

# **Pollination biology and fruit dispersal of *Alocasia sarawakensis* (M.Hotta) Araceae in a lowland rainforest in Borneo**

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## **Abstract**

This study investigated the reproductive ecology of *Alocasia sarawakensis* in Danum Valley Field Centre. Nothing is known about the pollination and dispersal biology of this species. Our investigation reports the first thermogenesis measurements and also observations on pollinators (Diptera, Drosophilidae). In addition unique spathe movements within this genus were recorded and are believed to act as a pollinator management device. Dispersal observations indicate that seeds of this species are exclusively dispersed by birds. A total of nine bird species were observed removing fruits, indicating *A. sarawakensis* is an important food resource for many species in lowland rainforest. Spiderhunters (Nectarinidae, *Arachnothera*) dominated in visitation rates and total seed removal. This may be due to the characteristic beak morphology of the genus allowing easy removal of often relatively inaccessible fruits, or possibly territorial exclusion of other species.

## **INTRODUCTION**

The family of Araceae is considered special for their inflorescence structure and flowering behaviour. Several clustered flowers form a spadix which is sheathed by a spathe. In spite of their constant inflorescence morphology, aroids have evolved many different strategies to attract insects to their blossoms and have established relationships with different insect groups. The attractant can be food (e.g. pollen or food bodies), shelter and, very unusually, warmth (Ivancic *et al.*, 2005) produced by thermogenesis. The common reward of nectar is never offered in this family but some flies are known to feed on stigma secretions. Some insects, especially flies, are known to use inflorescences also as brood site. There are, however, some aroids that dupe or trap visitors.

The present study focuses on chamber blossoms, which have a tube-shaped spathe but are not genuine trap flowers. In the genus *Alocasia* usually fruit flies are known to lay their eggs inside the inflorescence and while doing this act as pollinators (Ivancic *et al.*, 2005). The monoecious spadix is divided in four different parts starting with the female zone at the bottom followed by a sterile zone, a male zone and an appendix (Boyce, 2008). Although plants of the genus *Alocasia* are not known to trap their visitors, a spathe movement to protect the lower female part of the inflorescence before releasing the pollen is common. The species studied here seems to have a spathe movement which additionally manipulates the way the flies have to exit the inflorescence during male phase, which was never observed in this genus, but is known from the genus *Coryanthes* (Orchidaceae).

From the 105 genera and about 3,300 known species forming the Araceae family, pollinators are documented for only 49 genera and about 125 species (Gibernau, 2003). Among the already studied genera only a few species are surveyed for each of them. For 56 genera there are no records of pollinators. A generalisation from one species to a whole genus is hazardous since specialised plant pollinator interactions might be overlooked (Gibernau, 2003). In many cases it is not certain if the observed insects are pollinators or just visitors.

Likewise, nearly no studies have been done on fruit dispersal of Araceae. Present knowledge of frugivore animals visiting aroids come from single observations and generalization. Most aroids produce a high number of small fleshy fruits over a long period of time. Although this fruit type fits perfect to the avian fruit dispersal syndrome, the genus *Colocasia* is known to be mammalochor, having smelly inconspicuously coloured slimy fruits (Hay, 1996). Especially in tropical regions, where aroids have their main distribution, about 90% of all plants produce fleshy fruits which are dispersed by animals. There should be studies to broaden our knowledge about reproduction ecology of aroids and increase our understanding about the importance of their fruits in tropical food webs.

The present study focuses on pollination biology and seed dispersal of *Alocasia sarawakensis*, (Araceae), which is an endemic to Borneo. In particular, we study:

- How is the floral cycle of *Alocasia sarawakensis* organised and who are the pollinators?
- Is the inflorescence producing heat through thermogenesis as it is known from several other species and is this phenomenon connected to insect visitors?
- Which bird species visit the plant and which of them are potentially the most important for seed dispersal?

## **METHODS**

### **Study site and plants**

The present study was carried out in October 2010 at the (DVFC) Danum Valley Field Centre, which is situated in South-eastern Sabah, Malaysian Borneo (4°58'N and 117°35'E - 117°48'E, altitude ca. 170 m). The climate at Danum is equatorial with a mean annual temperature of 26.8 °C. Mean annual rainfall (1985-2006) is 2,825 mm.

20 individuals of *A. sarawakensis* were observed during the study. Three of them were flowering over the whole month and could be used for pollination as well as seed dispersal studies. The plants in the studied area are mostly growing in drainage channels along the road, in a very wet habitat. We also found one plant inside the forest on the bank of a stream, which may be its natural habitat.

## **Pollination biology**

Eleven inflorescences from three different individuals were observed in total.

