

8-23-1986

The Biomineralization in Social Wasps (Vespinae): The Presence of Statoliths

J. S. Ishay
Tel Aviv University

T. Shimony
Tel Aviv University

L. Arcan
Agriculture Research Organization

Follow this and additional works at: <https://digitalcommons.usu.edu/electron>



Part of the [Life Sciences Commons](#)

Recommended Citation

Ishay, J. S.; Shimony, T.; and Arcan, L. (1986) "The Biomineralization in Social Wasps (Vespinae): The Presence of Statoliths," *Scanning Electron Microscopy*. Vol. 1986 : No. 4 , Article 37.

Available at: <https://digitalcommons.usu.edu/electron/vol1986/iss4/37>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Electron Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



THE BIOMINERALIZATION IN SOCIAL WASPS (VESPINAE):
THE PRESENCE OF STATOLITHS

J.S. Ishay(*)¹, T. (Benshalom) Shimony¹ and L. Arcan²

¹Department of Physiology and Pharmacology, Sackler Faculty of Medicine,
Tel Aviv University, Ramat Aviv 69978

² and Volcani Center, Agriculture Research Organization,
Beit Dagan 50250, Israel

(Received for publication March 03, 1986, and in revised form August 23, 1986)

Abstract

In social wasps granular aggregates occur mainly in the head region and the body integument. In the former location they are present both on the outer and inner side of the frons. On the outer side of the frons, in a groove traversing its center, there is a deep pit surrounded by a membrane which forms a cyst-like sac. In the pit of the hornet *Vespa orientalis* (Linne, 1771), there are aggregates comprising silicon (Si), potassium (K), calcium (Ca) and iron (Fe), which are arranged in *morula*-like fashion. A granular material is secreted at the base of sensory hairs (sensilla), distributed on both sides of the groove, as well as along their shafts. Aggregates of silicon and calcium are also present on the inner side of the frons.

The integumental aggregates are comprised of yellow pigment granules. In the abdominal region these granules, which are concentrated in yellow stripes, assume a quasi-cylindrical shape, measuring 0.6 - 0.8 μm in length and 0.3 - 0.4 μm in diameter. Their structure is affected by caste, age, exposure to sunlight, and drugs introduced through feeding. Feeding on colchicine induces degeneration of the granules, whereas feeding on xanthines renders them refractory to the destructive action of colchicine. Their main metallic element is K.

We postulate that 1) the granules on both sides of the frons are part of a tympanic and static organ; 2) the yellow granules (YG) dispersed all over the external surface of the body possess properties of organic semiconductors; 3) 1 and 2 are piezoelectric, and are involved both in gravity orientation and transposition from photo to geotaxis.

KEY WORDS: Statocysts and Statoliths in Insects; Yellow Pigment Granules; Photo- and Thermoelectric Properties; Biomineralization; Bioaggregates containing Si, K, Ca, Fe; Gravity Orientation in Insects.

(*)Address for correspondence:

Jacob S. Ishay, Dept. of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel Phone No. (03) 420 863

Introduction

Only a few members of the Insecta have a developed statocyst: A cuticular nodule behind the eyes called Palmen's organ is found in the head of the adult and larva of Ephemeroptera; in *Dorymyrmex* (Hymenoptera) there is a statocyst above the coxa on the metathorax which is an invagination of the cuticle, lined with tactile hairs and possessing sand grains within the cavity. These organs probably function as gravity receptors (Marcus, 1956; Chapman, 1969).

In the order Hymenoptera (and others) some species are colored; in the social wasps (Vespinae), there is a predominantly yellow to green color found in the cuticula spread over various parts of the body (Kemper and Döhning, 1967; Guiglia, 1972; Spradbery, 1973; Edwards, 1980), which is of taxonomic importance: it is also believed to serve as a warning signal (Keeton, 1967). Such pigmented cuticular areas in Hymenoptera (as well as the colored wings in Lepidoptera) are formed by an infolding of the cuticula *inside* and the pigment aggregates are within *granules* composed of pteridines within the epidermal cells or above them, unlike the melanin which is embedded *inside* the cuticula (Becker, 1937; Descimon, 1965; Harmsen, 1966; Ziegler and Harmsen, 1969). These pigmented areas in social wasps have photoconductive as well as thermoconductive properties (Croitoru et al., 1978; Ishay and Croitoru, 1978; Ishay et al., 1982). Similar, although not identical, pigmented granules have also been observed in honeybees (J.S. Ishay, unpublished observation).

