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Sex pheromone mimicry in the early spider orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception

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Abstract We investigated the female-produced sex pheromone of the solitary bee *Andrena nigroaenea* and compared it with floral scent of the sexually deceptive orchid *Ophrys sphegodes* which is pollinated by *Andrena nigroaenea* males. We identified physiologically and behaviorally active compounds by gas chromatography with electroantennographic detection, gas chromatography-mass spectrometry, and behavioral tests in the field. Dummies scented with cuticle extracts of virgin females or of *O. sphegodes* labellum extracts elicited significantly more male reactions than odorless dummies. Therefore, copulation behavior eliciting semiochemicals are located on the surface of the females' cuticle and the surface of the flowers. Within bee and orchid samples, *n*-alkanes and *n*-alkenes, aldehydes, esters, all-*trans*-farnesol and all-*trans*-farnesyl hexanoate triggered electroantennographic responses in male antennae. Most of the alkanes and alkenes occurred in similar patterns both in the bees and orchids. *O. sphegodes* leaf extracts contained mostly the same compounds but in different proportions. In behavioral tests with synthetic compounds, blends of alkenes triggered significantly more approaches and pounces of the males whereas alkanes were not more attractive than odorless dummies. Since alkanes and alkenes together were most attractive, we conclude they constitute the bees' sex pheromone as well as the pseudocopulation-behavior releasing orchid-odor bouquet.

Key words *Andrena nigroaenea* · Sex pheromone · Chemical mimicry · Orchid pollination · Wax compounds

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Introduction

Nearly all species of the Mediterranean orchid genus *Ophrys* are pollinated by means of sexual deception (Pouyanne 1917; Kullenberg 1961; Paulus and Gack 1990). Their flowers mimic females of the pollinator species, usually solitary bees and wasps, of which only the males are attracted and try to copulate with the flower labellum (Fig. 1). During these so-called "pseudocopulations" the pollinia become attached to the bees and are transferred during further visits of the males on other flowers. Pollination in *Ophrys* is highly specific and each species is usually pollinated by only one or a few closely related insect species (Paulus and Gack 1990). Apart from visual and tactile signals, the scent of an *Ophrys* flower is most important for eliciting mating behavior in the pollinators (Kullenberg 1961). The odor is therefore thought to mimic the pollinator females' sex pheromone, which serves as model in this mimicry system. Previous investigations concerning the scent production of *Ophrys* orchids identified numerous compounds which partly occurred in the orchids as well as in their pollinators (Bergström 1978; Borg-Karlson et al. 1985; Borg-Karlson and Tengö 1986; Borg-Karlson 1990). Except for a beetle pollinator species, which was shown to be induced to copulate by a blend of synthetic alcohols (Borg-Karlson 1989), electrophysiological investigations and bioassays have so far not revealed compounds that elicited the full range of mating behavior in the pollinators (Kullenberg 1973; Priesner 1973; Tengö 1979; Ågren and Borg-Karlson 1984; Borg-Karlson and Tengö 1986).

Sex pheromone communication in bees is generally poorly understood, especially among the solitary species.

Fig. 1 Males of *Andrena nigroaenea* pseudocopulating with a flower of *Ophrys sphegodes*. Weikendorf (Lower Austria), May 1999. Photo by F.P. Schiestl

Whereas long-range pheromones have been identified in several species of solitary and social bees (summarized in Duffield et al. 1984; Free 1987; Engels et al. 1997), the compounds eliciting copulation behavior are, at least in solitary species, more or less unknown. Long-range sex pheromones may be important to maintain male aggregations, attract females, and enhance excitement and flight activity of males (Tengö 1979; Eickwort and Ginsberg 1980; Duffield et al. 1984). Such pheromones were frequently found to lure more than one species of a genus and in most investigations did not elicit copulation behavior (Free 1971; Kullenberg 1973; Tengö 1979; Borg-Karlson and Tengö 1986). Female-produced pheromones acting at close distance may therefore be responsible for species and sex recognition as well as eliciting copulation.

