REPRODUCTIVE BIOLOGY OF THE ENDANGERED AND ENDEMIC PALM JOHANNESTEIJSMANNIA LANCEOLATA (ARECACEAE)

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CHAN YM, LIM AL & SAW LG. 2011. Reproductive biology of the endangered and endemic palm, Johannesteijsmannia lanceolata (Arecaceae). The reproductive biology of the rare and endangered palm, Johannesteijsmannia lanceolata, was studied to provide basic but essential information that contributes to the conservation of the species. Floral phenology and visiting insects were observed in cultivated and wild populations. Pollen viability and stigma receptivity were tested for flowers of different ages. Thirty inflorescences were subjected to pollination experiments. Flowers of J. lanceolata were homogamous, with anthesis peaked from 0730 to 1100 hours. Pollen viability and stigma receptivity lasted one day. Small flies (Phoridae and Cecidomyiidae) and stingless bees (Trigona spp.) were the potential pollinators of the inflorescences. Flower abortion was high (> 90%) and seed set was very low (< 0.05%). The breeding system may be autogamy, geitonogamy or xenogamy. The species is self-compatible, indicating its ability to survive and persist in fragmented or isolated environment.

Keywords: Floral biology, breeding system, pollination, floral visitors, Coryphoideae

INTRODUCTION

Reproductive biology is essential for the survival of a plant species and yet, it is very little understood in palms especially for the non-economic species. It is important to combine phenology, pollinator activity and incompatibility systems as a whole approach in studying reproductive ecology (Búrquez et al. 1987). This approach allows us to understand the viability of a population and its gene flow, which is important in the conservation of rare species, as they are usually very restricted and habitat specific (Martén & Quesada 2001, Lee et al. 2006).

Many studies on the reproductive biology of palms are restricted to neotropical species. Although Malaysia is exceedingly rich and diverse in tropical palms with 398 indigenous species in 33 genera (Saw 1998), the study of the reproductive biology of the Malaysian palms is largely neglected and information on the breeding system is also lacking (Dransfield 1970, 1972, 1979, Fong 1978, Lee & Jong 1995, Lee et al. 1995). Most palms studied so far are self-compatible, but the compatibility in many major groups of palms is still not known (Henderson 2002).

In the recent Malaysia Plant Red List threat assessment on the rare Johannesteijsmannia, three species were assessed as endangered (J. lanceolata, J. magnifica and J. perakensis) and one vulnerable (J. altifrons). Restricted distribution...
and population decline caused by habitat loss and seed exploitation for the ornamental trade are imminent threats that need to be addressed in their conservation (Chan & Saw 2009). However, to formulate appropriate conservation measures, basic information on the reproductive biology of the targeted species is much needed. Therefore, we investigated the reproductive biology of *J. lanceolata*. This species from the subfamily Coryphoideae is an understorey hermaphrodite palm up to 3 m tall with tapering diamond-shaped leaves. The inflorescence is interfoliar, branched to the first order and bears 5–12 rachillae. The flowers are about 2 mm in diameter, crowded, sessile and spirally arranged in a rachilla. The species is endemic to Peninsular Malaysia and restricted to four localities in three states, i.e. Negeri Sembilan, Pahang and Selangor.

**MATERIALS AND METHODS**

**Study sites and materials**

All plant materials were sourced from plants cultivated at the Forest Research Institute Malaysia (FRIM), Kepong, Selangor (3° 14' N, 101° 38' E, 97 m above sea level) except for the pollination experiments which involved the wild population in the Angsi Forest Reserve (FR), Kuala Pilah, Negeri Sembilan (2° 43' N, 102° 04' E), about 70 km away from FRIM. The population in the Angsi FR grows in a lowland dipterocarp forest at 160–260 m above sea level, while that in FRIM grows in the Kepong Botanical Garden and in the main campus.

During the study period in the year 2004 and 2005, FRIM received > 2000 mm of annual rainfall with mean temperature 27.8 °C, while Kuala Pilah received 1500–1700 mm annual rainfall with mean temperature 26.9 °C (data from the Malaysian Meteorological Service Department, Malaysia). The wild population has two main flowering seasons per year, i.e. in March–May and October–December. Meanwhile, the cultivated population apparently flowers throughout the year (Chan 2009).

