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ORIGINAL PAPER

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Bumble bees alert to food with pheromone from tergal gland

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Abstract Foragers of *Bombus terrestris* are able to alert their nestmates to the presence of food sources. It has been supposed that this happens at least partially through the distribution of a pheromone inside the nest. We substantiate this claim using a behavioral test in which an alerting signal is transmitted from one colony to another by long distance air transport, so excluding all other modalities of information exchange. We then investigated the source of the pheromone and were able to show that a hexane extract from tergites V–VII of bumble bee workers elicits higher activity, like a successful forager does. Extracts from other glands, such as the mandibular, labial, hypopharyngeal, and Dufour's gland as well as extracts from other parts of the cuticle had no effect. This suggests that bumble bees possess a pheromone-producing gland, similar to the Nasanov gland in honey bees. Indeed, an extract from the honey bee Nasanov gland also proved to alert bumblebee workers, suggesting a possible homology of the glands.

Keywords Alerting · *Bombus* · Communication · Foraging · Tergal glands

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Introduction

Bumble bees are eusocial insects and live in colonies of up to a few hundred individuals. Nevertheless, they were long thought to be foraging by "individual initiative" (Heinrich 1979). This would mean that each bee essentially behaves like a solitary forager, making all decisions on the basis of information collected by itself. However, recent research has revealed a more complex picture. Bumble bees do not communicate spatial co-ordinates of food sources as honey bees do with the waggle dance, but successful bumble bee foragers do inform nestmates about the general availability and the scent of rewarding food sources (Dornhaus and Chittka 1999, 2001). This helps recruits to avoid searching for food when foraging conditions are unfavorable, as well as aiding in the discovery of rewarding flowers, which bees can recognize by the scent they have learned while in the nest.

How is this information about food availability communicated in the nest? Successful bumble bee foragers, when returning to the colony, often show a curious behavior consisting of excited runs with bouts of wing-fanning. The reaction shown by previously passive bees in the nest is to become active (show increased movement speeds; Dornhaus and Chittka 2001) and leave the nest in search for food. This increase in activity is transmitted from one colony to an adjacent one when air exchange is possible, but a single sheet of transparent plastic wrap disrupted signal transmission in a previous study (Dornhaus and Chittka 2001). This procedure would not disable visual communication or substrate vibrations, and we therefore concluded that a pheromone is the most likely means of alerting recruits (Dornhaus and Chittka 2001). However, the procedure in these experiments could not fully exclude other possibilities, such as air-movement perception or airborne sound (Oeynhausen and Kirchner 2001). We therefore conducted a new experiment to test more conclusively the hypothesis of the involvement of a pheromone in bumble bee forager alerting.

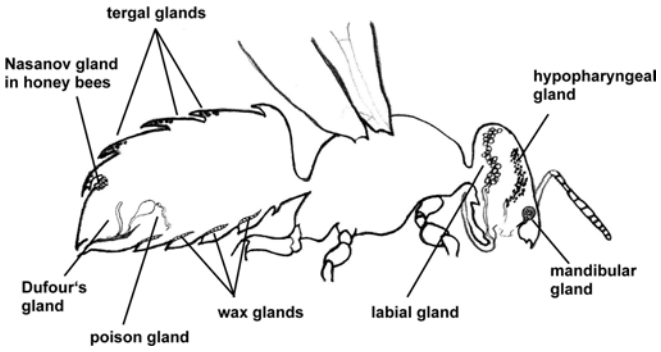

Fig. 1 Schematic drawing of pheromone-producing glands in social bees in head and abdomen (after Free 1987). In bumble bees, there are also wax glands associated with tergites (Cruz-Landim 1963)

A further test for the involvement of a pheromone is the isolation of a substance that can elicit the same reaction as the forager's behavior. We therefore attempted to isolate this putative pheromone and identify the site of its production by testing several potentially pheromone-producing glands known from bumble bees (Fig. 1). We used bioassays to test for the alerting function of various glandular extracts as well as different parts of the abdominal cuticle. Interestingly, an extract from the last couple of tergites elicited increased activity, like a successful forager does. In honey bees this is the site of the Nasanov gland (Snodgrass 1956) but the function of such a gland in bumble bees has not been described previously, although various small cuticular glands have been characterized morphologically (Hesselhaus 1922; Jacobs 1925; Altenkirch 1961; Cruz-Landim 1963).