Static sense organs composed of a hollow statocyst surrounded by sensory hairs and containing statoliths have been discerned in the heads of various species of Vespinae (Ishay et al., 1983; Ishay and Shimony, 1986). The present paper provides data on the variety of granules encountered in social wasps, and also speculates on their probable functions.

Materials and Methods

The majority of the observations were made on the adult population (workers, queens

and males) of the Vespinae species: on Vespa orientalis, collected from natural nests around the Tel Aviv area in Israel, and on V. crabro (Linne 1758), Paravespula germanica (Fabricius 1793), Dolichovespula media (Retzius 1783) and D. saxonica (Fabr. 1793) collected in Germany, as well as many other species of Vespinae and Polistinae (Ishay and Shimony, 1986). The hornets used for experiments were maintained in artificial breeding boxes (ABBs) (Ishay and Sadeh, 1975) in groups of 5-20, and kept on a regular diet of 30% sucrose solution and morsels of meat (for details, see J. Ishay, 1964).

Hornets and wasps were killed by freezing and stored until processing. Specimens were first observed under light microscopy for preliminary location of specific areas.

Scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDX)

Microstructural investigation as well as associated chemical information were carried out by JEOL-35 SEM, Cambridge Stereoscan 180S and Energy Dispersive X-ray Analysis System - PROXAN III equipped with a Si (Li) - Si2.5-4 high resolution detector.

For microstructural observation, the specimens were attached to aluminium or copper stubs with silver paint and sputter-coated by a 200-300 nm gold layer.

Parts of the internal surfaces in front were investigated after fixation for a minimum period of 4 h with 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.2, dehydrated through graded ethanol, then critical point dried (CPD) from liquid carbon dioxide and carbon and/or gold-sputtered.

Location of a number of elements presented in the frons area was investigated by x-ray microanalysis (EDX) on unfixed as well as on fixed tissues. Semi-quantitative measurements were performed on YGs using gelatin blocks as an organic matrix.

Stereo pictures at low and high magnification were performed in order to check the proper geometry for x-ray analysis. Minute parts of the above specimens (about 1-2 mm²) were tailored accordingly under a light microscope and attached to a carbon stub by carbon paint. The EDX was carried out at 15kV-20kV accelerating voltage with a 50 and 100 sec (lifetime) counting time for each analysis. Occasionally, a very thin layer of gold, insufficient to obscure the EDX peaks being studied, was necessary in order to suppress charging effects on some interesting areas.

Transmission Electron Microscopy (TEM)

For transmission electron microscopy, pieces from the head (frons area) were dissected out of fresh specimens and fixed in 5% glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 7.2). After fixation for 24 h at 4°C, the material was washed in buffer and secondarily fixed for 2 h in 2% osmium tetroxide buffered with cacodylate. Dehydration was accomplished with an ethanol series followed by embedding in Epon 812.

Thin sections were stained with 1.5% uranyl acetate and 0.3% lead citrate. Micrographs were obtained by a JEOL 100B electron microscope.

Changes in the size and shape of the yellow pigment granules were evaluated after one of the following treatments or conditions: a) exposure of hornet workers to continuous light for 14 days; b) the effect of age, i.e., the difference between young hornets (0-24 h), adults (several weeks old), and 'old' queens (one year of age); c) addition to the diet of caffeine, theophylline, allopurinol, or purine alone (in a dosage of about 5 µg/hornet/day) or together with colchicine for two weeks; d) deviation from the normal pattern due to an aberration caused either by excessive yellow pigment, or by its absence.

The photoelectric properties of the yellow abdominal stripes in situ (i.e., when connected to the intact body), or of the separated yellow pigment granules, were examined by observing the change in resistance under illumination and/or in the dark. This was achieved by exposing the hornet after each measurement to a low temperature (approximately 0°C), for 5-15 min. The change in resistance upon illumination was measured with an integral white light from incandescent bulbs of 100 W and 220 V at a distance of 20 cm from the specimen. The change in resistance as a function of illumination with white light was measured in two ways: 1) during 15-20 min of irradiation with light (until a saturation level was attained), and 2) by further exposure to a light/dark rhythm every 2 min (i.e., intermittent illumination). Control experiments were made to ascertain that the electrical connection, i.e. the silver paint, tungsten wires or the glass were not in any way photoconductive. (For further details, see Ishay and Croitoru, 1978; Ishay et al., 1980).

