ANIMAL BEHAVIOUR, 2005, **70**, 1411–1418
doi:10.1016/j.anbehav.2005.03.032

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Prey recognition by females of the European beewolf and its potential for a sensory trap

GUDRUN HERZNER, THOMAS SCHMITT, K. EDUARD LINSENMAIR & ERHARD STROHM
Department of Animal Ecology and Tropical Biology, Würzburg University

(Received 15 October 2004; initial acceptance 20 January 2005; final acceptance 30 March 2005; published online 7 November 2005; MS. number: 8307)

The asymmetry in mating strategies between males and females may influence the evolution of mate-signalling systems. Exploitation of pre-existing female preferences for certain visual or acoustic stimuli by male courtship signals has been reported from a variety of species. However, information on chemical communication systems is comparatively scarce. The sensory trap model of sexual signalling suggests that female preferences originated from and are maintained by selection pressures in a nonsexual context, e.g. prey recognition. We tested a key prediction from the sensory trap hypothesis for the evolution of the male sex pheromone in a solitary wasp, the European beewolf, *Philanthus triangulum*. Females hunt exclusively honeybees, *Apis mellifera*, as provisions for their larvae. Males mark territories with a pheromone to attract females. The co-occurrence of a long-chain alcohol, (*Z*)-11-eicosen-1-ol, in the male pheromone and on the cuticle of honeybees suggests that males might exploit a female preference for (*Z*)-11-eicosen-1-ol. We used behavioural assays with honeybees and honeybee dummies to investigate whether females use (*Z*)-11-eicosen-1-ol for prey recognition. Females used olfactory cues to find and identify honeybees and (*Z*)-11-eicosen-1-ol was an essential component of the prey recognition cue. Thus, female European beewolves have a high sensitivity for (*Z*)-11-eicosen-1-ol that probably evolved in the context of prey hunting. Therefore, males that included this compound in their sex pheromone probably attracted more females and experienced a selective advantage according to the sensory trap model.

The reproductive interests of males and females often differ dramatically. Females usually make larger parental investments than males, which invest more in mate attraction and mate encounter (Trivers 1972; Phelan 1992, 1997). To maximize their reproductive success females should evolve special sensory, neural, physiological and physical capabilities to locate and accumulate resources needed for provisioning offspring. Males, however, should evolve a high efficiency to locate or attract females to maximize the number of matings and thereby their reproductive fitness. This fundamental asymmetry in reproductive strategies may be a major determinant for the evolution of courtship signals. Therefore, male sexual signals are expected to track the females' response in evolutionary time (Phelan 1992, 1997).

Recognizing this asymmetry, the pre-existing biases (Basolo 1990) and sensory exploitation (Ryan 1990; Ryan et al. 1990) models of sexual signalling suggest that the evolution of male sexual signals is influenced by pre-existing characteristics of the females' sensory or neural systems. An expansion of this hypothesis is the sensory trap model (West-Eberhard 1984; Christy 1995) which takes into account how such pre-existing sensory sensitivities and female preferences may have evolved. It states that female preferences originate because they are selected for in at least one context outside mate choice, that is, in a natural selection context such as foraging. All three models propose that the female preference predates the preferred male trait and its use in sexual signalling.

There is a growing body of evidence that supports sensory traps as important factors for the evolution of visual and vibrational male courtship signals (e.g. Proctor 1991; Clark & Uetz 1992; Sakaluk 2000; Rodd et al. 2002;

Correspondence and present address: G. Herzner, Institute of Zoology, Regensburg University, D-93040 Regensburg, Germany (email: gudrun.herzner@biologie.uni-regensburg.de). E. Strohm is now also at this address. T. Schmitt is at the Institute of Biology I (Zoology), Freiburg University, Hauptstr. 1, D-79104 Freiburg i. Br., Germany. K. E. Linsenmair is at the Department of Animal Ecology and Tropical Biology, Biozentrum, Am Hubland, D-97074 Würzburg, Germany.

0003–3472/05/$30.00/0

Stålhandske 2002; Christy et al. 2003a, b; Madden & Tanner 2003). Although sexual signalling and mate choice frequently involve chemical communication, surprisingly little is known about possible evolutionary pathways of chemical communication systems and the role of sensory exploitation or sensory traps in shaping the composition of sex pheromones (Phelan 1992, 1997; Christy 1995). Selection for prey detection or recognition is an example of how selective forces in a context other than mate choice can influence female mating preferences (Proctor 1991; Christy 1995; Rodd et al. 2002). If females use odours to locate their food or prey, male signals that are sufficiently similar to this odour to attract females will be favoured by sexual selection (Christy 1995).

We tested a key prediction (see below) of the sensory trap model for the evolution of the sex pheromone of male European beewolves, *Philanthus triangulum* (Hymenoptera: Crabronidae). Female beewolves are strictly monophagous and hunt exclusively honeybee, *Apis mellifera*, workers. They search for honeybees on flowers, paralyse them, and bring them to their nest as provisions for their offspring (Strohm 1995). Beewolf females' reproductive success is limited by the number of bees they can secure for their progeny (e.g. Strohm & Linsenmair 1997, 1998; Strohm & Marliani 2002). Females can therefore be expected to have evolved special sensory, neural and physiological abilities to maximize their success in detecting and capturing honeybees. Tinbergen (1935) provided some evidence for the use of olfactory cues in the hunting behaviour of female European beewolves; however, the chemical nature of these cues has not been analysed. Many hymenopteran species use chemical cues for the location and/or identification of food sources. Predatory and parasitic species in particular rely on chemical stimuli associated with their prey or hosts (Dicke & Sabelis 1992; Godfray 1994; Stowe et al. 1995; Quicke 1997; Hendrichs & Hendrichs 1998). These so-called 'kairomones' can be either actively emitted signals intended for a different receiver, such as pheromones (Dunkelblum et al. 1996; Hendrichs & Hendrichs 1998; Hoffmeister & Gienapp 1999; Millar et al. 2001), or inadvertently provided cues, such as cuticular substances (Anton & Gnatzy 1998; Howard et al. 1998) or products such as faeces (Steidle & Ruther 2000; Schaffner & Müller 2001). In any case, kairomones usually reliably indicate the presence and identity of the host or prey.