The aim of our study was to identify the female sex pheromone of the solitary bee *Andrena nigroaenea* and to compare it with the behavior-releasing odor compounds of the early spider orchid (*O. sphegodes*) which is pollinated by *A. nigroaenea* males. We localized the source of the sex pheromone and behaviorally active orchid fragrances by conducting behavioral tests with different odor samples. We screened the volatile compounds of the attractive samples for biological activity at the antennal level of male bees by using gas chromatography with electroantennographic detection (GC-EAD). Compounds that released electroantennographic responses were identified by coupled gas chromatography-mass spectrometry (GC-MS) and their relative amounts calculated and compared in different behaviorally active and non-active samples. Subsequently, we tested different mixtures of corresponding synthetic compounds for behavioral activity in the field.

Materials and methods

Natural history

The early spider orchid (*O. sphegodes*) grows in ancient, short chalk grassland and calcareous limestone grassland (Hutchings 1987). Plants usually bear two to six flowers. Its only verified pollinator are males of *A. nigroaenea*, a solitary, possibly also communal bee (Westrich 1990). Males patrol in search for females at nonresource-based rendezvous sites, often at pine trees or *Ligustrum vulgare* shrubs, sometimes sympatric with other *Andrena* species (e.g., *A. flavipes*, *A. carbonaria*; F. P. Schiestl, unpublished observations). Females nest in sandy soils from mid-April to mid-June in southern Central Europe (Westrich 1990). In the area of our investigations, nests are usually not in dense aggregations, but more dispersed.

Sample collection

A. nigroaenea females were collected early in the flight season at nest locations in Weikendorf (Lower Austria). The bees were killed by freezing and the following pentane extracts were obtained from individual females; cuticle extracts: whole bodies were extracted in 400 µl for 1 min; head extracts: heads were extracted in 200 µl for 24 h. Intersegmental glands and Dufour's glands were taken out of the dissected bodies for extraction in 200 µl solvent. *O. sphegodes* plants were collected at the "Bisamberg" (Lower Austria) and flower labella and pieces of leaves of the rosette of equal size were extracted in 400 µl solvent for 24 h. Extracted labella were kept at high humidity (90–100%). From each individual sample, 75% of the total volume was used for behavioral tests. The rest of the samples was used for GC-EAD and GC-MS analyses.

Electrophysiology (GC-EAD)

One microlitre of each odor sample was injected splitless at 50 °C into a gas chromatograph (HP 6890) followed by opening the split valve after 1 min and programming to 310 °C at a rate of 10 °C min^{-1}. The GC was equipped with a DB-5 column (30 m × 0.32 mm); helium was used as the carrier gas. A GC

effluent splitter (press-fit-connection; split ratio 1:1) was used, and the outlet was placed in a purified and humidified airstream. A proper heat transfer from the heater of the outlet to the column proved to be essential for the experiments and was achieved by improving the insulation of the outlet heater and by inserting a metal tube into the space between the inner walls of the heater and the column. Additionally, the part of the column emerging from the heater in the airstream was kept as short as possible, otherwise most of the low-volatile compounds condensed at the end of the column and no responses could be recorded. The air containing the compounds that eluted from the column was directed over an *A. nigroaenea* male antenna prepared as follows. The tip of the excised antenna was cut off, and the antenna was mounted between two glass electrodes filled with insect Ringer solution. The electrode holding the base of the antenna was connected to a grounded Ag-AgCl wire. The distal end of the antenna was connected in the same way via an interface box to a signal acquisition interface board (IDAC; Syntech, Hilversum) for signal transfer to a PC. EAD signals and flame ionization detector (FID) responses were recorded simultaneously. Only such compounds were judged "physiologically active" which produced reproducible electroantennographic responses in multiple GC-EAD runs at the same retention time.