Methods for evaluating the thermoelectric properties, thermoelectric (Seebeck) effect and electrical capacitance of the yellow versus brown stripes of the hornet and wasp cuticle were also described earlier (Shimony and Ishay, 1981a,b; Ishay et al., 1982a; Shimony and Ishay, 1984). In this study individual hornets were attached with glue to a microscope slide between two silver paint electrodes. The yellow stripes on the abdominal segments were connected to the electrodes with thin tungsten wires. The ohmic properties of the contacts and their stability with time were ascertained (Ishay and Croitoru, 1978). The relative humidity in the test room was maintained at about 75% ($\pm 5\%$). Measurements of the electrical resistance of the cuticle were taken in total darkness in a thermostat in which the temperature was gradually altered within a range as needed, and the electric resistivity was gauged with an electrometer (Keithly Digital Electrometer, Model 616, which served as an ohmmeter) within resistance values of $0.3 \times 10^7 \Omega$ to $7.8 \times 10^7 \Omega$. Earlier studies also described the electrical resistance of the hornet cuticle and changes in resistance

induced by xanthines (Ishay et al., 1982b; Rosenzweig et al., 1985).

Results

SEM AND EDX on the center side of the frons

On the frons of *V. orientalis*' workers, a centrally-traversing canal called the frontal suture (or coronal suture) can be seen (Edwards, 1980), which is divided into three distinct structures: 1) The upper section; 2) the pit area; 3) the lower portion.

The upper section, situated close to the median ocellus, is rather shallow and essentially is comprised of a slightly recessed band devoid of sensilla. A pit can be found at the middle part of this structure in the form of an elliptical well approximately 30 μm deep, 60-70 μm long (externally) and 30-40 μm in maximal width (Fig. 1).

On the underlying surface of the frons the margins of this well protrude, similar to a cone. As a continuation of the well, the bottom of the canal elevates to a depth of only a few μm while its width increases. The upper part of the canal is not discernible in *V. crabro*, *D. media* and *P. germanica*, while in *D. saxonica* neither the upper nor the lower part of the canal is evident (although the well is visible). In *V. orientalis* there is a secretion from the upper rim and borders of the well, which accumulates in the following forms: a) as a thin smooth and rather solid membrane composed of an amorphous material which lines the inner surface of the well and also 'envelops' like a cyst wall sac, with a pore at its center (Fig. 1). In the well, alongside both its margins, there are numerous spinules and crests which at times anastomose. This cyst-like sac is located mainly in the deepest part of the well; b) as a granular accretion shaped like a mushroom or berry within the cyst-like sac (Fig. 2).

At times there are several accretions which are of varying sizes and shapes fastened to the borders of the well. There is an adhesive secretion which holds the granules together within the sac as well as on and above the margins of the well.

Sensory hairs. There are three clear categories of sensory hairs (sensilla), which extend over the entire length of the canal along the borders, and across the frons: 1) The marginal sensilla of the canal, which have accretions of Ca at the base, are 10-30 μm long and 1-3 μm thick at the base (Fig. 3). Occasionally, the secretion accumulates not only at the base but also along the shaft of the sensillum, and comprises a single bead or a string of several beads of secretion over its length (Fig. 4 for *P. germanica*). 2) The border sensilla of the frons, which number 12-16, are filiform and straight, about 100 μm long and 10 μm thick at the base, situated about 100 μm apart. 3) The ankyroid sensilla arranged across the frons above the region of the canal (below the median ocellus) whose length ranges between 200-300 μm with a thickness between

10-20 μm . Similar groups of sensilla, although of different dimensions, were discerned on the heads of hornets and wasps of all the species examined.

