Journal of Tropical Forest Science 38(2): 194–206 (2026)
https://doi.org/10.26525/jtfs2026.38.2.194
ISSN: 0128-1283, eISSN: 2521-9847

AN UPDATE ON POLLINATION AND RELATIVE ATTRACTION OF WILD FRUIT FLY MALES TO DACINIPHILOUS *BULBOPHYLLUM CHEÍRI* SUBSPECIES *CHEIRI* (ORCHIDACEAE) FLOWERS

Tan KH[1, 2, *], Ong PT[3] & Wee SL[4]

[1]*Mobula Research Sdn. Bhd., 20, Jalan Tan Jit Seng, 11200 Tanjong Bungah, Penang, Malaysia*
[2]*Academy of Sciences, Malaysia, MATRADE Tower, Jalan Sultan Haji Ahmad Shah,*
50480 Kuala Lumpur, Malaysia
[3]*Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia*
[4]*Centre of Insect Systematics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia*

**tan.kenghong@yahoo.com*

Submitted July 2025; accepted October 2025

The fruit fly orchid, *Bulbophyllum cheiri* subspecies *cheiri* is found in coastal vegetation and lowland tropical rain forest in Southeast Asia. Its flowers attract males of the Oriental fruit fly which are strongly attracted to methyl eugenol (ME) that is found in >450 plant species. This study shows that wild Oriental fruit fly males were not attracted to the non-resupinate fruit fly orchid flower via color nor shape. Instead, male flies were attracted solely to the flowers, when covered by cotton or synthetic fabric, via floral volatiles. Natural pollinarium removal and pollinia deposition were observed; and were exclusively performed by the oriental fruit fly males. Comparison of fly attraction to a day-one or day-two (d-1 or d-2) flower versus a Steiner trap, baited with 6 g commercial ME set 20 m apart in the field showed a significant difference between d-1 and d-2 flowers with ca. 29 and 18% of total captured flies per day, respectively.

Keywords: Floral synomone, methyl eugenol, orchid pollination, oriental fruit fly

INTRODUCTION

Flowers produced by angiosperms have amazing arrays of color and odor to attract and enhance pollination by insects. Flowers normally attract insects via a combination of olfactory and visual cues (Kevan 1983). Colour preferences evolved differently between bees and flies (Shrestha et al. 2019) and have also been shown to differ between dipteran families, e.g., blue in Bombyliidae (Kastinger and Weber 2001), red in Calliphoridae and Platystomatidae (Chen et al. 2015), and yellow in Syrphidae (Lunau 2014; An et al. 2018; Klecka et al. 2018). However, the daciniphilous *Bulbophyllum* orchid species were observed to solely attract true fruit flies belonging to the Tribe Dacini (Diptera: Tephritidae: Dacinae) solely via floral fragrance/chemical volatiles (Tan and Nishida 2012, 2013; Tan et al. 2014; Wee & Ong 2023). Among the many daciniphilous orchids are several *Bulbophyllum* species that possess methyl eugenol (ME, a phenylpropanoid) as a major component in their floral synomones, particularly in the fruit fly orchid (FFO) *Bu. cheiri* Lindl. (Greek *cheír* = hand) (Tan et al. 2002; Nishida et al. 2004; Nishida 2014).

Bulbophyllum cheiri is found in coastal vegetation (in mangrove forests and on cliffs) and lowland rain forests of Borneo, Peninsular Malaysia, and the Philippines (Vermeulen et al. 2015). Currently, this species has two subspecies - *Bu. cheiri* subspecies *cheiri* and subspecies *subuliferum* (Schltr.) J.J.Verm., P.O'Byrne & A.L.Lamb (Vermeulen et al. 2015). Flowers of the former, which produce ME, specifically attract key pest species, e.g. the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (OFF) and *Ba. umbrosa* (Fabricius) (Tan 2009, 2020). Flowers of the subspecies *subuliferum,* however, specifically attract *Ba. pendleburyi* (Perkins) (a non-pest species) which is known to respond only to zingerone (a phenylbutanoid) (Doorenweerd et al. 2018; Ong - personal communication). These observations strengthen the perspective that color, morphological and floral synomone variations do occur within a species e.g. *Bu. macranthum* (Nakahira et al. 2018) as well as within the same subspecies e.g. *Bu. ecornutum* subspecies *verrucatum* 'Nabawan' and 'Apin-Apin' ecotypes (Tan et al. 2021a) of daciniphilous *Bulbophyllum*.

