REPRINT from *Animal Behaviour*, **22**, 3 August, 1974

Anim. Behav., 1974, **22**, 695–704

BROOD RECOGNITION BY CONTACT PHEROMONE IN THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*

BY J. P. WALSH & W. R. TSCHINKEL

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Abstract. The rather unspecific fire ant brood pheromone assay of Glancey et al. (1970) could not be duplicated, probably because they confounded a food response with a brood response. A more specific assay for the brood recognition signal of the red imported fire ant, *Solenopsis invicta* Buren is described. Worker response in the assay was unaffected by altering social conditions in the colonies but demanded the presence of animal tissue in the ants' diet in order to be consistent. Evidence is presented for a non-volatile contact brood pheromone that is distributed evenly over the pre-adult cuticle and whose potency is abruptly reduced with the shedding of the pupal skin at eclosion. The signal is completely lost within 72 hr of death.

The constant licking and antennal stroking of ant brood by their nurses suggests that this intimate relationship is tightly bound to chemotactic tending releasers emitted by the brood. The possibility of brood pheromones in ants was first based on the observation that workers were attracted to larval oral secretions (Stager 1923; LeMasne 1953). The presence of large salivary organs in larvae of *Pachysima latifrons* Emery prompted Wheeler (1918) to suppose that they are the source of worker attractants; comparable 'exudatoria' were discovered by Menozzi (1930) in *Crematogaster rivai.*

Recently, more direct evidence for formicid brood pheromones has been reported. Watkins & Cole (1966) and Schneirla (1971) have demonstrated the attractiveness to workers of filter paper impregnated with brood odour in two army ant genera, and Schneirla (1971) has elicited worker attraction to the brood-containing arm of a Y-tube olfactometer. In fact, Schneirla (1945, 1971) believed that the stimulus to raid in army ants involves chemotactic arousal of workers by emerging larvae, as well as eclosing adults. Workers of the harvester ant, *Pogonomyrmex badius* Latreille show oriented placement of trophic eggs into the mouthparts of their larvae, guided probably by brood odour (Wilson 1971).

It seems likely that ant workers recognize conspecific brood by odour; however, only one report of experimentation in this area exists. Glancey et al. (1970) presented evidence for a hexane-soluble brood-recognition pheromone in the red imported fire ant, *Solenopsis saevissima* (F. Smith) (*S. saevissima* of Glancey et al.'s study was found later to be a new species, *S. invicta* Buren. *S. saevissima* is a Brazilian fire ant that has not reached North America. Consequently, both Glancey et al. and we studied *S. invicta*). They reported that various inanimate objects impregnated with brood extract were placed with the brood in their laboratory colonies and elicited tending behaviour in workers. In this paper we show, among other things, that because of faulty nest design and improper tests for food responses, these experiments are inadequate in demonstrating a brood pheromone. By altering nest design to include a separate brood chamber and by taking a closer view of worker behaviour, we achieved a more reliable assay for the brood signal, and have used this assay to describe the mode and characteristics of brood recognition in the imported fire ant.

Methods

Colony Maintenance

A sexual-producing mature colony of the red imported fire ant (referred to hereafter as the imported fire ant), *Solenopsis invicta*, was collected in April 1972, in Tallahassee, Florida, separated from the soil in the laboratory, and maintained in a 100-mm plastic petri dish nest with an entrance hole in the bottom half. Three plastic walls divided the nest into three lighted chambers and a darkened formicary (using red cellophane). Water was supplied to the formicary from a reservoir via a wooden plug. The nest rested on the bottom of a lidless plastic box, 19 × 27 × 9 cm, with sufficient area for the ants to forage for the food that was offered (an agar based ant medium (Bhatkar & Whitcomb 1970), tenebrionid beetle larvae, peanut butter, and 10 per cent glucose). The colony was maintained in constant light and temperature (19·5 to 24°C).

From this stock colony, 1000 randomly collected workers, a few males, and about sixty worker brood were placed into each of six nests identical to the stock nest, and under the same light and temperature regimes. A newly mated fire ant queen was added to each colony; in all cases, she was accepted and oviposited within a week. As in the stock colony, the brood were placed and tended exclusively in the darkened chambers. The first experimental colonies were used for about three months; a second colony also collected in Tallahassee, in June 1972, supplied the adults for the last 4 months of the study. The immature fire ants used, although collected from various *S. invicta* colonies from the Tallahassee area, were invariably accepted by the experimental workers.