Elemental analysis (EDX) revealed that aggregates in the cyst-like sac region as well as those at the base of the sensilla are composed of Si, K, Ca, Ti and Fe, as principal inorganic elements. The relative proportions of these elements have not yet been defined. We have focussed our efforts on qualitative aspects. An evident fact: in younger hornets (workers) there was a higher concentration in Ca versus Si (see J.S. Ishay et al., 1983) (Fig. 5a, b, c). Fig. 6a presents a view of the front of the head of a hornet displaying the frons region. Fig. 6b shows a cross-section in the tegumentary layer adjacent to the ocelli. In the upper part a separate mass is observable - the layered cuticle - and underneath it a layer of YGs are encased in delicate membranes. Thickness of the yellow granule layer in this region (see arrow) is 15-20 μm and the metallic element in these granules is K. Our EDS analyses were carried out at a qualitative level rather than quantitative. However, for the YGs semiquantitative data were obtained from gelatine blocks in which the yellow pigments, separated from the tissue matrix as well as standards, were embedded. Potassium (60%/-70%/-) and phosphorus (10%/-30%/-) were the principal elements detected in the x-ray spectra. A transmission electron micrograph showing the base of a sensillum and a layer of yellow granules on the inner side of the frons is presented (see Fig. 7) (J.S. Ishay et al., in preparation). Underneath this membrane is an additional, more internal membrane which is part of the layer lining the space and producing the 'strings' (see below). Underneath the frons and ocular sinus in all species of Vespinae examined there is a 'space' containing numerous strings (Fig. 6c). In *V. orientalis* the 'space' is harp-shaped and comprises an acoustic box (Ishay and Shimony, 1986). Inside the acoustic box there are some 120-150 strings stretched across its width from the frons to the lining above the protocerebrum (Fig. 6d). The individual strings, arranged helically, are about 10-50 μm apart with a length usually in inverse relation to their thickness. The short strings (see Fig. 6b) are 100-120 μm long and 8-15 μm in diameter, whereas the long strings (see Fig. 6c) attain 600-700 μm in length and 3-7 μm in diameter. When detached from their hold on the walls, they tend to coil and abbreviate like a spring (Fig. 6e). In *V. orientalis* the entire frons plate is studded (from the inside) with the YGs. Each granule is about 0.5 μm long and 0.4 μm in diameter (see Fig. 6f). A cross-section of one of the strings seen by TEM is presented in Figs. 8, 9). A section of an axon bundle originating from the protocerebrum and entering the acoustic box is shown (Fig. 10).

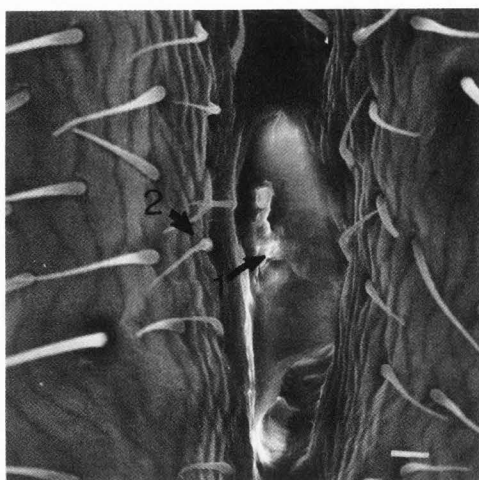


Fig. 1. Enlarged region of a *V. orientalis* worker in area of the well. The membrane covering the well appears in the center of the picture. Above the membrane, there are accretions of a granular material accumulated at the well's margins (see area marked by arrow no. 1), where there are relatively short sensilla with thickened bases (statolith-like sensilla). (See arrow no. 2). Bar = 10 μ m.

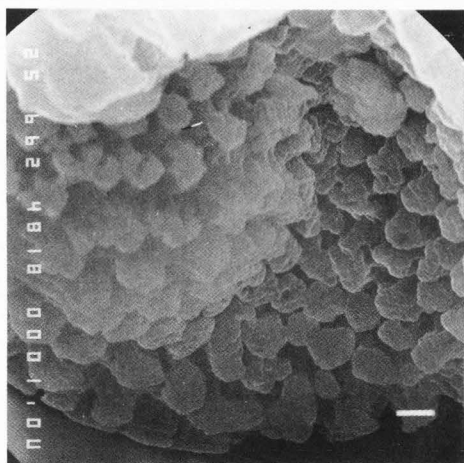


Fig. 2. View of the aggregated accretion in the form of a mushroom in *V. orientalis*, seen to protrude from the upper margins of the pit after removal of the enclosing membrane. The 'mushroom' hangs in the form of a cluster and is probably a consolidation of secretion exuding from this region. The material contains Si. Bar = 1 μ m.

Inside the acoustic box at the end of some strings there are aggregations containing Si (Fig. 11a,b). Between the cuticular layer and the YG layer there are gemulae-like structures containing Ca in an organic matrix (Fig. 12).

