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CHAPTER 33

Brood Pheromones In Ants

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Introduction

It has been known for a long time that worker ants are able to discriminate brood from adults as well as different categories of brood (see review in Le Masne 1953). It is also known that adult worker ants ingest regurgitated liquids produced by larvae, either spontaneously or after worker solicitation (Le Masne 1953). These products may be attractive to adults and involve salivary secretions, as suggested by Wheeler (1918) and Stager (1923). The above observations led to the hypothesis that ant brood produces pheromones that elicit specific adult behavior.

Terminology

Semiochemicals are chemicals that mediate interactions between organisms (Law and Regnier 1971). Within this broad category are allochemics and pheromones. The term pheromone is defined as a substance released by an organism to the outside that causes a specific reaction in a receiving organism of the same species (Nordlund and Lewis 1976). An allochemic is a substance produced or acquired by an organism that causes a specific non-food related reaction in a receiving organism of a different species (Wittaker 1970). The terms pheromone and allochemic have been further subdivided (see Nordlund 1981 for a review). In this review our primary interest is in releaser pheromones which, on perception by the recipient organism, causes an immediate behavioral response, as opposed to primer pheromones, which induce complex physiological changes that may not cause an immediate observable response.

Possible Brood Pheromones

To add more meaning to the term brood pheromone, the behavioral reaction being induced should be specified. We can hypothesize that brood pheromones may be subdivided into several categories as described below.

Brood recognition pheromone: Brood recognition implies that the surface chemistry of brood is recognized by adult ants as being different from that of adults. At a more refined level, each immature developmental stage may produce a specific pheromone that is distinguished by adult workers, leading to segregation of the immature stages in the nest. Species and colony recognition are confounding factors that have not been appropriately accounted for in brood pheromone studies.

Brood attractant pheromone: Brood may release a material that is volatile and that attracts workers. The bioassay used to measure this response has often been confounded by other behaviors. In several instances cited below, a bioassay was used to measure the response of worker ants to a spot of test material and cumulative worker counts were made over a series of time intervals to evaluate the bioassay. However, this method does not discriminate between attraction and aggregation. The workers may be staying at the spot simply because the extract tested has arrestant properties and is discovered during random foraging by the workers. The only way to measure attraction is via an olfactometer, which by definition measures the response only to volatiles. In ant species that have trail making and following capabilities the design of an olfactometer can be difficult. Walsh and Tschinkel (1974) used an olfactometer for the fire ant brood recognition pheromone in which they allowed only one ant to make a choice before cleaning the apparatus. This was done to avoid the possibility of the subsequent workers following the trail of the first ant. Obviously, this method becomes very tedious before the number of replicates reaches a statistically adequate level. Vander Meer *et al.* (in press) designed a Y-tube olfactometer that measured the first choice of the first 20 ants. Since fire ant trail laying occurs on the way back to the nest (Wilson 1962) and the olfactometer was designed to trap responding workers, we assumed that trail formation would not be a complicating factor. In spite of potential "follow the leader" effects, the system has worked well on research on the chemistry of the fire ant trail and queen pheromones. Glancey and Dickens (1988) subsequently used it to bioassay for volatiles from larvae.

Brood aggregation pheromone: This is a substance secreted by the brood that elicits a settling behavior in workers. For flying insects, an aggregation pheromone bioassay can be assumed to test also for an attractive element associated with aggregation; although for chemical isolation separate bioassays would have to be developed. In a bioassay that counts the number of worker ants aggregated around a spot of test material, it is not possible to assume associated attraction as discussed in the above section.

Brood tending pheromone: The term brood tending encompasses several adult behaviors, including feeding, grooming, and under disrupted colony conditions, retrieval back to the nest. Brood recognition is a prerequisite to these worker responses. Feeding and grooming have not been utilized in bioassays, since they can not be adequately observed with the surrogate brood required for demonstration of the existence of a chemical signal and the isolation of active chemicals from extracts. Brood retrieval has been used extensively in bioassays to measure tending. The major difficulty in this bioassay is the differentiation between a food response and a pheromone response. Several researchers have attempted to deal with this problem by 1) requiring for a positive result that treated particles are deposited in the brood chamber and 2) comparing the sample response to food controls. The problem with the first method is that workers feed the larvae and therefore must also bring food into the brood chamber. The food controls also present problems. For example, is the food value of the commonly used soybean oil control similar to the food value of extracted brood? The answer is no. Extracted brood contain a complex mixture of lipids, proteins, and carbohydrates, etc., whereas soybean oil contains primarily triglycerides. Ideally, one would like a food control that consisted of an equivalent amount of extract, by weight, of a non-ant larval form that was not currently in the colony diet. Trying to dampen out the food response by saturating the colony with a food control only increases the probability of workers responding to a novel food source; i.e. brood extract (Walsh and Tschinkel 1974).

Review and Critique of Brood Pheromone Related Literature

Brood pheromones have been investigated in several ant species, but most intensively in the red imported fire ant, *Solenopsis invicta*. We will critically review the non-fire ant literature followed by studies conducted on fire ants. We will then propose alternative hypotheses for the behavior of adult ants toward their brood.

Non-Fire Ant Literature

The first experimental work suggesting the existence of a brood pheromone was made on two army ant species, *Neivamyrmex opacithorax* and *N. pilosus* (Watkins and Cole 1966). The bioassay compared the number of ants on squares of chromatographic paper on which larvae or pupae had either rested for one hour or were smeared, to the number of ants on nearby control paper squares. *N. opacithorax* was reported to be attracted to secretions of larvae and pupae, but *N. pilosus* was not attracted to larvae or pupae. The authors did not report the amount of brood used in each test, nor whether or not the brood was from the same colony as the test workers. In addition, although the authors used the term "attraction", their bioassay measured *aggregation* on a spot of test material (see introduction). Schneirla (1971) also noted that army ant workers clustered on paper discs where larvae had rested and turned in greater numbers to a branch of a Y-tube through which larvae have been drawn rather than to the control branch. Again the clustering response is primarily aggregation, and the workers in the Y-tube responded to the equivalent of a larva-derived chemical trail rather than to volatiles in an air stream.

