

Chemical Ecology of Fruit Bat Foraging Behavior in Relation to the Fruit Odors of Two Species of Paleotropical Bat-Dispersed Figs (*Ficus hispida* and *Ficus scortechinii*)

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Abstract We investigated the fruit odors of two bat-dispersed fig species in the Paleotropics, in relation to the foraging behavior of fruit bats, to test the following hypotheses: 1) fruit odor plays a critical role for detection and selection of ripe figs by fruit bats; 2) bat-dispersed fig species are characterized by the same, or similar, chemical compounds; and 3) total scent production, in bat-dispersed figs, increases when fruits ripen. We performed bioassays to test the effect of both natural and synthetic fig fruit odors on the foraging behavior of the short-nosed fruit bat (*Cynopterus brachyotis*)—an important disperser of figs within the study area. Fruit bats responded to both visual and chemical (olfactory) cues when foraging for figs. However, the strongest foraging reaction that resulted in a landing or feeding attempt was almost exclusively associated with the presence

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of a ripe fruit odor—either in combination with visual cues or when presented alone. Fruit bats also used fruit odors to distinguish between ripe and unripe fruits. By using gas chromatography (GC) and GC/mass spectrometry (MS), a total of 16 main compounds were identified in the ripe fruit odor of *Ficus hispida* and 13 in the ripe fruit odor of *Ficus scortechinii*—including alcohols, ketones, esters, and two terpenes. Additional compounds were also recorded in *F. hispida*, but not identified—four of which also occurred in *F. scortechinii*. Total scent production increased in both species when fruits ripened. Both natural and synthetic fruit odors resulted in feeding attempts by bats, with no feeding attempts elicited by unscented controls. Reaction rates to natural fruit odors were higher than those to synthetic blends.

Keywords Seed dispersal syndromes · Frugivory · Old world tropics · Co-evolution · Olfaction · Foraging ecology · Moraceae · Chiroptera · Pteropodidae · Fruit scent · Sensory ecology

Introduction

Observations in tropical rain forests have revealed a broad dietary overlap between birds and mainly diurnal mammals when feeding on figs (Breitwisch 1983; Leighton and Leighton 1983; Shanahan et al. 2001a). This has led to the widespread belief that interactions between figs and their vertebrate seed dispersers are relatively generalized, as characterized by large crops of low-quality fruits consumed and dispersed by diverse and taxonomically disparate groups of frugivores. However, more recent studies on the feeding ecology of fruit bats, in both Neo- and Paleotropical rain forests, have revealed a distinct subset of fig species that are consumed and dispersed almost exclusively by nocturnal mammals—especially bats (Kalko et al. 1996; Korine et al. 2000; Shanahan and Compton 2001; Shanahan et al. 2001a; Hodgkison et al. 2003).

In contrast to diurnally dispersed figs, which are often characterized by visually conspicuous fruit displays, particularly bright colors that stand out against surrounding vegetation (e.g., orange and red), nocturnally dispersed species are often characterized by distinctive odors (Lambert 1987, 1989; Lambert and Marshall 1991; Kalko et al. 1996; Korine et al. 2000; Shanahan and Compton 2001; Shanahan et al. 2001a; Hodgkison et al. 2003). These adaptations in fruit display and fruit chemistry correspond closely with the sensory capabilities and foraging behavior of both groups of disperser—as birds and diurnal mammals have well-developed color vision, and locate fruits primarily by sight (Dominy et al. 2003; Smith et al. 2006; Schaefer et al. 2007), whereas fruit bats and other nocturnal mammals have an acute sense of smell, and locate fruits primarily by olfaction (Rieger and Jakob 1988; Laska 1990a, b; Acharya et al. 1998; Thies et al. 1998; von Helversen et al. 2000; Luft et al. 2003; Sanchez et al. 2004, 2006; Korine and Kalko 2005). Thus, many fig species are likely to be more specialized in their interactions with frugivores than previously thought (Kalko et al. 1996; Shanahan et al. 2001b; Hodgkison et al. 2003). The chemical compounds that mediate the interactions between figs and their vertebrate seed dispersers are currently unknown, although high levels of ethanol, which are often associated with overripe fruits, may act as a feeding deterrent to fruit bats (Sanchez et al. 2004).

The goals of this study were to investigate the fruit odors of two bat-dispersed fig species (*Ficus*) in the Paleotropics, in relation to the foraging behavior of fruit bats (Pteropodidae). We tested the following hypotheses: 1) fruit odor plays a critical role for the detection and selection of ripe figs by fruit bats; 2) bat-dispersed figs are characterized by

the same, or similar, chemical compounds; and 3) total scent production, in bat-dispersed figs, increases significantly when fruits ripen. We performed bioassays to test the effect of both natural and synthetic fruit odors on the foraging behavior of the short-nosed fruit bat, *Cynopterus brachyotis* (Müller), an important disperser of fig species within the study area (Hodgkison et al. 2003).

Methods and Materials

Study Site The field work was conducted at Kuala Lompat (3°43'N, 102°17'E), within the Krau Wildlife Reserve, Pahang, Peninsular Malaysia between March 2005 and August 2006. The Krau Wildlife Reserve consists of a large area of old-growth forest, which rises from 50 m (asl) at Kuala Lompat, to over 2000 m at the summit of Gunung Benom. The vegetation at Kuala Lompat can be described as lowland evergreen mixed dipterocarp forest (Raemaekers et al. 1980). However, compared to many other lowland dipterocarp forest sites in Malaysia, most of which have now been cleared for agriculture, this site is unusually rich in large Leguminosae. The site is also extremely rich in strangler and free-standing figs (*Ficus* spp.), with 41 species reported to date, at least 25 of which are consumed by birds and 10 by bats, with little overlap (Lambert 1987, 1989; Lambert and Marshall 1991; Hodgkison et al. 2003). The majority of fig species consumed and dispersed by birds at Kuala Lompat are characterized by visually conspicuous fruit displays that are produced as a result of distinct color changes upon ripening, often following the transition from green or yellow-green to bright orange or scarlet red (Lambert 1987, 1989). Figs consumed and dispersed by bats, by contrast, tend to be visually less conspicuous to humans, typically yellow-green, or dark red-brown, but are often characterized by the production of faint or strong odors when ripe. Several bat-dispersed fig species at Kuala Lompat are also caulicarpic, producing fruits on woody knobs or long runners that grow directly from the main trunk (Hodgkison et al. 2003).