Chemical identification

Most of the GC-MS analyses of GC-EAD active compounds were performed using a HP 5890 Series II GC-MSD system. The operating conditions were the same as those described for the GC-EAD experiments. Mass spectra (EI, 70 eV) were recorded from 40 amu to 550 amu. In addition, a coupled GC-MS system 8008/MD800 (Fisons Instruments) operating in EI-mode (70 eV, 50–600 amu) was used. The gas chromatograph was equipped with an Optima-5-MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25-m coating). Identification of target compounds was based on comparison of mass spectra with known data (McLafferty and Stauffer 1989) and on comparison of retention times using authentic reference substances. Position and stereochemistry of double bonds was determined by investigation of DMDS derivatives (Buser et al. 1983; Dunkelblum et al. 1985) of the natural products and comparison with those of synthetic compounds.

Bioassays

We tested behavioral responses of *A. nigroaenea* males to individual frozen virgin female bees, *O. sphegodes* flowers, odor samples of individual female bees and *Ophrys* flowers, and to different mixtures of synthetic compounds. Only mixtures of those compounds were tested which were found to be electrophysiologically active and occurred in both the bee and the orchid samples (14 hydrocarbons, see Table 2). The synthetic substances were mixed in relative proportions according to different cuticle and labella extracts (Fig. 3). The absolute amounts used were approximately 10 times higher than in the natural samples. Separate mixtures of the alkanes and of the alkenes were prepared according to the same samples. Odorless dummies and extracted flower labella were used as controls. We used dead *A. nigroaenea* females as dummies. These dummies had been Soxhlet-extracted in dichloromethane for 48 h, dried and fixed on insect pins. All field tests were performed in Weikendorf (Lower Austria) using a method modified after Kullenberg (1973) and Tengö (1979). In each test, a single odorless dummy was scented with a sample and offered to *A. nigroaenea* males in two different patrolling areas for 3 min. The position of the dummies within a test location was changed after each test, and the test location was changed after five tests. Four types of behavioral responses of male bees were recorded: approach to approximately 5 cm or less; short pounce on dummy; alight on dummy without copulation; copulation attempt with dummy.

Statistics

For comparisons of means of male reactions in behavioral tests, the Mann-Whitney *U*-Test was used. The significance level was calculated by dividing the 5% level by the number of comparisons within the groups. The reactions of the males to intact frozen bees, extracts of bees, and extracts of *Ophrys* flowers were compared with the reactions to odorless dummies, the reaction to intact flowers with the ones to odorless (extracted) flowers. Since the number of reactions to odorless dummies and odorless flowers showed no statistical difference, these groups were pooled and used as control group for the reactions to synthetic mixtures. For the comparisons of relative amounts of compounds in the various samples, an analysis of variance (one-way ANOVA) with a multiple comparison test (Scheffé) was performed (Norušis 1993).

Results

Significance and localization of attractive odor compounds

Odorless dummy-bees and solvent-extracted flower labella of *O. sphegodes* elicited only approaches of males, suggesting that visual cues alone have a low behavior-releasing capacity and do not elicit copulatory attempts (Table 1). An frozen intact virgin bee elicited significantly more copulation attempts than odorless dummies. Extracts of Dufour's glands and intersegmental glands trigged 4.73 ± 0.84 and 4.27 ± 0.73 ($n = 11$) male approaches, respectively, but no other reactions. Head extracts were little more attractive, especially in releasing more approaches. Among all bee odor samples tested, the most intensive reactions of males were elicited by the cuticle extracts. These samples released a number of approaches and alights significantly higher compared to the odorless dummy. We therefore concluded that the sex pheromone of *A. nigroaenea* is located on the surface of the cuticle (Schiestl et al. 1999). In *O. sphegodes*, extracts of flower labella (= *Ophrys* extract) triggered a significantly higher number of pounces and copulation attempts in the males than odorless dummies. Therefore, the behaviorally active odor compounds are apparently located on the surface of the labellum.

