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Sulphur-containing “perfumes” attract flower-visiting bats

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Abstract We tested the attractiveness of individual scent compounds of bat-pollinated flowers to their pollinators, small flower-visiting bats of the genus *Glossophaga* (Phyllostomidae). Twenty compounds belonging to four different chemical substance classes were tested, both in the laboratory and in the field. In the laboratory, the bats (*Glossophaga soricina*) approached odour sources spontaneously and without preceding experience. Without ever receiving any reward they preferred the scent of a sulphur-containing compound, dimethyl disulphide, to several other odour components emitted by bat-pollinated flowers, and to scentless controls. In the field, at La Selva station in the tropical lowland rain forest of Costa Rica, *G. commissarisi* were attracted by two sulphur-containing compounds, dimethyl disulphide and 2,4-dithiapentane, to visit artificial flowers filled with sugar water. Thus, in nectarivorous bats the sense of smell obviously plays an important role in searching for and localising food sources, and even single components of the scent bouquets of bat-pollinated flowers are attractive. The preference for sulphur-containing odours seems to be innate.

Key words Bat pollination · Olfactory orientation · Floral scents · Sulphur compounds · Dimethyl disulphide

Introduction

In the Old and New World tropics a considerable number of plant species are pollinated by flower visiting bats. Dobat (1985) counted more than 750 plant species in over 270 genera, and since then many more species have been discovered, especially in the Neotropics. These plants show a syndrome of adaptive traits (Vogel 1968, 1969a, b; Faegri and van der Pijl 1971; von Helversen 1993), amongst others a typical, sometimes intense, floral scent which is quite different from the smell of flowers visited by other pollinators, e.g. bees, moths or flies.

Even in the early studies of bat pollination, this peculiar flower scent was considered to be one of the most important indications for chiropterophily (Porsch 1931; van der Pijl 1936; Vogel 1958, 1968, 1969a, b). The following examples show how difficult it seems to describe these characteristic odours verbally and that most of the scents are perceived as unpleasant by humans: “peculiar, somewhat reminiscent to cabbage” (*Musa*, van der Pijl 1936); “unpleasantly sour” (*Ceiba pentandra*, van der Pijl 1936); “displeasent like mouse urine” (*Bassia latifolia*, van der Pijl 1936); “mild but skunk-like” (*Lemaireocereus thurberi*, Alcorn and Olin 1961); “smell of carcass” (*Adansonia digitata*, Porsch 1935; and *Weberocereus tunilla*, Bauer 1991).

Chemical analyses of floral scents of bat-pollinated plants have been carried out only recently for a small number of species (Kaiser and Tollsten 1995; Knudsen and Tollsten 1995; Bestmann et al. 1997). The most striking result of these studies was the presence of large amounts of sulphur-containing compounds in most of the scent bouquets analysed. Since these sulphur compounds (particularly dimethyl disulphide, dimethyl trisulphide and dimethyl tetrasulphide) are produced by many bat-pollinated plant species which are not related to each other, and are not or scarcely present in other floral scents (Knudsen et al. 1993), sulphur-containing flower scents seem to be the result of a convergent

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evolution within different plant families as an adaptation to bat pollination (Knudsen and Tollsten 1995).

The function of flower scents is to attract specific pollinators. Thus, these scents have to match the sensory capabilities of the respective pollinators.

Anatomical and physiological studies of olfactory organs (Bhatnagar and Kallen 1974, 1975) and the respective brain areas (Mann 1960; Stephan and Pirlot 1970; Baron 1973) showed that the development of olfactory structures and the feeding habits of bats are related. Frugivorous and nectarivorous bats have larger nasal epithelia and larger bulbi olfactorii and their sense of smell is more highly developed as compared to insect feeding bats. Also, the results of training experiments revealed low olfactory thresholds in *Desmodus*, *Phyllostomus*, *Artibeus* and *Carollia* (Schmidt and Greenhall 1971; Schmidt 1973, 1975, 1984; Laska 1990). This indicates that olfactory orientation is of special importance for foraging in these bats, a fact confirmed by direct observations: frugivorous and nectarivorous bats are able to locate food sources using olfactory cues alone (Möhres and Kulzer 1956; Vogel 1958; Schmidt 1984; Laska and Schmidt 1986; Rieger and Jakob 1988; Kalko et al. 1996; own observations).

The aim of the present study was to find out how nectar-feeding bats use their sense of smell to recognise and localise flowers, their primary source of food. In the laboratory, as well as in the field, we tested single floral scent compounds of bat-pollinated plants to find out which of these compounds or "smell types" are attractive.

Materials and methods

Laboratory experiments

Animals

Two groups of *Glossophaga soricina* (Glossophaginae: Phyllostomidae), which are kept in the Zoological Institute of the University of Erlangen in two air-conditioned greenhouses (area ca. 30 m² each), were used in our experiments. In these rooms, the bats could fly around completely unhindered. All animals were born in captivity. They were nourished at artificial "flowers" which are feeding bowls filled with watery solutions of honey, a hummingbird feed (NEKTAR-Plus), a nutritive complement (NutriComp) and suspensions of pollen. None of the above emits volatile components containing sulphur. Two groups of *Glossophaga* bats were used for the experiments, group 1 consisting of about 65 animals, descended from a few individuals caught near Caracas/Venezuela and in Jamaica, and group 2 consisting of about 100 bats, descended from animals caught in Puebla/Mexico.