**Floral phenology**

There were seven plants at FRIM but only five flowered during the phenological study. Detailed floral observations made on 20 inflorescences from June till August 2004 and in March 2005 included inflorescence development, number of open flowers per rachilla per day, time of flowering and duration, and flowering sequence (acropetal or basipetal). Flowers were observed at different times within a day for several days to determine the time of anthesis (i.e. dehiscence of anthers). The flowering sequence was recorded daily by marking open flowers with permanent markers of different colours for different days. The flowers were collected after 3–5 days and observed under a stereomicroscope for morphological changes, pollen quantity and stigma freshness. The time of anthesis was also observed in the wild population during the heavy flowering season, from 0630–1000 hours on 29 April and 6 May 2005. Of the 30 plants tagged, 12 were flowering but only 6 plants were observed because these were near to one another, enabling frequent observations at short time intervals (these same plants were observed for floral visitors).

**Pollen viability**

To determine pollen viability, fresh pollen just after anthesis was collected from five bagged inflorescences of *J. lanceolata* from four plants. The pollen was cultured using the sitting drop technique (Shivanna & Rangaswamy 1992) at room temperature (26–29 °C) in 10% sucrose + 0.01% boric acid solution, and scored for germination under a microscope after three hours. A pollen grain was considered germinated when the length of its tube was at least twice the diameter of the grain. The percentage of pollen germination was calculated as the total number of germinated grains/the total number of pollen grains × 100. The technique above was repeated for pollen from flowers aged 6 and 24 hours after anthesis (N = 5).

**Stigma receptivity**

To determine stigma receptivity, flower buds of *J. lanceolata* prior to, during and after anthesis were collected, fixed in Craf III solution, dehydrated through tertiary-butyl-alcohol (TBA) series (Johansen 1940), and critically point dried before being examined under a scanning electron microscope (model Jeol JSM-6400). Stigma receptivity was also determined by immersing some flowers in 3% aqueous hydrogen peroxide; bubbling indicates peroxidase activity and stigma receptivity (Carrington et al. 2003).
Floral visitors

At the Angsi FR, six flowering plants were observed for visiting insects from 0630–1000 hours on 29 April and 6 May 2005. At FRIM, peak flowering occurred in November 2005 and insects visiting the inflorescences were observed for their behaviour and relative abundance during peak hours of anthesis and at different times throughout the day. In both populations, some insects were captured by bagging and then preserved in 70% ethanol for later identification. Detailed observations were made at FRIM from 0810–1130 hours (N plant = 3, N inflorescence = 4). The number of visits by the insects was scored for 10 min on each plant at hourly intervals.

Breeding system

The pollen to ovule (P:O) ratio (i.e. the total number of pollen grains/the number of ovules per flower) was used to assess the breeding system of *J. lanceolata* based on Cruden’s (1977) classification. Six undehisced anthers from five mature buds (N inflorescence = 3, N plant = 2) were collected using fine forceps and each was transferred to a clean concave slide containing water and a drop of detergent. The anthers were squashed and scored for the number of pollen grains per anther under a microscope. The total number of pollen grains per flower was estimated by multiplying the mean number of pollen grains per anther with the total number of anthers in a flower. Five carpels were dissected transversely to determine the number of ovules in a flower.

Pollination experiments were also conducted to assess the breeding system from September 2004 until August 2005. Eighteen inflorescences from FRIM (N plant = 5) and 12 from the Angsi FR (N plant = 8) were tagged, with half of them bagged with organza mesh (0.5 × 0.5 mm) to exclude insect pollination, and the rest were left open as control. The inflorescences were bagged at initial flowering and any open flowers were removed. All treatments were carried out in the same individual whenever possible to eliminate genetic and environmental differences. The bags were removed after flowering. Fruit set was monitored and counted when fruits were visible (1–2 mm in diameter) and again scored for the final seed set when the fruits reached maturity (> 5 cm in diameter).