The Bioassay

The bioassay is based, in part, on observations of LeMasne (1953) and Wilson (1962) concerning placement of *Solenopsis* brood during alarm situations. The assayed sexual pupae or prepupae, used in the assays because of their large size and durability, were placed two at a time along a line 3 cm from the nest entrance (objects presented in the bioassay will be referred to hereafter as baits). A foraging worker that contacted the bait usually dragged it nestward after spending but a few minutes licking and antennating the cuticle. Once the worker and bait were inside the nest, the entire colony was alarmed by blowing air into the entrance once every 5 min for the duration of the trial, causing the hasty deposition of all brood into the formicary and maintaining a well-defined brood chamber during an experiment. Even under adverse conditions, such as starvation, the young in this chamber were treated amicably; whatever brood execution for food occurred, it took place in the outer chambers of the nest. A positive response was recorded (and the trial terminated) if a pupa or prepupa was placed in the brood chamber for at least 10 s within 50 min of initial worker contact with the bait in the foraging field. Correspondingly, one trial included the responses of the six colonies to two baits each, a total of twelve binominal responses in all. For a few trials, only one bait per colony was presented, giving six responses per trial. Placement of the bait in the formicary for even 10 s was evidence of permanent residence. The 50-min trial period represents twice the mean time that it took the experimental workers to place twenty-four live sexual prepupae in the brood chamber. When using other brood castes or stages, the trial time remained twice the time it took for retrieval of the corresponding live baits; baits were rarely brought in after this time.

As many as eight trials were conducted in one day between 08.00 and 21.00 hours with a minimum of 10 min rest period between trials. Statistical significance was determined by χ^2 test.

Results

The effects of various environmental and social conditions on worker-response were studied. An understanding of these influences was essential in maintaining a constant worker response throughout the study and maximizing the effectiveness of the bioassay.

Environmental Conditions Affecting the Response

Since light and temperature were held constant, they had no effect on differential worker-response. Also, there were no observed time dependent changes in worker behaviour during the study.

In fact, the only environmental factor that affected worker response was the absence of animal fats and proteins in their diet. If the colonies were not fed animal tissue, then workers responded positively to the body contents of larval *Zophobas* (a tenebrionid beetle) on bits of blotter paper; that is, they brought presented food into the brood chamber (mean 7·4 out of 12). Workers responded negatively to food baits (but still positively to presented brood), though, once *Zophobas* were added to their diet (mean 0 out of 12, $P<0{\cdot}001$, χ^2 test). Subsequently, *Zophobas* and other tenebrionid larvae became a source of animal fats and protein in the ants' diet, eliminating the possibility that worker responses to brood might be food responses.

Social Conditions Affecting the Response

It is possible that brood acceptance by workers is determined by social parameters. To assess the importance of maintaining social conditions in the experimental colonies during bioassay, the following experiments were performed:

Presence of the Queen. The queen in each of the six colonies was removed twice for periods of 48 hr, and live sexual prepupae were assayed at various times after queen removal. The mean number of positive responses in the queen-right assays (six trials) was 10·8/12; the mean in the

queen-less assays (nine trials) was 10·2/12 (P not significant, χ^2 test).

Worker number. Three queen-less colonies (housed in smaller versions of the previously described plastic nests) were established, each containing five worker pupae and either five, twenty-five or fifty randomly selected workers. Workers in all colonies retrieved all thirty-six of the introduced brood to their formicaries, as did the control colony with 1000 workers, suggesting that group effects play little role in worker behaviour toward introduced brood. Consequently, dead workers were not replaced in the experimental colonies after stocking.

Worker composition by size. The possibility that there exists a fire ant nurse caste based on size was investigated by establishing five colonies, each consisting of five worker pupae and twenty-five workers having a specified range of scape length. Therefore, the response of a certain size class of workers to introduced brood was compared to the control colony containing twenty-five randomly selected workers and five worker pupae. The baits presented were prepupae covered from the anterior end with paraffin wax (mean body area covered 31·7 per cent, SD 4·1 per cent). These modified baits measured worker thresholds to brood signals more accurately than uncovered brood. There was no significant differences between the number of brood out of eighteen retrieved by the control colony and any of the other colonies, indicating that fire ants do not possess an obligatory size-based nurse caste. Therefore, differential worker mortality in the experimental colonies would probably not result in a net difference in the colony's threshold to brood-emitted signals.

Amount of brood in the colonies. The entire brood and the queen were removed from each of the six colonies, and workers acclimated for 24 hr. Two sexual prepupae then were assayed per colony; assays followed the addition of one, ten, thirty, and sixty worker brood to each formicary, over a 4-day period. There was no significant difference between the mean number of brood retrieved at any time during the experiment by the experimental colonies (χ^2 test) and that retrieved by the control colony with sixty worker brood. Accordingly, brood was only qualitatively maintained in the experimental colonies.