Male beewolves establish and scent-mark territories with the secretion of a cephalic gland to attract females (Evans & O'Neill 1988; Schmitt et al. 2003). Remarkably, the major component of the males' pheromone, (*Z*)-11-eicosen-1-ol (Schmidt et al. 1990; Schmitt et al. 2003), is one of the major compounds of the alarm pheromone of honeybees, the exclusive prey of the females (Free et al. 1982, 1983; Pickett et al. 1982). Based on the sensory trap model (West-Eberhard 1984; Phelan 1992, 1997; Christy 1995), we propose a three-step scenario for the evolution of the male sex pheromone in *P. triangulum*. (1) Foraging honeybees should emit (*Z*)-11-eicosen-1-ol. (2) Since successful hunting is the major factor influencing their reproductive potential, beewolf females evolved a high sensory sensitivity for this characteristic component to locate or identify honeybees. (3) Males have evolved the production of (*Z*)-11-eicosen-1-ol as a pheromone component because of the high sensitivity of females for this substance. In the current study we focused on prediction 2. Predictions 1 and 3 will be studied and discussed elsewhere (T. Schmitt, G. Herzner, P. Schreier & E. Strohm, unpublished data; G. Herzner, T. Schmitt & E. Strohm, unpublished data).

We tested, by means of behavioural assays, whether beewolf females use olfactory cues, and in particular (*Z*)-11-eicosen-1-ol, to detect and identify honeybees. Females of the European beewolf are highly specialized and have probably evolved an accordingly highly specialized sensory-neural-motor system. In contrast to generalist species this specialization provides a good opportunity for a male to exploit effectively the females' sensory system and behavioural response. For the same reason, it should be easier to identify the stimuli necessary for prey location and identification in this species than in generalist species. Thus, owing to the females' extreme specialization, beewolves provide an exceptionally promising model system to test the sensory trap hypothesis.

METHODS

Beewolves

For a detailed description of the European beewolf and its behaviour see Strohm (1995). For the present study, females were either collected at various field sites in or close to Würzburg or obtained from a laboratory population reared at the Biocenter of the University of Würzburg (F1-generation of field-caught beewolves). They were brought into an environmental chamber and individually housed in sand-filled breeding containers (60 × 18 cm and 18 cm high) to which flight cages (15 × 18 cm and 18 cm high) were attached. These were lit by neon lamps (26:22 °C day:night 14:10 h light:dark cycle). For 5–7 days females were allowed to habituate to the laboratory conditions and provided with honey and honeybees ad libitum. During the following training and experimental period, they were provided with honey only and presented with differently manipulated honeybees or honeybee dummies (test prey).

Training

Beewolf females were trained to attack and paralyse honeybees that were offered at a specific spot in the foraging cage. For this purpose bees were anaesthetized with CO_2 and attached to commercial hairgrips (hairclips) by clamping one of the wings. Beewolf females that attacked the bees (which were then released from the hairgrips) were allowed to take them to their nests. After the females had reliably learned to accept the tethered honeybees (after approximately 1 week), freeze-killed and defrosted honeybees were offered. This step was included to eliminate the movement of the bees that could be a stimulus for prey detection by the females. Freezing does not alter the outer appearance or the odour bouquet of the bees (G. Herzner, unpublished data). Only females

that learned to accept the dead bees as prey were used for the bioassays described below.

During the initial training phase, females that attacked the tethered live or frozen bees showed a characteristic behaviour. After the first perception and localization of the bees (for which olfactory as well as visual cues were probably responsible, see also Tinbergen 1935), they hovered in front of the prey at a distance of approximately 10 cm for a few seconds before they finally pounced on it and stung it. Based on this behavioural sequence we assigned the females' response to one of the following categories during the subsequent bioassays: females either (1) did not hover at all, (2) hovered in front of the prey object but then did not pounce on it or (3) hovered and finally pounced on the prey. The first two categories were regarded as 'no attack', the third as 'attack'. An attack is thus defined as the pouncing of the female on the test prey, regardless of whether she tried to sting it or not.

Experimental Blocks

The role of olfaction

We first investigated whether olfaction plays a role in hunting by beewolf females. We tested whether the characteristic honeybee 'body odour', comprising the cuticular substances, is essential for releasing an attack. Therefore, we tested (1) odourless bees whose cuticular hydrocarbons were removed, (2) odourless bees that were rescented by contact with live honeybees, and (3) odourless bees that were rescented with a honeybee extract. To obtain odourless 'bees' we soaked freshly freeze-killed honeybees in acetone for 2 days and subsequently dried them in a drying oven at 70°C for 1 day. In this manner the characteristic bee odour was removed (this was verified with gas chromatography). After the initial training phase of the female beewolves, these odourless honeybees were offered. To obtain the first group of rescented bees, we stored odourless bees in a vial that contained 15 live honeybees for 1 day. The transfer of the cuticular substances to the odourless bees was again verified by gas chromatography. The rescented bees were taken out of the vial immediately preceding the test with a female and each rescented bee was used only once. For the second group of rescented bees we reapplied the cuticular substances to odourless bees by using an extract of honeybees. We obtained a honeybee extract by soaking three freshly freeze-killed honeybees in 2 ml of distilled hexane for 10 min (bee extract). Each extract sample was reduced in volume to approximately 50 µl and applied to an odourless bee with a pipette immediately before each test to avoid premature volatilization of substances. After the solvent had evaporated (after 1 min), the rescented bees were used for the bioassay. As a control, 50 µl of pure hexane was applied on odourless bees and presented to beewolf females.

To reduce visual stimuli further, we replaced the odourless honeybees by honeybee dummies. The dummies were made of dark-grey Teflon (because of its inertness to chemicals) and attached to thin metal rods. They were cylindrical and had the approximate size of honeybees (1.5 × 0.6 mm). We scented the dummies as described above for the odourless bees, either by placing them in a vial with live bees or by applying honeybee extract. We compared the number of attacks on odourless and rescented honeybees and on odourless and scented honeybee dummies.

