Variations in the Quantity of Attractants in Floral Odors and their Effects on Beetle Pollinator Arrivals in *Homalomena propinqua* (Araceae)

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Introduction

The Araceae represent one of the most diverse and abundant families in the humid tropics (Young 1986; Mayo et al. 1997), and their inflorescences are usually monoecious and protogynous (Bernhardt 2000). Beetle pollinators tend to arrive more frequently at the pistillate-phase inflorescences during the period of heat generation by the spadices than at the staminate-phase inflorescences, and this difference in timing can promote cross-pollination (Gibernau et al. 1999; Bernhardt 2000).

Homalomena propinqua (subfamily Aroidae) is a common understory herb found in lowland dipterocarp forests in Borneo, and two types of beetles (*Parastasia bimaculata* Guerin, Scarabaeidae; *Dercetina* sp., Chrysomelidae) are its main pollinators (Kato 1996; Momose et al. 1998; Kumano and Yamaoka 2006). Protogynous inflorescences of this species remain open for 3 days; the spathe is fully open by 0500 on the first day, and adhesive pollen begins to be shed on the morning of the second day. Beetle pollinators arrive at inflorescences mainly between 0700 and 1000, when the spadices generate endothermic heat (Kumano and Yamaoka 2006). Pollinators remain in the floral chamber with eating and/or mating, until the spathe closes tightly on the morning of the fourth day.

In our previous study (Kumano and Yamaoka 2006), we showed that the quantity of attractants released by the plant in the floral odors increased during the period of endothermic heat generation and proposed that the arrival of beetle pollinators may be affected by the increase in the quantity of these attractants. However, little information has been published about the attractiveness of floral odors even though beetle pollinators are attracted over long distances mainly by olfactory cues (Gottsberger and Silberbauer-Gottsberger 1991). In this study, we focused on variations in the quantity of attractants in floral odors to elucidate the relationship between quantity of attractants and arrivals of beetle pollinators in the *H. propinqua* pollination system. To do so, we first observed beetle pollinator arrivals during each floral stage, collected and analyzed floral odors, and then we conducted a bioassay of the main volatiles contained in these floral odors. We also investigated the relationships between variations in the quantity of attractants and the frequency of pollinator arrivals both during heat generation by the spadices and among floral stages. We use the results of these analyses to discuss the factors responsible for the reproductive success of *H. propinqua.*

Materials and Methods

Study site and materials

Our study was carried out in a lowland dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20′N, 113°50′E; 150 to 250 m a.s.l.). We found two *H. propinqua* populations along small streams, located 100 m apart and separated by a ridge. All examinations were conducted using these two populations.

Observation of pollinator arrivals during each floral stage

To determine the frequency of beetle pollinator arrivals during each floral stage, we used unpollinated inflorescences (40 inflorescences on the first day of flowering, 44 inflorescences on the second day, and 19 inflorescences on the third day). All inflorescences were covered with polyethylene bags the day before flowering and remained covered until our observations began. Bags were removed at 0600, and pollinators were then free to arrive at the inflorescences. Thereafter, we caught pollinators inside the floral chambers at 1030 hours using polyethylene bags. Differences in pollinator arrivals were compared using a two-way ANOVA followed by Schéffe's multiple-comparison test.

Bioassays using the main compounds found in floral odors

Bioassays were conducted using laboratory standard chemicals for the five main components of floral odor in *H. propinqua*, which together represented 88% of the floral odors compositions by mass on the first day(Kumano and Yamaoka, 2006). To test their effectiveness, we added 100 μg per hour of each test compound on a Petri dish placed below a plant with no inflorescences. We recorded the species and the total number of pollinators that flew in a zig-zag pattern above the Petri dish or that landed on the dish between 0630 and 1030. As a negative control, we used an empty Petri dish placed beside the Petri dish containing the test compound. As a positive control (intact flowers), we counted the total number of beetles that remained in open flowers once per week when we conducted the bioassays. To confirm differences in attractiveness of pollinators between test compounds and positive controls (intact flowers), we transformed the number of individuals to log (χ + 1) scale and subjected the resulting data to a one-way ANOVA, followed by Dunnet's test.