The total number of aborted flowers (A) in an inflorescence from the cultivated population was counted after the fruits had set. The number of fruits set (B) was also counted. Thus, the total number of flowers borne in the inflorescence was A + B. The initial and final fruit sets were calculated as thus:

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\text{Percentage of initial fruit set} = \frac{\text{total initial fruit} \times 100}{\text{total carpel}}
\]

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\text{Percentage of final fruit set} = \frac{\text{total mature fruit} \times 100}{\text{total carpel}}
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\text{Total carpel} = \text{total number of flowers} \times 3 \quad \text{(because each flower has three carpels)}
\]

For inflorescences in the wild population, direct counting of the total number of aborted flowers could not be done because many of the aborted flowers had dropped or were rotten after fruit had set due to the wet and humid environment. Thus, the total number of flowers in the inflorescences was estimated from a mixed-linear regression model (Pinheiro & Bates 2000) computed by R Version 2.3.1 (2006). The fitted model, \(Y = 126.02 + 43.81 \times X (R^2 = 98.6\% , p < 0.05)\), described the relationship between the rachillae length (X) and the number of flowers borne on it (Y), based on the data collected from measuring the rachillae length and counting all the buds borne on them (N rachilla = 58) (Chan 2009). These rachillae were from the inflorescences used in the pollination experiment.

Prior to statistical analyses, the fruit set data were transformed into square root, as the percentages of the fruit set were very low. The data were subjected to paired *t*-tests (for the cultivated population) and Mann–Whitney rank tests (for the wild population) to compare the open and bagged pollination treatments. The tests were computed using Statgraphics Plus for Windows Version 4.0 (1994–1999).
RESULTS

Floral phenology

New inflorescence bracts were flat when they emerged from the leaf sheath and rachillae were formed in about two weeks from the emergence of the bracts. The bracts were yellow green to brown and usually slit open apically or laterally one or two days before flowering. Initially the rachillae were erect and crowded in between leaf axils before elongating and branching out during flowering. The inflorescence was cream yellow. The total number of flowers per inflorescence ranged from 1500–3800 hours (mean = 2442, SD = 582, N inflorescence = 18). It was common to find 2–3 inflorescences in a plant with the flowering phase overlapping each other, and 3–4 plants flowering synchronously.

In an inflorescence, a few flowers opened on the first day with more opening on subsequent days. The majority reached anthesis on the fourth till the seventh day. The average number of opening flowers per rachilla per day ranged from 2–104 (N rachilla = 22). Flowering was acropetal in the inflorescence and rachilla, while flowers in a cincinnus opened asynchronously, i.e. each flower usually opened on a different day.

Flower anthesis was from 0700 hour (as early as 0600 hour in an observation) until about 1700 hour (Figure 1). The anthers started dehiscing introrsely and longitudinally when the petals opened. Peak hours of anthesis were from 0730–1100 hours. The flowers emitted a sweet sour smell that attracted insects. Full bloom was from 0900–1200 hours and anthers were fully dehisced, revealing shiny white pollen grains. The stigma was shiny and dry without any exudate. There was no nectar secretion in the flower. Rain did not seem to affect flower bloom. Very few or hardly any flowers opened in the evening. One day after anthesis (day 1), the stigma looked fresh (probably still receptive) whereas anthers apparently had very little or no pollen left, maybe due to rainwash. The flowers started to close from the evening of day 1 and were fully closed by 1200 hour on day 2. After that, the style and stamens degenerated and turned brown.

After one week of flowering, usually more than 50% of the flowers in an inflorescence had reached anthesis. The style and stamens wilted, turned dark brown and dropped off after the flower had closed. Petals started to turn brown and the flower became mouldy but persistent. The carpels looked fresh and eggs of insects were found deposited in some flowers. Flowering was somehow slowed down in the apical floral buds of the rachillae and it ended in about two weeks.

Qualitative observations of the anthesis time for the population at Angsi FR showed similar results to those in cultivation. The earliest observation was at 0630 hour when no flowers opened. The anthesis started at about 0715 hour with a few flowers opening and peaked from 0730–1000 hours.