The role of (Z)-11-eicosen-1-ol

To examine the role of (*Z*)-11-eicosen-1-ol in prey recognition, we conducted a second set of bioassays using the Teflon dummies. Not only is (*Z*)-11-eicosen-1-ol a major component in the alarm pheromone of honeybees, but it can also be found on honeybees' cuticles (T. Schmitt, G. Herzner, P. Schreier & E. Strohm, unpublished data). To determine the natural amounts of (*Z*)-11-eicosen-1-ol on honeybee cuticles we analysed honeybee extracts by combined gas chromatography and mass spectrometry (GC–MS). We found (*Z*)-11-eicosen-1-ol in varying amounts in all extracts. After the initial training phase of the females, three different kinds of scents were tested on dummies: (1) the normal honeybee extract (bee extract); (2) the pure hydrocarbon fraction of the honeybee extract containing no (*Z*)-11-eicosen-1-ol (HC); and (3) the hydrocarbon fraction of the bee extract to which (*Z*)-11-eicosen-1-ol was added (HC + Eicosenol).

To remove (*Z*)-11-eicosen-1-ol from the mixture of hydrocarbons, we washed 10 honeybees in 3 ml of distilled hexane for 10 min. The resulting extracts were loaded on to a silica gel column (Macherey and Nagel, Chromabond 500 mg) and eluted with 3 ml of hexane. The eluted fraction contained the whole set of hydrocarbons (HC: alkanes, methylalkanes and alkenes), but no (*Z*)-11-eicosen-1-ol. The HC solution was partitioned into three aliquots that were reduced in volume to approximately 50 µl and each aliquot was used for one dummy. To obtain solutions of the purified HC fractions that contained (*Z*)-11-eicosen-1-ol, we added commercially available (*Z*)-11-eicosen-1-ol (ICN Biomedicals, Irvine, CA, U.S.A.) in the amount found in the extracts of the bees before fractioning (HC + Eicosenol). This amount differed somewhat between extracts (between 3 and 8‰ of the amount of the main compound heptacosane). Therefore, the amount of (Z)-11-eicosen-1-ol that was added differed accordingly. The relative amount of (*Z*)-11-eicosen-1-ol in the bee extracts, the absence of (*Z*)-11-eicosen-1-ol in the HC, and the relative amount of (*Z*)-11-eicosen-1-ol in the HC + Eicosenol mix were determined by GC–MS. We compared the proportion of attacks on bee extract dummies with the HC dummies solution as well as the proportion of attacks on HC dummies with the HC + Eicosenol dummies. To avoid pseudoreplication, we used all individual prey objects only once.

Procedure

Hairgrips were thoroughly cleaned with acetone before all experiments. Every morning, each focal female was first offered a normal live honeybee attached to a hairgrip and allowed to paralyse it and take it to the nest. When the

female left her nest to forage again, a test prey was offered for 2 min and the response of the female (attack/no attack) was recorded. If the female attacked the test prey, we removed it and replaced it with a live honeybee that could be paralysed and brought to the nest. If the female did not attack the prey during the 2-min test phase, we immediately tested her motivation for foraging by offering a normal live honeybee. If the female attacked the bee within 2 min, she was considered to have been motivated during the bioassay and the previous test prey was categorized as 'not attacked'. If the female did not catch the live honeybee within 2 min, she was considered to have not been motivated to hunt and the previous trial was excluded from the analysis. Each motivated female was tested with different test prey categories, but only once with a particular test prey category to avoid pseudoreplication.

Chemical Analysis

For the GC–MS, we used an Agilent 6890N Series gas chromatograph (Agilent Technologies, Böblingen, Germany) coupled to an Agilent 5973 inert mass selective detector. The GC was equipped with an RH-5ms+ fused silica capillary column (30 m × 0.25 mm ID; $df = 0.25$ μm; temperature programme: from 60°C to 300°C at 5°C/min and held for 1 min at 60°C and for 10 min at 300°C). Helium was used as the carrier gas with a constant flow of 1 ml/min. A split/splitless injector was installed at 250°C and in the splitless mode for 60 s. The electron impact mass spectra (EI-MS) were recorded with an ionization voltage of 70 eV, a source temperature of 230°C and an interface temperature of 315°C. The software MSD ChemStation for Windows (Agilent Technologies, Palo Alto, CA, U.S.A.) was used for data acquisition. To identify the alkanes, alkenes and (*Z*)-11-eicosen-1-ol, we compared retention times and mass spectra of honeybee extracts with purchased substances or with data from a commercial library (NIST, Gathersburg, MD, U.S.A.; see also Schmitt et al. 2003).

Data Analysis

The data were analysed with Fisher's exact test (two tailed) using the statistics program BIAS for Windows version 7.07 (epsilon-Verlag GbR, H. Ackermann, Frankfurt/Main, Germany). Sample sizes were limited by the number of beewolf females available for the tests, the very time-consuming training of the females and the relatively long period of 4–5 weeks needed for the bioassays (this period corresponds to the females' average life expectancy). Some females did not learn to attack the tethered bees or did not attack the dead bees (olfaction experiment: 13/44; (*Z*)-11-eicosen-1-ol experiment: 9/28) and thus could not be used in the bioassays. Those that could be trained were not active outside their nests every day. Active females could usually be tested with only one or two prey objects on any one day, since they spent much of their time feeding or in their nests. Some of the females died before their response to all prey objects could be tested. Therefore, sample sizes differ between different tested stimuli.

RESULTS

The Role of Olfaction

Odourless honeybees ($N = 29$) and odourless honeybee dummies ($N = 17$) did not trigger the hovering behaviour and were (with one exception) not attacked (Fig. 1). By rescenting the previously odourless bees, we elicited the natural hovering and hunting behaviour. Prey objects that were rescented by contact with live honeybees were recognized as prey and attacked in 82–90% of the tests (Fig. 1). Similarly, the honeybee extracts applied to odourless bees and dummies elicited attacks in 75–81% of the tests (Fig. 1). After contact with the rescented bees, females showed the final stinging behaviour. Scented dummies, on the other hand, did not evoke stinging attempts but were thoroughly and excitedly antennated by the females. Since the proportion of hovering flights and predation attacks (stinging behaviour not included) on scented honeybees and dummies was similar and we wanted to reduce the influence of visual cues, we used only dummies for the subsequent tests.