Identification and relative amounts of electrophysiologically active compounds

In the GC-EAD experiments, 23 compounds in the attractive odor samples of female bees or orchid flowers triggered electrophysiological responses in male antennae (Fig. 2, Table 2). All compounds were identified by GC-MS analyses and coinjections with synthetic compounds. The electrophysiologically active compounds were shown to be *n*-alkanes and *n*-alkenes with chain lengths from C21 to C29, aldehydes, esters, all-*trans*-farnesol, and all-*trans*-farnesyl hexanoate. The following pairs of compounds could not be separated with the GC parameters used: docosane + farnesyl hexanoate, (*Z*)-12- +(*Z*)-11-heptacosene and the same isomers of

Table 1 Reactions of *Andrena nigroaenea* males in behavioral tests in the field to female bees, *Ophrys sphegodes* flowers, different odor samples, and synthetic compounds. A single test lasted for 3 min. Within the groups A, B, and C the Mann-Whitney *U*-test was used for statistical comparison

Test group	Reactions of males (mean ± SEM)				
	n	Approach	Short pounce	Alight on dummy without copulation	Copulation attempt
Group A					
(a) Odorless bee	13	5.85 ± 0.95	0	0	0
(b) Intact *Andrena*	16	8.06 ± 1.63	0.69 ± 0.34	0.25 ± 0.11	2.38 ± 0.71*
(c) Head extract	10	9.89 ± 1.43	0.05 ± 0.05	0.16 ± 0.12	0.11 ± 0.07
(d) Cuticle extracts	9	11.33 ± 1.39*	0.44 ± 0.24	0.56 ± 0.18*	1.00 ± 0.58
(e) *Ophrys* extract	12	11.83 ± 2.28	1.17 ± 0.42*	0.50 ± 0.26	1.50 ± 0.45*
Group B					
(a) Odorless *Ophrys*	9	7.44 ± 1.68	0	0	0
(b) Intact *Ophrys*	11	4.91 ± 1.66	0.09 ± 0.09	0.18 ± 0.18	5.45 ± 1.70*
Group C					
(a) Odorless dummies	22	6.50 ± 0.96	0	0	0
(b) Alkanes	16	8.88 ± 1.38	0	0	0
(c) Alkenes	21	14.10 ± 1.70*	0.48 ± 0.13*	0.05 ± 0.05	0.05 ± 0.05
(d) Alkanes + alkenes	31	14.06 ± 1.32*	0.81 ± 0.19*	0.26 ± 0.17	0.74 ± 0.23*

* Significant difference between the test group and the odorless bee/*Ophrys* group at the significance level of $P < 0.01$ (group A), $P < 0.05$ (group B), and $P < 0.02$ (group C)

nonacosene, as well as 2-nonyl hexadecanoate and dodecyl dodecanoate. Farnesyl hexanoate and farnesol, which are not indicated in Fig. 2, were found in very small amounts only. The aldehydes and esters were only detected in the orchid samples. Most of the hydrocarbons, farnesol and farnesyl hexanoate were found in the samples from both the bees and the orchids. We compared the relative amounts of the hydrocarbons in the bee-cuticle extracts, and extracts of orchid flowers and leaves Fig. 3). Of the 12 peaks investigated by statistical analyses, the relative amounts of 4 peaks were significantly different in the bee-cuticle extracts and flower extracts. The relative amounts of 6 peaks differed significantly in cuticle extracts and leaf extracts. In *Ophrys* flower and leaf extracts, the relative amounts of 8 peaks proved to be significantly different. Whereas the pattern of the alkanes and the alkenes showed striking

