Pollination Biology of the Genus *Artocarpus*, Moraceae

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ABSTRACT The pollination biology of *Artocarpus odoratissimus* and *A. integer* (Moraceae) was studied in Sarawak, Malaysia. Female and male heads of *A. odoratissimus* secreted liquid rewards containing fructose in nighttime, and were visited by diverse nocturnal insects (18 families of 4 orders) including flies, nitidulid beetles, moths and cockroaches. In *A. integer*, male heads offered only pollen as rewards, and female heads offered liquid rewards containing protein. They were visited by diverse nocturnal insects (11 families of 5 orders). The evidence for insect pollination of these two species of *Artocarpus* was from the existence of rewards, and that some insects carrying pollen grains were collected on female heads.

Key Words: *Artocarpus* / Moraceae / insect pollination / nocturnal flower / protein reward / Sarawak

The family Moraceae includes 48 genera, but pollination systems are known in only a few genera. The temperate genus, *Morus*, is considered to be wind-pollinated, because large amount of pollen is found in the air (Kinjo *et al.*, 1988). Some tropical genera in Australia (*Streblus* and *Malaisia*; Williams & Adam, 1993) and in the Neotropics (*Chlorophora* and *Trophis*; Bawa & Crisp, 1980; Bullock, 1994) are also wind-pollinated. *Ficus* is the only genus which has been revealed to be pollinated by animals (fig wasps; Galil & Eisikowitch, 1968).

The genus *Artocarpus* (Moraceae) is distributed in Indomalaysia and includes 31 species (Primack, 1983). Little information on pollination systems has been reported. Corner (1952) suggested that the genus *Artocarpus* includes both wind-pollinated and insect-pollinated species. If his suggestion is revealed to be true, *Artocarpus* is one of the very rare examples of both wind- and insect-pollinated species being included in the same genus (like *Espeletia*, Asteraceae; Berry & Calvo, 1989), so is good material for studying shift of pollination systems.

However, the evidence for insect pollination in *Artocarpus* is insufficient, because nobody has reported that insects carrying pollen grains visit female heads of *Artocarpus*. Corner (1952) noted that male heads of *A. heterophyllus* (jackfruit, cultivated), *A. integer* (cempedak, cultivated), and *A. dadah* were visited by flies and beetles in Malaya. He estimated that Malayan *A. rigidus* and *A. elasticus* were wind-pollinated, because male heads gave off clouds of pollen (ballistic pollen release). Primack (1983) observed *A. elasticus*, *A. kemando*, and *A. odoratissimus* in Sarawak, and found bees only on male heads. Male heads of the jackfruit, *A. heterophyllus*, cultivated in Australia were visited by flies,

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Drosophyla immigrans (Moncur, 1985). Brantjes (1982) reported that male heads of the breadfruit, *A. altilis* (= *A. communis*), cultivated in Brazil offered nectar, and were visited by bees, *Trigona fulventris* var. *guianae*. However, Primack (1983) noted that *A. communis* cultivated in Sarawak is wind-pollinated because of ballistic pollen release, and Singh et al. (1963) stated that fruits of *A. altilis* (= *A. communis*) cultivated in India set without pollination.

In this paper, we collected flower visitors and floral rewards from male and female heads of *A. odoratissimus* (terap or pingan) and *A. integer* (cempedak), examined pollen deposition on insects collected on flowers, and analyzed chemical contents of floral rewards, in order to show evidences of insect pollination of *Artocarpus*.

MATERIALS AND METHODS

Field observations and samplings were carried out in secondary forests around Lambir Hills National Park, Sarawak, Malaysia (4° 2'N, 113° 50'E, 150 m in altitude).

Artocarpus odoratissimus Blanco (voucher: T. Nagamitsu 156, KYO, SAR) is distributed in Borneo and the Philippines, and common in secondary forests. The tree is monoecious, up to 25 m tall. The male and female heads are similar in shape, size and location; ellipsoid, 9 cm x 3.5 cm, and located in leaf axes. The male head is green turning brown, and the female head is green in color of flowering. In Sarawak, it flowers at intervals of 2 or more years (K. Momose, personal observations).