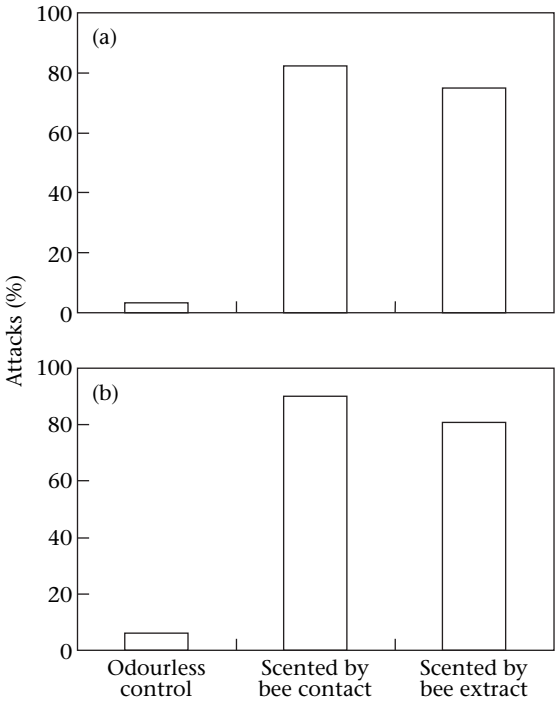

Figure 1. Percentage of attacks on differently treated prey objects. (a) Test prey were odourless control bees ($N = 29$), rescented bees that had been in contact with live honeybees (bee contact, $N = 34$) and bees treated with cuticle extracts of honeybees (bee extract, $N = 28$; difference from control: Fisher's exact test: $P < 0.0001$ for contact and extract). (b) Test prey were odourless control dummies ($N = 17$), scented dummies taken from a vial with live bees (bee contact, $N = 20$) and dummies to which a honeybee extract was applied (bee extract, $N = 17$; difference from control: Fisher's exact test: $P < 0.0001$ for contact and extract).

The Role of (*Z*)-11-eicosen-1-ol

The chemical profile of honeybee cuticles was dominated by alkanes and alkenes. (*Z*)-11-eicosen-1-ol was only a minor component. Figure 2 shows a typical total ion chromatogram of a honeybee worker extract containing (*Z*)-11-eicosen-1-ol and a chromatogram of this extract after removal of the (*Z*)-11-eicosen-1-ol. (*Z*)-11-eicosen-1-ol could be completely removed from the hydrocarbon fraction of the honeybee extract as can be seen in the overlay of the two chromatograms. The pattern of all other components was identical.

The attack rate on dummies scented with bee extract was lower in this second set of bioassays than in the first. This difference might have been caused by a disparity in the hunting motivation between the two sets of females (they were taken from different populations) or seasonal influences (the second set of bioassays was conducted later in the season). In contrast to the bee extract ($N = 19$), HC ($N = 14$) never elicited attacks on dummies (Fig. 3). HC was initially attractive to females; they hovered but did not attack the dummies. Notably, HC+Eicosenol ($N = 8$) was about as attractive as the bee extract and was significantly more attractive than HC to hunting beewolf females (Fig. 3). The bee extract and HC+Eicosenol triggered the normal sequence of the hunting behaviour comprising the hovering flight and the following attack.

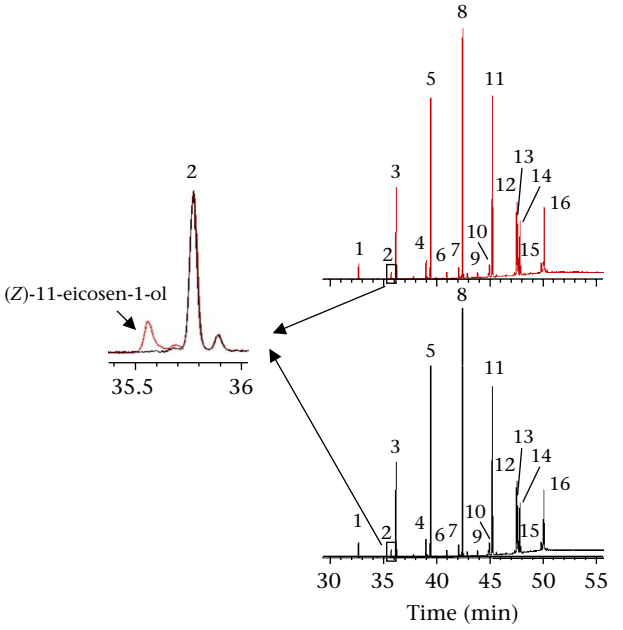

Figure 2. Comparison of the chromatograms of a cuticular extract of 10 honeybees (red) and the hydrocarbon fraction of the same extract (black). The chart on the left shows a magnified section of an overlay of both chromatograms. (For orientation: 1: heneicosane; 2: (*Z*)-9-tricosene; 3: tricosane; 4: (*Z*)-9-pentacosene; 5: pentacosane; 6: hexacosane; 7: (*Z*)-9-heptacosene; 8: heptacosane; 9: octacosane; 10: (*Z*)-9-nonacosene; 11: nonacosane; 12: (*Z*)-7-hentriacontene; 13: (*Z*)-9-hentriacontene; 14: hentriacontane; 15: (*Z*)-7-tritriacontene; 16: (*Z*)-9-tritriacontene.)

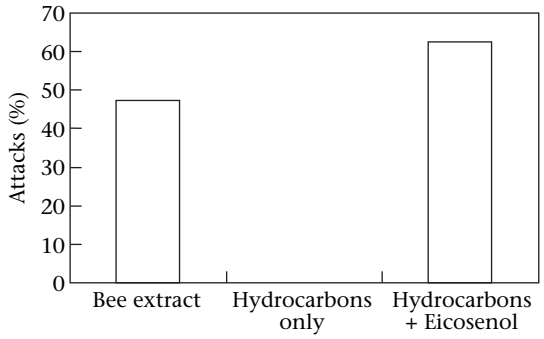

Figure 3. Percentage of attacks on honeybee dummies treated with differently processed honeybee extracts. Difference between natural honeybee extracts (bee extract, $N = 19$) and pure hydrocarbon extracts (from which (*Z*)-11-eicosen-1-ol had been removed by chromatography; $N = 14$): Fisher's exact test: $P = 0.002$. Difference between the hydrocarbon solution after the readdition of (*Z*)-11-eicosen-1-ol ($N = 8$) and pure hydrocarbon extracts: Fisher's exact test: $P = 0.002$.

