Overlapping flowering periods among Shorea species and high growth performance of hybrid seedlings promote hybridization and introgression in a tropical rainforest of Singapore

Kenzo, Tanaka; Kamiya, Koichi; Ngo, Kang Min; Faizu, Nik; Lum, Shawn Kaihekulani Yamauchi; Igarashi, Shuichi; Norichika, Yuki; Ichie, Tomoaki

2019

Kenzo, T., Kamiya, K., Ngo, K. M., Faizu, N., Lum, S. K. Y., Igarashi, S., . . . Ichie, T. (2019). Overlapping flowering periods among Shorea species and high growth performance of hybrid seedlings promote hybridization and introgression in a tropical rainforest of Singapore. Forest Ecology and Management, 435, 38–44. doi:10.1016/j.foreco.2018.12.038

<https://hdl.handle.net/10356/143896>

<https://doi.org/10.1016/j.foreco.2018.12.038>

© 2018 Elsevier B.V. All rights reserved. This paper was published in Forest Ecology and Management and is made available with permission of Elsevier B.V.

Downloaded on 26 Apr 2023 17:48:41 SGT

Overlapping flowering periods among *Shorea* species and high growth performance of hybrid seedlings promote hybridization and introgression in a tropical rainforest of Singapore

Tanaka KENZO^a, Koichi KAMIYA^{b,c,*}, Kang Min NGO^{b,d}, Nik FAIZU^b, Shawn Kaihekulani Yamauchi LUM^{b,d}, Shuichi IGARASHI^e, Yuki NORICHIKA^f, and Tomoaki ICHIE^f

^a Forestry and Forest Products Research Institute, Tsukuba, 305-8687, Japan

^b Center for Tropical Forest Science –Arnold Arboretum Asia Program, 1 Nanyang Walk, 637616, Singapore

^c Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-0865, Japan

^d Asian School of the Environment, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

^e The United Graduate School of Agriculture, Ehime University, Nankoku, 783-8502, Japan

^f Faculty of Agriculture, Kochi University, Nankoku, 783-8502, Japan

*Correspondence author at: Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-0865, Japan. E-mail address: kkamiya@agr.ehime-u.ac.jp (K. Kamiya)

Abstract

A considerable extent of interspecific hybridization among dominant canopy dipterocarp species was recently found in a tropical rain forest fragment in Singapore. However, information about the fertility of the hybrids and the growth of their successive hybrids remains limited. We studied the flowering phenology of four dipterocarp species in the genus *Shorea* (*S. curtisii*, *S. leprosula*, *S. parvifolia*, and *S. macroptera*) and two hybrids (*S. curtisii* × *S. leprosula* and *S. leprosula* × *S. parvifolia*) and studied the performances of seedlings reproduced from them. We observed that *S. macroptera* bloomed first, without overlap with congeneric species, followed by the other *Shorea* species and the two first filial (F₁) hybrids. *S. curtisii* and *S. leprosula*, the species forming hybrids are most likely to succeed in this forest, completely overlapped with their hybrids. Fruits collected from the hybrids were viable, and these hybrid seedlings showed greater survival and a higher growth rate than those from pure species under greenhouse conditions. They included various cross types such as a backcross with each parent species. These results may imply that successive hybrids and introgression spontaneously occur among *Shorea* species. It may be important to consider the risk of hybridization for the management of tropical forests, particularly in degraded tropical rainforests where mechanisms of ecological isolation between closely related species might be altered.

Key words: backcross; dipterocarp; forest degradation; general flowering; heterosis

1. Introduction

The extremely high species diversity of tropical rain forests in Southeast Asia is credited to the presence of large numbers of congeneric species that grow in sympatry (Ashton, 2014). A large proportion of the canopy trees in these forests belong to Dipterocarpaceae (dipterocarp trees, Ashton, 1982), and *Shorea* is one of the most speciose genera in this family (Ashton, 1982, 2014; Symington, 2004). Reproductive isolation and habitat specialization of dipterocarp trees in the forest are thought to prevent interspecific crossing (Ashton, 1969; 1982; Thomas, 2003; Kamiya et al., 2005; Ishiyama et al., 2008). However, in recent years, several hybrid trees of three closely related species of *Shorea* (*S. curtisii*, *S. leprosula*, and *S. parvifolia*) have been found in a small, isolated tropical rain forest in Bukit Timah Nature Reserve in Singapore (Kamiya et al., 2011; Kenzo et al., 2016). Surprisingly, approximately 20% of the hybrid trees that reached reproductive size (defined as diameter at breast height [DBH] > 30 cm) had two locally rare *Shorea* species (*S. leprosula* and *S. parvifolia*) as maternal parent species, but the frequency of hybridization of a much more abundant species, *S. curtisii*, was less than 2% (Kamiya et al., 2011). Moreover, the rate of F₁ seedlings and saplings of these species in Bukit Timah reached 40% (Kenzo et al., 2016).

Forests in Singapore have been subjected to anthropogenic fragmentation for more than 150 years, and the Bukit Timah forest (164 ha) is considered one of the oldest fragmented tropical rain forests in the area (Corlett, 1988). Although there are no clear factors in explaining this very high rate of hybridization in the forest, it is possible that forest fragmentation and degradation, which are also progressing rapidly throughout Southeast Asia (Hansen et al., 2013), may promote hybridization due to a decrease in mother tree density, pollinator overlap, and environmental changes (e.g. increased edge effects by forest fragmentation) that encourage the performance of hybrid seedlings (Rieseberg et al., 1989; Lamont et al., 2003; Field et al., 2008; Wong and Low, 2011; Kenzo et al., 2016).