Sometimes, a mature FFO bud, prior to flowering the next morning, attracts wild OFF males (Fig. 1). The FFO has solitary and non-resupinate flowers (with tips of petals and sepals generally are 1.0-2.5 and ca 1 cm apart, respectively) (Fig. 2A) resembling a 'close hand' with finger tips slightly apart. This arrangement of the perianth normally hinders detailed observations of actual pollinarium removal and/ or pollinia deposition on to the stigma of a flower (Fig. 2B). Each FFO flower possesses brownish red petals and sepals with yellowish bases (Fig 2C). The flower possesses a relatively high quantity of 0.6 mg ME (Tan et al. 2002, 2021b; Nishida et al. 2004), and many wild OFF males are attracted to a freshly bloomed flower in the morning (Fig. 2D). Although the flowers are attractive to fruit fly males for a short period of two days, general observations showed that day-one (d-1) flowers appeared to attract more flies than day-two (d-2) flowers. Despite, the chemical ecology of the OFF interactions with FFO, and OFF bearing pollinia (removal based on a wrong assumption that a fly moved backward from a temporary floral entrapment) have been reported (Tan & Nishida 2013), the actual processes of pollinarium removal and pollinia deposition by wild OFF males are not fully observed and understood. The aims of this study are to show and confirm that wild OFF males are: 1) strongly attracted to the FFO flowers via floral synomone (floral color plays little or no role in the attraction), and their ability to open the floral lip and successfully removed pollinarium is size (weight)-dependent; 2) involved in both pollinarium removal and pollinia deposition. In addition, since i) individual flowers remain attractive generally for 1-2 days after anthesis; ii) daily numbers of attracted wild flies vary considerably (making spatiotemporal-comparison of actual number of flies attracted to a flower might not be meaningful (Tan et al. 2021b); and iii) previous observations showed less flies were attracted to d-2 flowers, we compared between d-1 and d-2 flowers for their relative attractiveness of wild OFF males in the presence of a Steiner trap baited with a commercial wafer containing 6 g of synthetic ME (as an experimental control for comparison).

Figure 1 Lateral view of a *Bulbophyllum cheiri* subspecies *cheiri* bud with two *B. dorsalis* males at ca. 17:00 h (a day prior to anthesis). (Scale bar = 1 cm)

Figure 2 *Bulbophyllum cheiri* subspecies *cheiri* day-one flowers. (Scale bar = 1 cm) A. A freshly bloomed flower – column and lip-base not visible (lip in default close-position). B. A freshly bloomed flower – with lip forced open by an attracted *B. dorsalis* male. C. A freshly bloomed flower with several attracted *B. dorsalis* males with one moving along the lip (before 08:00 h). D. Many *B. dorsalis* males almost covering a flower at ca. 11:00 h.

MATERIALS AND METHODS

Plants

Two pseudobulb-clumps of *Bu. cheiri* subspecies *cheiri* (FFO) were obtained in 1989 from the Tenom Orchid Center, Tenom, Sabah. They were grown, each on a slab of a fern stem ca 20 × 20 × 2.5 cm3, *ex situ* near to a secondary rain forest (5°45’87.56” N, 100°29’19.68” E) in Tanjung Bungah, Penang, Malaysia.

Attraction of male fly to buds

Previous observations showed that some large buds did attract male flies. A developing bud (> 2 cm in length) was checked daily between 17:00 -19:30 h) for fly settling prior to blooming the next morning. Fifteen buds were observed.

Weight of pollinia package

A pollinarium, consisting of four pollinia and a viscidium, was removed within two hours after anthesis from a flower (n=14) using a fine entomological pin, weighed previously, and weighed using a torsion balance (White Electrical Instrument Company Ltd, Great Malvern, Worcester, UK, Model No. p927115E with capacity = 50 mg and 1division = 0.1 mg).

Additionally, after a wild fly had just removed the pollinarium from a flower, it was captured before it flew away. A wild OFF male bearing freshly removed pollinarium was captured and weighed after cold immobilization. The pollinia package was then removed from each fly with a fine entomological pin – leaving behind the viscidium residue on the thoracic dorsum; and its weight determined. A total of eight replicates were conducted.

The mean weight of pollinia packages extracted artificially was compared with that removed by attracted wild OFF males (from each the package of pollinia was extracted leaving behind most of viscidium on the thoracic dorsum).

Observation of wild fruit fly attraction by enclosing a flower in a dark-color fabric or in a reflective plastic cage with a white screened window

A freshly bloomed flower was enclosed in an acetate-sheet frame covered with a dark-color cotton fabric or stocking material and setup just prior to dawn (07:00 h). Five replicates were performed. To further show that attracted flies probe and feed on floral fragrance, a flower was enclosed in a clear plastic cage with a window (2 cm diameter) covered with a white fine-cotton screen. Attracted wild flies were allowed to settle on the screen for an hour in the morning 08:30 – 09:30 h. Saliva marks, if any, were observed 1- 4 h post fly exposure. Five replicates were conducted.