Robinson and Cherrett (1974) investigated the existence of a brood tending pheromone in the leaf-cutting ant, *Atta cephalotes*. Their bioassay utilized the fact that worker ants retrieve brood placed outside the nest. A positive response was recorded when the test object was brought back to the nest, regardless of its fate once in the nest.

The authors noticed that retrieval of live brood is a function of the distance to the nest. When a comparison was made between live and freeze-killed larvae or pupae, a significant difference was obtained only for larvae (live >dead). The authors attributed this result to physical damage due to freezing for larvae but not pupae, indicating that at least larval morphology may be important as a recognition cue. No difference was noted when alien and nestmate brood were compared, suggesting that there is no brood colony-specificity.

Pupae rinsed with hexane still induced positive responses; however, hexane rinses tested on filter paper at 1 pupa equivalent did not. Moreover, after a thorough succession of soaks and rinses in different solvents, dead pupae were retrieved as often as live ones. Vander Meer (unpublished) obtained similar results with *Solenopsis invicta* larvae. Hexane rinses of larvae were not retrieved either. The only treatment that increased pick up, although not retrieval to the nest, was a larval homogenate applied to filter paper disks. When this homogenate was added to sugar and tested against sugar, the sugar control elicited a greater response. The authors concluded that there was no evidence for the involvement of a pheromone in *Atta cephalotes* worker recognition of brood; however, physical properties might play a role.

At a more refined level, Brian (1975) studied *Myrmica rubra* worker discrimination of the following brood developmental stages: A) third (last) instar larvae (3IL) versus pharate pupae (PP), and B) worker (WB) versus queen-biased (QB) larvae. Bioassays consisted of paired observations of the retrieval of test objects and controls to the inside of the darkened brood chamber of small subcolony nests. Several physical properties of 3IL and PP were tested, which led to the conclusion that movement, shape and hairiness were not important for recognition. Only flaccidity significantly diminished retrieval. Alcohol and water-washed cuticles of 3IL and PP (i.e. eviscerated bodies filled with gelatin) gave results identical to controls. PP cuticles were found to be species-specific (tested with *M. scabrinodis*), but the species-specificity of 3IL was apparently not tested. No specific region of the larval or pupal body was found to be critical, although at least one half of the body had to be exposed to induce retrieval as quickly as controls.

Treatment of 3IL with chloroform, acetone or ether coupled with bioassays led to the conclusion that 3IL release a signal that can be removed by these solvents. However, the resulting extracts failed to elicit a positive response. The recognition cues released by 3IL or PP appear to be a combination of physical properties and non-volatile, heat stable chemical cues.

Queen-biased larvae are attacked and scarred by workers in queenright colonies of *Myrmica rubra* (Brian 1973). This treatment forces the QB larvae to lose their potential for becoming queens. Consequently, workers must be able to discriminate QB from WB larvae. Experiments demonstrated that size, growth rate, and morphological indicators of queen potentiality (i.e. ratio of leg:wing buds) were not used by workers for discrimination. Material collected on the ventral surface of isolated QB larvae elicited aggression when applied on WB larvae in queen-right colonies. Whatever chemical(s) is involved is trans-specific between *M. scabrinodis* and *M. rubra.*

Brian concluded that there was no need for chemical involvement in worker/brood discrimination, since the relative immobility and lack of movable appendages of the brood were sufficient to differentiate it from adults. However, it is likely that eggs, larvae (including pharate pupae), and pupae, have their own recognition features.

The following studies by Lenoir and collaborators did not address the questions of brood pheromones since they only used live brood in their bioassays. However, they provide some interesting information about brood recognition.

Lenoir (1981) designed a bioassay to test the colony-specificity of brood in *Lasius niger*. Ten alien and 10 nestmate pieces of worker brood were presented to workers and the order of retrieval was recorded. A) When one test was conducted on each of five colonies in summer, no significant difference was obtained. B) When 10 successive tests were conducted with larvae in summer on the same colony, four tests gave a significant difference (own brood retrieved first). C) When 10 successive tests with cocoons were made on the same colony at the same season, two tests gave a significant difference. It was not indicated if the same colony was used for both larvae and cocoons. D) In October, 11 colonies were tested once each and eight retrieved their nestmate brood before the alien brood. These results indicate a tendency towards brood colony-specificity and that worker discrimination may vary with the season. However, alien brood was always adopted even when rejected at first.

A second series of bioassays was made in order to test the preference between different brood stages: eggs, small larvae, large larvae and cocoons. Results showed that the oldest and largest stages were preferred, except in the case of large larvae versus cocoons where there was no significant difference. This series of tests confirmed that discrimination between immature stages exists, and that there is a preferential order of retrieval. However, the mechanism of discrimination was not addressed.

Lenoir (1981) also studied the influence of the deprivation of larvae in the adult worker environment on the ability of these workers to retrieve larvae. Larva-naive 15-day old workers, familiar to eggs, were tested in a larva/egg choice for one hour in a series of three experiments. In experiment one, eggs were retrieved first by workers in three of four colonies. After this experiment the larvae were removed and 24 hours later the second egg/larva choice was given to the same larva-naive workers. The egg preference was then significant in only one of the four colonies. After the second experiment, larvae were allowed to remain in the colonies. The third experiment was performed 24 hours later and no significant preference was noticed in any of the colonies. The effect of social experience on worker/brood discrimination was obvious, even when that experience only lasted one hour (second experiment).