Artocarpus integer (Thunb.) Merr. (voucher: K. Momose 7326, KYO, SAR) is distributed in the wild in Borneo, Sumatra, Sulawesi, Moluccas, and New Guinea, and widely cultivated in Southeast Asia. In Borneo, it is locally common in lowland dipterocarp forests. The tree is monoecious, up to 25 m tall. The male head is cylindrical, 3 cm x 1 cm, green, in the leaf axis. The female head is ellipsoid, 3.5 cm x 1.5 cm, green, cauliflorous. In Sarawak, it usually flowers at intervals of 4 or more years (S. Sakai *et al.*, unpublished).

Flower visitors of *Artocarpus odoratissimus* were collected at 20:00, 1:00, 6:00, 11:00 and 16:00, May 5-6, 1996. At each collecting time, flower visitors alighted on flowers were collected on two male heads and two female heads. The observed tree was located in a secondary forest. Flower visitors of *Artocarpus integer* were collected at 20:00-22:00, 4:00-6:00 and 12:00-14:00, May 22-23, 1996. At each collecting time, insects that visited flowers within two hours were collected on three male heads and three female heads. The observed tree was planted in a secondary forest near an Iban long house, ca. 2 km apart from a primary dipterocarp forest. The owner of the tree stated that the seed was originally brought from the primary dipterocarp forest near the long house. After flower visitors were pinned, we checked pollen depositions under a microscope.

Liquid rewards were collected using micro-capillaries (Drummond Scientific Co.). Reward samples were analyzed by high performance liquid chromatography (Column: COSMOSIL 5NH2-MS; Fluent: Acetonitrile / water = 75 / 25, Flow rate: 1.0 ml / min., Detection: RI). Protein was quantified in micro protein assay (CBBG-250 method).

RESULTS

In 1996, a population of *Artocarpus odoratissimus* flowered in May 1 - June 14. The longevity of male and female heads was more than 3 days (upper limit was not obtained). Male and female heads of *A. odoratissimus* started to emit odors and liquid rewards (for anatomical study of nectary, see Moncur 1985) from 19:00, and were visited by diverse insects: nitidulid beetles, blattellid

Fig. 1. Flowers and flower visitors of *Artocarpus odoratissimus* and *A. integer*. **A.** geometrid moth visited a female head of *A. odoratissimus*. **B.** A pyralid moth visited a female head of *A. odoratissimus*. **C.** A flesh male head of *A. odoratissimus* with a drop of liquid reward. **D.** A blattellid cockroach (center) and a nitidulid beetle (lower) visited a male head of *A. odoratissimus*. **E.** Female heads of *A. integer*. **F.** Flies of multiple families visited a male head of *A. integer*. **G.** A nitidulid beetle collected on a female head of *A. odoratissimus*. Pollen grains are found at ventral parts of the head and the thorax. **H.** A drosophilid fly collected on a female head of *A. odoratissimus*. Pollen grains are found on the eye and the forelegs.

Table 1. Insect families collected from female and male heads of *Artocarpus odoratissimus* and *A. integer*.
No. insects carrying pollen grains / No. collected insects.

