

FLORAL PHENOLOGY AND POLLINATION BIOLOGY OF *VATICA YEECHONGII* (DIPTEROCARPACEAE)

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The reproductive characteristics of *Vatica yeechongii* (Dipterocarpaceae), a critically endangered species, were investigated. *Vatica yeechongii* is a subcanopy dipterocarp species that flowers and fruits annually and some trees are capable of flowering twice a year. The species is outcrossing and self-incompatible. The study showed that apomixis did not occur in *V. yeechongii* and thus, pollination is required to produce fruits. The creamy white and strongly sweet-scented flower attracted insects and offered pollen as reward. Insects from the family Apidae carried abundant pollen on their hairy abdomen and legs, and were shown to be effective pollinators. Thrips were the most abundant visitors, but they were poor pollen-carriers and probably ineffective as pollinators. Pollen grains of *V. yeechongii* were viable almost for two days. Information regarding flowering phenology, floral biology and breeding system were used to propose appropriate conservation prescriptions.

Keywords: Breeding system, reproductive biology, critically endangered plant, flower visitors

INTRODUCTION

Studies on the floral phenology and pollination biology of plants include addressing the timing of recurring biological events such as flowering, fruiting and pollination. The duration and frequency are important in defining flowering and fruiting patterns especially for rare and threatened plant species. For example, results from the work on endemic and endangered palm *Johannesteijmannia lanceolata* and critically endangered medicinal plant *Swertia chirayita* allowed the understanding of its gene flow and regeneration in the community (Chan 2011, Ravinder et al. 2013).

Dipterocarps exhibit a unique reproductive phenology rhythm known as mast flowering, followed by mast fruiting (Wood 1956, Appanah 1985, Ashton et al. 1988, Sakai 2002). Masting is the intermittent production of large seed crops by a plant species and this is synchronised within a population (Kelly 1994).

General flowering involves the synchronisation of flowering and fruiting across diverse groups of plants, a phenomenon unique to the Asian dipterocarp forests (Ashton et al. 1988, Sakai 2002). General flowering events occurred at irregular intervals of 2 to 10 years, and during an event more than 80% of plant species

may produce flowers for about 4 or 5 months (Appanah 1985, 1993, Ashton et al. 1988, Curran and Leighton 2000, Numata et al. 2003). Based on the timing and frequency of flowering of 305 species from 56 families in Lambir Hills National Park, Sarawak, Sakai et al. (1999) was able to classify 257 species into general flowering, supra annual, annual and sub-annual flowering types.

Several meteorological factors may be involved in triggering general flowering (Sakai 2002). Temperature, rainfall, humidity and solar radiation are closely related, however, according to Sakai et al. (2006), drought is thought to trigger the initiation of general flowering in the aseasonal tropical forests. Based on 11 years of phenological observations, Ashton et al. (1988), supported by Yasuda et al. (1999), suggested instead a drop in night-time temperature. Brearley et al. (2007) concluded that drought periods are a more important cue to these general flowering events than low night temperatures. Sakai et al. (1999) noted that the decrease in solar radiation due to cloudiness may trigger general flowering in Sarawak. Other studies have shown strong correlation between drought and flowering (Augspurger 1981, Murali & Sukumar 1994).

Chan (1981) reported *Shorea macroptera*, *S. dasyphylla*, *S. lepidota*, *S. parvifolia*, *S. acuminata* and *S. leprosula* (section Muticae) as being highly self-incompatible. Self-incompatibility was also noted in *Shorea megistophylla*, *S. cordifolia*, *S. congestiflora*, *S. trapezifolia* and *Vateria copallifera* in Sri Lanka (Dayanandan et al. 1990). The wide spatial separation between anthers and stigma of *Dipterocarpus obtusifolius* is thought to be a factor leading to self-incompatibility (Ghazoul 1997).

Pollination is facilitated by energetically-limited thrips that use the flowers as their breeding ground and food resource (Chan & Appanah 1980, Appanah & Chan 1981). Kondo et al. (2016) investigated the pollinator of *Shorea curtisii* in a hill dipterocarp forest in Peninsular Malaysia and found that major visitors of *S. curtisii* in the hill dipterocarp forest were thrips and the predatory big-eyed bugs.

Apis dorsata and *A. indica* appeared to be the most effective pollinators, particularly because of their large body size, abundance of individuals and method of pollen collection (Dayanandan et al. 1990). Large moths (Sphingidae and Noctuidae) were recognised as night pollinators while butterflies (primarily Pieridae and Papilionidae) pollinated dipterocarp flowers during the day (Ghazoul 1997). During the 1996 general flowering event in Lambir Hills National Park, *Dipterocarpus crinitus*, *D. geniculatus*, *D. globosus*, *D. palembanicus* and *D. tempehes* were visited by large numbers of *A. dorsata* and one species, *D. pachyphyllus* was visited by geometrid moths (Harrison et al. 2005).

Studies on the reproductive biology of Dipterocarpaceae have focused on species from the genus *Shorea* section Muticae (*Shorea leprosula*, *S. macroptera*, *S. acuminata*, *S. parvifolia*, *S. lepidota* and *S. dasyphylla*) and *Dipterocarpus* (*Dipterocarpus globosus*, *D. geniculatus*, *D. obtusifolius* and *D. pachyphyllus*). However, little is known about the reproductive biology of *Vatica*. *Vatica yeechongii* is a new species described from Sungai Lalang Forest Reserve (FR), Selangor and also occurs in Setul FR in Negeri Sembilan. To date, there are 27 trees with diameter at breast height (DBH) more than 3 cm recorded from both sites. *Vatica yeechongii* is endemic to Peninsular Malaysia and is critically endangered (Chua et al. 2010).

