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Feeding Behaviour of *Cynopterus sphinx* (Pteropodidae) Under Captive Conditions

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Abstrak: Kajian terhadap kebolehan deria bau dan visual kelawar megachiroptera, *Cynopterus sphinx* telah dijalankan dengan mendiskriminasi bau dan bentuk pisang, *Musa sp.* Eksperimen telah dijalankan di dalam kurungan dengan memberikan beberapa pilihan makanan iaitu pisang masak, pisang yang dikisar dan pisang tiruan. Kelakuan kelawar-kelawar itu diperhatikan secara visual dan peratusan aktiviti dan rehat, tempoh percubaan pertama mencari makanan, bilangan percubaan untuk makan dan juga purata tempoh percubaan yang berjaya telah direkodkan bagi setiap kelawar. Kelawar-kelawar tersebut mempamerkan peningkatan pada bilangan kunjungan ke atas pisang masak dan juga pisang yang dikisar. Walau bagaimanapun, pisang tiruan tidak mengakibatkan sebarang tindakbalas. Kajian ini mencadangkan isyarat bau adalah lebih penting daripada isyarat visual untuk penentuan lokasi buah bagi *C. sphinx*.

Kata kunci: Kelawar Pemakan Buah, *Cynopterus sphinx*, Deria Bau, Penglihatan, Dalam Kurungan

Abstract: We examined the olfactory and visual abilities of megachiropteran bats, *Cynopterus sphinx,* for discrimination of the odour and shape of the banana fruit, *Musa sp.* We conducted the experiments in captive conditions by offering a selection of ripe bananas, blended bananas and artificial bananas. The behaviour of the bats was observed visually, and the percentage of activity and rest, duration of the first foraging bout, number of feeding attempts and the average duration of successful attempts was recorded for each bat. The bats exhibited an increased number of visits to ripe bananas and blended banana fruits. However, the artificial fruit did not evoke any response. Our study suggests that odour cues are more important than visual cues for the location of fruits by *C. sphinx*.

Keywords: Fruit-eating Bat, *Cynopterus sphinx*, Olfaction, Vision, Captive Condition

INTRODUCTION

Bats mainly depend on three important cues in the search for food: vision, olfaction and echolocation. Insect-eating bats, also known as microchiroptera, mostly depend on echolocation cues when searching for food (Swift & Racey 2002). With the exception of *Eonycteris* spp. and *Rousettus* spp., olfaction and vision are more important for fruit bats or megachiropterans during foraging. The Greater short-nosed fruit bat, *Cynopterus sphinx,* can learn to differentiate between olfactory cues and certain non-olfactory factors (Acharya *et al*. 1998).

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They also have the ability to make use of visual cues when searching for food in light or even in total darkness (Raghuram *et al*. 2009). Occasional observations have been made on the sensory cues that are involved in the feeding habits of a few species of megachiropteran, either in natural or captive conditions (Elangovan *et al*. 2000, 2006; Korine & Kalko, 2005; Raghuram *et al*. 2009). Few studies have addressed the importance of olfactory cues in fruit-eating bats in captive conditions (Acharya *et al*. 1998; Luft *et al.* 2003; Elangovan *et al*. 2006). Meanwhile, in more detailed studies, the use of vision, olfaction and echolocation in fruit-eating bats in captive conditions have been compared (Kalko & Condon 1998; Korine & Kalko 2005). Under natural conditions, the peak number of feeding visits of fruit bats has been found to occur at different times during the night depending on the type of fruit used (Elangovan *et al.* 2000). However, under captive conditions, the foraging behaviour of fruit bats was found to be different compared to natural conditions. Their foraging behaviour is composed of three distinct stages: a search or orienting flight, followed by approach behaviour and the final acquisition of ripe fruits (Korine & Kalko 2005).

The Greater short-nosed fruit bat, *C. sphinx* (Vahl) is a common frugivorous species in Southeast Asia. It feeds on a variety of wild and cultivated fruits (Bates & Harrison 1997). *C. sphinx* feeds on nectar from the flowers of *Musa paradisiaca* and *Bassia latifolia* (Elangovan *et al.* 2000). In our study, we aimed to determine the feeding habits of *C. sphinx* under captive conditions. The objective of this study was to determine the roles of olfaction and vision in the feeding habits of *C. sphinx* and also the response of *C. sphinx* to different experimental conditions in which we manipulated the odour, shape and size of the banana fruit, *Musa sp.*

MATERIALS AND METHODS

Study Site and Methodology

The study was conducted on Pulau Pinang from October 2012 to March 2013. Depending on space, we set three to four mist nets at eight locations around Pulau Pinang. Mist nets were opened between 18:30 hr and 23:00 hr and checked every hour for captured bats. Five adult *C. sphinx* were trapped and their forearms, ears, body and tail (if present) were measured to the nearest millimetre (mm), and their weight in grams (g) was recorded. Physical data, sex and reproductive conditions were also noted, and species identification was based on Francis (2008).