Some Characteristics of the Brood Signals

Two groups of twelve sexual prepupae were killed by freezing at −6°C for 30 min, returned to room temperature, and assayed at various times after death. The mean proportion of worker positive responses versus time after prepupae death is plotted in Fig. 1. The first significant decrease in prepupal attractiveness occurred at about 21 hr after death. By this time, shrivelling and dehydration had altered the cuticle somewhat, and the prepupae had begun to yellow in colour. The persistence of the signal for such long periods after death indicates either that the cue is present in large quantities, or is extremely potent and/or stable; also, auditory brood communication can be ruled out. It is not known if destruction of the post mortem signal is caused by loss of the signal per se, or if it is masked by decomposition.

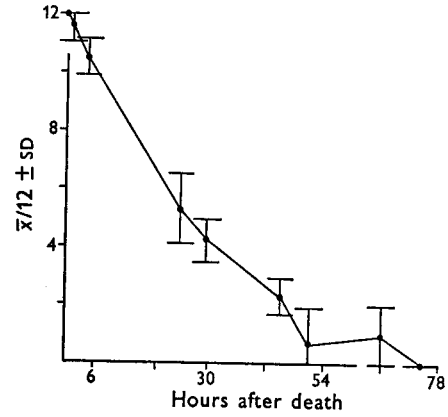

Fig. 1. Mean fraction of worker positive responses ($\bar{x}/12$, two trials) to dead sexual prepupae assayed at various times after death.

Localization of the signal. In order to localize areas of high concentrations of the brood signal on the cuticle (the most likely release point), sexual prepupae and pupae were dipped into hot paraffin to predetermined depths and then assayed. The paraffin hardened to an impermeable cast that masked whatever signal lay beneath it. Twelve prepupae per trial were dipped to one of five separate levels, including zero and 100 per cent coverage. The mean per cent body coverage was measured by tracing (under the microscope) the outlines of the covered bodies on graph paper, and converting the proportions of silhouettes covered to per cent body areas covered. The following partial coverages (plus zero and 100 per cent) were used in assaying pupae: head, head and thorax, gaster, and gaster and thorax. Because the coverages were not exact, the relation between

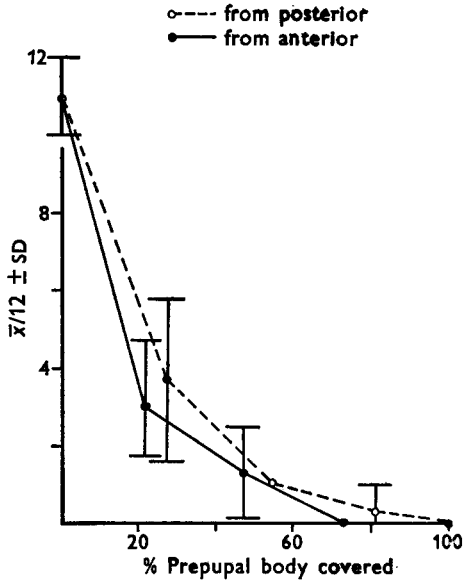

Fig. 2. Mean fraction of worker positive responses ($\bar{x}$/12, three trials) to sexual prepupae covered to various degrees with paraffin wax, from either anterior or posterior ends.

the mean areas covered, and the pupal body segments is indicated in Figs 2 and 3. In order to investigate the possibility of a signal gradient along the body, both the prepupae and pupae were covered, to different percentages, from both the anterior and posterior ends. A significant difference between the anterior and posterior coverage curves would indicate the presence of high-concentration areas. Each point on the graphs (Figs 2 to 4) represents the mean of three trials, each trial consisting of twelve responses. In both the prepupal and pupal curves, the differences between the two most divergent points are not significant (χ^2), suggesting that the signal is evenly distributed over the prepupal and pupal bodies. A comparison of the prepupal curves (Fig. 2) with the pupal curves (Figs 3 and 4) indicates that pupae are somewhat more potent than prepupae. Accordingly, the first significant decrease in retrieval of prepupae occurs at about 17 per cent coverage (from 11/12 to 5/12, $P<0{\cdot}05$), while 30 per cent pupa coverage was necessary to reduce worker response to the same level (from 11·5/12 to 5/12, $P<0{\cdot}05$).

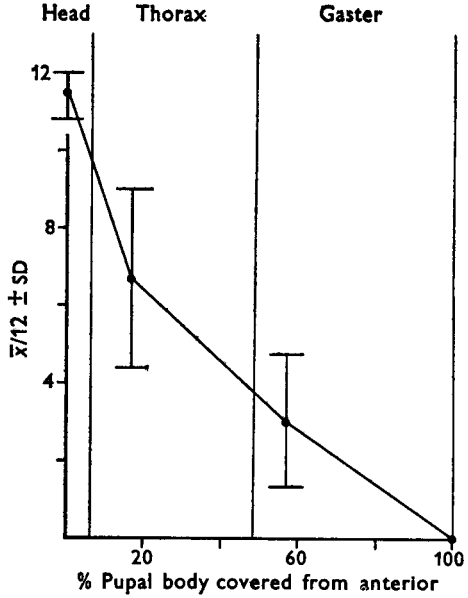

Fig. 3. Mean fraction of worker positive responses ($\bar{x}$/12, three trials) to sexual pupae covered with paraffin wax to various degrees from the anterior ends of the pupae. The proportion of the total body area represented by each of the body segments is designated by the vertical lines in the graph.

