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Beetle Pollination of *Vatica parvifolia* (Dipterocarpaceae) in Sarawak, Malaysia

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Abstract

Pollination of a canopy tree of two species, *Vatica* aff. *parvifolia* and *V. micrantha* (Dipterocarpaceae) was investigated in Sarawak, Malaysia. Flowers of the two species open in the evening, and last for + 2 days. Rewards are petal tissue and pollen. In *V.* aff. *parvifolia*, pollen was removed from anthers on the first day, and deposited on stigmas on the second day, which suggests that the flowers are protandrous. Most (76%) flower visitors were beetles (Chrysomelidae), 25% of which carried pollen. The beetles mated inside flowers and often touched anthers and stigmas. These suggest that the beetles are pollinators. Pollination of *V. micrantha* rarely occurred, because few pollen grains were removed from anthers and deposited on stigmas. Dominant (71%) flower visitors were weevils (Apionidae and Curculionidae), 9% of which carried pollen. The weevils laid eggs from outside flowers. Bagging treatment increased fruit set in *V. micrantha.* This suggests the negative effects, such as seed predation, of the weevils on fruit set.

Introduction

Subfamily Dipterocarpoidae (Dipterocarpaceae) in Asia comprises 13 genera and 470 species, classified into two tribes, the Dipterocarpae and Shoreae, based on phenotypic characters (Ashton, 1982). Pollinators of the Shoreae include various groups of insects, which differ in their ability to transport pollen: thrips (Appanah & Chan, 1981), beetles (Sakai *et al.*, 1999), and bees (Appanah, 1990; Momose *et al.*, 1996).

In the Dipterocarpae, pollinators known to date are butterflies, moths, and bees, all of which are capable of transporting pollen long distances. Honey bees have been reported to be the most effective pollinators of the self-incompatible *Vateria copallifera* in Sri Lanka (Dayanandan *et al.*, 1990). Stingless bees visit flowers of *Stemonoporus oblongifolius* in Sri Lanka (Ashton, 1988), which has a high (84%) outcrossing rate with potential of apomixis (Murawski & Bawa, 1994). Butterflies and moths pollinate nectar-

secreting flowers of *Dipterocarpus obtusifolius* in Thailand (Ghazoul, 1997). In Malaysia, geometrid moths pollinate *D. pachyphyllus*, and honey bees pollinate *D. geniculatus* (Momose *et al.*, 1998). However, pollinators of *Vatica*, the largest genus (65 species) in tribe Dipterocarpae, were previously unknown. Appanah (1987) predicted that pollinators of *Vatica* are tiny flower-feeding insects, such as thrips, beetles, bugs, and hoppers, according to floral traits of *Vatica*, such as nocturnal anthesis, poorly developed anthers, and no nectar production.

In this study, we observed abundance and behaviour of flower visitors of two species of *Vatica*, and examined the pollination process and the effect of exclusion of flower visitors on fruit set, in order to determine potential pollinators of *Vatica*.

Materials and Methods

The field research was conducted in the Lambir Hills National Park, Sarawak, Malaysia (4°20'N, 113°50'E, 150-200 m elevation). *Vatica parvifolia* Ashton is distributed in Sarawak and Burnei, and is rare on sandy ridges below 600 m elevation (Ashton, 1982). One tree, which flowered in September 1994, was investigated from the Operation Raleigh Tower in the park, 25 m height above ground. This particular tree had leaves with more distinct reticulation than *V. parvifolia*, so it is referred here as *V.* aff. *parvifolia* (voucher: *K. Momose 70*, KYO and SAR; Nagamasu & Momose, 1997). *Vatica micrantha* Slooten (voucher: *K. Momose 54,* KYO and SAR) in Borneo is widespread in mixed dipterocarp forest below 600 m elevation (Ashton, 1982). One tree, which flowered in October 1994, was investigated from the first aerial walkway in the Canopy Biology Plot at 20 m height above ground (Inoue *et al.*, 1995).