The bats were immediately captured and maintained in captivity in a separate facility that measured 3.1 x 2.4 x 2.5 m in height at the School of Biological Sciences, Universiti Sains Malaysia (USM), Pulau Pinang (5°21’N, 100°18’E) for behavioural experiments. Bats were exposed to two separate enclosures; one was designated as the test enclosure, while the other was considered a captive enclosure, where bats were maintained when they were not used for experiments. Only one individual was used during each observation period, while the others were kept in another enclosure. The temperature of both enclosures was maintained at 30±3°C. The captive enclosure was illuminated using a 15-W Philips light bulb. The light was on during the light period (06:00–18:00 h), and the light was turned off during the dark period (18:00–06:00 h). The bats were left undisturbed with an abundant supply of food, including ripe bananas, papayas and guavas, for at least one week to familiarise the bat with captivity conditions. All fruits offered were known to be consumed by *C. sphinx* in the wild. Food and water were placed in the cage every day. The cage was cleaned at 08:00 hr every morning, and all bats were released at the capture site after the experiment was completed. All techniques and regulations involving bats used in this research were in accordance with the general guidelines for maintenance of wild-caught mammals in captivity by Gannon and Sikes (2007), which are also advocated by USM policy.

Experimental Set Up

The experiment was performed with individual bats in a test enclosure with similar dimensions to the captive enclosure in which all bats were maintained. During the experiment, only one individual bat was brought into the test enclosure 30 min before the experiment began, and the other bats were maintained in a separate enclosure. The first set of experiments was conducted to investigate whether bats respond to olfactory cues when finding and locating food. The role of olfaction was tested by providing only the odour to the bat without any other cues. Ripe bananas were blended and offered by using cotton saturated with ripe banana juice. We learned to distinguish ripe and unripe bananas by estimating their hardness and colour (Luft *et al.* 2003). The cotton was placed in the test enclosure, and the response was evaluated. If bats responded to odour alone, they would approach the blended fruit.

The second experiment was conducted to test whether bats would detect and attempt to approach an object shaped like a banana fruit without any olfactory cues. The artificial banana fruit made of wax was hung in the test enclosure, and the response was evaluated. If bats responded to visual cues alone, they would approach the artificial banana fruit.

In the third experimental set up, the whole ripe banana was offered to the bats as a control. A ripe banana was hung in the test enclosure, and the response was evaluated. If bats responded to both olfactory and visual cues, they would approach ripe banana.

The individual bats were observed for six hr (from 18:00 hr until 00:00 hr) in each experiment after presenting the variables. The activities of the bats were observed under dim red illumination to minimise visual cues. The experimental setup was randomly changed between each set of experiments during each night, and the location of the variable used in the test enclosure was randomly changed to minimise possible learning effects. We recorded the following data during the observations: (a) percentage of activity and rest, (b) duration of the first foraging bout, (c) number of feeding attempts and (d) average duration of each successful attempt during each hour from 18:00 hr until 00:00 hr.

Statistical Analysis

All data were tested for normality using SPSS 20.0 Software. One-way ANOVA was used followed by Tukey's post hoc multiple comparison tests for normally distributed data. Data that was not normally distributed was analysed with the non-parametric Kruskal-Wallis test followed by Mann-Whitney U tests to compare the data among the three experimental variables (Zar 1999).

RESULTS

We observed several distinct behaviours of bats in the test enclosure: orientation flights, resting periods, exploration flights (approach phase) and also final approaches. When released in the test enclosure where the experiment was conducted, the bats typically flew for several minutes (2–15 min) along the walls of the test enclosure without aiming at a specific target. This behaviour was termed the orientation flight. The orientation flight was followed by a resting period where the bats hung from the roof of the test enclosure. While hanging, the bats scanned their surroundings for up to 5 min by continuously moving their head and ears for several minutes.