Materials and methods

Bumble bees

All experiments were performed with lab-reared colonies of *Bombus terrestris* (obtained from Koppert, The Netherlands). Each nest was contained in a wooden box (26 cm×14 cm×10 cm), which was connected to a foraging arena (40 cm×60 cm×30 cm) with a Plexiglas tube. Nest box and foraging arena had transparent Plexiglas covers, so that the behavior of the bees could be observed. Bees were fed by placing a dish filled with 2 mol l^{-1} sucrose solution (feeder) into the arena. Pollen was given directly into the nest box.

Nature of the alerting signal

As a confirmation that the alerting signal produced by foragers is indeed chemical and volatile, we tested whether the signal would pass from one colony to another if direct contact of bees was prevented, as a more rigorous version of the 2-colony experiment in Dornhaus and Chittka (2001). Here the setup was modified such that the signal between colonies had to pass through a 1.7-m-long glass tube (inner diameter 5 mm), rather than just through a double mesh (Fig. 2). Glass funnels on both sides of the tube were placed approx. 3 cm over the nest structures. In the middle of the glass tube, a pump was mounted, which created a flow of air from the "sender colony" to the "receiver colony" (strength of flow was 0.4 l min^{-1}).

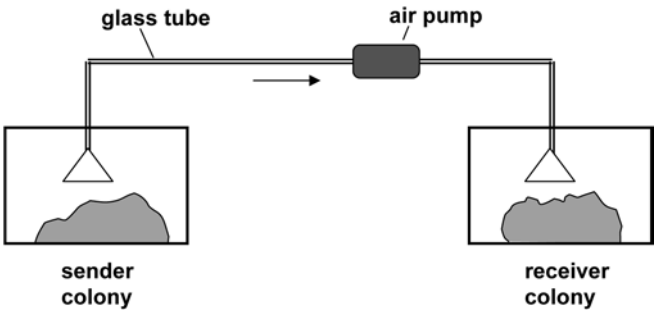

Fig. 2 Setup of experiment 1. The two bumble bee nest boxes are connected by a glass tube; air is pumped from the sender to the receiver colony by a pump

Activity of both colonies was measured by counting the number of bees leaving the respective nests in each 5-min interval through a control phase of 30 min and an experimental phase of 60 min. During the control phase, no food was available to any of the colonies. At the beginning of the experimental phase, a feeder was set up in the foraging arena of the "sender colony". Bees from that colony were allowed to forage freely, whereas bees from the "receiver colony" had no food available throughout the experiment. Changes in activity of the "receiver colony" which depend on activity of the "sender colony" during the experimental phase, but not the control phase, would then indicate that some information passed through the glass tube. The experiment was repeated 12 times on different days.

Source of the alerting pheromone

To identify the glands that are involved in the production of the alerting pheromone, we used a bioassay in which the effect of various gland extracts on the activity of a bumble bee colony was measured. Each experimental run consisted of 30 min control phase and 60 min experimental phase with one extract tested at a time. Activity of the colony was measured continuously as before. These time intervals were chosen because earlier studies had shown that full activation of foragers might take about 30 min (Dornhaus and Chittka 2001). By comparing the average activity during 30 min control phase and the average activity during the second half of the experimental phase (also a 30 min interval), this time-lag in forager reaction was accounted for. During the experimental phase, 10 μl extract was injected every 5 min onto a piece of filter paper placed in a little metal cage in the bumble bee nest. In some experiments, as detailed in the results, in addition to a control phase entirely without manipulation, the solvent (hexane) was injected onto the filter paper in the nest for an additional 60 min, to control for effects of the manipulation and the solvent. Each extract was tested in at least ten such experimental runs. Between two experimental runs there was a time interval of at least 2 h during which bees were not manipulated and not allowed access to food sources, to allow activity to calm down.

In a first set of experiments, Dufour's gland, mandibular, labial, and hypopharyngeal glands were tested using extracts made from glands of ten bees in 300 μl hexane (thus 1/3 bee equivalent was injected into the nest per 5 min). Also tested was an extract made from tergites VI and VII, which would include various glands on the cuticle of the bumble bees (see also Fig. 1). All extracts were always kept on ice and never used more than 24 h after preparation. Since the cuticular extract proved to be the most interesting, a second set of experiments was performed in which various parts of the cuticle were extracted. This was done to further localize the involved gland, which could be one of the various small cuticular glands described in the literature (Hesselhaus 1922; Jacobs 1925; Altenkirch 1961; Cruz-Landim 1963) or the effect might be due to

some component from the wax glands, which are located on all tergites and sternites in bumble bees (Hesselhaus 1922; Cruz-Landim 1963). We used extracts from sternites, anterior tergites, and posterior tergites. For the sternite extract, sternites V–VII were cut out, clipping off the joints to both tergites and the adjoining sternites. Likewise, tergites III–V (anterior tergites) and V–VII (posterior tergites) were cut out. In all cases, tracheae and inner organs were thoroughly removed. Sternites, respectively tergites, from ten bees were placed in 300 μl hexane.