Flower visitors

Flower visitors of *V.* aff. *parvifolia* were observed and collected at 11.00h, 16.00h, and 21.00h on 13 September, and at 6.00h, 8.00h, 10.00h, 14.00h, and 17.00h on 14 September, and those of *V. micrantha* at 9.00h, 13.00h, 15.00h, 17.00h, and 20.00h on 17 October, and at 6.00h on 18 October. We collected flower visitors for 10 min using hand nets. At first, we caught visitors flying around and coming to flowers, and later we swept visitors staying on the flowers into the nets. These insects were identified to family and superfamily levels. Pollen grains on their bodies were observed using both light and scanning electron microscopes.

Pollination process

In order to describe the pollination process, flowers of *V.* aff. *parvifolia* and *V. micrantha* were observed and collected after the collection of flower visitors. An additional collection of *V. micrantha* flowers was made at 11.00h on 17 October.

Some insects visiting flowers left feeding marks on the petals. Feeding marks were graded into four classes: Grade 0 = no feeding marks; Grade 1 = some petals with feeding marks; Grade 2 = all petals with feeding marks, but the feeding area of each petal less than half the petal area; Grade 3 = all petals with feeding marks, and at least one petal with more than half the area eaten.

Pollen grains were extracted from anthers of single flowers in 3N sodium hydroxide at 70°C, and dispersed in 1 ml of 20% ethanol. Pollen grains in 0.0032 ml sampled from the 1 ml ethanol solution were counted using a haematocytometer. Pollen grains on single stigmas were counted using a light microscope. Pollen tubes in single styles were stained with aniline-blue and counted using a fluorescine microscope (Alexander, 1987).

Fruit set

Effects of both exclusion of flower visitors and artificial self pollination on fruit set of *V. micrantha* were examined by a pollination experiment. (We did not conduct this experiment in *V.* aff. *parvifolia*, because the number of the manipulated flowers was not enough). Branches with flower buds were enclosed with fine mesh tetron bags (TORAY #9000). The number of both flower buds and flowers on the bagged and unbagged branches was counted on 10 October 1994 when flowering began. Artificial pollination with pollen of the same tree was conducted for flowers in some of the bags on 17 October. All bags were removed after flowering. To compare fruit sets among 1) control (neither bagging nor pollination treatment), 2) bagging treatment (no pollination treatment), 3) bagging and self-pollination treatment, fruits on the monitored branches were counted on 30 November and 23 December in 1994, and on 5 February in 1995.

Results

Flowers of *Vatica* aff. *parvifolia* (Fig. 1) have creamy-white petals, whereas those of *V. micrantha* have yellowish-white petals, outside suffused with red toward the base. In the two species, petals open from 16.00h to 21.00h. Anther dehiscence occurs before the petals open. Petals and anthers begin to fall two days after flower anthesis, depending on weather conditions.