These structures are more prominent in young hornets than in adults. Similar structures are present on the outer side of

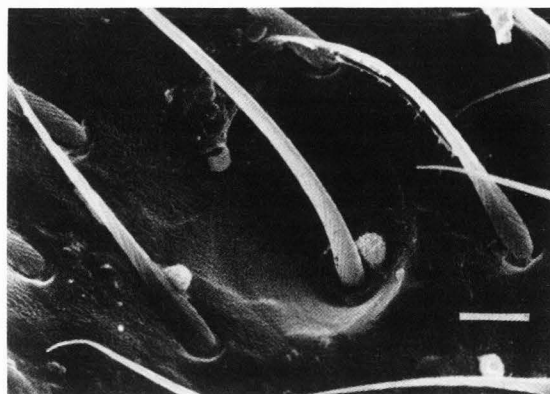


Fig. 3. Sensilla in the frons of a *V. orientalis* worker. Prominently displayed are some sensilla with accretion 'beads' close to their base. The accretions contain mainly Ca. Bar = 10 μ m.



Fig. 4. Lower region of the frontal suture in workers of *P. germanica*. Prominent are the sensilla bearing accretions arranged as 'beads' along the shaft. These accretions contain Si. Bar = 10 μ m.

the frons plate (Fig. 13), containing Si in the center and Ca at the 'envelope' of an organic matrix. The YGs from specimens of the same origin, i.e. species, caste, age or drug treatment vary but little in appearance (Shimony and Ishay, 1981a). However, the YGs from specimens of different ages are quite different in appearance. Fig. 14 shows the pigment granules from a queen pupa of *V. orientalis*. These granules are elongated-ovoid, not entirely uniform in shape, with a diameter smaller (0.1 μ m- 0.2 μ m) than in adult specimens. They do not fill the entire area but, rather, have spaces in between which are apparently filled with a cytoplasmic matrix. Inspection of the same area in a

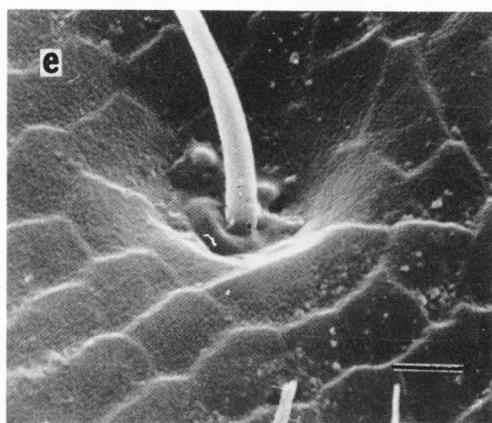
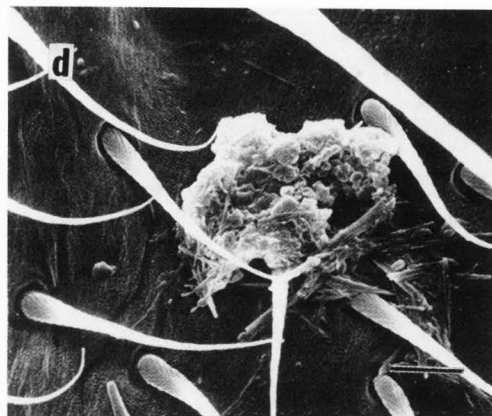
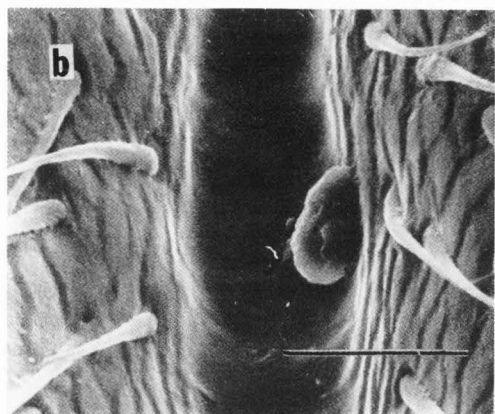
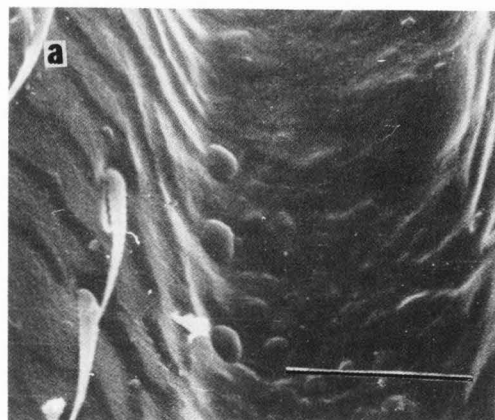


Fig. 5 a-c. SEM views of aggregates on the frons of young hornets. a. K accretion in lower sector of the groove. Bar = 30 μm . b. Same area Si aggregate associated with Ti and Fe. Bar = 30 μm . c. Closeup of Ca aggregates in the lower sector of the groove. Bar = 10 μm .