Similarity to honeybee Nasanov pheromone?

In honey bees, the Nasanov gland, used in the context of foraging, is located between tergites VI and VII. To investigate possible similarities between the Nasanov gland and potential glands in the same location in bumble bees, an extract of tergites V–VII of *Apis mellifera* bees was prepared in the same way as in experiment 2 with *B. terrestris*. The honey bees were taken from a large colony which was foraging outside. The effect of the extract on activity of a bumble bee colony was tested in the same manner as in experiment 2.

We also tested geraniol, the main component of the honey bee Nasanov pheromone, and citral, one of its most active components (Free 1987). These substances were diluted with hexane (1:1000) and, as in experiment 2, 10 μl per 5 min were injected into a bumblebee colony for 60 min, after a control phase of 30 min and a phase of hexane injection of 60 min (the solvent control).

Results

Nature of the alerting signal

An alerting signal was passed through 1.7-m glass tube. When the foragers of the ''sender colony'' started foraging, and presumably communicated to their nestmates the new availability of food, the ''receiver colony'' also showed a brief activity peak, usually lasting for about 5 min (Fig. 3). The activity in the ''receiver colony'' correlated significantly with the change in ''sender

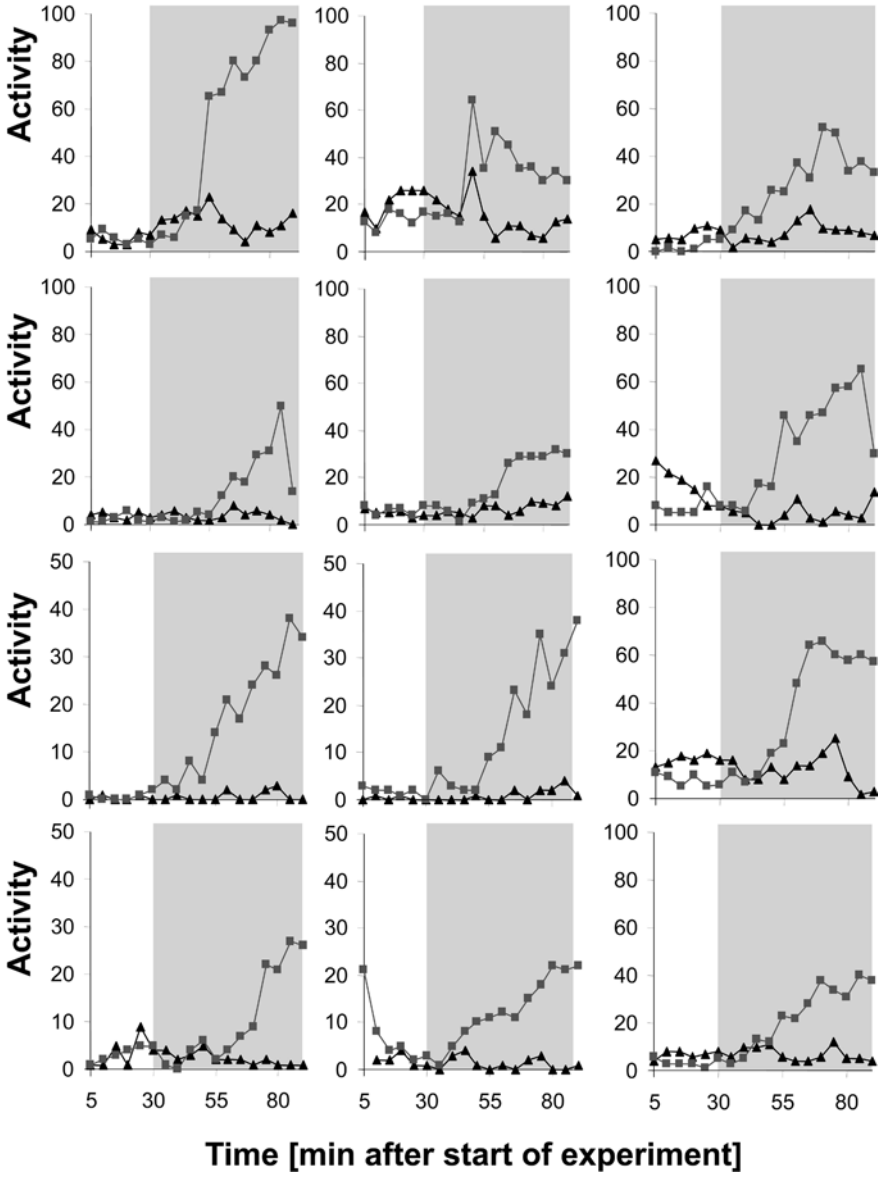

Fig. 3 Activity, i.e., the number of bees leaving the colony in the preceding 5-min interval, of the sender and receiver colonies during the control phase (from 0 to 30 min) and after the sender colony has started foraging (35–90 min, *shaded area*). The activity of the sender colony rises as more bees start foraging (*grey squares*). Receiver colony activity (*black triangles*) is very variable, but usually peaks when most foragers in the foraging colony become activated