The bat-dispersed species selected for sampling in the present study contrast in both color and odor production when ripe. *Ficus hispida* Linn. f. has a distinct color change upon ripening, from green to yellow, and emits a strong fruity odor as an attractant. *Ficus scortechinii* King, by contrast, exhibits only a slight color change upon ripening, from dark red-brown to slightly paler red-brown, and produces an odor, which, to a human nose, is almost completely imperceptible.

Fruit Odor Sampling Fruit odors of bat-dispersed figs were sampled in the field by using dynamic headspace adsorption techniques (Dobson et al. 2005; Kalko and Ayasse *in press*). Both ripe and unripe fruits were removed from plants and placed inside glass sample chambers, all of which were connected by equal lengths of silicon tubing to a single battery-operated pump (SKC, type 224). Air drawn into each glass sample chamber through a single inlet was filtered and cleaned of atmospheric pollutants by a cylindrical borosilicate glass cartridge packed with activated charcoal (Orbo-32, Supelco), and plugged with silanized glass wool (Raguso and Pellmyr 1998). Air drawn out of each chamber, through a single outlet, was sampled for volatile fruit odor compounds, by a sorbent tube containing a thin layer of activated charcoal (CLSA-Filter 1.5 mg). Both the sorbent tubes and charcoal filters were connected to the glass sample chambers by short lengths of Teflon tubing.

In total, four glass sample chambers were used to collect fruit odors during each sampling session. This allowed up to three fruits to be sampled simultaneously, along with a blank control. Each sampling session was conducted at night (between 1930 and 0500 hours)

with a flow rate of 100 ml min^{-1} per chamber and a total sampling time of 4 hr for *F. hispida* and 5 hr for *F. scortechinii*.

After sampling, all sorbent tubes were wrapped in aluminum foil, labeled, and sealed in airtight glass jars, with Teflon-coated lids, and then stored at -18°C . The samples were eluted the following day with 0.040 ml of 10:1 pentane/acetone. Eluted samples were sealed in small airtight borosilicate glass specimen tubes and returned for storage 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), and placed in a sterilizing oven set at 150°C for 1 hr. 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 Analysis of Compounds For quantitative analyses, $0.1 \mu\text{g}$ of *n*-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 ($30 \text{ m} \times 0.25 \text{ mm i.d.}$) 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. , $0.25 \mu\text{m}$ film thick, SGE). Mass spectra (70 eV) were recorded in full scan mode. Retention indices were calculated from a homolog series of *n*-alkanes. Esters and alcohols were purchased (Sigma-Aldrich, Schnellendorf, Germany) when commercially available. If not, target compounds were synthesized by conventional esterification by using an acid chloride and the respective alcohol under Schotten–Baumann conditions (Autorenkollektiv 1999). α -Bergamotene was identified by comparing the mass spectrum and retention index with the data of a critically evaluated database (Joulain and König 1998).

Behavioral Experiments Six behavioral experiments were conducted. The first set consisted of simple fruit detection tests, designed to assess the relative effect of three cues (color, shape, and odor) on the foraging behavior of captive bats. This involved four separate feeding trials, in which each bat was exposed to the following sequence of treatments: 1) ripe fruits, without modification; 2) unripe fruits, without modification; 3) ripe fruits, wrapped in a triple layer of 15 denier black nylon stocking; and 4) ripe fruit pulp smeared on an adhesive polyester patch and covered with a double layer of black stocking. The second set of experiments were two-choice feeding trials, designed to test the hypothesis that bats use fruit odors to distinguish between ripe and unripe fruits. This involved two separate feeding trials in which each bat was offered the following options: 1) an unripe fruit and a ripe fruit; and 2) two unripe fruits, one of which had been artificially scented with ripe fruit pulp. Bioassays were performed to test the effect of both natural and synthetic fruit odors on the foraging behavior of captive bats.

For bioassays, each bat was presented with a single unripe fig (control) or an unripe fruit that had been artificially scented with either a natural ripe fruit odor sample of *F. hispida* (collected by dynamic headspace adsorption techniques—see above) or a synthetic blend of the available compounds present and identified in the ripe fruit odor of the same species. The composition of the synthetic blend was mixed according to the relative proportions identified by gas chromatography. As the enantiomeric compositions of the chiral compounds were unknown, the synthetic blend used racemic (50:50) mixtures of both

enantiomers. The synthetic blend was diluted and applied to the figs at the same concentration as the dynamic headspace samples, using the solvent pentane. This concentration was equivalent to two fruits sampled for 4 hr. Compounds included in the synthetic blend are listed in Table 1. Control bioassays were also performed with pure pentane with no added ripe fruit odors (Nolte and Mason 1998).

All behavioral experiments and bioassays were conducted in a large flight cage (3×3×2.5 m-width × length × height), with newly captured and, hence, naïve bats. Bats were

Table 1 Comparisons of the relative proportions of 22 compounds present in the ripe and unripe fruit odors of *Ficus scortechinii* and *Ficus hispida*