Pollen viability and stigma receptivity

Most pollen grains lost their viability after 24 hours. Percentage germination of fresh pollen and those aged 6 and 24 hours after anthesis were 14.7 (SD = 21), 15.5 (SD = 35) and 0 respectively.

The stigma was dry throughout its receptive phase and positioned slightly higher (vertical distance of c. 0.2 mm) than the anthers. Stigmas of freshly open flowers at 0900 hour of an inflorescence showed active bubbling when immersed in 3% aqueous hydrogen peroxide solution. The bubbling slowed or reduced for flowers aged one day after anthesis. Flowers aged two days after anthesis were not tested as the petals had closed and the stigmas were considered not receptive. Scanning electron micrographs showed that the stigmas of mature flower buds prior to anthesis looked fresh and turgid (Figure 2). There was no opening in the stigma yet. Meanwhile, the anthers which were...
adpressed to the style started to split introrsely and longitudinally and eventually raised above the style.

When the petals opened, the stigma started swelling with a small opening in the middle. The stigma of open flower at 0830 hour continued to swell and the opening enlarged. By 1030 hour, the cells of the stigma had swollen to nearly twice of their original size. At 1330 hour, the stigma began to wilt and shrink although some stigmas were still receptive. The stigmas and styles of the closing or closed flowers started to wither and shrivelled one day after anthesis.

**Floral visitors**

Insects from three orders, i.e. Dictyoptera (cockroaches), Diptera (flies) and Hymenoptera (bees and ants) were observed and identified from both the cultivated and wild populations. The inflorescences received the most visitors in the morning and very rarely in the evening. From the detailed observations, the number of visits by insects increased and climaxed at about 1000–1110 hours, coinciding with peak anthesis (Figure 3). Flies from the families Phoridae and Cecidomyiidae (species indeterminate) were the most common and abundant visitors to the inflorescences, followed by black ants (Formicidae, species indeterminate) and stingless bees (Apidae, *Trigona* spp.). The ants were often seen crawling on the inflorescences but rarely on open flowers. The stingless bees foraged the open flowers for pollen but at a longer time interval and less frequent. A stingless bee could spend from a few seconds up to 10 seconds on an inflorescence. Other visitors observed on the inflorescences were a spider and some moth larvae (order Lepidoptera, family Lymantriidae). The spider was probably waiting for its prey while the latter damaged the buds and did not touch any of the open flowers. A cockroach (Blattidae, *Blatta orientalis*) was also found hiding or residing inside the inflorescence bracts.

**Breeding system**

The flower had six anthers and thus was estimated to have a total of 268 pollen grains (mean number of pollen grains per anther = 44.7, SD 15.6, N = 6). There were three ovules per flower, hence the P:O ratio was 268/3 = 89 for *J. lanceolata*. According to Cruden’s (1977) classification, the breeding system for this species was facultative selfing.

Flower abortion was very high, usually > 90% in all treatments. In both the cultivated and wild populations, open pollination gave higher fruit sets compared with bagged pollination, although the differences were not statistically significant, except for one (Table 1). Paired *t*-tests applied to the initial fruit set of the cultivated population

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**Figure 2** Scanning electron micrographs of stigmas of *Johannesteijsmannia lanceolata* at different hours: (A) mature flower buds prior anthesis, (B) opening flowers, (C–D) open flowers at 0830 hour, (E) at 1030 hour, (F) at 1330 hour and (G–H) closing and closed flowers after one day of anthesis.
was significantly different between the two treatments ($p = 0.004$). The same analysis on the final fruit set, however, showed no statistical difference ($p = 0.097$). The overall final fruit sets in all treatments were very poor, with the number of mature fruits produced per inflorescence ranging from 0 to 16 (mean = 1.5, SD = 3.6, N inflorescence = 30) (results not shown).