Order	Family	<i>Artocarpus odoratissimus</i>						<i>A. integer</i>			
		Female head			Male head			Total	Female	Male	Total
		20:00	1:00	6:00	20:00	1:00	6:00		20:00-22:00	20:00-22:00	
Orthoptera	Blattellidae	1/1			0/1	1/1	2/3			1/1	1/1
Coleoptera	Chrysomelidae					1/1	1/1				
	Curculionidae		0/1				0/1				
	Harpalidae					0/1	0/1				
	Nitidulidae	6/6	2/3	3/5	12/19	1/2	24/35				
	Pselaphidae							0/1			0/1
	Scarabaeidae								1/1		1/1
	Scolytidae				0/1		0/1				
	Tenebrionidae					1/1	1/1				
Lepidoptera	Geometridae	0/1					0/1		0/1		0/1
	Pyralidae				1/1	0/2	1/3		1/2		1/2
Diptera	Agromyzidae						1/1	1/1	1/1		1/1
	Cecidomyiidae								1/1		1/1
	Chironomidae	1/1					1/1				
	Culicidae				0/1	0/2	0/3				
	Drosophilidae	0/1	62/89	0/1	1/1	7/9	70/101	1/1			1/1
	Ephydriidae	1/1					1/1		1/1		1/1
	Lauxanidae			1/1		1/1	2/2				
	Phoridae				0/1		0/1				
	Sciaridae			0/1			0/1	1/1	0/1		1/2
	Trypetidae			0/1			0/1				
	Hymenoptera	Ichneumonidae							0/1		0/1
	Total	3/5	6/7	65/95	4/11	16/27	10/14	104/159	3/5	5/8	8/13

cockroaches, pyralid moths and geometrid moths (Table 1, Figs. 1. A, B, C, D). Although we could not determine the time when reward secretion was finished, reward was mostly dried up in the next morning (6:00). In the morning (6:00), drosophilid flies and nitidulid beetles visited flowers and fed on the remaining reward. In daytime (11:00 and 16:00), no visitors were found. Ballistic pollen release was not observed in male heads.

Pollen depositions were observed on 58 % (N= 52) of insects collected from male heads and 69 % (N= 107) of insects collected from female heads (Table 1, Figs. 1. G, H). The reward contained fructose (8.1 mg / ml in a male head, 1.5 mg / ml in a female head; Fig. 2), but glucose and sucrose were not included.

In 1996, a population of *Artocarpus integer* flowered in May 4 - June 1. The longevity of male and female heads was more than 3 days (upper limit was not obtained). Male and female heads of *A. integer* started to emit odors from 19:00, and were visited by diverse insects: flies of various families and blattellid cockroaches (Table 1, Figs. 1. E, F). In male heads, no liquid reward was secreted and flower visitors fed on pollen. Moths were collected on male heads, but they seemed to obtain no rewards. In female heads, liquid reward was secreted and fed on by flower visitors. In the morning (4:00-6:00) and daytime (12:00-14:00), odors were not detectable, no reward was secreted in female heads, and no visitors were found on male and female heads. Ballistic pollen release was not observed in male heads.

Pollen depositions were observed on 63 % (N = 8) of insects collected from male heads and 60 % of insects (N = 5) collected from female heads (Table 1). The liquid reward contained protein (2.3 mg / ml), but did not contain sucrose, fructose nor glucose (Fig. 2).

Fig. 2. HPLC analyses of liquid rewards of *Artocarpus odoratissimus* and *A. integer*. (Column: COSMOSIL 5NH2-MS; Fluent: Acetonitrile / water = 75 / 25; Flow rate: 1.0 ml / min., Detection: RI). **a.** Standard solution containing fructose, glucose and sucrose (30 mg / ml respectively); 1: H₂O, 2: fructose (6.0 min.), 3: glucose (7.0 min.), 4: sucrose (9.8 min.). **b.** Liquid reward collected from male heads of *A. odoratissimus*. A peak at 6.0 min. matched with the peak of fructose in the standard solution. **c.** Liquid reward collected from female heads of *A. odoratissimus*. A peak at 6.0 min. matched with the peak of fructose in the standard solution. **d.** Liquid reward collected from female heads of *A. integer*. A peak at 5.2 min. was revealed to be of protein from protein assay (CBBG-250 method).

DISCUSSION

Although insect visitations to *Artocarpus* flowers have been reported (Corner, 1952; Brantjes, 1982; Primack, 1983; Moncur, 1985), systematic observations in nighttime have not been carried out, and flower visitors have only been observed on male heads. Insect pollination of *Artocarpus* has not yet been demonstrated, because nobody reported that insects carrying pollen grains visited female heads. This study provides evidences that demonstrate that *A. odoratissimus* and *A. integer* provide rewards to attract diverse nocturnal insects and are pollinated by them. First, odors were emitted and rewards

were offered in nighttime. Second, male and female heads were visited by diverse insects in nighttime, and ones carrying pollen grains were collected on female heads.