# Compound	Mean Relative Amount (%)									
	<i>Ficus scortechinii</i>					<i>Ficus hispida</i>				
	Unripe (N=12)		Ripe (N=12)			Unripe (N=10)		Ripe (N=21)		
	Mean	SD	Mean	SD	U ^a	Mean	SD	Mean	SD	U ^a
1) Compound A	0.000	0.000	0.000	0.000	72	0.015	0.048	0.124	0.235	61
2) 2-Pentyl acetate ^b	3.948	5.319	0.425	0.611	56	3.137	4.892	13.638	7.884	20***
3) (<i>E</i>)-Pent-3-en-2-yl acetate ^b	2.436	8.438	0.000	0.000	66	0.000	0.000	6.966	5.692	5***
4) 2-Heptanone ^b	22.109	20.322	7.238	2.433	57	24.207	24.311	29.135	15.318	70
5) 2-Heptanol ^b	0.398	0.891	10.058	5.557	15***	0.559	1.184	11.937	7.113	7***
6) (<i>Z</i>)-Hex-3-enyl acetate ^b	0.000	0.000	0.000	0.000	72	0.298	0.632	0.282	0.469	54*
7) 2-Pentyl butanoate ^b	0.000	0.000	0.006	0.021	66	0.360	0.758	1.256	1.691	37**
8) 2-Heptyl acetate ^b	2.903	4.644	30.203	5.481	0***	2.149	2.383	21.472	14.020	22***
9) 2-Pentyl 2-methylbutyrate ^b	0.000	0.000	0.000	0.000	72	2.093	2.506	0.403	0.708	92
10) Furanoid linalooloxide	2.594	3.229	0.120	0.270	63	3.987	3.053	0.220	0.353	39**
11) 2-Nonanone ^b	0.450	1.492	8.233	1.439	0***	0.372	0.610	2.350	2.701	32**
12) 2-Nonanol ^b	25.923	15.108	12.329	5.171	27**	28.803	18.141	1.163	1.579	0***
13) Unknown Acetate	0.527	0.999	0.003	0.009	58	0.484	0.811	0.462	0.516	56
14) Unknown unsaturated Acetate	0.028	0.096	0.000	0.000	56	4.730	7.588	0.206	0.088	80
15) Unknown Acetate 2	0.700	1.285	0.020	0.038	58	2.127	2.810	0.216	0.361	98
16) 2-Pentyl hexanoate ^b	1.468	1.350	0.125	0.283	37*	1.694	1.179	0.196	0.307	22***
17) (<i>E</i>)-Pent-3-en-2-yl hexanoate ^b	0.720	0.974	0.138	0.262	60	1.272	1.072	0.106	0.113	37**
18) 2-Nonyl acetate ^b	5.444	9.740	25.793	9.130	11***	0.108	0.244	1.371	1.771	20***
19) 2-Undecanone ^b	0.354	0.843	0.261	0.247	34*	0.632	1.686	0.619	0.914	43*
20) α -Bergamotene	24.120	24.056	1.393	1.993	1***	11.865	9.260	1.705	4.739	30**
21) Compound B	5.354	6.071	2.371	2.992	52	10.701	7.614	5.189	8.132	51*
22) Compound C	0.524	1.031	1.285	0.947	27**	0.407	0.930	0.986	1.375	31**

^a Compounds that increase or decrease significantly in their relative proportions when fruits ripen, Mann-Whitney *U* test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

^b Compounds included in the synthetic mixture of *F. hispida*

obtained by using ground and canopy mist nets within the study area at Kuala Lumpur (Hodgkison et al. 2002; Kunz et al. *in press*). To facilitate the capture of specific fruit bat species that consume figs, mist nets were positioned adjacent to fruiting fig trees, opened at dusk (c. 1900 hours), and checked every 15 min for captured bats—with one individual bat retained for behavioral experiments or bioassays during each night of sampling. All newly captured bats were introduced to the flight cage on the night of capture, and then left undisturbed, with an abundant supply of food, until the following day. Immediately before dusk the following evening, any uneaten fruits were removed from the flight cage to prevent bats from becoming distracted during experiments.

Behavioral reactions of bats were recorded on videotape with the “nightshot” facility of a Sony digital video camera (Handycam DCR-PC350E). The effect of each treatment was quantified by the presence or absence of four types of behavioral response: circling, approaching, landing, and feeding. An “approach” was differentiated from circling, both by its greater precision in relation to the position of the fruit, and by a perceptible reduction in flight speed. A “feeding” attempt was scored when a bat attempted to bite and remove a fruit. Landing always occurred before feeding, and no fruit bat was observed to remove a fruit while in flight. To motivate the bats throughout the experiments, each bioassay was preceded by a feeding trial with a reward of one ripe fruit (*F. hispida*). Each experiment lasted for 20 min, with each individual bat exposed to up to six behavioral experiments or four bioassays per night. All fruits were presented to the bats in a natural way, by using wires to fix them to the trunks of small trees incorporated into the sides of the cage. To prevent bats from learning the spatial arrangements within the cage, all fruits were presented in different locations during each successive trial (Nolte and Mason 1998). The only artificial light source was an infrared beam emitted from the camera.

When experiments were completed, all bats were recaptured in the flight cage with a hand net, marked with an individually numbered stainless steel ball chain necklace (Ball-Chain Manufacturing Company, NJ, USA), and then released at the site of capture (Kunz and Weise *in press*). This ensured that no individual bat was exposed to a single experiment or bioassay on more than one occasion.

Data Analysis Total scent production and the relative amounts of each compound produced by the ripe and unripe fruits of *F. hispida* and *F. scortechinii* were compared by using the Mann–Whitney *U* test. The relative amounts of all compounds commonly found in both species of *Ficus* were compared with a discriminant function analysis (DFA). The standardized discriminant function coefficients and the factor loadings of each compound were used to assess the differences between the ripe and unripe fruits of both species. All statistical analyses of fruit odors were performed by using SPSS version 11.0.

The results of the behavioral experiments and bioassays were arranged on 2×2 contingency tables to express the observed frequencies of the four behavioral reactions in response to *n* treatments (i.e., approach vs. no approach × *n* treatments, and feeding vs. no feeding × *n* treatments). These results were analyzed with Fisher’s Exact Test of independence (Sokal and Rohlf 1995).

