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LARVAL DEVELOPMENT DURING THE NOMADIC PHASE OF A NEARCTIC ARMY ANT, *NEIVAMYRMEX NIGRESCENS* (CRESSON) (HYMENOPTERA: FORMICIDAE)

YVONNE J. WANG* and GEORGE M. HAPP†

Department of Biology, New York University, Bronx, N.Y. 10453, U.S.A.

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Abstract—A histological and histochemical study of larval development during the nomadic phase shows that the integument, tracheal system, alimentary canal, labial gland and the fat body were rich in nucleic acids, protein, glycogen, phospholipid, esterase and phosphatases. Pronounced changes in the labial gland suggest an important function of the gland during the nomadic phase as a source of stimulating substances; the tracheal system and the hindgut are other possible sources of stimulatory substances. It is concluded that results from this study are consistent with Schneirla's brood stimulation hypothesis.

Index descriptors (in addition to those in title): Pheromones, exocrine glands, histochemistry.

INTRODUCTION

THE ACTIVITY of a colony of social insects is the sum of countless individual interactions, many of which involve exchange of chemical substances. Some of these substances may be nutritive (as in regurgitative feeding of ants) while others may be specific chemical messengers or pheromones (Karlson and Butenandt, 1959). Numerous pheromones have been found in bees, ants, and termites, and very often the secretions are produced by distinct exocrine glands (Wilson, 1971).

The significance of stimulative relationships between adult social insects and their broods has been reviewed by Brian (1957, 1962) and Wilson (1965) for various ants, and by Schneirla (1938, 1946, 1957a, 1957b) for doryline ants specifically. Schneirla's extensive studies (1933–1968) on the behavior of several genera of army ants (principally *Eciton*, *Neivamyrmex*, *Aenictus*) have concentrated particularly upon analysis of the differences between the nomadic and statary phases of colonial activity. The nomadic phase is a period of high colonial activity in which successive daily raids typically end in emigration to new sites. This is followed by a statary phase when the workers of the colony are less active in raiding, and no emigration occurs. Durations of about 18 days in *Neivamyrmex* are characteristic for each of the nomadic and statary phases. Schneirla concluded that the maintenance of a high level of nomadic function depends upon summated stimulative events from the brood,

* Present address: Ophthalmology Department, George Washington University, Washington, D.C. 20037, U.S.A.

† Present address: Department of Zoology and Entomology, Colorado State University, Fort Collins, Colorado 80521, U.S.A.

and not a hypothetical "need for food" by the worker ants. On the basis of behavioral data (Schneirla, 1934, 1958, 1965, 1968; Schneirla and Reyes, 1966) Schneirla formulated the "brood stimulation hypothesis", which states that secretions produced by the larval brood are important regulators of colonial activity.

If the brood stimulation hypothesis is correct, one would expect that the onset and duration of the nomadic phase should have distinct morphological correlates in the exocrine glands of the larvae. Some histological information is available for the larvae of *Eciton* which supports this suggestion; Lappano (1958) found that the labial glands of *Eciton* increased significantly in size throughout the nomadic phase. However, since the labial glands of *Eciton* also produce silk for the pupal cocoon, their growth might merely reflect preparation for pupation and have nothing to do with brood stimulation.

It is the purpose of this paper to chronicle larval development in another army ant, *Neivamyrmex nigrescens*. In contrast to *Eciton*, there is no pupal cocoon. The labial glands, the integument, tracheal system, fat body and alimentary canal will be described at various points in the nomadic phase. By histochemical techniques, we will attempt to define precisely the functional activities of those tissues undergoing morphological changes; and finally, we will consider whether the morphological and histochemical evidence is consistent with Schneirla's brood stimulation hypothesis.

MATERIALS AND METHODS

Materials

Young larvae of *Neivamyrmex* were supplied by Drs. Schneirla and Topoff who studied the behavior of these ants and collected the young broods in the vicinity of Portal, Arizona. Larvae from the last statary day and nomadic days, 1, 3, 6, 9, 11, 14, 17, 18 were used. For all morphological and histochemical studies, larvae were pricked with sharp needles in the anteroventral region to facilitate penetration of fixatives.

Morphology

For general histology, whole larvae were fixed in Carnoy (6:3:1) and embedded in paraffin wax. Serial sections, cut at 5μ, were stained with Heidenhain's iron hematoxylin or paraldehyde fuchsin (Gomori, 1950a). For measurements of the volume of the reservoir of the labial gland, camera lucida tracings were made from 5μ serial sections and total volume was estimated by cutting out the tracings of each reservoir and weighing them.

Histochemistry

Larvae were fixed in cold (0–4°C) formol-calcium (Baker, 1944), embedded in gelatin (Pearse, 1960) and sections were cut on a cryostat microtome at −18°C. In other studies for carbohydrates, nucleic acids and general protein, 5μ paraffin sections of material fixed in Carnoy were used.

Lipids. Frozen sections were flooded with Oil red O in isopropanol (Lillie, 1954) or with Sudan black B in ethylene glycol (Chiffelle and Putt, 1951). Some larvae were chromed according to Baker's acid hematein (Baker, 1946). As a control, lipids were extracted with acetone or with pyridine (Baker, 1946).

Carbohydrates. For glycogen, paraffin sections were stained with the periodic acid-Schiff method (McManus, 1946). Control sections included the omission of the oxidation step or

pretreatment with diastase. For acid mucopolysaccharides, the alcian blue method (Steedman, 1950) was used on paraffin sections.

Nucleic acids. Methyl green-pyronin (Kurnick, 1955) was used for DNA and RNA staining. Ribonuclease-treated sections were used as RNA controls.