Weight of wild fruit flies attracted to flower and synthetic methyl eugenol (ME) sources

Preliminary observations showed that fly visitors varied in sizes. Therefore, random samples of flies were captured from an individual flower and a Clear trap (Tan et al. 2021b) baited with 1 ml ME; and were identified and weighed individually, after cold immobilization. Individual and range of fly weight were determined for comparison between flies captured from a flower and the Clear trap.

Pollinia removal

Due to difficulty in viewing the anther and base of a closed lip (Fig. 2), Tan et al. (2002) first reported pollinia removal by a male fruit fly attracted to a flower of *Bu. cheiri* based on a fly bearing pollinia. It was wrongly assumed that the fly 'presumably' moved backwards, after being tipped into the column cavity. For the specialized hinged see-saw lip (labellum) mechanism to work effectively before the fly can be tipped into the column cavity, the weight of a male fly must be sufficiently heavy to force the closed lip to an open position and eventually trapped temporarily between the lip and column cavity of the flower. To confirm the actual process of pollinarium removal, observations were conducted on eight flowers; and whenever possible to view, not blocked by tips of perianth, with video recordings.

Comparison of fly visitors to day-one (d-1) and day-two (d-2) flowers in the presence of a commercial source of methyl eugenol (6 g)

Flowers generally were attractive to fruit flies for two days and rarely attractive in subsequent days. Previous investigations showed that the daily numbers of flies trapped in traps and captured from flowers varied considerably (Tan et al. 2021b). Therefore, to overcome this uncertainty, fly visitors to d-1 and d-2 flowers were compared using a control trap, a standard Steiner-trap baited with a wafer containing 6 g of ME and a DDVP strip, placed 20 m apart at identical sites as described by Tan and coworkers (2021b). After each floral replication, the positions of flower and trap were interchanged. A flower replication was abandoned when half-hourly capture of flies was interrupted due to rain in the morning - especially, during the peak of fly attraction (between 08:00 and10:00 h). A total of 10 replicates were conducted to capture fly visitors on d-1 and d-2 flowers in the presence of the Steiner-trap.

Statistical analysis

Data obtained from i) weight of flies (between flower and lure visitors) and ii) flies attracted to d-1 and d-2 were tested for normal distribution using Shapiro-Wilk's Normality test, at $p = 0.05$ and were subjected to Student's *t*-test if distributed normally, otherwise by Mann-Whitney Rank Sum Test ($p = 0.05$).

RESULTS

Fly attraction

Figure 1 shows the lateral view of a mature bud, without an obvious split or gap between petals and sepals, and yet flies were attracted at dusk, and settled for the night on the mature bud prior to blooming the next morning. Four of fifteen matured buds attracted OFF males at dusk prior to anthesis the next morning. Anthesis normally started between 05:00-06:00 h. Initially, 1-3 males were attracted between 07:30-07:45 h (dawn breaks at 07:00 h), and one of them would open the lip and probe as well as move toward the column (Fig. 2A). As the morning progressed, more male flies were attracted to a point of almost covering the whole flower (Fig. 2D; 2-3 h after blooming).

Figure 3A and 3B show attracted OFF males settling on dark thick and stocking fabrics, respectively, enclosing a FFO flower. The OFF flies settled on the fabric for more than 2 hours, if undisturbed, and they appeared to be probing on the fabric. When a white fabric was used as a window screen, attracted flies were actually feeding on floral volatiles, absorbed and/or adsorbed on the fabric screen, and leaving saliva marks (Fig 4).

Weight of wild male fruit flies

The mean (+ s. e.) weight of an OFF males (floral visitors - 12.13 ± 0.16 mg) and that captured in the ME-baited Clear trap (12.39 ± 0.23 mg) showed no significant difference between the two groups of trapped flies (Mann-Whitney Rank Sum Test; $p > 0.05$) (Table 1). Figure 5 shows three specimens of OFF males, simultaneously captured in a clear plastic bag from a FFO flower, with distinct differences in size and weight. Some of the smaller sized flies were unable to force open the hinged see-saw lip when in a closed position (normal after anthesis).

Weight of pollinia package removed artificially and from fly-pollinator The mean (± s. e.) weight of a pollinia package artificially removed as pollinarium from a FFO flower was 2.46 ± 0.09 mg (n = 14), compared to 2.11 ± 0.01 mg (n = 8) from those removed by OFF males [t 1, 21) = 1.22 with p-value 0.118. The slight lost in weight, due to remnant of viscidium left on the OFF males, was not sufficient to result in a significant difference in the weight of the pollinia. The mean weight of the OFF males, that bore remnants of the viscidium after the pollinia were removed, was 13.84 ± 0.62 mg (range 11.6-16.5 mg; n = 8). The mean weight of flies that successfully removed pollinarium was significantly heavier than the mean weight (12.13 ± 0.16 mg) of general floral visitors ($p < 0.05$).

