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The orientation inducer pheromone of the fire ant *Solenopsis invicta*

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Abstract. Foraging ants recruit nestmate workers to food sources by a variety of mechanisms. We report that one behavioural subcategory of the recruitment pheromone complex of *Solenopsis invicta* Buren involves orientation induction. The orientation inducer pheromone exerts its effects by changing the physiological state of the recipient rather than by releasing a measurable behaviour. Some ant species use a physical 'waggle' behaviour to motivate (change physiological state) nestmate workers to follow their chemical trail. The orientation inducer pheromone can be interpreted as a chemical analogue of the physical 'waggle' inducing effects. This behaviour is not elicited by the recruitment pheromone components responsible for orientation and/or attraction. Each of these behavioural categories is mediated by a different blend of chemicals from the Dufour's gland. Activity-concentration thresholds indicate that the attraction and inducer part of the recruitment pheromone require about 250 times more worker equivalents for a response than the orientation pheromone. Therefore, the recruitment sub-categories are differentially activated by the amount of Dufour's gland material released.

Key words. *Solenopsis invicta*, orientation, pheromone, recruitment, behaviour, physiology, induction, primer.

Introduction

Ants have evolved a variety of mechanisms to recruit and orient workers to food sources. These mechanisms range from primitive tandem calling in *Leptothorax acervorum* (Möglich *et al.*, 1974) to the complex recruitment systems of the African weaver ant, *Oecophylla longinoda* (Hölldobler & Wilson, 1978). In the case of *L.acervorum*, a forager attracts a nestmate with chemicals from its sting, and then physically guides it to the food source. In contrast, workers of the weaver ant, *O.longinoda*, release multiple glandular secretions combined with a variety of tactile signals in order to cope with a variety of context-related recruitment situations (Hölldobler & Wilson, 1978). In other ant species, foraging workers lay chemical trails: however, motor displays and mechanical signals are necessary to stimulate actual trail following; e.g. *Formica fusca* scouts must perform a waggle display to stimulate nestmates to follow their trail (Möglich & Hölldobler, 1975).

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In the more advanced recruitment system of *Solenopsis invicta* Buren, the contents of the Dufour's gland elicit all of the behaviours associated with mass recruitment (Wilson, 1959, 1962). Recruitment can be divided into two behavioural stages defined as attraction to and orientation along the trail. The isolation and identification of components of the recruitment pheromone of *S.invicta* have enabled us to unravel the intricate association between the chemicals produced by the Dufour's gland and mass recruitment behaviours (Vander Meer *et al.*, 1981, 1988; Alvarez *et al.*, 1987).

The chemical components of the recruitment orientation pheromone were isolated and identified as two alphafarnesenes and two homofarnesenes (Vander Meer *et al.*, 1981; Alvarez *et al.*, 1987). The bioassay used to guide the isolation specifically measured the response of ants already following a natural trail to test materials substituted for a portion of this trail (Barlin *et al.*, 1976; Jouvenaz *et al.*, 1978). Quantitatively, Z,E-alpha-farnesene was both the major component in the Dufour's gland, and the component to which trailing workers were most sensitive. In addition, since Z,E-alpha-farnesene and an equivalent amount of Dufour's gland extract (based on gas chromatography) elicited identical activity in the orientation bioassay, it was concluded that this compound was solely responsible for orientation.

Worker attraction is also associated with the contents of fire ant Dufour's glands (Wilson, 1962). However, in contrast to activity found with Dufour's gland extracts, neither Z,E-alpha-farnesene nor the other three active trail orientation components singly or in any combination elicited attraction when presented to non-trailing ants in either point source or olfactometer bioassays (Vander Meer *et al.*, 1988). Using appropriate bioassays, Vander Meer *et al.* (1988) subsequently discovered that attraction was obtained when a yet unidentified tricyclic homosesquiterpene monoene ('C-1'; present at approximately 75 pg per Dufour's gland) was added to Z,E-alpha-farnesene (*c.* 6 ng per Dufour's gland). When the combination of these two components was compared in an olfactometer bioassay with an equivalent amount of Dufour's gland extract (Vander Meer *et al.*, 1988), no significant difference in the response of the ants was found. Remarkably this mixture, which represents the orientation and attraction components of the recruitment pheromone, did not initiate orientation in foraging workers that were not trailing. We concluded that there must be other components to the recruitment pheromone, which are required to initiate trailing.