Test apparatus

The test apparatus consisted of a tripod (height ca. 1.5 m) with a horizontally rotating sample holder on the top, which consisted of eight radially arranged metal rods. Specimen tubes containing the scent compounds to be tested (each filled with 200 µl of pure liquid substance), were fixed with metal clamps at the ends of the rods. The sample holder was slowly but continuously rotated by an electric motor. Twenty scent compounds were selected from the results of our own previous headspace analyses of the scent com-

pounds of bat-pollinated flowers (Bestmann et al. 1997; for method see also Brunke et al. 1992). The specimen tubes were closed by screw caps, through the centre of which a hole had been bored and fitted with a fine-meshed wire net. Thus, free diffusion of the scent compounds into the environment was possible while the animals were prevented from coming into direct contact with the substances.

The flight behaviour of the animals and the approaches to the specimen tubes were recorded by an infrared-sensitive video camera connected to a video recorder. Subsequently, the bat's visits to each specimen tube were counted. When a bat's snout was observed to be at a distance of 2 cm or less from the specimen tube, this was counted as a "visit". Discrimination between individual bats was not possible.

Experimental procedure

The test apparatus was installed 5 days before the beginning of a series of tests in order to familiarise the animals with the new object. For an experiment, six of the eight specimen tubes of the test apparatus were filled with pure scent compounds, and the two remaining specimen tubes were empty and served as a scentless control. Thus, in each single trial, six scents could be offered simultaneously to the animals. In total, 20 different flower scent compounds were tested on 52 nights; in each trial the scent compounds were arranged in new combinations. A total of 7551 approaches were counted (corresponding to an average number of 145 approaches per night).

Since we wished to observe the spontaneous reaction of the bats to the scents, it was very important to avoid training them to the scents or the positions of the scent sources. For this reason, the approaches to the scent sources were not rewarded and the sample holder was rotated. The direction of the rotation was changed every 2.5 min. The duration of the tests were limited to 15 min per night, as the bats' interest in the scents weakened about 10–15 min after the beginning of the test due to the lack of rewards.

The relative preference of a scent was determined as follows: in every trial a "preference factor" was calculated for each of the tested substances, which describes the preference for the respective scent when compared with the scentless control tubes. This factor was derived by dividing the number of approaches to the scent by the average number of approaches to both of the non-smelling samples. Thus, for each scent compound 13–14 values were obtained over the complete experiment, which were averaged to obtain an average preference factor.

Field experiments

Study site

The field experiments were carried out at La Selva Biological station. The station is located in the Atlantic rain forest at the confluence of the Río Sarapiquí and the Río Puerto Viejo in the province of Heredia, Costa Rica, and operated by the Organization for Tropical Studies (OTS).

Animals

The most abundant bat species at the study sites was *G. commisaris*; all photographs taken at the artificial flowers showed this species. It cannot be excluded, however, that individuals of *Hylomycteris underwoodi* and *Lichonycteris obscura* also visited the artificial flowers.

Field experiment 1

Experimental procedure

Fourteen artificial flowers were set up along the edge of a rain-forest clearing at regular intervals of about 10 m. The artificial

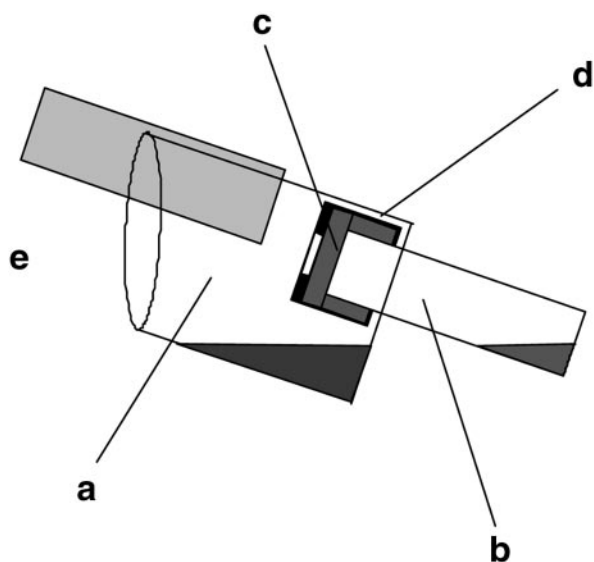


Fig. 1 Schematic drawing of an artificial flower for field experiments. *a* plastic cylinder containing sugar water; *b* specimen tube with liquid scent compound; *c* fine-meshed wire net; *d* bored screw cap; *e* plastic cover over the opening of the artificial flower to prevent rain water running into the cylinder. *Photo: Glossophaga commissarisi* visiting an artificial flower (at dimethyl disulphide; field experiment November 1995, La Selva/Costa Rica)

flowers (Fig. 1) consisted of small plastic cylinders closed on one side (length 40 mm, 30 mm inner diameter). A specimen tube (length 40 mm, 15 mm diameter) filled with 1 ml of a scent compound was inserted through a hole at the bottom of the cylinder. The specimen tube was closed from the inner side of the cylinder by a screw cap with a hole bored through and fitted with a fine-meshed wire net. A plastic cover was fixed over the opening of the cylinder to prevent rain water from running into the interior. These "flowers" were fixed at the ends of wooden sticks (length 1.5 m) using metal clamps, so that they could easily be removed and changed. One of the 14 artificial flowers was always scentless. At the beginning of a test night (17 00 hours) the flowers were filled with 1 ml of sugar water (20% w/w concentration); the amount of sugar water remaining in each tube was measured on the following morning. The wooden sticks were treated with Tanglefoot Insect Trap to prevent ants invading the flowers. To avoid learning of a position, the positions of the scents relative to each other were changed every night. In addition, bat approaches were documented photographically.