Lenoir (1984) used *Cataglyphis cursor* to investigate the colony-specificity of brood. In this species brood-exchange between neighboring colonies has been observed in nature. The activities of callow workers introduced into an alien colony were compared to those of resident workers of the same age. The only significant difference occurred with brood-tending during the first five days following adoption. Adoptees spent less time interacting with brood than did controls. Thus, Lenoir concluded that larval recognition is to a certain extent colony-specific. The absence of a difference after the sixth day was rationalized by the adoptee becoming familiar with their new colony brood. An alternative hypothesis is that the adopted callow workers were disturbed by their introduction into the alien colony, and some aspects of their social behavior (i.e. broodtending) were temporarily altered. Support for this possibility comes from Lenoir et al. (1982), who found on the same species that adopted callow workers are less integrated into the colony than controls.

In a study demonstrating pre-imaginal learning of colony odor in *Cataglyphis cursor*, Isingrini et al. (1985) showed that larvae are recognized by workers as nestmates or non-nestmates. Ten-day old callow workers were tested with three larvae from their colony and three larvae from an alien colony. In group G1, callow workers were individually isolated from eclosion to the time of the test. In group G2, callow workers were placed individually with two adults and two larvae from the alien colony for ten days. In group G3, callow workers were maintained in groups from eclosion to the time of the test. G1 and G3 workers tended nestmate larvae significantly more than alien larvae. There was also a significant difference between G1 and G3, with G3 workers tending nestmate brood more than G1 workers. For G2 workers, no significant difference was recorded. The authors suggested that recognition of nestmate larvae cannot be reversed by post-imaginal experience, although the results indicate that it can be altered. A complicating factor is that the tested callow workers spent their first eight hours of adult life in their own colony, which could be sufficient to induce a lasting preference for their nestmate larvae. It has been shown that the first few hours of ant adult life can be sufficient to insure normal development of social behaviors (Morel 1983a, 1985, in press).

Fire Ant Literature

Glancey et al. (1970) used brood retrieval by workers to investigate the existence of a brood tending pheromone in *Solenopsis invicta*. Crushed worker and sexual larvae or pupae were extracted with hexane. Larval extracts applied on corn grits induced retrieval, but pupal extracts were ineffective. Within 24 hours, treated grits were discarded, indicating, according to the authors, that the pheromones are volatile. Grits treated with vegetable oil were not retrieved which verified, according to the authors, that the responses obtained were not food responses. The authors concluded that larvae release hexane-extractable volatile chemicals that induce retrieving and specific behaviors directed toward brood. Several criticisms can be made of the methodology used by Glancey et al. (1970). First, no indication of the concentration of extracts or controls was given. Comparison of the responses to extracts with responses to food should have been done in the same test. In addition, a better control would have been a larva-equivalent of an insect extract rather than vegetable oil (see introduction). Consequently, the food value of brood extracts has not been eliminated. Moreover, the fact that workers discarded the grits treated with larvae extracts within 24 hours at refuse sites can be attributed to the loss of food value through worker consumption or decomposition of lipids inducing necrophoric behavior (Howard and Tschinkel 1976).

A few years later, research on brood pheromones of the same species was published by Walsh and Tschinkel (1974). These authors designed bioassays to obviate two major criticisms they made of the work of Glancey et al. (1970): 1) possible food response and 2) poor nest design that did not provide a separate brood chamber.

They used sexual pupae and prepupae because of their size and durability. In addition, other brood stages required twice as much time to be retrieved. Objects to be tested were placed two at a time in front of a sub-colony entrance. Once the objects were retrieved into the nest, alarm behavior was induced by blowing air through the nest entrance. A positive response was recorded when the test object stayed at least 10 sec. in the brood chamber during the first 50 min. after the release of alarm behavior.

Several social conditions were tested: presence of the queen, worker number (5, 25, 50, 1000), worker size and amount of brood in the colony (from 0 to 60). No significant difference was obtained under these different social conditions (note that queens in each sub-colony were alien colony-founding queens, and that a different result may have been obtained with physogastric mother-queens).

Bioassays were then conducted to elucidate some characteristics of the brood signal. Worker response to freeze-killed prepupae decreased significantly 21 hours after death. The authors concluded that the signal was present either in large quantity or very potent and stable. The signal may not be lost after 21 hours, but simply masked by decomposition products. By dipping different parts of the pupae into paraffin, it was established that the signal was evenly distributed on the cuticle, which was also the case for *Myrmica rubra* larvae (Brian, 1975; see above). The first significant decrease in positive responses was reached when 17% of the prepupae, or 30% of the pupae was covered. The signal apparently is lost after eclosion since young adult sexuals are attacked and injured. However, this phenomenon may be specific to sexuals since it is well known that newly eclosed workers are readily adopted between colonies.

A hexane extract of about 2,000 brood individuals was applied to 60 pieces of paper. None of these baits gave a positive response. Extracts with other solvents also failed to elicit a response. To test the volatility of the signal, five live prepupae were placed into one arm of a Y-tube olfactometer which had a constant air flow and was connected to sub-colony nests. A positive response was recorded if the first worker moving into the Y-tube entered the brood-containing arm and walked towards the prepupae. No significant difference was obtained indicating that no volatile was emitted by the brood.