Temporal separation of flowering timings is a key mechanism that isolates species of Dipterocarpaceae from each other (Ashton, 1982, 2014; Ashton et al., 1988; Ghazoul, 2016), but the separation might be incomplete as several hybrid *Shorea* species have been found (Kamiya et al., 2011). Most dipterocarp species bloom simultaneously during the general flowering period (~6 months), which is observed at 2- to 10-year intervals in the region (Ashton, 1982; Sakai et al., 2006), but the flowering peak for each dipterocarp species occurs sequentially (Ashton et al., 1988; Ghazoul, 2016). In the genus *Shorea*, the possibility of interspecific crossing exists due to the behavior of common pollinators including thrips, stingless bees, honeybees, and beetles (Appanah and Chan, 1981; Momose et al., 1998; Sakai et al., 1999; Ashton, 2014). If the hybrid trees are fertile and their flowering periods overlap with those of the parental species, successive hybridization and introgression would be promoted in *Shorea* species. However, even if hybrid trees are fertile, it is possible that their descendants may be maladaptive. Therefore, it is also important to assess the initial performances of parent species and their hybrid offspring at the seedling stage in understanding and predicting the progress of future hybridization (Arnold, 2006). Although naturally occurring F₁ hybrid seedlings in *Shorea* species have similar growth performances to those of their parent species in the forest understory (Kenzo et al., 2016), successive hybrids, such as those of backcrosses and F₂ generations, may have high growth performances that are advantageous to regeneration.

For example, Schweitzer et al. (2002) found that F₁ hybrids demonstrated relatively low fitness, but that naturally occurring backcrosses (BC) and the experimentally produced second generation of backcross (BC₂) had relatively high fitness. If seedlings which are derived from F₁ hybrid mother trees are viable and show growth comparable to that of parent trees, high rates of hybridization among dipterocarp species and the existence of adult hybrid individuals at high frequency in the forest might eventually lead to loss of species through the consolidation of distinct gene pools by introgression (Levin et al., 1996; Orians et al., 1999; Arnold, 2006; Kettle et al., 2012). However, few studies have examined the ecological traits of hybrids and their offspring (Kamiya et al., 2011; Kenzo et al., 2016).

In this study, we addressed two questions: Is there a difference in the flowering period between adult F₁ hybrids and their parent species?; and do seedlings derived from the mother trees of parent species and F₁ hybrids have different growth performances and viabilities? To answer these questions, we observed the flowering phenology of four species in the section *Mutica* of the genus *Shorea* and two pairs of their hybrids in the Bukit Timah forest. In addition, we collected seeds from hybrid mother trees and parent species and evaluated the initial growth performances at the seedling stage under nursery conditions.

2. Materials and Methods

2.1 Study site

Our study was conducted in a coastal hill dipterocarp forest in the Bukit Timah Nature Reserve (1°21' N, 103°46' E) in Singapore. The area has a humid tropical climate with no clear seasonal patterns in rainfall (Fig. 1, annual mean: 2,473 mm) nor temperature (annual mean: 27°C) (Lum et al., 2004). The forest is a fragment that has persisted in a landscape impacted by anthropogenic activities for more than 150 years (Corlett, 1988). It presently has an area of 164 ha, of which 48-71 ha is intact dipterocarp forest and the remaining parts are degraded secondary forest (Corlett, 1988; Ngo et al., 2016). Because it contains approximately half of the plant and animal species that have been recorded in Singapore, the reserve is considered an important site for conservation (Turner et al., 1994). Canopy height varies from 30 to 45 m, and the upper canopy consists of mixed species dominated by the family Dipterocarpaceae. *S. curtisii* and *D. caudatus* are locally abundant in the forest, and about 24% of the large trees (DBH > 30 cm) in the entire forest are *S. curtisii* (Lum et al., 2004; Ngo et al., 2013).

2.2 Plant material

There are four closely related *Shorea* species of the section *Mutica* (*S. curtisii*, *S. leprosula*, *S. macroptera*, and *S. parvifolia*) in the Bukit Timah forest (Kamiya et al., 2011). Although 23 species of this botanical section occur in the Malay Peninsula, Bukit Timah contains only these 4 species. The number of mature trees (>30 cm DBH) in the entire forest fragment (164 ha) varied greatly according to the species (*S. curtisii*, 975; *S. leprosula*, 84; *S. macroptera*, 24; and *S. parvifolia*, 10). All the morphologically identified hybrids were confirmed by the DNA sequence analyses of Kamiya et al. (2011). In that study, the authors counted 18 F₁ hybrids of *S. curtisii* × *S. leprosula*, but those of *S. curtisii* × *S. parvifolia* (one individual) and *S. leprosula* × *S. parvifolia* (two individuals) were rare. We were not able to find any hybrids of *S. macroptera* and the other three in the forest. There was a difference in morphological and ecological traits between the four *Shorea* species studied (Symington, 2004; Ashton, 1982, 2014). *S. curtisii* is dominant on ridges in hill dipterocarp forests and is occasionally found in coastal hill dipterocarp forests (Burgess, 1972, Kamiya et al., 2012), whereas *S. leprosula* and *S. parvifolia* are more common on well-drained soil in lowland and hill dipterocarp forests (Symington, 2004). *S. macroptera* is widely distributed on clay and sandy clay soils, from 0 to 900 m above sea level. These species coexist on some coastal hills and inland mountain ranges in Malaysia (Symington, 2004).