General protein. Paraffin 5μ serial sections were stained with mercuric-bromphenol blue (Bonhag, 1955).

Carboxylic esterases. Frozen sections were dried onto slides and treated with naphthol AS-D acetate at pH 7·1 for a 1–6 hr incubation at 25°C (Burstone, 1962); liberated naphthol was simultaneously coupled with Garnet GBC or Fast blue RR. Another substrate used was 5-bromo-indoxyl acetate (Holt, 1958) incubated in 0·2 M Tris buffer, pH 7·1 at 37°C. Control procedures involved either the omission of substrate and/or pretreatment with inhibitors [(NaF (10^{-2}M) or silver nitrate (10^{-2}M)].

Phosphatases. Frozen sections were incubated, after air-drying on the slides with the phosphates of naphthol AS-BI for acid phosphatase at pH 5·0, and of naphthol AS-MX for alkaline phosphatase at pH 8·3; upon hydrolysis the naphthols were simultaneously coupled with Red Violet LB (Burstone, 1962) and incubated 1–6 hr at 37°C. The Gomori metal salt method (Gomori, 1950b, 1952) with glycerophosphate as substrate was also used both for acid and alkaline phosphatases at pH 5·0 and pH 9·0 respectively. Incubations were carried out at 37°C for 1–14 hr. In all cases, control slides were used with inhibitor NaF (10^{-2}M) or with the omission of the substrate.

OBSERVATIONS

General development of the larvae

The larvae examined from the last statary day were still in the embryonic stage; there are no clearly differentiated internal tissues but protein granules are visible (Fig. 1). From the first day of the nomadic phase (designated ND 1) through the end of this phase (ND 17 or 18, depending on the colony), the integument, tracheal system, alimentary canal, labial gland, fat body and muscle are easily demonstrated in fixed material. By ND 17 or 18, larvae developed into the prepupal stage (Table 1).

TABLE. 1. BODY LENGTH OF LARVAE DURING NOMADIC PHASE

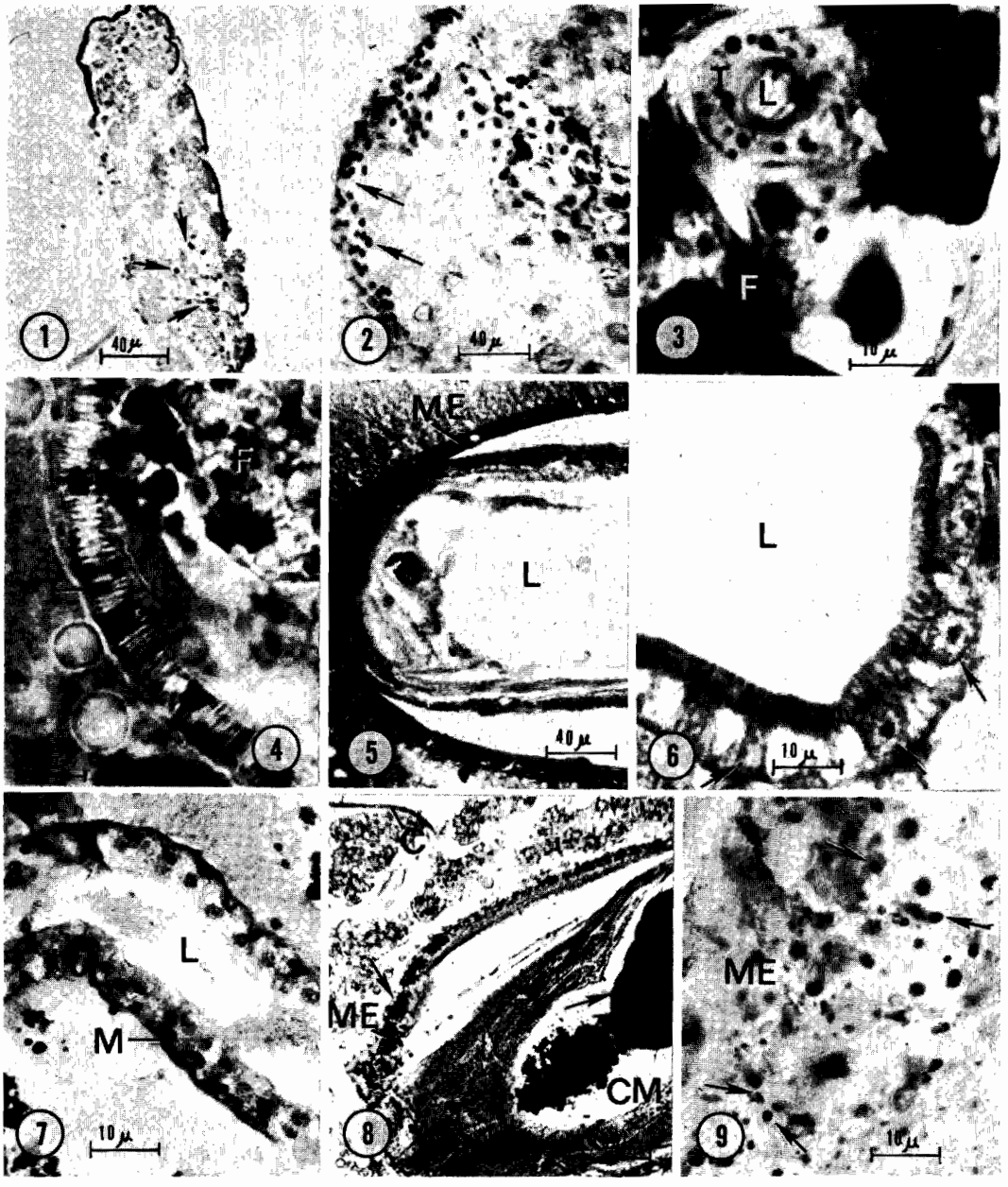
Developmental stage	Body length (mm)*
ND 1	0·78 ± 0·10
ND 3	1·49 ± 0·05
ND 6	2·54 ± 0·32
ND 9	3·13 ± 0·03
ND 14	3·42 ± 0·46
ND 18	4·28 ± 0·05

* Average measurements of five larvae for each stage of development.

