., Chrysomelids, (Kishimoto-Yamada and Itioka, 2008). Second, *S. laxa* has physiological mechanisms to reduce

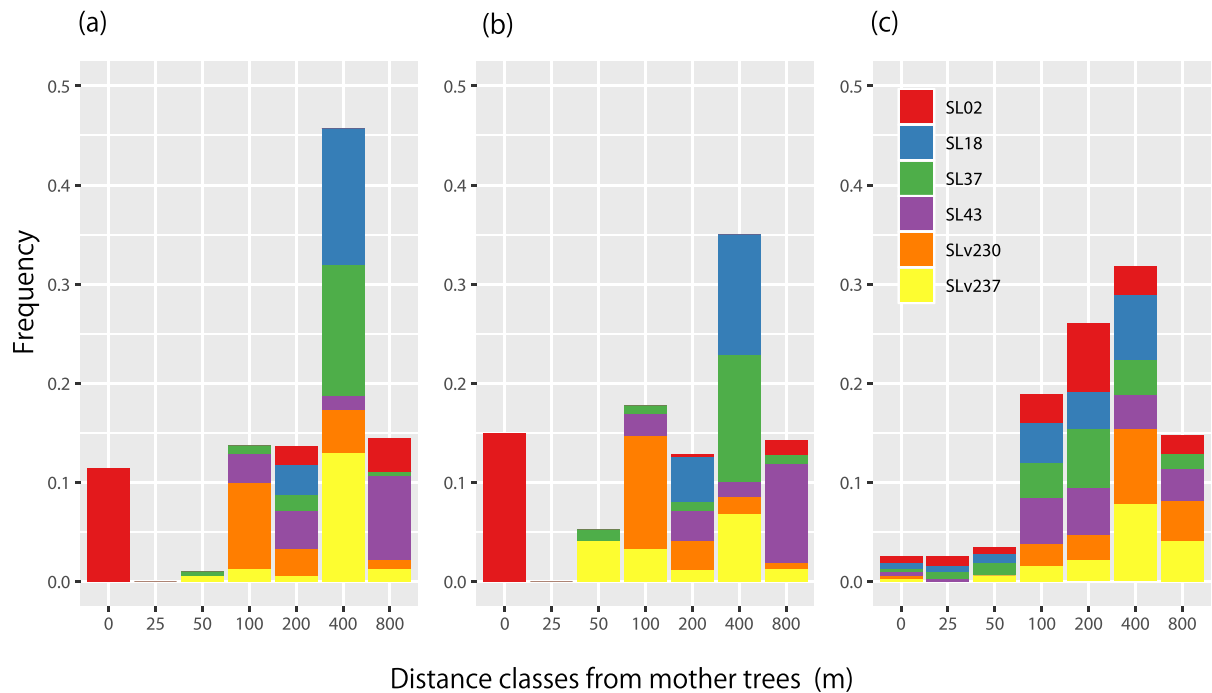


Fig. 2. Total frequency of (a) raw pollen dispersal distances, estimated from parentage analysis; (b) dispersal distances assuming uniform distribution of candidate pollen parent along the distance from mothers, i.e., (a) divided by (c); and (c) pairwise spatial distances between target mother trees and all candidate pollen parents. The color difference indicates the mother tree difference.

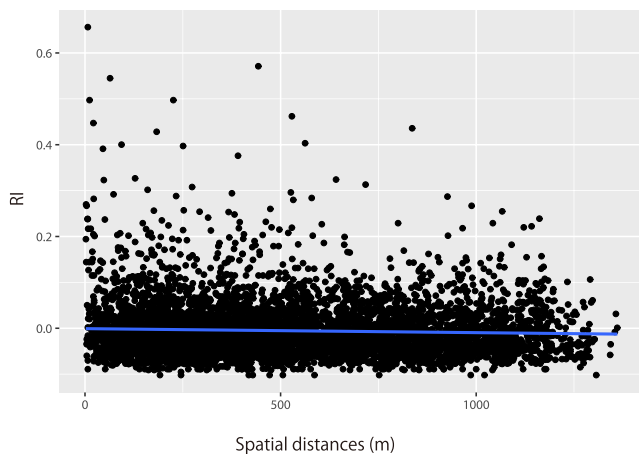
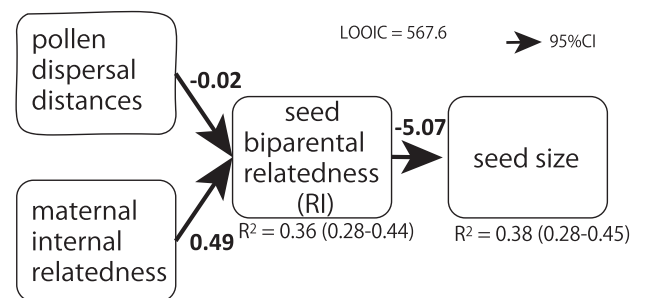