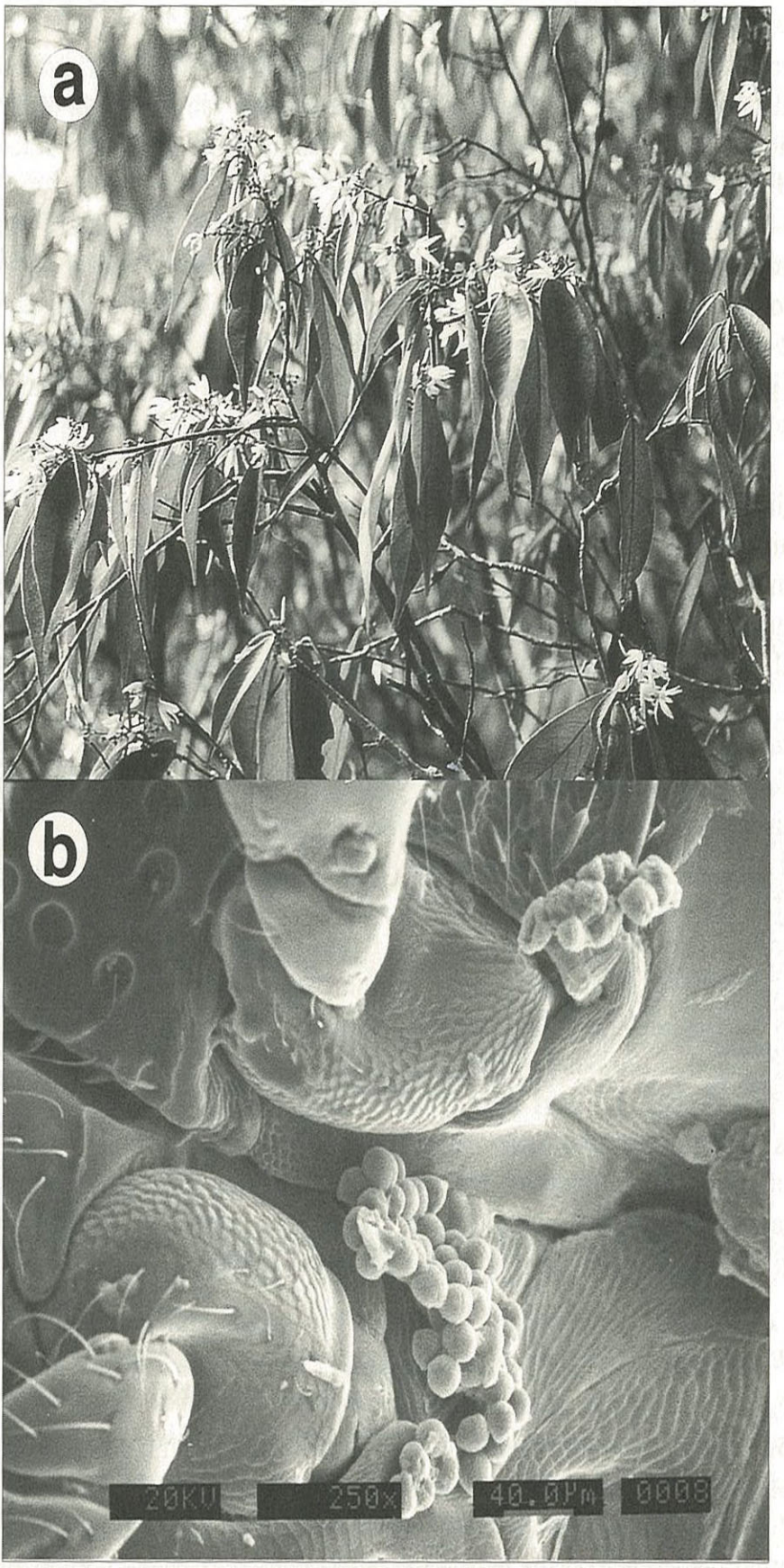

Figure 1: a) A flowering tree of *Vatica* aff. *parvifolia*; b) A chrysomelid beetle, *Oomorphus* sp. (Lamprosomatinae), collected from a *V.* aff. *parvifolia* flower. Pollen grains are found on its ventral thorax.

Fresh and one-day-old flowers can be distinguished by the degree of petal opening and connectivity of filaments to petals. No liquid reward was observed in flowers of either species. Pollen and petal tissue are the rewards for flower visitors.

Flower visitors

Most flower visitors collected from *V.* aff. *parvifolia* were beetles (Chrysomelidae dominated by *Oomorphus* spp.; 76%, n = 179; Fig. 2a), while those from *V. micrantha* were weevils (Apionidae and Curculionidae; 71%, n = 461; Fig. 2b). The composition of the flower visitors of the three most abundant families differed between *Vatica* species (χ^2 test, $P < 0.001$). The composition of beetles and weevils temporally changed in *V. micrantha*, but was stable in *V.* aff. *parvifolia* (χ^2 test, $P < 0.001$ and $P = 0.539$, respectively). Chrysomelid beetles on *V. micrantha* flowers were more abundant at night and in the morning than in the afternoon (Fig. 2b). Other visitors include species of Scarabaeidae and Cucujidae (Coleoptera); Cicadellidae, Fulgolidae, Cimicidae, and Plataspidae (Hemiptera); Blaconidae, Chalicidoidea, Trigonaloidea, Formicidae, and Vespidae (Hymenopreta); Culicidae (Diptera); Blattellidae (Blattodea); and Acromantidae (Mantodea). In addition to these insects, thrips (Thysanoptera) were found in 10.2% (n = 177) and 1.4% (n = 140) of the collected flowers of *V.* aff. *parvifolia* and *V. micrantha*, respectively.