Fig. 5 d-f. SEM views of aggregates on the frons of young hornets. d. Si in organic matrix outside the groove. Bar = 10 μm . e. Sensillum with Si accretion at its base. Bar = 10 μm . f. Detailed view of Si-Ca complex in organic matrix. Bar = 10 μm .

freshly-ecloded queen revealed that the pigment granules had undergone thickening to gradually assume a cylindrical shape. In a mature queen (several weeks of age) the pigment granules occupying all the available spaces are typically cylindrical in shape, most commonly 0.6 μm -0.8 μm long and 0.3 μm in

diameter. What strikes the eye, however, is that some of the granules are elongated whereas others are short. Since the Vespinae are annual insects, the queen survives for not more than one year, while a worker will live at best a number of months. In both aging queens and workers the yellow color tends to

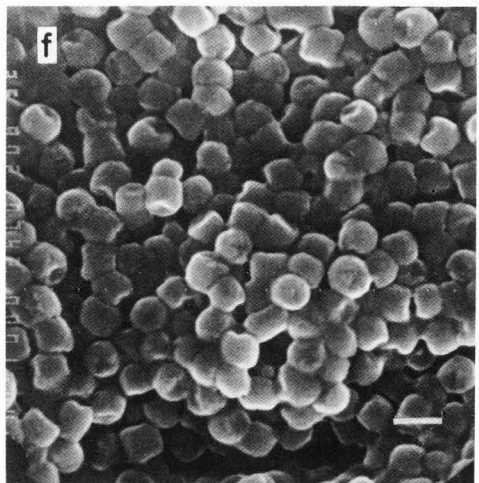
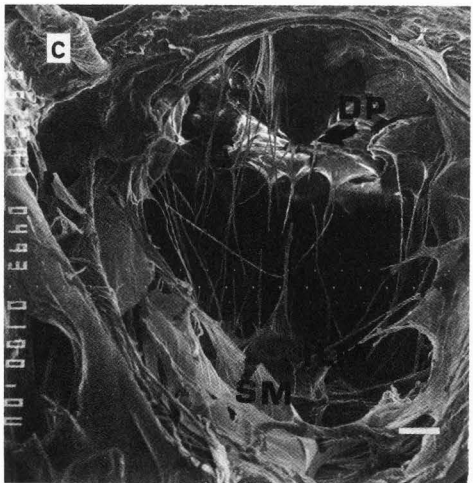
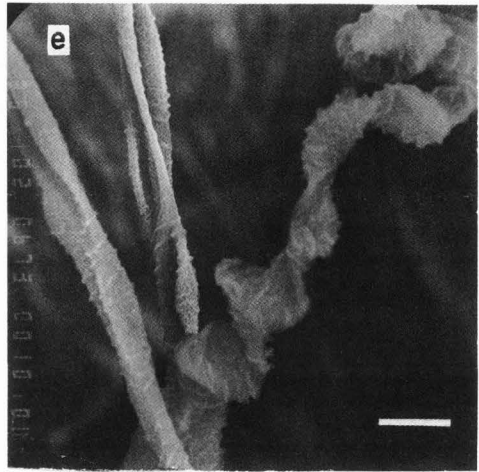
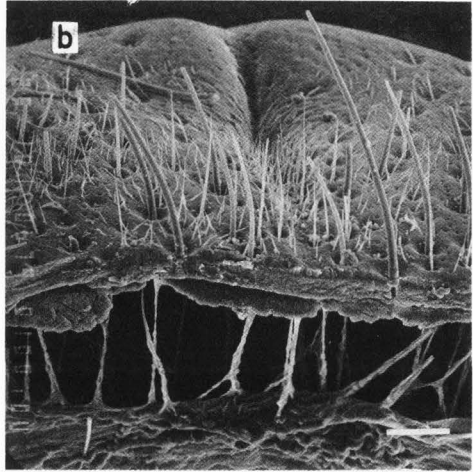
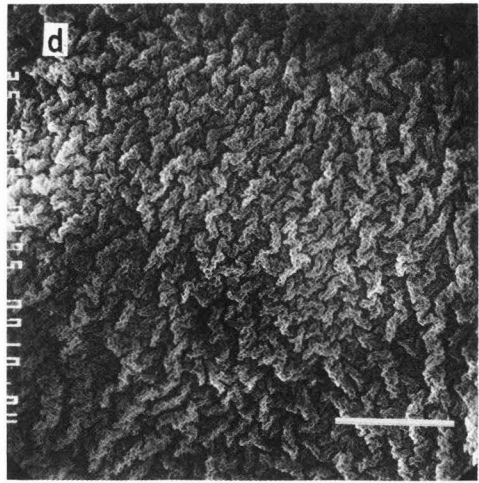
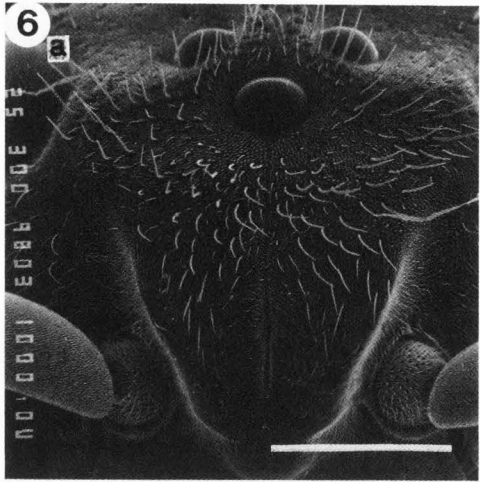