2.3 Observation of flowering phenology

We observed the flowering of the mature individuals of four parent species (*S. curtisii*, *S. leprosula*, *S. macroptera*, and *S. parvifolia*) and three F₁ hybrids (*S. curtisii* × *S. leprosula*, *S. curtisii* × *S. parvifolia*, and *S. leprosula* × *S. parvifolia*) during the general flowering event in 2009 (Table 1). The last general flowering prior to this event was in 2005. We targeted individual trees beyond reproductive size (defined as DBH > 30 cm) for the study (Kamiya et al., 2011). All the individuals of the three hybrid combinations and *S. parvifolia*, and about 80% of those of *S. macroptera* were observed during the event (Table 1). Because of large numbers of individuals of *S. curtisii* and *S. leprosula* in the forest, 14% and 30% of those were selected respectively (Table 1). A phenological observation about their flowering was made almost every weekday (except rainy days) from the end of February to the end of May 2009. We checked the bud stage, flowering, peak flowering (defined as 80% flowering on the branches), and the end of flowering with binoculars and by inspecting fallen flowers.

2.4 Initial seedling performances and estimation of hybrid seedling genotype

We collected seeds from the mother trees of *S. curtisii*, *S. leprosula*, *S. macroptera*, *S. parvifolia*, and the *S. curtisii* × *S. leprosula* hybrid. The seeds were sowed into a large plastic vat filled with well-watered river sand. After germination, each seed was transplanted into a plastic pot in August 2009. There were 96, 52, 66, 54, and 43 seedlings of *S. curtisii*, *S. leprosula*, *S. macroptera*, *S. parvifolia*, and *S. curtisii* × *S. leprosula* hybrids respectively. The potting media consisted of river sand (approx. 30%) and forest soil (approx. 70%). All the seedlings were nursed in a greenhouse covered with shade cloth (25% shade), and were watered every morning. We measured the diameter at base and height, and counted the number of leaves of each seedling 7 months after germination. We also recorded the survival of seedlings 7, 11, 15, and 17 months after germination in the nursery. The genotypes of all the seedlings obtained from the mother trees of a *S. leprosula* × *S. curtisii* hybrid were also identified by DNA analysis. The DNA was isolated from all the seedlings of the hybrid descendants, and species-specific differences in nucleotide in the chloroplast region of *trnL-trnF* were determined to identify their maternal species (Kamiya et al., 2011). Next, we genotyped the individuals to identify post-F₁ hybrids using eight microsatellite markers (shc03, shc04, shc07 and shc09 in Ujino et al., 1998; sle105, sle392, sle562 and sle384 in Lee et al., 2004) and used them for clustering analyses by STRUCTURE (Pritchard et al., 2000).

2.5 Statistical analyses

We used a one-way analysis of variance (ANOVA) with Tukey's HSD test to analyze interspecific differences in seedling growth traits (height, diameter, and leaf number). The survival of seedlings of each species was analyzed by using a generalized linear model (GLM) with binomial error distribution and logit link functions; the survival rate of each species was tested by using the likelihood ratio test. The R function 'glm' was used to build a model, and the 'analysis of variance' was made for the likelihood ratio test (R Development Core Team 2011).

3. Results

3.1 General flowering in Bukit Timah in 2009

We observed a total of 208 trees during the general flowering event in 2009 (Table 1). The percentage of flowering trees was less than 30% in *S. curtisii*, *S. leprosula*, and *S. macroptera*. Only 2 of the 18 *S. leprosula* × *S. curtisii* hybrids and 1 of the 2 *S. leprosula* × *S. parvifolia* hybrids flowered, whereas the only *S. curtisii* × *S. parvifolia* hybrid did not flower (Table 1). Nine out of ten (90%) *S. parvifolia* trees flowered. The peak flowering of *Shorea* occurred from March to May (Fig. 2). In addition to *Shorea* in Bukit Timah, many other trees in Singapore, e.g. in the forests of the MacRitchie area and Singapore Botanical Garden, flowered between the end of February and July 2009 (Kamiya et al., unpublished data). Before the flowering began, the minimum daily temperature from December 2008

to March 2009 was higher than 22.3 °C (Fig. 1A, Meteorological Service Singapore, <http://www.weather.gov.sg/climate-historical-daily/>). On the other hand, there was severe drought two months before the flowering (Fig. 1B). For example, the precipitation was 38 mm in January 2009, which was significantly less than the average for January in other years (246 mm from 1981 to 2010) (Fig. 1C).

Table 1 Number of observed and flowering individuals during the 2009 general flowering event in Bukit Timah Nature Reserve, Singapore. Numbers in parentheses indicate the total recorded. There was a significant difference in the flowering rate between species ($P < 0.001$, Fisher's exact test).

Species	Number of trees observed	Number of trees flowering	Floweri
<i>S. curtisii</i>	133 (975)	33	25
<i>S. leprosula</i>	25 (84)	6	24
<i>S. macroptera</i>	19 (24)	3	16
<i>S. parvifolia</i>	10 (10)	9	90
Hybrid (<i>S. curtisii</i> × <i>S. leprosula</i>)	18 (18)	2	11
Hybrid (<i>S. leprosula</i> × <i>S. parvifolia</i>)	2 (2)	1	50
Hybrid (<i>S. curtisii</i> × <i>S. parvifolia</i>)	1 (1)	0	0

3.2 Flowering patterns of four species of *Shorea* (section *Mutica*) and their hybrids

The flowering of the four species of *Shorea* (section *Mutica*) (*S. curtisii*, *S. leprosula*, *S. macroptera*, and *S. parvifolia*) occurred sequentially from early March to late May 2009 and the duration of the flowering was about 2 to 4 weeks (Fig. 2). *Shorea macroptera* bloomed first, followed by *S. parvifolia*, *S. curtisii*, and *S. leprosula*, and its period did not overlap with theirs. The flowering periods of these three species overlapped each other (Fig. 2). The peak flowering of *S. parvifolia* was slightly earlier than that of the first two species, *S. curtisii* and *S. leprosula*, with no temporal isolation from each other.