To test the hypothesis that a contact chemical signal was involved, worker responses to physically treated prepupae were tested. A hexane, but not a water rinse, significantly decreased worker responses to freshly killed prepupae. No significant difference was found between whole cuticle and freshly killed prepupae (6/12 vs. 10/12); however, it is possible that this difference would have been significant with more than four trials. Hexane significantly removed the signal that caused worker responses to prepupae cuticle, as it did for the whole prepupae. Cuticle sandwiched in tissue gave a significantly reduced response compared to control cuticle, showing that contact with the cuticle was necessary. Body contents of prepupae were tested against freshly killed prepupae and body contents of tenebrionid larvae and adult *S. invicta* males. No difference was found between the prepupae body contents and the freshly killed prepupae control. However, here again the difference was 6/12 vs. 10/12 which is indicative of the need of a larger sample size. Prepupae body contents elicited a significantly more positive response than the body contents of both tenebrionid larvae and adult males.

Body contents of tenebrionid were tested as a control for food response, and body contents of adult males were used as a control for colony response. These two controls seem inappropriate. The food response should have been tested with an insect absent from the ants' laboratory diet. The authors demonstrated in this paper that positive responses were obtained with novel insect (tenebrionid larvae) food baits and that this response could be dampened by adding that insect to the ants' diet. Therefore, the body contents of prepupae may simply represent a novel food item. Using adult male body contents as a colony response odor does not take into account that the colony discrimination cues are very likely to be on the cuticle (Wilson 1971). Moreover, colony-discrimination occurs principally between workers, and may be applied in a different way, or not be applied at all, to adult sexuals. Consequently, the authors' conclusion that there is evidence for the existence of a contact brood pheromone is not strongly supported.

Bromine, which attacks double bonds, was the only chemical reagent to diminish the response to brood. But since other double bond specific reagents did not induce a significant difference, it is possible that the bromine itself had a residual repellent effect.

Bigley and Vinson (1975) attempted to extract and characterize the *S. invicta* brood-tending pheromone reported by Glancey et al. (1970). Sexual pharate pupae were preferred for their studies over other immature forms because: 1) the gut contents have been expelled and can not contaminate the extract, and 2) they were retrieved to the nest more rapidly, which is in agreement with Walsh and Tschinkel (1974).

Homogenized pupae were extracted with chloroform:methanol (2:1), a standard solvent mixture for total lipid extraction (Christie 1973). Either field colonies or one-year old (i.e. not yet producing sexual brood) lab colonies were used in bioassays. Extracts were tested on filter paper disks placed in the foraging arena. The concentration and the quantity of the extract applied on each disk, i.e. its pupa-equivalent was not given. Soybean oil in hexane was used as a food response control (concentration and the amount applied unknown). As already mentioned for Glancey et al. (1970), an insect extract would have been more appropriate for this control. A positive tending response was recorded when "workers approached the treated disk and exhibited rapid palpitations of the antennae" or if the disk remained in the nest for 24 hours. These criteria are not specific to brood in ant colonies.

Pupal-extract treated disks elicited significantly more positive responses than soybean oil or solvent control. Discs treated with sex pupae extracts were not placed with the live worker brood, but apart where some live sex pupae given to the colonies were placed. This result led the authors to suggest that worker and sex brood produce different chemicals. However, since social experience has been shown to influence brood-directed behavior in at least one species (Lenoir 1981), it is possible that the sex brood-naive workers put treated disks and live sex pupae together because they were perceived as different from the familiar worker brood. A field test was made with 40 pupa-treated disks and 10 soybean oil-treated disks on a fire ant mound. All pupae-treated disks were retrieved into the nest where they remained over one day. About 20% of the soybean oil-treated disks were retrieved but they were found on the trash pile the next day. As in the laboratory test, not knowing the comparative quantitative and qualitative food value of the pharate pupae extract and soybean oil control makes an evaluation of the results impossible.

Bigley and Vinson (1975) proceeded to fractionate the pharate pupae extracts by thin layer chromatography (TLC) and to isolate a bioassay active spot. They then assigned that spot the specific structure of triolein based on the fact that a triolein standard had a similar Rf value (distance of the spot from origin : distance of the solvent from origin in TLC). The active fraction lost activity on saponification and a comparison of the recovered acid fraction with oleic acid by TLC showed that they had the same Rf value. The authors concluded that the active fraction was triolein. The flaws in their chemical argument have been discussed elsewhere (Vander Meer 1983). Briefly, standard silica gel TLC separates the simple lipids into spots of chemicals of similar class (e.g. triglycerides or fatty acids). Within each class there are usually many individual components. For example the Rf value of many triglycerides will be identical. Structural assignments can not be based solely on TLC.

If Bigley and Vinson were correct, it would mean that the pharate sex pupae produce a single triglyceride, triolein, one of a multitude of triglycerides. This would be extremely unusual (Christie 1973). We tested this hypothesis by isolating the active TLC fraction of pharate sex pupae of *S. invicta* as described by Bigley and Vinson. We then used argentation TLC to separate triglycerides based on the number of double bonds as described by Christie (1973). Using triolein as a standard we demonstrated that there were at least eight categories of triglycerides present in the pharate sex pupae extract, none of which corresponded to triolein (Vander Meer unpublished).

The positive bioassay results of Bigley and Vinson (1975) for 1% solutions of triolein and diolein may well reflect phagostimulant activity rather than that of a brood pheromone. Diolein has been implicated in the retrieval of plant seeds by *Aphaenogaster rudis* (Marshall et al. 1979). Seed retrieval is similar to brood retrieval but is clearly a food response. The net result is that the question of a brood pheromone in *S. invicta* is still open.

LaMon and Topoff (1985) observed that *S. invicta* pupae need adult assistance to break their cuticle and emerge as adults. With the previously published brood pheromone work as a background, they looked for chemicals that indicate to adult fire ant workers that a pupa is ready to emerge and requires help (social facilitation of eclosion). These chemicals would be a recognition signal for this special brood stage.