### **Anthesis**

Flowering inflorescences were observed four times a day (early morning between 05:00 and 07:00; late morning between 09:00 and 12:00 (noon); afternoon between 16:00 and 19:00; during the night between 23:00 and 02:00). Different stages of opening of spathe were photographed and scent production was tested by smelling different parts of the inflorescence. Stigma receptivity was tested using peroxidase test (McInnis *et al.*, 2006). Pollen release was determined by observation as the pollen grains could easily be seen with naked eye.

### **Insect visitors**

First arrival time, leaving time and behaviour during the visitation of flies were recorded. Macro photographs were taken to look for pollen covering the animals. Insects were collected from the spathe and the spadix using an exhaustor. One inflorescence was covered with a fine mesh bag during the night so that any insects already inside the floral chamber got trapped upon leaving the inflorescence next morning. The insects were counted and identified to family level. Their behaviour inside the floral chamber was observed through a hole cut into the lower part of the spathe using a knife.

Post-anthesis inflorescences were cut after one, four and eight days and dissected under a microscope, to look for insect eggs and larvae placed on the spadix. Larvae were conserved in 70% ethanol for further identification. Unripe and ripe infructescences were opened and inspected for adult flies which might have developed from these larvae.

### **Thermogenesis measurements**

Temperature was measured in four inflorescences from two different individuals using a “Thermofox” data logger connected to a multisensory thermo element with four external temperature sensors. The data logger saves the temperature in a five minute interval. During the evening of the first opening, three sensors were inserted: one inside the appendix, one inside the upper male part and one inside the lower male part. The fourth sensor measured air temperature a few centimetres next to the inflorescence. Temperature was always measured for at least three days (72 hours) to cover the whole flowering period and one additional day as a control measurement. “Thermofox” software was used to transfer the measured data from the data logger to a computer.

## **Dispersal biology**

### **Patch sampling and rate of dehiscence**

Twenty individual plants were sampled from six patches of varying plant densities ranging from one to six individuals per patch. All open infructescences were labelled, and the time of opening noted. The degree of opening (dehiscence) of the infructescence was measured by recording the distance between the base of the infructescence and the opening. These measurements were repeated three times a day for seven days typically at 06:00, 13:00 and 17:30. The rate of dehiscence was calculated as distance (cm) opened per day.

### **Fruit removal**

The structure of the infructescence prevented a simple count of the number of seeds removed. We therefore measured the distance along the peduncle devoid of fruits. These measurements were repeated three times a day for seven days, typically at 06:00, 13:00 and 17:30. After most or all of the fruits had been removed, the infructescence was cut and brought to the lab. Here the distance measurements were converted to actual fruit removal by counting the numbers of empty seed bracts within the measured distance. Fruit removal was then calculated as a function of numbers of fruits removed per day.

### **Disperser observations**

*A. sarawakensis*, fruits were regularly observed to determine its seed dispersers. There were three observation periods of ninety minutes each day for seven days: Early morning (06:00-07:30), late morning (08:30-10:00) and afternoon (15:30-17:00). The largest patches were used for observation to increase chances of observing a visitor and to monitor as many individual plants as possible. Observations were made at a distance or with the use of a hide to avoid influencing visitation. At each observation the visiting species were identified. In addition, the plant visited, the duration of visit and the numbers of fruits removed were recorded.

## **RESULTS**

### **Pollination**

#### **Anthesis and insect behaviour**

The flowering cycle of *Alocasia sarawakensis* lasts for about 48 hours (Table 1). The spathe starts to open in the afternoon of day one at about 16:00. The opening process requires nearly the whole night. During this stage, small unidentified bluish flies were already sitting at the lower part of the spathe and on the peduncle. Before dawn of day two the spathe is eventually fully opened and starts to produce an apple scented washing powder-like odour that is mostly emitted from the appendix. At

around 06:00 the first flies of a different species (*Drosophilidae*) arrive, settle down on the spathe and most of them immediately enter the floral chamber where the female flowers are situated. The number of flies increases continuously to reach a peak at about 07:00. At this time the upper part of the spathe and the inside of the chamber is covered with flies. The vast majority consists of small similar looking flies however there are also some individuals of an unidentified larger fly.

At this stage of the flowering cycle stigma receptivity was confirmed. All stigmata were producing a great amount of secretion and flies were observed feeding on it while moving between the pistils. Copulation takes place on the inner surface of the spathe inside the chamber. During this activity the dorsal part of the male individual comes in contact with the stigmata. The insects spend the whole day somewhere on or in the inflorescence. The upper part of the spathe starts bending backwards and showing signs of senescence. At around 18:00, when it is getting dark, the flies move into the chamber to spend the night there. None of the bigger flies were seen acting like this. They probably leave before sunset.