On the bodies of these insects collected from the both *Vatica* species, pollen grains were observed on 25% of beetles (Chrysomelidae, n = 103; Fig. 1b), and 9% of weevils (Apionidae and Curculionidae, n = 142). No pollen grains were found on the bodies of the other insects (n = 7). There was no significant difference in the proportion of individuals with pollen grains between beetles and weevils (Fisher's exact probability test, $P = 0.790$ in *V.* aff. *parvifolia* and $P = 0.127$ in *V. micrantha*), and between *Vatica* species ($P = 0.259$ in weevils and $P = 0.052$ in beetles). Female chrysomelid beetles were observed feeding on both petals and pollen, and staying inside the base of petal cups. On the other hand, male beetles were observed feeding on petals, and waiting for females on the apical part of the petals, where they mated. We found that apionid and curculionid weevils laid eggs from outside the flowers into the petals and ovaries. The weevils were observed feeding on both petals and pollen, but their mating was rarely observed.

Pollination process

Feeding marks on petals of both *V.* aff. *parvifolia* and *V. micrantha* increased with time after flower anthesis (Fig. 3a and 3b). Feeding marks in fresh flowers of *V.* aff. *parvifolia* increased between 6.00h and 8.00h (Kraskal-

Wallis test, P = 0.039). One-day-old flowers had more feeding marks than fresh flowers in both *V.* aff. *parvifolia* and *V. micrantha* (Mann-Whitney test, P = 0.002 and P = 0.003, respectively).

The number of pollen grains in anthers of *V.* aff. *parvifolia* was higher in fresh flowers than in one-day-old flowers, but that of *V. micrantha* did not differ (Mann-Whitney test, P < 0.001 and P = 0.850, respectively; Fig. 3c and 3d). The number of pollen grains in anthers of fresh flowers of *V.* aff. *parvifolia* decreased between 8.00h to 14.00h (Kraskal-Wallis test, P = 0.002; Fig. 3c).

The number of pollen grains on stigmas of *V.* aff. *parvifolia* was higher in one-day-old flowers than in fresh flowers, but that of *V. micrantha* did not differ (Mann-Whitney test, P < 0.001 and P = 0.121, respectively; Fig. 3e and 3f). The number of pollen grains on stigmas of one-day-old flowers of *V.* aff. *parvifolia* increased throughout the day (Kraskal-Wallis