We observed how the two F₁ hybrids (*S. curtisii* × *S. leprosula* and *S. leprosula* × *S. parvifolia*) flowered and the duration of the flowering was about 2 to 3 weeks (Fig. 2 and 3). The flowering periods of each hybrid and its parental species overlapped considerably (Fig. 2), though the peak flowering periods of these hybrids were between those of *S. parvifolia* (earlier flowering species) and *S. curtisii* or *S. leprosula* (later flowering species).

3.3 Genotypes and initial seedling performance under nursery conditions

STRUCTURE analysis based on eight SSR markers showed that the seedlings collected from the mother trees of a F₁ hybrid (*S. curtisii* × *S. leprosula*) continuously changed in the probability of their belonging to either a *S. curtisii* or *S. leprosula* cluster (Fig. 4).

Seedling performances such as growth and survival rates significantly varied according to the species (Table 2; Fig. 5). Most of the seeds from the pure species and the hybrid were viable and germinated immediately after their collection. The seedlings from the hybrid mother trees (*S. curtisii* × *S. leprosula*) had greater height and higher diameter growth rates than the species studied (Table 2), and also had a larger number of leaves than the parental species (Table 2). The survival of the hybrid seedlings was higher (84% at 17 months) also than the other species (65% in *S. curtisii* and 50% in *S. leprosula* and *S. parvifolia*) (Fig. 5).

Table 2 The number of seedlings (n), diameter (D), height (H), and the number of leaves per seedling from the mother trees of four *Shorea* species and one hybrid (*S. curtisii* × *S. leprosula*) 7 months after germination under nursery conditions. Different letters indicate significant differences between species as assessed by ANOVA followed by the Tukey's HSD test ($P < 0.05$) ± standard error

Mother tree species	n	D (mm)	H (cm)	Number of leaves
<i>S. curtisii</i>	85	1.7 ± 0.1 ^c	15.0 ± 0.7 ^{bc}	5.0 ± 0.2 ^b
<i>S. leprosula</i>	37	1.7 ± 0.1 ^c	17.0 ± 0.8 ^{bc}	4.5 ± 0.2 ^{bc}
<i>S. macroptera</i>	55	2.0 ± 0.1 ^b	13.7 ± 0.4 ^{bc}	3.8 ± 0.2 ^c
<i>S. parvifolia</i>	50	1.6 ± 0.1 ^c	13.2 ± 0.6 ^c	5.4 ± 0.3 ^{ab}
Hybrid (<i>S. curtisii</i> × <i>S. leprosula</i>)	38	2.6 ± 0.1 ^a	23.2 ± 1.5 ^a	6.2 ± 0.3 ^a

4. Discussion

4.1 General flowering in Singapore

The severe drought which occurred in January 2009 might have induced the flowering of dipterocarp trees in Singapore as a trigger. Recent studies also reported that severe drought prior to flowering had acted as a trigger for general flowering in the Malay Peninsula and Borneo (Sakai et al., 2006; Ashton, 2014; Ghazoul, 2016). Corlett (1990) also reported from Bukit Timah that they had observed a severe drought in February 1987 (rainfall; 24.5 mm between 30 January and 3 March) and this may have caused a general flowering event between March and May 1987. Although low temperature stress (usually below 21°C) is also considered as a trigger for the general flowering of dipterocarp species (Ashton et al., 1988; Sakai et al., 2006; Ashton, 2014), no distinct low temperature was recorded, e.g. daily minimum temperature was above 22°C.

The observed general flowering was relatively on a large scale in terms of participating species numbers. We observed 92% of the dipterocarp species (11 out of 12 species) flowering in this study in Bukit Timah and more than 70% in other forests in Singapore, including the MacRitchie area and Singapore Botanical Garden, during the general flowering period in 2009 (Kamiya et al., unpublished data). Both percentages were comparable to those during the previous general flowering observed in other areas, e.g. 81% of the dipterocarp species (33 out of 41 species) flowering in Sarawak, Malaysia in 1996 (Sakai et al., 1999).

