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Alarm Recruitment, Attack Behavior, and the Role of the Alarm Pheromone in *Polybia occidentalis* (Hymenoptera: Vespidae)

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Summary. 1. Defensive behavior in the social wasp *Polybia occidentalis* involves two steps: first, in response to jarring of the nest, large numbers of adults are recruited rapidly to the outer surface of the envelope. Second, a fraction of these wasps may fly out to attack the intruder.

2. Observations suggest that the first step, alarm recruitment, is released by a signal given inside the nest. Experimental introduction into the nest of the odors of various glands and body parts show that venom and venom-bearing structures (venom sac, sting apparatus) elicit alarm recruitment that is indistinguishable from that caused by jarring the nest. The wing buzzing that accompanies alarm recruitment is experimentally shown not to communicate alarm.

3. Outside the nest, the odor of venom greatly reduces the threshold for release of attack behavior, but is not itself a releaser of attack. The release of attack behavior requires an appropriate visual stimulus. Dark color of models elicited more attacks than did movement.

Introduction

Compared to our knowledge of chemical communication in the ants and bees, that of wasps (Vespidae) is in a primitive state. In the best known group, the highly eusocial subfamily Vespinae, there is evidence for three pheromones: a queen substance (Ishay et al. 1965), a thermoregulatory pheromone (Ishay 1972, 1973; Koeniger 1975) and an alarm pheromone (Maschwitz 1964; Edwards 1980). The first two have been chemically identified (Ikan et al. 1969; Veith and Koeniger 1978), and the alarm pheromone is known to be contained in the venom (Maschwitz 1964).

The other major subfamily of social wasps, the Polistinae, has been little explored in this regard, despite the fact that it contains more than 85% of the species of social Vespidae (Jeanne 1980). *Polistes canadensis* (tribe Polistini) has recently been shown to possess an alarm pheromone (Jeanne, in preparation), and swarms of *Polybia sericea* (tribe Polybiini) are guided to new nest sites by means of a trail pheromone (Jeanne 1981). For other species in this large tropical tribe some observations suggest that there is chemical communication of alarm by stinging workers (Naumann 1970; Schremmer 1972; Overal et al. 1980), but this has not been demonstrated experimentally. On the other hand it has been suggested that the sound of wing buzzing by alarmed wasps may also communicate alarm to nestmates (Naumann 1970; Chadab 1979; West Eberhard 1969)

In the present study I provide experimental evidence for an alarm pheromone in the social wasp *Polybia occidentalis*, the first such demonstration for a member of the tribe Polybiini. I also analyze the steps in the defensive response of this species and investigate the releasers of each step.

Materials and Methods

Polybia o. occidentalis (Olivier) is a common wasp distributed from northern South America to northern Argentina (Richards 1978). In Brazil, where the study was carried out, it forms colonies of several hundred adults. Nests are 10–25 cm long and contain 2–8 combs.

The study was done at Fazenda Taperinha, 40 km east of Santarém, Pará, in October and November, 1978, and September and October, 1980 (dry season). All observations and experiments were done on colonies in the field, except when draft-free conditions (experiment 1) or movement or manipulation of nests (experiments 2–4) were required. These experiments were performed on colonies collected at night and transplanted into a sunny room open to the outside during the day. These colonies were provided with sugar water and typically survived from one to several weeks. When experiments were in progress the windows were closed to eliminate drafts. Detailed methods used in each experiment will be given in the relevant section below.

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Fig. 1. Alarm recruitment in *Polybia occidentalis*. The nest has just been jarred, eliciting alarmed exiting by a large fraction of the colony. The line indicates the nest entrance

Results

Description of Alarm and Defensive Behavior

There are two stages to defense of the nest against an intruder.

Alarm Recruitment. If a nest of *Polybia occidentalis* is jarred the wasps inside respond with a sudden burst of wing-buzzing lasting for less than a second. Almost immediately large numbers of wasps pour from the nest entrance and fan out over the surface of the envelope. As they run, their wings are raised and spread at a 45° angle, and the gaster tip is dragged on the surface of the envelope. The movement of exiting wasps is rapid at first, then gradually slows until it stops after 5–8 s ($n=7$). Later emerging wasps move in behind them until the entire nest is covered with motionless wasps, all with wings raised (Fig. 1).