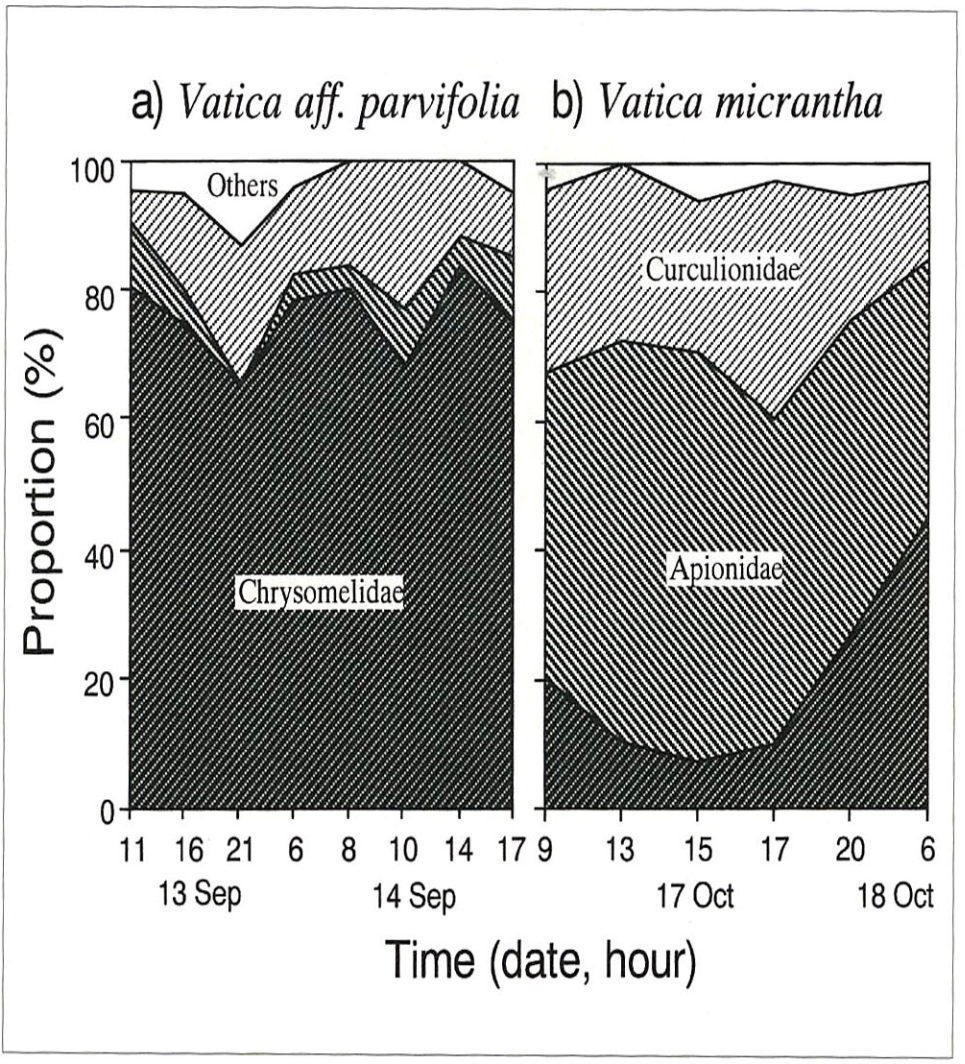

Figure 2: Temporal changes in the proportion of four taxa of flower visitors (Chrysomelidae, Apionidae, Curculionidae, and other taxa) collected from a) *Vatica* aff. *parvifolia* and b) *V. micrantha.*