Integument

Through the nomadic phase, the epidermal cells of the larvae are stained by acid hematein, bromphenol blue, methyl green-pyronin and PAS, thus indicating the presence of phospholipid, protein, nucleic acids and carbohydrates, respectively. Non-specific carboxylic esterases are demonstrated by naphthol and indoxyl methods throughout most of

the nomadic days. The cuticle is PAS-negative, but stains with bromphenol blue. No esterases are found in the epidermis during ND 18; however at this stage acid phosphatases can be demonstrated by naphthol AS-BI and Gomori methods. This may suggest the autolysis of certain epidermal cells prior to pupation (Fig. 2).



FIGS. 1–9.

Tracheal system

The major tracheal trunks, found within each segment, run from the body wall, and are connected to the well-developed tracheal system of the larvae. The secondary and tertiary tracheal trunks are intimately associated with the fat body. Each tracheal trunk is composed of a cuticular intima and cuboidal cells beneath it; acidophilic substances are found in the lumen of the trunk. The results show that the cells of the tracheal trunk are highly basophilic. Lipid droplets containing both neutral fat (Oil red O) and phospholipid (acid hematein) are present throughout most of the nomadic phase (Figs. 3 and 4) except for the last nomadic day (ND 18). Esterase (naphthol AS-D and indoxyl), acid phosphatase (naphthol AS-BI) and alkaline phosphatase (naphthol AS-MX) are very abundant in these cells. The presence of lipids, esterases, and phosphatases throughout the nomadic phase may be related to secretory activity of these cells. The product could be discharging to the larval surface via the spiracles.

Alimentary canal

The alimentary canals of the larvae consist of foregut, midgut and hindgut. Throughout almost all of the nomadic phase, the midgut is very prominent (Fig. 5). Its epithelium consists of cells with large oval nuclei, granular cytoplasm and a distinct brush border. Over the first 16 days of the nomadic phase the basophilia of the epithelial cells increases. Within the tunica are acidophilic substances and many layers of peritrophic membrane.

The lumen of the midgut is not continuous with that of the hindgut during most of the nomadic phase. The highly basophilic columnar epithelial cells of the hindgut and the many vacuoles present may suggest that this structure is a possible source of exocrine secretions during the nomadic phase (Fig. 6). At ND 17, the midgut morphology is strikingly modified (Fig. 7); it becomes continuous with the hindgut and its contents are apparently evacuated. Peritrophic membranes are no longer visible, and the sac of the midgut is greatly diminished in size. The conspicuous wavy membrane of the midgut epithelium suggests its contraction during this last nomadic day.

FIG. 1. Larva from last statary day. Dark granules are probably protein (arrows). Mercuric-bromphenol blue stained, paraffin section.

FIG. 2. ND 18 larva, showing positive acid phosphatase staining in epidermal cells (arrows). Gomori, gelatin, frozen section.

FIG. 3. ND 6 larva, showing fat droplets in tracheal trunk (T) and near lumen (L). Fat body (F). Sudan black B stained, gelatin, frozen section.

FIG. 4. ND 9 larva, showing positive fat staining in tracheal system (T). Fat body (F). Sudan black B stained, gelatin, frozen section.

FIG. 5. ND 9 larva, showing prominent rounded midgut sac. Multilayer peritrophic membrane can be seen inside midgut. Midgut epithelium (ME) Lumen (L). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 6. ND 14 larva, showing columnar epithelial cells (arrows) of hindgut. Lumen (L). Mercuric-bromphenol blue stained, paraffin section.

FIG. 7. ND 17 larva, showing strikingly modified contracted midgut (M) with relatively narrow lumen (L). Fat body (F). Heidenhain's iron haematoxylin stained, paraffin section.

FIG. 8. ND 9 larva, indoxyl acetate test for carboxylic esterase. Activity is highest in midgut epithelium (ME) and in center of the midgut (CM). Some activity is also present in peritrophic membrane. Fat body (F). Formol-calcium, gelatin frozen section.

FIG. 9. ND 14 larva, naphthol AS-BI phosphatase (pH 5·0). Positive "acid phosphatase" (arrows) reaction in midgut epithelium (ME) is resistant to inhibition of sodium fluoride. Formol-calcium, gelatin, coupled with Red Violet L.B.

In the early nomadic days neutral fats (Oil red O) are present in the midgut epithelium and in the lumen of the gut. From ND 9 onward the concentration of neutral fat in the midgut region is progressively reduced. By ND 17, only very faint traces of neutral fat are found. The results of Sudan black B stain also demonstrate abundant deposits of lipid in the alimentary canal throughout most of the nomadic phase, but at ND 17 or ND 18 the intensity of staining declines. The phospholipid detected by Baker's acid hematein test in the midgut epithelium is extractable with pyridine. Esterases (indoxyl and naphthol AS-D), and acid phosphatases (naphthol AS-MX and Gomori) are very abundant in the alimentary canal system, especially in the midgut region where they are present not only in the huge epithelial cells but also inside the gut (Fig. 8). The esterases are inhibited by silver nitrate (10^{-2}M). The "acid phosphatase" is resistant to sodium fluoride (10^{-2}M) (Fig. 9). During the last nomadic day (ND 17), esterases are greatly reduced in the midgut epithelium and scarcely seen inside the gut. The alkaline phosphatase in the midgut epithelium also declines but its activity is still high in the lumen.