Sampling of floral odors

To examine temporal variation in the quantity of attractants during the morning pollination period, we used eight unpollinated inflorescences per day from the first day to the third day of flowering. To confirm the variation in attractant quantities among floral stages, we used 10 unpollinated inflorescences and sampled consecutively from the first to the third day of flowering between 0700 and 0800 hours, when both of the main beetle pollinators visit most frequently (Kumano and Yamaoka, 2006). The methods used for collection and chemical analyses of the floral odors are described by Kumano and Yamaoka (2006). Temporal variations in the quantity of attractants were analyzed using the Kruskal-Wallis test combined with Scheffé's test. Differences among floral stages were tested using Friedman's test combined with a Steel-Dwass test.

Results

Observation of pollinator arrivals at each floral stage

The mean number of pollinator arrivals at each floral stage is shown in Fig. 1. The mean number of P. bimaculata arrivals decreased significantly from day 1 to day 3 (Schéffe's test, $P < 0.01$). On the other hand, the mean number of Dercetina sp. arrivals showed no significant differences among floral stages ($P > 0.05$).

In addition, the number of P. bimaculata arrivals on the third day was significantly lower than the number of Dercetina sp. arrivals at every floral stage ($P < 0.05$).

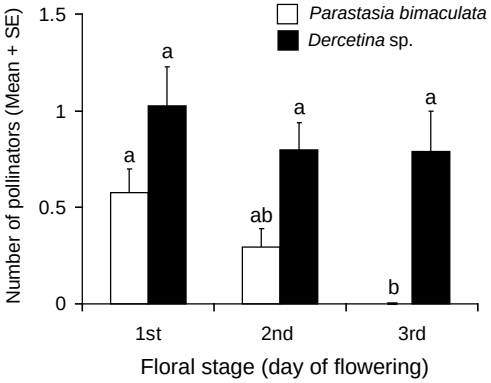

Fig. 1 Mean number (+ 1SE) of pollinators (*Parastasia bimaculata* and *Dercetina* sp.) arrivals on each floral stage inflorescence (the first day = 40 inflorescences, the second day = 40, the third day = 19). Different letters indicate significant differences (Sheffé test, $P < 0.05$). Open bars show *P. bimaculata*, and solid bars show *Dercetina* sp.

Table 1 Attractiveness of five main compounds in floral odors of *H. propinqua* and mixtures of them toward beetle pollinators (*Parastasia bimaculata* and *Dercetina* sp.).

Attractants [2]	Number of pollinator (mean ± SE)[1]	
	P. bimaculata	*Dercetina* sp.
α-Pinene (P)	0	0 *
2-Butanol (B)	0	0 *
2-Methyl-3-buten-2-ol (M)	0.13 ± 0 .13	0 *
2-Heptanol (H)	0	0 *
Veratrole (V)	0	1.71 ± 0.52
BP	0	0 *
BM	0	0.29 ± 0.18
BH	0	0.14 ± 0.14 *
BV	0.57 ± 0.30	2.29 ± 1.30
VM	0.29 ± 0.29	3.71 ± 1.43
VP	0	4.00 ± 1.09
VH	0	1.71 ± 0.47
BVP	0.29 ± 0.18	0.57 ± 0.20
BVM	0.14 ± 0.14	0.57 ± 0.20
BVH	0.86 ± 0.40	0.29 ± 0.18
BVPH	0.50 ± 0.31	1.20 ± 0.39
BVMH	0.13 ± 0.13	0.75 ± 0.25
BVPMH	0.60 ± 0.25	4.60 ± 1.44
Positive control (intact flowers)	0.65 ± 0.15	2.70 ± 0.59