The spathe remains open until the early morning and starts closing between 04:00 and 05:30. When sun comes out the opening is already closed except for a small hole which is now the only exit for the flies. This is also the time when pollen is released from the male part of the spadix. While forcing themselves through this hole the animals have to pass the male flowers and so come in touch with the pollen. The pollen covered flies do not fly off immediately after leaving the chamber but they remain on the free part of the spadix which also contains male flowers and pollen. Later in the morning all flies are gone. A total of 178 individuals of *Drosophilidae* were trapped using a bag covering one inflorescence.

The dissection of inflorescences after anthesis revealed larvae of *Diptera*, feeding on the senescing and rotting parts of the inflorescence. Two types of larvae, feeding on different parts, could be distinguished. Bigger maggots were found in the upper section that was previously the male part and much smaller ones were found in decomposing stigmata and pollen grains at the female part. None of the carpels were found to be damaged. Just one day after the arrival of flies there were already very small larvae present.

**Table 1. Main events during the 48 hour anthesis of *Alocasia sarawakensis*, showing the activity of different inflorescence zones and behaviour of visitors**

<b>Time</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>
<b>05:00 – 07:00</b>	Spathe closed	Fully open, scent release, flies arrive, stigmata receptive	Pollen release, spathe closed except for a small hole
<b>08:00 – 12:00</b>	Spathe closed	Flies on all parts, scent still being released	Flies taking off, small hole still open
<b>16:00 – 02:00</b>	Spathe opening	Upper spathe senescing, flies inside the floral chamber, spathe still open	Upper spathe continues senescing

### **Thermogenesis measurements**

The thermogenic activity lasts approximately 35 hours (Figure 1). The spadix starts to increase in temperature during the night after the first opening going up to 30 °C until about 06:00. Then the appendix heats up rapidly reaching a peak of 43 °C at about 6:45 (air temperature 22 °C). The temperature of the male part at the same time is 35°C. After this peak the whole spadix temperature decreases to 31 °C, which is 4 °C warmer than air temperature over the day. During the following night the lower male part holds this 31 °C having another warming up to 36 °C at 06:50 the next morning (air temperature 22 °C). At 09:00 all measured parts are already similar to air temperature. No further thermogenesis was measured the following night.