We report now that the specific Dufour's gland chemicals responsible for worker attraction and worker movement along a trail (orientation) do not by themselves, or in combination, induce workers to follow a trail. Non-trailing fire ant workers do not automatically follow a trail. Instead, they require a pheromone-induced change in physiological or motivational state, which allows them to respond to the orientation component of their recruitment pheromone.

Materials and Methods

Source colonies. Monogyne *S.invicta* colonies were established in the laboratory either from colonies collected in the field or by rearing them from newly mated queens collected near Gainesville. Standard laboratory rearing methods were practiced (Banks *et al.*, 1981) and only mature colonies were used as sources for workers and brood.

The orientation inducer bioassay. The bottom of a plastic Petri dish (7.5 cm diameter) was covered with red cellophane. The dish was inverted and the edges balanced on two small vial caps in the centre of a colony rearing tray (7 × 44 × 56 cm). This elevated the dish about 1 cm above the tray floor (Fig. 1). A group of 500–1000 workers (foragers and reserves; see

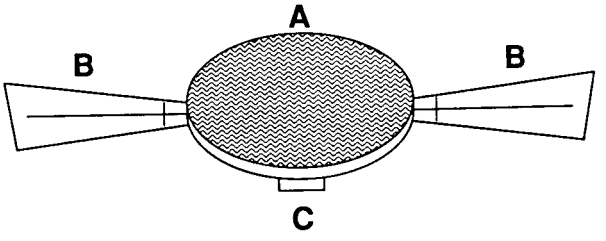

Fig. 1. Diagram of the orientation bioassay. A = Petri dish with red cellophane covering; B = test papers with 10 cm line: first cm treated with test material, the last 9 cm treated with the orientation pheromone, Z,E-alpha-farnesene; C = one of two caps used to elevate the Petri dish.

Mirenda & Vinson, 1981) from a source colony were placed in the tray. A small amount of brood was placed under the dish to induce the worker ants to aggregate at that location.

The behaviour of worker ants to various artificial trails was evaluated using wedge-shaped pieces of paper 12.5 cm long, with maximum and minimum widths of 7.5 and 2.5 cm. Test trails were marked on these papers with a 10 cm pencil line drawn along the midline of the paper starting at the narrow end. The line was divided into sections of 1 cm and 9 cm starting at the narrow end. The samples to be tested and controls were applied to the line using a 10 µl or 25 µl syringe. In all cases experimental trails were prepared by treating the 9 cm part of the line with the orientation pheromone, Z,E-alpha-farnesene, while the first centimetre was treated with defined concentrations of the control (Z,E-alpha-farnesene), or a hexane extract of Dufour's gland, or the attractant pheromone mixture of Z,E-alpha-farnesene and 'C-1'. Each test consisted of a direct comparison of the response of the ants to one of the experimental trails and a control trail, with the exception of the test series in which the comparison was between the attractant pheromone and Dufour's gland extract. The two trails were presented simultaneously to the ants aggregated under the Petri dish by placing the narrow ends of the two test papers adjacent to, but on opposite sides of the ants (Fig. 1). This was done very carefully to avoid physical excitement of the ants. The quantitative measure of the ants' responsiveness was based on the number of ants able to traverse the complete 10 cm trail in 2 min. The data were analysed by either paired *t*-tests or the Newman-Keul's test multiple comparison procedure.

