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**BROODING BEHAVIOR
OF THE CUBAN BURROWING COCKROACH
BYRSOTRIA FUMIGATA (BLABERIDAE, BLATTARIA)**

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SUMMARY

First instar nymphs of *Byrsotria fumigata* aggregate under and around their mother until the second stage. Brooding behavior is exhibited by a female parent *B. fumigata* by raising her body to allow nymphs access to the aggregation space beneath. Stimulus for aggregation is a pheromone present in and extractable from feces. Contact chemoreception was found to be the major sensory modality used by first instar nymphs in orientation to a pheromone source. No interspecific attraction to the aggregation pheromone from mandibular glands of *Blaberus craniifer* was observed.

RÉSUMÉ

**Relations « mère-jeune » chez la Blatte cubaine
Byrsotria fumigata (Blaberidae, Blattaria).**

Les nymphes du premier stade de *Byrsotria fumigata* s'agrègent sur la mère et autour d'elle jusqu'au deuxième stade. Une mère de *B. fumigata* manifeste son comportement parental en soulevant le corps pour permettre aux nymphes de s'agréger sous elle. Le stimulus responsable de l'agrégation est une phéromone qu'on peut extraire des fèces. C'est par chémoréception de contact que les nymphes du premier stade s'orientent par rapport à la phéromone. Nous n'avons pas observé d'attraction interspécifique à la phéromone d'agrégation produite par les glandes mandibulaires de *Blaberus craniifer*.

INTRODUCTION

The Cuban burrowing cockroach *Byrsotria fumigata* is an ovoviviparous blaberid that displays brood care. ROTH and WILLIS (1960) mention 15 other species of cockroaches, the majority ovoviviparous, that probably brood their young. Brood care is generally exhibited by aggregation of newly hatched offspring around their mother, the mother to some extent facilitating the nymphal aggregation by remaining immobile for varying periods of time. The duration of the relationships varies from the extremes of *Leucophaea maderae* nymphs accompanying their mother on food foraging trips (SEIN, 1923; PÉSSOA and CORRÊA, 1928) to that of *Blatella vega* nymphs, an oviparous species, remaining under their mother only until their cuticle darkens after which they disperse (ROTH and WILLIS, 1954).

These postparturient associations have not been documented previously by studies of the behavioral parameters involved or the duration of the associations. Using *Byrsotria fumigata* the following aspects were investigated: the attractiveness of a female to its progeny, the duration of the familial association, the extent of the female's participation in brooding, and the sensory modalities used by nymphs in locating their mothers, and the source of the attractant. Also investigated was the interspecific attraction of *B. fumigata* nymphs by the aggregation pheromone of a related species, *Blaberus craniifer* (BROSSUT, 1970, 1973).

MATERIALS AND METHODS

Insect Material.

Colonies of the Cuban cockroach *Byrsotria fumigata* were maintained with a 12 : 12 LD photoperiod and an average temperature of 23 °C with access to water and Purina laboratory chow. To obtain first instar nymphs, gravid females were isolated in 900 ml plastic beakers with food and water; beakers were checked daily for newly hatched nymphs. Females and their newly hatched progeny, the primary experimental animals, were kept isolated until the young had reached the third instar at which time the adult and immatures were returned to one of the colonies. In certain experiments nymphs were taken directly from the colonies, provided it was not necessary to know the exact age or female parent of the nymphs.

Behavioral assays.

Pheromone extracts of feces were prepared by homogenizing feces in solvent followed by filtration of the homogenate through Whatman Grade 1 filter paper. Tissue extracts were prepared by grinding in solvent with a homogenizer and filtering as described

above. Concentrated extracts were prepared in some instances by vacuum evaporation at room temperature using a Buchler Roto-vac apparatus. Extracts were usually tested the same day they were prepared and none was used later than 48 hours after preparation. In instances where extracts were used the next day, they were refrigerated over night at 0 °C. and then allowed to equilibrate to room temperature before testing.