colony'' activity ($P < 0.01$, $r = 0.25$, $n = 143$). This means that an increase in activity in the ''sender colony'', presumably because of foragers alerting their nestmates, resulted in higher activity in the ''receiver colony''. There was no such correlation in the control phase ($P = 0.50$, $r = 0.09$, $n = 60$).

Source of the alerting pheromone

The only extract that seemed to have any effect in the first set of experiments was the cuticular extract made from tergites VI and VII, which induced a significantly higher activity in bumble bee colonies. The median (between runs) number of bees leaving the nest per 5-min interval increased from 10.4 during the control phase to 24.3 during the experimental phase ($P < 0.01$, $n = 10$ experimental runs; Wilcoxon test). When Dufour's gland was used, the activity also increased, from 9.8 during the control to 14.2 during the following experimental phase, but this was not significant ($P = 0.42$, $P = 11$; Wilcoxon test).

The glands from the head did not seem to cause any change in activity. Median activity when only the solvent was injected into the nest was 4.8, which is not significantly different from the control phase without manipulation (median activity 3.9, $n = 10$, $P = 0.31$). Activity during the phase when mandibular gland extract was injected (median activity 2.4) was also not different from the solvent control ($n = 10$, $P = 0.72$; Wilcoxon test); the same is true using labial glands (median activity 5.5, $n = 13$, $P = 0.20$) and using hypopharyngeal glands (median activity 4.0, $n = 13$, $P = 0.83$). Thus, the first set of experiments already indicated that the alerting pheromone might be produced by a gland associated with the cuticle.

The second set of experiments, comparing different parts of the cuticle, showed that only the extracts from the posterior tergites (V–VII) induced bumble bees to leave the nest; the solvent or other cuticular extracts had no effect (Fig. 4). The activity during the experimental phases when the posterior tergite extract was injected into the nest was again significantly higher than the activity during the respective control phases ($P < 0.05$, $n = 14$; Wilcoxon test). Activity after injection of extract from sternites did not differ from activity during control phases ($P = 0.21$, $n = 15$). When extract from anterior tergites was used, activity did not increase significantly either, although there seemed to be a trend ($P = 0.07$, $n = 13$). We conclude that the gland eliciting the ''food alert'' in bumble bees is present only in the posterior tergites; however, it cannot be excluded that the gland cells are also present to a lesser degree in the anterior tergites. It is also possible that some of the alerting pheromone from the posterior tergites was smeared onto the other tergites. Whichever is the case, only the extract from the posterior tergites produced a significant effect, so they seem to contain the main source of the pheromone.

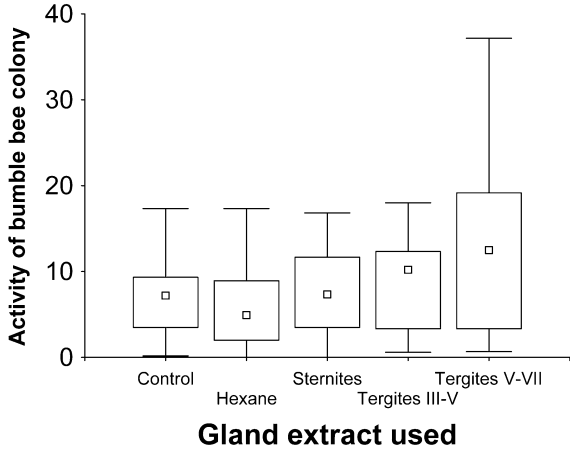

Fig. 4 Injecting the solvent or extract from different glands or parts of the cuticle has no effect on the activity of the bumble bee colony. Only an extract from tergites V-VII elicits higher activity. Shown are medians, 1st and 3rd quartiles (*boxes*) and ranges (*error bars*)

Similarity to honey bee Nasanov pheromone?

