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Group Response to Alarm Pheromones in Social Wasps and the Honeybee

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Abstract

The reaction of social wasps and honeybees to alarm pheromones was quantified using a metabolic bio-assay. Groups of workers placed in an air flow-through system were exposed for 60 s to the odour of a squashed venom sac and sting apparatus. The reaction of the workers was a stereotyped short term increase in their metabolic activity which was monitored with an infrared CO2-analyzer. Group size had considerable influence on the reaction of each individual in the group. In honeybees, isolated workers showed weak responses to the alarm stimuli and with increasing group size the reaction per individual rose. Wasps showed the opposite behaviour. Isolated wasp workers generally reacted more intensely than workers in groups. This phenomenon is particularly well expressed in small species (e.g. Paravespula vulgaris). The large workers of Vespa crabro only showed this phenomenon if they were exposed to high doses of 2-methyl-3-butene-2-ol, the major compound of their alarm pheromone. We could not elicit any alarm reactions in Polistes gallicus with this metabolic bioassay.

Introduction

Insect societies rely heavily on the cooperative behaviour of individuals aggregated in groups. Actions and interactions among the members of the group will result in the expression of an overall group phenotype. Often groups can express behaviour which cannot be expressed by individuals. For example, the ability to regulate the temperature of nests of social insects is often only evident if workers are in groups. Isolated workers behave like poikilothermic organisms and have only limited abilities to thermoregulate (Heinrich 1985).

Another typical group character is the defensive behaviour of colonies of social insects. The use of alarm pheromones to alert other nest mates is common in most social wasps, ants and honeybees. Pheromonal action, by definition (Karlson & Butenandt 1959), requires a group of at least two individuals, where
we have one individual releasing a pheromone and another one receiving the pheromonal signal. If group sizes are larger, the dynamics of the group reaction will be more complex, with possible secondary pheromonal, tactile, optical, or acoustical interactions among the group members. All of these stimuli have been shown to play important roles in the mode of group defense in social insects (Blum 1969; Hermann & Blum 1981).

A vast literature is available concerning the defensive behavior of honeybees, which probably is the best understood social behavior of insects in general (see Seeley 1985 for a recent review). Honeybee colonies which are exposed to alarm pheromone show a typical defense reaction. Individually foraging honeybee workers, however, show no behavioral response to the alarm pheromone (Maschwitz 1964). This field observation was supported by a metabolic bio-assay used by Southwick & Moritz (1985). The reaction of honeybee workers to alarm pheromone was quantified by measuring the resulting increase in oxygen consumption of the bees. Single bees responded to high doses of the major compound of the honeybee's alarm pheromone, isopentyl-acetate, only weakly (Boch et al. 1962). Groups of honeybee workers, however, generally showed the typical short term increase in their metabolic activity.

Defensive behaviors of social wasps have been documented in detail (Maschwitz 1964; Edwards 1980; Jeanne 1981). Alarm semiochemicals are known to be effective and release alarm behavior throughout the eusocial group of Vespinae (Maschwitz 1964, 1984; Edwards 1980). In the other major subfamily of social wasps, the Polistinae, alarm pheromones have been found (Jeanne 1982; Post et al. 1984), although some Polistes species seem to have no alarm pheromone (Freisling 1943; Maschwitz 1964; West-Eberhard 1969). Also, the tropical Polybia occidentalis has been found to have an alarm pheromone (Jeanne 1981).

In the genus Vespa few attempts have been made to characterize the chemical structure of alarm pheromones (Saslavsky & Ishay 1973; Wheeler et al. 1983). For the European hornet, V. crabro, a major compound of the alarm pheromone has recently been identified (Veith et al. 1984). 2-Methyl-3-butene-2-ol proved to elicit 74% of the defense reaction compared with that released by the venom itself in field tests with V. crabro nests.