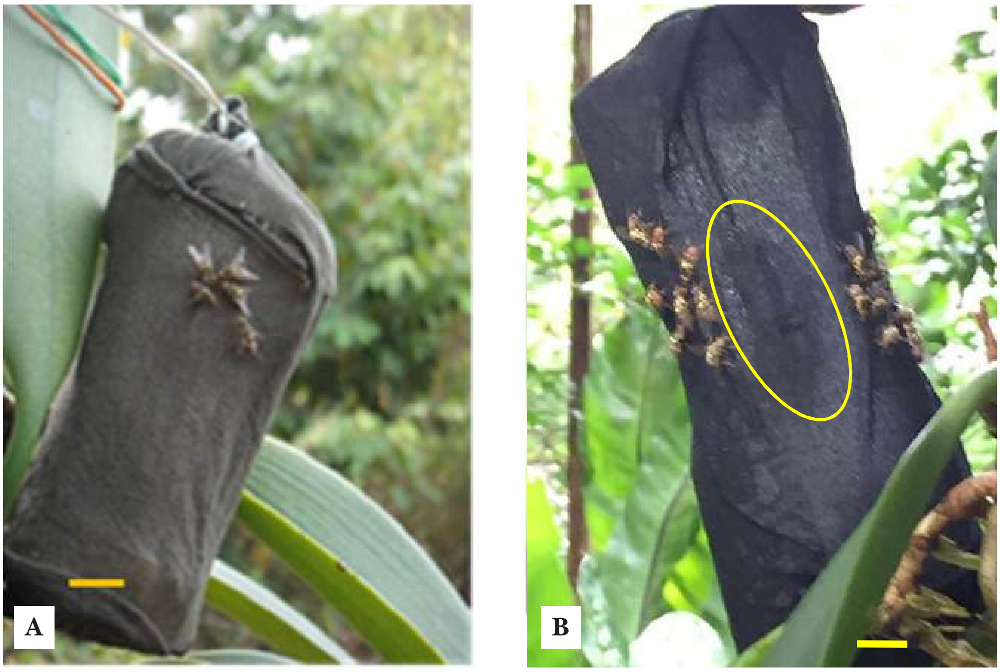


Figure 3 *Bactrocera dorsalis* males attracted to and settled (probing) on black-color fabric covering a *Bulbophyllum cheiri* subspecies *cheiri* flower. (Scale bar = 1 cm) A. Flies on a relatively thick cotton fabric. B. Flies on a fine and thin stocking fabric – silhouette of flower within yellow oval line.

Table 1 Mean (± s. e.) weight and weight range of individual wild *Bactrocera dorsalis* male captured from *Bulbophyllum cheiri* subspecies *cheiri* flowers and in a Clear trap baited with 1 mL methyl eugenol (20 m apart).

	Attracted to flowers	Captured in Clear trap
Mean fly weight (mg)	12.13	12.39
Standard error (±)	(0.16)	(0.23)
Number (n)	319	138
Range of fly weight (mg)	4.9 – 20.3	4.8 – 19.2

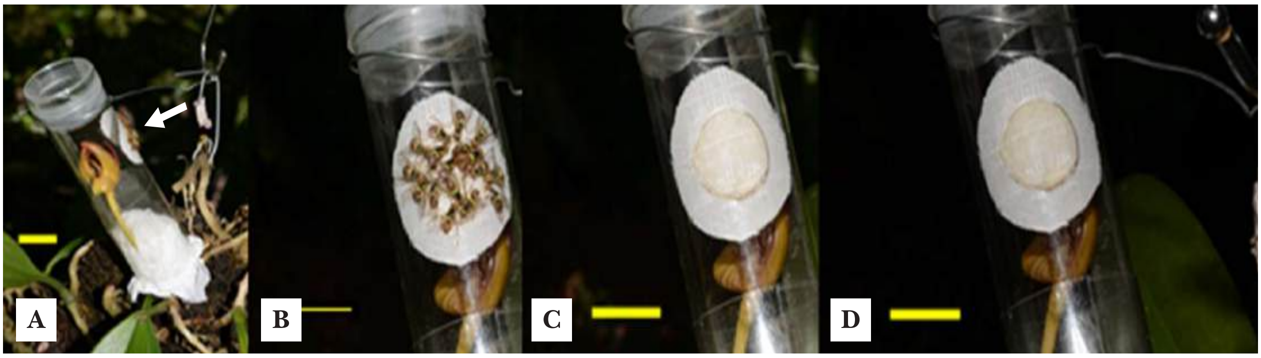


Figure 4 *Bactrocera dorsalis* males attracted to and feeding on window-screen (white cotton cloth) of clear plastic cage enclosing a *Bulbophyllum cheiri* subspecies *cheiri* flower, and leaving saliva marks. (Scale bar = 2 cm) A. A flower in a plastic cage with flies on window screen (white arrow). B. *Bactrocera dorsalis* males on window-screen of cage (ca 1 h exposure). C. One hour-post fly exposure - window-screen turned yellow due to saliva marks. D. Four hour-post fly exposure - color of window-screen turned faint brownish.