4.2 Overlap between the flowering periods of hybrids and their parent species

Although the flowering of parent species and their hybrids occurred in order, the flowering periods of the hybrids overlapped each other and those of their parent species. The sequence of the peak flowering periods of the four species of *Shorea* (section *Mutica*) (*S. curtisii*, *S. leprosula*, *S. macroptera*, and *S. parvifolia*) was consistent with that observed in Peninsular Malaysia and Borneo; *S. macroptera* bloomed first, followed by *S. parvifolia*, *S. curtisii*, and *S. leprosula*, and its period did not overlap with theirs. (Tamari, 1976; Chan and Appanah, 1980; LaFrankie and Chan, 1991; Brearley et al., 2007; Ghazoul, 2016; Chen et al., 2017). The consistent patterns of sequential flowering in different forests suggest that the timing of flowering induction in these species may be genetically controlled by similar environmental cues (Kobayashi et al., 2013; Ashton, 2014; Yeoh et al., 2017). It is noteworthy that the two ecologically different species, *S. curtisii* and *S. leprosula*, were in no temporal isolation for flowering from each other, and they occasionally coexist in coastal hill dipterocarp forests in Malaysia and Singapore (Symington, 2004). In the duration of flowering there was a great similarity between these species and the overlap in their flowering periods was observed in hill dipterocarp forests in the Malay Peninsula (Medway, 1972; Yap and Chan, 1990). This phenomenon is consistent with the fact that these two species can hybridize in nature, and that they make the most common hybrid combination in the forest (Kamiya et al., 2011; Kenzo et al., 2016), which suggests that habitat isolation (e.g. *S. curtisii* and *S. leprosula* mainly distributed over the ridges and lower slopes respectively) may be a more significant barrier between the two species (Kamiya et al., 2011; Ashton, 2014). On the other hand, earlier flowering in *S. parvifolia* than in *S. leprosula* and *S. curtisii* may imply the rarity of hybrids between *S. parvifolia* and *S. leprosula* and between *S. parvifolia* and *S. curtisii* in Singapore (Kamiya et al., 2011; Kenzo et al., 2016). The temporal isolation of flowering is an important factor in preventing hybridization between these three species, although it is not completely effective because of some overlap of flowering periods. In addition, significant overlap in flowering periods between each hybrid and its parental species, together with the fact that thrips are common pollinators of the section *Mutica* in Peninsular Malaysia (Appanah and Chan, 1981; Ashton, 2014), may enable the hybrids to cross with their parent species. We also found several mature F₁ hybrid (*S. curtisii* × *S. leprosula*) trees in three isolated forests logged before 1970's in the Malay Peninsula, and observed overlapped flowering periods between F₁ hybrids and their parent species in Recreational Forests of Pasir Panjang in Negeri Sembilan and of Tanjung Tuan in Malacca in June 2014, and of Sungai Udang in Malacca in June to July 2016 (Kenzo et al. unpublished data). Although further detailed study is needed, these observations may be evidence that hybridization of *Shorea* species occurs in other degraded forests as well.

4.3 Occurrence of backcross and high growth performance of hybrids

Most of the hybrid individuals collected from the mother trees of each F₁ hybrid were confirmed as not assigned to a single species by STRUCTURE analysis and this suggests the occurrence of backcrossing between the parent species and/or the F₁ hybrid. It should be noted that by the same STRUCTURE analysis, the samples which were of a F₁ hybrid of *S. curtisii* × *S. leprosula* as were described in Kamiya et al. (2011) were genetically assigned a 50% probability of their belonging to *S. curtisii* or *S. leprosula* (data not shown). Hence, the continuous change in probability of identifying species suggests that the hybrid could have a chance of intercross with the parental species (*S. curtisii* and *S. leprosula*) and the other F₁ hybrid individuals (*S. curtisii* × *S. leprosula*) naturally. The presence of viable seeds from the hybrid mothers also implies the occurrence of introgression through hybridization (Anderson, 1948; Rieseberg, 1995; Arnold, 2006). In addition, the significantly smaller population size of *S. leprosula* compared to that of *S. curtisii* may lead to loss of species through the consolidation of distinct gene pools by introgression (Levin et al., 1996; Orians et al., 1999; Kettle et al., 2012), even though the habitat boundaries between these species still exist in the Bukit Timah forest (Kamiya et al. 2011).

The higher growth and survival rates of the hybrid seedlings suggest the occurrence of heterosis and of successive generation hybrids, though evidence was obtained from one combination for F₁ hybrids (*S. curtisii* × *S. leprosula*). Heterosis has been noted in hybrids of several plant species, including some tree species such as *Acacia* and *Pinus* spp. (Dungey, 2001; Arnold, 2006; Lu and Sinclair, 2006; Tullus et al., 2007; O'Reilly-Wapstra et al., 2014), which is consistent with the present study. A large number of leaves in the hybrid seedlings contribute to much carbon absorption by high photosynthetic productivity, so it may explain the higher growth rate of the hybrids (Larcher, 2003). Irino et al. (2004) also found a positive relation between the number of leaves and the growth rate in dipterocarp seedlings under nursery conditions. The bright nursery environment rather than the dark forest understory may allow a higher survival rate to the present hybrid seedlings, commensurate with better survival of the F₁ hybrid seedlings (*S. curtisii* × *S. leprosula*) being promoted by canopy openness in the forest (Kenzo et al. 2016). In the previous study, however, heterosis expressed by better growth rates was not obvious in F₁ hybrids under natural forest conditions (Kenzo et al., 2016). Limited light conditions in the natural forest understory may also render the effects of heterosis in F₁ hybrid seedlings not so obvious. In addition, the rooting ability of cuttings of F₁ hybrids also lack obvious heterosis under low light conditions in a nursery (Kenzo et al., 2019). On the other hand, despite their high growth performances under nursery conditions, the successive hybrid seedlings are rare in Bukit Timah compared with a large proportion of the F₁ hybrid seedlings (Kamiya et al., 2011; Kenzo et al., 2016). Although it is not clear why the formation or survival of successive hybrid populations seems lower in the field, this could be due to some ecology-dependent selection, for example, by disease or particular edaphic conditions occurring (Arnold, 2006).