Extractions were made on worker pupae about to eclose and young or mature worker pupae by soaking at least 50 of each (no upper limit given) in 0.5 ml methylene chloride for 24 hours. Preliminary water or ethanol extracts did not give positive results. Bioassays were conducted in the foraging arena, which is curious since foragers are not expected to be the most motivated to help in emergence of young adults.

Worker "attraction" was tested to live pupae of various stages and to a piece of cotton soaked in 0.2 ml of extract (at least 20 pupa-equivalents, or PE, per bait). Objects were placed in pairs 7 cm apart in such a way that ants could only touch them with the tip of their antennae. For each pair of stimuli, the number of ants was recorded in two tests on two colonies. The data were not analyzed statistically and the results are not always clear-cut. The authors concluded that live eclosing pupae or their extract are the most attractive. But paired observations between live eclosing pupae and their extract were not made. Moreover, the bioassay does not unambiguously measure attraction, because of the confounding aggregation factor (see introduction). In addition, a 7 cm separation of the test samples may not be sufficient to keep the two samples independent.

Cotton thread baits were soaked for 5 min. in the extract (presumably at least 20 PE per thread). Freshly killed pupae were used as a control. Each bait type was tested separately, using 10 baits three successive times. Observations were made for 30 min. after the baits were placed in the brood chamber. Results showed that eclosing pupae, alive or dead, or their extracts stayed in the brood chamber after 30 min., as did live early pupae. The negative result obtained with the early pupal extract may have been due to the small number of replicates or to missing cues. Dead early pupae were not tested. Since the tests were not paired, the order in which they were made or time-related changes in the activity of the colony could account for the observed differences.

The limited response induced by non-eclosing pupal extracts is in contradiction with the previous results on the same species by Walsh and Tschinkel (1974) and Bigley and Vinson (1975), but not with Glancey et al. (1970). It is difficult to rationalize the difference in results, but worker pupae may release different signals than sex pupae. However, both worker and sex pupae probably need help from workers to become adults, and no difference in signal would be expected for the two categories. Physical cues, such as pigmentation and movement, can not be *a priori* eliminated. The main conclusion of LaMon and Topoff, i.e. that "facilitation of eclosion by adult workers is ... under the control of a chemical stimuli", is not supported by the experiments presented in their paper, and the bioassays used were inappropriate to test their hypothesis.

The most recent paper about the brood pheromone in *S. invicta* (Glancey and Dickens, 1988) intends to present new behavioral evidence for the existence of contact and non-contact tending pheromones released from larvae.

Rinses were obtained from field-collected 3rd and 4th instar worker larvae. Preliminary spot bioassays showed that at least 500 larva-equivalents (LE), i.e. the rinse of 226 mg of brood, was necessary to elicit a response. A cockroach (*Periplaneta americana*) rinse was prepared, but the equivalence was not in terms of a total food value or weight of material in each extract. There is much more surface area on 226 mg of larvae than on the same weight of cockroach.

A spot "attraction" bioassay used a piece of filter paper divided into 4 parts. In the center of each part was tested either a larval rinse, roach rinse, solvent, or the secretions left by 300 larvae during 15 min. The filter paper was placed in a Petri dish with 20 brood tender workers, and counts of the number of ants within 1/2 cm of the test spots were made every minute. The brood tenders had two different diets, with or without roach. In both cases, they were significantly more attracted to the larval rinse spot than to the other test spots (secretions from live larvae came second). No indication of the number of tests is given.

The production of a volatile attractant was tested with an olfactometer (Vander Meer *et al*, in press; see also introduction). Fifty brood-tender workers were placed at the entrance of the Y-tube and 300 live larvae were placed in one arm of the tube. Results of six replicates showed significant attraction to the brood-containing arm, regardless of whether the brood was nestmate or alien. Therefore, 300 live larvae released a non-colony specific worker attractant. The requirement of at least 300 live brood pieces to elicit an olfactometer response throws doubt as to what the worker ants were actually responding to. Just the respiration products from so many larvae may make the air different from the blank control. Also, workers do respond to individual immatures; i.e. in a disrupted colony situation. These results represent an effect of the group of brood, which may be real in a colony situation where brood is concentrated together. However, the effect is not pheromonal. The reasons why Walsh and Tschinkel's (1974) olfactometer experiment failed may be due to the different brood stages tested, to the different amount of brood tested, or to the olfactometer design.

A surrogate-brood bioassay consisted of placing brood rinse-treated rubber septum in a Castone bottom Petri dish with 20 brood-tenders, from either a roach or roach-free diet. The number of ants clustering in the 2 cm^2 area around the surrogate was counted every minute. Workers responded to the brood-rinse treated surrogate significantly more than to the roach-rinse or solvent treated septum. In a second bioassay, pieces of paper treated with different rinses (larva, roach, and solvent) were placed in a large tray with a disrupted colony. One hour later, their positions in the colony were recorded. Of five colonies, each tested once, only the brood rinse treated papers were retrieved. The LE of each surrogate is not known.

Finally, electroantennograms (EAG) were recorded for larval extracts of 1, 10, and 100 LE concentrations. Three ant antennae preparations were tested from the lowest concentration to the largest, with exposure to a hexanol standard (100 μg) in between each. The distance from antenna to the test sample was one cm. The response to the test extracts was given as the percent of the response to the standard. Only two concentrations gave significant differences between each other. One LE gave a response of about 5% of hexanol and 10 and 100 LE gave about 25% of the hexanol response. The authors concluded that, in addition to the olfactometer results, this is evidence for a volatile component in the brood pheromone. But the highest mean EAG response was only -0.1 mV. Ant pheromone EAG responses below /0.5/ mV are questionable (personal communication, C. Masson) and a response of ca. /0.5/ mV was obtained for 0.2 queen equivalent of fire ant queen pheromone (Vander Meer, unpublished). Hexanol is a component of the odor of green leaves (Visser et al. 1979). It seems extraordinary that the EAG response to hexanol was higher than was the response to brood volatile. One would expect that a component which is omnipresent and does not play a role in the ant's life (leaves are neither a major part of the fire ant diet nor their nest) to induce a low response. The results of this experiment do not appear strong enough to support the authors' contention that brood rinse volatiles give a pheromonelike response.