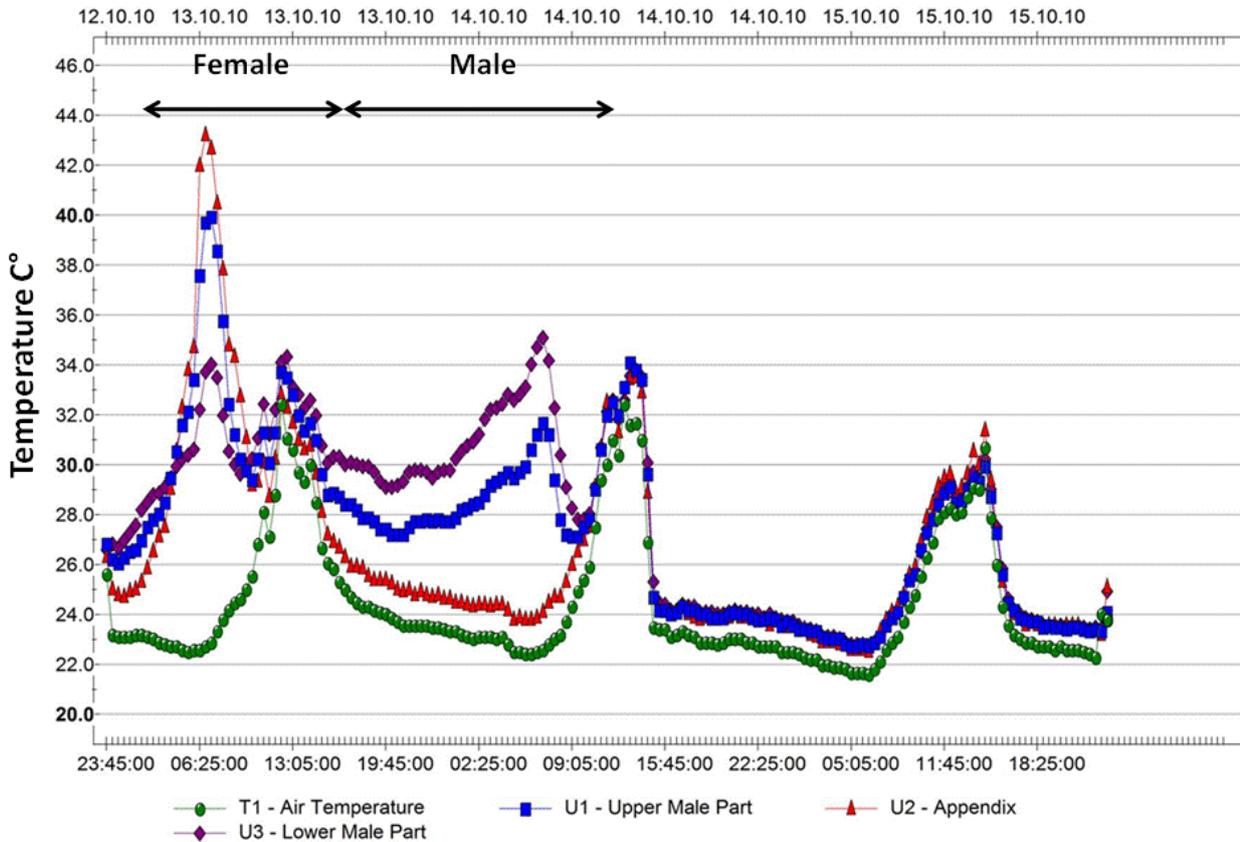


Figure 1. Temperature of three floral parts of *Alocasia sarawakensis* and air temperature during a period of 72 hours. The 43 °C peak of the appendix at 6:50 in the first morning, as well as the 36 °C peak of the lower male part at 6:50 the next morning, are linked with the female and the male phase of anthesis.

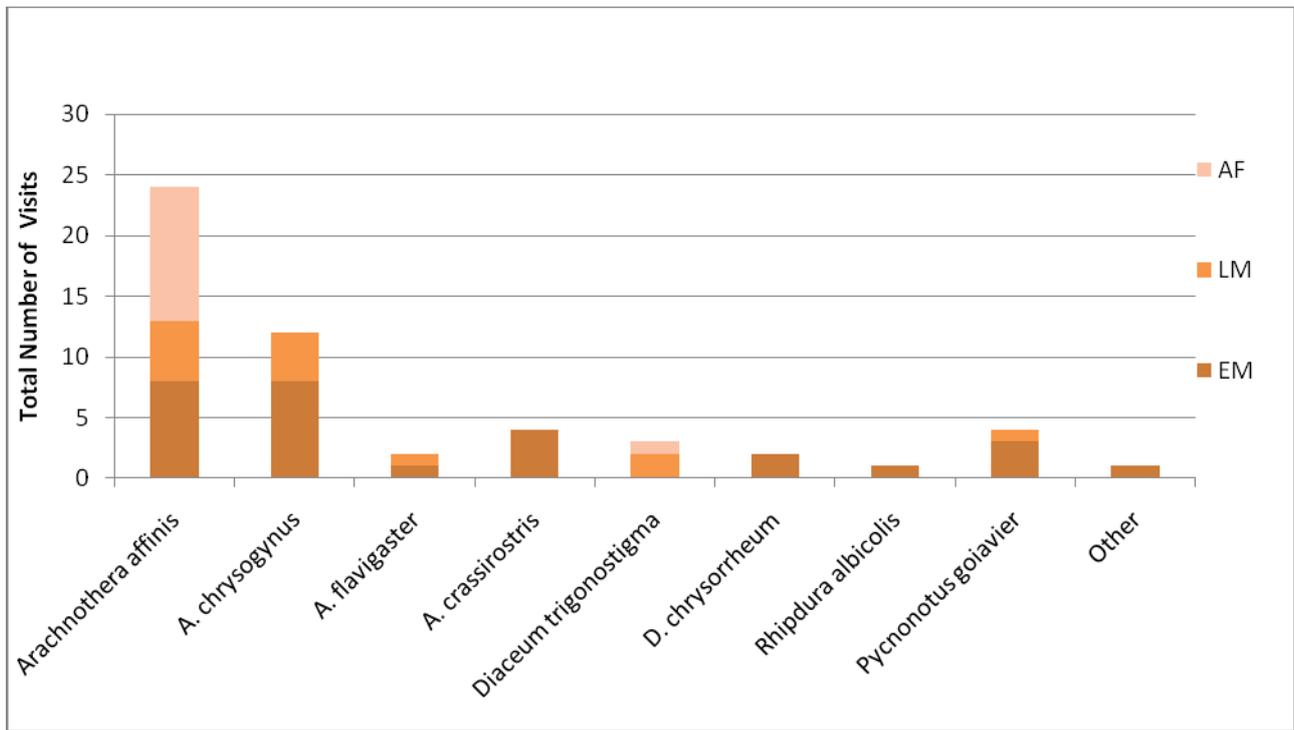
## Dispersal

### Observed species

There was a total of nine bird species observed visiting *A.sarawakensis* during a seven day period (Table 2).