For certain tests, components of the *Blaberus craniifer* aggregation pheromone reported by BROSSUT (1970, 1973), undecane, tetradecane, and ethyl hexanoate (Eastman Kodak and Matheson Scientific), were used to determine if these compounds elicited aggregation behavior in the related species *Byrsotria fumigata*.

Statistical tests used include the G-test of independence with the Yates correction and the Wilcoxon two sample test (SOKAL and ROHLF, 1969).

RESULTS

1. First instar aggregation site preferences.

Preliminary observations of *Byrsotria fumigata* nymphs and brooding mothers suggested that nymphs aggregate beneath or in the vicinity of their own mother. To test this assumption, nymphs from a single brood and other mothers were employed in a behavioral assay to determine if nymphs locate and aggregate beneath their mother when given the choice of their mother, another *B. fumigata* female, or a mimic (constructed to visually represent a female *B. fumigata*).

The assay employed a round arena 40 cm in diameter with plastic sides to confine the cockroaches. The arena was transected by lines into four sectors of equal size. The mimic or an adult female cockroach were placed in opposite sectors and were rotated between the sectors for each individual trial within a replicate. For the first set of replicates, cylinders (fitted with supports to allow free movement of nymphs), confined females (or mimics) to the centers of the opposing sectors; the nymphs were released in the center of the arena (fig. 1). After 20 minutes the number of nymphs in each of the four sectors was counted. Each replicate was carried out in a dark room, with illumination by a G. E. Ruby photography lamp (BARTH, 1964) at the time observations were made.

The results (fig. 2 a, b) indicate that 24 hour old first instar nymphs can locate and to prefer to aggregate beneath their own mother as compared to an inanimate object or another female ($p < .001$). Similar results were obtained when nymphs were given the choice between their mother and a *Leucophaea maderae* female; nymphs preferred their mother ($n = 373$, $p < .001$).

2. Duration of nymphal aggregating behavior.

As nymphs mature, their tendency to aggregate beneath or near their mother seems to decline. Figures 2 a, b, c, and d show that the tendency of newly hatched nymphs to aggregate beneath or near their mother decreased markedly

after 15 days ($n = 56$) or after molting to the second instar ($n = 19$) so that they showed no preference for their mother over an unrelated female. It is not certain why 15 days old first instar nymphs exhibited less orientation toward both their mother and an unrelated female than did second instar nymphs, but it is possible that they have reduced chemical and tactual receptor acuity.

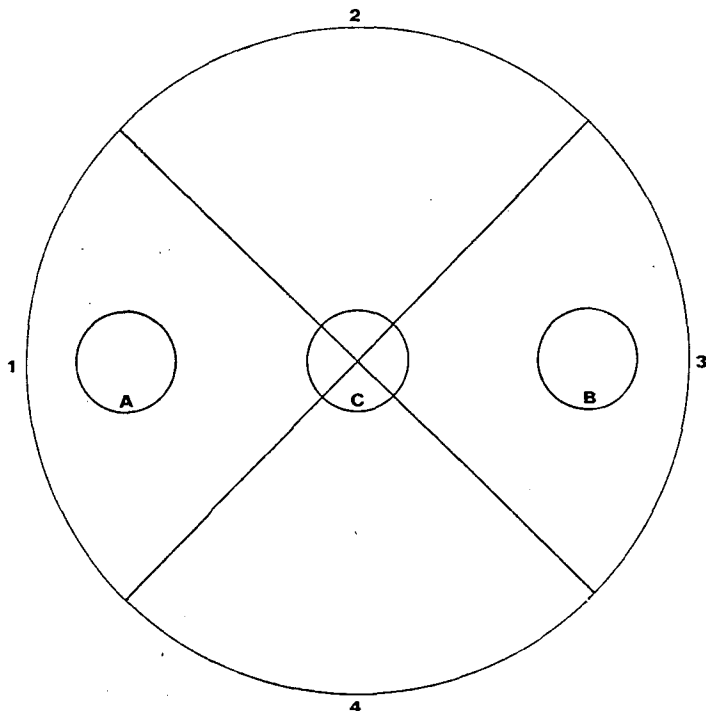


FIG. 1. — Bioassay arena 40 cm in diameter. 1, 2, 3, 4 : equal size sectors; A, B : confinement zones of adults and mimic; C : zone of nymph introduction.