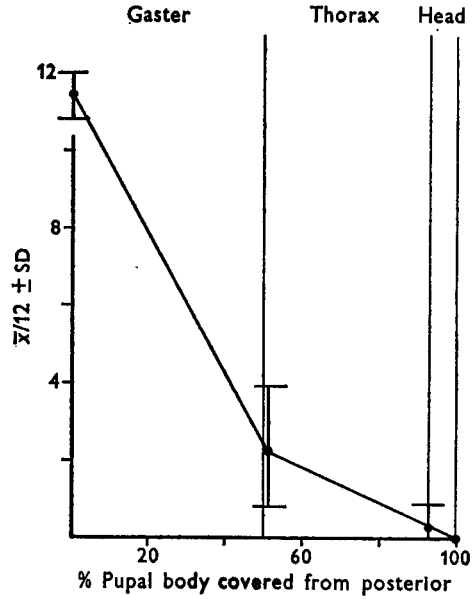

Fig. 4. Mean fraction of worker positive responses ($\bar{x}$/12) three trials) to sexual pupae covered with paraffin wax, to various degrees from the posterior ends of the pupae. The proportion of the total body area represented by each of the body segments is designated by the vertical line in the graph.

Loss of signal after eclosion. Workers do not retrieve freshly killed adult sexuals from their own colony when presented in the bioassay, evidence that the brood signal disappears some time between eclosion and maturation. To determine when this change occurs, twenty-four sexual pupae, twelve male and twelve female, near eclosion, were presented, one at a time, to the six colonies, until they were no longer retrieved. After the experiments were terminated, the trial times were grouped according to time intervals relative to eclosion and the corresponding worker responses listed (Fig. 5). Not only did the pupae lose their potency at eclosion, they were also attacked and dismembered, indicating that the brood signal is drastically modified by the loss of the pupal cuticle. Four pre-eclosion pupae were destroyed by workers and hence could not be included in the Figure.

Nature of the Signal

Hexane extract experiment. The extraction method of Glancey et al. (1970) which was claimed to demonstrate the presence of a brood pheromone in the imported fire ant was duplicated: 1 g of fire ant brood was ground in 1 ml of hexane, and the supernatant was poured over thirty bits of filter paper in one trial, and thirty bits of blotter paper in the other. The bits were then tested in our assay. None of the sixty surrogates presented were brought to the brood chamber or even into the nest. Failure in getting a positive response to these extracts using our more specific assay, casted doubt on the evidence for an extractable brood pheromone and suggested that alternative methods would have to be used to understand the mode of the signal.

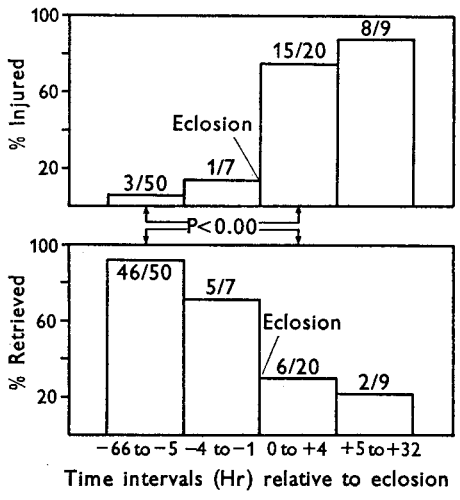

Fig. 5. The responses of workers to eclosing pupae. The lower histogram shows the proportion of sexual pupae (male and female) retrieved during various time intervals relative to eclosion (−before, +after eclosion). The upper histogram, which is on the same time scale as the lower, indicates the proportion of worker-caused injuries to pupae relative to the eclosion time. As is indicated in the figure, there are significant differences in worker responses to the pre-eclosion versus the eclosing and post-eclosion group (χ^2 test).