Field experiment 2

Experimental procedure

Eight artificial flowers were set up along the edge of a rain forest clearing (setup and scent amounts as for field experiment 1) at regular intervals of ca. 1.5 m. An infrared photoelectric light trap was installed directly in front of the flower's opening, so that a bat visit led to an interruption of the light beam. The time and the duration of the interruptions were registered by a computer. The number of artificial flowers in this test series had to be limited to eight as the computer used was equipped with only eight data channels. The wooden sticks were again treated with Tanglefoot Insect Trap. The experiment was run for 8 nights (11.12.96–18.12.96). Occasionally bat visits were directly observed with a night-vision scope.

In all field experiments the bats had to be rewarded; otherwise the visits would have been too rare to give statistically significant results. At the beginning of the night the flowers were filled with 1 ml of sugar water (20% w/w concentration). Every night, the relative positions of different scent stimuli were changed. To reduce the effect of training to the less attractive scents, 2–3 nights with reward were followed by 1–2 nights without reward. On these nights all approaches of bats to the (now empty) flowers were also registered.

Results

Plants and scent composition

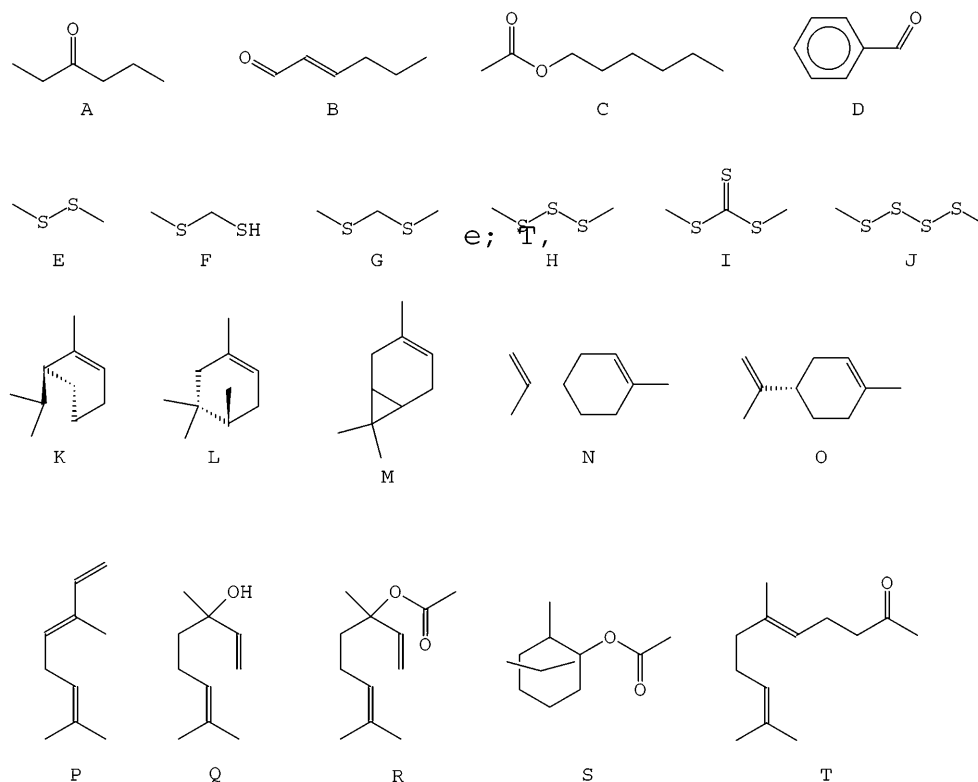
In La Selva (Atlantic rain forest, Costa Rica) a total of 21 bat-pollinated plant species is known (M. Tschapka, personal communication). Data on floral scent analyses exist for the following eight of these species (Bestmann et al. 1997; Winkler 1998): *Arecaceae: Calyptrogyne ghiesbreghtiana*; *Bignoniaceae: Crescentia cujete*; *Bombacaceae: Ceiba pentandra, Ochroma pyramidalis*; *Bromeliaceae: Vriesea gladioliflora*; *Cactaceae: Weberocereus tunilla*; *Musaceae: Musa × paradisiaca*; *Solanaceae: Markea neurantha*. The identified flower scent compounds of the bat-pollinated plants can be divided into four substance classes: (1) aliphatic compounds; (2) aromatic compounds; (3) sulphur-containing compounds; and (4) terpenoid compounds.

In Fig. 2 and Fig. 3 the major scent compounds of these eight plant species from La Selva, and from four further species from Brazil, Costa Rica and Mexico are listed. The test substances for the experiments were selected from these. In addition we tested whether the animals could distinguish between the two stereoisomers of *a*-pinene and limonene.

Choice experiments in the greenhouse

The test apparatus was set up at places where unhindered approaches to each of the eight specimen tubes were possible. All approaches were spontaneous, as rewards were never offered together with any of the scents. The duration of the experiments was limited to 15 min per day (see Materials and methods) in order to prevent the bats from learning too fast that a visit to a scent tube in this situation would never lead to a reward. Of course,

Fig. 2A–T Structural formulae of the 20 tested compounds found in the headspace of the flowers of bat-pollinated plant species. **A** 3-hexanone; **B** (*E*)-2-hexenal; **C** hexyl acetate; **D** benzaldehyde; **E** dimethyl disulphide; **F** 2-thiopropane-1-thiol; **G** 2,4-dithiapentane; **H** dimethyl trisulphide; **I** dimethyl trithiocarbonate; **J** dimethyl tetrasulphide; **K** (+)- α -pinene; **L** (-)- α -pinene; **M** δ -3-carene; **N** (*R*)-limonene; **O** (*S*)-limonene; **P** ocimene; **Q** linalool; **R** linalyl acetate; **S** bornyl acetate; **T** geranylacetone



after some time this induced the animals to probe all possible “flowers”, even the scentless controls. However, the frequency of visits to the different scents remained selective.