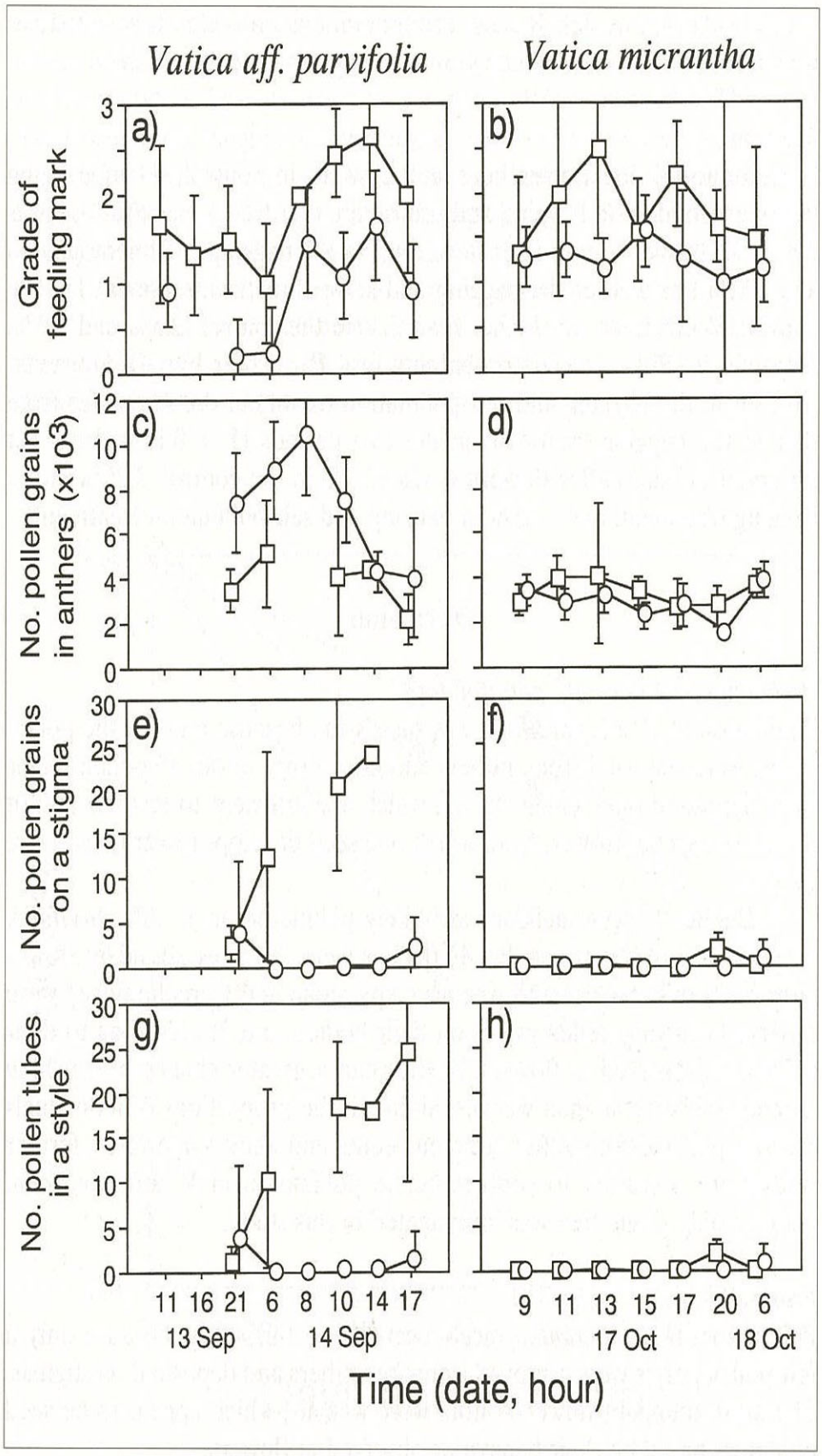

Figure 3: Temporal changes in the grade of feeding marks on petals (a-b), the number of pollen grains in anthers (c-d), the number of pollen grains on a stigma (e-f), the number of pollen tubes in a style (g-h), of flowers of *Vatica* aff. *parvifolia* (a, c, e, and g) and *V. micrantha* (b, c, f, and h). Circles and squares show fresh and one-day-old flowers, respectively.

test, P = 0.014; Fig. 3e). Results of observations on pollen tubes in styles were similar to those of pollen grains on stigmas (Fig. 3g and 3f).

Fruit set

We monitored 568 flower buds and flowers in control, 89 in bagging treatment, and 90 in bagging and self-pollination treatment. Fruit set was observed 50 and 72 days after flowering. In the bagging treatment (27.0% and 20.2%) as well as the bagging and self-pollination treatment (15.6% and 11.1%) fruit set was higher than that in the control (2.5% and 1.9%, respectively; Fisher's exact probability test, $P < 0.001$; Fig. 4). However, fruit set in the bagging and self-pollination treatment did not differ from that in the bagging treatment in the two periods ($P > 0.154$). Fruit set observed 117 days after flowering was 0.35% in the control, 2.25% in the bagging treatment, and 2.22% in bagging and self-pollination treatment.

