Olfaction in the fruit-eating bats *Artibeus lituratus* and *Carollia perspicillata*: an experimental analysis

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**ABSTRACT**

Studies suggest that frugivorous bats search and select fruit mainly by olfaction so that they can be attracted by smell alone. The aim of this study was to evaluate, in captivity, the behavioural response (number of foraging attempts) of *Artibeus lituratus* and *Carollia perspicillata* offered essential oils extracted from ripe fruit of *Ficus insipida* (Moraceae) and *Piper hispidum* (Piperaceae) as well as intact fruit wrapped in gauze to attract bats with reduced visual stimuli. Based on previous reports, we hypothesized that *A. lituratus* would exhibit preference for *Ficus* fruits/oil while *C. perspicillata* would prefer *Piper* fruit/oil. Four arrangements of these attractants were tested in triplicate: *P. hispidum* fruit vs. *F. insipida* fruit, *P. hispidum* oil vs. *F. insipida* oil, *P. hispidum* oil vs. *F. insipida* fruit and *P. hispidum* fruit vs. *F. insipida* oil. As expected, in all tests, *A. lituratus* showed the highest number of foraging attempts in *F. insipida* while *C. perspicillata* in those of *P. hispidum*. Based on the number of foraging attempts both species exhibited a positive response to their favorite fruit genera, though the differences were not always statistically significant. The results confirm the importance of smell in fruit choice by these species.

**Key words:** bats, essential oils, food preference, fruits, smell.

**INTRODUCTION**

Phyllostomidae is a family of bats that is known for its richness (approximately 160 species) (Simmons 2005) and also for the importance of its species interactions with the environment (Kunz and Fenton 2003). Olfaction combined with echolocation and vision, form the set of senses that enable bats to orient in space and search for food (Fleming 1988, Thies et al. 1998). In fact, laboratory experiments have shown that some frugivorous Phyllostomidae (e.g. *Artibeus jamaicensis* Leach, 1821) posses increased numbers of nasal structures compared to essentially insectivorous species, such as the genus *Myotis* Kaup, 1829 (Bhatnagar and Kallen 1975). Furthermore, studies have shown that olfaction...
plays an important role in the selection of mature fruits by species such as *Artibeus watsoni* Thomas, 1901, *Carollia perspicillata* (Linnaeus, 1758), *C. castanea* (H. Allen, 1890) and *Vampyressa pusilla* (Wagner, 1843) (Laska 1990, Mikich et al. 2003, Korine and Kalko 2005).

Following this reasoning, Mikich et al. (2003) and Bianconi et al. (2007) evaluated the attraction of frugivorous phyllostomids to essential oils extracted from ripe chiropterocochic fruit. The results proved so efficient that the same authors proposed a tool for forest restoration based on the attraction of bats to degraded areas and the increase of seed rain (Bianconi et al. 2007, Bianconi et al. 2010). Yet it remains to be proven whether this tool leads to increased seedling recruitment and establishment (Reid and Holl 2013).

Underlying these bat-plant relations, there is an interesting point regarding the preferential consumption of fruits in nature - evidenced when an animal, provided with a choice, uses a particular food source (*sensu* Chensson 1983). In this approach, the aforementioned phyllostomid genera are classic examples of the Neotropical region. According to many authors (e.g. Fleming 1988, Kalko et al. 1996, Wendeln et al. 2000, Mikich 2002), *Artibeus* Leach, 1821 shows marked preference for fruits of *Ficus* L. and *Carollia* Gray, 1838 for those of *Piper* L. These findings are generally supported by analyses of faeces and/or direct observations in shelters and/or feeding perches. However, these studies lack the simultaneous assessment of the availability of resources in the area. The number of studies adopting this approach is still small (Fleming 1988, Giannini 1999, Thies and Kalko 2004, Pereira et al. 2010, Mello et al. 2011, Sánchez et al. 2012), which reinforces the need for further studies, especially those that control fruit supply.

So here we tested the response of captive *Artibeus lituratus* (Olfers, 1818) and *Carollia perspicillata* offered controlled quantities of olfactory stimuli from two fruit species, *Ficus insipida* and *Piper hispidum*, and their respective essential oils. Our hypothesis was that *A. lituratus* would exhibit preference for *Ficus* fruit/oil while *C. perspicillata* would prefer *Piper* fruit/oil.

