Two dispersers are better than one: a 'bird-fig' attracts bats via nocturnal scent

Simon P. Ripperger[a,b,*], Saskia Rehse[a,#], Stefanie Wacker[a], Elisabeth K. V. Kalko[a,c], Stefan Schulz[d], Bernal Rodriguez-Herrera[e], Manfred Ayasse[a]

[a]Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Helmholtzstr. 10-1, Containerstadt, 89081 Ulm, Germany

[b]Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Invalidenstraße 43, 10115 Berlin, Germany

[c]Smithsonian Tropical Research Institute, P.O. Box 0843-03092, Balboa, Panama

[d]Institute of Organic Chemistry, Technische Universität Carolo-Wilhelmina zu Braunschweig, Hagenring 30, 38106 Braunschweig, Germany

[e]Escuela de Biologia, Universidad de Costa Rica, P.O. Box 11501-2060, San Pedro, Costa Rica

[#]current address: Leibniz-Institut für Gewässerökologie und Binnenfischerei Müggelseedamm 310, 12587 Berlin, Germany

*corresponding author, E-mail: simon.ripperger@mfn-berlin.de, phone: ++49 (0)30 2093 8468, fax: ++49 (0)30 2093 8565

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Abstract

The plant genus *Ficus* is a keystone resource in tropical ecoystems. One of the unique features of this group is the modification of fruit traits in concert with various dispersers, the so-called fruit syndromes. The classic example of this is the strong phenotypic differences found between figs with bat and bird dispersers (color, size, and presentation). The 'bird-fig' *Ficus colubrinae* represents an exception to this trend since it attracts the small frugivorous bat species *Ectophylla alba* at night, but during the day attracts bird visitors. Here we investigate the mechanism by which this 'bird-fig' attracts bats despite its morphology which should appeal solely to birds. We performed feeding experiments with *Ectophylla alba* to assess the role of fruit scent in the detection of ripe fruits. *Ectophylla alba* was capable of finding ripe figs by scent alone under exclusion of other natural sensory cues. This suggests that scent is the key signal in the communication between *Ectophylla alba* and *Ficus colubrinae.* Analyses of odor bouquets from the bat- and bird-dispersal phases (i.e. day and night) differed significantly in their composition of volatiles. This indicates that an olfactory signal allows a phenotypically classic 'bird-fig' to attract bat dispersers at night thus to maximizing dispersal.

Key words

Ficus colubrinae; *Ectophylla alba*; seed dispersal syndromes; sensory cues; fruit volatiles; diel differences; neotropics

FRUITING PLANTS NEED TO ENSURE THAT THEIR SEEDS ARE TRANSPORTED AWAY FROM THEIR POINT of origin in order to increase survival probability by avoiding competition and reaching advantageous environments for germination (Howe & Smallwood 1982). Common ways of seed dispersal include self-dispersal by explosive fruits, dispersal by wind or the production of fleshy fruits to promote dispersal by animals (Willson & Travaset 2000). Animal dispersal, or zoochory, frequently consists of a mutualistic relationship between plants and animals where animals are rewarded with edible, fleshy fruit parts for their service of transporting seeds away from the parental plant (Herrera 2002).

Bats and birds are very important vertebrate seed dispersers in tropical ecosystems (Galindo-González *et al.* 2000,Fleming & Kress 2013). Fruits, however, that are consumed by either bats or birds may vary strongly in their appearance as a consequence of the contrasting life histories of the associated dispersers (Hodgkison *et al.* 2013). Diurnal birds mainly rely on vision while foraging and hence prefer conspicuous fruits that contrast with the foliage (Gautier-Hion *et al.* 1985,Wheelwright & Janson 1985,Burns & Dalen 2002). On the contrary, bat fruits are frequently cryptic green and produce strong odors to attract their nocturnal dispersers (Thies *et al.* 1998,Korine *et al.* 2000,Korine & Kalko 2005). Additionally, bat dispersed plants present fruits on erect spikes or pendulous structures in order to facilitate close distance detection by echolocation (Kalko & Condon 1998,Thies *et al.* 1998). While bats are able to consume larger fruits piecemeal by using their teeth, fruit size may be challenging to birds since they are limited by gape width (Wheelwright 1985,Lomáscolo *et al.* 2008).