DISCUSSION

Prey Recognition in Beewolves

The results of our behavioural assays clearly show that beewolf females use olfactory cues for prey identification. In accordance with Tinbergen (1935) we conclude that the hunting behaviour of beewolf females consists of three distinct steps and involves different sensory modalities. The hovering flight in front of the potential prey at a distance of approximately 10 cm seems to be an important step in the hunting sequence in which the female decides to attack or to ignore a potential prey. The hovering flight was elicited by bee extracts as well as by the hydrocarbon solutions (with or without (*Z*)-11-eicosen-1-ol). This implies that females rely on 'bee-like' odours for the first detection and localization of the potential prey. White dummies treated with honeybee extract could not be localized and were not attacked by females (G. Herzner, unpublished data). Thus, both visual and olfactory cues are essential for initial prey detection.

The actual identification of the prey and the decision to attack seem to take place during the hovering and are obviously mediated by olfactory cues. Notably, the hydrocarbon fraction alone did not elicit attacks. Only honeybee extracts containing (*Z*)-11-eicosen-1-ol, as in either the bee extract or in the HC+Eicosenol solution, elicited attacks. Thus, (*Z*)-11-eicosen-1-ol is an essential cue for prey recognition and attack.

The final stimuli that evoke the stinging behaviour seem to comprise both gustatory and tactile cues. Rescented honeybees were stung by beewolf females, indicating that all necessary cues were present. Dummies bearing the same odour were not stung, most probably because they had the 'wrong' shape and surface. Such a multisensory detection, localization and acceptance of prey or hosts, involving visual, olfactory, gustatory and tactile cues, has been described for other hymenopteran species, such as the digger wasp *Liris niger* (Anton & Gnatzy 1998) and two species of aphid parasitoids (Battaglia et al. 2000; Völkl 2000).

The sensory equipment responsible for prey detection and recognition in *P. triangulum* has not yet been investigated in detail. We found a high diversity and density of presumably olfactory and gustatory sensilla on the antennal flagella of European beewolves (Herzner et al. 2003). One type of these sensilla, the multiporous large sensillum basiconicum is present only on the antennae of female beewolves. This sensillum type has a role in the discrimination between potential prey species in the digger wasp *L. niger* (Anton & Gnatzy 1998), and may serve a similar function in *P. triangulum*.

(*Z*)-11-eicosen-1-ol as a Recognition Cue

Predators or parasitoids with a broad prey or host range usually use cues that are common to many potential prey or host species (Lewis et al. 1971; Schaffner & Müller 2001; but see Steidle & van Loon 2003). Specialized predators, such as the European beewolf, however, usually locate or identify their prey with the help of infochemicals (or mixtures thereof) that are more or less unique to the prey (Bargen et al. 1998; Bernays 1998; De Moraes et al. 1998; Powell et al. 1998; Al Abassi et al. 2000; Steidle & van Loon 2003).

Beewolf females flying through their hunting grounds are exposed to an enormous number of chemical stimuli. Owing to their monophagy, females must be able to filter out reliably those stimuli that are characteristic for their honeybee prey. Alkanes, methylalkanes and alkenes, which are the prominent compounds found on honeybee cuticles (Francis et al. 1985; Salvy et al. 2001), are widespread among the Hymenoptera (e.g. *Lasioglossum malachurum*: Ayasse 1991; several bumblebee species: Oldham et al. 1994; the leafcutter bee, *Megachile rotundata*: Paulmier et al. 1999; *Andrena nigroaenea*: Schiestl et al. 1999; the almond seed wasp, *Eurytoma amygdali*: Krokos et al. 2001; three species of decorator wasps *Eucerceris*: Clarke et al. 2001; and the wheat stem sawfly, *Cephus cinctus*: Bartelt et al. 2002; *Polistes fuscatus*: Panek et al. 2001; the European hornet, *Vespa crabro*: Ruther et al. 2002) and other insect orders (e.g. Diptera: Ishii et al. 2001; Coleoptera: Nelson et al. 2002; Lepidoptera: Guo & Blomquist 1991; Heteroptera: Drijfhout & Groot 2001). Hence, they cannot easily be used as reliable cues for honeybee prey recognition by female European beewolves. (*Z*)-11-eicosen-1-ol, however, is very scarce in the Hymenoptera and has hitherto not been reported from nonhymenopteran species. Besides its occurrence in *A. mellifera* and in the pheromone of *P. triangulum* males (Schmitt et al. 2003; T. Schmitt, G. Herzner, P. Schreier & E. Strohm, unpublished data), it has been described as a major component of the venom of the Asian honeybee *Apis cerana* (Schmidt et al. 1997), the Dufour's gland secretion of the neotropical stingless bee *Frieseomelitta varia* (Patricio et al. 2003), and in the thoracic glands of male carpenter bees, *Xylocopa micheneri*, from Arizona (Andersen et al. 1988). Thus, (*Z*)-11-eicosen-1-ol has not been described in species other than *Apis mellifera* in the distribution range of the European beewolf and might hence be an ideal cue for largely unequivocal prey recognition by beewolf females.

Removal of (*Z*)-11-eicosen-1-ol from the honeybee extracts rendered them unattractive to foraging females. It is a well-known but little understood phenomenon that odour blends lose or change their information content by only slight changes in their composition. In several bee species, females become unattractive to males after mating owing to the removal (Ayasse et al. 1999), addition (Schiestl & Ayasse 2000) or removal and addition (Simmons et al. 2003) of certain components from or to the odour bouquets. Although (*Z*)-11-eicosen-1-ol is only a very minor component of the chemical cuticular profile of honeybees, its presence is essential for prey recognition; it can thus be regarded as a discriminator or recognition substance (Hölldobler & Michener 1980).