Olfactometer experiments. The possibility that the brood release a volatile pheromone was examined using a series of olfactometers connected directly to small fire ant colonies (Fig. 6). Each of the five closed nests consisted of two chambers, the more humid queen-brood chamber and the foraging field. A hole cut through a thick piece of plastic formed the walls of each chamber; attached plastic floors and glass tops sealed the system. A tunnel connected both chambers and opened to the outside at either

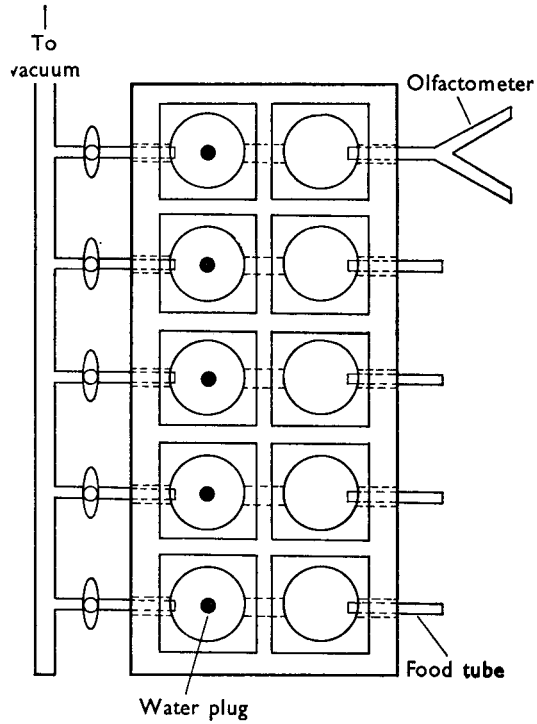

Fig. 6. Schematic diagram of apparatus used in assaying for a volatile brood pheromone. Food tubes are in place in four of the colonies; the olfactometer is inserted in the fif h. Air flow was equalized by stopcocks connected to a common manifold. See text for further description.

end of the nests (Fig. 6). The tunnel exposing the queen-brood chamber to the outside was screened to prevent escape and connected to a vacuum line via a manifold that equalized the air flow through each colony. A food tube inserted into the open end of the tunnel connecting the foraging area with the outside prevented escape and supplied food. Each nest was stocked with 200 workers, a fertilized queen, and a small brood, and given 2 days before beginning experiments. At the beginning of a trial, a Y-tube, containing from one to five live sexual prepupae in one arm (the arms being alternated) was inserted in place of the food tube. The other arm contained a wooden surrogate to equalize the air resistance in both arms. During a trial, air was drawn evenly through the two arms of the olfactometer, and through the foraging area and the queen-brood chamber. If a volatile brood pheromone was released from an arm of the olfactometer, it would be carried along the air stream into the colonies where workers could respond.

A positive response was recorded if the first worker into the olfactometer entered the arm containing the brood and walked to the prepupa. Discovering workers unfailingly antennated and licked the prepupae, and many attempted to drag it back to the nest. There was no time limit, since a trial rarely took more than 5 min. Out of sixty-seven trials, workers chose the brood arm of the olfactometer twenty-seven times (P not significant using χ^2 test); if the brood communicates chemically, it is not by a volatile pheromone.

Worker Response to Physically Treated Brood

The results of the olfactometer experiments do not eliminate the possibility of a short distance or even contact pheromone, which might be suspected given the close contact that exists between adult and immature fire ants in their subterranean formicary. The following experiments provide some evidence for a cuticular brood pheromone of extremely low or no volatility.

Table I lists the responses of workers to various treatments of the brood cuticle. Live baits of all brood castes were retrieved almost unanimously. These baits, after being killed by freezing at −6°C for 30 min and returned to room temperature, were also accepted, eliminating the possibility of auditory brood communication. The freshly killed baits were then soaked, twelve at a time, in 0·2 ml hexane for two periods of 30 min each. The soaked brood were dried, first in laboratory tissue, then in an airstream for 2 min, before being presented, two at a time, in the foraging fields. The hexane-washed brood did not repel workers, an indication that the hexane had been sufficiently removed. Using the χ^2 test, the differences in the proportions of

Table I. Mean Fraction of Worker Positive Responses to Variously Treated Food

Treatment	No. of trials	Mean $\bar{x}/N$	Control	Control $\bar{x}/N$	*P versus Control
Live	4	11/12	—	—	—
Freshly killed	4	10/12	Live	11/12	NS**
Fresly killed hexane washed	4	1/12	Freshly killed	10/12	$P<0{\cdot}002$
Freshly killed water washed	2	8/12	Freshly killed	10/12	NS
Cuticle	4	6/12	Freshly killed	10/12	NS
Hexane-washed cuticle	3	0·7/12	Cuticle	6/12	$P<0{\cdot}05$
Prepupal body contents on blotter	5	6/12	Freshly killed	10/12	NS
			Zophobas on blotter (food response)	1/12	$P<0{\cdot}04$
			adult sexual body contents on blotter (colony response)	1/12	$P<0{\cdot}04$
Cuticle in tissue	2	0/12	Cuticle	6/12	$P<0{\cdot}02$

*χ^2 test. **Not significant.

positive responses between the freshly killed, and the freshly killed and hexane-washed brood, were significant (Table I). Water failed to destroy the signal and re-application of the hexane washings failed to return the activity. However, this does not detract from the finding that the brood recognition signal is a property of the cuticle.