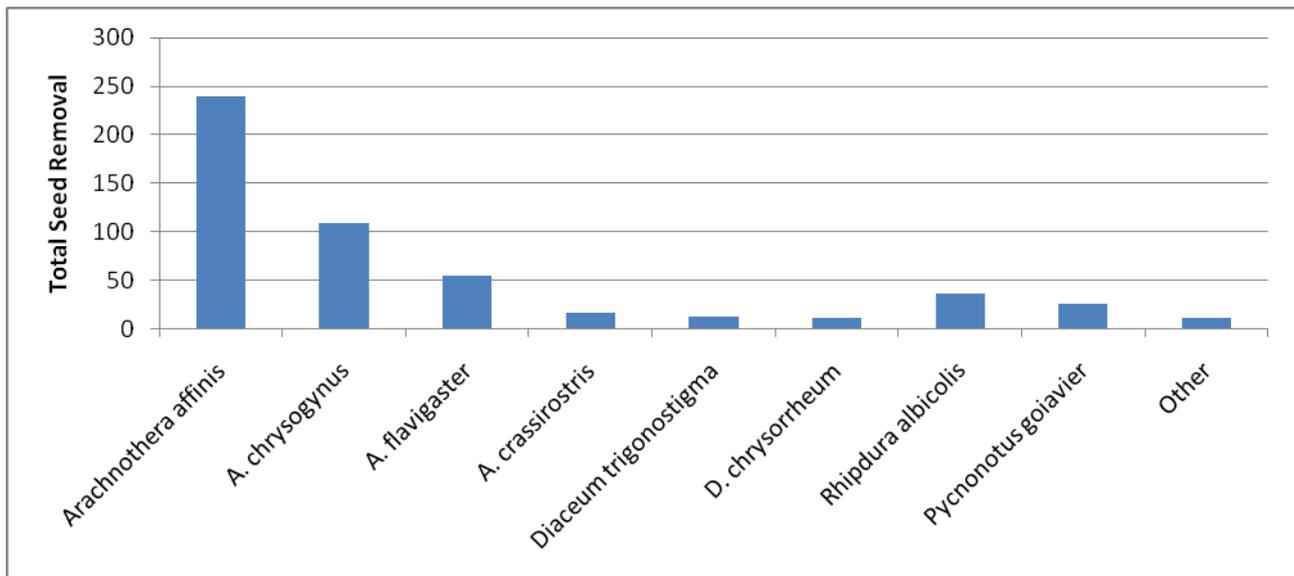
Table 2. Species observed visiting the fruits of *A. sarawakensis* at Danum Valley Field Centre

Frequency of Visitation Rank	Species
1	Streaky Breasted Spiderhunter ( <i>Arachnothera affinis</i> )
2	Yellow Eared Spiderhunter ( <i>Arachnothera chrysogenys</i> )
3	Yellow Vented Bulbill ( <i>Pycnonotus goiavier</i> )
3	Thick Billed Spiderhunter ( <i>Arachnothera crassirostris</i> )
5	Orange Bellied Flowerpicker ( <i>Diaceum trigonostigma</i> )
6	Spectacled Spiderhunter ( <i>Arachnothera flavigaster</i> )
6	Yellow Vented Flowerpecker ( <i>Diaceum chrysorrheum</i> )
8	White Throated Fantail ( <i>Rhipdura albicolis</i> )
9	Other (Unidentified species)