Such different requirements of disperser groups drove the development of so-called dispersal syndromes, trait combinations that show a correlated evolution (van der Pijl 1982,Janson 1983,Howe & Westley 1988). The existence of dispersal syndromes has been discussed for a long time and was confirmed by a comprehensive study of the plant genus *Ficus*

(Lomáscolo *et al.* 2008,Lomáscolo *et al.* 2010) a keystone resource for many tropical frugivores including bats and birds (Korine *et al.* 2000,Shanahan *et al.* 2001). In detail, bird dispersed figs or 'bird-figs' from both New and Old World tropics tend to be smaller, stronger contrasting to the foliage, less odorous, and arise from branches. On the contrary, figs dispersed mainly by bats or 'bat-figs' are larger, more cryptic relative to the foliage, have an aromatic scent, and are frequently presented on the trunk (Hodgkison *et al.* 2007,Hodgkison *et al.* 2013).

However, not all species of the genus *Ficus* are clearly classifiable as 'bat- or bird-figs'. Intermediate phenotype combinations exist and are frequently associated with dispersal by both bats and birds (Lomáscolo *et al.* 2010). Trait expression may even vary temporally. The Paleotropical fig species, *Ficus benghalensis*, has been shown to produce significantly different odor bouquets during day and night, probably in order to attract nocturnally foraging bats by scent, while diurnal birds are attracted by visual cues (Borges *et al.* 2011). Unfortunately, the appeal of the altered scent on the nightly dispersers has not been studied in experimental setups. The importance of olfaction for fruit detection in bats has been demonstrated in feeding trials for several frugivorous species of the Neotropical bat family Phyllostomidae (Thies *et al.* 1998,Korine & Kalko 2005,Hodgkison *et al.* 2013). These studies show that the examined bat species are able to localize fruits by either olfaction alone or in combination with echolocation. This dominant role of olfaction in the foraging behavior of frugivorous bats may enable plants that phenotypically match the bird-dispersal syndrome to expand seed dispersal into the night by nocturnal production of volatiles that attract bats or other nocturnal mammals.

The Mesoamerican fig species *Ficus colubrinae* is an excellent study organism to investigate the mechanisms of attracting nightly dispersers despite heavy bird visits during day. The phenotype of *F. colubrinae* clearly matches the bird-dispersal syndrome with very small fruits which are bright red colored when ripe and presented on the branches (Burger

1977,Galindo-González *et al.* 2000). While birds extensively visit these fig trees during day, the small phyllostomid bat *Ectophylla alba* feeds heavily on fruits of *F. colubrinae* at night (Brooke 1990). In the present study we assess the role of fruit odor in the attraction of *E. alba* to ripe fruits of *F. colubrinae*. In detail we test the following hypotheses: (1) olfaction plays a major role for the detection of ripe fruits in *Ectophylla alba*; (2) odor bouquets of fruits change when the fruits ripen and vary among day and night in ripe fruits, and (3) ripe fruits will shift production and release of volatiles during night in favor of substances that are known from published studies to be dominant in 'bat-figs'. In order to test these hypotheses we combine semi-natural behavioral experiments with wild bats and chemical analyses of fig scent.

METHODS

STUDY SITE—Our study was conducted at „La Tirimbina Rainforest Center" (TRC) in the province Heredia in Costa Rica (10°26' N, 83°59' W). The study site is located in the Caribbean lowlands of Costa Rica. Annual precipitation averages at 3900 mm. Behavioral experiments were performed during May and June 2010 and sampling of fig scent from February to May 2011.

STUDY ORGANISMS—*Ficus colubrinae* (Moraceae) is a Neotropical fig species. Its fruiting phenology is characterized by asynchronous fruit crop production of small fruits (diameter < 0.8 mm, mass 0.3 g) that are presented on the branches and turn dark red while ripening (Burger 1977,Korine *et al.* 2000). On Barro Colorado Island in central Panama *F. colubrinae* draws little attention of frugivorous bats and is hence considered to be mainly bird-dispersed (Kalko *et al.* 1996,Korine *et al.* 2000). However, farther north where *F. colubrinae* occurs in sympatry with *Ectophylla alba* this particular bat species shows a dietary specialization on *F. colubrinae* (Brooke 1990).

STUDY ANIMAL—*Ectophylla alba* is a small-bodied leaf-nosed bat species (Phyllostomidae) that is distributed from northern Honduras to north-eastern Panama (Rodriguez-Herrera *et al.* 2008). It modifies leaves, predominantly of plants of the genus *Heliconia*, to construct shelters where it roosts in social groups of typically four to eight individuals (Brooke 1990).