The study investigated whether the flowering phenology, pollination and breeding system of *V. yeechongii*, an understorey dipterocarp, behaved in the same manner as *Shorea* and *Dipterocarpus*,

all of which occupy the emergent layer. A high conservation value forest (HCVF) has been declared for the population at Setul FR. The present study was aimed at understanding its floral phenology, breeding system and pollination biology. This information is crucial for predicting population viability and survival and identifying factors in flowering and pollination that could potentially lead to bottleneck events. For the HCVF to be an effective conservation approach, this information is required when developing the management strategies.

MATERIALS AND METHODS

Study area

The study was conducted at (i) Sungai Tekala recreational forest park, which is located in Sungai Lalang FR, Selangor (3° 03.485' N, 101° 52.373' E, 79 m asl), a protected area gazetted for water catchment and recreational under National Forestry Act 1984, where seven individuals with diameter greater than 2.0 cm are growing beside a stream at the campsite, and (ii) Setul FR, Negeri Sembilan (2° 46.937' N, 101° 55.069' E, 192 m asl). The population occurs in a very small isolated forest fragment at the margin of the Mantin-Seremban main road. There are fewer than 35 trees with diameter greater than 2.0 cm and the largest diameter recorded is about 32.0 cm.

The Malaysian Meteorological Department provided meteorological data for the years 2012–2014. Rainfall and minimum temperature data were collected from the weather stations of Empangan Air Sungai Semenyih (N 2° 56', E 101° 52', 233.3 m asl., distance 2 km from Sungai Lalang FR and Hospital Seremban (N 2° 43', E 101° 56', 64.1 m asl, distance 8.5 km from Setul FR).

Figure 1 provides the daily rainfall and minimum temperature at both weather stations for the years 2012 to 2014. In 2014, Empangan Air Semenyih recorded the lowest minimum temperature of 20.0 °C on 31 January 2014 and there were six consecutive days that had a minimum temperature of between 20.0–20.8 °C. The Seremban Hospital recorded the lowest minimum temperature of 20.9 °C on 4 February 2014. During the study period, February 2014 and September 2014 were the driest months in Setul FR and Sungai Lalang

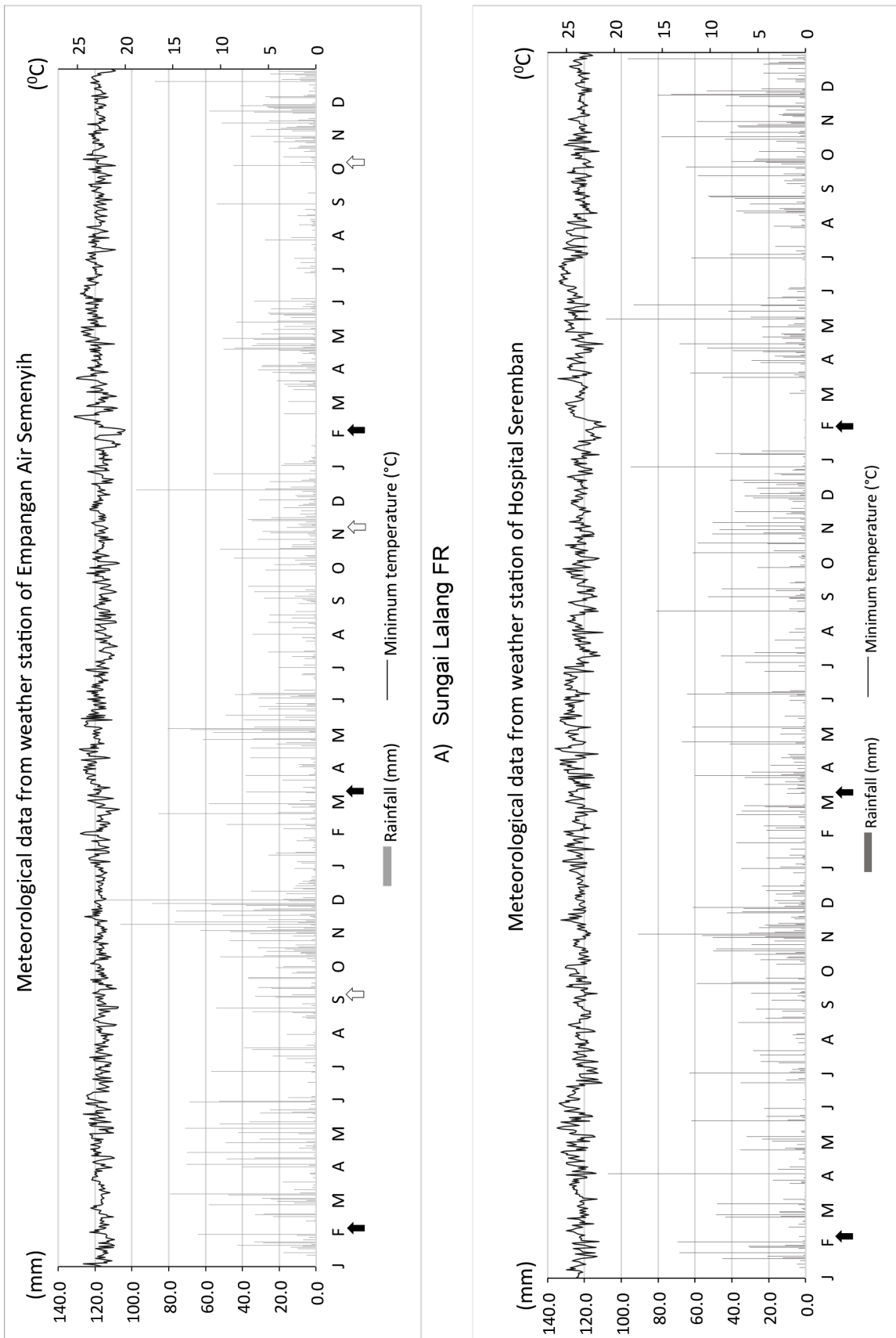


Figure 1 Records of daily rainfall and daily minimum temperature from January 2012 until December 2014; solid arrows below the graphs indicate the first flowering event and open arrows indicate the second flowering event in Sungai Lalang FR and Setul FR; FR = forest reserve