Sample preparation. Dufour's glands were excised from worker ants, transferred to a vial containing hexane (Burdick & Jackson, HPLC grade, Muskegon, Michigan) and crushed to facilitate extraction of the total recruitment pheromone. Solvent volume was adjusted with hexane to give a specific number of worker equivalents per µl (WE/µl). Z,E-alpha-farnesene was isolated and synthesized as previously described (Vander Meer *et al.*, 1981) and diluted with hexane to give a concentration equivalent to the amount of Z,E-alpha-farnesene in the corresponding Dufour's gland extract. The amount of Z,E-alpha-farnesene in the extracts was determined by gas chromatography (Varian 3700 gas chromatograph, Sunnyvale, California, equipped with a flame ionization detector and a DB-1 (J&W Scientific Inc., Folsom, California) fused silica column, 0.322 mm i.d. × 15 m, 0.25 film thickness, coupled with a data processor (Varian vista 401). The recruitment attractant pheromone component, 'C-1', was isolated from extracts of workers as previously described (Vander Meer *et al.*, 1988). The compound was quantified by gas chromatography as described above, and concentrated (under a stream of nitrogen) or diluted with hexane as required to give the appropriate WE/µl.

Results

The addition of 0.05 WE/cm of Dufour's gland extract to the first centimetre of a 9 cm artificial trail of Z,E-alpha-farnesene significantly increased the mean (±SE) number of trailing workers when compared to a paired trail of only Z,E-alpha-farnesene (Fig. 2A; 24.8±5.0 *v.* 4.6±2.1; $n=8$; $P = 0.0017$). In contrast, the addition of the attractive elements of the recruitment pheromone (Z,E-alpha-farnesene and 'C-1') to the first cm of a Z,E-alpha-farnesene trail did not significantly increase the number of workers following the trail (Fig. 2B; 1.6±0.8 *v.*

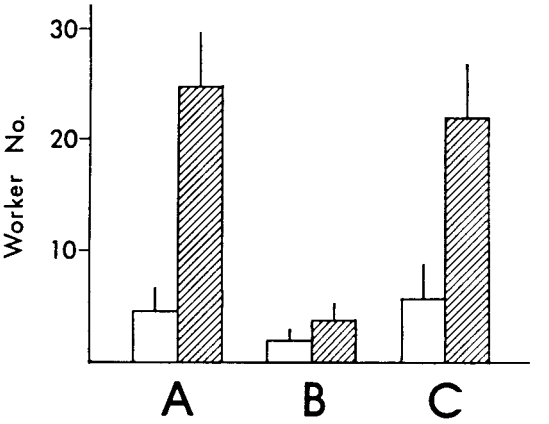

Fig. 2. Mean number of workers (±SE; $n=10$) following a Z,E-alpha-farnesene trail when the first centimetre of the trail is treated with: (A) Z,E-alpha-farnesene (white) versus Dufour's gland extract (stripes); 0.05 WE, $P<0.002$; (B) Z,E-alpha-farnesene (white) versus attractant pheromone (stripes); 0.02 WE, $P < 0.1$; (C) attractant pheromone (white) versus Dufour's gland extract (stripes); 0.02 WE, $P<0.02$.