BEHAVIORAL EXPERIMENTS—We captured groups of *Ectophylla alba* from roosts in *Heliconia* leaves in the area of TRC and selected single males for the feeding experiments in order to prevent lactating or pregnant females or juveniles from isolation of the social group. All individuals that were not considered for further experiments were set free immediately in close proximity to the roost. Following the capture, a single male was released into a flight tent (Eureka; ground area 4 x 4m, height 2.5m) several hours before sunset. At nightfall we installed a freshly cut branch of *Ficus colubrinae* that yielded a range of fruits of different stages of maturity into the flight tent. In order to adjust to the foraging situation we allowed the bat to feed on ripe fruits. After the consumption of five fruits we started choice trials in order to test whether *E. alba* relies mainly on olfaction or echolocation/vision for the short-range localization of ripe fruits. On one side of the branch we presented a strong olfactory cue to the bat that lacked visual or echo-acoustic properties of natural figs, i.e. we presented a tissue bag that was filled with ten ripe figs (similar methods have been used to test for the response of bats to olfactory cues in absence of natural fruit shape or surface structure: Kalko and Condon (1998) presented cotton saturated with juice of cucurbit fruits to bats; Hodgkison *et al.* (2007) wrapped ripe figs in several layers of nylon stockings). Simultaneously we presented on the other side of the branch fig models made from red clay that were similar to natural *F. colubrinae* fruits in terms of form, color, and fruit presentation (in branch forks). We rated *E. alba*'s behavior as a positive response to the presented object when repeated approximation flights to or a landing next to the object followed by a directed movement to it occurred. In total, we tested six individual bats. Every bat was tested only once in order to avoid bias caused by learning effects. It was not possible to record data blind because our study involved focal animals. We documented bat behavior using an infrared camera (Sony Night-Shot DCR-HC42E, Sony, Japan) that was connected to a video recorder (GV-D 900E, Sony, Japan). We stored recordings on MiniDV video tapes (DVM60PR3, Sony, Japan).

SAMPLING OF FIG SCENT—We sampled volatiles of *Ficus colubrinae* fruits based on dynamic headspace adsorption techniques (Hodgkison *et al.* 2007,Kalko & Ayasse 2009,Hodgkison *et al.* 2013). Three categories of fruits were sampled: (1) unripe during night, (2) ripe during day, and (3) ripe during night. Single fruits were collected from five individual fig trees and placed in glass chambers. Four glass chambers were connected to a single battery operated membrane pump. Every individual glass chamber was connected via a Teflon tube to an adsorbent tube containing activated charcoal (activated charcoal, Supelco, Orbo 32 large) that was installed upstream in order to filter-clean the pulled atmospheric air. After passing the glass chamber containing the fruit, the air exit through a glass sampling cartridge packed with 5mg Super Q (Waters Division of Millipore) in order to collect volatiles. The sampling cartridges were twice y-connected to the pump via silicone tubing. Two such setups were run simultaneously allowing for the collection of seven samples at a time along with one blank control that consisted of an empty glass chamber. Each sampling session was started at 2000 h for nightly sampling, or 0800 h for daily sampling, respectively, and lasted for eight hours with a flow rate of ca. 100mL min^{-1}. After sampling, all sorbent tubes were eluted with 0.050 ml of 10:1 pentane/acetone. Eluted samples were sealed in small airtight borosilicate glass specimen tubes and stored in the freezer at −18°C. After each sampling session, all glassware was thoroughly cleaned three times with ethanol (Absolute Alcohol, Hayman Ltd., Essex, UK), acetone (LiChrosolv, Merck, Darmstadt, Germany), and pentane (SupraSolv, Merck). Sorbent tubes were cleaned three times with ethanol, dichloromethane (LiChrosolv, Merck), and pentane, and then wrapped in aluminum foil and stored for future use in airtight glass jars with Teflon-coated lids.

CHEMICAL ANALYSES OF COMPOUNDS: GC-RUNS, QUANTIFICATION & MS-ANALYSES—For quantitative analyses, 0.1 µg of octadecane was added as an internal standard to each of the eluted fruit odor samples collected by dynamic headspace adsorption (see above). All samples were analyzed with an HP5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a DB5 capillary column (30 m × 0.25 mm i.d.) that used hydrogen as the carrier gas (2 ml min−1 constant flow). One microliter of each sample was injected splitless at 40°C. After 1 min, the split valve was opened and the temperature increased by 4°C min^{-1} until reaching a temperature of 300°C.GC/MS analyses were carried out on an HP 6890 Series GC connected to an HP 5973 mass selective detector (Hewlett-Packard) fitted with a BPX5 fused-silica column (25 m, 0.22 mm i.d., 0.25 µm film thick, SGE). Mass spectra (70 eV) were recorded in full scan mode. Retention indices were calculated from a homolog series of n-alkanes. Structural assignments were based on comparison of analytical data obtained with natural products and data reported in the literature (McLafferty & Stauffer 1989,Hodgkison *et al.* 2007,Hodgkison *et al.* 2013), and those of synthetic reference compounds. Structures of candidate compounds were verified by co-injection.