FR, respectively. For Sungai Lalang, of the three years 2014 was the driest with a total rainfall of 2129.1 mm followed by 2013 (2755.3 mm) and 2012 (3519.2 mm), while for Setul, 2013 was the driest year with a total rainfall of 2387.8 mm followed by 2012 (2560.2 mm) and 2014 (2870.1 mm).

Flowering and fruiting phenology

Seven individuals in the Sungai Lalang population and twenty five individuals in the Setul population with diameter more than 3 cm were observed from early January 2012 to late December 2014. The crown of each tree was scanned twice a week on a bi-monthly frequency using binoculars. The crown was divided into four sections, and for each section a gauge of < 25%, 25–50%, 50–75% and > 75% of crown in flower and fruit was used. Additional information was obtained by observing the fallen corollas or fruits on the ground.

Floral morphology and pollen viability

For the Sungai Lalang population, the development of thirty flower buds (10 per tree for three trees) was observed weekly beginning from their first appearance on the inflorescences. Selection of flower buds was based on the availability and accessibility of inflorescences. Daily observations were conducted from 16–21 March 2014 at Sungai Lalang population to determine the time of flower opening, anther dehiscence and stigma receptivity. The floral parts were measured with digital calipers. Stigma receptivity was determined using the method of Carrington et al. (2003). This was repeated every 30 minutes for about five hours beginning with the first floral opening. Pollen from dehisced anthers was collected and observed under Scanning Electron Microscope (SEM) for reference purpose.

To determine pollen germination, pollen was randomly collected from five flowers of three trees. The number of pollen grains used was between 70 and 300 and pollen grains aged 2, 4, 8, 14, 27 and 42 hours after anthesis were tested using the method of Shivanna and Rangaswamy (1992). The length of the pollen tube was measured using light microscope. The pollen grains were considered germinated when length of its tube was equal to, or greater than the diameter of the pollen grain.

Breeding system

Three trees at Sungai Lalang FR were selected for the pollination experiments and the selection of trees was based on the availability and accessibility of inflorescences for pollination. Five treatments were applied on each tree, namely, open pollination (inflorescences tagged without any manipulation), control for selfing (flowers bagged with no manipulation), self-pollination (flower were emasculated prior to anthesis, pollen from other flowers of the same tree applied and bagged), cross-pollination (flower were emasculated prior to anthesis, fresh pollen from another tree in the same population was applied and bagged) and emasculation (flowers were emasculated and bagged). Each treatment had 20 replicates per tree. One flower per inflorescence was a replicate. Organza of mesh size 0.2 mm was used for bagging. Prior to establishing the control for selfing, cross-pollination, self-pollination and emasculation treatments, any open or immature flowers were removed from the inflorescences. In the process of emasculation, all stamens were carefully removed using a pair of fine forceps and pollen was applied with a fine tip brush. Different brushes were used for each treatment to ensure that the brush was free from pollen grains. The flowers were kept bagged for two weeks. Fruit set was monitored and counted. The treatments were considered successful when the fruit nut reached maturity.

The index of self-incompatibility (ISI) for *V. yeechongii* was determined using the formula proposed by Zapata and Arroyo (1978):

$$\text{ISI} = \frac{\text{Fruit set from self-pollination}}{\text{Fruit set from cross-pollination}}$$

The values of the ISI correspond to the breeding system as such:

> 1	self compatible
> 0.2 < 1	partially self-incompatible
< 0.2	mostly self-incompatible
0	completely self-incompatible

The ratio of pollen grains to ovules (P:O) in flowers were also used to indicate the breeding system of the species, based on Cruden's (1977) classification. The P:O ratio is calculated by dividing the total pollen grains per flower with the number of ovules in a flower. Each flower bud has fifteen anthers. Fifteen undehisced anthers were randomly collected from ten mature buds of

three trees and transferred to a clean concave slide containing a drop of detergent and water. The anther was crushed and viewed under a light microscope to calculate the number of pollen grains per anther. The average number of pollen grains per anther was multiplied by the total number of anthers in a flower to obtain the total number of pollen grains per flower.

Floral visitors

Casual observations were done on flower visitors from 16–20 March 2014 for three trees at Sungai Lalang FR. Sample collection started from 0530 hours when the flowers begin to open and continued until 1200 hours when all petals had fallen. Detailed observations were made on one tree on 17 March 2014 by counting the frequency of insect visitation for five minutes at 30 minutes interval for about 6 hours. The observations were done on a selected branch comprising five flowers; the selection of branch was based on the availability of flowers and accessibility. The visitors were caught using hand nets and stored in 90% alcohol. Small flower visitors such as thrips were caught by collecting the flowers into small plastic vials. All thrips seen were recorded and no difference made between adults and juveniles. Length of visits, feed characteristics, flight and search patterns of visitors were recorded. Where possible the insects were identified up to species level. Specimens were observed under Scanning Electron Microscope (SEM) for the presence of pollen grains on their bodies. The presence of

pollen grains on the bodies was compared with reference specimens of pollen from *V. yeechongii* flowers.