Next, the body contents of sexual prepupae were removed by suction, and the remaining cuticular sacs were presented two at a time in the foraging fields of the six experimental colonies (Table I). Another group of skins was washed for two 30 min periods in 0·6 ml of hexane, dried, and presented two at a time. In another experiment, the body contents of prepupae were applied to bits of blotter paper and presented two at a time. Finally, prepupal skins were tightly sandwiched in four plies of laboratory tissue and presented. These last baits were not significantly larger than the skins and could be retrieved by a single worker. The mean fractions of positive responses and the statistical significance versus the appropriate controls for each experiment are listed in Table I. Retrieval of prepupal skins, especially body contents, in proportions not significantly different from the whole prepupa provides evidence that the brood signal is chemical rather than tactile and is concentrated in the cuticle. The loss of activity in tissue-wrapped skins indicates that the brood pheromone is non-volatile and must be contacted to be perceived. As in the intact prepupae, washing the skins destroyed their activity.

Solvent extract experiments. The failure of the concentrated hexane washings to elicit a positive response prompted a search for a suitable solvent and substrate system with which to demonstrate further the chemical nature of brood recognition. Hexane, di-ethyl ether, methanol, and benzene were used as solvents in extracting from either whole sexual prepupae or prepupal skins. From one to six prepupal equivalents of the concentrated washings were applied per substrate (either freshly killed and washed prepupae or bits of blotter paper). Not a single positive response resulted from the presentation of these surrogates, although all of the solvents except water destroyed the signal.

Preparative column chromatography was then performed on a small Florisil 115/150 mesh column using the concentrated hexane washings of twelve sexual prepupae in one trial and, in another trial, washing from twelve prepupal skins. Hexane, benzene, and ether (20 ml each) fractions were eluted, concentrated under nitrogen, applied to pieces of blotter paper (two equivalents per bait) and presented two at a time (twelve per trial). None of the fractions elicited a positive response.

Mechanical 'extraction' experiments. A few attempts were made to remove the surface chemical by rubbing. In one case, pieces of blotter paper were rubbed against sexual prepupae (two prepupae per piece), and presented to the six colonies; none of the six surrogates were retrieved. In another experiment, a piece of blotter paper was placed for a week on the floor of the formicary of a stock fire ant colony with a large brood. The brood were dumped on the paper, and tended there all week. After this time, the paper was cut into small pieces and presented to the colonies, two at a time. None of the baits was harvested.

Removal of the signal from intact brood by chemical reagents. The evidence acquired to this point strongly suggests that the brood communicate to workers by a contact system that likely involves a pheromone, that is either extractable from the cuticle in organic solvents, or is destroyed or masked in situ by the solvents.

More evidence for a pheromone was sought by exposing freshly killed sexual prepupae and pupae to different chemical reagents, hoping to reduce the activity of the signal by altering the chemical structure of the pheromone.

Twelve sexual brood (prepupae and pupae) per trial were exposed to the chemicals, which, except for ozone-saturated ethanol and aqueous hydrogen bromide, were in the vapour phase. All of the reagents tested were known to react rapidly with the functional groups they attacked. The exposure varied with reagent, but in all cases, was the maximum time before there was noticeable alteration in the cuticular structure, as observed under a dissecting microscope. The brood were washed in water for 10 min after exposure, then dried for 1 hr in air, before being presented two at a time to the six colonies. The effect of each reagent was assayed twice. The control for all of the vaporous reagents was freshly killed sexual prepupae; for ethanolic ozone, the control was equal exposure of sexual brood to oxygen-saturated ethanol, and for aqueous HBr, equal exposure to water. Table II lists the reagents used, the functional groups they most rapidly attacked under experimental conditions, exposure times, and the results of

Table II. Responses of Fire Ant Workers to Brood Previously Exposed to Various Chemical Reagents

Reagent	Functional groups most rapidly attacked	Exposure time	Mean $\bar{x}N$	Control $\bar{x}/N$	*P versus control
Bromine	Double bonds	1 min	1/12	11/12	$P<0{\cdot}001$
Acetyl chloride	Amines, acids, alcohols	40 s	9·5/12	11/12	NS
Silyl reagents	Amines, acids, alcohols	1 min	9/12	11/12	NS
Iodine	Double bonds, weakly	30 s	9/12	11/12	NS
Hydrogen cyanide	Aldehydes, ketones	10 min	8/12	11/12	NS
Ozone	Double bonds	5 min	3·5/12	6/12	NS
Hydrogen bromide	Double bonds	10 s	8/12	11/12	NS

*χ^2 test.

the assays. The only reagent to reduce significantly the brood signal was molecular bromine, implying that a functional group of a pheromone had been attacked and the pheromone thereby deactivated.