STATISTICAL ANALYSES—We performed principal component analysis (PCA) on the relative amounts of fruit scent compounds using SPSS 17. We used the resulting principal components (PCs) with an eigenvalue above one to run a discriminant function analysis (DFA) in order to test for differences in the scent composition between (1) unripe fruits during night, (2) ripe fruits during day, and (3) ripe fruits during night. We used the factor loadings after varimax rotation and the standardized discriminant function coefficients to assess the importance of individual compounds. Factor loading above 0.5 were considered high. Finally, we compared relative amounts of single compounds of ripe fruits during day and night (groups 2 and 3) using Mann-Whitney U-tests in R 2.15.3 (R Developing Core Team 2015).

RESULTS

ACCUSTOMING PHASE IN THE FLIGHT TENT AND EXPERIMENTAL TRIALS—After releasing captured bats into the flight tent, the bats performed circular inspection flights for several minutes before they roosted in a corner of the flight tent until dusk. Shortly before dusk we installed a natural branch of *F. colubrinae* with several ripe and unripe fruits. All six bat individuals performed search flights that lasted between less than one minute and almost two hours (mean ± standard deviation: 32 ± 43 minutes, n = 6) until the bats approached the branch for the first time. Then the bats conducted two to nine approximation flight towards the branch over a period of one to 91 minutes (mean ± standard deviation: 19 ± 36 minutes, n = 6) before they landed and consumed a fig either directly on the branch or on the wall of the tent.

After the consumption of five ripe figs we started the behavioral experiments by presenting to the bat red modelling clay fig dummies on a natural branch of *F. colubrinae* and a tissue bag filled with 10 ripe *F. colubrinae* figs. None of the tested bats showed a clear positive response to the modelling clay figs. We did neither observe repeated approximation flights nor landing in the proximity of the models which represented an echo-acoustic/visual cue similar to natural figs (a red, similar sized sphere presented in branch forks). On the contrary, five out of six individuals responded to the bag filled with ripe figs representing a strong olfactory cue. After a period of six to 48 minutes (mean ± standard deviation: 16 ± 21 minutes, n = 5, see Table 1) and one to five approaches the bats either landed on or right next to the bag or landed more than 5 cm away and move hand over hand along the branch towards the bag. Subsequently the bats bit open the bag and consumed a fig.

COMPARISON OF ODOR BOUQUETS—In the chemical analyses we registered 14 distinct peaks that were attributed to 17 individual substances, again 13 of which were unambiguously identified by mass spectrometry (Table 2). Nonanal and 1-tetradecanol contributed the largest share to the overall bouquet (Fig. 1, Table S1). Three further substances could be assigned to substance classes, however, so far not identified and one substance could not be classified. The identified substances belonged to different compound classes: aliphatic compounds derived from the fatty acid biosynthetic pathway (here shortly named fatty acid pathway compounds, FAPCs), sesquiterpenenes, and aromatic compounds. In three cases, two substances contributed to a single peak in the GC-analysis. In those cases the overlapping substances were represented by a single value for the following analyses. Two of the identified substances, indene and anthracene, have a main relevance in industrial applications and were therefore excluded from all further analyses. They were considered environmental pollutants that accumulated on the outside of the fruits over time since our field site was closely located to human structures including infrastructure and industry. There were no significant differences in relative amounts of indene and anthracene among day and night in ripe fruits. Medians were lowest in unripe fruits and rising over time while ripening (Fig. S1 & Fig. S2).

We performed a PCA that included 12 individual values for the relative amounts of the remaining 15 chemical compounds from the three tested groups of figs ((1) unripe fruits at night, (2) ripe fruits during day, and (3) ripe fruits during night). Four PCs with an eigenvalue above one accounted for 76.2 % of the total variation. The DFA that used the four PCs as variables resulted in two discriminant functions (DFs) and showed significant differences between the tested groups (function 1: $\chi^2 = 78.9$, df = 8, $p < 0.001$; function 2: $\chi^2 = 24.9$, df = 3, $p < 0.001$;

Fig. 2). The highest coefficient for DF 1 was attributed to PC2 which in turn had high factor scores on the sesquiterpenes α-copaene and δ-cadinene + calamenene (Table S2 & Table S3). For DF 2, PC1 and PC3 had the highest coefficients. PC1 had high factor loading on sesquiterpene A, β-copaene + naphthalene derivative, α-cubebene + 1,1'-biphenyl and the FAPCs nonanal and decanal. 1-dodecanol and 1-tetradecanol loaded high on PC3. Seventy-five percent of the original grouped cases were correctly classified (72.5 % of cross-validated grouped cases).