Discussion

Pollination in Vatica aff. parvifolia

Pollination of *V.* aff. *parvifolia* was successful, because most of the pollen grains were removed from anthers and on average about 20 pollen grains were deposited on a single stigma, which are sufficient to fertilize one of the six ovules per flower, from which one seed develops (Swarupanandan, 1986).

Beetles (Chrysomelidae) are likely pollinators in *V.* aff. *parvifolia*, based on the observations that 1) beetles were the most abundant flower visitors, 2) only beetles and weevils (Apionidae and Curculionidae) were observed carrying pollen grains on their bodies, and 3) according to their behaviour observed at flowers, beetles had a greater chance of touching anthers and stigmas than weevils, although the proportions of individuals carrying pollen did not differ between beetles and weevils. However, further studies are necessary to confirm beetle pollination in *V.* aff. *parvifolia*, because only single tree was investigated in this study.

Fruit set in Vatica micrantha

Pollination of *V. micrantha* rarely occurred in this study, because only a few pollen grains were removed from the anthers and deposited on stigmas. The most abundant flower visitors were weevils, which appear to be seed predators based on their behaviour observed at flowers.

The result of the pollination experiment showed that bagging treatment increased fruit set. This unusual result suggests at least two causes: 1) negative effects of flower visitors on fruit set, and 2) pollination

mechanisms in the bagged flowers. One of the negative effects of flower visitors is seed predation. Weevils, the dominant flower visitors, are common seed predators of dipterocarps (Daljeet-Singh, 1974; Toy, 1991). Thus, it is plausible that the bagging treatment prevented the weevils from predating the seeds. Pollination in the bagged flowers might be achieved by thrips or through autogamy, otherwise apomixis could occur. There is a possibility that thrips entered and pollinated the bagged flowers (Appanah & Chan, 1981), because neither insecticide before bagging nor glue at the tied mouths of the bags were used. Autogamy and apomixis have been shown in some other dipterocarps (Chan, 1981; Kaur *et al.*, 1986; Dayanandan *et al.*, 1990; Murawski & Bawa, 1994).