Although the lumen of the midgut is PAS-negative the cells are very rich in glycogen (Fig. 11) but by ND 17 or ND 18 PAS-positive material is greatly reduced. The many layers of peritrophic membranes, which are present inside the midgut, appear to be acid mucopolysaccharide in nature since they are stained by alcian blue.

The labial gland

Larvae of *Neivamyrmex nigrescens* possess a pair of labial glands. Each gland consists of a secretory portion, reservoir, labial duct, and common efferent duct. The secretory portion extends from about the fifth abdominal segment forward to the prothorax where it empties into a reservoir. From the reservoir, a labial duct runs to the common efferent duct through which both glands convey their products to the labium (Figs. 10 and 12). The

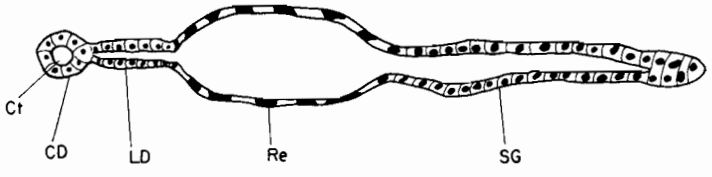


FIG. 10. Labial gland of *Neivamyrmex* larva. Cuticular intima (Ct), common duct (CD), labial duct (LD), reservoir (Re), secretory portion of labial gland (SG).

common duct and the labial duct of each labial gland are composed of cuticular intima surrounded by cuboidal cells (Fig. 13). Throughout the nomadic phase, the duct cells show no changes in cytology. However, the labial duct itself increases in size from 6μ in diameter at ND 1 to its greatest diameter 27μ at ND 11. When the larvae reach ND 14, the lumen size decreases, and by ND 18 the lumina of both the labial and common ducts are collapsed (Figs. 14 and 15). The reservoir of the labial gland consists of a thin cuticular intima around which are squamous epithelial cells. This reservoir is first seen at ND 3. By ND 6, it is greatly enlarged, and is filled with acidophilic substances (Fig. 16). At ND 9, 11 and 14 this reservoir is smaller (Fig. 17) presumably due to the release of secretory product from the labial gland. By ND 17 or 18, the reservoir is collapsed (Fig. 18).

The nuclei of the secretory cells of the labial gland grow in size, from 7μ in diameter at ND 1 to 16μ at ND 6. By ND 9 onward, nuclei show a significant change; the chromosomes

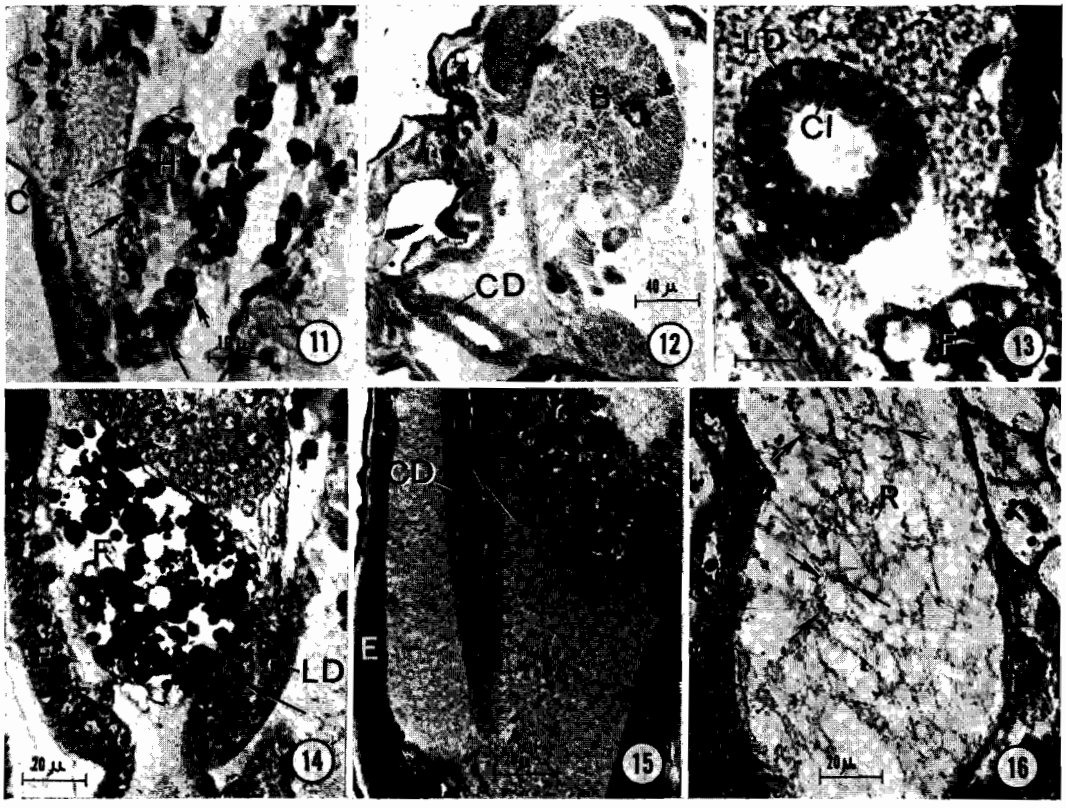


FIG. 11. ND 1 larva, PAS-positive staining (arrows) showing glycogen in hindgut (H). The reaction is prevented by diastase treatment. Cuticle (C). Periodic acid-Schiff stained, paraffin section.