DAILY DIFFERENCES OF SINGLE COMPOUNDS IN RIPE FRUITS—All scent compounds analyzed were present in diurnal and nocturnal scents. In general, fatty acid pathway compounds dominated both diurnal and nocturnal scents (Fig. 1). However, relative amounts of sesquiterpene compounds increased at night and FAPCs decreased, except the two long-chain alcohols (Table 2). Six out of twelve day/night comparisons of relative amounts of single scent components showed significant differences. The aldehydes nonanal and decanal and one unclassified substance accounted for a significant greater share during day, while three sesquiterpene compounds in combination with aromatic compounds (sesquiterpene A, β-copaene + naphthalene derivative, α-cubebene + 1.1-biphenyl) had significantly higher proportions during night (Table 2).

DISCUSSION

Our study shows that scent is an important signal in the communication between *Ectophylla alba* and *Ficus colubrinae*. *Ectophylla alba* was capable during experimental trials to find ripe figs by scent alone under exclusion of other natural sensory cues. Odor bouquets of figs undergo significant changes with regard to the relative amounts of compounds during the process of maturation and bouquets of ripe figs differ significantly in the composition of volatiles during day and night. Nightly changes in scent composition show a pattern that contrasts with other 'bat-figs'. We suggest that this strategy of *Ficus colubrinae* is an adaptation towards dispersal by small bats such as *Ectophylla alba* rather than towards bat dispersal in general, since odor may be an ideal signal to attract a specific group of bat species.

Semi-natural feeding trials showed that phyllostomid bats locate fruits by echolocation (Kalko & Condon 1998) or olfaction (Thies *et al.* 1998,Korine & Kalko 2005,Hodgkison *et al.* 2013) as the primary sensory cues. Our results from the feeding experiments show that *E. alba* conforms to the latter foraging strategy. The tested bats only showed strong responses to the tissue bag that gave a strong olfactory cue but lacked natural texture, shape, size, or presentation of figs that might be of importance for detection by echolocation. Therefore, we assume that echolocation may not play such a dominant role for *E. alba* in fruit detection as it does for other bat species. The Neotropical bat *Phyllostomus hastatus* feeds on fruits of a Cucurbitaceae that are borne on pendulous structures (Kalko & Condon 1998). This style of fruit presentation facilitates detection by echolocating bats because the fruit represents a clutter free target. In general, flagellichory or cauliflory (pendulous or trunk-borne presentation of fruits that reduce the presence of foliage close to the fruit) are widespread adaptations of plants to chiropterochory (Van der Pijl 1957). Korine and Kalko (2005) argue that detection of fruits by downwards frequency modulated signals which are typical for phyllostomid bats is possible but largely depends on the fruit presentation and the complexity of the surrounding clutter. However, *F. colubrinae* presents its fruits sessile, usually paired at the node (Burger 1977), thus in a highly cluttered environment making detection by echolocation difficult. Hence, we conclude that based on *F. colubrinae*'s way of fruit presentation only olfaction qualifies as primary cue for detecting figs, at least until *E. alba* gets very close to the figs.

Olfactory cues enable plants to signal the readiness of fruits for dispersal. Accordingly, temporal changes in the volatile profile of fruits are common during the process of ripening (e.g.

(Lalel *et al.* 2003,Obenland *et al.* 2012,Li *et al.* 2013)) and have also been documented for wild, bat-dispersed fig species (Hodgkison *et al.* 2007). Our data is consistent with a change in the overall composition of the scent bouquet during the process of ripening. Additionally we observed significant changes among day and night, caused by day-time specific scent production. Circadian changes in the volatile profile of fruits seem to be a much rarer phenomenon. To our knowledge, only Borges *et al.* (2011) observed diel differences in the volatile signal in Old World figs of the species *F. benghalensis*. These fruits are consumed by birds during the day and by bats during the night. Dispersal by both, birds and bats, is not uncommon within the genus *Ficus*, yet this dispersal mode usually concurs with fruit phenotypes that are considered intermediate between the bird and the bat syndrome (Lomáscolo *et al.* 2010). While most fruit traits in *F. colubrinae* match the bird-syndrome, scent alone is sufficient for *Ectophylla alba* to detect the ripe fruits as shown by our behavioral experiments. Hence, a nightly shift in volatile production may enable 'bird-figs' to additionally attract certain bat species as dispersers and hence allow for dispersal during the daytime and at nighttime. To achieve seed dispersal by distinct animal taxa may result in multiple benefits to a reproducing plant. The contribution to overall seed rain by birds or bats, respectively, may vary quantitatively across seasons (Galindo-González *et al.* 2000). Microhabitat deposition also strongly depends on the disperser since birds tend to disseminate seeds when perched while bats usually defecate seeds during flight. The resulting seed rain can be dominated by chiropterochorously dispersed seeds at forest edges and open areas, while most ornithochorous seeds reach forest sites (Charles-Dominique 1986,Gorchov *et al.* 1993). An all-season reproducing plant species like *F. colubrinae* that may develop both, epiphytic and solitary life forms (Burger 1977), may in particular benefit from the attraction of both bats and birds. This way the plant may maximize dispersal rates of the year-round produced fruits and seeds may arrive in a more heterogeneous range of microhabitats for germination.