**DISCUSSION**

**Floral biology**

*Johannesteijsmannia lanceolata* is considered homogamous with male anthesis overlapping stigma receptivity. In Coryphoideae, protandry is reported in *Serenoa repens* (Carrington et al. 2003), *Licuala* spp. (Barfod et al. 2003) and *Thrinax parviflora* (Henderson 1986), and protogyne in *Cryosophila albida* and *Sabal palmetto* (Henderson 1986). In all these species anthesis was observed to occur in the morning, except in *Cryosophila albida* which reached anthesis at night. The floral biology of *J. lanceolata* shares some similarity with its sister group, *Licuala*. The inflorescence of *Licuala* also flowers during the rainy season, showing acropetal flowering sequence with male anthesis beginning from 0600 to 1200 hours while female receptivity is initiated 12 hours later as each flower lasts for two days (Barfod et al. 2003). The inflorescence of *Licuala* lasts longer, for about one month (Barfod et al. 2003) compared with two weeks in *J. lanceolata*.

The pollen viability of *Johannesteijsmannia* lasts only one day and this was also reported in *Astrocaryum mexicanum* (subfamily Arecoideae) (Búrquez et al. 1987). The low percentage...
of pollen germination in this study could be attributed to the large number of degenerated pollen grains and short incubation period. In addition, the medium used was probably not optimum.

The stigma may be receptive up to two days in *Phoenix* (Henderson 1986) and three days in *Licuala* (Barfod et al. 2003) compared with only one day in *Johannesteijsmannia*.

**Pollination**

*Johannesteijsmannia lanceolata* is unlikely to be wind-pollinated; the flowers emit a sweet sour scent, the P:O ratio is low, the pollen is sticky, the stigmatic surface is very small, the inflorescences are obscured by debris or leaf litter most of the time, and the plant is an understorey in a closed canopy forest where there is hardly any strong wind (YM Chan, personal observation). Water pollination may be possible as *J. lanceolata* flowers during the rainy season and the distance between the anther and stigma is very close in a flower. Drops of rainwater may fill a flower and pollen that floats on the surface may eventually reach the stigma. Although rare, water pollination does occur in terrestrial plants through rainwater (Faegri & van de Pijl 1966).

Contrary to *J. lanceolata*, most of the dieocious *Chamaerops* species including *C. alienans*, an understorey rainforest palm, and *C. radicalis* (Arecoideae), an understorey montane palm, are primarily wind-pollinated (Otero-Arnaiz & Oyama 2001, Berry & Gorchov 2004). The small flowers (2–3 mm in diameter, similar in size to those of *J. lanceolata*) have numerous dry pollen grains with exposed stigmas and anthers but lack of scent to attract pollinators and open during dry season (Berry & Gorchov 2004). Similarly, the dwarf palm, *Chamaerops humilis* (a common understorey of evergreen oak forest) produces large amounts of powdery pollen which are readily dispersed by wind though the female flowers may secrete nectar (Herrera 1989).

*Johannesteijsmannia lanceolata* is entomophilous and is mostly visited by flies and ants. Bees rarely visit the flowers of *J. lanceolata* (Figure 3); perhaps the pollen quantity per flower is low and there is no nectar reward and hence, it is not energy efficient for the bees to visit these flowers. Energy which is needed for travelling to food source and maintaining body heat is compensated or gained from rewards of nectar, pollen or food bodies of plants. Some insects such as social bees may not forage at all on cold days or when rewards are not profitable in order to maximise energy efficiency (Richards 1986). On the other hand, in *Euterpe precatoria* large numbers of pollen grains are produced in the male phase (> 21 000 per flower) and more concentrated nectar is available in the female flower to attract pollinators such as beetles and bees (Küchmeister et al. 1997). Similar observations have also been reported in *Geonoma irena* (Borchsenius 1997) and *Licuala distans and L. peltata* (Barfod et al. 2003) in which bees are the main pollinators.

Small flies are less specialised and restricted to primitive flowers. Their activity is irregular and inefficient but they may be important pollinators in certain weather conditions when no other pollinators are available as they are found all year round (Faegri & Van de Pijl 1966). As flies are very common and abundant in many habitats, their numbers may offset the inefficiency at pollination (Ghazoul 1997). Thus, in *J. lanceolata*, flies may be more important than bees in pollinating the flowers which open during rainy seasons. Moreover, flies were the most frequent and abundant visitors observed. The flies were attracted by the strong smell emitted by the inflorescences.