FIG. 12. ND 9 larva, showing anterior part of larva, brain (B), common efferent duct (CD) opening to labium (white arrow). Heidenhain's iron haematoxylin stained, paraffin section.

FIG. 13. ND 6 larva, cross section of labial duct (LD) of labial gland showing cuboidal cells and cuticular intima (CI). Fat body (F). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 14. ND 18 larva, showing labial duct (LD) in last nomadic day with lumen (arrow) collapsed. Epidermis (E), fat body (F). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 15. ND 18 larva, showing common duct (CD) in last nomadic day with closed lumen (arrow). Epidermis (E). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 16. ND 6 larva, reservoir (R) of labial gland is filled with acidophilic substances (arrows). Note squamous cell of reservoir (SR) at this stage. Fat body (F). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

become enlarged and separated from one another, suggesting that endomitosis (Grosch, 1950) has occurred and that, in fact, these chromosomes are polyploid (Fig. 19). Associated with the nuclear changes is the appearance of many basophilic granules and vacuoles in the cytoplasm (Fig. 20) which disappear at ND 17 (Fig. 21).

Secretory cells of the labial glands are Oil-red-O-negative throughout the nomadic phase. However, lipid is present; following chromation, both Sudan black B and acid hematein methods reveal lipid granules (Fig. 22). In contrast, the gland cells are rich in glycogen,

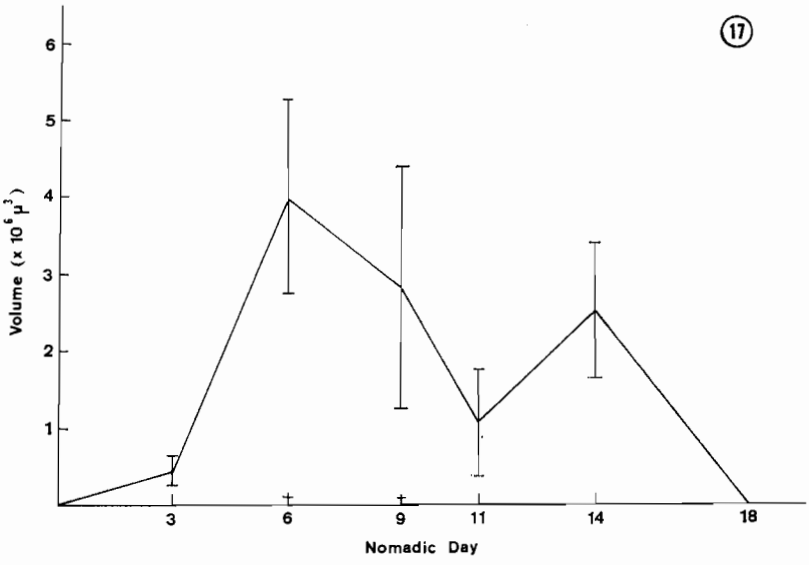


FIG. 17. Changes of actual volume of the reservoir of labial gland of larvae during nomadic phase.

protein, DNA and RNA. During ND 9, 11 and 14, methyl green-pyronin staining showed RNA in the cytoplasm of the secretory lobe (Fig. 23) and the positive pyronin staining can be prevented by digestion with ribonuclease (Fig. 24). At ND 18 cytoplasmic RNA staining is greatly decreased. Esterases are generally present. These can be seen by the indoxyl or naphthol AS-D method (Figs. 25 and 26). Acid phosphatases, which are resistant to sodium fluoride, are present at ND 14 (Fig. 27) and the reaction is more intense at ND 18 (Fig. 28). Thus from the ND 1 through ND 14, the cells show increasing signs of secretory activity and the reservoir of the gland contains product; and at ND 17 or 18, secretory activity has ceased.

Fat body

Early in the nomadic phase, fat cells become organized into segmental clusters, and over most of the nomadic phase, the fat body grows in volume. A major aspect of its growth is the abundance of the basophilic globules (Fig. 29) and of droplets of neutral fat which are seen to be arranged around the periphery of the fat cells (Fig. 30). The basophilic globules increase in number and in size until the end of the nomadic phase, when many appear to be freed and pass into the heamolymph. Until ND 14, the globules contain PAS-reactive material which is diastase-resistant (Fig. 31). At this stage, the globules also stain with acid hematein, suggesting that they are heterogeneous, containing lipid, carbohydrate and perhaps protein.

The results of both azo-dye and indoxyl methods show a marked increase of non-specific esterases in the fat body from ND 6 to ND 14; they are localized between globules and fat droplets (Fig. 32). Alkaline phosphatases are present through the first 14 days of the nomadic phase (Fig. 33) while acid phosphatases are also present through ND 14. At ND 17 or 18 acid phosphatases appear to be absent according to the AS-BI method; but, Gomori's metal salt method still shows some activity. This lack of agreement between the 2 techniques