RESULTS

Flowering and fruiting phenology

During the observation period, trees of *V. yeechongii* in both populations flowered every year. However, the flowering period in 2014 recorded the highest number of trees in flower compared with 2012 and 2013. Four trees in Setul FR (16%) and two trees in Sungai Lalang FR (29%) flowered annually but there were trees that flowered only in 2014. During the study, three individuals in Sungai Lalang FR produced flowers twice a year while no trees flowered twice a year in Setul FR. These trees would produce new flowers almost immediately after the maturation of fruits derived from the previous flowering. Flower buds were produced in February–March and September–November.

The first flowering season (February–March) in year 2014 was the most intense for both populations where 40% and 100% of the observed trees in Setul and Sg. Lalang FR respectively had between 50–75% and > 75% of their crowns in flower (Table 1). In 2013, Sungai Lalang and Setul populations had low flowering intensity with only 29% and 16% trees flowering with < 25% flowering intensity respectively. During the second flowering period in each year, none of the trees had flowering intensity more than 25%.

Table 1 The number of flowering/fruiting individuals and the range of flowering/fruiting intensity during first (February–March, 1) and second (September–November, 2) flowering periods

		2012		2013		2014	
		1	2	1	2	1	2
Setul FR (N = 25)	No. tree flowering/fruiting	4 (16)	0 (0)	4 (16)	0 (0)	10 (40)	0 (0)
	Flowering intensity	i	0	i	0	ii–iv	0
	Fruiting intensity	i	0	i	0	i–iii	0
Sungai Lalang FR (N = 7)	No. tree flowering/fruiting	5 (71)	3 (43)	2 (29)	2 (29)	7 (100)	2 (29)
	Flowering intensity	i–iv	i	i	i	ii–iv	i
	Fruiting intensity	iii	i	i	0	i–iii	i

i = < 25%, ii = 25–50%, iii = 50–75% and iv = > 75%, of the crown; value in parenthesis is percentage

The duration of flowering to fruit maturity was 21–22 weeks. Mature fruit fall for the first flowering season began in July and August, and for the second season, the following February. During the first flowering period in 2014, flowering was followed by high fruiting intensity (Table 1). During the second flowering period in 2012 and 2014, low intensity flowering resulted in limited fruit set. In 2013, no fruit set was recorded following the second flowering season at Sungai Lalang FR even though the trees had flowered before. During the same period, none of the trees at Setul FR had flowered and fruited.

Floral morphology and development

The inflorescence bud is firm and covered with light brown densely rufous stellate hairs, and after 4–7 days it breaks exposing the inflorescence. Young flower buds become visible approximately 10–14 days after the appearance of the inflorescence bud. These floral buds continued to grow in a sigmoid-curve pattern (Figure 2) changing from green into creamy white when mature. The initial growth was slow, but increased rapidly after 10 days and required between 26 to 32 days to mature. The mean number of flowers per inflorescences was 23.40 ± 11.73 ($N = 30$, range 1–56) and the flowers matured synchronously.

Flowers of *V. yeechongii* are creamy white, funnel-shaped and have five oblong twisted petals which overlap or arranged alternate at the base

(Figure 3). The flower has fifteen stamens of about 1.3 mm long and arranged in a whorl at the base of the flower. The arrangements of stamens are slightly curved, and turned inward toward the axis. The stigma is located close to the mouth of the opening flower and positioned higher than the anther (c. 0.8 mm at vertical distance).

Flowers of *V. yeechongii* opened around 0530–0600 h. They measured 17.0–23.0 mm in diameter when opened and exuded a strong sweet scent which attracted insects. The anthers dehisced longitudinally and exposed white pollen grains upon floral opening. A small portion of pollen grains is shed onto the petals. The stigma became receptive 30 to 60 minutes after the flower opened and remained so for about two to three hours. In an inflorescence, all flowers opened synchronously, and the anthers and stigma remained fresh for about four to five hours. Dehiscence of petals and anthers began as early as 0930 h.

The mean number of pollen grains per anther was 1036.40 ± 93.33 . Since a flower has fifteen anthers, it is estimated to carry a mean number of 15,546 pollen grains. The number of ovules per flower was 6 and therefore the ratio of pollen grains to ovules was 2591:1.

The pollen grain of *V. yeechongii* was viable for almost two days and performed well in the medium 15% sucrose + 0.01% boric acid solution. Pollen aged 4 hours after anthesis showed healthy germination in both media compared to pollen of other ages (Figure 4). Highest mean tube

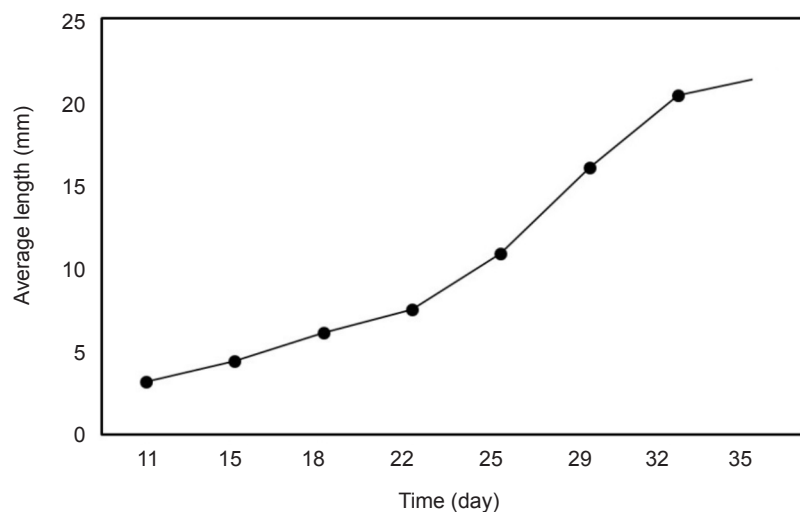


Figure 2 Average length of in *Vatica yeechongii* after the appearance of a floral inflorescence bud on the branch, $n = 30$