Within 15–30 s after the beginning of the response, if the nest is not disturbed again, the wasps fold their wings and re-enter the nest, beginning with those nearest the nest entrance. The last to fold their wings and begin moving are those at the edges of the array – i.e. the first ones out of the nest. These may hold their alarm posture for 60–75 s or more before resuming normal activity.

Attack. If the initial disturbance to the nest is a strong one, e.g. a very violent jarring, several or many of the recruited wasps may fly at the intruder and attempt to sting it. Attack flights are rapid and direct, and the attacker typically hits the intruder with considerable force. Moving objects near the nest are particularly subject to attack.

The Release of Alarm Recruitment

If a nest of *P. occidentalis* is lightly disturbed mechanically (tapping or shaking) there is often no response at first, but with increasingly vigorous stimulation a number of alarmed adults exit suddenly from the nest as if on cue. A similar response may be elicited by ants on the nest or by return to the nest of a worker that has been manhandled or has just stung an intruder. These observations suggest that the wasps are responding to an alarm signal rather than directly to the disturbing stimulus. The following experiments were designed to test this hypothesis.

Is Alarm Recruitment Released by a Chemical Signal? Body fluids or crushed body parts of single, lightly etherized *Polybia occidentalis* workers were placed on the end of the plunger of a 1 ml. glass syringe. The plunger was then inserted halfway into the syringe, and the syringe fitted to a no. 20 hypodermic needle passing through the nest envelope and into the lower chamber of an active nest. The needle was clamped rigidly to a tripod so that the syringe could be attached and removed from it without jarring the nest. The 0.5 ml of air in the syringe was then injected via the needle into the nest. Following each test the plunger was withdrawn partway to remove the residue of injected air from the needle. The syringe and plunger were then cleaned in isopropyl alcohol and allowed to dry before the next test. As controls, injections of air were interspersed between injections of tested substances.

The following body parts and substances were tested: head, thorax, fifth gastral sternite (bearing Richards' gland at its base), hindgut, venom sac, sting apparatus (venom sac and duct removed), venom gland, Dufour gland, venom leaked from sting, acetic acid and formic acid. Fifteen trials were performed with each substance.

Response to the injected substances was measured in two ways. First, the number of alarmed wasps recruited from the nest was counted. Second, the strength of the response was judged as 'weak', 'medi-

Table 1. Responses of *Polybia occidentalis* to injection into the nest of 0.5 ml of air with the indicated body part or substance. Data are numbers of trials yielding an alarm response of each intensity. In parentheses are the numbers of alarmed wasps exiting from the nest, summed for the trials shown

Body part or substance	No. of colonies tested	Strength of response				Total numbers responding
		None	Weak	Moderate	Strong	
Head	3	15				0
Thorax	4	15				0
5th sternite	2	14	1 (1)			1
Hindgut	3	13	2 (9)			9
Dufour gland	2	14	1 (1)			1
Venom gland	2	10	5 (22)			22
Venom sac	6	1	3 (22)	1 (5)	11 (166)	193
Sting apparatus	2	1	5 (38)	4 (77)	5 (31)	146
Venom	4	2	3 (11)	5 (85)	5 (62)	158
Acetic acid	1	13	2 (4)			4
Formic acid	1	13	2 (7)			7
Air	6	40				0

um', or 'strong' on the basis of the rapidity of movement of the responding wasps, independently of their numbers.

Of the injected substances only venom-containing body parts (venom sac, sting apparatus) regularly produced moderate or strong alarm responses involving large numbers of wasps (Table 1). Head and thorax yielded no response. The few weak responses to 5th sternite, hindgut, and Dufour gland were probably the result of accidental contamination when venom leaked from the sting onto the parts during dissection. I conclude that the venom contains a pheromone capable of eliciting alarm recruitment when perceived inside the nest. The negative response to acids shows that alarm is not merely a general response to any strong chemical.

Can Wing-Buzzing Release Alarm Recruitment? Single female wasps, both intact and with sting apparatus removed, were held in forceps close to (1 cm) or touching the envelope at the nest entrance of unalarmed lab colonies to determine whether air- or substrate-borne sound of wing buzzing can release alarm.