Results

Chemical Composition of Fruit Odors Several odor compounds were identified by GC/MS by comparing their mass spectra and retention times with those of authentic reference

samples. This revealed a series of odd numbered 2-alkanones, 2-alkanols, and their respective acetates, butanoates, and hexanoates (Table 1). Compound 3 (Table 1) showed a mass spectrum similar to that of pent-3-en-2-ol. However, a higher molecular mass, by 42 Da, and a more intense ion m/z 43 suggested an acetate. Comparisons with synthetic samples of all possible pentenol acetates revealed this compound to be (*E*)-pent-3-en-2-yl acetate (Fig. 1). The corresponding hexanoate was identified by using the same method. Compound 9 (Table 1) proved to be an ester of 2-pentanol with a C_5 -acid. Comparisons with authentic reference samples of 2-pentyl pentanoate, 3-methylbutyrate, and 2-methylbutyrate revealed the natural compound to be the latter. The only terpenes found were the furanoid form of linalooloxide and α -bergamotene. Structures of the identified compounds are shown in Fig. 2.

A total of 16 compounds were identified in the ripe fruit odor of *Ficus hispida*, whereas six additional compounds remained unidentified. Twelve of the identified, and four of the unidentified, compounds also occurred in the ripe fruit of *Ficus scortechinii* (Table 1), producing a reduced but similar bouquet (Fig. 3). 2-Heptyl acetate was particularly prominent in the ripe fruit odors of both species, and accounted for ca. 30% of total scent production in *F. scortechinii*, and 21% in *F. hispida* (Table 1). Other prominent compounds included 2-hepanol and 2-heptanone (Table 1).

Total scent production of ripe fruits was significantly greater than that of unripe fruits (Fig. 4), and included a greater number of compounds (Fig. 3). On average, total scent production per fruit of *F. scortechinii* increased from $0.008 \pm 0.017 \mu\text{g hr}^{-1}$ (mean \pm SD) to $0.148 \pm 0.162 \mu\text{g hr}^{-1}$ upon ripening ($N=12$ in both cases); whereas total scent production per

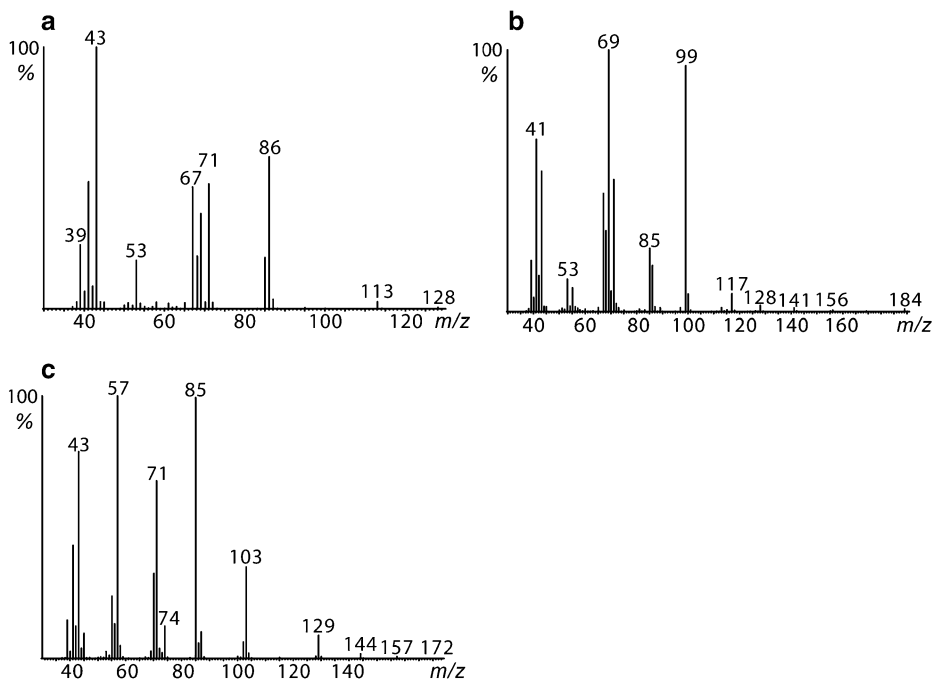
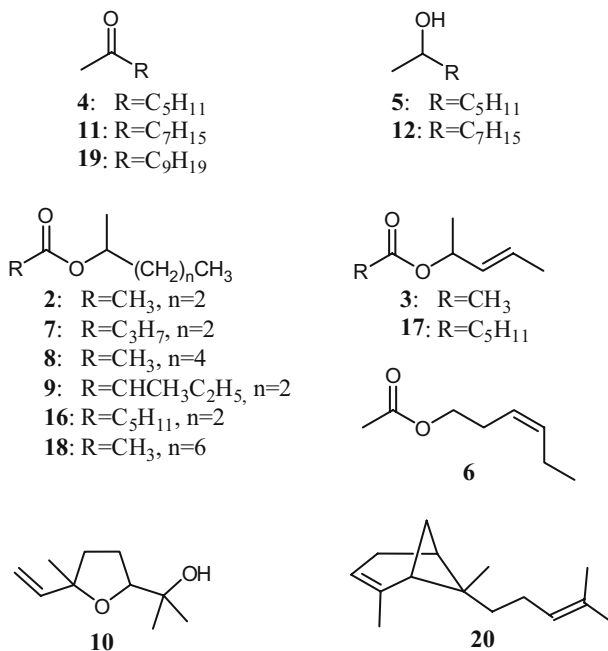


Fig. 1 Mass spectra of (*E*)-pent-2-en-3-yl acetate (a), (*E*)-pent-2-en-3-yl hexanoate (b), and 2-pentyl 2-methylbutyrate (c)

Fig. 2 Structures of compounds identified in the ripe fruit odors of *Ficus scortechinii* and *Ficus hispida*. Numbers refer to entries in Table 1



fruit of *F. hispida* increased from $0.017 \pm 0.020 \mu\text{g hr}^{-1}$ to $0.507 \pm 0.546 \mu\text{g hr}^{-1}$ ($N=10$ and 20 , respectively). Relative amounts of several compounds also increased (e.g., 2-heptanol and 2-heptyl acetate) or decreased significantly (e.g., 2-nonanol and α -bergamotene) when fruits ripened (Table 1). No compounds were associated exclusively with unripe fruits of either fig species.