Although qualitative aspects of alarm behavior and some biochemical background of the alarm reaction of wasps are well understood, little is known concerning quantitative aspects of the alarm reaction. For the study of group-individual interactions, which are crucial for the understanding of group behavior, a quantitative approach, as previously used for honeybees (Moritz et al. 1985), was thought to be more useful. Since the social structures of wasp colonies are very different from those of honeybee colonies, and the forms of sociality are highly variable among species, group interactions in wasps might be different, reflecting the various social complexities of wasp colonies. In this paper we study the impact of group size on the alarm reaction of individual members of the group in various social wasps in relation to the alarm reaction shown in honeybees. We use a bio-assay, in which CO₂ production of groups of wasps and groups of honeybees is measured to quantify the metabolic activity.
Material and Methods

Nests of Vespa crabro, Paravespula vulgaris, Dolichovespula media, Dolichovespula saxonica, and Polistes gallicus were collected in early June. The nests were established in the field in the vicinity of the laboratory at the Institute für Bienenkunde in Oberursel for easy access to experimental wasps. Workers were caught at the entrance to the nests and placed into airtight containers (100 ml volume) without using any narcotics. Group sizes in the test containers ranged from 1 to 20 individuals. Honeybee workers were collected from the honey combs of a colony of Apis mellifera carnica. They were placed in the same 100-ml containers as the wasps. Group sizes, however, ranged from 1 to 70 workers.

The test groups were kept for at least 1 h in the dark at 25 °C. The containers were supplied with candy as a food source and constantly ventilated at a rate of 100 ml/min with a suction pump. This pre-test phase allowed the test groups to calm down and reach their resting metabolic rate.

The containers with the test groups, which remained in the dark, were then connected with tygon tubing to a flow through system as shown in Fig. 1. The air was pulled through the system at a

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**Fig. 1:** A group of workers was placed into an airtight container [TEST] which was connected to the above flow-through system (arrows indicate the direction of air flow; 100 ml/min). The air was dried [DRY] before measuring the CO2-concentration. A timer controlled T-valve allowed for the selection of fresh air or pheromone from a sting apparatus with venom sac or synthetic pheromone.

**Fig. 2:** Typical metabolic reaction of a group of 5 V. crabro workers to a 1-min exposure to alarm pheromone. Arrow: time at which the stimulus was presented. The reaction was quantified by measuring Δ CO2, the difference between resting metabolic rate and max. CO2-production.
rate of 100 ml/min using a suction pump. Air entered the system via an electronic T-valve which allowed for selection of fresh air or air which had passed through a small tube (1.5 ml) containing a single squashed sting apparatus and a venom sac. A timer controlled the T-valve so that the test groups were continuously exposed to fresh air and received only a 60-s dose of pheromone. In another set of experiments with V. crabro, air saturated with synthetic 2-methyl-3-buten-2-ol (MBO) was used as an alarm stimulus for 5 s. The air which had passed the test group was dried (CaCO₃) and led through an infrared analyzer (Hartmann & Braun, URAS 2) to determine the CO₂ concentration. The results were recorded on line with an HP 3390 A integrator.

The difference between the amount of CO₂ produced at rest and maximal metabolic activity as response to the pheromone (ΔCO₂) was used to quantify the alarm reaction (see Fig. 2). The groups were narcotized with CO₂ after the test and weighed in order to determine the weight specific metabolic reaction (μlCO₂/min/mg). Each test group was used only once to have statistically independent observations and to avoid possible effects of conditioning.

Results

1. Reaction of Wasps to Sting and Venom Gland

A typical reaction of a test group after the 60-s exposure to the volatile compounds of a single sting apparatus and venom sac is shown in Fig. 2. After a steep increase in the CO₂-production, reflecting a high metabolic activity, the groups slowly return to the resting rate. The short delay between setting of the timer and actual reaction is a result of the time taken by the stimulus to be drawn through the tubing between the pheromone container, test flask, and infrared analyzer. At the flow rate used it arrived after 10 s at the test container and after another 5 s at the CO₂ measuring cell of the analyzer.

All tested species, except P. gallicus, responded to the stimuli used in our bio-assay. Since P. gallicus did not react and other investigators could find no evidence for the existence of alarm pheromones in this species (Freising 1943; Maschwitz 1964), we eliminated P. gallicus from the further analyses of group effects. Though these wasps did not respond to the sting apparatus or the venom sac, this does not mean that they generally do not show responses to other volatile odours presented. P. gallicus regularly showed strong metabolic reactions to the odour of unspecific dead tissue from other insects (e.g., squashed bee thoraces).