FIG. 1. — L'aire d'expérimentation de 40 cm de diamètre. Les secteurs 1, 2, 3 et 4 sont égaux; A, B : zone de confinement des adultes et zone comportant les leurres; C : zone d'introduction des nymphes.

Further information was obtained on the duration of first instar nymphal attraction to their mother by observing two broods of cockroaches isolated in rectangular containers (18×28 cm). The two broods were observed periodically and counts made of the number of nymphs aggregated around their mother or elsewhere in the container. For brood number one, observations started when the nymphs were 9 days old and ceased when 27 of the 31 nymphs had molted to the second instar (21 days after hatching). For brood number two observations started when the nymphs were 18 days old and ceased when all 27 nymphs

had molted to the second instar (32 days after hatching). Observations were made at the beginning of the light period when the cockroaches are least active.

Brood number one aggregated around their mother until 14 days after hatching (Table I). At 21 days after hatching, 27 of the 31 nymphs had ecdysed

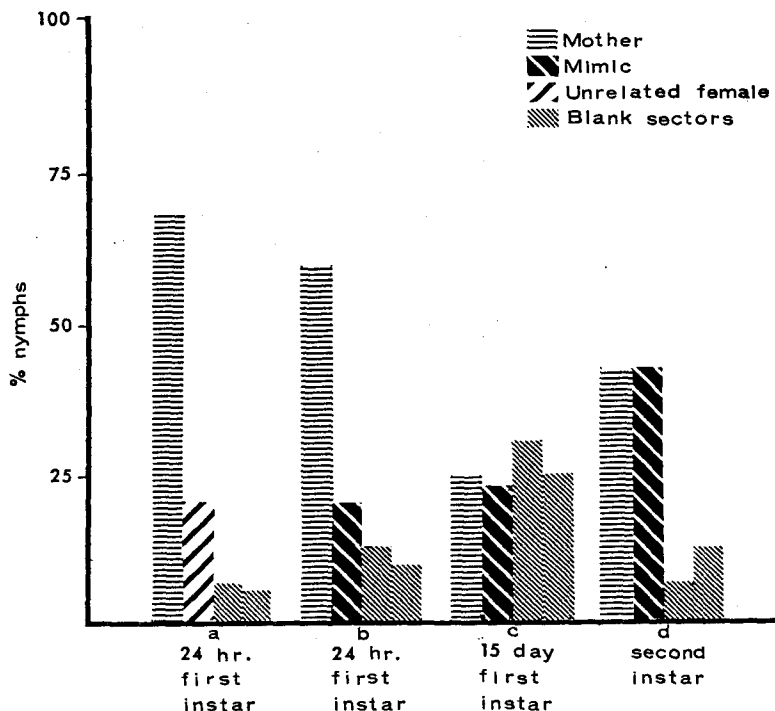


FIG. 2. — Aggregation of nymphs of *Byrsotria fumigata* at different ages. a : 24 hour old first instar nymphs (n = 73); b : 24 hour old first instar nymphs (n = 110); c : 15 days old first instar nymphs (n = 56); d : second instar nymphs (n = 19).

FIG. 2. — Agrégation de nymphes de *Byrsotria fumigata* à des âges différents. a : nymphes du 1^{er} stade âgées de 24 heures (n = 73); b : nymphes du 1^{er} stade âgées de 24 heures (n = 110); c : nymphes du 1^{er} stade âgées de 15 jours (n = 56); d : nymphes du second stade (n = 19).

to second instar and 20 of the 27 second instar, continued to aggregate at the female along with the four remaining first instar nymphs. At the beginning of observations of brood number two, three second instar nymphs were already present. As the number of first instar nymphs decreased the number of nymphs congregating around the female decreased until at 32 days of age all were found dispersed in small groups, none around the female (Table I).

TABLE I. — Duration of brooding behavior in two isolated groups of female and progeny.

TABLEAU II. — Durée des relations « mère-jeune » dans 2 groupes de femelles isolés avec leur progéniture.