Discussion

The experiments of Glancey et al. (1970) were dissatisfying for two reasons: First, the nests they used were unpartitioned with very little difference in interior microclimate; hence, brood was scattered with no specific tending area (D. Jouvenaz, personal communication). Whether workers place a bait with their brood or just randomly drop a bait near a brood pile seems too subjective a criterion for brood retrieval. Secondly, vegetable oil was used to test for a food response. Although vegetable oil on blotter was never retrieved in our assay, the highly lipoidal body contents of larval *Zophobas* were, if the colony diet was lacking in animal food. The proper food control should have been animal fats and proteins, rather then vegetable oil.

There exists substantial evidence for a brood pheromone. The retrieval of skins and larval body contents on blotter, the persistence of the signal for such long periods after death despite disfigurement of the larval cuticle, and the ability of organic solvents to destroy the signal without visibly altering the cuticle are compelling evidence for a pheromone. In addition, the exclusive ability of molecular bromine to remove the signal in vivo indicates the disruption of a pheromone by alteration of some functional group.

There is also sufficient evidence that the brood pheromone is non-volatile, and transmits information by contact. Workers showed no signs of orientation prior to contacting a live bait in the foraging field. The random responses of workers in the olfactometer experiment demonstrate the pheromone's low vapour pressure. That the cuticle must be contacted to be recognized was shown clearly by the failure of workers to retrieve skins wrapped in extremely thin, porous laboratory tissue.

The difficulty in obtaining direct evidence for contact pheromones, as first predicted by Wilson (1965), probably lies in the ease with which these chemicals are masked in extraction processes. It is not surprising, then, that the signal could be so easily destroyed by organic solvents, but could not be successfully re-applied to substrates. Conventional extraction techniques are inadequate in dealing with these elusive messengers.

Although the list of insect pheromones has increased steadily, reports of contact chemoreception are less than common. Wilson (1962) notes a contact pheromone releasing grooming in adult workers of *Solenapsis invicta*. The male of the satyriid butterfly, *Eumenis semele* (L) utilizes a contact pheromone during courtship (Tinbergen et al. 1943). The oviposition markers of the apple maggot, *Rhagoletis pomonella* (Walsh) (Prokopy 1972), the Australian sheep blowfly *Lucilia cupria* (Wied) (Barton et al. 1969), and the desert locust *Schistocerca gregaria* (Forsk.) (Norris 1970) operate by contact. Among social insects, the queen of the ant *Myrmica rubra* (L.) maintains her retinue by means of a contact pheromone (Brian 1970), and Free (1967) has discovered a contact brood

pheromone in bumblebees that alters worker foraging behaviour.

The fire ant brood pheromone is found in greatest activity in the cuticle and is evenly distributed over the body surface with no noticeable gradient. As in other ants (LeMasne 1953; Weir 1959), fire ant microlarvae are not removed from the egg pile until they have grown considerably (and probably moulted) (Walsh, unpublished). The pheromone, which is first produced in these young larvae, performs its recognition function until eclosion, when the signal is lost with the pupal skin and the emerging adults, if from another colony, are attacked (Fig. 5). Curiously, workers attack neither narcotized adults from foreign colonies, nor domestic eclosing pupae. Why, then are foreign emerging sexuals attacked? The answer probably lies in the molecular differences between pupal and adult cuticles and the totally obscure mechanism of colony recognition of adult fire ants. The invariable acceptance of foreign brood by fire ants workers is also curious. Sudd (1967) believes that the ability to accept foreign broods is widespread in ants, suggesting an adaptive significance for the family. Perhaps close taxonomic relationship that exists between slave-making ants and their slaves is indication that the origins of slave-making can be traced to the ability of workers to perceive the brood recognition signals of closely related species as conspecific. Evidence for such a phenomenon was discovered even in non-slave making ants by Plateux (1960), who conducted larval adoption experiments between different genera and allowed workers to rear orphaned brood to adulthood. He found that workers of *Leptothorax nylanderi* Foerster would rear *Solenopsis fugax* Latreille brood to the imago stage only if there were no *L. nylanderi* brood present; if *L. nylanderi* larvae were present, the *S. fugax* brood were destroyed, and the *L. nylanderi* brood were tended, suggesting that the distinction between brood signals of the two myrmicines is so fine that *L. nylanderi* must contact its own brood to perceive the difference.