The extract from *A. mellifera* tergites results in an increase in activity of a bumble bee colony: from 2.1 bees per 5-min interval during control (and 2.7 during injection of hexane) to 5.7 when *Apis* extract was injected ($P < 0.05$, $n = 10$ experimental runs; Wilcoxon test). It is thus likely that the extract made from honey bee tergites, and thus probably the honey bee Nasanov gland, contained substances that are also present in the bumble bee alerting pheromone. Geraniol and citral did not result in an increase in activity ($P = 0.21$ and $P = 0.29$, $n = 9$ and $n = 12$, respectively; Wilcoxon test); this in turn means that the active substances were neither geraniol nor citral, at least not in their pure form. It is possible that bumble bees are only alerted by a mixture of these or other substances which are contained in the honey bee Nasanov gland.

Discussion

A volatile chemical is used by successful bumble bee foragers as a signal to alert nestmates to the availability of food. While our experiments do not exclude the possibility that signals of other modalities, such as acoustic signals (Oeynhausen and Kirchner 2001) may also be employed, the passage of the signal through a 1.7-m glass tube and a vibrating and noisy pump demonstrates that at least part of the alerting effect is due to a pheromone. Furthermore, the reaction of passive bumble bees to a successful forager increased mobility, and bees leaving the nest, presumably to search for food, could also be elicited by a chemical alone, namely an extract from the cuticle of the posterior tergites (V–VII) of the bumble bees' abdomen.

In honey bees, the Nasanov gland is located in the anterior part of tergite VII. This gland consists of a large number of Leydig cells which open into a groove between tergites VI and VII (Jacobs 1925; Renner 1960). Honey bees expose the Nasanov gland, thereby releasing the secretion, to attract nestmates; they do so when swarming, at the nest entrance, and sometimes at very rewarding food sources (Renner 1960). The behavior of exposing the gland by stretching the abdomen (called ''sterzeln'' in German) is not known in bumble bees. Bumble bees also do not possess the morphological structures associated with the Nasanov gland in honey bees, which vary even within the genus *Apis* (Jacobs 1925). However, clusters of secretory gland cells have also been described in bumble bees (Hesselhaus 1922; Jacobs 1925). These occur on all tergites and also on sternites (Jacobs 1925; Altenkirch 1961). It has been speculated that some of the more dispersed gland cells serve to lubricate the joints between the tergites (''Schmierdrüsen''; Hesselhaus 1922), but to our knowledge there is no evidence for this function, and most authors classify their function as ''unknown'' (Jacobs 1925; Altenkirch 1961; Duffield et al. 1984). In *B. terrestris*, Jacobs (1925) finds structures on the intersegmental membrane which he interprets as being adapted to facilitate evaporation of glandular secretion; because of this he supposes that these bumble bees have scent glands on the anterior and posterior sides of tergite VI (Jacobs 1925). Cruz-Landim (1963) claims that bumble bees possess a ''scent gland'' in the same location, and in a more recent review bumble bees are even said to possess a Nasanov gland (Duffield et al. 1984).

We have demonstrated that an extract from tergites VI and VII induces higher activity in a bumble bee colony, whereas an extract from other tergites has little, if any, effect, and an extract from sternites has no effect. This indicates that the glands on the posterior tergites contain a pheromone which is produced to a much lesser extent, if at all, in the other segments. The responsible gland cells might then be homologous to the Nasanov gland in honey bees. Our experiments show that bumble bees can be alerted by an extract from honey bee tergites containing the Nasanov gland. Thus, the gland used by bumble bees in alerting produces at least partly the same or similar components as the Nasanov gland in honey bees. Bumble bees are, however, not alerted by geraniol or citral. This could either mean that only a mixture of substances is recognized by bumble bees as an alerting pheromone, or that one of the other components of the Nasanov pheromone is the alerting substance. One other alternative explanation would be that honey bees possess an undescribed small gland in this location in addition to the Nasanov gland.

Although the similarity between the bumble bee tergal gland and the honey bee Nasanov gland could be an example of convergent evolution, it could also mean that these glands have a common origin. If that is the case, bumble bees use their ''Nasanov gland'' to produce a pheromone, not for attraction or recruitment, but in a similar context for alerting nestmates to the presence of rewarding food sources.

Alerting signals are not uncommon in social insects. However, inside the nest motor signals are commonly used by foragers to activate nestmates. This has been described in ants (for a review see Hülldobler and Wilson 1990) as well as stingless bees, where a returning bee also performs excited runs in the nest (Lindauer and Kerr 1960; Nieh 1998). Bumble bees might be unusual in that they can use a pheromone inside the nest for the purpose of motivating nestmates. On the other hand, similar pheromones in other bees may simply have not been looked for and still await discovery.

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