The discriminant function analysis (DFA) of fruit scents produced four chemically distinct clusters, which corresponded with the ripe and unripe fruits of both fig species (Fig. 5). The first discriminant function, which accounted for 66.5% of the total variance, was mainly important for discriminating between the unripe fruits of *F. scortechinii* and the ripe fruits of *F. hispida* (DF1: Chi-square=267, $df=45$, $P<0.001$). The second function, which accounted for 31.6% of the total variance, was mainly important for discriminating between the ripe fruits of *F. scortechinii* and the unripe fruits of *F. hispida* (DF2: Chi-square=129, $df=28$, $P<0.001$). The third function was not statistically significant. Compounds with the highest standardized canonical discriminant function coefficients were pentyl acetate, compound 15, and (*E*)-pent-3-en-2-yl hexanoate (in DF1), and 2-undecanone and compound 22 (in DF2). Of the 54 samples used in the DFA, 53 (98.1%) were correctly assigned to groups—with a cross-validated accuracy rate of 83.3%.

Fruit Bat Foraging Behavior Fruit bats responded to both visual and chemical (olfactory) cues when foraging for fruits. However, in the absence of a ripe fruit scent, the proportion of individuals that approached, or attempted to land or feed on unripe fruits, was significantly lower than on the ripe fruit control ($P>0.01$, in each case). Of the 20 individual bats tested, only 70% of individuals approached unripe fruits, 15% attempted to land, and only 5% attempted to feed ($N=20$). By contrast, approach rates of 100% were recorded for all scented treatments, with landing and feeding rates ranging from 95 to 100% (Table 2). In two-choice feeding trials, bats showed a strong preference for ripe fruits over

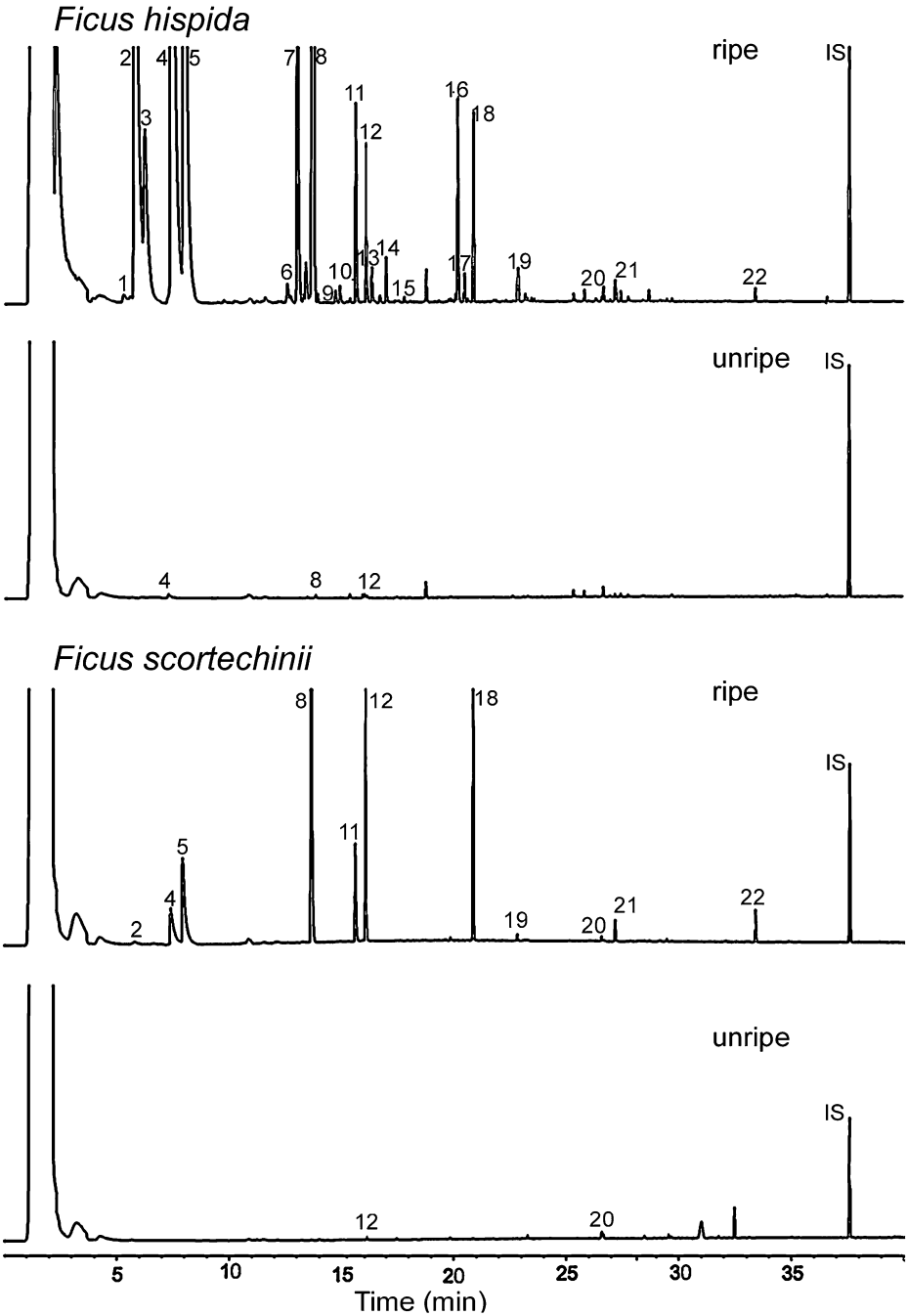
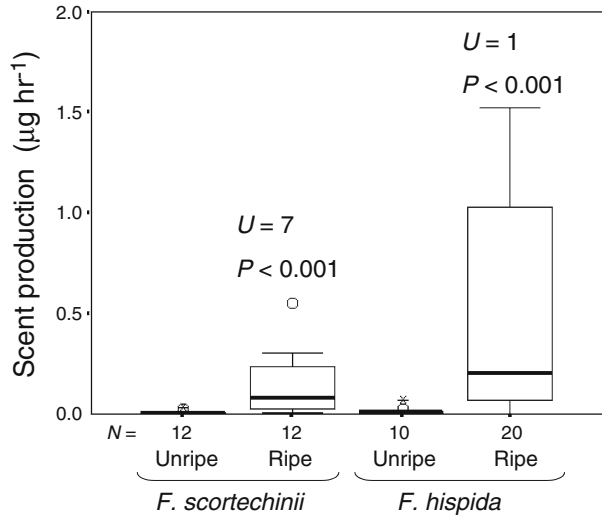