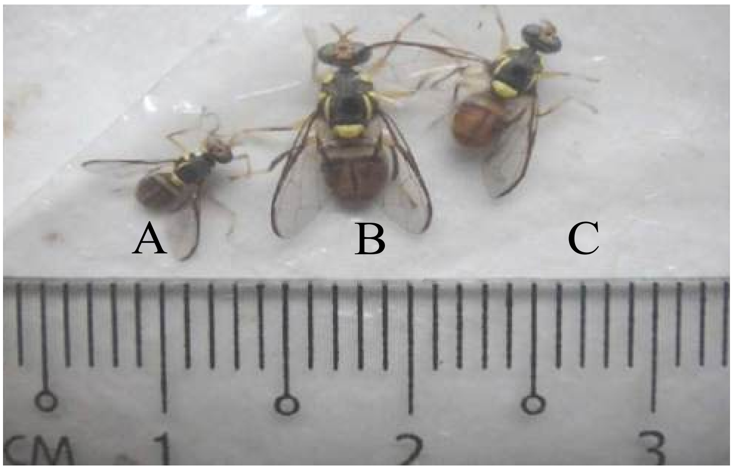
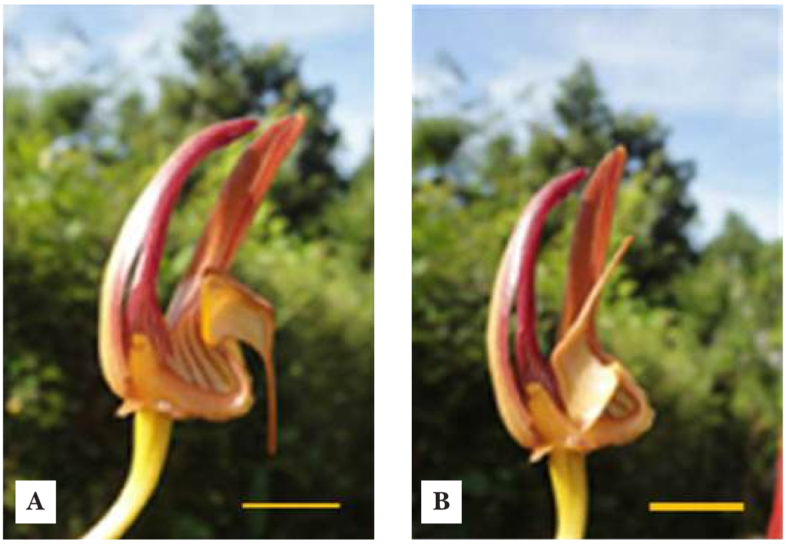


Figure 5 Three different-sized *Bactrocera dorsalis* males simultaneously captured (in a clear plastic bag) while probing on a *Bulbophyllum cheiri* subspecies *cheiri* flower. (Inset ruler scale bar - 1 division = 1 mm) Fly wet weight (mg) - A = 5.4, B = 19.3,and C = 12.1.

Pollinia removal

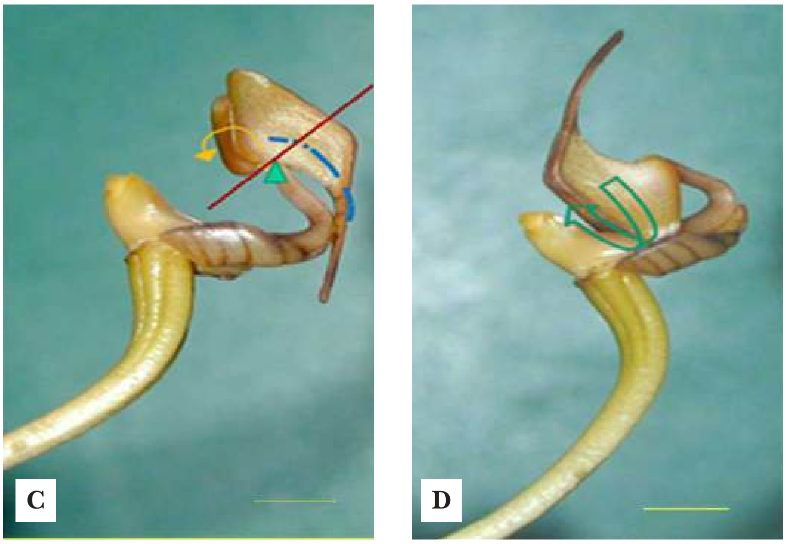
After anthesis (usually between 07:30 and 07:45 h), an attracted male fly (one of the first few visitors) would eventually move on to the hinged see-saw lip, which was forced from the close (Fig. 6A) to the open position (Fig. 6B). An attracted fly would either move to other parts of the flower or continue feeding on the lip adaxial surface and move towards the base of the lip. In the latter situation, when the fly moved pass the lip hinge (or fulcrum of the see-saw lip – Fig. 6C), it would suddenly be tipped in to the floral column cavity by the lip closing instantaneously due to an imbalance resulting from a shift of the fly's weight (Fig. 6D). The fly was trapped in