Fig. 6a. Presented is a view of the head of a hornet displaying the frons region, which is the prominent plate in the center of the photograph. Displayed in the basal part on both sides are the bases of the antennae and, in the upper part, the three ocelli. Visible on the frons are large spines 200-600 μm in length, and the groove - sutura coronalis in the center. The entire plate is elevated, protruding in a wing-like structure towards the middle part of the compound eye. (Bar = 1000 μm)



Fig. 6b. Represents a cross-section through the integument, and through the space underneath the frons in the region adjacent to the ocelli. Here one can clearly discern the groove - sutura coronalis - in the upper part of the frons, as well as the various setae protruding from the frons. Visible underneath are the short strings transversing the 'space' whose length in this region is 100-150 μm , with a diameter of 8-15 μm . Most of these strings are straight, although some are branched, but all lie 50-100 μm apart, bridging the inner surface of the frons with the membrane above the protocerebrum. (Bar = 100 μm)

Fig. 6c. Shows a cross-section of the 'space' along an imaginary band passing through the upper portion of the base of the two antennae. The space is always dry, i.e., no fluid is to be found in this cavity, even in freshly prepared specimens. Visible in the upper part center is a cone-like recession (see arrow marked by the letters DP), which is the invagination of the 'deep pit' in the center of the frons. From this 'cone' and other parts of the roof comprising the inner surface of the frons plate, strings project which originated from the inner lining membrane (ILM) of the 'space'. This ILM is a more internal membrane than the membrane which enfolds a layer of yellow granules. The strings (at least those that did not break off in the process of fixation) stretch across the 'space' to its other bank. The maximal height of the 'space' at this region is 600-700 μm while the length of the strings is proportionate to this height. Clearly exposed to view are the strings displaying intermittent thickenings along their length so that their diameter ranges between 3-7 μm . The 'roof' of the 'space' is comprised of arcs and concavities. The inner lining enfolds the entire 'space' on all sides while at the bottom it comprises a septum separating between the 'space' - henceforth to be designated 'acoustic box' - and the area below it, i.e., the inter-antennary triangle plate of the hornets. This membrane is designated as a separating membrane (SM), traces of which are evident in the photograph. The 'acoustic box' is revealed only after removal of the SM. (Bar = 100 μm)


Fig. 6d. Offers a magnification of a section of the inner lining with the delicate structure appearing to resemble lacework. (Bar = 10 μm)

Fig. 6e. A number of strings can be seen. Note that the right one, which had been detached from its hold on the roof, is now coiled and abbreviated, with the coiling occurring in the narrower parts along its length. Next to this string are two stretched strings with spindle-shaped folds along their length. (Bar = 10 μm)