Discussion And Conclusions

As previously defined, a brood pheromone is a chemical or mixture of chemicals released by the brood that elicits a behavioral response in adult ants of the same species. Studies reviewed in this paper demonstrated that adults distinguish brood from workers, but none have demonstrated the existence of a brood pheromone.

It is clear that one of the most confounding factors to deal with is the differentiation of a food response from that of a brood pheromone. The wealth of pheromone research in ants and other insects has shown that chemicals active as pheromones are present in minute quantities relative to the individual's biomass. For example, the queen attractant pheromone in fire ants is present at only about 10 ng per queen (Glancey 1986) and the trail orientation and recruitment pheromones are present at only 6 and 0.075 ng per worker, respectively (Vander Meer et al. 1981; Vander Meer et al. in press). Behavioral responses to these fire ant pheromones are obtained with one or less body-equivalent. Consequently, serious questions arise when investigations of a brood pheromone require whole body extracts or a large number of brood in order to elicit worker responses. In addition, discrimination cues, such as a brood recognition pheromone, are most likely to be found on the cuticular surface rather than from the inner body (Wilson 1971). None of the reviewed studies used a whole adult ant control, either worker or sexual. Therefore it is impossible to assess whether or not the behaviors elicited were general responses obtainable from any colony group.

Proposition I: *Yes, brood pheromones do exist*. However, technical difficulties both in the development of bioassay and/or chemical methodologies have so far precluded their clear demonstration. Perhaps this paper will stimulate further investigations of brood pheromones that avoid the problems discussed in the review of past work.

Proposition II: *Brood pheromones do not exist*. This view is, in our opinion, more strongly supported by the literature reviewed, from which the following can be concluded about brood/adult interactions:

- Body shape can be an important cue used by adults to distinguish brood from adults.
- The "I am brood" signal is not localized on any part of the brood's body.
- Brood is successfully adopted into other colonies of the same species or in colonies of closely related species (see Jaisson 1971 for review).
- The preceding statement also applies to callow workers (see Jaisson 1971 for review).

The above information implies that the differential treatment of brood by adults depends on more than cuticular chemicals and that the chemicals involved may not be classified as pheromones. We would like to explore the possibility that adult behavior toward brood is moderated by at least the following factors: A) morphology, B) behavior, and C) cuticular chemicals.

A: There is no question that brood morphology is basically different from adult morphology. Within the three main stages of brood: eggs, larvae, and pupae (with or without cocoon), the morphological differences are also obvious. Within the larval stage, each instar may also have a characteristic morphology, as is found in S. *invicta* (Petralia and Vinson 1979). The 1st instar larvae are hairless; the 2nd instar larvae have a few simple hairs; the 3rd instar larvae have numerous short simple and bifid hairs and the 4th instar larvae have long simple hairs on the head and the antero-ventral surface and bifid hairs on the rest of their body. In addition, 4th instar larvae are the only stage to have sclerotized mandibles. Although it is only conjecture that fire ant workers use morphological features to distinguish larvae, it is known that workers preferentially feed solid food to the specially adapted 4th instar larvae (Petralia and Vinson 1978). Modification of brood morphological features had an effect on brood/adult interactions in two studies (Robinson and Cherrett 1974; Brian 1975).

B: Adult and brood behavior in ants is clearly distinguishable. Eggs and pupae are characterized by a lack of behavior, and in most ant species the behavior of larvae is limited primarily to food-related movements. The difference between worker/worker and brood/worker interactions could be due in part to the dissimilarity in the associated behaviors. Contrary to other brood stages, *Solenopsis invicta* or *Camponotus vagus* eclosing pupae can be identified by the onset of gross uncoordinated movements (LaMon and Topoff 1985; Morel 1983b). This phenomenon may be general to ants. The dramatic change in pupal behavior may be enough to elicit initial worker facilitation of eclosion.

C: The third factor involved in brood/worker interactions is chemical cues. From the studies on brood pheromones reviewed above, there is no conclusive evidence for the involvement of species-specific chemicals acting at a pheromonal level. The chemical factors included in brood/worker interactions may be directly related to nestmate recognition cues, which may include exogenous and/or endogenous odors (Vander Meer, in press). In addition, it is conceivable that chemical cues are involved in the differential treatment of brood stages. Brian (1975) demonstrated that *Myrmica* queen-biased larvae produced a non species-specific substance that induced the loss of their queen potentiality in queen-right colonies due to worker aggression. Although LaMon and Topoff (1985) did not show that social facilitation of eclosion in fire ants is under chemical control, they described the eclosing pupae as "covered with a sticky, viscous exudate". It has not been demonstrated whether or not workers are responding to the exudate as a food material, as a specific behavior-releasing chemical, or simply because it is different from the normal pupal cuticular chemicals. In any event the exudate may represent a chemical discriminatory cue.

Different chemical factors have been proposed to account for the acceptance of callows in alien colonies. Jaisson (1972) proposed that very young adults produce a substance which both attracts mature workers and inhibits their aggressivity. Based on unpublished experimental data, Holldobler and Michener (1980) suggested that at least a part of the "brood pheromone" is contained in the exuvial liquid which coats the young adult during eclosion. These hypotheses have yet to be supported by further experimental evidence.