A - see-saw lip in open position.

B – see-saw lip in close position (default during anthesis).

Bottom row - floral column and see-saw lip -



C – Open lip (green triangle – lip hinge; green dash line – likely path of a probing fruit fly; red line - extrapolated plane of lip at hinge; pink arrow – path of lip closing caused by an imbalance (resulting from a fly's center of gravity passing the hinge).

D – Close lip (green arrow - U-turn path of a trapped fly within a temporary floral entrapment (formed between column and U-shaped end of lip (when viewed abaxially) to emerge always head first (at the pointed tip of green arrow; also see Fig. 8C) with thoracic dorsum facing the anther.

Figure 6 Side view of a *Bulbophyllum cheiri* subspecies *cheiri* flower. (Scale bar = 1 cm) Top row - Left petal and left lateral sepal removed.

an enclosed space formed between the closed lip and the floral column. It then presumably made a U-turn in the entrapment (Fig. 6D) and eventually emerged, always headfirst, through a small U-shaped gap (ca. 2 mm wide), temporarily formed between the longitudinal groove of hinged see-saw lip and the anther. To illustrate further, we describe the actual pollinarium removal by an attracted OFF male from a flower (with tips of perianth further apart than normal) that allowed clear visibility of the anther and lip movements for detail observations. Initially, a male Artocarpus fruit fly, *Ba. umbrosa* Fabricius opened the close lip and followed by an OFF male climbing on to the base of the open lip. The OFF male was probing on the lip next to the column (Fig 7A) when the *Ba. umbrosa* male dismounted from the lip to continue probing at the base of the flower (Fig. 7B), and the see-saw lip instantaneously closed and tipped the OFF male in to the column cavity due to a weight imbalance. As the temporarily trapped OFF male emerged headfirst out of the entrapment (Fig. 7C), its thoracic dorsum would touch the sticky viscidium, which was not visible (Fig 7D) and the package of four pollinia was partially loosened (Fig. 7E) and, after much struggling, subsequently detached from the anther (Fig. 7E). The pollinia package was invariably attached to the thoracic dorsum of the fly that eventually crawled out from the temporary floral entrapment on to the lateral sepal (Fig. 7G) and then moved to the base of the flower (Fig. 7H) before flying away.

A - A *Ba. dorsalis* male (green arrow) at the U-shaped end (viewed adaxially), just before being tipped, and a *Ba. umbrosa* male at the straight end (bottom) of the see-saw lip.

B - The *Ba. dorsalis* male (not visible) had been tipped into a temporary floral-trap after the *Ba. umbrosa* male dismounted from the straight end of lip causing the see-saw lip to close suddenly.

C - After making a U-turn within the floral-trap, the male's head (arrow) emerged first with thoracic dorsum facing the anther.

D - Male momentarily held back when it touched the viscidium of pollinarium.

E - After struggling for ca a minute, the pollinia (red arrow) were dislodged from the anther.

F - Male crawling out of floral-trap with pollinia (red arrow) attached to its thoracic dorsum.

G - Male bearing the freshly removed pollinia (red arrow) out of the floral-trap.

H - Male (with the pollinia [red arrow] firmly stuck to thoracic dorsum) ready to take off from the flower.

Figure 7 Removal of *Bulbophyllum cheiri* subsp. *cheiri* pollinarium by a *Bactrocera dorsalis* male. (Scale bar = 1 cm)

Pollinia deposition

After pollinia removal, a fly pollinator bearing a package of pollinia would fly off, and sometimes, would subsequently be captured in a ME-baited trap (Fig. 8), especially when there were no suitable flowers available. However, in a rare event to complete floral pollination, a fly was observed to have deposited the pollinia and struggling to free itself (Fig. 9).