Dimethyl disulphide had the highest olfactory attractiveness of all tested scent compounds (Fig. 4) reaching an average preference factor of 2.1. The observation of the bats flight behaviour of the bats close to the specimen tube also confirmed this result: individuals often turned back after having passed dimethyl disulphide at a distance to inspect the specimen tube, and in other cases the animals returned immediately after a first approach to visit the scent sample a second time.

Most scent compounds had a tendency to be approached more often than the scentless controls. However, when a Bonferroni correction was made (in a *t*-test for paired samples), this was significant only for dimethyl disulphide ($P < 0.001$), and for 2,4-dithiapentane, (*E*)-2-hexenal and delta-3-carene ($P < 0.05$). In addition, when all possible combinations were tested, dimethyl disulphide was significantly preferred to all other substances.

According to the number of approaches, the animals did not distinguish between the two stereoisomers of α -pinene and of limonene, respectively.

Experiments in the field

The field experiments took place in the Costa Rican lowland rain forest. Although the basic idea of attracting bats by scent in the field may be simple, in practice

we encountered unexpected difficulties: sometimes we put up our series of artificial blooms, but no bats appeared within a period of 3–4 nights. In four series of tests the animals found our feeding grounds, but the data obtained could not be analysed quantitatively due to heavy rains which caused interruptions of the photoelectric light beams, or, in addition, the rain water filled the artificial flowers so that a determination of the amount of sugar water intake was impossible. In one of the first series of tests (before the artificial flowers were treated with Tanglefoot) ants found the sugar water sources and exploited them completely.

Field experiment 1 (November 1995)

Along the edge of a rainforest clearing we offered 13 different scent compounds and a scentless control in

Fig. 3 Tested scent compounds and their occurrence in the floral scents of different bat-pollinated plants. The scent compounds are divided into four chemical substance classes: aliphatic [3-hexanone, (*E*)-2-hexenal, hexyl acetate]; aromatic (benzaldehyde); sulphur-containing (dimethyl disulphide, 2-thiopropane-1-thiol, 2,4-dithiapentane, dimethyl trisulphide, dimethyl trithiocarbonate, dimethyl tetrasulphide); and terpenoid compounds (*K–T*). The height of the columns corresponds to the respective relative concentration of the compound (determined by capillary gas chromatography). Arrows indicate low but significant concentrations. The plant species are *Hippeastrum calyptratum*, *Calypstrogyne ghiesbreghtiana*, *Crescentia cujete*, *Parmentiera alata*, *Ceiba pentandra*, *Ochroma pyramidale*, *Vriesea gladioliflora*, *Weberocereus tunilla*, *Bauhinia unguilata*, *Musa* sp., *Cobaea scandens* and *Markea neurantha*