	Nymph age in days	First instar		Second instar	
		% w/♀♀	% elsewhere	% w/♀♀	% elsewhere
Brood # 1 n = 31.	9	80.6	19.4	—	—
	10	80.6	19.4	—	—
	12	96.8	3.2	—	—
	13	74.2	25.8	—	—
	14	90.3	9.7	—	—
	21	12.9	0	64.5	22.6
Brood # 2 n = 27.	18	33.3	55.6	0	11.1
	19	33.3	44.4	0	22.2
	21	11.1	48.1	14.8	25.9
	22	48.1	11.1	11.1	29.6
	23	29.6	18.5	18.5	33.3
	25	0	18.5	18.5	63.0
	32	—	—	0	100.0

3. Female brooding behavior.

To probe the possibility of active brooding behavior by *B. fumigata* mothers, females were either anesthetized and bound to a piece of hardware cloth (allowing no movement) or placed in a hardware cloth cage (allowing leg movement outside the cage). When mothers were constrained to prevent all movement and placed in the arena, their progeny still preferred them over the mimic (Fig. 3 a) ($p < .001$). However, nymphs were pushed away and prevented from aggregating beneath their mother when the female restrained but allowed leg movements (Fig. 3 b) ($P < .001$). Thus, the mother does not actively aid nymphs in attempts to aggregate beneath her, except perhaps to elevate her body, but she must passively accommodate aggregation attempts.

4. Pheromone perception by nymphs.

Since nymphs locate and aggregate beneath their mother in the dark, the cues employed by nymphs must be either chemical or tactile. Perception of female odors seem to depend on contact chemoreception, since, when mothers were placed in containers 15 mm above the arena floor, and nymphs were given the choice of the elevated mother versus an elevated empty container, preferences for either container were not statistically significant ($n = 125$). The height of 15 mm prevented nymphs from contacting the female with antennae, therefore providing only possible olfactory cues.

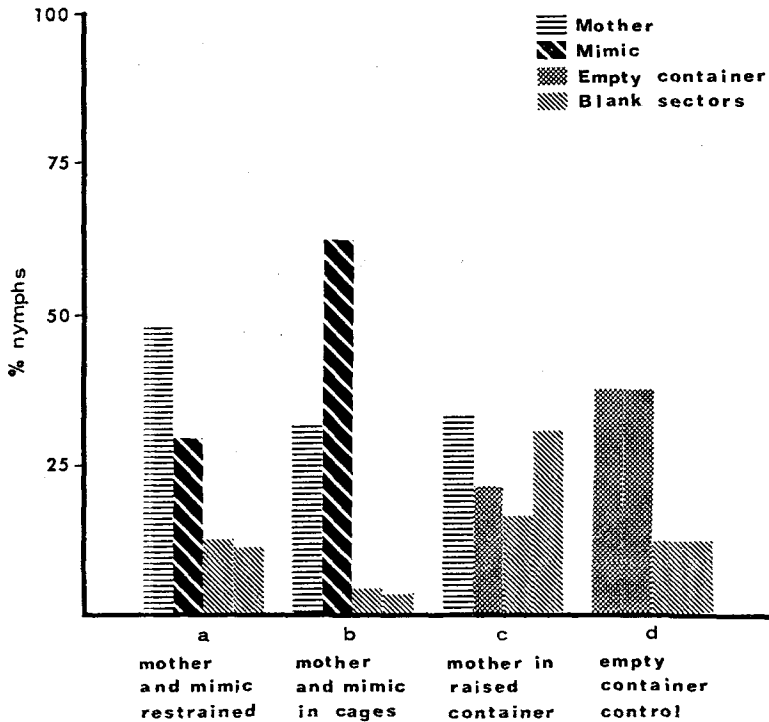


FIG. 3. — Maternal participation in aggregation of first instar nymphs of *Byrsotria fumigata*. a : mother completely restrained (n = 103); b : mother able to exhibit escape movements (n = 109); c : aggregation of nymphs to mother resulting from olfactory cues (n = 125); d : two raised empty container control (n = 16).