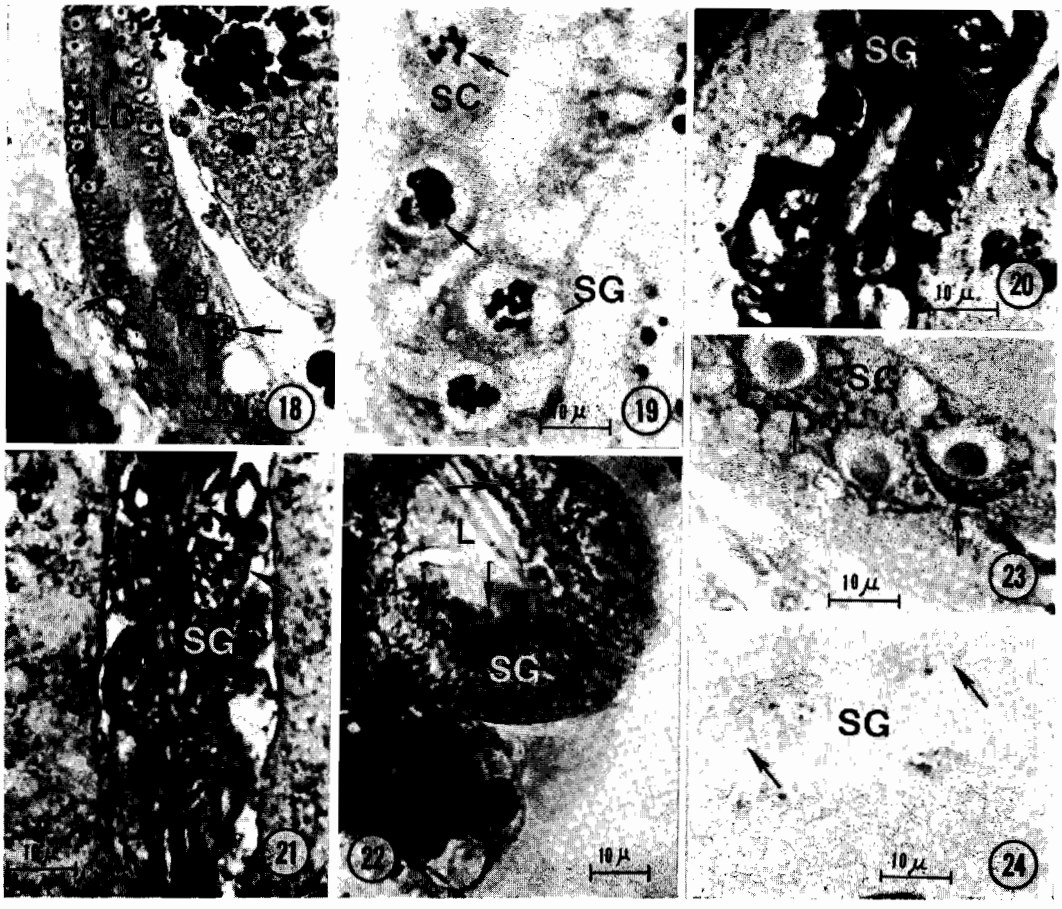


FIG. 18. ND 17 larva, reservoir (R) part of labial gland at this stage is collapsed. Note that cells of reservoir at this stage become cuboidal (arrows). Labial duct (LD). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 19. ND 9 larva, secretory portion of labial gland (SG) showing presumed endopolyploidy in nuclei (arrows) of the secretory cells (SC). Heidenhain's iron haematoxylin stained, paraffin section.

FIG. 20. ND 9 larva, showing secretory portion of labial gland (SG) with highly basophilic cytoplasm (white arrows). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

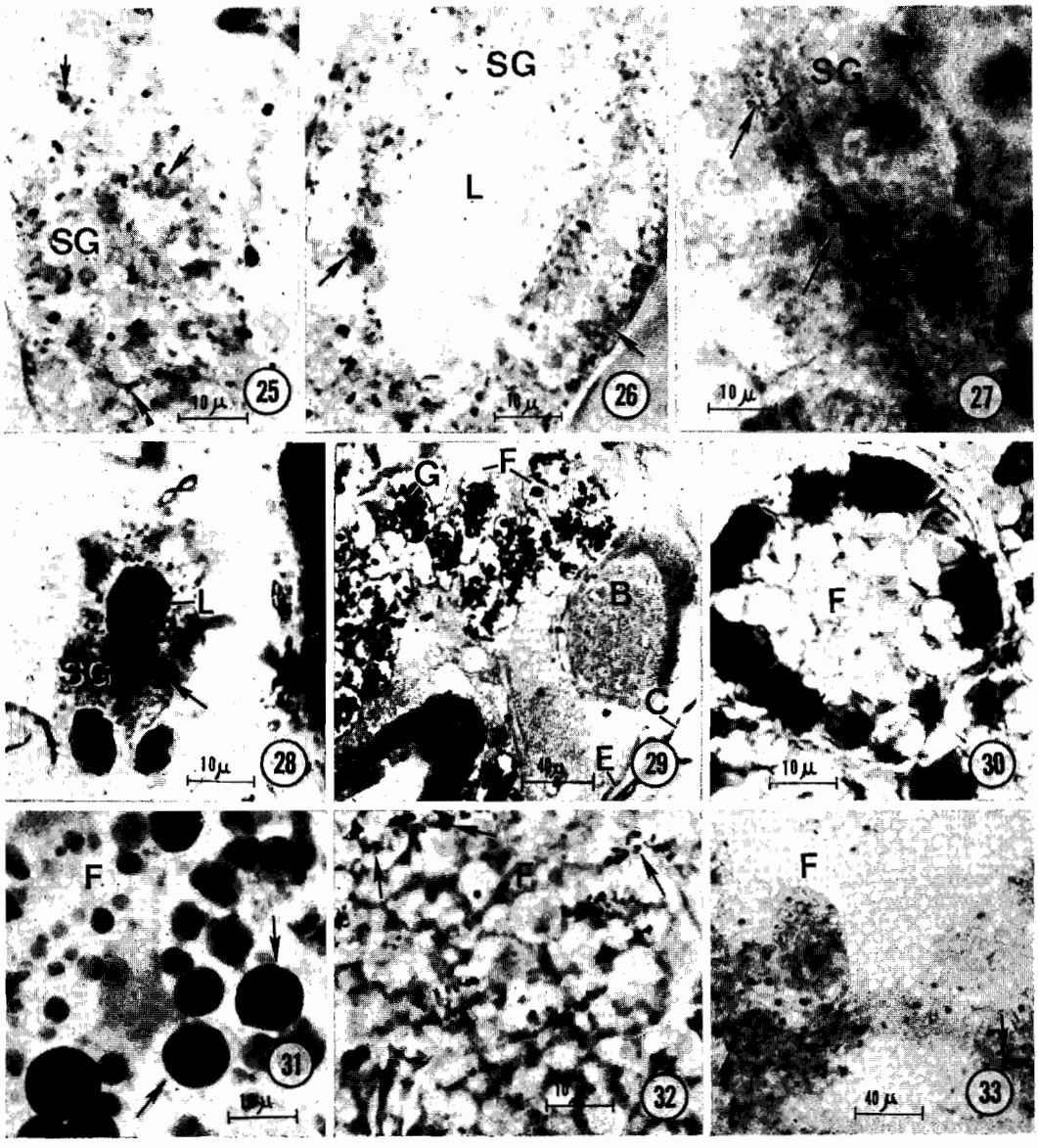
FIG. 21. ND 17 larva, showing secretory portion of labial gland (SG) at last developmental stage, with markedly decreased basophilic substances in cytoplasm (arrows). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 22. ND 14 larva, acid hematein test showing deposits of lipids throughout secretory part of the labial gland (SG), some lipid granules (arrows) are present near lumen (L). Formol-calcium, chromed, gelatin frozen section.