Figure 3 Flowers of *Vatica yeechongii*

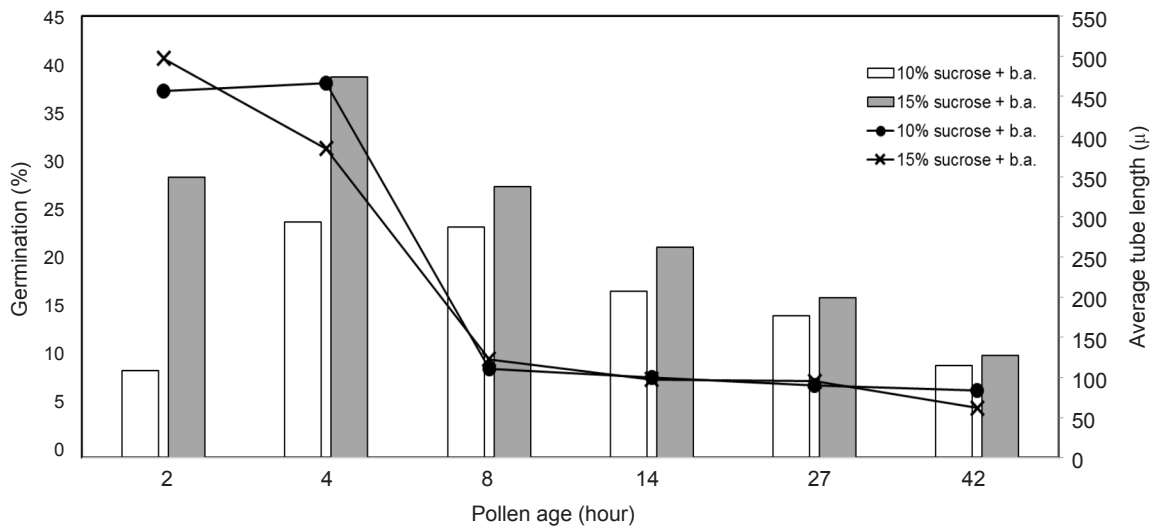


Figure 4 Percentage pollen germination (bar graph) and pollen tube length (line graph) in two different media at different age after anthesis

length was observed for pollens aged 2 hours incubated in the medium of 15% sucrose + 0.01% b.a (497.08 µm, SD 205.36), followed by pollens aged 4 hours incubated in 10% sucrose + 0.01% b.a (466.37 µm, SD 201.53). The length of pollen tube reduced rapidly four hours after anthesis.

Breeding system

Open-pollinated flowers produced the highest fruit set (28.3%) followed by cross-pollination (13.3%). There was no fruit set following emasculatation of the anthers, indicating that apomixis did not occur for *V. yeechongii*. Bagged flowers without any manipulation and self-

pollination treatment also did not produce mature fruits. Based on these results, *V. yeechongii* is considered self-incompatible and therefore the self-incompatibility (ISI) value for *Vatica yeechongii* is 0. The breeding system for *Vatica yeechongii* is obligate outcrossing or xenogamy.

Floral visitors

Throughout the study period, insect visitors from the families Apidae and Thripidae were often seen while visitors from the family Tephritidae were rare (Figure 5). Other families such as Formicidae and Chrysomelidae were also observed.

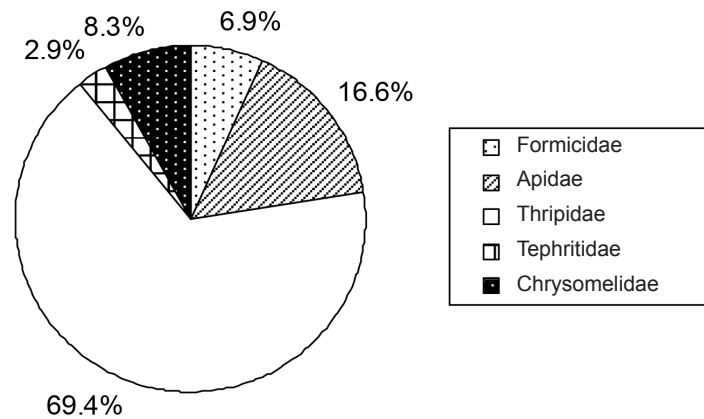


Figure 5 Percentage of floral visitors, n = 75

Generally, the insects began visiting at around 0630 h. The number of visits increased between 0700–0800 h (Table 2), coinciding with stigma receptivity. Apart from thrips and bees, no insects were observed between 1030 and 1200 h when petals and anthers dropped.

Most thrips observed on the opened flowers were *Thrips florum*, *T. hawaiiensis*, *T. palmi* and *Thrips* sp. Some were found inside the opened flower, either residing or hiding inside the floral parts and foraging the pollen. Despite their abundance, only two out of 27 specimens examined were found to carry pollen grains on their abdomens (Figure 6), and each had only one grain.

Trigona bees from the subfamily Apinae visited flowers and inflorescences within and between trees. They remained on the open flowers for about 5–30 seconds, and sometimes up to 60 seconds. In order to obtain pollen grains that are located at the base of floral bracts, the bees had to insert their head and abdomen deep into the flower. While foraging on other flowers they would subsequently transfer pollen onto the stigma. Twelve specimens for all three species (*Geniotrigona thoracia*, *Heterotrigona itama* and *Tetragonula laeviceps*) carried a large amount of pollen on their mouth parts, legs and abdomen.