The other tested wasp species could be successfully used in investigations of the effect of the group on individual reactions. Fig. 3 shows that the reaction of the individual strongly depends on the number of members in the group. Particularly in the small species (e.g., P. vulgaris), isolated individual workers showed greater responses than workers in larger groups. This can be tested statistically by analyzing the corresponding regression equations in Table 1. Since they are of the type of

\[ y = a + \frac{b}{x} \]

\( y \) expresses the metabolic reaction [μl CO₂/min/mg] for large groups [x] (b/x → 0 for large x). The regression coefficient, b, expresses the impact of the group size on the alarm reaction of the individual group member. If b is close to zero, as in V. crabro, group size plays no role in the reactivity of the individual. Thus the overall group reaction will linearly increase with the number of wasps in the group. The regression coefficient, b, and consequently the effect of the group on individual activity gets smaller the larger the insect.
Fig. 3: Effect of group size on individual metabolic reaction to physiological concentrations of alarm pheromones for four different social wasp species. Circles: $\bar{X} \pm$ S.E.

For single wasps there is a strong negative correlation between body mass and reaction as shown in Fig. 4. Also, the variance of the reaction gets smaller whereas the variance of body mass gets larger with increasing weight of the workers. The various species form distinct clusters which do not overlap in their 90% confidence limits. For large groups there is no significant difference in the reaction per body mass among all tested species.

2. Exposure of V. crabro to 2-Methyl-3-butene-2-ol

When test groups of V. crabro were exposed for 5 s to air which was saturated with 2-methyl-3-butene-2-ol, the results were different from those with the squashed sting apparatus. In this test series we found the typical hyperbolic regression of reaction on group size (1) as found for the other wasps when exposed to sting apparatus and venom (Fig. 5). The effect of the group size on the individual reaction was almost nine fold stronger than in the experiments with V. crabro in which a single venom sac was used as pheromone source. Consequently,
the regression coefficient, \( \beta \), is significantly larger indicating a stronger impact of the group structure (\( t \)-test: \( t = 2.94 \ p < 0.01 \)). The reactivity of large groups to the 5-s exposure is in the same range as the reaction to the volatile compounds released by sting apparatus and venom sac (\( \bar{a}_{\text{venom}} = 0.048 \pm 0.020 \), \( \bar{a}_{\text{MBO}} = 0.039 \pm 0.022 \), \( t = 0.303 \), n.s.).

3. Reaction of Honeybees to Alarm Pheromones

In *A. mellifera*, the effect of group size on the reaction of individual workers to the pheromones released by their sting apparatus was strikingly different from that for wasps. Fig. 6 shows that group size has the opposite effect on the metabolic response from that observed for the wasp species tested. Single
Table 1: Regression of individual metabolic reaction, $y$ [μl/min/mg], on group size, $x$, for the various species tested. Generally the non-linear regression (hyperbolic or logarithmic) gives a significantly better fit to all data, except in the case of *V. crabro* when exposed to venom. The $r^2$ for the linear regression is given in the last column. * $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$

<table>
<thead>
<tr>
<th>Species</th>
<th>Regression equation</th>
<th>N</th>
<th>$r^2$</th>
<th>$r^2_{[\text{linear}]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vulgaris</em></td>
<td>$y = 0.033 + 0.212/x$</td>
<td>70</td>
<td>0.29***</td>
<td>0.03 n.s.</td>
</tr>
<tr>
<td><em>D. saxonica</em></td>
<td>$y = 0.057 + 0.097/x$</td>
<td>70</td>
<td>0.21***</td>
<td>0.06 n.s.</td>
</tr>
<tr>
<td><em>D. media</em></td>
<td>$y = 0.038 + 0.078/x$</td>
<td>70</td>
<td>0.40***</td>
<td>0.19**</td>
</tr>
<tr>
<td><em>V. crabro</em> venom sac</td>
<td>$y = 0.048 + 0.006/x$</td>
<td>70</td>
<td>0.00 n.s.</td>
<td>0.00 n.s.</td>
</tr>
<tr>
<td><em>V. crabro</em> synthetic</td>
<td>$y = 0.039 + 0.051/x$</td>
<td>60</td>
<td>0.38***</td>
<td>0.05 n.s.</td>
</tr>
<tr>
<td><em>A. mellifera</em></td>
<td>$y = 0.035 + 0.023 \ln x$</td>
<td>70</td>
<td>0.31***</td>
<td>0.15**</td>
</tr>
</tbody>
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Fig. 6: Reaction of groups of honeybees to the sting apparatus. There is a positive effect of group size on reactivity. Circles: $X \pm$ S. E.

individuals occasionally showed a weak response, but most (63%) isolated workers did not increase their metabolic activity at all. For larger group sizes however, the reaction per individual worker increased, following a logarithmic regression function (see Table 1) which gives a significantly better fit than a linear regression model (F-test, $F = 1.52; p < 0.05$). The alarm reaction of individuals in large groups of honeybees per body mass is similar to those found for the highly reactive individual *P. vulgaris* workers.

