1 H. Gloor and H.R. Kobel, Revue suisse Zool. *73*, 229 (1966).
2 J. Derksen, L. Wieslander, M. van der Ploeg and B. Daneholt, Chromosoma *31*, 65 (1980).
3 C.J. Grond, J. Derksen and M. van der Ploeg, in preparation.
4 D.L. Lindsley and L. Sandler, Phil. Trans. R. Soc. Lond. B *277*, 295 (1977).
5 O. Hess, in: The genetics and biology of *Drosophila*, vol. 1c, p. 1343. Eds M. Ashburner and E. Novitsky. Academic Press, New York 1976.
6 H. Frei, Arch. Gen. *49/50*, 42 (1976, 1977).
7 G. Lefevre, Genetics *67*, 497 (1971).
8 H.D. Berendes, Chromosoma *14*, 195 (1963).

Evidence for an alarm substance in *Polistes canadensis*[1]

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Summary. In this tropical social wasp the odor of venom elicits an alarm response, reduces the threshold for attack, and acts as an attractant. Attack is released visually; dark colored objects were shown to be more effective releasers than were moving objects.

In recent years chemical communication of alarm has been documented in a wide range of higher social Hymenoptera[2,3]. In the primitive social wasp genus *Polistes*, however, evidence for alarm pheromones has not been found. Maschwitz tested body substances and found none that would elicit alarm in *Polistes dubius* Kohl[2]. In addition, Freisling showed for *P. gallicus* (L.) and *P. nimpha* (Christ) that wing buzzing by a disturbed wasp would excite others on the nest to an alert posture, whether the buzzing wasp was intact or had had its abdomen removed[4]. It has therefore been concluded that these wasps rely on communication of alarm via substrate vibration[4,5]. In this paper I report the discovery of chemical communication of alarm in *Polistes canadensis* (L.). This is the first evidence of a pheromone in this primitively eusocial wasp genus.

Polistes canadensis occurs from Arizona through Central and South America to Paraguay, Bolivia, and northern Argentina[6]. At Santarém, Pará, Brazil, where the present study was carried out, colonies are locally extremely common and have the unusual habit of building multiple combs, rather than just one as is typical of the genus[7]. The observations and experiments reported below were performed on colonies in situ.

Wasps on the nest respond to movements of nearby large objects with mild alarm behavior: they turn to face the disturbance, raise the anterior end of the body, wave the forelegs, spread and elevate the wings, and increase ventilatory pumping of the gaster. If the disturbance is more violent (e.g. if wasps are pulled from the combs, or if combs are removed from the nest), alarm intensifies: the wings are buzzed, the sting chamber is held slightly open, and the tip of the gaster is flexed to one side. This may be followed by attack: one or more wasps fly at the intruding object and attempt to sting it.

I found that once an object has been attacked, merely passing it upwind of the nest elicits a wave of renewed attacks. If a female is held in a pair of forceps, she struggles and attempts to sting. When such a struggling wasp is held upwind of a nest, the colony becomes strongly alarmed. These observations suggest the presence of an alarm pheromone. The following experiment was carried out to test this possibility and to determine the effectiveness of color and movement in eliciting attack behavior.

The search for the alarm pheromone was made by crushing female body parts onto a piece of white filter paper folded around the end of a 75 cm dowel and held in place by a paper clip. The paper was then presented upwind of an unalarmed colony. Tested materials included:

1. Venom sac. 1 per trial.
2. Dufour gland. 2 per trial.
3. Hemolymph squeezed from gaster after removal of sting apparatus and venom sac. 2 wasps per trial.
4. Glacial acetic acid. Several drops.
5. Formic acid. Several drops.
6. Clean, dry filter paper (control).

2 wasp equivalents of Dufour gland and hemolymph were used per trial because each yielded smaller quantities of material than did the venom sac. The purpose was simply to determine whether each body part contained alarm pheromone or not, and not to determine the relative response per wasp equivalent.

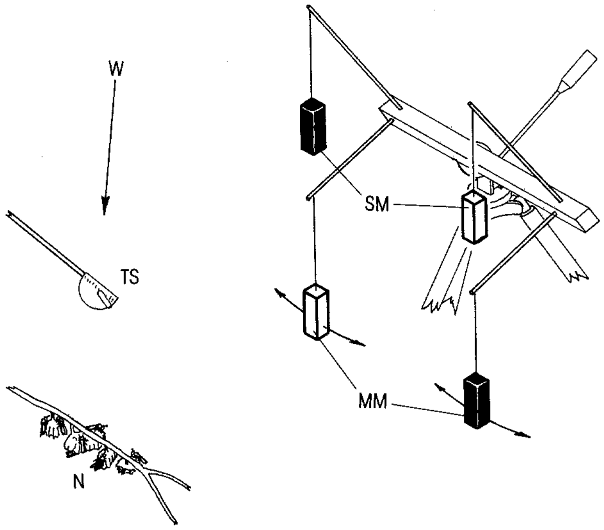
Responses by *Polistes canadensis* to 4 visual models and the filter paper bearing the tested substance. Data are total numbers of wasps responding to each object. Numbers of trials yielding a response are given in parentheses. All responses to the visual models were stinging attacks. Most responses to the odor source were calm inspection

Tested substance	No. trials	Visual models Moving Dark	Light	Non-moving Dark	Light	Filter paper
Clean control	9	0	0	0	0	0
Acetic acid	5	0	0	0	0	0
Formic acid	5	0	0	0	0	0
Hemolymph from gaster	6	0	0	0	0	0
Dufour gland	5	2(1)	0	0	0	0
Venom sac	5	64(5)	0	2(1)	0	5(3)
Venom sac and sting apparatus from:						
2 wasps	11	79(6)	3(1)	12(4)	0	27(8)
3 wasps	5	50(5)	2(1)	10(4)	0	16(5)

The alarmed wasps were allowed to attack 4 models with the following characteristics: dark, moving; light, moving; dark, stationary; light, stationary. Models consisted of 60 ml square plastic bottles wrapped in black or white paper. Each model presented an area of approximately 28 cm^2. Reflectance of the black paper was 4-5% at all wavelengths (290-700 nm). Reflectance of the white paper was 80-90% in the 450-700 nm range, then dropped to 30-70% in the UV (290-400 nm). The background against which the models were presented was an open, sunlit area of sand and short grass.