All unambiguously identified compounds except 1-dodecanol, 1-tetradecanol, and calamenene have been documented to be produced by *Ficus* spp., either by floral stages (Grison-Pigé *et al.* (2002): α-cubebene, α-, β-copaene, β-selinene, δ-cadinene, decanal) or by fruits (Hodgkison *et al.* (2013): α-, β-copaene, δ-cadinene; Borges *et al.* (2011): nonanal, decanal, α-copaene, δ-cadinene). The scent bouquet of *F. colubrinae* fruits, which was dominated by fatty acid pathway compounds, was more similar to 'bat-figs' from the Old World tropics (Hodgkison *et al.* 2007,Borges *et al.* 2008,Borges *et al.* 2011) than to Neotropical bat-dispersed fig species that were characterized by high proportions of monoterpenes (Hodgkison *et al.* 2013). Monoterpenes were completely missing in our samples. This result was surprising since feeding trials showed that fruit scents, which were dominated by monoterpenes were highly attractive to the phyllostomid bat *Artibeus jamaicensis* (Hodgkison *et al.* 2013). Instead, in our samples sesquiterpenes increased throughout and in parts significantly during night, while fruit scents that were dominated by sesquiterpenes were rejected by *A. jamaicensis*. The day-round changes in the scent production of the Paleotropical *F. benghalensis*, were also in contrast to our observations, despite similarities in the overall bouquet. In *F. benghalensis* relative amounts of fatty acid pathway compounds significantly increased during the nocturnal bat-dispersal phase and sesquiterpenes contributed significantly higher proportions during day (Borges *et al.* 2011). The reverse pattern we observed indicates that certain sesquiterpenes may play an important role in the attraction of *E. alba*. Paleotropical bats and even larger-bodied Neotropical species, however, go for different substance groups.

Those fundamental differences observed among figs that attract bats point towards different olfactory preferences in bats that have different diets, as it was already proposed by Hodgkison *et al.* (2013). Kalko *et al.* (1996) found that fruit size in Panamanian fig species correlates with the body size of the associated bat species. *Ficus culubrinae* has small fruits and is visited mainly by *E. alba*, at least in the study area. Occasionally another small bat species (*Mesophylla macconnelli*) can be netted at fruiting trees and rarely also medium-sized bats like *Plathyrrinus helleri* and *Uroderma bilobatum* (pers.obs, BRH). To our knowledge it has never been studied how fig trees attract the respective size class of bats that feeds on their fruits. Similarities in the scent bouquet of equally sized fruits may be a possible signaling strategy. This may explain the contrasting odor profile of the 'bat-figs' investigated in Panama (Hodgkison *et al.* 2013) that are medium- to large-sized and attract much larger bat species than *E. alba* (Kalko *et al.* 1996). Interestingly, sesquiterpenes, including α- and β-copaene, dominated the bouquet of the only small sized Neotropical fig species (*F. costaricana*) in the sample of Hodgkison *et al.* (2013). Fruit scents of *F. costaricana* were rejected in feeding trials with the large Phyllostomid bat *A. jamaicensis*, but seeds of this 'bird-fig' can occasionally be found in the feces of small bat species (Kalko *et al.* 1996,Giannini & Kalko 2004). In general, there are only few data available on volatile composition of fruits that attract small-bodied bats. The sesquiterpenes we detected (calamenene, α-copaene and β-selinene) have been identified from the scent of inflorescences of *Calyptrogyne ghiesbreghtiana* (Knudsen 1999). This palm is visited by bats including small *Artibeus* species (*watsoni/phaeotis*) (Tschapka 2003), which also feed on small-sized figs (Kalko *et al.* 1996). This may be a hint for different plant species using similar olfactory cues to attract a similar disperser spectrum.