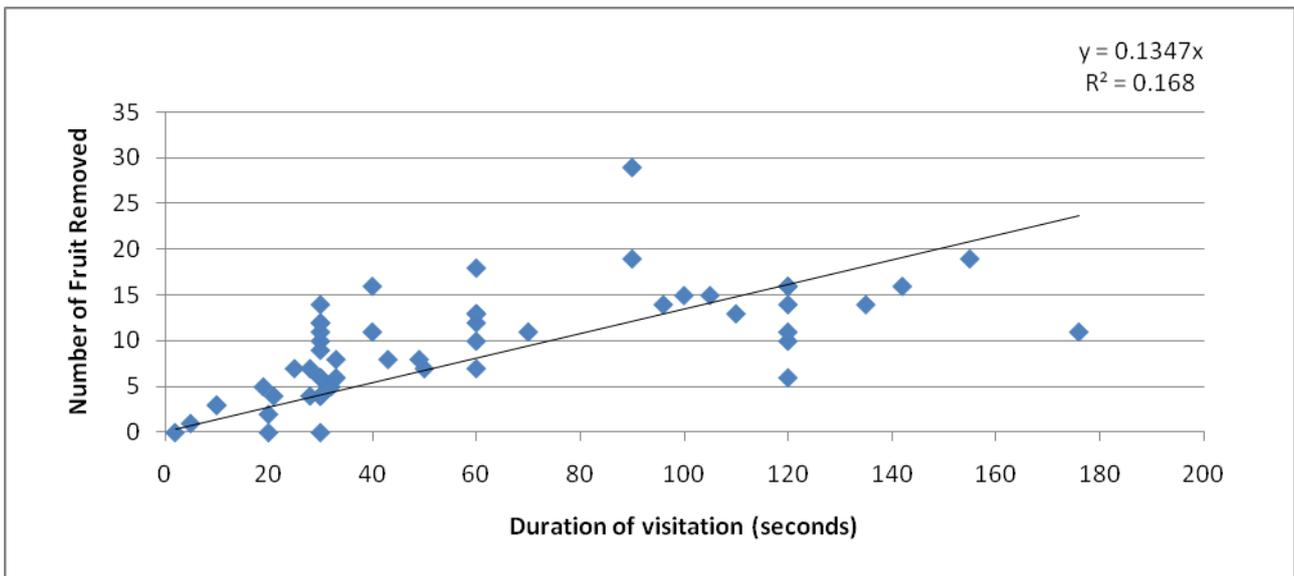
**Figure 2. Total number of visits from each species observed in the early morning (EM), late morning (LM) and afternoon (AF).**

*A. affinis* was observed visiting *A. sarawakensis* the greatest number of times, eight times in the early morning, five times in the late morning and eleven times in the afternoon. *A. chrysogenys* was the second most frequent visitor, observed eight times in the early morning and four times in the late morning. All other species were observed infrequently and generally most often in the early morning.



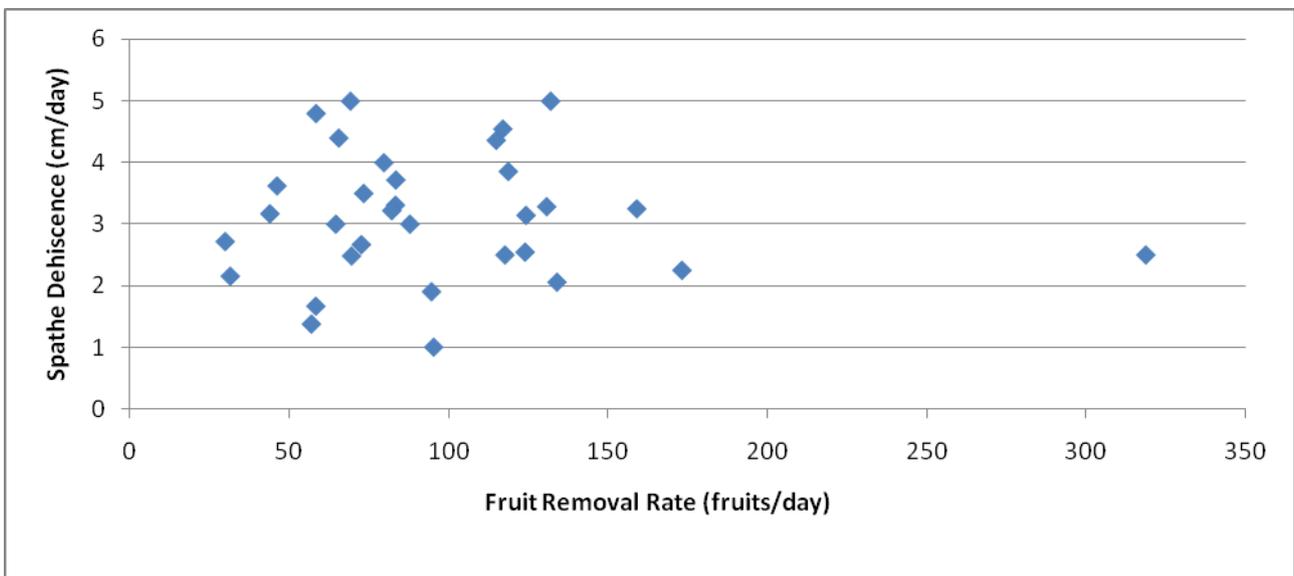
**Figure 3. The total number of fruits removed from *A. sarawakensis* by each observed bird species.**

*A. affinis* removed the greatest number of fruits (240) from *A. sarawakensis* over the total observation period. *A. chrysogenys* removed 109 fruits, and *A. flavigaster* 54, while the remaining species removed relatively few in comparison (Figure 3).