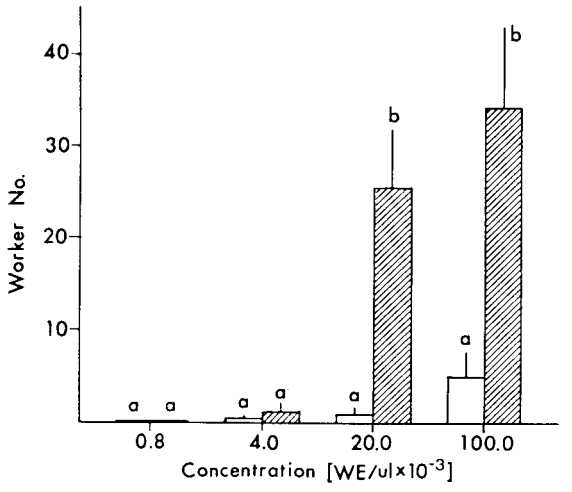

Fig. 3. Effect of increasing concentrations of Dufour's gland extracts applied to the first centimetre of a 10 cm Z,E-alpha-farnesene trail (stripes). The white bars represent results when equivalent amounts of Z,E-alpha-farnesene were applied to the first centimetre of the trail. The values shown are the means and standard errors of five replicates. Results for samples where the letter above the mean is different indicates a significant difference, $P<0.05$, Newman-Keul's test.

3.4±1.7; mean ±SE, $n=5$; $P = 0.08$; 0.02 WE/cm). Also in matched tests, Dufour's gland extract was significantly more effective than the attractant pheromone (Z,E-alpha-farnesene plus 'C-1') in eliciting orientation to Z,E-a-farnesene (Fig. 2C; 22.0±4.9 *v.* 7.6±3.2; mean ±SE, $n=5$; $P = 0.02$).

In separate tests, we found that the number of ants following a Z,E-alpha-farnesene trail was influenced by the concentration of Dufour's gland extract applied to the first centimetre of the trail (Fig. 3). For example, no trailing response was induced at 0.0008 or 0.004 WE/cm of Dufour's gland extract; however, a significant increase was observed at 0.02 WE/cm, and the effects increased further at 0.1 WE/cm.

Trailing activity on the Z,E-alpha-farnesene control varied from test to test depending on the sample with which it was matched, since it was impossible to eliminate 'telegraphing' effects of an active orientation inducer test sample to ants located on the opposite side of the Petri dish test arena. This effect is apparent in the control results in Fig. 3, where the number of ants responding to the control correlates with an increase in Dufour's gland concentration.

Discussion

The test results demonstrate that non-trailing *S.invicta* workers do not respond to artificial trails of Z,E-alpha-farnesene unless they are first exposed to extracts of Dufour's gland. The recruitment attractant pheromone (Z,E-alpha-farnesene + 'C-1') did not enhance worker orientation when placed at the beginning of a Z,E-alpha-farnesene trail. Therefore, the chemical components in the Dufour's gland that induce worker ants to orient along a trail are not Z,E-alpha-farnesene and/or 'C-1', although these components may be a part of a mixture required for orientation induction. The fact that the Dufour's gland extract dramatically enhanced fire ant worker sensitivity to the orientation pheromone led us to postulate that unknown components of the Dufour's gland alter the physiological or motivational state of the recipient worker. In the presence of a recruitment trail workers orient along the trail.

There are many physical analogues of what we report as chemical activation: in *Messor rufitarsis* recruitment of workers to food is enhanced by stridulatory activity (Hahn & Maschwitz, 1985); physical contact is required prior to trail orientation by *Myrmica rubra* (Cammaerts, 1978); rapid locomotion and vigorous antennation were pre-trailing cues observed for *Iridomyrmex humilis* (van Vorhis Key & Baker, 1986); tournament recruitment by *Myrmecocystus mimicus* scouts is initiated by a jerky motor display, which alerts workers (Hölldobler, 1976); *Neivamyrmex nigescens* adults use mechanical stimulation to arouse callow workers to follow trails during emigration (Topoff & Mirenda, 1978).

Under certain circumstances the orientation inducer pheromone may be considered a modulator. Modulatory communication is defined as a 'low efficiency' communication system that does not force the receiver into a determined behavioural channel, but shifts the probability for the performance of other behavioural acts (Hölldobler, 1978). The *S.invicta* orientation inducer pheromone and the chemicals that release attraction and orientation are all produced by the Dufour's gland and must be simultaneously emitted; therefore, in the context of trail formation the receiver is led directly to orientation. However, we realize that the

release of the Dufour's gland contents by a worker in a non-trailing context may lead to heightened worker responsiveness to a variety of stimuli; e.g. to intruders, injured nestmates, nest repair, and mating flights. The Dufour's gland contents have also been reported to release alarm behaviour (Wilson, 1962). Under these circumstances the inducer pheromone may be thought of as a modulator.