5. Conclusions

We found that the flowering periods of the F₁ hybrids (*S. curtisii* × *S. leprosula* and *S. leprosula* × *S. parvifolia*) overlapped with those of their parent species (*S. curtisii*, *S. leprosula*, and *S. parvifolia*). This may imply that the F₁ may be able to hybridize further with their parental species, resulting in their increased introgression. This is concordant with the fact that the seeds collected from the *S. curtisii* × *S. leprosula* hybrid were viable and their seedlings had high growth performance. Although there were no obvious intrinsic (gametic) isolating barriers in the F₁ hybrids and their parents, successive hybrid individuals under natural rainforest conditions were rare (Kamiya et al., 2011; Kenzo et al., 2016). This rarity is possibly due to extrinsic (ecological) constraints, such as disease, and unsuitable environment for the hybrids, and such conditions might limit the possibilities for successive hybrid generations in the forest. However, when ecologically-maintained isolating barriers and micrometeorological conditions are changed by forest degradation and impactful logging, this may allow outcrossing and survival of hybrids, and then selfing and hybridization extents may increase with further changes in the composition and abundance of local species (Lamont et al., 2003; Field et al., 2008; Schaberg et al., 2008; Cannon and Lerdau, 2015). Although the role of multiple mating behavior in the *Shorea* species remains unknown, understanding of interspecific hybridization between the sympatric species of *Shorea* can be important particularly in a more proactive tropical forest management that considers conservation of rare species and small populations on both contemporary and evolutionary scales. Further studies are needed on hybrids between other closely-related dipterocarp species in various degraded forests, and their ecological traits such as ecophysiological and reproductive properties to understand the risk of hybridization in degraded tropical rainforests in Southeast Asia.

Acknowledgements

We thank the National Parks Board, Center for Tropical Forest Science – Arnold Arboretum Asia Program, Nature Heritage Group in the Japanese Association Singapore, and the National Institute of Education, Nanyang Technological University, Singapore, for their kind support of this study. This study was conducted under permission from National Parks Board, Singapore (NP/RP 857 and 972-1 to 972-7). This work was partly supported by JSPS KAKENHI Grants (No. 21780155, No. 21688011, No. 24405032, No. 24688017, 16K07795, 17H04602, and 17H04623), and by the Environment Research and Technology Development Fund (RF-1010, S-9) of the Ministry of the Environment, Japan.

References

- Anderson, E., 1948. Hybridization of the habitat. *Evolution* 2, 1–9.
- Appanah, S., Chan, H.T., 1981. Thrips: the pollinators of some dipterocarps. *Malay. For.* 44, 234–252.
- Arnold, M.L., 2006. *Evolution through genetic exchange*, Oxford University Press, Oxford.
- Ashton, P.S., 1969. Speciation among tropical forest trees: some deductions in the light of recent evidence. *Biol. J. Linn. Soc. Lond.* 1, 155–196.
- Ashton, P.S. 1982. Dipterocarpaceae. *Flora Malesiana* 9, 237–552.
- Ashton, P.S., Givnish, T.J., Appanah, S. 1988. Staggered flowering in the Dipterocarpaceae: new insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *Am. Nat.* 132, 44–66.
- Ashton, P.S., 2014. *On the forests of tropical Asia: lest the memory fade*. Kew Publishing, London
- Brearley, F.Q., Proctor, J., Nagy, L., Dalrymple, G., Voysey, B.C., 2007. Reproductive phenology over a 10-year period in a lowland evergreen rain forest of central Borneo. *J. Ecol.* 95, 828–839.
- Burgess, P.F. 1972., Studies on the regeneration of the hill forests of the Malay Peninsula. *Malay. For.* 35, 102–123.