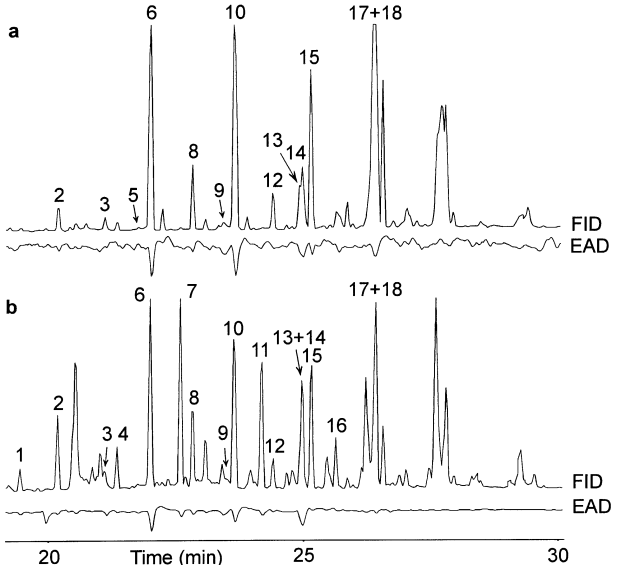

Fig. 2 Gas chromatographic analyses with electroantennographic detection (GC-EAD) of (**a**) cuticle extract of an *Andrena nigroaenea* female and (**b**) labellum extract of an *Ophrys sphegodes* flower. Flame ionization detector (FID) and electroantennographic detector (EAD) responses were simultaneously recorded using antennae of *A. nigroaenea* males. Numbered peaks correspond to compounds that elicit electroantennographic responses. The names of the compounds are indicated in Table 1. In order to detect all "physiologically active" compounds and discriminate electrophysiological responses from noise we performed five to ten GC-EAD runs for each type of sample and checked the reproducibility of all reactions at the same retention times

Table 2 Electrophysiologically active compounds in cuticle extracts of virgin *Andrena nigroaenea* females and extracts of *Ophrys sphegodes* flower labella

No[1]	Name	Occurrence	
		Andrena	*Ophrys*
1	Octadecanal		X
2	Heneicosane	X	X
3	Docosane	X	X
4	Eicosanal		X
5	(*Z*)-9-Tricosene	X	
6	Tricosane	X	X
7	2-Nonyl tetradecanoate		X
8	Tetracosane	X	X
9	(*Z*)-9-Pentacosene	X	X
10	Pentacosane	X	X
11	2-Nonyl hexadecanoate		X
	Dodecyl dodecanoate		X
12	Hexacosane	X	X
13	(*Z*)-12 + (*Z*)-11-Heptacosene	X	X
14	(*Z*)-9-Heptacosene	X	X
15	Heptacosane	X	X
16	Dodecyl tetradecanoate		X
17	(*Z*)-12 + (*Z*)-11-Nonacosene	X	X
18	(*Z*)-9-Nonacosene	X	X
	Farnesol[2]	X	X
	Farnesyl hexanoate[2]	X	X