FIG. 23. ND 9 larva, methyl green-pyronin staining showing RNA (arrows) in cytoplasm of secretory portion of labial gland (SG). Methyl green-pyronin (Kurnik) stained, paraffin section.

FIG. 24. ND 9 larva, methyl green-pyronin staining in secretory portion of labial gland (SG) (see Fig. 23), ribonuclease control (arrows). Paraffin section.

may indicate that the lead precipitate from the Gomori's incubation at this stage is an artifact. Furthermore, the acid phosphatase activity, at all stages, with either substrate, proves insensitive to sodium fluoride. This finding suggests that the enzyme or enzymes responsible are not merely simple lysosomal acid phosphatases.



FIGS. 25–33.

DISCUSSION

Development of Neivamyrmex *larvae during the nomadic phase*

The development of *Neivamyrmex* larvae during the nomadic phase can be divided into 3 stages:

(1) First or early stage of development—ND 0–6.
(2) Second stage of development—ND 7–16.
(3) Third or last stage of development—ND 17 and 18.

All the tissues studied changed significantly during these 3 stages of development. The first stage consists of a period of general differentiation: the integument, tracheal system, alimentary canal including the labial glands, fat body and the larval musculature develop. In this stage the tracheal system may contain some secretory products. In stage 2, the metabolic and secretory activities of the larvae increase greatly and seem to reach the peak of their development. All the tissues studied showed increases in functional activity. Although no dermal glands have been seen in larvae of *N. nigrescens*, the present study of the histochemical characteristics of the epidermis shows that there is synthetic activity throughout the nomadic phase, and thus the whole layer of epidermal cells may function in the secretion of behaviorally active products as well as in the periodic molts. Especially of interest as sources of secretion are the labial glands, the tracheal system and the hindgut. Characteristic secretory structures have been found in the tracheal systems leading to the spiracles of nymphs and adults of other insects (Roth and Stay, 1958). The labial glands, which are highly active are similar to those found in *Eciton* (Lappano, 1958). The histological findings of endopolyploidy (Painter, 1945; Grosch, 1950), the highly basophilic vacuolized cytoplasm, and the histochemical data indicate that the glands are engaged in active secretion. By stage 3 of development, most of the tissues appeared to be less active. This last stage of development is characterized by the degeneration of the labial gland and the contraction of the alimentary canal.

FIG. 25. ND 9 larva, indoxyl acetate test for carboxylic esterase showing reactions (arrows) in cytoplasm of the secretory portion of labial gland (SG). Formol-calcium, gelatin frozen section.

FIG. 26. ND 14 larva, naphthol AS-D test for carboxylic esterase showing reactions (arrows) in cytoplasm and near lumen (L) of secretory portion of labial gland (SG). Formol-calcium, gelatin frozen section, coupled with Fast blue BB.

FIG. 27. ND 14 larva, naphthol AS-BI test for acid phosphatase at pH 5·0 in secretory portion of labial gland (SG), positive activity (arrows) is not inhibited by NaF. Formol-calcium, gelatin frozen section, coupled with Red Violet LB.

FIG. 28. ND 18 larva, Gomori test for acid phosphatase at pH 5·0, intense activities are seen in cytoplasm of secretory cells (arrows) and in lumen (L) of secretory portion of labial gland (SG). Formol-calcium, gelatin frozen section.

FIG. 29. ND 9 larva, showing anterior part of the larva, well developed fat body (F) with numerous basophilic globules (G) in fat body. Cuticle (C), epidermis (E), foregut (Fg), brain (B). Paraldehyde fuchsin/haematoxylin stained, paraffin section.

FIG. 30. ND 14 larva, Sudan black B test showing fat globules arranged in periphery of a cell of fat body (F). Formol-calcium, gelatin frozen section.