Fig. 3 Gas chromatograms of the ripe and unripe fruit odors of *Ficus hispida* and *Ficus scortechinii*. All labels correspond with the numbers in Table 1. IS = internal standard (octadecane). N.B. compounds 10, 15, 16, and 17 occurred only in trace amounts in *F. scortechinii*

Fig. 4 Total scent production in ripe vs. unripe fruits of *Ficus scortechinii* and *Ficus hispida*. Included are medians, mid-quartiles, 90th and 10th percentiles and outliers. There was a significant increase in total scent production in both species when fruits ripened (Mann–Whitney U test)



unripe fruits, and scented (i.e., manipulated) unripe fruits over unscented (i.e., unmanipulated) unripe fruits (Table 3). Responses to ripe and manipulated unripe fruits, smeared with ripe fruit pulp, were not significantly different ($P=0.43$), with feeding attempts by 100 and 93% of the individuals tested, respectively (Table 3).

Feeding attempts by fruit bats were elicited by both natural fruit odors and synthetic blends. However, a greater proportion of bats reacted to natural headspace samples of *F. hispida* than to synthetic blends of the same species (55 and 15% respectively, $N=20$, $P=0.019$). Compared to the two unscented controls, the synthetic blend of *F. hispida* had no significant affect on the proportion of bats attempting to feed ($P=0.23$). Of the 20 bats tested, only 15% attempted to feed on fruits treated with the synthetic blend, whereas no bats attempted to feed on untreated unripe fruits or on unripe fruits treated with pure pentane.

Fig. 5 Discriminant Function Analysis of the ripe and unripe fruit odors of *Ficus hispida* (*Fi. hi.*) and *Ficus scortechinii* (*Fi. sc.*). This analysis was based on the relative proportions of 15 compounds that commonly occurred in the headspace samples of both species (see Fig. 3 and Table 1). Both discriminant functions accounted for 98.1% of the total variance

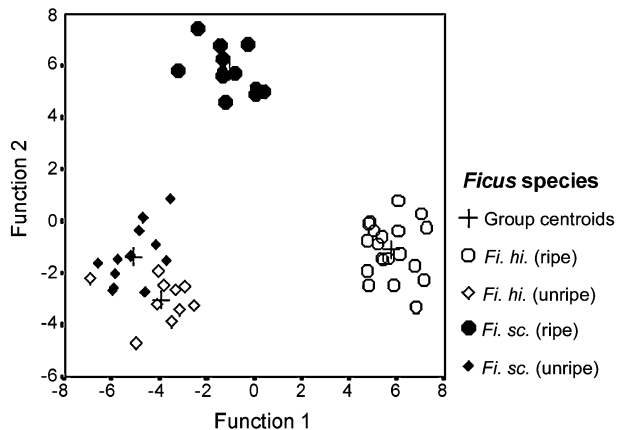


Table 2 The relative effect of three cues (color, shape, and odor) on the foraging behavior of four species of pteropodid bat—*Balionycteris maculata* ($N=1$), *Chironax melanocephalus* ($N=1$), *Cynopterus brachyotis* ($N=18$), and *Cynopterus horsfieldi* ($N=2$)

Treatment	Sensory Cue	N	Frequency of Behavioral Response (%)			
			Circling	Approaching	Landing	Feeding
1) Ripe fruit	Color, shape, odor	22	100	100	100	100
2) Unripe fruit	Color, shape	20	85	70	15	5
3) Ripe fruit in black stocking	Shape, odor	20	100	100	95	95
4) Smearred fruit pulp	Odor	12	100	100	100	100

^a N indicates the number of individual bats tested

Discussion

Fruit bats responded to both visual and chemical (olfactory) cues when foraging for figs. However, the strongest foraging reaction, resulting in a landing or feeding attempt, was almost exclusively associated with the presence of a ripe fruit odor, either in combination with visual cues, or when presented on its own. Thus, no visual cues were required for a fruit bat to locate the source of an odor. Fruit bats also used fruit odors to distinguish between ripe and unripe fruits. These results support the conclusions of previous studies (Acharya et al. 1998; Luft et al. 2003), that Old World fruit bats use mainly chemical, rather than visual, cues for the location and acquisition of food.

A total of 16 compounds were identified in the ripe fruit odor of *F. hispida*, and 13 in the ripe fruit odor of *F. scortechinii*—including alcohols, ketones, and esters. All 13 compounds that were identified in the ripe fruit odor of *F. scortechinii* were also present in the ripe fruit odor of *F. hispida*. This large overlap in fruit odor chemistry suggests that both species use the same compounds for bat attraction. However, none of the compounds identified in the fruit odors of either species is known to be associated with the attraction of bats (Bestmann et al. 1997; von Helversen et al. 2000; Knudsen and Tollsten 1995; Knudsen et al. 1993; Pettersson and Knudsen 2001; Pettersson et al. 2004). Indeed, many compounds that are commonly associated with Neotropical bat-pollinated flowers (e.g., sulfur-containing compounds) were completely absent (Bestmann et al. 1997; von Helversen et al. 2000).