Wasps held in forceps buzzed their wings and intermittently tried to bite and sting the forceps. When intact buzzing wasps were held facing the nest entrance, no alarm was elicited among wasps in or on the nest ($n=9$). This was true for wasps held 1 cm from the nest as well as for those held in contact with it. When intact wasps were reversed to face away from the nest, however, clear-cut alarm recruitment resulted ($n=12$). No alarm response was obtained when stingless females were held facing away from the nest ($n=10$). These results are consistent with the conclusion that the alarm was elicited by the odor of venom wafted onto the nest on the backwash of the wings of intact wasps, and was not caused by the sound of wing buzzing.

In order to check whether wing buzzing performed by wasps held in forceps is of the same quality as the buzzing produced by alarmed wasps two nests maintained in the laboratory were placed in firm contact with one another. The odor of venom was injected into one of them, producing alarm recruitment accompanied by wing buzzing. If the wing buzzing were providing an alarm signal, wasps from the second nest should also respond with alarm.

No alarm response could be obtained from the second nest, despite the clear alarm recruitment elicited from the first by the injection of venom ($n=6$).

It is possible that contact between the two nests at only one point was not sufficient to carry a strong enough signal to the second nest to alarm its inhabitants. To test this, a clear, round plastic disk 7 cm in diameter was taped to one end of a paper cylinder 5 cm long. The other end of the cylinder was carefully trimmed to fit tightly against the side of one of the lab reared nests. This windowed chamber could be placed carefully over the wasps resting on that portion of the envelope without alarming them or other wasps on the nest, and held in place with a rubber band. The odor of venom was then used to alarm the wasps outside the chamber. When the odor of venom was wafted over the outside of the nest, wing buzzing and alarmed running were elicited among all those outside the paper chamber, but did not spread to those inside ($N=11$).

As an alternative design to test the effect of wing buzzing in the absence of any accompanying chemical signal, I plugged the passageway connecting the two lower chambers with a wad of cotton using colonies in the field. I then inserted a hypodermic needle, rigidly mounted on a tripod, into the upper of the two chambers. Finally, I broke an artificial 'entrance' through the envelope into this upper chamber on the side of the nest opposite the real entrance, which opens into the lower chamber. Thus the air in each chamber was isolated from that in the other, and the wasps in each had separate exits to the outside of the nest. When the colony had recovered from the trauma of this operation, I injected the odor of venom via the needle into the upper chamber.

In each of four trials with two nests, wing buzzing and alarm recruitment (via the artificial entrance) were elicited among wasps in the upper chamber, but those in the lower chamber showed no response whatsoever.

These experiments leave little doubt that the wing buzzing that typically precedes alarm recruitment does not communicate alarm to nestmates. The conclusion is that the alarm pheromone contained in the venom is the sole alarm signal.

Do Alarmed Wasps Release Venom Inside the Nest? I hypothesize that when the nest is disturbed, one or more wasps inside respond by releasing a small amount of venom from the tip of the sting. Wing buzzing may serve to disperse the pheromone. When the odor is perceived by others inside the nest, alarmed exiting is released. The hypothesis predicts that air inside a nest whose inhabitants have just been alarmed will contain the odor of venom. To test this a tripod-mounted hypodermic needle was inserted into the lower chamber of a nest in the field. A second needle, fitted with a 5 ml syringe, was inserted into a second nest located nearby. The second nest was then rapped sharply, provoking a strong alarm response. A second or so after the initiation of the response air was withdrawn from the nest via the needle into the syringe. The syringe was then quickly removed and mounted on the other needle and its load of air injected into the unalarmed nest. In eight rials alarm was elicited only once – three wasps exited in a very weak response. Either the hypothesis in incorrect, or the test was technically inadequate. It is possible that the air withdrawn from the alarmed nest was not taken from a region of high venom odor concentration. The concentration of venom odor inside the syringe would have been further diluted when the sample was injected into the test nest. For either or both reasons the concentration of pheromone by the time it reached the wasps in the second nest may have been below the threshold for releasing alarm.

Attack Behavior

Once alarmed wasps have been recruited to the outside of the nest, the next step is response to the external threat. If the threat is posed by a vertebrate attacker, the response is to attack and attempt to sting it. The following experiments were undertaken to determine the releasers of attack behavior.

In order to investigate the roles of movement and color these two variables were combined in four visual models: moving black, moving white, stationary black, and stationary white. The models were 60 ml Nalgene square plastic bottles wrapped in black or white paper. The black paper used absorbs 95–96% of incident light at all wavelength (290–700 nm), while the white reflects 80–90% in the range 450–700 nm, but drops to 30–70% reflectance in the UV range (290–400 nm). Thus, assuming that the visual sensitivity of *Polybia occidentalis* is similar to that of *Apis mellifera* and *Vespula germanica*, these papers probably appeared to the wasps black and light bluegreen, respectively (Frisch 1967; Beier and Menzel 1972).