The moving vials were hung from nylon threads 13 cm long; the stationary models were fixed to stiff wires of the same length. The 4 models were suspended from a framework of dowels to form a square 30 cm on a side (fig.). The apparatus was placed 50-60 cm from a nest and crosswind so that the odor of venom left on the models during an attack would not reach the nest. A trial was initiated by swinging the 2 moving models in a plane perpendicular to the line of sight of wasps on the nest, then slowly and smoothly moving the filter paper bearing the test substance into the airstream 10-20 cm upwind of the colony (fig.). Thus the chemical and visual stimuli were in different directions from the nest, so that the responses to each could be distinguished. Responses were scored as the number of attacks or landings on each of the 4 models and on the filter paper. Each trial continued as long as there were attacks (usually less than 60 sec). For each new trial the papers covering the models were replaced and the position of each model on the apparatus was randomized by flips of a coin. 3 mature colonies of 50-70 adult wasps each were used in the experiment.

The wasps responded with alarm and attack behavior only in the presence of the crushed venom sac (table). This result strongly suggests that there is an alarm pheromone in the venom. The 2 attacks in 1 trial with Dufour gland were probably due to inadvertent leakage of venom onto the gland during dissection. The lack of any response to acetic or formic acid indicates that alarm behavior is not elicited simply by any strong chemical odor.



Apparatus used to test for alarm substances and the effect of movement and color in releasing attack behavior in *Polistes canadensis*. Wooden framework bearing black and white stationary models (SM) and black and white moving models (MM) is attatached to tripod and placed so that models are 50-60 cm from active wasp nest (N). Trial begins when moving models are swung from side to side and filter paper bearing tested substance (TS) is placed so that prevailing wind (W) carries the odor of the substance across the nest. Wasps attacking each model are counted.

Once the alarm pheromone was localized in the venom, additional trials were run in which crushed venom sac and sting apparatus were presented in order to obtain data on the relative numbers of wasps attacking each of the 4 models. 2 or 3 wasps were used in each trial so as to yield a strong alarm response. The results of these tests are included in the table. The differences in numbers of wasps attacking the 4 models indicate that in the presence of venom and against a heterogeneous sunlit background, a black object was a stronger releaser of attack than was a moving object ($\chi^2 = 16.03$, d.f. = 1, $p \ll 0.005$). This is the reverse of what has been found for yellowjackets (*Vespula* spp)[2].

There was a striking difference in how the wasps responded to the visual models vs the filter paper bearing the odor of venom. All responses to the models were attacks: rapid, direct flight onto the model, producing a loud tap, followed by stinging attempts that often left wet spots of venom on the paper. In contrast, wasps were simply attracted to the filter paper: they approached it from downwind with slow, hovering flight, then landed quietly and walked over the paper while antennating and sometimes mouthing the crushed parts. The few attacks that did occur usually followed an inadvertent rapid movement of the filter paper.

These results suggest that the odor of venom has 3 effects on the wasps: it elicits alarm behavior, reduces the threshold for attack, and it acts as an attractant. Attack itself is released only by an appropriate visual stimulus, a criterion met by the black or moving models but not by the white, immobile filter paper bearing the odor. This is the same as concluded previously for bees *(Apis mellifera)* and yellowjackets *(Vespula vulgaris)*[2]. In a real situation the odor of venom would be left on an intruder following the initial stinging attacks, and this would combine with the visual stimulus of the intruder to attract more attacking wasps. The fact that dark models released more attacks than did light ones can perhaps be interpreted as an adaptation to dark-bodied vertebrate predators, or that attacks are directed toward darker parts of the body, such as the noses of mammals.

The precise role of the venom in coordinating defense of the nest in a natural situation remains unclear. Specifically, it is not known whether intense alarm is first elicited by the odor of venom released incidentally during the initial attempts to sting an intruder, or whether wasps on the nest release venom from the sting prior to sting attempts. The latter would mean that production of the signal communicating alarm has been freed from the original specific context (stinging) in which it evolved. That this is the case is suggested by the observation that the sting chamber is held open during intense alarm.

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2 U. Maschwitz, Z. vergl. Physiol. *47*, 596 (1964).
3 B. Hölldobler, in: How Animals Communicate, p. 418. Ed. T.A. Sebeok. Indiana University Press, Bloomington 1977.
4 J. Freisling, Z. Tierpsychol. *5*, 439 (1943).
5 M.J. West Eberhard, Misc. Publs Mus. Zool. Univ. Mich. *140*, 1 (1969).
6 O.W. Richards, The Social Wasps of the Americas Excluding the Vespinae. British Museum (Natural History), London 1978.
7 R.L. Jeanne, Behav. Ecol. Sociobiol. *4*, 293 (1979).