Fig. 3. Relationship between pairwise RI and pairwise spatial distance for all adult trees ($N = 109$). The significant negative correlation was detected by Mantel test (Mantel $r = -0.046$, $p < 0.01$) with 9999 permutation.

inbred offspring selectively before the seed stage, which results in high outcrossing rate and long pollen dispersal. Some dipterocarp species employ self-incompatibility (Chan, 1981; Dayanandan et al., 1990; Sakai et al., 1999; Kenta et al., 2002) and inbreeding depression (Naito et al., 2005), which explains high outcrossing rate in low flowering intensity events (Naito et al., 2008b; Tani et al., 2009). Selfing rate was also low in the fragmented forest (0% to 2.3%, Takeuchi et al., 2013), indicating there were enough flowering trees within the forest. On the other hand, we previously high selfing rates in forest-edge trees (Takeuchi et al., 2020), likely caused by outcrossed pollen limitation. The results indicate that the high outcrossing rate of *S. laxa* depends on sufficient outcrossed pollen, which is affected by physical and phenological conditions as well as pollinator availability.

This study detected the varying range of pollen dispersal distances, consistent with previous studies in natural populations of other *Shorea*

(a) Primary forest



(b) Fragmented forest

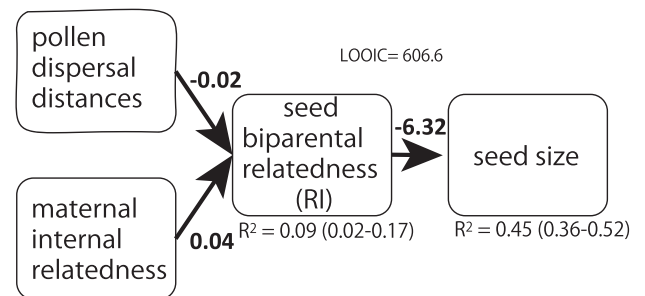


Fig. 4. Structural equation models (SEM) depicting direct and indirect factors of seed size in (a) primary forest and (b) fragmented forest. For each path, the regression coefficient (95% CI) is shown in black. Bayesian LOOIC and R^2 (95% CI) values are also shown for each model.

species (e.g., ~1 km and ~700 m in *Shorea leprosula*, Fukue et al., 2007; Tani et al., 2009, respectively; ~700 m, Naito et al., 2008a; ~450 m in *Shorea parvifolia*, Tani et al., 2009; ~660 m in *S. maxwelliana*, Masuda et al., 2014). Some studies have suggested different pollen dispersal

curves with different flowering tree density based on community pollinator population and availability (Tani et al., 2009; Masuda et al., 2014). Our previous study suggested that pollen dispersal was limited only within the forest, which indicates the forest edge acts as a barrier to pollinators' movement. Pollen dispersal range is affected by contemporary resource availability for flowers and pollinators in the community and by spatial distribution of the species. In other words, the degree of spatiotemporal isolation from other conspecific flowering trees may be a crucial determinant of pollen dispersal distance and outcrossing of this species (Naito et al., 2008a).

4.2. Fine-scale genetic structure and biparental inbreeding depression

We found the significant but weak genetic structure in the adult tree population of *S. laxa* contrary to expectation based on limited seed dispersal. Species with limited seed dispersal tend to have stronger genetic structure, while wind-dispersal (long-distance seed dispersal) species and/or taller tree species have weaker genetic structure (Takeuchi et al., 2004; Harata et al., 2012). Weak genetic structure is the result of constant high outcrossing and long-distance pollen dispersal across events (Hardy et al., 2006; Smith et al., 2018).

SEM indicated significant biparental inbreeding depression on seed size. To our knowledge, this is the first report to directly detect biparental inbreeding depression in a natural population of a tropical tree in SE Asia. Seed inbreeding was influenced by both the mother tree's IR and the pollen parent's spatial distance. This suggests that long-distance pollen dispersal is effective to avoid inbreeding between genetically related neighbors and prevent biparental inbreeding depression. Inbreeding depression was expressed as reduction in seed size, which results in lower seedling survival. The seeds of *S. laxa* were starch-rich (Nakagawa and Nakashizuka, 2004), and the amount of starch likely determines subsequent seedling performance such as germination (Domic et al., 2020) and survival (Bonfil, 1998; Khan, 2004). In fact, we previously reported that inbred seedlings tended to have smaller initial seedling height, and that smaller initial seedling height decreased seedling survival (Takeuchi et al., 2020), indicating that biparental inbreeding depression is expressed in an early life stage, as is inbreeding depression (Ghazoul et al., 1998; Nagamitsu et al., 2000; Hufford and Hamrick, 2003; Naito et al., 2005; Tani et al., 2015). Based on the results of this study and previous studies of indirect estimation of biparental inbreeding depression in tropical trees (Stacy, 2001; Kenta et al., 2004; Castilla et al., 2019), we believe biparental inbreeding depression is common in natural populations of tropical tree species. The presence of a system to reduce inbred individuals, i.e., inbreeding depression, would be a key mechanism to enhance overall population fitness, and might partly explain why tropical forest trees are predominantly outcrossing and have long-distance pollen dispersal.