After a series of orientation flights and resting periods, *C. sphinx* began exploration flights oriented towards the experimental variable. Typically, a bat would circle several times around the variable and direct its approach closer and closer to the variable. This phase was termed the approach phase. The exploration flights, where bats approached the fruit multiple times, ended in a final approach when the bat hovered near the fruit and finally landed on it. They usually licked the fruit, swallowed it, ingested the juice and spat out the remnants. On a few occasions, they also bit off pieces of fruit and continued consuming the fruit at their roosts in the experimental room. This usually occurred when the whole ripe banana was offered to them. However, when cotton saturated with ripe banana juice was presented, the same behaviour was detected, and they usually sucked the cotton for several minutes (1–29 min). However, the artificial banana fruit did not evoke any response.

None of the data was normally distributed. We attempted to transform the data using a log transformation but failed to achieve normality, except for data regarding the average duration of successful attempts. The percentage activity of *C. sphinx* was highest when blended fruit was offered (Table 1). However, the percentage activity of bats when fresh fruit (control), blended fruit and artificial fruit (Kruskal-Wallis test, $\chi^2 = 3.863$, $df = 2$, $p>0.05$) was offered was not significantly different. There was also no significant difference in the number of attempts ($F2, 27 = 1.603$, $p>0.05$) between the variables, but there was a significant difference for the average duration of successful attempts (Kruskal-Wallis test, $\chi^2 = 10.148$, $df = 2$, $p<0.05$) between the variables. The average duration of successful attempts for the artificial fruit differed significantly when compared to fresh fruit (Mann-Whitney U test, $Z = -2.796$, $p<0.05$) and blended fruit (Mann-Whitney U test, $Z = -3.104$, $p<0.05$). There was no significant difference in the average duration of successful attempts between fresh fruit and blended fruit (Mann-Whitney U test, $Z = -0.348$, $p>0.05$).

Table 1: Behavioural responses of *C. sphinx* to fresh fruit, blended fruit and artificial fruit in captive conditions.

Experiments	Activity (%)	Rest (%)	No. of attempts	Duration of the first attempt (min)	*Average duration of successful attempts (min)
Fresh fruit (control)	9.08	90.92	7.79±1.05	10.25±5.36	3.97±7.17
Blended fruit	9.44	90.56	7.09±2.66	7.78±10.75	4.43±7.24
Artificial fruit	2.92	97.08	7.79±0.97	7.25±8.30	0.00

Notes: Data are shown as mean±SD (n=5); *$p<0.05$.

DISCUSSION

Our results indicate that *C. sphinx* is able to detect and locate fruits by using olfactory cues. This is shown by the high percentage of activity observed in bats when the blended and ripe bananas were offered. We acknowledge that our experimental area was enclosed, thus it remains unclear whether fruit odours can also be attractive from longer distances. Elangovan *et al*. (2006) stated that *Cynopterus spp.* was able to discriminate different odours of substances in a complex olfactory environment. The main olfactory bulb, which is important for localisation of food in Pteropodidae, is large in size and facilitates navigation in large and more complex habitats such as forests (Safi & Dechmann 2005).

Reliance on odour as a primary cue for detecting fruits has also been reported for other pteropodid bats such as *Pteropus pumilus*, *Pteropus jagori* (Luft *et al*. 2003) and *Cynopterus brachyotis* (Hodgkison *et al*. 2007). The current knowledge of fruit bats, which normally rely on a variety of plants from different families, suggests a highly flexible use of olfactory cues while foraging. This suggests that the response of fruit bats may vary mainly based on the olfactory stimuli of species of fruits and flowers. *C. perspicillata* for example, depends on olfactory cues to detect the essential oils of the Piper species (Mikich *et al*. 2003).

However, it remains unclear which active component produces the odours that can attract fruit bats. Although only non-natural odours were tested, fruit bats can still adapt their feeding behaviour to new odours in a short time (Acharya *et al*. 1998). Hodgkison *et al.* (2007) tested a total of 16 main compounds in the ripe fruit odour of *Ficus hispida* and 13 compounds in the ripe fruit odour of *Ficus scortechinii*. They found that *C. brachyotis* responded to both natural and synthetic fruit odours, with higher reaction rates towards natural compared to synthetic fruit odours. The relatively larger number of responses of *C. sphinx* to ethyl acetate followed by isoamyl acetate, benzaldehyde, limonene and pinene indicate that these chemicals may be the predominant components in the fruit species that are usually visited by bats (Elangovan *et al*. 2006). However, *C. sphinx* exhibited the least number of responses to dimethyl disulfide (von Helversen *et al*. 2000). This result suggests that the response of bats may vary based on the olfactory stimuli of the species of fruits and flowers.

CONCLUSION

Our study showed that *C. sphinx* mainly uses olfactory compared to visual cues to locate fruits. Further studies need to be conducted to determine the ability of this species to detect and localise the odour cues of other fruit types.

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