- Cannon, C.H., Lerdau, M., 2015. Variable mating behaviors and the maintenance of tropical biodiversity. *Front. Genet.* 6, 183.
- Chan, H.T., Appanah, S., 1980. Reproductive biology of some Malaysian dipterocarps I: flowering biology. *Malay. For.* 43, 132–143.
- Chen, Y.Y., Satake, A., Sun, I., Kosugi, Y., Tani, M., Numata, S., Hubbell, S.P., Fletcher, C., Supardi, M.N.M., Wright, S.J., 2017. Species-specific flowering cues among general flowering *Shorea* species at the Pasoh Research Forest, Malaysia. *J. Ecol.* 106, 586–598.
- Corlett, R.T., 1988. Bukit Timah: the history and significance of a small rain-forest reserve. *Environ. Conserv.* 15, 37–44.
- Corlett, R.T., 1990. Flora and reproductive phenology of the rain forest at Bukit Timah, Singapore. *J. Trop. Ecol.* 6, 55–63.
- Dungey, H.S., 2001. Pine hybrids—a review of their use performance and genetics. *For. Ecol. Manage.* 148, 243–258.
- Field, D.L., Ayre, D.J., Whelan, R.J., Young, A.G., 2008. Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregata*. *J. Ecol.* 96, 1198–1210.
- Ghazoul, J., 2016. *Dipterocarp Biology, Ecology, and Conservation*. Oxford University Press, Oxford.
- Hansen, M.C., Potapov, P.V., Moore, R., Hancher, M., Turubanova, S.A., Tyukavina, A., Thau, D., Stehman, S.V., Goetz, S.J., Loveland, T.R., Kommareddy, A., Egorov, A., Chini, L., Justice, C.O., Townshend, J.R.G., 2013. High-resolution global maps of 21st-century forest cover change. *Science* 342, 850–853.
- Irino, K.O., Iba, Y., Ishizuka, S., Kenzo, T., Ripot, S., Kendawang, J.J., Miyashita, N., Nara, K., Hogetsu, T., Ninomiya, I., Iwasaki, K., Sakurai, K., 2004. Effects of controlled-release fertilizer on growth and ectomycorrhizal colonization of pot-grown seedlings of the dipterocarp *Dryobalanops lanceolata* in a tropical nursery. *Soil Sci. Plant Nutr.* 50, 747–753.
- Ishiyama, H., Inomata, N., Yamazaki, T., Ab Shukor, N., Szmidt, A.E., 2008. Demographic history and interspecific hybridization of four *Shorea* species from Peninsular Malaysia inferred from nucleotide polymorphism in nuclear gene regions. *Can. J. For. Res.* 38, 996–1007.
- Kamiya, K., Harada, K., Tachida, H., Ashton, P.S., 2005. Phylogeny of *PgiC* gene in *Shorea* and its closely related genera (Dipterocarpaceae), the dominant trees in Southeast Asian tropical rain forests. *Am. J. Bot.* 92, 775–788.
- Kamiya, K., Gan, Y.Y., Lum, S.K.Y., Khoo, M.S., Chua, S.C., Faizu, N.N., 2011. Morphological and molecular evidence of natural hybridization in *Shorea* (Dipterocarpaceae). *Tree Genet. Genomes* 7, 297–306.
- Kamiya, K., Nanami, S., Kenzo, T., Yoneda, R., Diway, B., Chong, L., Azani, M.A., Majid, N.M., Lum, S.K.Y., Wong, K.M., Harada, K., 2012. Demographic history of *Shorea curtisii* (Dipterocarpaceae) inferred from chloroplast DNA sequence variations. *Biotrop.* 44, 577–585.
- Kenzo, T., Ichie, T., Norichika, Y., Kamiya, K., Nanami, S., Igarashi, S., Sano, M., Yoneda, R., Lum, S.K., 2016. Growth and survival of hybrid dipterocarp seedlings in a tropical rain forest fragment in Singapore. *Plant Ecol. Divers.* 9, 447–450.
- Kenzo, T., Ichie, T., Kamiya, K., Ngo, K.M., Lum, S.K., 2019. Rooting ability of leafy-stem cuttings of hybrid *Shorea* (Dipterocarpaceae). *J. Trop. For. Sci.*, in press.
- Kettle, C.J., Maycock, C.R., Burslem, D., 2012. New directions in dipterocarp biology and conservation: a synthesis. *Biotrop.* 44, 658–660.
- Kobayashi, M.J., Takeuchi, Y., Kenta, T., Kume, T., Diway, B., Shimizu, K.K., 2013. Mass flowering of the tropical tree *Shorea beccariana* was preceded by expression changes in flowering and drought - responsive genes. *Mol. Ecol.* 22, 4767–4782.
- LaFrankie, J.V., Chan, H.T., 1991. Confirmation of sequential flowering in *Shorea* (Dipterocarpaceae). *Biotrop.* 23, 200–203.
- Lamont, B.B., He, T., Enright, N.J., Krauss, S.K., Miller, B.P., 2003. Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. *J. Evol. Biol.* 16, 551–557.
- Larcher, W., 2003. *Physiological Plant Ecology*. 4th Edn. Springer-Verlag, New York.
- Lee, S.L., Tani, N., Ng, K.K.S., Tsumura, Y., 2004. Isolation and characterization of 20 microsatellite loci for an important tropical tree *Shorea leprosula* (Dipterocarpaceae) and their applicability to *S. parvifolia*. *Mol. Ecol. Notes* 4, 222–225.
- Levin, D.A., Francisco-Ortega, J., Jansen, R.K., 1996. Hybridization and the extinction of rare plant species. *Conserv. Biol.* 10, 10–16.
- Lu, P., Sinclair, R.W., 2006. Survival, growth and wood specific gravity of interspecific hybrids of *Pinus strobus* and *P. wallichiana* grown in Ontario. *For. Ecol. Manage.* 234, 97–106.
- Lum, S.K.Y., Lee, S.K., LaFrankie, J.V., 2004. Bukit Timah Forest Dynamics Plot, Singapore. In: Losos EC, Leigh J, Giles E. (Eds.), *Tropical forest diversity and dynamism: Findings from a large-scale plot network*. University of Chicago Press, Chicago, pp. 464–473.
- Medway, L., 1972. Phenology of a tropical rain forest in Malaya. *Biol. J. Linn. Soc.* 4, 117–146.
- Momose, K., Yumoto, T., Nagamitsu, T., Kato, M., Nagamasu, H., Sakai, S., Harrison, R.H., Hamid, A.A., Itioka, T., Inoue, T., 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the