FIG. 3. — Rôle de la mère dans l'agrégation des nymphes du 1^{er} stade de *Byrsotria fumigata*. a : mère totalement entravée (n = 103); b : mère pouvant avoir des mouvements de fuite (n = 109); c : agrégation de nymphes à leur mère sous l'effet de facteurs olfactifs (n = 125); d : 2 groupes de contrôle élevés dans des récipients vides.

5. Determination of perception mechanisms using an olfactometer.

In a further effort to determine if nymphs are attracted to a female pheromone, a T-tube olfactometer was employed. The T-tube (15 mm I.D.) consisted of an 8 cm entry tube terminating in left and right 9 cm exit tubes. Nymphs were introduced individually from a 3 cm³ chamber attached to the entry tube. Clean air or air containing odors was introduced into one of the exit tubes at 10 cm³/sec. Since previous experiments (BELL *et al.*, 1972) indicated that *B. fumigata* nymphs were attracted to odors of feces, air passing over feces was introduced into either the left or right exit tube. After each nymph was tested, the source of

odor was changed to the opposite exit tube. The results indicated no difference between the number of nymphs moving toward the source and those moving toward clean air ($n = 74$). These results support the conclusion that olfaction is not a major modality employed by nymphs in locating their mother, but can be further defined as contact chemoreception.

6. Effect of ablation of sensory organs.

Evidence for aggregation to colony odor was obtained using the bioassay of ISHII and KUWAHARA (1967) and BELL *et al.* (1972) with three vertical folded filter papers in a 10 cm diameter arena. One paper was impregnated with a methanol feces extract, one received only methanol and the third remained untreated. The feces extract significantly attracted first instar nymphs (Table II *a*) ($n = 36$, $p < .001$). Since this assay permitted nymphs direct contact with the three filter papers in the arena, the following experiment was designed to determine which sensory organs perceived the odor. Ablation of antennae, maxillary or labial palps was performed on two groups of 18 first instar nymphs. Each group (one antennectomized, one depalped) was then tested by the (three-filter-paper) assay. Neither group of nymphs discriminated between control papers and papers impregnated with the extract (Table II *b, c*). The evidence

TABLE II. — Orientation of first instar nymphs to aggregation pheromone after ablation of sensory organs.

TABLEAU II. — Orientation de nymphes du premier stade par rapport à la phéromone d'agrégation après ablation d'organes sensoriels.

Treatment	n	Percent nymphs aggregating to pheromone after 20 min.			
		Hours post-operation			
		1 hour	2 hours	17 hours	21 hours
a. Control	36	75.0	*	*	75.0
b. (—) antennae	18	22.2	33.3	33.3	55.5
c. (—) maxillary and labial palps	15	16.6	8.3	27.2	40.0
* Not tested.					

is ambiguous because nymphs not only failed to select the extract impregnated paper, but a statistically significant number from both groups failed to select any paper ($p < .001$ for both groups). Ablation of either antennae or palps is probably too traumatic to be of any use in determining which chemosensory structures are sensitive to the aggregation odor.

7. Isolation of pheromone secretory site.

BROSSUT (1970, 1973) isolated the aggregation pheromone from the mandibular glands of a closely related species, *Blaberus craniifer*. This source seemed likely for *B. fumigata* also because feces extracts might have been contaminated with secretions of mandibular glands. However, ISHII and KUWAHARA (1968) and BLOCK and BELL (1974) found the source of aggregation pheromones of *Blattella germanica* and *Periplaneta americana*, respectively, to be rectal pads in the gut. Therefore, both mandibular and rectal tissues were tested for aggregation pheromone activity.