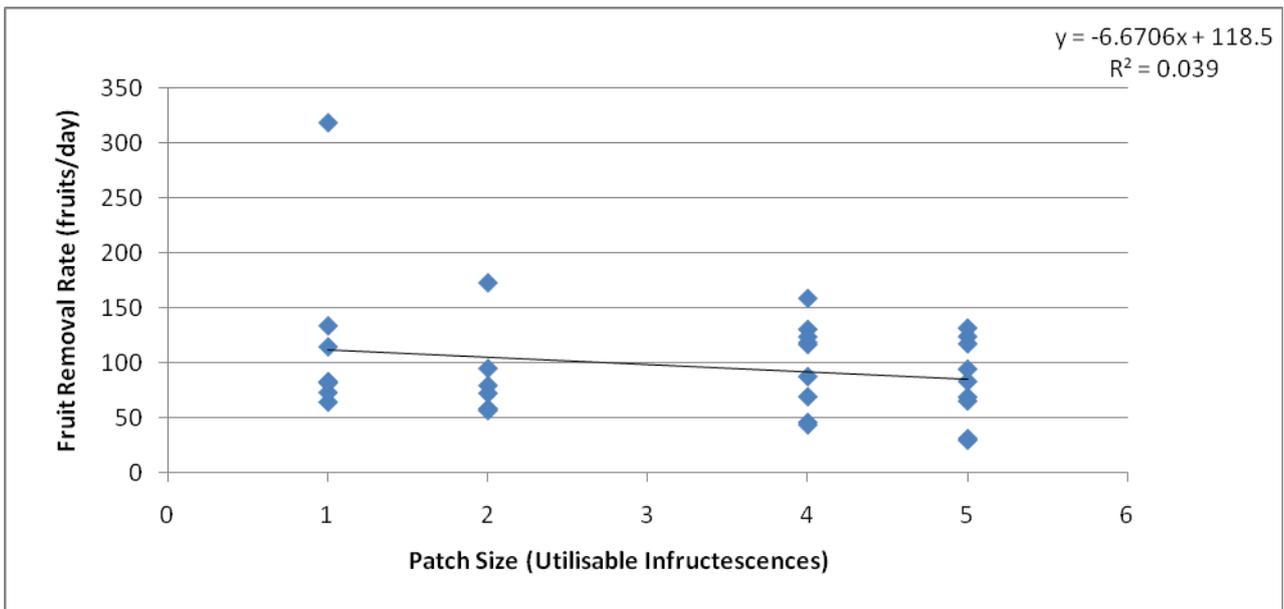
**Figure 4. Relationship between the number of fruits removed and the duration of the visit.**

There was a positive linear relationship between the number of fruits removed at each visit, and the duration of that visit, with 17% of the variability in seed removal explained by the duration of the visit (Figure 4). With an average of 146 fruits per infructescence, and with the average number of fruit removed being twelve, then each visitor typically removes 8% of the fruit store.



**Figure 5. Relationships between the rate of fruit removal (fruits/day) and the rate of fruit dehiscence (cm/day) of *A. sarawakensis*.**

There was no relationship between fruit removal rate and the rate of spathe dehiscence (Figure 5).



**Figure 6.** Relationship between rate of fruit removal from *A. sarawakensis* and patch size (the number of infructescences available to visitors).

There was also no relationship between the rate of fruit removal from *A. sarawakensis* and the number of infructescences that can be utilised by the visitor in a given patch (Figure 6).

**Table 3.** Pearson’s product moment correlation between Number of Available Fruits, Fruit Removal Rate, Rate of Dehiscence, and Total number of Visitors/Patch .  $P < 0.1$  for all correlations

	Number of Fruits Available (Patch size)	Fruit Removal Rate	Rate of Dehiscence
Fruit Removal Rate	-.220	*	*
Rate of Dehiscence	.209	-.052	*
Total Number of Visitors /Patch	.178	.164	.276

The number of fruits, fruit removal rate, rate of dehiscence and total number of visitors/patch were not mutually correlated (Table 3).

## DISCUSSION

### Pollination biology

The observed events during anthesis are all related to pollinator management which brings advantages to the plant by manipulating the pollinator’s behaviour. They include scent production, temperature changes and spathe movements.

Like in most other Araceae the warming of the spadix goes together with odour production. As visitors arrive at the same time it is very likely that olfactory cues are important to attract flies. The appendix with a peak of 43 °C is 20 °C above air temperature and this might increase the intensity and distance of fragrance dispersal. The strong scent is recognisable over a few metres even for humans.