CONCLUSION—Taking the results from behavioral trials and chemical analyses together, our study suggests that the 'bird-fig' *Ficus colubrinae* attracts nightly dispersers by altered scent production. Daily variation in the volatile profile of fruits may be more common than previously thought, but widely overlooked until very recently, since it has now been documented in both the New and the Old World tropics. Generally, volatile ecology in the genus *Ficus* seems to be complex and seems to be worth to receive further attention. The description of 'bat-figs' as fragrant is just as simplified as calling 'bird-figs' odorless. Scent may possibly be a qualitative adaptation to a certain disperser spectrum. However, to prove the latter hypothesis, a genus-wide identification of fig scents would be necessary along with multi-species feeding trials across frugivorous bat families.

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DATA AVAILABILITY STATEMENT

Data will by archived upon article acceptance.

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TABLE 1. Parameters measured during behavioral trials on six individuals of *Ectophylla alba* that were subjected with fig clay dummies and a bag filled with real figs of *Ficus colubrinae*

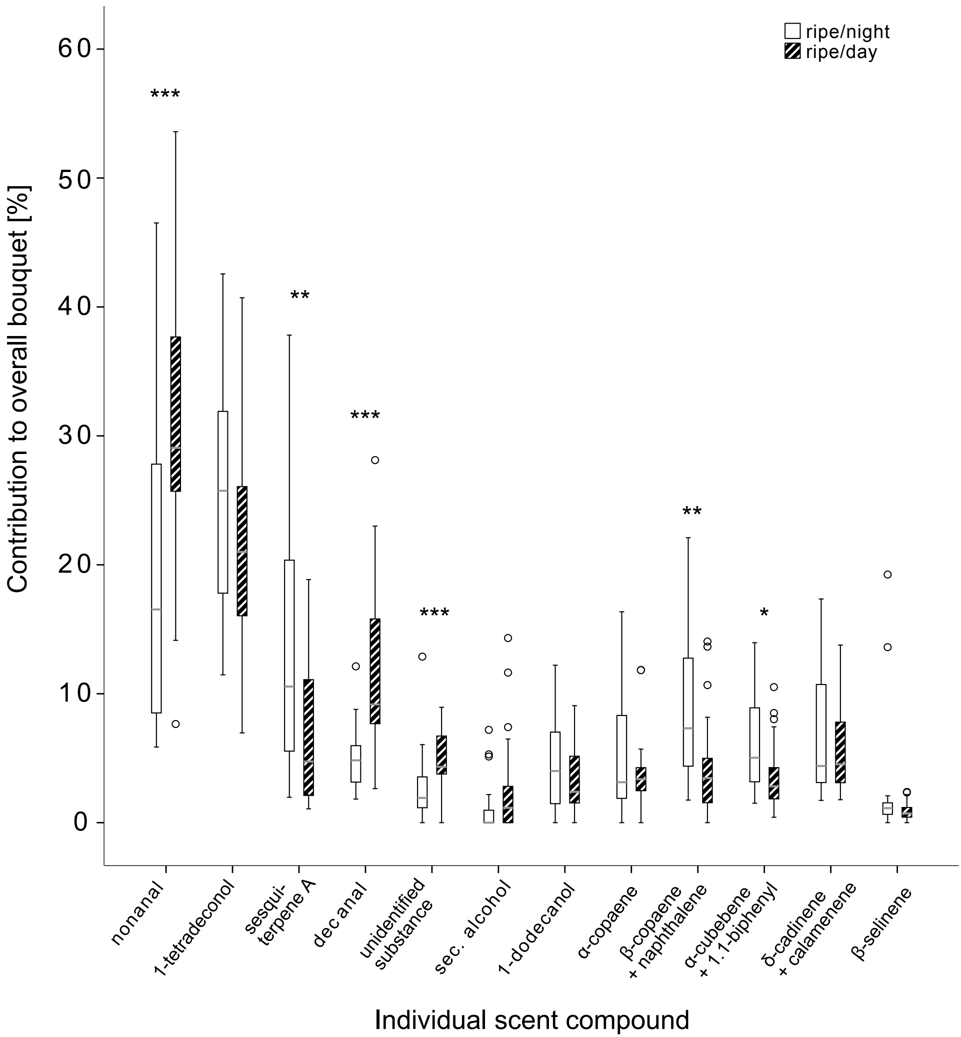
		Reaction to bag with figs		
Bat individual	Reaction to clay dummies	Overall reaction	Time until first landing [min]	# approaches before first landing
1	-	+	19	1
2	-	+	14	5
3	-	-	-	-
4	-	+	48	1
5	-	+	17	1
6	-	+	6	4