It seems that no or few pollen grains of *A. odoratissimus* and *A. integer* are carried by wind, because ballistic pollen release was not observed. However, experimental studies on the role of wind as a pollen vector in *A. odoratissimus* and *A. integer*, as well as *A. rigidus* and *A. elasticus*, which have been suggested to be wind-pollinated (Corner, 1952), are required.

Little information is available on the pollination of tropical nocturnal flowers. Most neotropical nocturnal flowers are pollinated by specialized pollinators like bats, moths or beetles (Bawa, 1990). In contrast, the two *Artocarpus* species studied in this study were pollinated by diverse nocturnal insects in a number of insect families of several orders (moths, cockroaches, flies and beetles). According to recent studies, some of these insects were also found on other nocturnal flowers in lowland dipterocarp forests: *Uvaria* (Annonaceae) is pollinated by cockroaches (Nagamitsu & Inoue, 1997) and *Gnetum* (Gnetaceae) is pollinated by flies or moths (Kato *et al.*, 1995); but flower visitors of *Artocarpus* are much more diverse.

Artocarpus odoratissimus secreted a liquid reward containing fructose (0.15–0.8 mg / ml). Nectar in diurnal flowers and moth- or bat-pollinated nocturnal flowers contain a much higher density of sugars (2.2–30.5 mg / ml in flowers of Lambir, Sarawak; A. Hatada *et al* unpublished) in a mixture of fructose, glucose and sucrose (Freeman *et al.*, 1992, A. Hatada *et al* unpublished). The liquid reward of *A. odoratissimus* is characterized by the thin sugar content and the simple sugar component.

Male heads of *Artocarpus integer* do not have liquid rewards, and only pollen is offered to visitors. Female heads offer protein-rich liquid rewards without sugar. The reward of *A. integer* is unique in two points. First, the phenomenon that only female flowers have a liquid reward is very strange. Second, although proteins or amino acids are often contained in nectar (Baker, 1986), protein-rich liquid rewards without sugar have not been reported.

In unisexual flowers, if only pollen is offered as a reward in male flowers, female flowers usually deceive insects as observed in *Myristica* (Armstrong & Irvine, 1989), *Begonia* (Agren & Schemske, 1991), *Sterculia* and *Diospyros* (K. Momose, personal observations). In these cases, insects stay on female flowers during a much shorter time than on male flowers. Unlike female flowers of *Myristica*, etc., female heads of *Artocarpus* contain many stigmas. Thus, if pollinators stay for a long time on female heads, successful pollination of multiple stigmas is to be expected. Liquid reward is considered to encourage visitors to stay long on female heads.

When the reward of male flowers is only pollen, pollinators mainly obtain nitrogen (protein) from male flowers rather than energy (carbohydrate) (Roubik, 1989). The protein-rich liquid reward of female heads of *Artocarpus integer* seems to offer similar resources for pollinators as the pollen of male flowers.

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百瀬邦泰, 畑田彩, 山岡亮平, 井上民二 *Artocarpus* 属 (クワ科) の送粉様式について

マレーシア, サラワク州で *Artocarpus odoratissimus*, *A. integer* の 2 種について送粉過程を調べた。*A. odoratissimus* では, 雄花序, 雌花序の両方で, 夜間にフルクトースを含んだ報酬が分泌され, ガ, ゴキブリ, ハエ, ケシキスイなど 4 目 18 科の昆虫が訪花した。*A. integer* では雄花序は花粉のみが報酬で, 雌花序ではタンパク質を含んだ報酬が分泌されていた。*A. integer* の雌雄の花序には, 夜間に 5 目 11 科の昆虫が訪花した。これら 2 種は, 報酬が存在することと, 雌花序でそれぞれの花粉を付着させた昆虫が採集されたことから, 虫媒であることが証拠づけられた。