We would like to propose two alternative hypotheses that involve the morphology, behavior and cuticular chemicals of brood and its differential treatment by adults.

In the first, we define the cuticular chemicals involved in worker/brood interactions as nestmate recognition cues. The cues are transferred between workers and may also be transferred to brood. As discussed above, the morphology and behavior are clearly different between adult and brood. Their combination with nestmate recognition cues could be sufficient to account for the differential treatment of brood by adult ants. Brood-derived nestmate recognition cues could account for all bioassays made with brood secretions or extract-treated baits (although results with brood extracts are most likely food responses). The contribution of each factor may vary from species to species. As seen in the literature reviewed, modification of any of the factors may affect the behavior of workers towards brood.

The second hypothesis is based on an associative learning process between a characteristic (morphological, behavioral, or chemical) of brood and a "reward" obtained by adult workers from brood. Learning in ants has been known for a long time (Alloway 1972; Menzel 1985). If only chemicals are used, they have to be specific to the brood. Workers have been described as ingesting oral secretions and excretory products from larvae (see Le Masne 1953 for review). In addition, 4th instar larval regurgitated products play an important role in the colony nutrition (Tschinkel, in press). There is precedence for the necessary involvement of larvae in colony nutrition in *Vespa orientalis* (see in Wilson 1971). This last hypothesis readily accounts for the relations between workers and larvae and possibly eclosing pupae. The benefits of the associative learning could be extended from larvae to eggs and pupae, although they do not offer a reward.

Invoking multiple factors in brood/worker interactions, based on either of the above hypotheses, offers an interesting explanation for the success of the adoption of brood into alien colonies. Aggressive behavior between two workers of two colonies of the same species or two different species involves recognition and a hierarchy of agonistic behaviors in both partners. Since brood lacks the ability for agonistic display, it would be predicted that aggressive behavior toward them would be moderated even if their nestmate recognition label did not match that of the receiving colony (see Breed and Bennett, 1987 for a review of nestmate recognition). Once inside the foreign colony, the successful adoptee can begin to acquire a new colony odor through social interactions. This situation may be analogous to the passive integration mechanism used by a myrmecophilous beetle (Vander Meer and Wojcik 1982). Based on nestmate recognition studies in ants, the ultimate acceptance of brood and/or the resulting adult workers may be dependent on the acquisition of the nestmate recognition cues of their adopted colony. The limitation of the inter-specific adoption of brood to closely related species (in Jaisson 1971) indicates that species differences in one or some combination of the three factors (most likely morphology and/or cuticular chemistry) may negatively influence interspecific brood/worker interactions. The reduced behavioral repertoire and acquisition of nestmate recognition cues by callow workers may also contribute to their acceptance in alien colonies as cited by Jaisson (1985).

The above hypotheses need to be supported by experimental evidence. We hope that the critical review of brood pheromone literature and the alternative propositions stimulate interest and research in this extremely complicated, but fascinating area.

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Literature Cited

Alloway, T. M.
1972 Learning and Memory in Insects. Ann. Entomol. Rev. 17, 43-56.

Bigley W. S. and Vinson S. B.

1975 Characterization of a brood pheromone isolated from the sexual brood of the Fire Ant, *Solenopsis invicta.* Ann. Entomol. Soc. Am., 68, 301-304.

Breed M. D. and Bennett B.

1987 Kin recognition in highly eusocial insects. In: Kin Recognition in Animals, D. J. C. Fletcher and C. D. Michener eds., John Wiley and Sons, New York, pp. 243-286.

Brian M. V.

1973 Queen recognition by brood-rearing workers of the ant *Myrmica rubra* L. Anim. Behav., 21, 691-698.

1975 Larval recognition by workers of the ant *Myrmica.* Anim. Behav., 23, 745-756.

Christie W. W.

1973 Lipid Analysis. Pergamon Press, Oxford, 338 p.

Glancey B. M.

1986 The queen recognition pheromone of *Solenopsis invicta.* In: Fire Ants and Leaf-Cutting Ants: Biology and Management, C. S. Lofgren and R. K. Vander Meer eds., Westview Press, Boulder, CO, pp.223-230.

Glancey B. M., Stringer C. E., Craig C. H., Bishop P.M. and Martin B.B.

1970 Pheromone may induce brood tending in the fire ant, *Solenopsis saevissima.* Nature, London, 226, 863-864.

Glancey B. M. and Dickens J.C.

1988 Behavioral and electrophysiological studies with live larvae and larval rinses of the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). J. Chem. Ecol., 14,463-473.

Hölldobler B. and Michener C. D.

1980 Mechanisms of identification and discrimination in social Hymenoptera. In: Evolution of Social Behavior: Hypotheses and Empirical Tests, H. Markl ed., (Dahlem Konferenzen 1980), Verlag Chemie, Weinheim, pp. 35-58.

Howard D. F. and Tschinkel W. R.

1976 Aspects of the necrophoric behavior in the red imported fire ant, *Solenopsis invicta.* Behaviour, 56, 157-180.

Isingrini M., Lenoir A. and Jaisson P.
1985 Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. Proc. Natl. Acad. Sci. USA, 82, 8545-8547.

Jaisson P.
1971 Experiences sur l'agressivite chez les Fourmis. C. R. Acad. Sci., Paris, ser. D, 273, 2320-2323.
1972 Nouvelles experiences sur l'agressivite chez les Fourmis: Existence probable d'une substance active inhibitrice de l'agressivite et attractive secretee par la jeune Formicine. C. R. Acad. Sci., Paris, ser. D, 274, 302-305.
1985 Social behavior. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, G. A. Kerkut and L. I. Gilbert eds., Pergamon Press, Oxford, vol. 9, pp. 673-694.