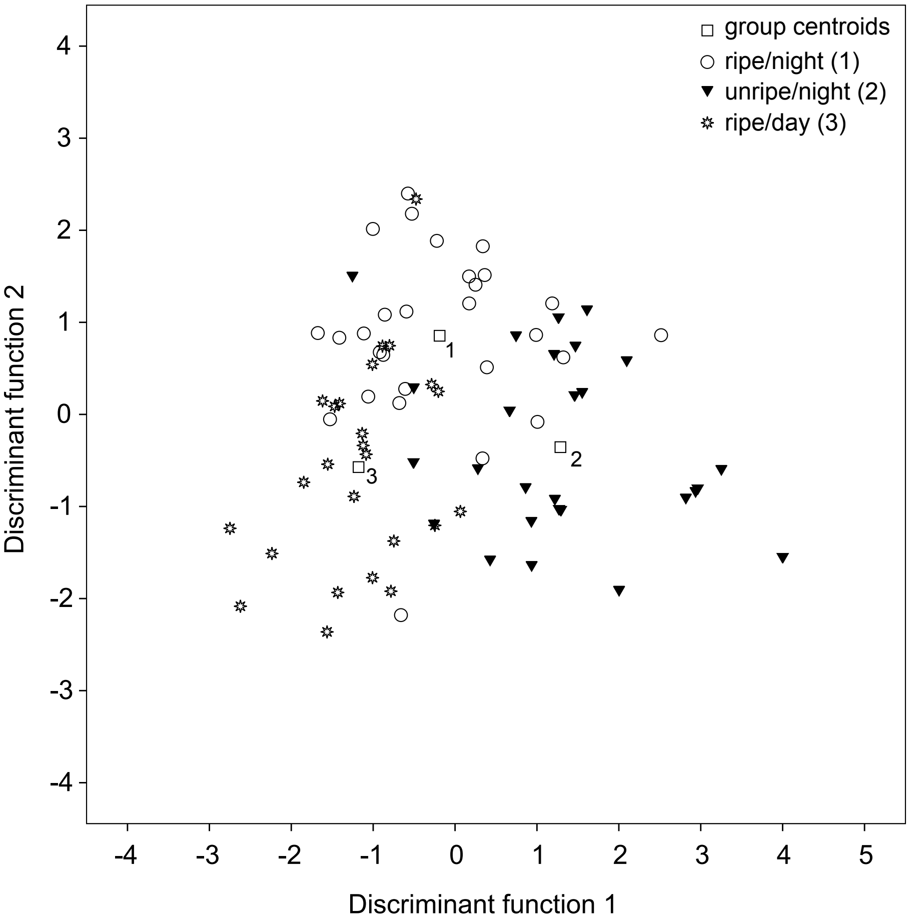
TABLE 2. Comparison of individual chemical scent compounds of ripe fruits during day and during night based on relative amounts; substance were attributed to the following classes: *fapc* fatty acid pathway compounds, *st* sesquiterpenes, *ac* aromatic compounds, *uk* unknown

Compound	substance class	higher during	p	Mann-Whitney U
1-dodecanol	fapc	night	0.449	307.5
1-tetradecanol	fapc	night	0.105	259
secondary alcohol	fapc	day	0.052	247
nonanal	fapc	day	<0.001	145
decanal	fapc	day	<0.001	67
unidentified substance	uk	day	<0.001	139.5
α-copaene	st	night	0.845	339
β-copaene + naphthalene derivative	st + ac	night	0.001	164
α-cubebene + 1,1'-biphenyl	st + ac	night	0.022	221
sesquiterpene A	st	night	0.006	197
β-selinene	st	night	0.084	253
δ-cadinene + calamenene	st	night	0.643	324

FIGURE 1 Relative amounts of compounds that contribute to the separation of ripe figs during daytime and night. Asterisks indicate significance based on the following α-levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

FIGURE 2 Comparison of scent bouquets produced by unripe fruits at night, ripe fruits at night and ripe fruits during day based on the composition of their chemical compounds using canonical discriminant function analysis (DFA)


ripe/night
ripe/day
Contribution to overall bouquet [%]
0
10
20
30
40
50
60
***
**
***
***
**
*
nonanal
1-tetradeconol
sesqui-
terpene A
decanal
unidentified
substance
sec. alcohol
1-dodecanol
α-copaene
β-copaene
+ naphthalene
α-cubebene
+ 1.1-biphenyl
δ-cadinene
+ calamenene
β-selinene
Individual scent compound



group centroids
ripe/night (1)
unripe/night (2)
ripe/day (3)
1
2
3
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4
5
Discriminant function 1
Discriminant function 2