Figure 8 A wild Bactrocera dorsalis male, bearing a set of pollinia (green arrow) on its thoracic dorsum, in a plastic bag of Clear-trap baited with methyl eugenol. (Scale bar = 1 cm)

Figure 9 Deposition of pollinia - Bactrocera dorsalis male (arrow shows abdomen) struggling to free itself (while pollinia still stuck between the fly's thoracic dorsum and sigma) by attempting to move backwards. (Scale bar = 1 cm)

Comparing number and percentage (captured from a flower/total captured from either day-one (d-1) or day-two (d-2) flowers and ME-baited Clear trap) of flies

Of fourteen flowers investigated, four of them did not attract flies on d-2, while day-3 flowers did not attract any flies. Table 2 shows that there was no difference in flies captured in the traps; but the numbers of fly visitors to d-1 and d-2 flowers were significantly less than that captured in the 6 g ME baited trap, respectively. Furthermore, there was significant difference (p=<0.05) between mean (± s. e.) number of flies captured per day from d-1 (55.4 ± 11.8) and d-2 (22.6 ± 7.6) flowers; and similarly for percentage (relative proportion) of flies captured per day between d-1 (29.4%) and d-2 (17.7%) flowers (Table 3).

Table 2 Mean (± s. e.) number/day of wild *Bactrocera* dorsalis males captured from day-one and day-two *Bulbophyllum* cheiri subspecies *cheiri* flowers in the presence of a methyl eugenol (6 g) baited Steiner trap (20 m apart) (n = 10).

	Mean	(± s. e.)	t-test *t* 1,18) =	*p*-value
Total flies captured/day	187.4	30.66		
day-one flower	55.4	11.80		
			3.108	0.0061
Trap baited with ME	132.8	21.93		
Total flies captured/day	158.1	34.79		
day-two flower	22.6	8.62		
			3.100	0.0062
Trap baited with ME	135	35.78		

Table 3 Comparing numbers and percentages (flies attracted to flower/total captured per day) of *Bactrocera dorsalis* males captured between day-one and day-two *Bulbophyllum cheiri* subspecies *cheiri* flowers (n = 10) in the presence of a methyl eugenol (ME - 6 g) baited trap (20 m apart).

Captured/day	Flowers	Total	Mean	+ s. e.	Statistical tests
Number of flies	Day-one	554	55.4	11.80	*t*-test: $t_{1,18} = 2.49$, $p = 0.01$
- " -	Day-two	226	22.6	7.62	
Percentages of total flies	Day-one	294.1	29.4	3.47	Mann-Whitney test: *Z-score = 2.04,* $p = < 0.05$
- " -	Day-two	176.9	17.7	3.67	

DISCUSSION

This study further corroborates with the earlier observation that males of OFF are attracted to a FFO flower neither because of structure/form nor color (Tan *et al.* 2002). The attraction that is unrelated to color was also corroborated by a study in Hawaii that showed clear traps caught significantly more OFF flies than yellow, green, blue and red traps (Howarth and Howarth 2000). Further, the FFO being an epiphyte bearing solitary flowers in their natural costal mangrove forest and lowland rainforest/jungle habitats is normally hidden/blocked from the fly's visual perception by thick tree canopies. Therefore, floral volatiles from a solitary FFO flower, which contains a large content of ME and other ME derivatives, can only be detected and accurately located by the OFF males via their specifically attuned olfactory perception to these floral compounds.