**MATERIALS AND METHODS**

**CAPTURE AND CARE OF BATS**

Bats for the experiments were captured during February 2008 in Atlantic Forest fragments in southern Brazil. Soon after capture, the animals were placed in cotton bags and transported to a facility located in the Scientific Breeding of Wild Animals of the Museu de História Natural Capão da Imbuia, Curitiba – state of Parana, Brazil. The bats were kept in an enclosure with two rooms (2.8 m x 2.5 m x 2.8 m in height, each) covered with screen. While the bats were not being used in the experiments, they were fed a variety of different fruits. All procedures were carried out according to the international practices for animal use and care under the control of an internal Animal Ethics Committee of the Pontifícia Universidade Católica do Paraná, Brazil (Protocol n. 315).

**FRUITS**

Ripe fruit of *Ficus insipida* Willd. (Moraceae) and *Piper hispidum* Sw. (Piperaceae) used in the experiments were collected at the same locations and periods in which the bats were caught, thus ensuring a prior knowledge of this resource by the animals. The fruits were frozen in -15 °C for a maximum of two months until their use in testing or extraction of their essential oils. *Ficus* is a genus of canopy tree widely distributed in the Neotropics, from Mexico to Paraguay (Banack et al. 2002). *Ficus* species exhibit a “boom” of maturation, but are generally asynchronous within species (Figueiredo and Sazima 1997, Morrison 1978). *Ficus insipida* produces fruits (syconia), approximately 24 mm long by 26 mm wide, containing 100 to 300 seeds (Bianconi et al. 2007, Mikich and Silva 2001). The genus *Piper* is
Pantropical and includes herbs, shrubs and small trees, which are very common pioneers in degraded rural areas (Fleming 1988, Mikich et al. 2003, Thies and Kalko 2004). Their inflorescences are drupes with characteristic chiropterocoric fruit traits i.e., green colour, strong odour and exposure on the outside of leaves (van der Pijl 1957). However, unlike Ficus spp, Piper have sequential ripening fruit within the canopy, offering few mature fruits per night (Dumont 2003), plus a sequential addition of fruit availability between different species of the community (Fleming and Heithaus 1986). The fruits of Piper hispidum are approximately 100 mm long by 8 mm wide and have 1000 to 2000 seeds (Mikich and Silva 2001).

ESSENTIAL OIL EXTRACTION

Essential oils from two species of fruits were extracted by hydrodistillation with a modified Clevenger apparatus (Bianconi et al. 2008). To obtain each ampoule of crude essential oil a total of 300 g of ripe fruits were boiled for 4 hours (Mikich et al. 2003, Bianconi et al. 2007).

CAPTIVE EXPERIMENTS

The tests consisted of the simultaneous offering two olfactory attractants, fruit and/or essential oils, hanging by a nylon thread at a height of 50 cm from the ceiling of the enclosure and separated by 90 cm. For treatments using essential oils, an ampoule was opened at the beginning of testing and dissolved in 2 mL of diethyl ether, to impregnate two rubber septa (Sigma, 6 mm long). In the case of fruits, the attractant consisted of one whole fruit wrapped in gauze, so that the bats could not actually see the fruit but could potentially smell it. Accordingly, the rubber septum was also wrapped in gauze when tested against fruit (see arrangements 3 and 4 below) to standardize the stimuli.

Each bat, eight individuals of Artibeus and six of Carollia, was observed across the four different arrangements of paired attractants, each measured three times: 1) P. hispidum fruit and F. insipid fruit; 2) P. hispidum oil and F. insipida oil; 3) P. hispidum oil and F. insipida fruit and 4) P. hispidum fruit and F. insipida oil.

Irrespective of the arrangement tested, the following steps were followed: i) the stimuli were arranged in the enclosure, ii) one individual was released; iii) foraging attempts were recorded for 10 minutes (see below) right after the individual’s first exploration flight; v) the individual was removed from the enclosure; and vi) a new individual was released. The position of the stimuli was switched among the replicates to avoid learning and to randomize sampling.

The observations were done according to the method “all occurrences sampling” (Altmann 1974), by measuring the number of foraging attempts of each individual towards the two different attractants for 10 minutes. The experiments were always conducted at night and viewed from the outside of the enclosure, with the aid of an infrared video camera (SONY DCR-HC28) to reduce disturbances.

DATA ANALYSIS

A chi-square test was used to examine independence of the number of foraging attempts on each attractant, and the significance level was 0.05.