In the army ants, the survival of the young depend on the queen's presence. Her removal results in abandonment of the brood by workers. Furthermore, foreign brood are accepted and tended only after first being exposed to the resident queen's odour (Schneirla 1971). The queen in this case seems able to alter worker perception of brood signals to an extraordinary degree. Fire ant workers, on the other hand, accepted and tended brood under a variety of social conditions, including in queen-less colonies, suggesting that brood recognition by fire ant workers is much less dependent on social factors than in dorylines. However, the constant presence of the *S. invicta* queen in the brood chamber (personal observation), a trait that is characteristic of *Solenopsis* queens (LeMasne 1953), implies that she may play an important role in brood rearing and may affect worker perception of brood age, sex, or caste.

Acknowledgments

The authors wish to express their thanks to the members of the Fire Ant Research Team of the Department of Biological Science, Florida State University, for their help in many areas of this study. Special thanks are due to Ms Cheryl Curington for her efforts in taking worker scape measurements. Financial support for J.P.W. was supplied by National Science Foundation Grant No. GB-31698. We are grateful for the contribution. This paper fulfilled the thesis requirement of J.P.W. for the Master of Science Degree from the Department of Biological Science, Florida State University.

REFERENCES

Barton Browne, I., Bartell, R. J. & Shorey, H. H. (1969). Pheromone-mediated behaviour leading to group oviposition in the blowfly, *Lucilia cuprina*. *J. Insect. Physiol.*, **15**, 1003–1014.

Bhatkar, A. & Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *Fla. Entomol.*, **53**, 229–232.

Brian, M. V. (1970). Communication between queens and larvae in the ant, *Myrmica*. *Anim. Behav.*, **18**, 467–472.

Free, J. B. (1967). Factors determining the collection of pollen by honeybee foragers. *Anim. Behav.*, **15**, 134–144.

Glancey, B. M., Stringer, C. E., Craig, C. H., Bishop, P. M. & Martin, B. B. (1970). Pheromone may induce brood tending in the fire ant, *Solenopsis saevissima*. *Nature, Lond.*, **226** (5248), 863–864.

LeMasne, G. (1953). Observations sur les relations entre le vouvain et les adultes chez les fourmis. *Annls Sci. nat.*, **15**, 1–56.

Menozzi, C. (1930). Formiche della Somalia Italiana meridionale. *Memorie Soc. ent. ital.*, **9**, 76–131, in Wilson, E. O. (1971).

Norris, M. J. (1970). Aggregation response in ovipositing females of the desert locust, with special references to the chemical factor. *J. Insect Physiol.*, **16**, 1493–1515.

Plateux, L. (1960). Adoptions experimentales de larves entre des fourmis de genres differents. *Leptothorax nylanderi* Forster and *Solenopsis fuga* Latreille. *Insectes Soc.*, **7** (2), 163–170.

Prokopy, R. J. (1972). Evidence for marking pheromone deterring repeated oviposition in apple maggot flies. *Environ. Entomol.*, **1**, 326–332.

Schneirla, T. C. (1945). The army ant behavior pattern: nomad-statary relations in the swarmers and the problem of migration. *Biol. Bull.*, **88,** 188–193.

Schneirla, T. C. (1971). *Army Ants: A Study in Social Organization* (Ed. by Howard T. Topoff). San Francisco: W. H. Freeman.

Stager, R. (1923). Resultate meiner Beobachtungen und Versuche an *Aphaenogaster testaceo-pilosa* Lucas, *spinosa* Emery, var. *nitida* Emery. *Z. wiss. Insektenbiol.*, **18,** 351–356.

Sudd, J. H. (1967). *An Introduction to the Behaviour of Ants.* London: Edward Arnold Ltd.

Tinbergen, N., Meeuse, B. J. D., Boerema, L. K. & Varossieau, W. W. (1943). Die Balz des Samtfalters, *Eumenis* (=*Satyrus*) *semele* (L.). *Z. Tierpsychol.*, **5,** 182–226. In: Myers, J. (1972). Pheromones and courtship behavior in butterflies. *Am. Zool.*, **12,** 545–551.

Watkins, J. F. & Cole, T. W. (1966). The attraction of army ant workers to secretions of their queens. *Tex. J. Sci.*, **18,** 254–265.

Weir, J. S. (1959). Egg masses and early larval growth in *Myrmica. Insectes soc.*, **6,** 187–201.

Wheeler, W. M. (1918). A study of some ant larvae, with a consideration of the origin and meaning of the social habit among insects. *Proc. Am. phil. Soc.*, **57,** 293–343.

Wilson, E. O. (1962). Chemical communication among workers of the fire ant, *Solenopsis saevissima* (Fr. Smith). 3. The experimental induction of social responses. *Anim. Behav.*, **10,** 159–164.

Wilson, E. O. (1965). Chemical communication in the social insects. *Science, N.Y.*, **149,** 1064–1071.

Wilson, E. O. (1971). *The Insect Societies.* Cambridge, Mass.: The Belknap Press of Harvard University Press.

(*Received* 19 *February* 1973; *revised* 1 *September* 1973; *MS. number:* A1415)