(*Z*)-11-eicosen-1-ol and the Sensory Trap

The very small amounts of (*Z*)-11-eicosen-1-ol and its low volatility suggest that beewolf females possess high sensory (olfactory) and neural abilities that evolved to maximize their success in detecting and identifying honeybees. The neural hypothesis (Bernays & Wcislo 1994; Bernays 1998, 2001) states that resource specialization, which is usually associated with strong sensory and neural focusing, leads to more economic information acquisition and processing, which allows for faster and more effective search and recognition behaviours. Such a fast and accurate assessment and identification of the potential prey is crucial to females' survival and reproductive success. The resulting strong restriction to only one or a few very particular host cues by females may act as an important selective force for the evolution of the males' sexual signals ('sensory drive', see e.g. Endler 1992). Thus, a highly specialized, and therefore highly sensitive, prey recognition mechanism should be more prone to exploitation by male signalling than a less fine-tuned system.

Our results clearly support our second prediction that follows from the sensory trap model. (*Z*)-11-eicosen-1-ol is used as an essential cue for prey recognition and has therefore a high potential to function as a sensory trap. Males that incorporate it in their pheromone may evoke an out-of-context feeding response of females to attract them (West-Eberhard 1984; Christy 1995) thereby increasing their reproductive success.

Acknowledgments

We thank Sabine Förtsch, Matthias Mösl, Mascha Bischoff, Carolin Hefter, Joachim Haug, Wolf-Christian Saul and Martin Kaltenpoth for their help during the course of the bioassays. Norbert Schneider is acknowledged for the construction of the breeding cages and dummies. We thank Annett Endler for supplying the silica gel column. This study was supported by the German Science Foundation, DFG, Bonn (SFB 554, TP B3).

References

Al Abassi, S., Birkett, M. A., Pettersson, J., Pickett, J. A., Wadhams, L. J. & Woodcock, C. M. 2000. Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. *Journal of Chemical Ecology*, **26**, 1765–1771.

Andersen, J. F., Buchman, S. L., Weisleder, D., Plattner, R. D. & Minckley, R. L. 1988. Identification of thoracic gland constituents from male *Xylocopa* spp. Latreille (Hymenoptera: Anthophoridae) from Arizona. *Journal of Chemical Ecology*, **14**, 1153–1163.

Anton, S. & Gnatzy, W. 1998. Prey specificity and the importance of close-range chemical cues in prey recognition in the digger wasp, *Liris niger*. *Journal of Insect Behavior*, **11**, 671–690.

Ayasse, M. 1991. Chemische Kommunikation bei der primitiv eusozialen Furchenbiene *Lasioglossum malachurum* (Halictidae): Ontogenese kastenspezifischer Duftstoffbouquets, Paarungsbiologie und Nesterkennung. Ph.D. thesis, Universität Tübingen.

Ayasse, M., Engels, W., Lubke, G., Taghizadeh, T. & Francke, W. 1999. Mating expenditures reduced via female sex pheromone modulation in the primitively eusocial halictine bee, *Lasioglossum (Evylaeus) malachurum* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, **45**, 95–106.

Bargen, H., Saudhof, K. & Poehling, H. M. 1998. Prey finding by larvae and adult females of *Episyrphus balteatus*. *Entomologia Experimentalis et Applicata*, **87**, 245–254.

Bartelt, R. J., Cosse, L. A., Petroski, R. J. & Weaver, D. K. 2002. Cuticular hydrocarbons and novel alkenediol diacetates from wheat stem sawfly (*Cephus cinctus*): natural oxidation to pheromone components. *Journal of Chemical Ecology*, **28**, 385–405.

Basolo, A. L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science*, **250**, 808–810.

Battaglia, D., Poppy, G., Powell, W., Romano, A., Tranfaglia, A. & Pennacchio, F. 2000. Physical and chemical cues influencing the oviposition behaviour of *Aphidius ervi*. *Entomologia Experimentalis et Applicata*, **94**, 219–227.

Bernays, E. A. 1998. The value of being a resource specialist: behavioral support for a neural hypothesis. *American Naturalist*, **151**, 451–464.

Bernays, E. A. 2001. Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology*, **46**, 703–727.

Bernays, E. A. & Wcislo, W. T. 1994. Sensory capabilities, information processing, and resource specialization. *Quarterly Review of Biology*, **69**, 187–204.

Christy, J. H. 1995. Mimicry, mate choice, and the sensory trap hypothesis. *American Naturalist*, **146**, 171–181.

Christy, J. H., Backwell, P. R. Y. & Schober, U. 2003a. Interspecific attractiveness of structures built by courting male fiddler crabs: experimental evidence of a sensory trap. *Behavioral Ecology and Sociobiology*, **53**, 84–91.

Christy, J. H., Baum, J. K. & Backwell, P. R. Y. 2003b. Attractiveness of sand hoods built by courting male fiddler crabs, *Uca musica*: test of a sensory trap hypothesis. *Animal Behaviour*, **66**, 89–94.

Clark, D. L. & Uetz, G. W. 1992. Morph-independent mate selection in a dimorphic jumping spider demonstration of movement bias in female choice using video-controlled courtship behaviour. *Animal Behaviour*, **43**, 247–254.

Clarke, S. R., Dani, F. R., Jones, G. R., Morgan, E. D. & Schmidt, J. O. 2001. (*Z*)-3-hexenyl (*R*)-3-hydroxybutanoate: a male specific compound in three North American decorator wasps *Eucerceris rubripes*, *E. conata* and *E. tricolor*. *Journal of Chemical Ecology*, **27**, 1437–1447.

De Moraes, C. M., Lewis, W. J., Pare, P. W., Alborn, H. T. & Tumlinson, J. H. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature*, **393**, 570–573.

Dicke, M. & Sabelis, M. W. 1992. Costs and benefits of chemical information conveyance: proximate and ultimate factors. In: *Insect Chemical Ecology* (Ed. by B. Roitberg & M. Isman), pp. 122–155. New York: Chapman & Hall.

Drijfhout, F. P. & Groot, A. T. 2001. Close-range attraction in *Lygocoris pabulinus* (L.). *Journal of Chemical Ecology*, **27**, 1133–1149.