RESULTS

After 64 h of observations, conducted during 37 non-consecutive nights in March, April, July and September 2008, there were 923 bat foraging attempts on the attractants. Artibeus lituratus (eight individuals) totalled 211 attempts while Carollia perspicillata (six individuals) totalled 712 (Table I).

Piper fruit vs. Ficus fruit

Artibeus lituratus exhibited a low number (N=11) of foraging attempts on whole fruit so that even though the number of attempts on Ficus were higher.
### TABLE I

Number of responses of *Artibeus lituratus* and *Carollia perspicillata* in tests with paired attractions offered in captivity. Ph = *Piper hispidum* and Fi = *Ficus insipida*.

<table>
<thead>
<tr>
<th>Attraction pair</th>
<th>Number of foraging attempts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artibeus lituratus</td>
</tr>
<tr>
<td></td>
<td>Ph</td>
</tr>
<tr>
<td>1) <em>P. hispidum</em> fruit vs. <em>F. insipida</em> fruit</td>
<td>3</td>
</tr>
<tr>
<td>2) <em>P. hispidum</em> oil vs. <em>F. insipida</em> oil</td>
<td>9</td>
</tr>
<tr>
<td>3) <em>P. hispidum</em> oil vs. <em>F. insipida</em> fruit</td>
<td>28</td>
</tr>
<tr>
<td>4) <em>P. hispidum</em> fruit vs. <em>F. insipida</em> oil</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
</tr>
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(N=8) than on *Piper* (N=3), the difference was not statistically significant ($\chi^2_{(1)}=2.27$, $P=0.13$). As for *Carollia*, the total number of foraging attempts was higher (N=162) and statistically significant for *P. hispidum* ($\chi^2_{(1)}=30.24$, $P<0.05$).

**Piper oil vs. Ficus oil**

*Artibeus lituratus* showed a significant preference for *Ficus* essential oil ($\chi^2_{(1)}=11.3; P<0.05$). Approximately 57% of *Carollia perspicillata* attempts occurred on *P. hispidum*, but the difference was not significant ($\chi^2_{(1)}=3.18$, $P=0.07$).

**Piper oil vs. Ficus fruit**

*Artibeus lituratus* exhibited a significant preference for *Ficus* ($\chi^2_{(1)}=11.04$, $P<0.05$) and *C. perspicillata* for *Piper* ($\chi^2_{(1)}=8.83; P<0.05$).

**Piper fruit vs. Ficus oil**

Even though *A. lituratus* invested 60% of the foraging attempts on *F. insipida* oil there was no significant difference ($\chi^2_{(1)}=2.64$, $P=0.10$) between these attractants. *Carollia perspicillata* on the other hand invested significantly more in the fruit of *P. hispidum* ($\chi^2_{(1)}=5.63$, $P<0.05$).

**DISCUSSION**

The majority of *Artibeus lituratus* foraging attempts suggested a preference for *Ficus* even though in two out of four arrangements tested the difference was not statistically significant. In this interpretation, the results support the various studies that indicate *Ficus* spp. as the most common item in *Artibeus* spp. diet (e.g. Morrison 1978, Handley et al. 1991, Mikich 2002, Giannini and Kalko 2004, Olea-Wagner et al. 2007). Bonaccorso and Gush (1987), in tests with captive *Artibeus* spp. [*Artibeus phaeotis* (Miller, 1902), *A. toltecus* (Saussure, 1860) and *A. jamaicensis* Leach, 1821] and fruits of *Ficus ovalis* (Liebm.) Miq, also obtained positive attraction responses to species such as *Piper amalago* L. and *Muntingia calabura* L. Morrison (1978) noted that on Barro Colorado Island *A. jamaicensis* continued to feed on *Ficus* trees even when other fruits were abundant. The same was observed in this study for *A. lituratus* which chose *Ficus* fruits even with the increased supply of *Piper* (septum with essential oil extracted from approximately 300 g of fruit). For Dumont (2003) and S.M.C. Francener (Unpublished data), the preference for this Moraceae is related to its high fibre content and low nitrogen and lipid concentrations. To compensate for the low nutrient content, bats feed on large quantities of *Ficus* fruits and supplement their diet with other species (Bonaccorso and Gush 1987, Dumont 2003).