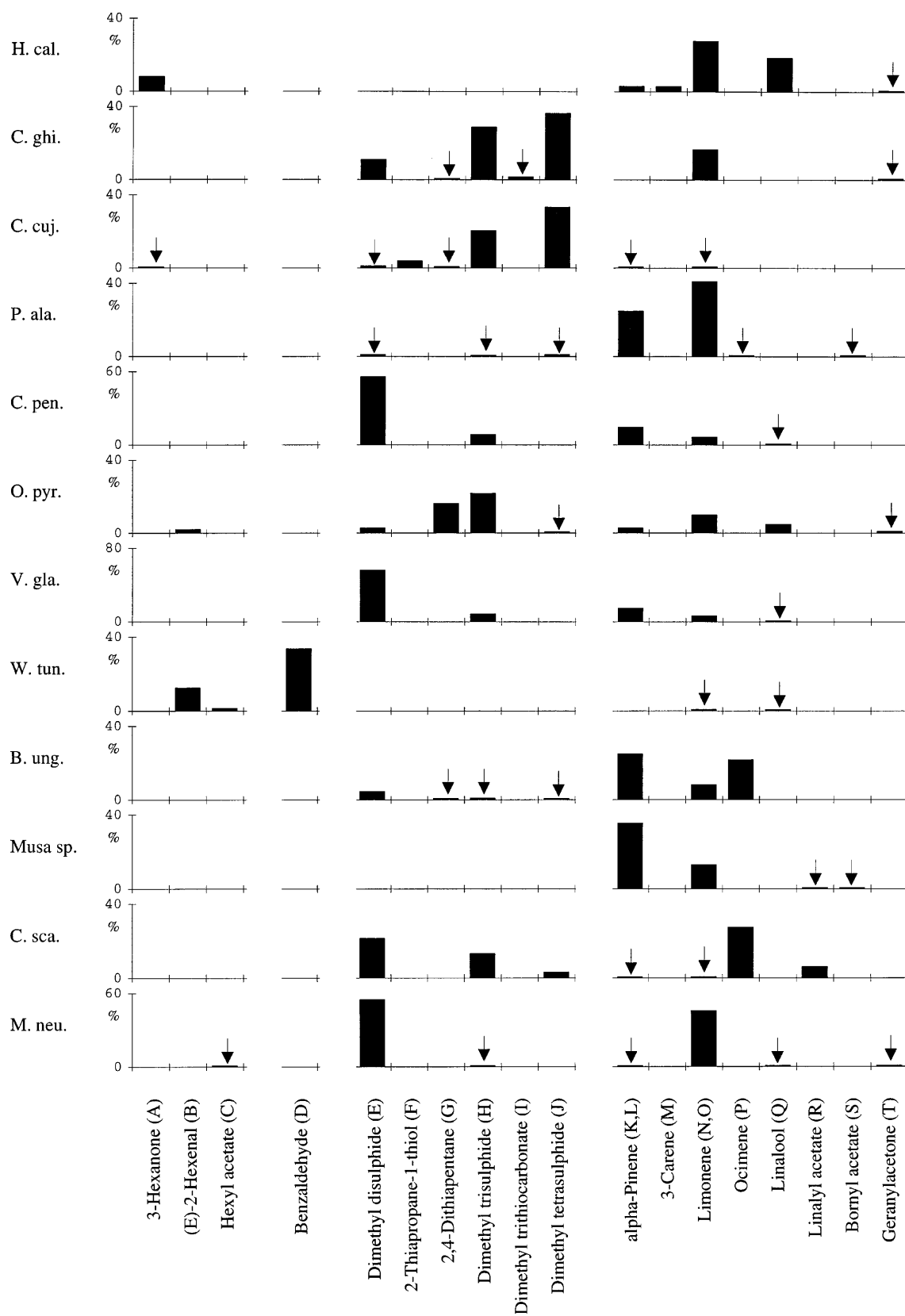
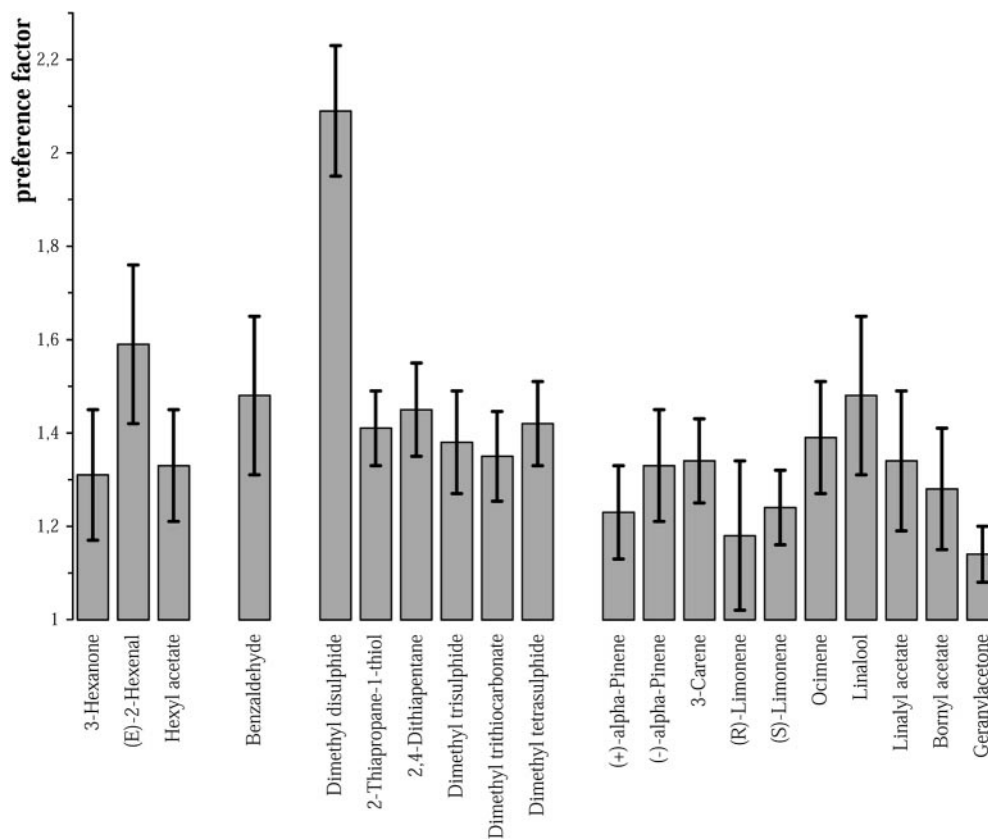


Fig. 4 Relative olfactory attractiveness of 20 flower scent compounds in spontaneous choice experiments conducted in the laboratory. Means and standard errors (SE) of the preference factors. The preference factor describes the relative preference of the respective scent in comparison to the scentless control, see text. A preference factor larger than 1.0 indicates that the scent compound was preferred compared to the scentless reference; a factor value of less than 1.0 would mean a repellent effect of the scent compound. Tests were carried out on a total of 52 nights, the number of counted visits was 7551. In total, 18 scent compounds were tested, each 14 times; 2-thiapropane-1-thiol and dimethyl tetrasulphide were tested only 13 times



artificial flowers during 4 consecutive nights (see Materials and methods). During the 1st night only the two flowers containing sulphur compounds (dimethyl disulfide and 2,4-dithiapentane) were visited; during the 2nd and 3rd nights, bornyl acetate was also attractive, but none of the other compounds. On the 3rd and 4th nights we installed a camera with a flash light close to the flower opening in order to be sure of the fact that bats exploited the artificial flowers. The photographs clearly showed *G. commissarisi* as a visitor (Fig. 1). On the 4th night the bats also visited some of the flowers previously ignored, probably because they had learned the positions and the shape of the artificial flowers. The substances never visited during the first three nights were 3-hexanone, (*E*)-2-hexenal, hexylacetate, benzaldehyde, (+)- and (-)-alpha-pinene, (*r*)- and (*S*)-limonene, linalool and linalyl acetate.

Field experiment 2 (December 1996) with seven scent compounds and a scentless reference

The eight artificial flowers were set up along the edge of the forest at regular intervals of 1.5 m. With the help of photoelectric light-traps all approaches (times and durations of the hovering flights) could be registered.