Although ME is a major component of the FFO flowers, additional six ME-analogs, namely eugenol, (*Z*)-methyl isoeugenol, (*E*)-methyl isoeugenol, 2-ally-4,5-dimethoxyphenol (DMP), 5-allyl-1,2,4-trimethoxybenzene (euasarone), and (*E*)-3,4-dimethoxycinnamyl acetate, have been detected as minor compounds (Nishida *et al.* 2004). For OFF males, DMP has been shown to be as strong an attractant as ME (Nishida *et al.* 1988). A single orchid flower could attract many flies in the presence of flanking ME traps (ca. 14% of total trapped flies was from a d-1 flower vs two Steiner traps, each baited with a 6-g ME wafer) (Tan et al. 2021b). The proportion of flies captured at each d-1 flower in this study is double (29%) that obtained in the previous study by Tan et al. (2021b). The possible reasons to explain this unique phenomenon, though speculative, is the *in situ* biosynthesis and emission of floral volatiles occur very rapidly during and after anthesis during the day time. In addition, since an average ME quantity per flower is less than 1 mg, mentioned above, and together with a cocktail of other floral volatiles, the single flower may have its own entrapment tactic to lure some OFF males away from the relatively large quantities (ca 6000/0.6 = 10,000 x) of the highly potent synthetic ME source. In suggesting the former, the rhythmicity of floral scent emission has been shown to occur in many plant species and is often correlated with the activity of the respective pollinators (Matile and Altenburger 1988; Raguso 2004; Muhlemann *et al.* 2014). Thus, this also demonstrates how plants can mediate insect pollinator-plant interactions via the production of floral odor according to its biosynthetic activity (Beyaert and Hilker 2014). Hence, to account for this unique floral attraction phenomenon, it may be due to a) the right blend of floral volatiles in appropriate ratios or b) the rate of release by the FFO flower or c) a combination of both the factors. Furthermore, the right blend of the floral volatiles may have to include DMP which together with trans-coniferyl alcohol are the two major male sex pheromonal components of OFF males (Tan and Nishida 1996, 1998; Tan *et al.* 2011, 2013, 2014) responsible for attracting conspecific females – particularly the latter component (Tan and Nishida 1996, 1998; Hee and Tan 1998; Khoo *et al.* 2000). These require further laborious investigations to find the right blend of chemicals, and also the relative ratio of release of each of the components. Eventually, such investigations may lead to a possible attractant that will be more effective than the already very potent ME for ME-responsive *Bactrocera* species, especially OFF. Nonetheless, we envisage that due to probable difficulties encountered, i.e., similar to the prospective use of pheromone of *Bactrocera* species in traps for surveillance, control, management or eradication of OFF (Tan et al. 2014; Vargas et al. 2014), further investigations may be stalled due to a lack of long-term stability of chemical blends (except for ME) and different rates of release of individual components. This conundrum may be further compounded by insufficient interest and research funding.

In the presence of a ME (6 g) baited Steiner trap set 20 m apart from d-1 or d-2 flowers attracted significantly higher relative numbers (%) of OFF males than that previously captured from d-1 flowers – when each was placed between two flanking Steiner traps baited with the same commercial dispenser (20 m apart as mentioned above) (Tan et al. 2021b). Based on the highest trap density (20 traps/km^2) recommended by FAO/IAEA (2018), the distance between two adjacent traps is ca. 225 m. This is more than 10 folds the distance of 20 m used in this study. Therefore, on a one to one basis, an individual FFO flower may attract for capturing as many OFF males in comparison with the Steiner trap baited with the 6 g ME bait when the distance between them is more than 100 m. Would a distance of >100 m from the trap when the commercial ME source has little or no effect on floral attraction of the OFF males? To answer this intriguing question, further field research is warranted.

Based on our observation, certain mature buds of FFO attracted OFF males prior to or during dusk and the flies settled on them for the night prior to anthesis the next morning. On some occasions, the mature buds did show initial sign of petals and sepals separating slightly from each other, later in the night during darkness. This suggests that the flower buds had already synthesized the attractive floral volatiles, and minute quantities might have diffused out of the buds, and thus, attracting the very highly responsive OFF males (ED_{50} = ca 300 ng ME) (Wee et al. 2002).

Field observations showed that some small size OFF males were unable to open the close lip after anthesis. This phenomenon is like the dynamic lip mechanism of *Bu. hortorum* non-resupinate flowers where the relatively much smaller close-spring lip and indirectly filtered out light-weight individual flies and thus allowing the appropriately sized individuals (> 11 mg) to be potential pollinators (Tan et al. 2023). Selected flies of the optimal size are not only enabling successful removal of a pollinarium from a flower; but also, to ensure the pollinarium (equivalent to 10-20 % of fly's weight) loaded on the fly's thoracic dorsum would not interfere its flight activity. Although in this current study, there is a large variation in the size/weight of wild OFF males. However, the average weight of the pollinarium might be 25-50% of a small fly weighing less than 11 mg. Therefore, a relatively small fly might not be able to fly long distances if it was able to remove and bear a pollinarium, and thus not a good/potential pollinator of FFO flowers. This aspect was not the objective of the current study and should be investigated further.

CONCLUSIONS

This study further shows and confirms that a true mutualistic interaction exists between the fruit fly orchid, *Bu. cheiri* subspecies *cheiri* and the Oriental fruit fly, *B. dorsalis* whereby the male flies are the exclusive vectors for the orchid's pollination; and visual cues, i.e., floral color and form play no role in attracting *B. dorsalis* for pollination of the fruit fly orchid flowers. Day-1 flowers attract more wild male flies than day-2 flowers in the presence of 6g methyl eugenol-baited traps at a distance of 20 m.

ACKNOWLEDGEMENTS

The assistance and moral support provided by L.C. Sim are gratefully appreciated. We are grateful to the late Anthony Lamb of the Tenom Orchid Center, Tenom, Sabah, for providing the initial plant cuttings of *Bu. cheiri* subspecies *cheiri* in 1989.

Funding: The authors declare that no funds or other financial support were received for the research performed.

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