[1]Means in the same column with asterisk are significantly different between the positive control based on Dunnet test ($P < 0.05$). Each trial conducted on the separate day.
[2]Capital letters mean abbreviated names of five compounds. P: α-pinene, B: 2-butanol, M: 2-methyl-3-buten-2-ol, H: 2-heptanol, V: veratrole

Bioassays using the main components of the floral odors

The results of our bioassay are shown in Table 1. The positive controls (intact flowers) showed that *Dercetina* sp. arrived at inflorescences four times more frequently than *P. bimaculata*. No pollinators were attracted by empty Petri dishes (the negative controls). *Parastasia bimaculata* was attracted by 2-methyl-3-buten-2-ol (M), veratrole (1,2-dimethoxybenzene; V) plus M, and all mixtures containing 2-butanol (B) plus V thus the minimum components necessary for the attraction of *P. bimaculata* were M and BV. In contrast, *Dercetina* sp. was attracted by V, all mixtures containing V, and BM, which therefore appear to be the minimum components required for the attraction of *Dercetina* sp. Of these minimum components, BV and veratrole were the more efficient attractants for *P. bimaculata* and *Dercetina* sp., respectively.

Variations in the quantity of attractants in floral odors

Temporal variations in the quantity of the minimum components of attractants are shown in Table 2. On the first day of flowering, quantities both of BV and V increased significantly between the 0530 to 0630 period and the 0700 to 0800 period, then remained above the initial level (but not significantly) until the 10:00 to 11:00 period (Schéffe's test, $P < 0.05$). On the second day, the pattern was similar, but the quantity

Table 2 Time variations in the quantity of attractants in floral odors of *H. propinqua* from 05:30 to 12:30 hours over the flowering periods (µg/ h / flower).

flowering day		Time				
		5:30-6:30	7:00-8:00	8:30-9:30	10:00-11:00	11:30-12:30
Attractants of *P. bimaculata*						
BV (2-butanol + Veratrole)						
1st	Median	**13.64** [a]	**97.54** [b]	**49.53** [ab]	**28.08** [ab]	**13.67** [a]
	Quatile	5.64 - 33.97	55.19 - 117.41	25.66 - 80.97	16.86 - 49.34	7.12 - 15.71
	Min - Max	2.38 - 57.92	52.26 - 194.08	11.88 - 141.39	13.54 - 80.62	6.33 - 63.25
2nd	Median	**41.32**	**77.76**	**41.76**	**38.69**	**11.7**
	Quatile	17.25 - 99.48	42.24 - 128.31	27.98 - 90.54	16.75 - 102.96	6.74 - 42.45
	Min - Max	2.38 - 456.27	29.23 - 172.37	22.89 - 121.78	5.28 - 106.98	2.73 - 85.00
3rd	Median	**11.89**	**12.36**	**14.53**	**15.51**	**3.66**
	Quatile	8.58 - 16.51	6.22 - 44.43	3.13 - 18.13	10.06 - 20.48	2.61 - 4.27
	Min - Max	2.69 - 23.93	7.89 - 163.61	0.81 - 19.85	1.74 - 23.82	1.21 - 4.78
Attractant compound of *Dercetina* sp.						
Veratrole						
1st	Median	**0.67** [a]	**16.29** [b]	**10.23** [ab]	**3.55** [ab]	**2.08** [a]
	Quatile	0.25 - 1.14	10.06 - 27.59	2.59 - 20.21	1.63 - 4.62	1.05 - 2.37
	Min - Max	0 - 6.06	5.50 - 29.55	1.78 - 34.95	1.49 - 5.34	0.46 - 5.40
2nd	Median	**6.46**	**18.02**	**8.06**	**4.44**	**3.9**
	Quatile	1.88 - 19.45	9.82 - 38.99	5.73 - 12.66	3.27 - 23.57	1.94 - 7.95
	Min - Max	0.29 - 205.95	2.51 - 53.19	2.10 - 21.93	2.86 - 25.50	0.91 - 18.25
3rd	Median	**1.66**	**7.02**	**4.05**	**1.91**	**1.88**
	Quatile	1.29 - 6.81	0.83 - 19.92	0.95 - 7.03	0.92 - 3.47	1.4 - 2.11
	Min - Max	0.21 - 15.73	0 - 141.25	0.11 - 9.28	0.14 - 3.87	0.13 - 2.63