Inflorescences which have female and male phase separated in time face a problem: If visitors are only attracted to the female phase of the anthesis, they would not remain until the pollen release and never come in touch with pollen. These insects would not be useful as pollen vectors. The studied species evolved a way to manage this task. As most diurnal insects hide during night the simple offering of a chamber that can be used as a hiding place is one reason for insects to stay at this place. Most insects have to regulate their body temperature utilising external energy sources to heat up their body. Diurnal insects mostly use the power of the sun, so over night they are facing a lack of energy. *Alocasia sarawakensis* employs two strategies that work in favour of its pollinators and with this increases the success of pollination.

The heat production during the night is an additional reward to the hiding place. The thermogenesis measurements show that during night only the lower male part of the spadix produces heat. This part is situated inside the floral chamber where the flies are staying. This targeted usage of thermogenesis might influence more flies to stay overnight rather than leaving before sunset.

The peak of 36 °C in the morning is the second way to manipulate the flies. They do not need to wait until ambient air in the morning is warming up to their optimal flight temperature. Their body already has optimal flight temperature, so they can immediately fly to another inflorescence which starts the female phase of anthesis.

Another part of pollinator management is the spathe movement which is connected with the male phase. Some Araceae are known to close the spathe to trap insects for as long as the plant needs to release the pollen. Here we have a special case of this strategy. Rather than trapping the flies, they are forced to use a particular exit which leads exactly over the male part. This exit could act as insurance for the plant that every single fly is covered with a huge amount of pollen when it leaves. That hypothesis would need further experiments to test it. The closing process could be manipulated to keep the spathe open. Flies would be collected after exiting manipulated and not manipulated inflorescences. Pollen grains on the insect body would be counted. So it would be clearer which impact this mechanism has on the amount of pollen transported on each insect.

Another possible function would be a filter exiting mechanism that helps to exclude other visitors except the legitimate pollinators. The bigger flies for example which were observed to visit the flowers during the day cannot use the chamber over night because in the morning they would be unable to leave.

A similar way of pollinator management is known from two other genera of Araceae and could be an example for convergent evolution. It might be that the way of freeing the insects is as important as trapping them.

## **Dispersal**

### **Primary dispersers**

After a total of 2,160 minutes of observation, it was clear that the most frequent visitor and presumably the one most important for dispersal was *A. affinis*, with *A. chrysogynus* also potentially making a large contribution to dispersal. It is important to note however that we could not confirm that the visiting species were actually legitimate dispersers or seed predators.

The observed affinity of *Arachnothera* for *A. sarawakensis* may be explained by either behavioural or morphological traits of this bird genus. Territoriality may explain the disproportionate visitation of *Arachnothera* species if the sample sites happened to be in close proximity to *Arachnothera* nesting sites. *Arachnothera* birds may be actively fending off competition for the fruit resource within their territory. This territorial behaviour in *Arachnothera* species has been documented when defending floral nectar resources (Johansen, unpublished data).

*Arachnothera* genus has a prominent beak effective for catching insects and accessing nectar from long tubular corollas. We observed that it is also perfectly suited for fruit removal from the cylindrical protective lower spathe of *A. sarawakensis*. We witnessed that numerous short-beaked birds e.g. *Diaceum* species, were unable to access some of the fruits positioned on the lower section of the peduncle while *Arachnothera* birds with their elongated, decurved beak could easily reach the lower fruits. This observation may explain why there was no relationship between how quick the protective spathe opens and the fruit removal, since the spathe did not represent any obstacle to the most frequently visiting bird species.

### **Removal rate and patch size**

Our study examined some factors which we thought may influence the removal rate of the fruits from the infructescence, namely duration of visit, patch size and the rate of dehiscence. Perhaps not

surprisingly we found a strong linear relationship between duration of visit and seed removal. In contrast, patch size i.e. the number of infructescence available for utilisation in a given area, was not related to the rate of seed removal per individual plant. There is thus no advantage for an individual of *A. sarawakensis* to occur in a large patch. However our study only looked at four different patch sizes with no replication which could have influenced our results.

### **Ecological importance of fruits**

Although *Arachnothera* were the dominant visitors, our study showed they are certainly not the exclusive dispersers. Nine species were observed utilising the fruits of *A. sarawakensis*, five of which were from other genera than *Arachnothera*, including two *Diaceum* species, *Rhipdura albicollis*, *Picnonotus goiavier* and one other unidentified species. In total 5,446 fruits were removed over a seven day period. This illustrates the importance of this food resource for numerous bird species. The large number of fruits removed indicates *A. sarawakensis* has a considerable energy input into the local food web.

### **Further research**

Our study was limited to one study site and habitats mostly outside tropical forest. The observed patterns in *A. sarawakensis* thus need to be verified in other areas of Borneo, particularly within primary forest.

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