Ant species *Dolichoderus thoracicus* and *Pseudolasius* sp. from the family Formicidae were observed crawling on the tree trunk, branches, inflorescences and open flowers, but were rarely seen inside the flowers. Ants occurred in great numbers as they nest on branches of *V. yeechongii*, and only one out of five specimens of *Pseudolasius* sp. carried a few pollen grains on the tip of its abdomen.

Two species of leaf beetles from the family Chrysomelidae also visited the opened flowers of *V. yeechongii*. The smaller species fed on and destroyed flower petals. During the forage, they sometimes crawled into the flower and possibly ate the pollen. This foraging behaviour may lead to contact with an active stigma. The larger beetle species ate the petals only and did not probe the area where the anthers are located. This beetle is large in size and does not fit the corolla tube. These leaf beetles also carried a very few pollen grains on their abdomens. Other visitors that were attracted by the sweet floral scent included *Bactrocera* flies from the family Tephritidae, but no pollen was present on its mouth and body parts.

DISCUSSION

Trees of *V. yeechongii* may flower annually or semi-annually. The semi-annual to annual flowering pattern is the first reported for a Dipterocarp species. Heavy flowering followed by profuse fruiting took place in both populations in February 2014. During this year, other tree species in Sungai Lalang FR such as *S. leprosula*, *S. bracteolata*, *S. parvifolia*, *Saraca cauliflora* (Leguminosae), *Parkia* sp. (Leguminosae), *Canarium* sp. (Burseraceae) were also flowering with high intensity. During the dry season of February to April 2014, general flowering occurred in several forest reserves in Terengganu, Johor, Selangor, Pulau Pinang and Perak (Lau 2015). The *V. yeechongii* masting in 2014 appeared to be in synchrony with general flowering.

The flowering in 2014 was in response to the occurrence of low rainfall in both study

Table 2 Frequency of insect visitation on open flowers, from 0530–1200 hours, 17 March 2014

Family	Hour		Frequency of visit										
	0530-0600	0600-0630	0630-0700	0700-0730	0730-0800	0800-0830	0830-0900	0900-0930	0930-1000	1000-1030	1030-1100	1100-1130	1130-1200
Formicidae	-	-	-	1	1	3	-	1	2	1	-	-	-
Apidae	-	-	1	1	3	5	8	7	4	2	1	1	-
Thripidae	-	2	5	5	7	10	11	13	9	4	3	-	-
Tephritidae	-	-	-	-	-	-	1	-	1	1	-	-	-
Chrysomelidae	-	-	-	1	1	2	3	2	2	1	-	-	-

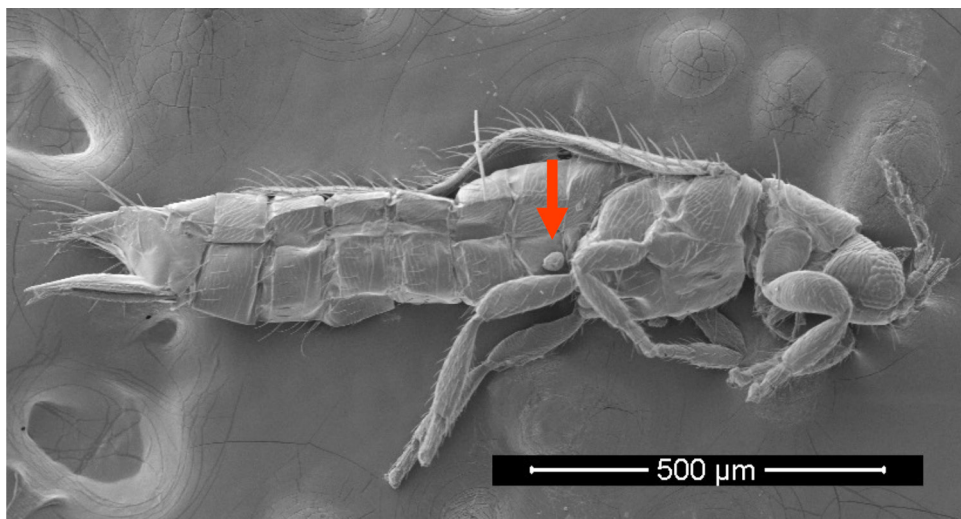


Figure 6 Thrip carrying a pollen grain on its abdomen

areas as well as other areas in the peninsula. This flowering also coincided with the northern hemisphere dry monsoon. The second flowering event in the year is possibly a response to the southern hemisphere dry monsoon, during which time masting in the southern latitudes was regularly observed. A drop in the daily minimum temperature that occurred about one week before the appearance of the first inflorescence bud possibly triggered flowering initiation in *V. yeechongii*. This result corroborates the hypotheses of Ashton et al. (1988), Yasuda et al. (1999), Sakai et al. (1999) and Numata et al. (2003). However, this association was not observed for the Setul population. A longer monitoring period is therefore needed to conclude whether both factors are associated with masting in *V. yeechongii*.

Cross-pollination is needed for fruit set and apomixis did not occur in *V. yeechongii*. The species is self-incompatible, outcrossing and pollinators are required for successful pollen transfer. The synchronised floral and

fruit maturity in the populations is necessary for effective cross-pollination. Apart from maximising opportunities for outcrossing, synchronisation in blooming and the emittance of scents will simultaneously increase the chances for gene exchange. Such synchronisation is particularly important for outcrossing species with low population densities (Sakai 2002). The timing overlap between anther dehiscence and stigma receptivity may induce self-pollination but the failure of self-pollinated flowers to set fruits indicates that there are chemical and genetic incompatibilities.