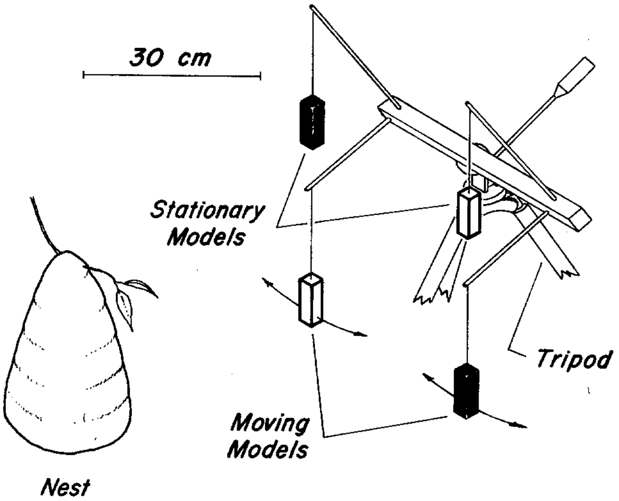

Fig. 2. Apparatus used to test the effectiveness of color and movement in eliciting attack in *Polybia occidentalis*. Black and white, moving and stationary visual models face the nest at a distance of 30–50 cm. The moving models are swung, then the nest is tapped to elicit alarm and attack on the models. Positions of the models are changed at random with each trial

The stationary models were hung from stiff wires 13 cm long, while the moving models swung at the ends of nylon monofilament line of the same length. The wires and lines were attached to the ends of sticks projecting forward from a wooden base and arranged so that the four models formed the corners of a vertical square 30 cm on a side. The position of each model in the square array was randomized for each trial by flips of a coin. The array was mounted on a tripod and placed 30–50 cm from the nest with the center of the square at the same height as the nest entrance (Fig. 2). The backgrounds against which the models were seen by the wasps on the nests were sunlit areas of low herbaceous vegetation.

Each trial was begun by swinging the two moving models from side to side, in a plane perpendicular to the line of sight of wasps on the nest, then tapping the twig bearing the nest to stimulate alarm. The number of wasps attacking each of the four models was tallied. The paper wrapping of each vial attacked during a trial was changed prior to the next trial. Seven colonies in the field were used in the experiment.

No attacks occurred on the models until the nest was tapped. In 36 trials 103 wasps attacked the moving black model, 8 the moving white, 31 the stationary black, and 0 the stationary white. Attack behavior consisted of rapid, direct flight from the nest to the model, the wasp hitting the model with such force as to produce an audible rap. Stinging behavior followed.

The effects of movement and color are independent of one another (contingency table: $\chi^2 = 2.37$; $df = 1$; $0.05 < P < 0.01$). This result should be treated cautiously, however, in view of the observation that in a given trial the same wasp may have attacked the models more than once. The numbers of attacks on the black stationary model and on the white moving model are significantly different from one another ($\chi^2 = 13.56$; $P \ll 0.005$). This means that against a distant background of heterogeneous vegetation black color in the absence of movement is a stronger releaser of attack behavior than is movement in the absence of black color. Since the background for the presentation of the models was not controlled, these results do not separate the effect of darkness of the object per se from the effect of contrast of the object with the background.

In order to determine whether the odor of venom on an object increases the likelihood that it will be attacked two stationary models were placed at the same height and 30 cm from the nest on a level with the nest entrance. Both were of the same color, either black or white. On one of the two models (chosen randomly) the freshly dissected sting apparatuses and venom sacs from three wasps were crushed. Immediately thereafter the nest was lightly tapped, eliciting alarm recruitment. The number of wasps subsequently attacking each model was counted.

In 19 trials using black models 317 attacks were made on the venom-scented model and 72 were made on the unscented model. Thus an object bearing the odor of venom is more likely to be attacked than one lacking the odor. When the models were upwind of the nest (12 of 19 trials) the odor of venom alone was sufficient to elicit alarm, i.e. no tapping of the nest was necessary.