**Discussion**

Though the bio-assay gives highly accurate quantitative results for the metabolic reaction to pheromones, one has to keep in mind that they indeed are *quantitative* and tell us nothing about the *quality* of a particular behaviour which is released by pheromone action. In the present study, we used well documented alarm pheromones, for which the releasable behavioural patterns had been described in detail (Maschwitz 1964; Jeanne 1981, 1982; Veith et al. 1984). Such
behavioural analyses, used to determine the quality of a behaviour which is released by a pheromone, have to be carried out before a quantitative approach, such as ours, can be meaningful. The present bio-assay is *not* applicable to search for new pheromonal compounds, since insects usually have a well developed olfactory sense and will unspecifically respond to odors of various kinds (Southwick & Moritz 1985).

**Positive Group Effects**

The alarm reaction in honeybees is a typical social behaviour in its classical sense. Single individual workers do not respond to pheromonal cues and only in groups can alarm be elicited. The results found in the present study confirm those of Southwick & Moritz (1985), who found that in honeybees, single workers exposed to high doses of synthetic isopentyl acetate did not respond, whereas with increasing group size the reaction of the individual workers increased logarithmically. Moritz et al. (1985) showed that this group effect is not due to alarm pheromones secondarily produced by the tested bees. Besides effects released by other semiochemicals, also physical contact among the group members might affect group dynamics. "Critical mass" phenomena for group behaviour, as observed in the present study, have been reported repeatedly for honeybees in longevity experiments. Chauvin and coworkers (Chauvin 1980, 1981, 1985; Grasse & Chauvin 1944; Stibon 1967a, b) found that workers in groups had a longer lifetime. Arnold (1979) and Stibon (1971) reported that the neurosecretion in the pars intercerebralis is dependent on group size. Hence the social context of a group seems to be a general factor, affecting not only behavioural but also various physiological conditions in honeybees.

**Polistes gallicus**

In *P. gallicus* the alarm reaction seems to be controlled in a different manner. Workers did not respond to any volatile compound in the sting apparatus and the venom sac. This observation is in line with previous observations (Freising 1943) in which *P. gallicus* became alerted by wing buzzing only, regardless of whether the buzzing wasp was intact or had had its abdomen removed. The absence of an efficient alarm pheromone in the venom is particularly supported by the reaction to unspecific insect tissue. The strong increase in metabolic activity after exposing *P. gallicus* workers to freshly squashed thoraces of honeybees and the other wasp species used in our experiment, probably resulted from a necrophoretic reaction. However, since we only can test quantity of the reaction and not the quality, other reasons for this strong reaction cannot be excluded. Regardless of the actual origin of the metabolic response, this shows that the wasps were highly sensitive to physiological concentrations of volatile compounds in the present test setup. Therefore the lack of response to the volatile compounds of the sting apparatus and venom sac is unlikely to be an artifact of the test conditions.

The lack of a reaction in our bio-assay, however, does not mean that in *P. gallicus* there is no effect of the group on the individual alarm reactions. Our bio-assay simply did not release any alarm behaviour and, therefore, this species does
not qualify for the documentation of any group effects with the present experimental setup. Another quantitative bio-assay has to be developed, in order to analyze social behaviour which is not releasable by volatile semiochemicals.

**Negative Group Reaction in Vespinae**

Generally workers of the tested species of the subfamily Vespinae were affected in their alarm response by the group size. However, they reacted strikingly different from the honeybee workers. Single isolated wasps were more irritable than when gathered in groups. Since workers of all tested species are known to release alarm pheromones when alerted (Maschwitz 1964), this shows that effects of secondarily released alarm pheromones play only a minor role in the present bio-assay. Semiochemicals, other than alarm pheromones, dispersed by the workers, or tactile interactions between the group members might affect the irritability of the individual worker.