The spider recorded was not a pollinator and was most probably foraging for insects, while moth larvae were pests as they chewed the flower buds. Black ants were abundant and they nest inside the inflorescence bracts. Their presence served as an additional defence to the plant against predators. The ants may be potential pollinators as they brush through the pollen and pollinate flowers when crawling on the inflorescences. Cockroaches also resided in the bracts but were unlikely pollinators as they did not go near the flowers.

Numerous studies have shown that palms have diverse floral visitors and more than one pollinator. For example, *Licuala spinosa*, *L. peltata* and *L. distans* are visited by flies, bees, wasps, ants, beetles, spiders, moths and birds but calliphorid and tachinid flies, halictid and *Trigona* bees, and eumenid wasps are the suggested pollinators (Barfod et al. 2003). *Cryosophila albida* is pollinated by beetles although bees (*Trigona* spp.) are common visitors to the inflorescences (Henderson 1986). Henderson (1986, 2002) has reported various pollination syndromes in Coryphoideae; from anemophily (*Trinax*
parviflora) to cantharophily (Rhapidophyllum, Cryosophila), mellitophily (Sabal palmetto) and zoophily (Pritchardia), but most members are entomophilous.

Breeding system

The higher fruit sets in the open inflorescences compared with those of bagged inflorescences imply that visiting insects enhance pollination. On the other hand, the ability of bagged inflorescences of J. lanceolata to set fruit in the absence of pollinators indicates autogamy and self-compatibility. Autogamy is possible as the flowers are homogamous and the anther and stigma are in close contact. The low P:O ratio also indicates that the species is facultatively selfing. Self-compatibility was evident when isolated individuals planted at FRIM and University of Malaya, Kuala Lumpur produced viable seeds which germinated and grew into seedlings (YM Chan, personal observation).

The dense and crowded flowers of J. lanceolata easily allow pollinators to visit and pollinate the open flowers within the same rachilla or inflorescence, encouraging geitonogamy. The overlapping of flowering phase of several inflorescences in a plant also increases geitonogamy as pollinators would likely travel less and visit more flowers or inflorescences within the same plant (Richards 1986). However, the low-food reward and the steady, long flowering duration (with few flowers open per day) in the inflorescence of J. lanceolata may increase the chances of xenogamy by forcing pollinators (especially bees) to forage further to other nearby conspecifics. Cross-pollination was not conducted to test xenogamy because emasculation of stamens is impractical as the flowers are very small and an inflorescence bears 1500–3800 flowers. However, the Analysis of Molecular Variance (AMOVA) done by Look (2007) using amplified fragment length polymorphism (AFLP) fingerprinting suggests that J. lanceolata is possibly outbreeding since it shows high genetic variation (85%) within populations, and low (14.8%) genetic variation among populations which indicates moderate gene flow. However, these findings are not substantial enough for drawing solid conclusion as the sampling size was small (8–9 plants per population) and thus the mating system should be investigated further. In summary, J. lanceolata may exhibit autogamy, geitonogamy or xenogamy, and pollination is facilitated by insects.

Other species within Coryphoideae also show similar breeding systems as J. lanceolata, i.e. Thrinax and Licuala are self-compatible (Barfod et al. 2003) and Serenoa repens is facultatively xenogamous (Carrington et al. 2003).

Implications for conservation

Selfing would be advantageous to J. lanceolata when no pollinator is available and it also reduces the problem of getting a mate when the population density is low (Wilson 1983). The species would be able to survive and persist in fragmented habitats or in small population size. However, inbreeding leads to lack of genetic variability and could in time severely restrict a population to small niches. The inbred plants may be in critical danger when there is adverse change in habitat or environment, or deleterious genes persist in progenies in the long run. As J. lanceolata may favour xenogamy (Look 2007), it is important to keep whole populations intact to preserve the genetic diversity and to reduce inbreeding depression. Since the species is only found in four localities with rather small population size, all the populations should be conserved, with the biggest population having the highest genetic diversity be given the utmost priority.

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