Table 1 shows that, already on the 1st night, 9 out of 13 visits occurred at dimethyl disulphide, and one further approach was registered at 2,4-dithiapentane. On the following night the number of visits at the artificial

flowers emitting sulphur containing scents went up to 22 and 24, respectively. The total number of visits on the 3rd night was nearly the same as on the preceding night, but now the approaches were distributed among more or less all the artificial flowers, probably because the bats had learned that they all contained sugar water. For this reason, the visits were not rewarded on the following 2 nights. On the first night without reward the number of visits went up to 74; the sulphur-containing scent compounds were clearly preferred. On the second night without rewards the number of visits dropped to 15 – probably due to the lack of reward. On the 6th and 7th nights the visits were rewarded again. As a result, the number of approaches increased (45 and 150, respectively). Again most visits were to the flowers containing sulphur compounds (23 and 14, 37 and 37 visits, respectively). On the 7th night, many approaches (22) were also registered at bornyl acetate. A possible reason for this was the position of the flower, which was situated between the two sulphur compounds on this night (see Fig. 5b). As a consequence of the lack of reward, the total number of visits decreased again on the 8th night.

As the animals were not marked, it could not be determined whether the flowers were visited by one single bat, a small number of individuals, or by many different individuals each of which approached the flower only a few times. The time sequences of visits, registered by the computer, suggested that often single individuals visited the flowers for 2–3 min and repeatedly approached the same flowers (Fig. 5). This type of

Table 1 Sugar water intake and number of visits by wild bats in the lowland rainforest of Costa Rica at La Selva Biological station. Artificial flowers were emanating seven different scents, and an eighth flower served as a scentless control. Flowers were set up in a line, at the edge of a rainforest clearing, with a distance of about 1.5 m each. (*Pos* position of the scent at the respective night, *n* number of visits recorded automatically, *ml intake* amount of sugar water removed from the individual flower during the night, the flowers having been filled with 1 ml of 20% sugar solution at dusk). At the 4th and 5th night and in the last night no sugar water was offered as a reward

Scent compound	12/11/1996			12/12/1996			12/13/1996			12/14/1996		
	Pos	<i>n</i>	ml Intake	Pos	<i>n</i>	ml Intake	Pos	<i>n</i>	ml Intake	Pos	<i>n</i>	ml Intake
Dimethyl disulphide	2	9	0.00	6	22	1.00	2	12	1.00	7	13	* no reward
2,4-Dithiapentane	7	1	0.00	8	24	0.90	5	2	0.20	3	31	*
Linalool	8	0	0.00	1	1	0.00	4	1	1.00	2	10	*
Bornyl acetate	3	3	0.00	3	1	0.15	8	12	0.50	6	4	*
Hexyl acetate	4	0	0.00	5	0	0.00	7	1	0.00	1	7	*
Benzaldehyde	6	0	0.00	4	0	0.00	3	4	0.15	8	8	*
(<i>E</i>)-2-Hexenal	1	0	0.00	7	1	0.00	1	2	0.00	5	1	*
No scent	5	0	0.00	2	0	0.00	6	9	0.40	4	0	*
Sum		13	0.00		49	2.05		43	3.25		74	*
Scent compound	12/15/1996			12/16/1996			12/17/1996			12/18/1996		
	Pos	<i>n</i>	ml Intake	Pos	<i>n</i>	ml Intake	Pos	<i>n</i>	ml Intake	Pos	<i>n</i>	ml Intake
Dimethyl disulphide	4	1	* no reward	1	23	1.00	6	37	0.95	2	38	* no reward
2,4-Dithiapentane	1	6	*	5	14	0.60	4	37	0.90	5	11	*
Linalool	8	3	*	3	2	0.00	8	10	0.10	7	1	*
Bornyl acetate	3	2	*	7	1	0.00	5	22	1.00	6	15	*
Hexyl acetate	7	0	*	6	2	0.00	3	17	0.60	4	6	*
Benzaldehyde	5	0	*	2	0	0.00	1	4	0.90	1	1	*
(<i>E</i>)-2-Hexenal	6	1	*	8	1	0.15	2	1	0.10	3	3	*
No scent	2	2	*	4	2	0.00	7	22	0.90	8	7	*
Sum		15	*		45	1.75		150	5.45		82	*