In previous studies we found that Dufour's gland concentrations required to release attraction were two orders of magnitude higher than that required to release orientation (Vander Meer *et al.*, 1981, 1988). Therefore it was of interest to study the effects of increasing concentrations of Z,E-alpha-farnesene and Dufour's gland extracts in the orientation inducer bioassay. The minimum orientation inducer pheromone concentration required for a significant response was over 250 times the minimum Z,E-alpha-farnesene concentration required to elicit orientation in workers already following a trail (Vander Meer *et al.*, 1981). This concentration is approximately equal to the minimum concentration of Dufour's gland extract required to give a statistically significant attraction response in an olfactometer bioassay (Vander Meer, unpublished results). Therefore, not only does *S.invicta* use a hierarchy of recruitment sub-categories (attraction, orientation induction, and orientation), but the differential activation of these sub-categories is effected by the amount of Dufour's gland contents released. This conforms with our knowledge of fire ant recruitment behaviour and biology.

The vast majority (90%) of fire ant workers reside within the colony as nurses and reserves (Mirenda & Vinson, 1981). Reserves may perform a variety of functions, including recruitment to food sources. A foraging worker will lay a trail back to its nest if it finds a food source too large to carry back itself. The recruitment process is directed at reserve workers rather than other foraging workers. This is sensible, since the reserves represent a concentrated source of recruits. In addition, there are usually many potential food sources in a given area and foraging efficiency would be adversely affected if a trail attracted and diverted other foraging workers from hunting. It also makes sense energetically for workers to be highly sensitive to the orientation part (0.4 pg/cm) of the recruitment pheromone, since trails may be several metres long. Our results suggest that *S.invicta* foraging workers lay orientation trails back to their nests that do not have to be of sufficient concentration to attract workers. However, once they contact workers at the nest, high concentrations must be released both to attract and induce workers to follow the trail. Therefore, it is logical mechanistically and energetically that attraction and orientation induction require a significantly higher release rate of Dufour's gland contents than actual trail orientation. The different behavioural sensitivity thresholds of fire ant workers to specific concentrations of Dufour's gland extracts support this scheme. After initial trail formation it is possible that a heavily reinforced trail elicits full recruitment (Wilson, 1962). In *Acromyrmex landolti* (Jaffe *et al.*, 1985) the recruitment process is elicited by the contents of the poison gland. It was found that for artificial trails the orientation signal lasted up to 15 h, whereas the attractive component lasted only about 7 min. The different activity times for these behaviours may reflect differential worker sensitivity to pheromone component concentrations. An alternative explanation involves different pheromone component volatilities. The latter explanation is not the case for *S.invicta*, as demonstrated in this and other papers (Vander Meer *et al.*, 1981, 1988). For *S.invicta* we have the great advantage of having access to the actual components responsible for attraction and orientation.

In conclusion, *S.invicta* has a highly evolved recruitment strategy that uses pheromones to elicit attraction, orientation induction, and orientation. Some ant species utilize different pheromone glandular sources for these behavioural stages (Cammaerts-Tricot, 1974; Maschwitz & Schonegge, 1983), but *S.invicta* falls into the category in which a single glandular source is responsible for all mass foraging activities; consequently, the different components can not be applied separately or varied independently. Within this constraint there are at least three possibilities: a single chemical or blend is required for the three recruitment pheromone stages, or each stage has its own exclusive component or blend of components, or the intermediate possibility where some components are common elements in eliciting each of the recruitment stages but each stage has its own non-exclusive chemical blend. It appears that

S.invicta most closely fits the third case, since attraction and orientation require different chemical blends but have common components. Detailed chemical studies will determine whether or not the orientation inducer pheromone has common or unique components.

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