Of the 16 compounds identified in the ripe fruit odors of figs, the methyl ketones and the secondary alcohols and their acetates are common fruit odor components within a wide

Table 3 Discrimination between ripe and unripe fruits (*Ficus hispida* and *Ficus scortechinii*) by four species of fruit bat (*Balionycteris maculata*, *Chironax melanocephalus*, *Cynopterus brachyotis*, and *Cynopterus horsfieldi*) in relation to fruit odor

Treatment	N ^a	Food Choice	Frequency of Feeding Attempts (%)
1) Unripe fruit vs. ripe fruit	20	Unripe fruit	0
		Ripe fruit	100
2) Unripe fruit vs. unripe fruit smearred with ripe fruit odor (from fruit pulp)	15	Unripe fruit	0
		Unripe fruit-smearred with ripe fruit odor (from fruit pulp)	93

^a N indicates the number of individual bats tested

range of plant taxa (e.g., Laska 1990b; Jordan et al. 2001). In contrast, butyrates and hexanoates are much rarer. Interestingly, (*E*)-2-penten-3-yl esters, which were present in both species, have not, to the best of our knowledge, been reported from nature before. This suggests that a single, unique, bat-attracting compound could be present within the two fig species analyzed in this study. Alternatively, a combination of other compounds, particularly those that increase in production when fruits ripen (e.g., 2-heptanol and its acetate), could also function as attractants or markers, allowing the bats to differentiate between different fig species.

Both natural and synthetic fruit odors of *F. hispida* resulted in feeding attempts by fruit bats during bioassays. However, reaction rates to natural fruit odors were significantly higher than those displayed toward synthetic blends. Thus, the fruit bats could either detect subtle differences in chirality between the two treatments, or some additional compounds that are attractive to bats were missing from the synthetic blend. In the case of the former hypothesis, the use of racemic mixtures of 12 chiral compounds could at least partially explain the lower reaction rates of bats toward the synthetic blend used. Given the chiral nature of the receiving proteins, it seems likely that bats, and many other mammals, can distinguish between enantiomers of many different compounds (e.g., compounds 2, 3, 5, 7, 8, 9, 10, 12, 16, 17, 18, and 20, Table 1). Hence, the enantiomeric composition that occurs in nature could be of great importance to bat foraging behavior. However, the effect of enantiomeric composition on the foraging behavior of Old World fruit bats is currently unknown.

Spatial information on the precise location of fruits could also depend to some extent on the presence of low volatility compounds, such as sesquiterpenes (e.g., α -bergamotene), which were omitted from the synthetic blend used here. Compared to relatively high-volatility compounds, such as ketones and alcohols, sesquiterpenes are likely to produce a more localized plume of scent, which could allow bats to discriminate between ripe and unripe fruits situated in close proximity.

Further studies on the composition of fruit odors and the foraging behavior of fruit bats are needed to identify the key attractive compounds, and also to assess the occurrence of these compounds, more generally, within the genus *Ficus*. However, the results of this study have demonstrated the key importance of fruit odors for the mediation of interactions between bats and figs in the Paleotropics, and have also revealed many compounds of interest for future studies.

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References

- ACHARYA, K. K., ROY, A., and KRISHNA, A. 1998. Relative role of olfactory cues and certain non-olfactory factors in foraging of fruit-eating bats. *Behav. Processes* 44:59–64.
- AUTORENKOLLEKTIV. 1999. *Organikum*, 20th edition. Wiley-VCH, Weinheim.
- BESTMANN, H. J., WINKLER, L., and VON HELVERSEN, O. 1997. Headspace analysis of volatile flower scent constituents of bat-pollinated plants. *Phytochemistry* 46:1169–1172.
- BREITWISCH, R. 1983. Frugivores at a fruiting *Ficus* vine in a southern Cameroon tropical wet forest. *Biotropica* 15:125–128.