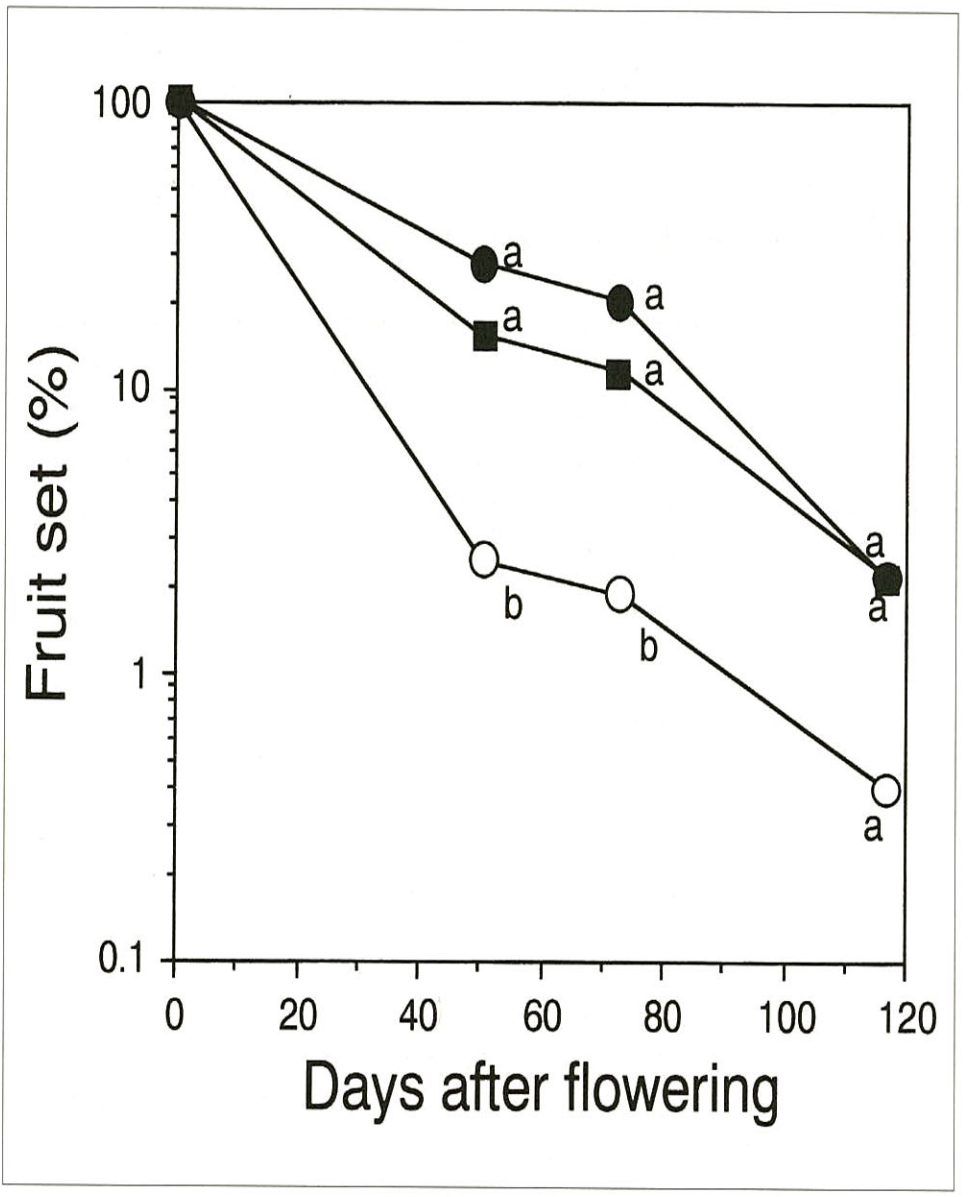

Figure 4: Temporal changes in fruit set of *Vatica micrantha*. Fruit set is compared among control (open circles), bagging treatment (closed circles), and bagging and self-pollination treatment (closed squares). Different letters show significant differences ($P < 0.001$).

Beetle pollination syndromes in dipterocarps

Appanah (1987) predicted that *Vatica* flowers could be pollinated by tiny flower-feeding insects, such as thrips, beetles, bugs, and hoppers. The present study suggests that the potential pollinators of *Vatica* aff. *parvifolia* are chrysomelid beetles, which supports the prediction of Appanah (1987).

Chrysomelid beetle pollination in subfamily Dipterocarpoidae has also been demonstrated in *Shorea parvifolia* (Sakai *et al.*, 1999), although there are differences in pollinator behaviour and phenological traits of flowers between *Shorea* and *Vatica*. Pollinators of *S. parvifolia* are dominated by *Monolepta* spp. (Galerucinae), which visited flowers at night, while those of *V.* aff. *parvifolia* and dominated by *Oomorphus* spp. (Lamprosomatinae), which visited flowers throughout the day, and left feeding marks on petals in the morning. Pollen removal from anthers of *V.* aff. *parvifolia* was also observed in the morning. Pollen deposition on stigmas was observed at night in *S. parvifolia*, but throughout the day in *V.* aff. *parvifolia*. These lines of evidence suggest that beetle pollinators of *S. parvifolia* exhibited clear nocturnal activity, whereas those of *V.* aff. *parvifolia* seemed most active in the morning.

In accordance with the difference in daily activity of pollinators, floral phenology differed between *S. parvifolia* and *V.* aff. *parvifolia* (Sakai *et al.*, 1999). The lifetime of most flowers is one day in the former, and two days in the latter, even though flowers of the both species open in the evening. Pollination of flowers of *S. parvifolia* occurs at the first night after flowering, while flowers of *V.* aff. *parvifolia* seem protandrous with pollen removal on the first day and pollen deposition on the second day, although lack of stigma receptivity in fresh flowers has not been confirmed. These two types of temporal matching between pollinator activity and floral phenology observed in *Shorea* and *Vatica* suggest divergent syndromes in chrysomelid beetle pollination in dipterocarps.

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