Experiments with *C. perspicillata* showed a high number of foraging attempts on fruit and essential oil of *P. hispidum*, even when the offer...
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of *Ficus* was greater (*Piper* fruit *x* *Ficus* oil tests). This pattern is similar to what is found in the wild, where *Piper* provides few mature fruits per night when compared with the Moraceae genus (Dumont 2003). The relationship of this phyllostomid bat with *Piperaceae* is well documented (e.g. Fleming 1988, Charles-Dominique 1991, Sánchez et al. 2012), with studies suggesting co-evolution between these groups (Thies and Kalko 2004). Bonaccorso and Gush (1987) reported for captive *C. perspicillata* and *C. subrufa* (Hahn, 1905), a marked preference for fruits with a high nutritional value, in the case when *Piper amalago* L. was offered simultaneously with *Ficus ovalis* fruits. According to authors, the strategy of these bats is to feed on fruits which are rich in protein and low in fibre (Herbst 1986, Bonaccorso and Gush 1987, Fleming 1988), a condition potentially found in *Piperaceae* (Fleming 1988, Thies and Kalko 2004).

Tests with essential oils showed 191 responses by both bat species, which may indicate that these bats are guided only by the odor to select the preferred ripe fruits. From a more conservative approach, our results also validate studies that indicate olfaction as one of the main sense used by fruit bats for obtaining food (Laska 1990, Thies et al. 1998, Mikich et al. 2003). Additionally, our findings reinforce the hypothesis proposed by Laska (1990), which associates the feeding specificity of bats to olfaction. Similarly, Thies et al. (1998) evaluated the attraction of *Carollia* spp. (*C. perspicillata* and *C. castanea*) to variations in texture, shape, maturity status, position and odor of *Piper* fruits (*P. aequale* Vahl., *P. dilatatum* Rich., *P. grande* Vahl., *P. marginatum* Jacq). Of all the treatments used, the bat species only exhibited a positive response to stimuli containing odor of ripe fruit.

In forest tests conducted by Mikich et al. (2003), *C. perspicillata* responded strongly to *P. gaudichaudianum* oil even when there was an abundance of fruit availability of this and other *Piper* species available in the environment. Bianconi et al. (2007), in turn, described the efficacy of essential oils in attracting fruit bats in open areas. Different from that observed by Mikich et al. (2003), oil of *P. gaudichaudianum* mostly attracted *A. lituratus*. In this case, the authors suggest that in areas without resources, as in the case of open pastures and crops, bats can be attracted to odors of other fruits in addition to their preferred choice. It is possible that this attraction is in some way related to similarities in the composition of the essential oils consumed between species and genera.

The experiments in this study demonstrated that *Artibeus lituratus* responded more frequently to *Ficus insipida* whereas *C. perspicillata* responded more to *Piper hispidum*. The number of foraging attempts of both species shows preference for their favorite fruit genera. Moreover, these animals responded positively to odorous stimuli, reinforcing the importance of olfaction in their foraging activity. In this sense, indications were also obtained that these bats may be guided only by odor to select and pick ripe fruit.

**RESUMO**

Estudos sugerem que morcegos frugívoros buscam e selecionam o fruto utilizando-se principalmente do olfato, podendo ser atraídos apenas pelo cheiro. O objetivo deste estudo foi avaliar, em cativeiro, a resposta comportamental (número de tentativas de forrageamento) de *Artibeus lituratus e Carollia perspicillata* diante da oferta de óleos essenciais extraídos de frutos maduros de *Ficus insipida* (Moraceae) e *Piper hispidum* (Piperaceae), bem como de frutos intactos envoltos em gaze para atrair morcegos com estímulo visual reduzido. Baseados em estudos anteriores, nós hipotetizamos que *A. lituratus* iria preferir frutos/óleo de *Ficus* enquanto *C. perspicillata* iria preferir frutos/óleo de *Piper*. Quatro arranjos desses atrativos foram testados, em triplicatas: fruto de *P. hispidum* vs. fruto de *F. insipida*, óleo de *P. hispidum* vs. óleo de *F. insipida*, óleo de *P. hispidum* vs. fruto de *F. insipida*, e fruto de *P. hispidum* vs. óleo de *F. insipida*. Como esperado, em todos os testes, *A.
lituratus apresentou o maior número de tentativas de forrageamento em F. insipida, enquanto C. perspicillata em P. hispidum. Com base no número de tentativas de forrageamento, ambas as espécies exibiram uma resposta positiva aos seus gêneros de frutos preferidos, ainda que as diferenças não tenham sido estatisticamente significativas sempre. Os resultados confirmam a importância do olfato na escolha de frutos por essas espécies.

**Palavras-chave:** morcegos, óleos essenciais, preferência alimentar, frutos, olfato.

**REFERENCES**


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