The relation between pollination systems and multiple floral characters such as colour, shape and odour play an important role in attracting potential pollinators (Faegri and Pijl 1979). The funnel-shaped, creamy white flowers and high flower number per inflorescence provide a visual contrast against green foliage. In addition, the emission of a strong scent enhances its ability to attract pollinators.

The *V. yeechongii* is most probably pollinated by bees from the family Apidae. Despite the abundance of thrips, the presence of pollen on their abdomens was too low to be of significance. Being minute and energetically limited in flight (Appanah and Chan 1981, Kondo et al. 2016), they may have limited opportunities to conduct pollen transfer during the short stigma receptivity period. In addition, the anthers of *Vatica* bear short stubby appendages unlike the awn-like *Shorea* section *Mutica* appendages, which bend back at anthesis to trap thrips, together with the dehisced anthers, in a distinct chamber formed by the overlapping petal bases. The structure of *Vatica* flowers appears to be less effective in serving thrip pollination. This result contrasted those of Appanah and Chan (1981) who reported that floral morphology and flowering behaviour of *Shorea* species (section *Mutica*) appeared to be highly adapted for pollination by thrips. Momose et al. (1998) reported Chrysomelids beetles as being major pollinators at the Lambir study site while Sakai et al. (1999) noted that the introduction of thrips to bagged flowers did not increase fruit set when compared to beetles. Kondo et al. (2016) suggested that that big-eyed bugs and flower thrips are major and indirect pollinators respectively for *Shorea curtisii* at Semangkok FR.

Apidae visitors have the highest potential to be effective pollinators for *V. yeechongii* based on the presence of high pollen load on their mouth parts, abdomen and legs. The reward, either nectar and/or pollen grains, are important attractants. *V. yeechongii* produced a large number of pollen grains per flower and several trees flowered annually. High pollen load offering a large reward is important during times when many other species in the area are not flowering. The fairly long viability of its pollen grains is an added advantage in that it provides a longer window of opportunity for pollen transfer by pollinators thus affecting cross pollination. Elsewhere, bees (*Trigona*, *Apis*, *Braunsapis*) are recognised as important pollinators for many tropical plants (Ghazoul & McLeish 2001, Momose et al. 1998, Corlett 2004, Ghazoul 1997).

Beetles from the family Chrysomalidae also have the potential to be pollinators for *Vatica yeechongii* but their low numbers and frequency of visits indicate that they may not be efficient pollinators. Momose et al. (1998), Nagamitsu et al. (1999) and Sakai et al. (1999) noted that

multiple species of beetles fed on the petals, pollen and pistils of 20 species of *Hopea*, *Shorea* and *Vatica* in the lowland forests of Sarawak. *Vatica yeechongii* flowers produced a strong scent and may attract beetles.

Vatica yeechongii is critically endangered because of its narrow geographical distribution and small population sizes. The legal protection accorded to the forest habitat and ecosystem in Sungai Lalang FR is appropriate and necessary to ensure no further loss of trees within the population and more importantly safeguard the dynamics of ecosystem functions within the landscape. *V. yeechongii* is strictly dependent on insects for pollination and reproduction – clearly the survival of populations is dependent upon the complex dynamics of functions that maintain the food web, predation and selection pressures.

Vatica yeechongii in Sungai Tekala recreational forest park may face local extinction if prescriptions that mitigate loss are not implemented. The area occupied by the trees also serves as camp sites and clearing of the undergrowth and cleaning are done daily to maintain cleanliness. This activity will negatively impact seedling regeneration. The loss of seeds is further aggravated by the fact that *V. yeechongii* favours gentle earth banks near the stream, and fruits are often lost through stream flow. In the long term, there will be inadvertent negative impacts on the regeneration, establishment and survival of the population. Understanding phenological patterns allows the estimation of timing for mature fruit collection thereby facilitating *ex-situ* conservation measures. A cleaning schedule that is environmentally friendly can be introduced.

The population in Setul FR is located on the margin of a very small isolated forest fragment, thus edge effects are likely to severely impact the population in the long term. Relocation of trees to similar sites in adjacent forest reserves may be necessary in the near future to ameliorate the deleterious impacts of edge effects. Establishing a germplasm collection is therefore necessary.

CONCLUSIONS

Vatica yeechongii is a dipterocarp species that flowers and fruits annually and some trees are capable of flowering twice a year. The pattern of flowering was about the same every year whereby flowering usually begins in February or March and coincided with dry months. The

flowers of *V. yeechongii* are protandrous and offer pollen as reward. The species is outcrossing and self-incompatible. Apomixis did not occur in *V. yeechongii* and pollinating agents are required to produce fruits. It is speculated that floral visitor from the family Apidae has the highest potential to be effective pollinators because their hairy abdomen and legs enable them to carry more pollen load.