[1] Numbers according to Fig. 2 and Fig. 3
[2] Not indicated in Fig. 2

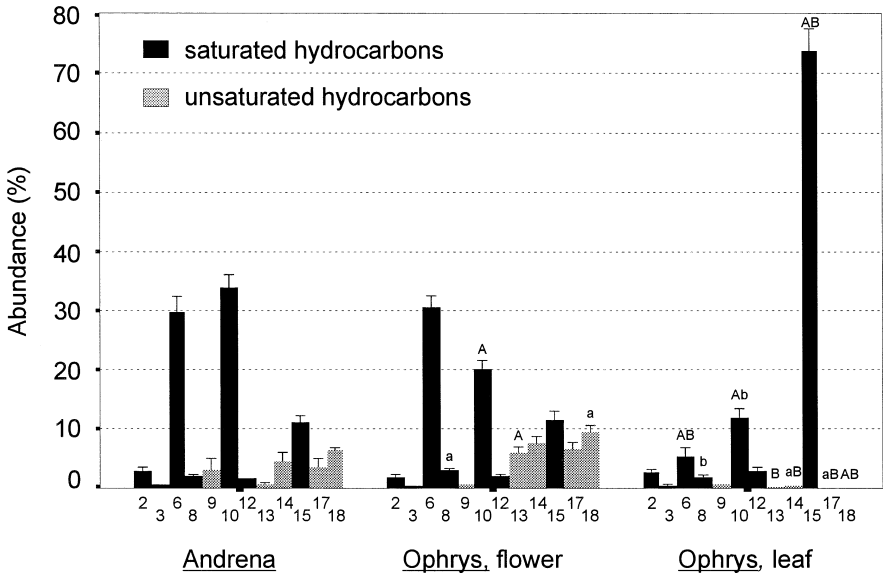

Fig. 3 Mean relative amounts (+SEM) of physiologically active hydrocarbons in *Andrena* cuticle extracts, *Ophrys* flower and leaf extracts. Names of compounds (*numbers below bars*) according to Table 2. *Letters* on the bars indicate statistically significant differences between the respective group and *Andrena* cuticle extracts (*A*,*a*) and *Ophrys* flower extracts (*B*,*b*) at the significance level of $P < 0.05$ (*a*,*b*) and $P < 0.001$ (*A*,*B*)

similarities in *Andrena* cuticle extracts and *Ophrys* flower extracts, especially the alkenes were found in much lower amounts in the *Ophrys* leaf extracts.

Identification of behaviorally active compounds

The blend of synthetic alkanes alone was not significantly more attractive than the control group consisting of the reactions to the odorless dummy-bees and flowers (Table 1). Alkenes elicited significantly more approaches and pounces than the control group. By using all 14 co-occurring alkanes and alkenes, more approaches, pounces, and copulation attempts than the control group could be elicited. These tests with synthetic compounds showed that the hydrocarbons constitute a mating behavior-eliciting odor signal. Farnesyl hexanoate and farnesol were found to inhibit copulation attempts in males of *A. nigroaenea* (F.P. Schiestl and M. Ayasse, accepted for publication).

Discussion

Significance of olfactory cues in mating behavior

Our data suggest a superior importance of olfactory over visual or tactile stimuli for eliciting mating behavior in *A. nigroaenea* males. Odorless dummies clearly stimulated fewer male reactions than intact females or dummies scented with certain female cuticle extracts (Table 1). In accordance with our results, most studies investigating odor communication in bee mating behavior have proven the significance of chemical stimuli (Barrows 1975; Duffield et al. 1984; Wcislo 1987; Ayasse and Dutzler 1998; Ayasse et al. 1999). Butler (1965), however, suggested the body odor of female *A. flavipes* bees to be of minor importance for recognition of females by males because dried specimens, which he presumed had lost most of their odor, were as attractive as freshly killed ones. The odor signal in *A. nigroaenea*, however, is low in volatility and probably outlasts the drying process of a dead individual. This could be true for *A. flavipes* as well and, therefore, have accounted for Butler's (1965) findings.

In *O. sphegodes*, flower labella, which had been extracted in solvent and obviously lost most of their odor, triggered only approaches, whereas intact flowers were highly attractive and elicited numerous copulation attempts. Labella extracts released a greater number of most male-reaction types than the odorless controls. Therefore, odor also seems to be most important for releasing the pollinators' copulatory behavior in *O. sphegodes*, which had also been shown before in several other *Ophrys* species (Kullenberg 1961; Bergström 1978; Borg-Karlson 1990).