Medians with different letter are significantly different among five collection times in each day (Sheffé test, $P < 0.05$).

didn't vary significantly among times for either attractant (Kruskal-Wallis test, $P > 0.05$). On the third day, the pattern was similar to that on the second day, but with a lower quantity of attractants, and quantities didn't vary significantly among the five collection periods (Kruskal-Wallis test, $P > 0.05$). Variations in the

quantity of attractants among floral stages are shown in Fig. 2. The quantity of BV decreased steadily over time, and the difference compared with the first day became significant on the third day (Steel-Dwass test, $P < 0.05$). The quantity of veratrole also decreased slightly from the first day to the third day, but the difference was not significant (Friedman's test, $P > 0.05$).

Discussion

In general, the pistillate phase (stigma receptivity) and the staminate phase (anther dehiscence) of protogynous Araceae inflorescences do not overlap, therefore obligate outcrossing seems to be the general rule (Mayo et al. 1997). Our results showed that *P. bimaculata* tended to arrive during the pistillate phase, when heat generation occurs; in contrast, *Dercetina* sp. arrived at inflorescences regardless of the floral stage (Fig. 1). To confirm the relationship between these behaviors and variations in the quantity of

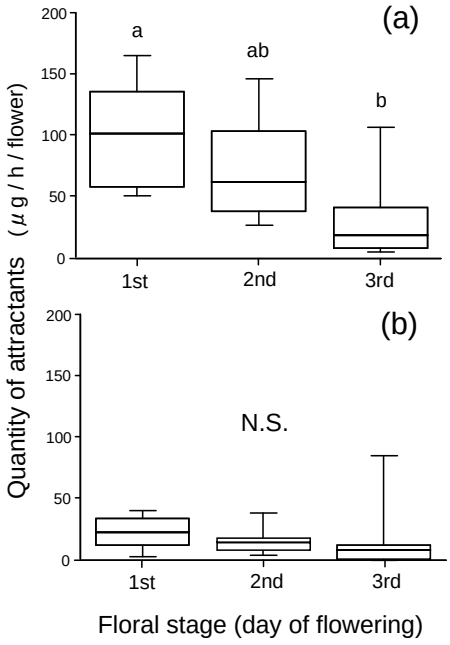

Fig. 2 Variations in the quantity of attractants among floral stages. Maximum and minimum values for each sample are shown at upper and lower ends of the vertical bars, respectively, and 75% and 25% points are given by the upper and lower ends of the box, respectively. The middle bar indicates median (each n = 10). Different letters indicate significant differences (Steel-Dwass test, $P < 0.05$). N.S. means no significant differences (Steel-Dwass test, $P > 0.05$). (a) BV (2-butanol + veratrole), (b) veratrole

attractants, we examined the variations in quantities of BV and veratrole, and these were consistent with the frequency of arrivals of both insects during heat generation (Table 2) and among floral stages (Fig. 2). These results demonstrated that *P. bimaculata* is attracted by inflorescences that produce more BV when the volatilization of BV increases due to heat production by the spadices, whereas *Dercetina* sp. is attracted to even relatively small amounts of veratrole, and can thus arrive at inflorescences both during and after heat generation. Thus, *P. bimaculata* appears to have adapted to the protogynous *Homalomena* inflorescence. However, the number of *Dercetina* sp. arrivals was approximately four times that of *P. bimaculata* (Table 1, positive control), thus *H. propinqua* may be capable of attracting both specialized and general anthophagous pollinators to promote both the female and the male reproductive success.

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