Dunkelblum, E., Mendel, Z., Gries, G., Gries, R., Zegelman, L., Hassner, A. & Mori, K. 1996. Antennal response and field attraction of the predator *Elatophilus hebraicus* (Hemiptera: Anthocoridae) to sex pheromones and analogues of three *Matsucoccus* spp (Homoptera: Matsucoccidae). *Bioorganic and Medicinal Chemistry*, **4**, 489–494.

Endler, J. A. 1992. Signals signal conditions and the direction of evolution. *American Naturalist*, **139**, S125–S139.

Evans, H. E. & O'Neill, K. M. 1988. *The Natural History and Behavior of North American Beewolves*. Ithaca: Comstock Publishing Associates.

Francis, B. R., Blanton, W. E. & Nunamaker, R. A. 1985. Extractable surface hydrocarbons of workers and drones of the genus *Apis*. *Journal of Apicultural Research*, **24**, 13–26.

Free, J. B., Williams, I. H., Pickett, J. A., Ferguson, A. W. & Martin, A. P. 1982. Attractiveness of (*Z*)-11-eicosen-1-ol to foraging honeybees. *Journal of Apicultural Research*, **21**, 151–156.

Free, J. B., Ferguson, A. W., Simpkins, J. R. & Al-Saíad, B. N. 1983. Effect of honeybee Nasonov and alarm pheromone components on behaviour at the nest entrance. *Journal of Apicultural Research*, **22**, 214–223.

Godfray, H. C. J. 1994. *Parasitoids*. Princeton, New Jersey: Princeton University Press.

Guo, L. & Blomquist, G. J. 1991. Identification, accumulation, and biosynthesis of the cuticular hydrocarbons of the southern armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera, Noctuidae). *Archives of Insect Biochemistry and Physiology*, **16**, 19–30.

Hendrichs, M. A. & Hendrichs, J. 1998. Perfumed to be killed: interception of Mediterranean fruit fly (Diptera: Tephritidae) sexual signaling by predatory foraging wasps (Hymenoptera: Vespidae). *Annals of the Entomological Society of America*, **91**, 228–234.

Herzner, G., Schmitt, T., Linsenmair, K. E. & Strohm, E. 2003. Flagellar sensilla in male and female European beewolves, *Philanthus triangulum* F. (Hymenoptera: Sphecidae). *Entomologica Fennica*, **14**, 237–247.

Hoffmeister, T. S. & Gienapp, P. 1999. Exploitation of the host's chemical communication in a parasitoid searching for concealed host larvae. *Ethology*, **105**, 223–232.

Hölldobler, B. & Michener, C. D. 1980. Mechanisms of identification and discrimination in social Hymenoptera. In: *Evolution of Social Behavior: Hypotheses and Empirical Tests* (Ed. by H. Markl), pp. 35–58. Weinheim: Verlag Chemie.

Howard, R. W., Charlton, M. & Charlton, R. E. 1998. Host-finding, host-recognition and host-acceptance behavior of *Cephalonomia tarsalis* (Hymenoptera: Bethylidae). *Annals of the Entomological Society of America*, **91**, 879–889.

Ishii, K., Hirai, Y., Katagiri, C. & Kimura, M. T. 2001. Sexual isolation and cuticular hydrocarbons in *Drosophila elegans*. *Heredity*, **87**, 392–399.

Krokos, F. D., Konstantopoulou, M. A. & Mazomenos, B. E. 2001. Alkadienes and alkenes, sex pheromone components of the almond seed wasp *Eurytoma amygdali*. *Journal of Chemical Ecology*, **27**, 2169–2181.

Lewis, W. J., Sparks, A. N. & Redlinger, L. J. 1971. Moth odor: a method of host-finding by *Trichogramma evanescens* (Hymenoptera – Trichogrammatidae). *Journal of Economic Entomology*, **64**, 557–558.

Madden, J. R. & Tanner, K. 2003. Preferences for coloured bower decorations can be explained in a nonsexual context. *Animal Behaviour*, **65**, 1077–1083.

Millar, J. G., Rice, R. E., Steffan, S. A., Daane, K. M., Cullen, E. & Zalom, F. G. 2001. Attraction of female digger wasps, *Astata occidentalis cresson* (Hymenoptera: Sphecidae) to the sex pheromone of the stink bug *Thyanta pallidovirens* (Hemiptera: Pentatomidae). *Pan Pacific Entomologist*, **77**, 244–248.

Nelson, D. R., Olson, D. L. & Fatland, C. L. 2002. Cuticular hydrocarbons of the flea beetles, *Aphthona lacertosa* and *Aphthona nigriscutis*, biocontrol agents for leafy spurge (*Euphorbia esula*). *Comparative Biochemistry and Physiology B, Biochemistry and Molecular Biology*, **133**, 337–350.

Oldham, N. J., Billen, J. & Morgan, E. D. 1994. On the similarity of the Dufour gland secretion and the cuticular hydrocarbons of some bumblebees. *Physiological Entomology*, **19**, 115–123.

Panek, L. M., Gamboa, G. J. & Espelie, K. E. 2001. The effect of a wasp's age on its cuticular hydrocarbon profile and its tolerance by nestmate and non-nestmate conspecifics (*Polistes fuscatus*, Hymenoptera: Vespidae). *Ethology*, **107**, 55–63.

Patricio, E., Lopez, L. C., Maile, R. & Morgan, E. D. 2003. Secretions of stingless bees: the Dufour glands of some *Frieseomelitta* species (Apidae, Meliponinae). *Apidologie*, **34**, 359–365.

Paulmier, I., Bagneres, A. G., Afonso, C. M. M., Dusticier, G., Riviere, G. & Clement, J. L. 1999. Alkenes as a sexual pheromone in the alfalfa leaf-cutter bee *Megachile rotundata*. *Journal of Chemical Ecology*, **25**, 471–490.

Phelan, P. L. 1992. Evolution of sex pheromones and the role of asymmetric tracking. In: *Insect Chemical Ecology* (Ed. by B. Roitberg & M. Isman), pp. 265–314. New York: Chapman & Hall.