Localization of the sex pheromone

Some studies concerned with the production site of female sex pheromones in bees suggest their source to be the cephalic or, more specifically, the mandibular glands (*Andrena*: Tengö 1979; *Bombus*: Free 1971; van Honk et al. 1978; *Eucera*: Kullenberg 1973; *Nomia*: Wcislo 1992; *Panurgus*: Tengö et al. 1988). In *Lasioglossum zephyrum* and *L. malachurum* it was shown that the sex pheromone is located on the surface of the cuticle (Barrows 1975; Wcislo 1987; Ayasse et al. 1999). Similar results were obtained in other bee species of various genera (*Dasypoda*: Bergmark et al. 1984; *Eucera*: Shimron and Hefetz 1985; *Osmia*: Ayasse and Dutzler 1998). Batra (1980) reported a possible contact sex pheromone located on the thorax in *Colletes thoracicus* and mandibular gland secretions eliciting general attraction and increased flight activity.

In our experiments, cuticle extracts of the whole female body clearly released the most intense behavioral reactions of males. Neither extracts of intersegmental glands nor of Dufour's glands extracts triggered more male reactions than the odorless dummies. Tengö (1979) also found *A. haemorrhoa* males not to be attracted to extracts of Dufour's glands of females. Our data lead to the conclusion that the sex pheromone of *A. nigroaenea* is located on the cuticle surface. Further investigations will be necessary to determine the production site of the signal. Shimron and Hefetz (1985) assumed that small glands associated with abdominal tergites produce the aphrodisiac in *Eucera palestinae*. Such glands have also been found in *Andrena* (Altenkirch 1962) and could play a role in the sex pheromone production of *A. nigroaenea*.

Chemistry of the sex pheromone

Chemical analyses of behavior-releasing compounds have been conducted in only a few bee species. Among the social "non-*Apis*" bees, female sex pheromones have been chemically identified in the stingless bee *Scaptotrigona postica* (Engels et al. 1997) and social sweat bees (Smith et al. 1985; Ayasse et al. 1999). In solitary bees, pheromones that attracted males were chemically identified (Tengö 1979; Cane and Tengö 1981; Bergmark et al. 1984; Borg-Karlson 1990), but more intense behavioral reactions could only rarely be triggered. In *Andrena*, Tengö (1979) found that alkanols from the mandibular glands attracted males. Mixtures of compounds proved to be more attractive than single compounds.

In our study, a mixture of synthetic compounds elicited even copulation behavior in the male bees. These compounds are low-volatile saturated and unsaturated cuticular hydrocarbons with a carbon chain length of 21–29. Hydrocarbons are significant constituents of insect cuticular lipids whose primary function is desiccation resistance (Blomquist et al. 1993; Singer 1998). They are used as sex pheromones in several insects, especially among the Diptera (summarized in Howard and Blomquist 1982; Blomquist et al. 1993; Howard 1993). Undecane proved to be the sex pheromone of the formicine ant *Formica lugubris* (Walter et al. 1993). In bees, the role of female-produced hydrocarbons in attracting males has recently been shown in the sweat bee *L. malachurum* (Ayasse et al. 1999). Although numerous studies deal with the biological significance of cuticular hydrocarbons in insect chemical communication (summarized in Howard and Blomquist 1982; Howard 1993; Eigenbrode and Espelie 1995; Singer 1998), only few investigators proved their detection by electrophysiological studies (den Otter and Saini 1985; Ågren and Borg-Karlson 1984).

In our behavioral experiments with synthetic mixtures, the alkenes proved to be more attractive than the alkanes. Mixtures of both unsaturated and saturated hydrocarbons, however, showed a clearly greater attractiveness, especially in eliciting copulation attempts. A synergistic effect between alkanes and alkenes therefore seems to play a role in releasing mating behavior. Similar results were obtained by several studies investigating dipteran sex pheromones (summarized in Howard and Blomquist 1982).

The low volatility of the female sex pheromone relates to the mating system of *A. nigroaenea*. The males patrol for females at scent-marked rendezvous sites (Haas 1960; Tengö 1979). Males and probably also females are attracted to these locations by the odor marks. In such a mating system, where the sexes meet at rendezvous sites, female-produced long-distance pheromones are unnecessary (Alcock et al. 1978).