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REFERENCES

- APPANAH S. 1985. General flowering in the climax rain forest of Southeast Asia. *Journal of Tropical Ecology* 1: 225–240.
- APPANAH S. 1993. Mass flowering of dipterocarp forests in the aseasional tropics. *Journal of Biosciences* 18: 457–474.
- APPANAH S & CHAN HT. 1981. Thrips: The pollinators of some dipterocarps. *Malaysian Forester* 44: 234–252.
- ASHTON PS, GIVNISH TJ & APPANAH S. 1988. Staggered flowering in the Dipterocarpaceae: new insights into floral induction and the evolution of mast fruting in the aseasional tropics. *American Naturalist* 132: 44–46.
- AUGSPURGER CK. 1981. Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinator and seed predators on *Hybanthus prunifolius* (Violaceae). *Ecology* 62: 775–788.
- BREARLEY FQ, PROCTOR J, SURIANTATA, NAGY L, DALRYMPLE G & VOYSEY BC. 2007. Reproductive phenology over a 10-year period in a lowland evergreen rain forest of central Borneo. *Journal of Ecology* 95: 828–839.
- CARRINGTON ME, GOTTFRIED TD & MULLAHEY JJ. 2003. Pollination biology of saw palmetto (*Serenoa repens*) in Southwestern Florida. *Palms* 47: 95–103.
- CHAN HT. 1981. Reproductive biology of some Malaysian dipterocarps. III. Breeding systems. *Malaysian Forester* 44: 28–36.
- CHAN HT & APPANAH S. 1980. Reproductive biology of some Malaysian dipterocarps. I: flowering biology. *Malaysian Forester* 43: 132–143.
- CHAN YM, LIM AL & SAW LG. 2011. Reproductive biology of the endangered and endemic palm *Johannesteijsmannia lanceolata* (Arecaceae). *Journal of Tropical Forest Science* 23: 213–221.
- CHUA LSL, SUHAIDA M, HAMIDAH M & SAW LG. 2010. Malaysia plant red list. Peninsular Malaysia Dipterocarpaceae. *Research Pamphlet No. 129*. Forest Research Institute Malaysia, Kepong.
- CORLETT RT. 2004. Flower visitors and pollination in the Oriental (Indomalayan) Region. *Biological Review* 79: 497–532.
- CRUDEN RW. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 3: 32–46.
- CURRAN LM & LEIGHTON M. 2000. Vertebrate responses to spatiotemporal variation in seed production of mast-fruited Dipterocarpaceae. *Ecological Monographs* 70: 101–128.
- DAYANANDAN S, ATTYGALLA DNC, ABEYGUNASEKERA AWWL, GUNATILLEKE IAUN & GUNATILLEKE CVS. 1990. Phenology and floral morphology in relation to pollination of some Sri Lankan dipterocarps. In Pp 103–134K. S. Bawa & M. Hadley (eds) *Reproductive Ecology of Tropical Forest Plants*. Parthenon, London.
- FAEGRI K & VAN DE PIJL L. 1979. *The Principles of Pollination Ecology*. 3rd Edition. Pergamon Press, Oxford.
- GHAZOUJ. 1997. *Field Studies of Forest Tree Reproductive Ecology. A Manual*. ASEAN Forest Tree Seed Centre Project, Saraburi.
- GHAZOUJ & MCLEISH. 2001. Reproductive ecology of tropical forest trees in logged and fragmented habitats in Thailand and Costa Rica. *Plant Ecology* 153: 335–345.
- HARRISON RD, NAGAMITSU T, MOMOSE K & INOUE T. 2005. Flowering phenology and pollination of *dipterocarpaceae* (Dipterocarpaceae) in Borneo. *Malayan Nature Journal* 57: 67–80.
- KELLY D. 1994. The evolutionary ecology of mast seeding. *Trends in Ecology and Evolution* 9: 465–470.
- KONDO T, OTANI T, LEE SL & TANI N. 2016. Pollination system of *Shorea curtisii*, a dominant species in hill dipterocarp forest. *Journal of Tropical Forest Science* 28: 318–323.
- LAU KH. 2015. Agarwood flowering: Masting or coincidence? *Conservation Malaysia Bulletin* 20: 1–2
- MURALI KS & SUKUMAR R. 1994. Reproductive phenology of a tropical dry forest in Mudumalai, southern India. *Journal of Ecology* 82: 759–767.
- MOMOSE K, YUMOTO T, NAGAMITSU T ET AL. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. *American Journal of Botany* 85: 1477–1501.
- NAGAMITSU T, HARRISON RD & INOUE T. 1999. Beetle pollination of *Vatica parvifolia* (Dipterocarpaceae) in Sarawak, Malaysia. *Gardens' Bulletin Singapore* 51: 43–54.
- NUMATA S, YASUDA M, OKUDA T, KACHI N & SUPARDI MNN. 2003. Temporal and spatial patterns of mass flowerings on the Malay Peninsular. *American Journal of Botany* 90: 1025–1031.
- RAVINDER R, PRABHURAJ P, YASH PS & ROMESH CR. 2013. Reproductive biology of *Swertia chirayita* – a temperate critically endangered medicinal plant. *Caryologia* 66: 12–20.

- SAKAI S. 2002. General flowering in lowland mixed dipterocarp forests of South-East Asia. *Biological Journal of the Linnean Society* 75: 233–247.
- 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. *American Journal of Botany* 86: 62–69.
- SAKAI S, MOMOSE K, YUMOTO T, NAGAMITSU T, NAGAMASU H, HAMID AA & NAKASHIZUKA T. 1999. Plant reproductive phenology over four years including an episode of general flowering in a lowland dipterocarp forest, Sarawak, Malaysia. *American Journal of Botany* 86: 1414–1436.
- SAKAI S, HARRISON RD, MOMOSE K ET AL. 2006. Irregular droughts trigger mass flowering in aseasonal tropical forests in Asia. *American Journal of Botany* 93: 1134–1139.
- SHIVANNA KR & RANGASWAMY NS. 1992. *Pollen Biology-A Laboratory Manual*. Springer-Verlag, Berlin.
- WOOD GHS. 1956. Dipterocarp flowering season in Borneo. *Malaysian Forester* 19: 193–201.
- YASUDA MJ, MATSUMOTO J, OSADA N ET AL. 1999. The mechanism of general flowering in Dipterocarpaceae in the Malay Peninsula. *Journal of Tropical Ecology* 15: 437–449.
- ZAPATA TR & ARROYO MTK. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10: 221–230.