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Source and Possible Nature of the Odor Trail of Fire Ants

Abstract. Experimental evidence shows that the odor trail of the fire ant *Solenopsis saevissima* (Fr. Smith) is produced as a secretion of the accessory gland of the poison apparatus and released through the extruded sting. Preliminary studies suggest that this substance may be chemically allied to or even identical with the toxic principle of the venom.

Chemical trails laid down by worker ants are essential mechanisms in the organization of foraging and colony migration in many ant species. Yet only recently has much careful attention been paid to the topographic form of these trails, to their anatomical source, and to their chemical nature (*1, 2*). Chemical analyses conducted by Carthy show that in the formicine species *Lasius fuliginosus* (Latreille) the trail substance is a water-soluble anal emission containing uric acid, polysaccharides, and proteins (*2*). These data suggest that the bulk of the material is normal excretory and fecal matter rather than a special glandular secretion. But they do not exclude the possibility that special secretory products, serving as releasers of trail-following behavior, may be present in small amounts.

Now it is possible to show that in the myrmicine species *Solenopsis saevissima* (Fr. Smith) the essential trail substance is produced as a glandular secretion and is released through the sting. Workers of this species lay trails by dragging the tips of their abdomens over the ground with the stings fully extruded. To determine whether venom passed from the sting can induce trail following, a series of experiments was performed in which artificial trails of freshly extracted venom were drawn in the vicinity of foraging workers from a captive colony. In separate trials, venom was either collected directly on sharpened cork tips or (to reduce the possibility of contamination) collected by "milking" the ants with fine capillary tubes and then transferring the fluid to cork tips.

In preliminary tests, foraging workers could almost always be diverted to the artificial venom trails, although their response was generally weaker than it was to true trails laid in the same area by living minor workers. Under the experimental conditions described below, three such trails produced from venom collected directly on cork tips brought forth 6, 8, and 18 workers, respectively, the duration of the effect extending between 1 and 2.5 minutes. Numerous artificial trails made under the same conditions from the wall and contents of the principal gut divisions, as well as from crushed tissue and hemolymph of head, alitrunk, and abdomen (gaster), evoked various intensities of alarm, circling, antennal palpation, and even feeding (in the case of the crop contents), but no distinct trail following.

An attempt was next made to localize the source of the critical trail substance. Three organs are known to empty material through the sting or in its immediate vicinity: the hind-gut, the "true" poison glands (which are paired and empty their contents into the poison vesicle), and the accessory gland of the sting. In a series of experiments these organs were dissected out of freshly killed major and media workers, separated, doubly washed in insect Ringer's solution, and then crushed on separate cork tips to make artificial trails. All three organs were taken from each ant killed, and these were presented in varying sequences to eliminate possible bias in the results due to special sequential effects. As much uniformity as possible was obtained with respect to the number of foraging workers and their trophic "mood" by first allowing masses of workers to accumulate around a freshly killed meal worm (larva of *Tenebrio molitor*) pinned at the edge of the glass plate on which the trails were drawn. Under the particular conditions prevailing at the time of the experiments, a relatively stable concentration of 150 to 200 workers was reached within 10 minutes after the meal worm had been found by the first foraging worker. At this time a fringe of more or less idle workers milled in a tight group around the edge of the meal worm, and it was to these that the artificial trails were drawn.

The results, presented in Table 1, show clearly that the trail substance is concentrated in the accessory gland of the sting. The fact that both the hind-gut and the true poison glands (with vesicle) frequently gave quite negative results suggests that these structures do not normally contain any of the releaser substance at all, only picking it up by contamination during dissection. The relatively high variation in response to the accessory gland preparation may be explained, at least in part, by two irregularities difficult to control: variation in leakage of gland contents during dissection and variation in responsiveness of worker groups. It was noted that when several trials were made on the same day, responsiveness tended to decline progressively.

Table 1. Response of fire ant workers to artificial trails made from various abdominal organs of ten freshly killed workers. The positive responses recorded are those in which workers ran at least half the length of the artificial trails, or approximately 8 cm. The duration is the time interval from the first positive response observed to the last and is given to the nearest half minute.

No. of workers responding		Duration of group effect (min)	
Range	Mean ± standard error	Range	Mean
Hind-gut			
0–18	2.3 ± 1.7	0–1.5	0.4
Poison glands plus poison vesicle			
0–26	8.2 ± 2.8	0–4	1.4
Accessory gland			
31–164	107.5 ± 14.4	3.5–7	5.9

The foregoing results lead to the question: Does the accessory gland substance serve as both releaser and orientator, or is it only a releaser, with venom from the true poison glands functioning as the orienting agent in the trails? To solve this problem, the following experiment was devised. After the experimental conditions described previously had been arranged, an *accessory* gland preparation was drawn in a short sidewise stroke next to the peripheral group of ants, while simultaneously an artificial trail made from the *poison* glands (plus vesicle) was drawn outward from them. In each of five such trials, the ants showed intense excitement, spreading outward in random looping movements, and many new workers were attracted to the scene. But in only one case was the poison-gland trail followed, and then by the relatively small force of 20 workers, representing less than 30 percent of the outward-moving swarm of foragers.

It thus appears that the accessory gland secretion functions as both a releaser and an orientator of trail following. On the other hand, it was noted that occasionally when workers were following accessory-gland trails in large numbers they would also follow nearby old

poison-gland trails that had been ignored previously. The implication seems to be that workers will follow other odor leads if there is some "knowledge" that a true (accessory-gland) trail exists. It also follows that only a small amount of the accessory-gland secretion need be in a trail to induce trail following. In fact, the venom from the true poison glands may be serving as a diluent for the accessory-gland secretion, although there is at present no direct evidence to support such a hypothesis (*3*).

