Sulphur-containing “perfumes” attract flower-visiting bats

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); “displeasing 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
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 bulb 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 compounds 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 compounds 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 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 Rio Sarapiqui and the Rio 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. commissaris; all photographs taken at the artificial flowers showed this species. It cannot be excluded, however, that individuals of Hylonycteris 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 rainforest clearing at regular intervals of about 10 m. The artificial