4.3. Conservation remarks for *S. laxa*

This study confirmed that long-distance pollen dispersal is essential for reproductive and recruitment success in *S. laxa*. To secure the robust regeneration process in this species, reproductive isolation must be avoided. These findings could aid in the development of an effective management plan for *S. laxa* and related species in both *in situ* and *ex situ* conservation, as discussed below.

For *in situ* conservation, protected areas (PAs) play a major role to conserve the population of the species; *S. laxa* currently exists mainly within PAs (Julia et al., 2019). Even within PAs, mismanagement can result in population fragmentation and isolation due to logging, agricultural activities, and local use of forest resources (Laurance et al., 2012). Management of PAs should ensure the reproductive range of the species (~1 km) and reduce the edge effect in order to avoid the reproductive isolation. Extreme climate, including higher temperature and severe drought, will also affect the flowering phenology, since GF plants use environmental cues such as drought and low daily temperature to

synchronize flowering within the population (Sakai, 2002; Kobayashi et al., 2013; Ushio et al., 2020). Pollinator behavior also responds to environmental factors and flowering intensity in a community, and will be affected by climate change. This could result in reproductive isolation, i.e., pollen dispersal limitation, causing fitness reduction due to strong inbreeding depression. Continued monitoring of the phenological and ecological behaviors of this *S. laxa* population is necessary to investigate the effects of climate change. In the fragmented forest, *S. laxa* suffered from limited pollen dispersal within the population, and biparental inbreeding depression occurred. Forest fragmentation is known to affect the reproductive ecology of plants; in fragmented forests, the outcrossing rate and pollen dispersal distance both decrease (Aizen and Feinsinger, 1994; Nason and Hamrick, 1997; Takeuchi et al., 2013; Breed et al., 2015), possibility in connection with the mobility of pollinators (Breed et al., 2015; Castilla et al., 2017) and/or change in flowering behavior (Fuchs et al., 2003). In addition to any effect on reproductive and regeneration processes factors like inbreeding depression and genetic loads within the population affect species fitness under these threats. Different populations need to maintain connectivity (Castilla et al., 2017) to increase outcrossing with distant individuals and prevent inbreeding depression.

Ex situ conservation of tropical trees is becoming increasingly important to prevent loss of genetic resources, and may be a strategy for climate change adaptation. This study suggests that larger seeds in a mother tree be collected to help prevent inbreeding depression. Seeds from primary forests rather than fragmented forests will have greater genetic diversity, which will also minimize inbreeding depression and genetic erosion (Tito de Moraes et al., 2015). Moreover, this study and our prior study (Takeuchi et al., 2020) suggest that *S. laxa* is exposed the strong inbreeding depression in inbred and biparental inbred offspring, and has genetic loads. More research is needed on the genetic background of genetic loads and the effective gene flow to effectively sustain the population in both natural and logged forests.

5. Conclusions

In this study, we detected biparental inbreeding depression to seed size in *S. laxa* via positive maternal IR and negative pollen dispersal distance. We also found high outcrossing rate, frequent long pollen dispersal (~1 km), and genetic structure in the adult tree population. In the population of a fragmented forest, where pollen dispersal is limited, biparental inbreeding depression was also detected. These results show that long-distance pollen dispersal is essential to prevent biparental inbreeding depression in *S. laxa*. Species with genetic load and fine-scale genetic structure, when reproductively isolated, are more susceptible to inbreeding depression. Ensuring the reproductive range of the species and minimizing edge effect are important for *in situ* management of PAs to avoid the reproductive isolation. For *ex situ* conservation of tropical trees, larger seeds in a mother tree should be used to avoid inbreeding depression. Both *in situ* and *ex situ* comprehensive strategic plans are necessary for sustainable population management and for maintaining genetic resources in this species under the increasing threats of habitat fragmentation and climate change.

CRedit authorship contribution statement

Yayoi Takeuchi: Conceptualization, Methodology, Investigation, Writing - original draft. **Bibian Diway:** Writing - review & editing.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2021.119063>.

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