Pollinator-attracting compounds in *O. sphegodes*

In *Ophrys* orchids, chemical mimicry was first proposed by Kullenberg (1961). Since then, it has been an intriguing challenge to find the compounds in this mimicry system that are responsible for eliciting mating behavior in the pollinators. Bergström (1978) discusses mainly two possibilities concerning the biologically active volatiles involved in the chemical mimicry of *Ophrys* orchids: (1) the orchid mimics an aphrodisiac produced by the females, and (2) *Ophrys*-orchids make use of "extranormal stimuli", i.e., compounds usually not produced by a female, which hit existing receptors associated with sexual behavior. Previous investigations provided support for both hypotheses. Priesner (1973) reported unspecific electroantennographic reactions in pollinator males to most odor compounds of the investigated *Ophrys* flowers. Only γ-cadinene elicited high and specific EAG reactions in male *Eucera* bees. This compound was, however, only identified in odor samples of *Ophrys* flowers and never in the pollinating *Eucera* bees (Kullenberg and Bergström 1976; Borg-Karlson 1990). Support for hypothesis 1 is provided by Borg-Karlson et al. (1985) and Borg-Karlson and Tengö (1986), who reported aliphatic primary alcohols and methylcarbinols as well as several terpenes, occurring in flowers and bee secretions, to be the key components in *O. lutea* pollinated by *Andrena*-bees. However, although males frequently approached a dummy scented with these synthetic compound blends in bioassays, copulation attempts could rarely be elicited. Borg-Karlson et al. (1987) found the hydrocarbon patterns of the Dufour's glands of two *Argogorytes* species to resemble *O. insectifera* flowers, which are pollinated by them. Hydrocarbons also elicited EAG responses in *Argogorytes* males (Ågren and Borg-Karlson 1984). In field test however, synthetic copies of these compounds were not attractive to the males.

In *O. sphegodes*, we showed that the pollinator attraction and the release of copulation behavior is ensured by the same compounds that constitute the sex pheromone of the pollinator species (Schiestl et al. 1999). Not only do the same compounds occur among

the orchid volatiles, but these compounds also show similar quantitative proportions. A comparison with the hydrocarbon pattern of the orchid leaf extracts elucidates this similarity. These samples also contain most of the compounds, but their relative amounts are considerably different. As the orchid leaves do not attract the pollinators, the specific pattern is probably a key factor for releasing male mating behavior. Several cases of chemical mimicry have been suggested to be mediated by a similar hydrocarbon pattern (Stowe 1988; Dettner and Liepert 1994). Preliminary tests with the electrophysiologically active aldehydes and esters occurring only in the *Ophrys* samples indicated that these compounds alone do not trigger more intense behavioral reactions (e.g., copulation attempts) in the males. Semiochemical functions of these compounds are discussed elsewhere (Ayasse et al. 2000).

In plants, cuticular waxes typically consist of alkanes, wax esters, free fatty alcohols, and free fatty acids (Hadley 1981; Baker 1982; Eigenbrode and Espelie 1995). They primarily prevent the loss of water and also mediate interactions between plants and herbivores (Eigenbrode and Espelie 1995). *n*-Alkanes of odd-numbered chain length are usually the most abundant components, whereas alkenes are generally less ubiquitous. In *O. sphegodes*, the greatest differences between the flower and the leaf extracts was found among the alkenes, especially among the isomeres of heptacosene and nonacosene, which occur much more abundantly in the flowers. This considerably greater abundance corresponds fairly well with the high amounts of these compounds in the cuticle extracts of the bees. In our behavioral tests, the alkenes of the orchid and bee cuticle proved to be behaviorally more active than the alkanes. The specific adaptation of *O. sphegodes* flowers for pollinator attraction seems therefore to be a higher production of specific isomeres of alkenes. The evolution of the pollination mechanism of sexual deception, which involves a mimicry of the pollinators' sex pheromone, has apparently been mediated by a change in the hydrocarbon pattern in the wax layer of the flowers.

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