First, whole head extracts were tested using diethyl ether solvent (as employed by BROSSUT). Negative results were obtained when the extract was tested in the three-filter-paper bioassay. Methanol was effective as a solvent for feces extracts; therefore, head homogenates were made in methanol but the extract showed negative results when tested in the bioassay. Similar results were obtained with extracts of heads in chloroform and acetone. Next, adult *B. fumigata* were dissected, the intestines removed, and divided into three sections: Rectal-anal, ileum with malpighian tubules, ventriculus and gastric caecum. Three portions from each section were homogenized in 7 ml methanol and 0,5 ml of each homogenate was tested in the three-filter-paper bioassay. Extracts of all gut sections proved to be inactive. Finally, a cuticular wash was made of two females using 5 ml methanol as solvent. This too was found to be inactive. The aggregation pheromone is, therefore, present in the feces but has not been extracted in active form from potential secretory sites.

8. Attractiveness of *Blaberus craniifer* aggregation pheromone.

Another experiment was conducted using pure *Blaberus craniifer* aggregation pheromones (BROSSUT, 1970, 1973). The active ingredients, tetradecane and undecane, and an inactive component ethyl hexanoate were tested in a two filter paper bioassay for evaluation of attractiveness to *Byrsotria fumigata* nymphs. The chemicals were tested individually and in combination (tetradecane + undecane, 31 % : 38 %; tetradecane + ethyl hexanoate, 31 % : 17 %; undecane + ethyl hexanoate, 31 % : 17 %; tetradecane + undecane + ethyl hexanoate, 31 % : 38 % : 17 %). All chemicals, tested either singly or in combination, repelled ($n = 20$, $p < .05$ for each test) rather than attracted the nymphs.

DISCUSSION

WILSON (1970) classified the social structure of some cockroach species as subsocial or quasisocial on the basis of brood care and gregarious behavior, attributes not common to most insects. ROTH and WILLIS (1960) mention 15 cockroach species that have been observed to care for broods in laboratory colonies or in the field. SEIN (1923) and PÉSSOA and CORRÊA (1928) observed *Leucophaea maderae* nymphs accompanying their mother on night foraging trips. This is probably the ultimate in brood care or familial association in cockroaches, with the most common behaviour being aggregation of nymphs under and around their mother only until their cuticle hardens after which the nymphs disperse. Newly hatched *Nauphoeta cinerea* nymphs crawl beneath the female's body and wings (WILLIS *et al.*, 1958), but the association lasts less than a day (EWING, 1973).

Gregarious behaviour of nymphs is at least partly attributable to aggregation to a feces pheromone (BELL *et al.*, 1972). The present study failed to elucidate the origin of the pheromone, which seems not to be the gut or head structures previously shown to supply aggregation in other species. This study did, however, show that females secrete distinct pheromones which are perceived by nymphs through contact chemoreception and lead to aggregation of nymphs beneath their own mother. Individual variation among pheromones may account for individual recognition of mothers by nymphs, or perhaps two pheromones are involved, one a general aggregation pheromone and the other a pheromone which differs among females.

A female assumes a passive but facilitating role in brooding situations by raising her body, allowing the progeny to move underneath; she remains in the raised position for an hour or longer if undisturbed. Similar behavior has been observed in other ovoviviparous cockroaches (ROTH and WILLIS, 1960). However if the female is allowed escape movements, but otherwise constrained, nymphs do not aggregate under their female parent, apparently being pushed away by the moving legs.

CHOPARD (1938) suggested that gregarious behaviour in Orthoptera varies in intensity according to the age or physiological state of the insect. This was found to be true of *B. fumigata* with the mother-seeking behavior of first instar nymphs decreasing as they approach the second instar. Second instar nymphs aggregate under their mother and another female *B. fumigata* in equal numbers.

The vulnerability of nymphs at birth to predation and harmful elements of the environment is obvious. Brooding behavior would seem to be a favorable adaptation because the female, having selected a place of safety, would shelter and hide the nymphs. Why, however, is this adaptation lacking in the behavior of oviparous cockroaches, which select an oviposition site and abandon the oötheca and thus the nymphs? Oviparous cockroaches reproduce more rapidly,

producing more offspring per unit time than ovoviviparous cockroaches (WILLIS *et al.*, 1958). The advantage for brooding cockroaches would be a lower mortality rate among the nymphs, thus balancing the advantages of a high reproductive rate. Paucity of ecological data and observations of *B. fumigata* in nature, however, preclude definitive analysis of the ecology of brood care.

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