This surprising group effect was most evident in the workers of the small yellow jackets (P. vulgaris) and was reduced in species with increasing body mass. Workers of V. crabro were not significantly affected by group size when exposed for 1 min to the odours of the sting apparatus and venom sac. Regardless of their social environment they showed a constant response to the alarm stimulus. Only when V. crabro workers were exposed to unphysiologically high concentrations of synthetic MBO did they react similarly to the other wasp species.

The correlation between reactivity of single workers and body mass found support in preliminary field observations where also the large species showed a lower defense activity than the small species. This recalls a general biological rule (Kleiber 1947), that small organisms have larger relative metabolic rates than large organisms of the same order.

**Sociobiological Consequences**

The various group effects on individual behaviour may be a reflection of the social organizations of the species tested. Defensive situations arise when colonies of social insects are disturbed by predators. Honeybees are organized in large perennial colonies. Honeybee workers never encounter a pheromonal released defensive situation without the social contact with numerous nest-mates. Only in the social context of a group will a defensive reaction be meaningful. Single honeybees on foraging flights are unlikely to become alarmed by pheromones since there is no colony to defend. If predators are encountered at the feeding site, foragers tend to avoid the aggressor instead of attacking.

Colonies of social wasps in the temperate zones have to be established by individual queens in spring. In the most critical initial stage early in the season, when nests are most vulnerable only very few workers will be available for colony defense. Workers of social wasps, therefore, will frequently encounter nest defense situations while in very small groups. From this point of view, it should be crucial for wasps to react to alarm pheromones in a way that is independent of group size. This hypothesis, however, would only explain the phenomenon we found for V. crabro, where large group sizes did not reduce the reaction of the
individual. It does not explain why the reactivity of the workers decreases with increasing group size in the other species tested.

This problem indeed leaves an open question. Why is the single wasp worker more reactive than when within a group? Several speculations could be tested in further studies. One explanation for the high irritability of single individual wasps might be the different behaviour of wasps and bees foraging for protein. Foraging wasps operate as individual hunters (Spradbery 1973), also predateing on other Hymenoptera which have highly efficient defense mechanisms. For example V. orientalis is known to predate on honeybees (Ishay et al. 1967) as does P. germanica (Kemper & Döhring 1962). In this situation, where the hunter easily becomes the hunted, it may well pay off to be alert and show rapid responses to changes in the odoural environment. Wasps occasionally use their sting and venom to paralyze prey (Pack-Beresford 1931), thus releasing alarm pheromone in foraging situations. Furthermore single wasps tend to behave aggressively when disturbed at feeding sites (Chulun 1984). Consequently isolated wasps in our bio-assay should be highly irritable and show strong responses to changes in environmental odours. The group might provide a social context in which reactivity could be kept at a lower level.

Honeybees forage exclusively on floral carbohydrate and protein sources and they do not predate on other species. Therefore, the behaviour of individual honeybee workers in foraging situations should differ substantially from that of wasps. Even in cases of extreme foraging competition individual honeybees have not been observed to use their sting for killing other insects in order to defend the nectar source. In groups, however, they may show aggressive behaviour e.g. when robbing another colony (Koeniger 1982).

Another explanation may be that other pheromones which are released by workers in groups, not only enhance the alarm reaction but also may have attenuating effects. Francke et al. (1978) found that targets were not attacked by wasps in field tests when treated with a mixture of certain spiroketals.

Regardless of the actual origin of the negative group effect on the reactivity found in wasps, we have definitely shown that the group is a crucial factor in the expression of social behaviour for both wasps and honeybees. Such group effects can be enhancing or attenuating, depending on the social organization of the species tested. We would expect also social behaviour, other than the alarm behaviour, to be subject to similar non-linear group effects as found in the present study.

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Literature Cited


Ishay, J., H. Bytinski-Salz, & A. Shilo, 1967: Contributions to the bioeconomics of the oriental hornet (Vespa orientalis Fab.). Israel. J. Entomol. 2, 45—106.


Pelumi, W., 1984: Papierwespen im Konflikt zwischen Angriffs- und Saugtendenz. Versuche an einer künstlichen Futterquelle. Z. Tierpsychol. 64, 147—162.


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