- plant-pollinator community in a lowland dipterocarp forest. *Am. J. Bot.* 85, 1477–1501.
- Ngo, K.M., Turner, B.L., Muller-Landau, H.C., Davies, S.J., Larjavaaram, M., bin Nik Hassan, N.F., Lum, S., 2013. Carbon stocks in primary and secondary tropical forests in Singapore. *For. Ecol. Manage.* 296, 81–89.
- Ngo, K.M., Davies, S., Nik, Hassan, N.F., Lum, S., 2016. Resilience of a forest fragment exposed to long-term isolation in Singapore. *Plant Ecol. Divers.* 9, 397–407.
- O'Reilly-Wapstra, J.M., Miller, A., Potts, B.M., 2014. Variable patterns of inheritance of ecologically important plant secondary metabolites in an inter-specific eucalypt hybrid. *For. Ecol. Manage.* 318, 71–77.
- Orians, C.M., Bolnick, D.I., Roche, B.M., Fritz, R.S., Floyd, T., 1999. Water availability alters the relative performance of *Salix sericea*, *Salix eriocephala*, and their F1 hybrids. *Can. J. Bot.* 77, 514–522.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- R Development Core Team, 2011. R: A language and environment for statistical computing. Version 2.13.0. R Foundation for Statistical Computing, Vienna.
- Rieseberg, L.H., Zona, S., Aberbom, L., Martin, T.D., 1989. Hybridization in the island endemic, catalina mahogany. *Conserv. Biol.* 3, 52–58.
- Rieseberg, L.H., 1995. The role of hybridization in evolution: old wine in new skins. *Am. J. Bot.* 82, 944–953.
- Sakai, S., Momose, K., Yumoto, T., Kato, M., Inoue, T., 1999. Beetle pollination of *Shorea parvifolia* (section *Mutica*, Dipterocarpaceae) in a general flowering period in Sarawak, Malaysia. *Am. J. Bot.* 86, 62–69.
- Sakai, S., Harrison, R.D., Momose, K., Kuraji, K., Nagamasu, H., Yasunari, T., Chong, L., Nakashizuka, T., 2006. Irregular droughts trigger mass flowering in aseasonal tropical forests in Asia. *Am. J. Bot.* 93, 1134–1139.
- Schweitzer, J.A., Martinsen, G.D., Whitham, T.G., 2002. Cottonwood hybrids gain fitness traits of both parents: a mechanism for their long-term persistence? *Am. J. Bot.* 89, 981–990.
- Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Nijensohn, S.E., 2008. Anthropogenic alterations of genetic diversity within tree populations: implications for forest ecosystem resilience. *For. Ecol. Manage.* 256, 855–862.
- Symington, C.F., 2004. Foresters' manual of dipterocarps, Revised by Ashton, P.S., Appanah, S., Malaysian Forest Records No. 16. Forest Research Institute Malaysia, Kepong.
- Tamari, C., 1976. Phenology and seed storage trials of dipterocarps. Research Pamphlet No. 69. Forest Research Institute, Peninsular Malaysia, Kepong.
- Thomas, S.C., 2003. Comparative biology of tropical trees: a perspective from Pasoh. In: Okuda, T., Manokaran, N., Matsumoto, Y., Niiyama, K., Thomas, S.C., Ashton, P.S. (Eds.) Pasoh: ecology of a lowland rain forest in Southeast Asia. Springer Tokyo, Tokyo, pp 171–194.
- Tullus, A., Tullus, H., Vares, A., Kanal, A., 2007. Early growth of hybrid aspen (*Populus* × *wettsteinii* Hämet-Ahti) plantations on former agricultural lands in Estonia. *For. Ecol. Manage.* 245, 118–129.
- Turner, I.M., Tan, H.T.W., Wee, Y.C., Ibrahim, A., Chew, P.T., Corlett, R.T., 1994. A study of plant species extinction in Singapore: lessons for the conservation of tropical biodiversity. *Conserv. Biol.* 8, 705–712.
- Ujino, T., Kawahara, T., Tsumura, Y., Nagamitsu, T., Yoshimaru, H., Ratnam, W., 1998. Development and polymorphism of simple sequence repeat DNA markers for *Shorea curtisii* and other Dipterocarpaceae species. *Heredity* 81, 422–428.
- Wong, K.M., Low, Y.W., 2011. Hybrid zone characteristics of the intergeneric hybrid bamboo x *Gigantocalamus malpenensis* (Poaceae: Bambusoideae) in Peninsular Malaysia. *Gard. Bull. Singapore* 63, 375–383.
- Yap, S.K., Chan, H.T., 1990. Phenological behavior of some *Shorea* species in Peninsular Malaysia. In Bawa, K.S., Hadley, M. (Eds.) Reproductive ecology of tropical forest plants, UNESCO, Paris, pp 21–35.
- Yeoh, S.H., Satake, A., Numata, S., Ichie, T., Lee, S.L., Basherudin, N., Muhammad, N., Kondo, T., Otani, T., Hashim, M., Tani, N., 2017. Unravelling proximate cues of mass flowering in the tropical forests of South - East Asia from gene expression analyses. *Mol. Ecol.* 26, 5074–5085.

Fig. 1

Climate data from 2008 to 2009 in Singapore; minimum temperature (A), running total rainfall for 30 days (B), and monthly rainfall and average monthly rainfall during the last three decades (C). An arrow indicates drought in January 2009 (38 mm for the month). Horizontal bars indicate flowering periods of *Shorea* species studied in Bukit Timah.

Fig. 2
Percentage of flowering individuals and flowering periods of four *Shorea* species and two F1 hybrids (*S. leprosula* × *S. curtisii* and *S. leprosula* × *S. parvifolia*) in Bukit Timah Nature Reserve, 2009.

Fig. 3
Flowers of *S. curtisii*, *S. leprosula*, and their F1 hybrid in 2009.

Fig. 4
Genotypes of seedlings obtained from a mother F1 hybrid (*S. leprosula* × *S. curtisii*) tree according to probability analysis using STRUCTURE. Bars indicate individuals ($n = 42$). Filled bars indicate the probability of *S. leprosula* and open bars indicate that of *S. curtisii*. In theory, seedlings of backcross with *S. leprosula* have a 75% probability of *S. leprosula*, seedlings of backcross with *S. curtisii* have a 75% probability of *S. curtisii*, and seedlings of crossing with a F1 hybrid (*S. leprosula* × *S. curtisii*) have a 50% probability of either.

Fig. 5
Cumulative survival of seedlings germinated from four *Shorea* species and one F1 hybrid. *, statistically significant (likelihood ratio test, $P < 0.001$); ns, non-significant difference ($P > 0.05$).