When the test was repeated using white models a striking change in behavior was noted. Although a few wasps flew headlong onto the scented models and attempted to sting in a typical attack response, the majority flew slowly toward the venom odor source and hovered downwind or landed and antennated or chewed at the crushed venom sac as though simply attracted by the odor. In eight trials 111 wasps were so attracted to the scented model and seven to the unscented model.

This difference between the responses to black and to white scented models suggested that the chemical and visual cues play different roles in eliciting attack behavior. To distinguish these roles more clearly by spatially separating the chemical from the visual stimulus, the visual stimulus, consisting of a single black moving model of the type described above, was placed 25–30 cm from the nest entrance, and crosswind to it. The odor source was placed on a bit of white filter paper on the tip of a stick and presented slowly 30 cm upwind of the nest. The odor source presented a much smaller visual stimulus than did the visual model, and every effort was made to minimize movement of the odor source. As a source of venom, venom sacs and sting apparatuses from three wasps were crushed onto the filter paper for each trial. Formic acid and acetic acid were also tested. Controls consisted of presenting clean filter paper in an identical manner. The numbers of wasps attacking and landing on the visual model and on the filter paper bearing the odor were counted.

No attacks were elicited by the controls (13 trials) or by acetic or formic acid (5 trials with each). In contrast, alarm behavior and attack flights were released among wasps on the envelope as soon as air carrying the scent of venom passed over the nest. Virtually all of the 211 responses to the moving model were clear-cut attacks – rapid, audible hits onto the paper followed by stinging attempts (18 trials). In contrast, very few of the 90 wasps attracted to the odor source attacked it in this way. Instead, most flew slowly upwind toward it, often hovered, then landed quietly on the paper, where they walked about, often antennating and chewing at the crushed sting apparatuses. Rarely were sting attempts observed.

These results indicate that the odor of venom alone does not release attack. It must be coupled with the appropriate visual stimulus. The lack of effect of formic and acetic acid supports the conclusion that the alarm response is specific to the odor of venom and not merely to any strong chemical odor.

Discussion

The alarm pheromone has a different effect in each of the two stages of defensive behavior. Inside the nest it elicits alarm recruitment, quickly bringing a force of workers to the outside, where the source of the threat to the colony can be perceived directly. The hypothesis that venom is released by alarmed wasps inside the nest remains unconfirmed by direct test but is supported circumstantially by the evidence that an alarm signal is given inside the nest, by the fact that the alarm recruitment response elicited mechanically is indistinguishable from that elicited by injecting the odor of venom, and by the failure to show that wing buzzing provides the signal.

When perceived outside the nest, the alarm pheromone releases flying, hovering, landing, and inspection at the odor source and it reduces the threshold for attack. Attack itself appears to be released only by an appropriate visual cue, and then only if the wasps have been alarmed by the odor of venom. Thus the chemical signal in the venom is integrated with the visual stimulus of the target object to yield defensive behavior (Kennedy 1978); neither the chemical nor the visual cue is alone sufficient to elicit the full sequence of alarm recruitment followed by stinging attack.

As alarmed wasps emerge from the nest and spread out over the nest envelope they drag the tip of the gaster. The function of this behavior is unknown, but it is conceivable that venom is being spread on the nest envelope, perhaps to maintain a state of alarm or to communicate alarm to wasps already on the outside of the nest.

The alarm response in *Polybia occidentalis* differs in two ways from that of *Vespula vulgaris* and *V. germanica* as described by Maschwitz (1964). First, the fanning out over the nest surface by alarmed *P. occidentalis* does not occur in *Vespula*. The latter fly directly from the nest entrance at the intruder. *V. vulgaris* and *V. germanica* nest underground or in otherwise enclosed cavities; moving onto the nest envelope would not put them in a position to see and respond to a predator. The advantage to *P. occidentalis* of covering the nest envelope may come in the event that the attackers are ants, which can then be detected and removed before they reach the nest entrance. Ants are probably more serious enemies of *Polybia* than of *Vespula* (Jeanne 1975, 1979).

The second difference is that in *Vespula* movement is a stronger releaser than is dark color (Maschwitz 1964), while in *Polybia* it is the reverse. This difference may be an artifact of differences in the techniques used in the two studies. Maschwitz does not specify the reflectance of the black and white objects used, the background against which they were presented, or the nature of the movement of the moving models. Only carefully controlled, standardized tests performed on both genera will reveal whether the indicated differences are real.

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