Phelan, P. L. 1997. Evolution of mate-signaling in moth: phylogenetic considerations and predictions from a asymmetric tracking hypothesis. In: *The Evolution of Mating Systems in Insects and Arachnids* (Ed. by J. Choe & B. Crespi), pp. 240–256. Cambridge: Cambridge University Press.

Pickett, J. A., Williams, I. H. & Martin, A. P. 1982. (*Z*)-11-eicosen-1-ol, an important new pheromonal component from the sting of the honeybee, *Apis mellifera* L. (Hymenoptera, Apidae). *Journal of Chemical Ecology*, **8**, 163–175.

Powell, W., Pennacchio, F., Poppy, G. M. & Tremblay, E. 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control*, **11**, 104–112.

Proctor, H. C. 1991. Courtship in the water mite *Neumania papillator*: males capitalize on female adaptations for predation. *Animal Behaviour*, **42**, 589–598.

Quicke, D. 1997. *Parasitic Wasps*. London: Chapman & Hall.

Rodd, H. F., Hughes, K. A., Grethor, G. F. & Baril, C. T. 2002. A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proceedings of the Royal Society of London, Series B*, **269**, 475–481.

Ruther, J., Sieben, S. & Schricker, B. 2002. Nestmate recognition in social wasps: manipulation of hydrocarbon profiles induces aggression in the European hornet. *Naturwissenschaften*, **89**, 111–114.

Ryan, M. J. 1990. Sexual selection, sensory systems and sensory exploitation. *Oxford Surveys in Evolutionary Biology*, **7**, 157–195.

Ryan, M. J., Fox, J. H., Wilczynski, W. & Rand, S. 1990. Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature*, **343**, 66–67.

Sakaluk, S. K. 2000. Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. *Proceedings of the Royal Society of London, Series B*, **267**, 339–343.

Salvy, M., Martin, C., Bagneres, A. G., Provost, E., Roux, M., LeConte, Y. & Clement, J. L. 2001. Modifications of the cuticular hydrocarbon profile of *Apis mellifera* worker bees in the presence of the ectoparasitic mite *Varroa jacobsoni* in brood cells. *Parasitology*, **122**, 145–159.

Schaffner, U. & Müller, C. 2001. Exploitation of the fecal shield of the lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), by the specialist parasitoid *Lemophagus pulcher* (Hymenoptera: Ichneumonidae). *Journal of Insect Behavior*, **14**, 739–757.

Schiestl, F. P. & Ayasse, M. 2000. Post-mating odor in females of the solitary bee, *Andrena nigroaenea* (Apoidea, Andrenidae), inhibits its male mating behavior. *Behavioral Ecology and Sociobiology*, **48**, 303–307.

Schiestl, F. P., Ayasse, M., Paulus, H. F., Löfstedt, C., Hansson, B. S., Ibarra, F. & Francke, W. 1999. Orchid pollination by sexual swindle. *Nature*, **399**, 421–422.

Schmidt, J. O., McDaniel, C. A. & Simon Thomas, R. T. 1990. Chemistry of male mandibular gland secretions of *Philanthus triangulum*. *Journal of Chemical Ecology*, **16**, 2135–2143.

Schmidt, J. O., Morgan, E. D., Oldham, N. J., Do Nascimento, R. R. & Dani, F. R. 1997. (*Z*)-11-eicosen-1-ol, a major component of *Apis cerana* venom. *Journal of Chemical Ecology*, **23**, 1929–1939.

Schmitt, T., Strohm, E., Herzner, G., Bicchi, C., Krammer, G., Heckel, F. & Schreier, P. 2003. (*S*)-2,3-dihydrofarnesoic acid, a new component in cephalic glands of male European beewolves *Philanthus triangulum*. *Journal of Chemical Ecology*, **29**, 2469–2479.

Simmons, L. W., Alcock, J. & Reeder, A. 2003. The role of cuticular hydrocarbons in male attraction and repulsion by female Dawson's burrowing bee, *Amegilla dawsoni*. *Animal Behaviour*, **66**, 677–685.

Stålhandske, P. 2002. Nuptial gifts of male spiders function as sensory traps. *Proceedings of the Royal Society of London, Series B*, **269**, 905–908.

Steidle, J. L. M. & van Loon, J. J. A. 2003. Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata*, **108**, 133–148.

Steidle, J. L. M. & Ruther, J. 2000. Chemicals used for host recognition by the granary weevil parasitoid *Lariophagus distinguendus*. *Journal of Chemical Ecology*, **26**, 2665–2675.

Stowe, M. K., Turlings, T. C. J., Lougrin, J. H., Lewis, W. J. & Tumlinson, J. H. 1995. The chemistry of eavesdropping, alarm, and deceit. *Proceedings of the National Academy of Sciences, U.S.A.*, **92**, 23–28.

Strohm, E. 1995. *Allokation Elterlicher Investitionen Beim Europäischen Bienenwolf Philanthus triangulum* Fabricius (Hymenoptera: Sphecidae). Berlin: Verlag Dr Köster.

Strohm, E. & Linsenmair, K. E. 1997. Low resource availability causes extremely male-biased investment ratios in the European beewolf, *Philanthus triangulum* F. (Hymenoptera: Sphecidae). *Proceedings of the Royal Society of London, Series B*, **264**, 423–429.

Strohm, E. & Linsenmair, K. E. 1998. Temperature dependence of provisioning behaviour and investment allocation in the European beewolf *Philanthus triangulum* F. *Ecological Entomology*, **23**, 330–339.

Strohm, E. & Marliani, A. 2002. The cost of parental care: prey hunting in a digger wasp. *Behavioral Ecology*, **13**, 52–58.

Tinbergen, N. 1935. Über die Orientierung des Bienenwolfes. II. Die Bienenjagd. *Zeitschrift für Vergleichende Physiologie*, **21**, 688–716.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136–179. Chicago: Aldine.

Völkl, W. 2000. Foraging behaviour and sequential multisensory orientation in the aphid parasitoid, *Pauesia picta* (Hym., Aphidiidae) at different spatial scales. *Journal of Applied Entomology*, **124**, 307–314.

West-Eberhard, M. J. 1984. Sexual selection, competitive communication and species-specific signals in insects. In: *Insect Communication* (Ed. by T. Lewis), pp. 283–324. New York: Academic Press.