LaMon B. and Topoff H.
1985 Social facilitation of eclosion in the Fire Ant, *Solenopsis invicta*. Dev. Psychobiol., 18, 367-374.

Law J. H. and Regnier F. E.
1971 Pheromones. Ann. Rev. Biochem., 40, 533-548.

Le Masne G.
1953 Observations sur les relations entre le couvain et les adultes chez les Fourmis. Annls. Sc. Nat., 15, 1-56.

Lenoir A.
1981 Brood retrieving in the ant *Lasius niger*. Sociobiology, 6, 153-178.
1984 Brood-colony recognition in *Cataglyphis cursor* worker ants (Hymenoptera: Formicidae). Anim. Behav., 32, 942-944.

Lenoir A., Isingrini M. and Nowbahari M.
1982 Le comportement d'ouvrieres de *Cataglyphis cursor* introduites dans une colonie etrangere de la meme espece. Colloque S.F./U.I.S.S.I. "La communication dans les societes d'Insectes", Barcelone, 107-114.

Marshall D. L., Beattie A. J., and Bollenbacher W. E.
1979 Evidence for diglycerides as attractants in an ant-seed interaction. J. Chem. Ecol., 5, 335-344.

Menzel R
1985 Learning in honey bees in an ecological and behavioral context. *In:* Experimental Behavioral Ecology and Sociobiology. B. Holldobler and M. Lindauer, eds., Gustav Fischer Verlag, Stuttgart, 488 p.

Morel L.

1983a Relation entre comportement agressif et privation sociale précoce chez les jeunes immatures de la Fourmi *Camponotus vagus* Scop. C. R. Acad. Sci., Paris, Ser. III, 296, 449-452.

1983b Contribution à l'étude des interactions sociales chez les jeunes ouvrieres de la Fourmi *Camponotus vagus* Scop.: Développement du comportement trophallactique et régulation de l'agressivité. Thèse IIIe cycle, Aix-Marseille II, 164 p.

1986 Ontogenesis of the antennal activity associated with food transfer in the callow worker ant. Dev. Psychobiol., 19, 413-426.

Ontogenesè de la reconnaissance des membres de la société chez *Camponotus floridanus* (Hymenoptera: Formicidae). Role de l'expérience sociale précoce-Biol. Behav., in press.

Nordlund D. A.

1981 Semiochemicals: A review of terminology. In: Semiochemicals: Their Role in Pest Control, D.A. Nordlund, R. L. Jones and W. J. Lewis, eds., John Wiley and Sons, New York, pp. 13-28.

Nordlund D. A. and Lewis W. J.

1976 Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. J. Chem. Ecol., 2, 211-220.

Petralia R. S. and Vinson S. B.

1978 Feeding in the larvae of the Imported Fire Ant, *Solenopsis invicta*: Behavior and morphological adaptations. Ann. Entomol. Soc. Amer., 71, 643-648.

1979 Developmental morphology of larvae and eggs of the Imported Fire Ant, *Solenopsis invicta*. Ann. Entomol. Soc. Amer., 72, 472-484.

Robinson S. W. and Cherrett J. M.

1974 Laboratory investigations to evaluate the possible use of brood pheromones of the leaf-cutting ant *Atta cephalotes* (L.) (Formicidae, Attini) as a component in an attractive bait. Bull. ent. Res., 63, 519-529.

Schneirla T. C.

1971 Army Ants: A study in social organization, H. T. Topoff ed., W. H. Freeman, San Francisco, 349 p.

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

Tschinkel, W. R.,
Social Control of egg laying rate in queens of the fire ant, Solenopsis invicta, Physiol. Entomol., in press.

Vander Meer R. K.
1983 Semiochemicals and the Red Imported Fire Ant (*Solenopsis invicta*) Hymenoptera: Formicidae. Fla. Entomol., 66, 139-161.
Behavioral and biochemical variation in the Fire Ant, *Solenopsis invicta*. In: Interindividual Behavioral Variability in Social Insects, R. L. Jeanne ed., Westview Press, Boulder, CO, in press.

Vander Meer R.K. and Wojcik D.P.
1982 Chemical mimicry in the myrmecophilous beetle *Myrmecaphodious excavaticollis*. Science, 218, 806-808.

Vander Meer R. K., Williams D. F. and Lofgren C. S.
1981 Hydrocarbon components of the trail pheromone of the Red Imported Fire Ant, *Solenopsis invicta*. Tetrahedron Lett., 22, 1651-1654.

Vander Meer, R. K., Alvarez, F. M. and Lofgren, C. S.
Isolation of the trail recruitment pheromone of *Solenopsis invicta*, J.Chem. Ecol., in press.

Visser J. H., Van Straten S. and Maarse H.
1979 Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. J. chem. Ecol., 5, 13-25.

Walsh J. P. and Tschinkel W. R.
1974 Brood recognition by contact pheromone in the Red Imported Fire Ant, *Solenopsis invicta*. Anim. Behav., 22, 695-704.

Watkins J. F. and Cole T. W.
1966 The attraction of army ant workers to secretions of queens. Tex. J. Sci., 18, 254-265.

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.

Whittaker R. H.
1970 The biochemical ecology of higher plants. In: Chemical Ecology, E. Sondheimer and J. B. Simeone, eds., Academic Press, New York, pp. 43-70.
Wilson E. O.
1962 Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 1. The organization of mass-foraging. 2. An information analysis of the odour trail. 3. The experimental induction of social responses. Anim. Behav., 10, 134-164.
1971 The Insect Societies. Belnap Press of Harvard University, Cambridge, MA. 548 p.