- DOBSON, H. E. M., RAGUSO, R. A., KNUDSEN, J. T., and AYASSE, M. 2005. Scent as an attractant, pp. 197–230, in A. Dafni, P. G. Kevan, and B. C. Husband (eds.). *Practical Pollination Biology*. Enviroquest Ltd., Cambridge, Ontario, Canada.
- DOMINY, N. J., SVENNING, J. C., and LI, W. H. 2003. Historical contingency in the evolution of primate color vision. *J. Hum. Evol.* 44:25–45.
- HODGKISON, R., AHMAD, D., BALDING, S. T., KINGSTON, T., ZUBAID, A., and KUNZ, T. H. 2002. Capturing bats (Chiroptera) in tropical forest canopies, pp. 160–167, in A. W. Mitchell, K. Secoy, and T. Jackson (eds.). *Techniques of Access and Study in the Forest Roof: The Global Canopy Programme Handbook*. Global Canopy Programme, Oxford.
- HODGKISON, R., BALDING, S. T., ZUBAID, A., and KUNZ, T. H. 2003. Seed dispersal by fruit bats (Chiroptera: Pteropodidae) in a Malaysian lowland rain forest. *Biotropica* 35:491–502.
- JOULAIN, D., and KÖNIG, W. A. 1998. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*, E.-B. Verlag, Hamburg.
- JORDAN, M. J., TANDON, K., SHAW, P. E., and GOODNER, K. L. 2001. Aromatic profile of aqueous banana essence and banana fruit by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). *J. Ag. Food Chem.* 49:4813–4817.
- KALKO, E. K. V., and AYASSE, M. In press. Study and analysis of odor involved in the behavioral ecology of bats, in T. H. Kunz, and S. Parsons (eds.). *Ecological and Behavioral Methods for the Study of Bats*, 2nd Edition. Johns Hopkins University Press, Baltimore.
- KALKO, E. K. V., HERRE, E. A., and HANDLEY, C. O. 1996. Relation of fig fruit characteristics to fruit-eating bats in the New and Old World tropics. *J. Biogeogr.* 23:565–576.
- KORINE, C., and KALKO, E. K. V. 2005. Fruit detection and discrimination by small fruit-eating bats (Phyllostomidae). *Behav. Ecol. Sociobiol.* 59:12–23.
- KORINE, C., KALKO, E. K. V., and HERRE, E. A. 2000. Fruit characteristics and factors affecting fruit removal in a Panamanian community of strangler figs. *Oecologia* 123:560–568.
- KNUDSEN, J. T., and TOLLSTEN, L. 1995. Floral scent in bat-pollinated plants: a case of convergent evolution. *Bot. J. Linn. Soc.* 119:45–57.
- KNUDSEN, J. T., TOLLSTEN, L., and BERGSTROM, L. G. 1993. Floral scents—a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* 33:253–280.
- KUNZ, T. H., and WEISE, C. 2008 (in press). Methods of marking bats, in T. H. Kunz and S. Parsons (eds.). *Ecological and Behavioral Methods for the Study of Bats*, 2nd Edition. Johns Hopkins University Press, Baltimore.
- KUNZ, T. H., HODGKISON, R., and WEISE, C. 2008 (in press). Methods for capturing bats, in T. H. Kunz, and S. Parsons (eds.). *Ecological and Behavioral Methods for the Study of Bats*, 2nd Edition. Johns Hopkins University Press, Baltimore.
- LAMBERT, F. R. 1987. Fig-eating and seed dispersal by birds in a Malaysian lowland rain forest. PhD dissertation, University of Aberdeen, Scotland.
- LAMBERT, F. R. 1989. Fig-eating by birds in a Malaysian lowland rain forest. *J. Trop. Ecol.* 5:401–412.
- LAMBERT, F. R., and MARSHALL A. G. 1991. Keystone characteristics of bird-dispersed *Ficus* in a Malaysian lowland rain forest. *J. Ecol.* 79:793–809.
- LASKA, M. 1990a. Olfactory sensitivity to food odor components in the short-tailed fruit bat. *Carollia perspicillata* (Phyllostomatidae, Chiroptera). *J. Comp. Physiol. A* 166:395–399.
- LASKA, M. 1990b. Olfactory discrimination ability in short-tailed fruit bat, *Carollia perspicillata* (Phyllostomatidae, Chiroptera). *J. Chem. Ecol.* 16:3291–3299.
- LEIGHTON, M., and LEIGHTON, D. R. 1983. Vertebrate responses to fruiting seasonality within a Bornean rain forest, pp. 181–196, in S. L. Sutton, T. C. Whitmore, and A. L. Chadwick (eds.). *Tropical Rain Forest Ecology and Management*. Blackwell Scientific Press, Oxford.
- LUFT, S., CURIO, E., and TACUD, B. 2003. The use of olfaction in the foraging behaviour of the golden-mantled flying fox, *Pteropus pumilus*, and the greater musky fruit bat, *Ptenochirus jagori* (Megachiroptera: Pteropodidae). *Naturwissenschaften* 90:84–87.
- NOLTE, D. L., and MASON, J. R. 1998. Bioassays for mammals and birds, pp. 327–395, in K. F. Haynes, and J. G. Millar (eds.). *Methods in Chemical Ecology*, Vol. 2. Bioassay Methods. Kluwer Academic Publishers, Boston.
- PETERSSON, S., and KNUDSEN, J. T. 2001. Floral scent and nectar production in *Parkia biglobosa* Jacq. (Leguminosae: Mimosoideae). *Bot. J. Linn. Soc.* 135:97–106.
- PETERSSON, S., ERVIK, F., and KNUDSEN, J. T. 2004. Floral scent of bat-pollinated species: West Africa vs. the New World. *Biol. J. Linn. Soc.* 82:161–168.
- RAEMAEKERS, J. J., ALDRICH-BLAKE, F. P. G., and PAYNE, J. B. 1980. The forest, pp. 29–61, in D. J. Chivers (ed.). *Malayan Forest Primates*. Plenum Press, New York.

- RAGUSO, R. A., and PELLMYR, O. 1998. Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos* 81:238–254.
- RIEGER, J. F. and JAKOB, E. M. 1988. The use of olfaction in food location by frugivorous bats. *Biotropica* 20:161–164.
- SANCHEZ, F., KORINE, C., PINSHOW, B., and DUDLEY, R. 2004. The possible roles of ethanol in the relationship between plants and frugivores: first experiments with Egyptian fruit bats. *Integr. Comp. Biol.* 44:290–294.
- SANCHEZ, F., KORINE, C., STEEGHS, M., LAARHOVEN, L. J., CRISTESCU, S. M., HARREN, F. J. M., DUDLEY, R., and PINSHOW, B. 2006. Ethanol and methanol as possible odor cues for Egyptian fruit bats (*Rousettus aegyptiacus*). *J. Chem. Ecol.* 32:1289–1300.
- SCHAEFER, H. M., SCHAEFER, V., and VOROBYEV, M. 2007. Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? *Am. Nat.* 169 (supplement):159–169.
- SHANAHAN, M., and COMPTON, S. G. 2001. Vertical stratification of figs and fig-eaters in a Bornean lowland rain forest: how is the canopy different? *Plant Ecol.* 153:121–132.
- SHANAHAN, M., HARRISON, R. D., YAMUNA, R., BOEN, W., and THORNTON, I. W. B. 2001a. Colonization of an island volcano, Long Island, Papua New Guinea, and an emergent island, Motmot, in its caldera lake. V. Colonization by figs (*Ficus* spp.), their dispersers and pollinators. *J. Biogeogr.* 28:1365–1377.
- SHANAHAN, M., SO, S., COMPTON, S. G., and CORLETT, R. 2001b. Fig-eating by vertebrate frugivores: a global review. *Biol. Rev.* 76:529–572.
- SMITH, A. C., BUCHANAN-SMITH, H. M., SURRIDGE, A. K., OSORIO, D., and MUNDY, N. I. 2006. The effect of colour vision status on the detection and selection of fruits by tamarins (*Saguinus* spp.). *J. Exp. Biol.* 206:3159–3165.
- SOKAL, R. R., and ROHLF, F. J. 1995. Biometry. 3rd Edition. W.H. Freeman and Company, New York.
- THIES, W., KALKO, E. K. V., and SCHNITZLER, H.-U. 1998. The roles of echolocation and olfaction in two Neotropical fruit-eating bats, *Carollia perspicillata* and *C. castanea*, feeding on *Piper*. *Behav. Ecol. Sociobiol.* 42:397–409.
- VON HELVERSEN, O., WINKLER, L., and BESTMANN, H. J. 2000. Sulphur-containing “perfumes” attract flower-visiting bats. *J. Comp. Physiol.* 186:143–153.