The artificial trails made from accessory-gland preparations provide supernormal stimuli that attract far more workers than normal trails laid under similar circumstances by single living workers. The chemical nature of the releaser substance has not yet been precisely determined. However, the following data may be considered suggestive. A petroleum ether extract of steam distillate of whole ants prepared by M. S. Blum and his associates (*4*) produced trail-following responses of nearly comparable magnitude to those produced by accessory-gland preparations when it was tested under the experimental conditions described above. The number of workers drawn out by contact with the distillate was at least equal to the number attracted by the accessory-gland preparations, but orientation along the trails was somewhat less consistent. Blum *et al.* have shown that the infrared spectra of the distillate and of whole venom contain the same carbonyl band. On the basis of preliminary investigations, these authors have suggested that the carbonyl band is exhibited by the toxic principle itself, and that this constituent is manufactured by the accessory gland (*5*). It remains to be proved that the toxic principle and the trail-following releaser are one and the same (*6*).

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4. I am indebted to Dr. Blum for supplying me with the fire ant extract used in this study and for granting permission to use unpublished data pertaining to it.
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6. It is interesting to note the significant observation by G. W. K. Cavill and D. L. Ford [*Chem. & Ind. (London)* 1953, 351 (1953)] that workers of the dolichoderine species *Iridomyrmex detectus* (Fr. Smith) follow artificial odor trails made from the steam distillate of other *detectus* workers. These authors have identified the distillate as 2-methylhept-2-en-6-one.

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Redox Absorption Spectra from Single Pigment Cells of Squid

Abstract. Single pigment cells from the squid *Loligo forbesi* have been studied by microspectrophotometry. The absorption spectra obtained show characteristic changes on reduction and oxidation which are compatible with those found in ommochromes. The presence of melanoid substances, however, cannot be excluded.

In several cephalopods, such as *Sepia officinalis, Octopus vulgaris*, and *Eledone moschata*, and also in arthropods such as Crustacea and Arachnoidea, a peculiar group of pigments, the ommochromes, has been found (*1, 2*). One of the significant properties of most ommochromes is that there is a characteristic change in the absorption spectrum on oxidation and reduction, although a few ommochromes do not behave in this manner (*3*). Pigments closely related are the ommatins (*3, 4*) and insectorubin (*5*), the latter being found in locusts and other insects.

In contrast to other investigations reported in the literature, the studies presented in this report were carried out on single pigment cells in the cutis of a cephalopod, *Loligo forbesi*, caught in the North Sea. The tissue was fixed in 4-percent Formalin, and sections were rinsed for 2 hours and immersed for 24 hours in a (reducing) 0.05*M* solution of $Na_2S_2O_5$. Microspectrophotometric measurements were made by comparing substrate and blank at each wavelength. The single pigment cells were magnified about 150 times. The absorption spectrum obtained after reduction is shown in Fig. 1 (curve 1). A maximum is found between 525 and 540 mμ, representing, when compared with measurements by Schwinck (*2*), a slight shift toward the longer wavelengths. This shift may be due in part to light scattering (*6*) or fixation. After oxidation for 24 hours in 7-percent H_2O_2, the maximum at 525 to 540 mμ essentially disappears (Fig. 1, curve 2).

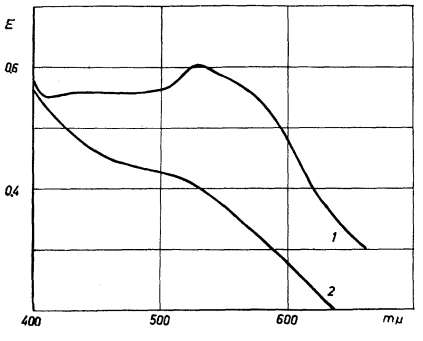

Fig. 1. Absorption spectra of a single pigment cell of *Loligo forbesi* after reduction (curve 1) and oxidation (curve 2) for 24 hours.

These results are in general agreement with bulk analyses on ommochromes reported by Becker (*1*) and Schwinck (*2*). They do not exclude, however, the presence of melanin or melanoid substances which show a gradually increasing absorption to the shorter-wavelength range (*7*); nor should it be postulated that the pigment found is identical with others already known. This study may merely show that, with suitable technique, redox absorption spectra can be obtained even from a single pigment cell and, thus, compared with analyses on extracted material.

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17 October 1958

Blood Groupings in Marshallese

Abstract. The absence of the Diego blood factor, the extremely low incidence of the *M* gene, and the unusually high R^1 gene frequency of the Marshallese more nearly resemble the blood groupings of the people of the western islands of Indonesia than the blood groupings of the Amerindians.

During March 1958, the annual medical survey of the Marshallese people of Rongelap Island was carried out, 4 years after they were accidentally exposed to radioactive fallout (March 1954) (*1*). These annual surveys are carried out by Brookhaven National Laboratory under the direction of R. A. Conard and are sponsored by the Atomic Energy Commission with the collaboration of the Department of Defense. During the course of these studies it became of interest to determine the blood groupings in the Marshallese people as an index of their origin and homogeneity. Blood samples were obtained by the survey team for this purpose.

The frequent movement of the Marshallese people among the various islands of Micronesia and, to a lesser extent, of Melanesia and other adjacent areas precludes any such concept as "pure" Marshallese. However, these people have lived for an estimated 2000 years on these islands with fewer outside contacts, perhaps, than most other groups. The findings presented consist of the