FIG. 31. ND 18 larva, periodic acid-Schiff positive globules in fat body (F) of the larva. At this stage of development, globules (arrows) are resistant to diastase treatment. Periodic acid-Schiff test with diastase as control. Paraffin section.

FIG. 32. ND 9 larva, indoxyl acetate test for carboxylic esterase in fat body (F). Activity is localized between fat droplets and globules. Formol-calcium, gelatin frozen section.

FIG. 33. ND 14 larva, naphthol AS-MX alkaline phosphatase (pH 8·3); activity (arrows) is localized between fat droplets and globules in fat body (F). Formol-calcium, gelatin frozen section, coupled with Red Violet LB.

Comparison of Neivamyrmex *with other social Hymenoptera*

In general, *Neivamyrmex* larvae have longer developmental stages than *Eciton* or *Apis*. The longer and more active functional activity of the midgut may reflect the differences in social organization of these genera. Unlike *Apis*, *Formica* and *Eciton*, the labial glands of *Neivamyrmex* produce no silk for a cocoon. The prolonged activity of these glands throughout the nomadic phase of the functional cycle may be correlated with stimulation of the workers.

The development of the "albuminoid granules" in the fat body of larval Hymenoptera has been described previously but no general pattern seems to emerge from the data available at present (Schneider, 1928). In *Eciton*, there are no basophilic granules even at ND 12 which is the day preceding pupation. In *Neivamyrex*, *Formica* and *Apis*, albuminoid globules are present. The globules of *Neivamyrmex* (basophilic) and *Formica* (acidophilic) (Oertell, 1930), appear in the first half of larval life and persist throughout, whereas those of *Apis* (basophilic) (Bishop, 1922) appear only after pupation. These abundant "albuminoid granules" may function in nutrient storage or may play other roles.

Neivamyrmex *larvae as a source of social regulatory substances*

The function of exocrine glands in social insects as sources of chemical regulatory signals has been demonstrated by many workers (see Wilson, 1971 for references): the "queen substances" of the honey bee are produced by the mandibular glands; the Dufour's gland secretion of the fire ant, *Solenopsis saevissima* functions in trail laying; and the mandibular gland is the source of an alarm releaser for *Pogonomyrmex badius*.

Schneirla and his co-workers have suggested that larval secretions are responsible for the heightened activity of worker *Neivamyrmex* during the nomadic phase (Schneirla, 1958). In behavioral studies, Schneirla reported that, during the nomadic phase, workers constantly licked and manipulated the larvae. Topoff (1968) also has shown that workers removed from nomadic colonies have a higher rate of oxygen uptake than workers taken from statary colonies. Thus, heightened activity is reflected in both behavior and physiology. On morphological and histochemical grounds, the present study suggests several possible sources of stimulatory substances in larval *Neivamyrmex*. These are the labial gland, the tracheal system, the hindgut and perhaps the epidermal cells of the integument. The development and histochemical characteristics of the labial gland in the nomadic phase are consistent with a major role as a regulator of colonial activities. The early drop in worker activity during the first few days in the nomadic phase (Schneirla, 1958) correlates well with the early stage (ND 0 – ND 6), when the reservoir increases to its maximum size but little secretion seems to be expelled. It is after this stage that the reservoir apparently releases its product while secretory cells remain active (ND 7–16). This second stage (ND 7–16) of the labial gland is correlated with the heightened activity of the worker (Schneirla, 1958). The volume of the reservoir of the labial gland drops to almost zero at the last stage (ND 17 and 18) as worker activity declines just before the statary phase. At this point, degeneration of the labial gland occurs, since the labial gland is not involved in silk production.

In studying the sources of larval salivary gland secretion in the dipteran *Chironomus* (Doyle and Laufer, 1969) it was concluded that proteins are synthesized originally in larval tissues other than the salivary gland and are then released into the haemolymph, from which the salivary gland sequesters and subsequently secretes them. In *Neivamyrmex*, the labial gland may function in a similar manner: one or more components of its secretory product may be derived from the haemolymph. In fact, they may be of quite large molecular

weight and could be produced by such tissues as fat body. The stimulatory substance itself could be such a large molecule. It is unusual for a large molecule (e.g. protein) to serve as a pheromone. However, in this situation, where transmission is by contact and/or ingestion, a pheromone need be neither volatile nor non-polar; rather it need only be soluble and interact with a specific receptor surface. For example, water-soluble substances which release an alarm reaction have been reported in snails (Snyder, 1967) and other organisms (Wilson, 1970).

The tracheal system of *Neivamyrmex* may be another source of stimulatory substances: since this system starts functioning in the early stages of development, and its cells appear to be secretory; it could produce "surface pheromones" which are connected with the early activities in the nomadic phase of the workers. The alimentary system may be yet another possible source of stimulatory substances. Either the midgut (by regurgitation) or hindgut could produce such molecules.

In the third stage of larval development, not only do the morphological and histochemical data indicate a decline in the secretory activities of the midgut and hindgut, but also there is less "food" (primarily lipid) in the midgut.

In balance, how do these observations bear on the "brood stimulation hypothesis" of Schneirla? Certainly, several secretory systems, which could be the sources of excitatory pheromones, have been described, and furthermore the secretory cycles parallel the waxing and waning of the colonial activity during the nomadic stage. It is tempting to invoke specific glandular systems, such as labial gland, as direct regulators of colonial activity and thus the functional cycle. Yet without isolation of secretory products and experimental analysis of the effects on worker behavior, the data obtained in this study can best be regarded as suggestive of possible sources for chemical stimulation by the brood as predicted by Schneirla's hypothesis.

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