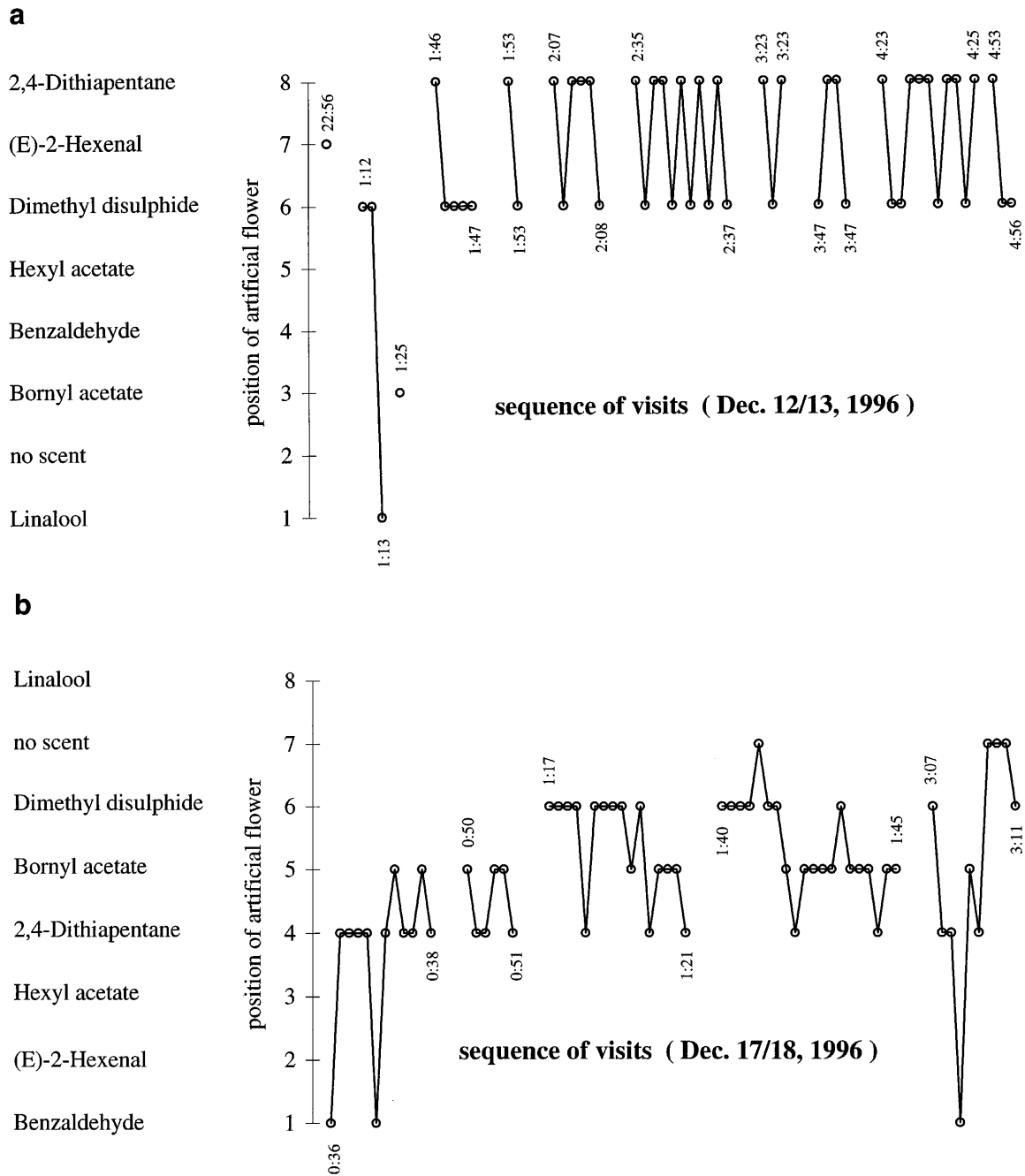


Fig. 5a, b Two examples of the visiting sequences of bats in the field experiment 2. Time at the beginning and end of each “bout of visits” is given. Although the bats sometimes visited flowers with “unattractive” scents at the beginning (or even during) of individual flight sequences, they always returned to one of the flowers emitting a sulphur-containing scent compound. The artificial flowers were lined up at the edge of the forest numbered sequentially from 1 (left outer side) to 8 (right outer side). **a** December 12 1996, 2nd night of experiment. All visits and their times are given. **b** December 17 1996; examples for five bouts of visits are given

behaviour could also be observed directly with a night-vision scope.

An analysis of such sequences of visits showed that sometimes a less attractive scent was approached, but then the bat immediately continued to repeat its visits at

one of the sulphur compounds (Fig. 5). Very often, during a series of visits, the bats approached the two sulphur-containing scents alternately.

The most frequently visited flowers sometimes were completely exploited during the course of a night (Table 1). As a consequence, the bats found empty flowers at the most attractive scents, especially after midnight, but flowers with less attractive scents still contained sugar water.

In Fig. 6 the total amounts of sugar water the bats had removed over the 8 nights and the total number of visits for the seven different scents and the control are shown. Statistical analysis [one-way ANOVA and Least Significance Difference (LSD/Bonferroni) test with sig-

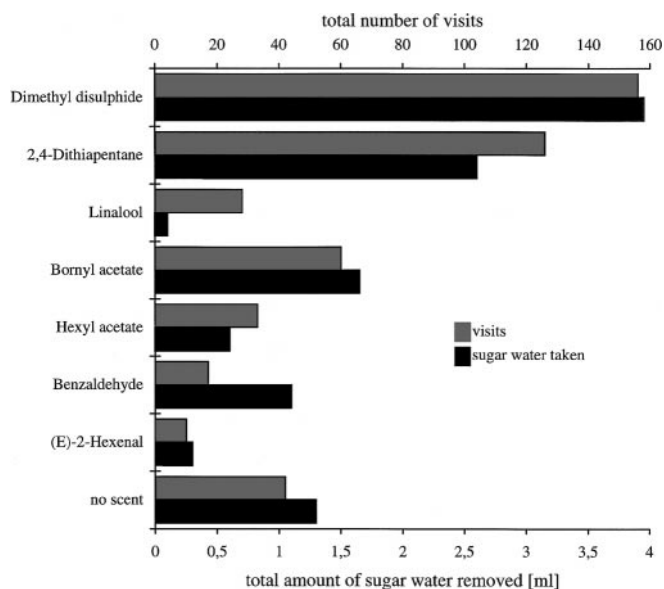


Fig. 6 Total number of visits and amount of sugar water removed by wild bats on the eight test nights of field experiment 2. The flowers emitting the scent of a sulphur compound were clearly preferred by the bats. As in the laboratory experiment, the most attractive scent compound was dimethyl disulphide (156 visits; 3.95 ml sugar water imbibed), followed by 2,4-dithiapentane (126 visits; 2.60 ml); bornyl acetate was the most attractive scent of all the other test compounds (60 visits; 1.60 ml), but was much less attractive than the two sulphides

nificance level 0.05] showed that dimethyl disulphide was significantly preferred over the scentless reference and all other tested smells except for 2,4-dithiapentane; the latter was significantly preferred over benzaldehyde and (*E*)-2-hexenal. The other scent compounds were not significantly more attractive than the scentless reference. A dependence of the number of visits on the position of a test scent could not be found.

Discussion

Our observations show that, in the field, flower-visiting bats are able to detect and localise a source of food *guided only by the scent*. Sulphur-containing compounds – especially dimethyl disulphide – seem to play an especially important role in the olfactory orientation of nectar-feeding bats. These sulphur compounds are effective as single compounds, without the naturally occurring complex scent bouquet of bat-pollinated flowers.

The similar results obtained in field and laboratory experiments (where the bats were born and raised in captivity and never exposed to natural bat-pollinated plants) indicates that the preference for dimethyl disulphide is innate.

Spontaneous choice behaviour

Flower-visiting bats can easily be trained to discriminate different smells (own observations). We were, however,

interested in the question whether these scents are attractive in the absence of training; therefore, in the greenhouse experiments the bats were *never* fed in the presence of a scent compound, neither in the course of the tests nor at any other time. This, on the other hand, might indicate avoidance training in the course of the experiment: scent-oriented approaches to the artificial flowers were always unsuccessful, and so the animals could learn that the respective scent did not lead to any reward.

Flower visitors are, however, generally relatively resistant to negative experiences, as an empty flower always could have been exploited by another individual a short time before, and this experience should not prevent subsequent approaches to the same flower or flower type.

In the field experiments, rewards were indispensable at the artificial flowers, otherwise the number of visits would have been too small. For the sugar water used in the tests we selected a higher sugar concentration (20% saccharose) than the natural nectar concentration (a maximum of 15–16% w/w) of bat-pollinated flowers blooming at the same time at La Selva (mainly *Vriesea gladioliflora*, *Mucuna holtonii*, *Markea neurantha* and *Calyptrogyne ghiesbreghtiana* were in bloom). Rewarding, of course, increased the risk that the bats would also approach less attractive-smelling flowers and would then be trained on the respective scents. In fact, the selectivity for scents decreased after a few days (Table 1). Therefore, we inserted unrewarded test nights in our test paradigm. In all cases the sulphur-containing compounds were clearly preferred.

Long and short distance attraction

It has been suggested that the scent production of bat-pollinated plants is mainly important for long-distance attraction (Vogel 1958). Certainly this is true for many bat-pollinated plants, as even humans with their weak sense of smell perceive some of these flower scents over long distances (up to 20 meter or more); however, our results show that the olfactory orientation also permits the *precise* localisation of a flower.

The observations shown in Fig. 5 indicate that the scent of a flower might also determine a “taste quality” of the nectar for the bats: in our field experiments the bats repeatedly visited artificial flowers (either first, or several times in the course of a sequence of visits) that contained less attractive scents and were rewarded there, but then they directly switched back to flowers emitting sulphur smells. As all artificial flowers had the same sugar water as reward, the bats could also have learned the less attractive smells after their first visits, but they still preferred the sulphuric smells. Either a very strong innate preference for sulphur-containing scent components dominated learning, or the “taste” of the sugar water with sulphur-containing scent compounds was especially attractive to the bats.

The evolution of the floral scents of bat-pollinated plants

The recognition of smells that serve as signals for long-living pollinators (with widely overlapping generations) need not necessarily be inborn. It is also conceivable that each individual bat learns the smells of its food plants. In this case it can be expected that plant species that are in the processing of evolving bat-pollination should develop flower scents similar to already existing plants; otherwise these plants might not be found fast enough by the bats. The specific smell of bat-pollinated plants could then serve as a “trademark” and be transmitted by learning (von Helversen 1993).

The existence of a characteristic smell common to bat-pollinated plants could, however, more easily be explained if innate preferences of the pollinator were the driving force for evolution. Our experiments indicate that this may be the case – at least as far as sulphur-containing scent compounds are concerned.

To date, the scent compounds of 22 bat-pollinated plant species have been analysed. Sulphur compounds were found in the floral scent bouquet of 16 species (Kaiser and Tollsten 1995; Knudsen and Tollsten 1995; Bestmann et al. 1997). This was a striking result, especially as sulphur-containing compounds have so far only rarely been detected in floral scents (Knudsen et al. 1993). It is possible that not all plant families which have evolved bat-pollinated species have been able to synthesise sulphur compounds, and therefore had to mimic the characteristic smell with other combinations of scent compounds. Such scents – for bats as well as for man – are similar to the “sulphur” smells.

The attractiveness of sulphur-containing compounds to bats may reflect the role of such odours in their social behaviour. Smells play an important role in the mating behaviour of bats (Häussler 1989; Gebhard 1997), in the recognition of mother and young (Kolb 1957, 1977; Kulzer 1958; Nelson 1965; Wilson 1971) and in social and territorial behaviour (Bradbury and Emmons 1974; Höller and Schmidt 1993; Schmidt 1984; C. Voigt and O. von Helversen, unpublished observations).

It would be interesting to investigate whether the conspicuous preference for dimethyl disulphide and 2,4-dithiapentane is also reflected in low detection thresholds for these scent compounds. In the case of some fruit-eating bat species (*Carollia perspicillata*, *Phyllostomus discolor* and *Artibeus jamaicensis*) and the sanguinivorous vampire bat *Desmodus rotundus* the detection thresholds for several scent compounds, especially from fruits, were determined (Schmidt 1973, 1975; Laska 1990); unfortunately no analogous experiments have yet been carried out with nectarivorous bats and for sulphur compounds.

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