Fig. 6f. Shows the yellow granules occurring between the cuticular layer of the frons plate, and the membrane lining the acoustic box. These granules are 0.5-0.7 μm long and 0.4-0.5 μm in diameter. K is the dominant metallic element in the lining membrane, the string and the yellow granules. (Bar = 1 μm)

fade (Spradbery, 1973). Fig. 15 shows the pigment granules in a worker over 2 months of age, which had been kept under regular daylight illumination. It can be seen that the pigment granules had undergone partial degenerative changes, evident both lengthwise - usually it is the short ones which occur - and crosswise, where the granules are seen to have lost their typically smooth and rounded appearance, splitting up into smaller subunits. The degenerative changes revealed a whorl-like structure in the inner part of the granules. A similar picture can occur in a hornet only 8-10 days old, provided that it had been exposed to constant illumination throughout its life. However, in hornets exposed to daylight for a couple of months (i.e., the old ones), there are no degenerative changes in the pigment granules if the diet contained one of the xanthines (theophylline, caffeine or like substance). In hornets fed on allopurinol, there seemed to be few granules, and those that were present showed signs of disintegration, even though their diameter was at least twice that of normal hornets. From the manner of disintegration of these granules, it would seem that they are helical in structure. Hornets fed on colchicine are short-lived (not more than 7-9 days), and their pigment granules are considerably broader (0.5-0.6 μm in diameter), displaying raspberry-like grooves and breaking up differently than in older workers (Fig. 16), that is, the breaking up of the granules occurs lengthwise rather than crosswise, like a log of wood splintering into longitudinal chips. Hornets that received colchicine in their diet together with one of the xanthines survived similar to hornets receiving xanthines only (up to several weeks), and their pigment granules did not show the transformation induced by colchicine alone (Ishay et al., 1981).

In one colony of hornets (out of hundreds), a pigment aberration was observed in

Fig. 9. TEM cross-section of one of the strings at an area showing an epithelial cell nucleus. (Bar = 2 μ m). 

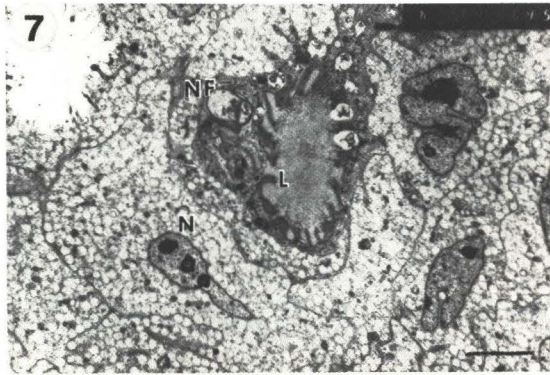


Fig. 7. Transmission electron micrograph (TEM) showing the base of a sensillum and a layer of yellow granules in the frons of a *V. orientalis* worker. In the center - the base of the sensillum. Toward the 11 o'clock position from the center, a section through a nerve fiber is visible (NF). At the periphery of the sensillum base stacked membrane systems (lamellae) are discernible (L). Outside the base the field is loaded with vesicles containing the yellow pigment granules. The borders of three cells and their nuclei (N) are visible; on the top left there is a space left after the mineral aggregate (dissolved during specimen preparation). (Bar = 4 μ m.)

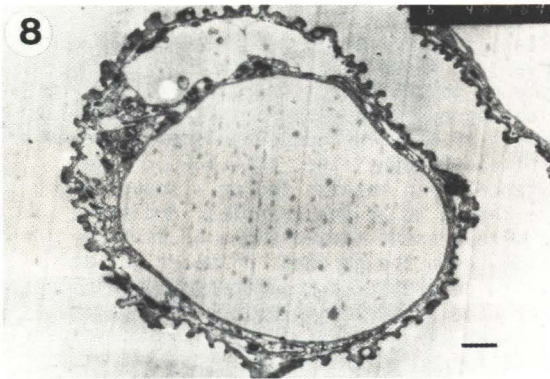



Fig. 8. TEM cross-section of one of the strings in the 'acoustic box'. At the periphery underneath the cuticle layer there is a tracheal epithelial sheath. A nucleus of this epithelium is shown in Fig. 9. The section reveals two spaces which are separated by a septum. (Bar = 1 μ m).

Fig. 11 a,b. The underlying surface of the same frons area as in Fig. 5 showing some aggregates. a. Ultrastructure of Si-Ca aggregation. Individual granules of Si are easily identified by EDX with the organic matrix. Bar = 30 μ m. b. Closeup of Si aggregates near the groove area. Bar = 30 μ m. 

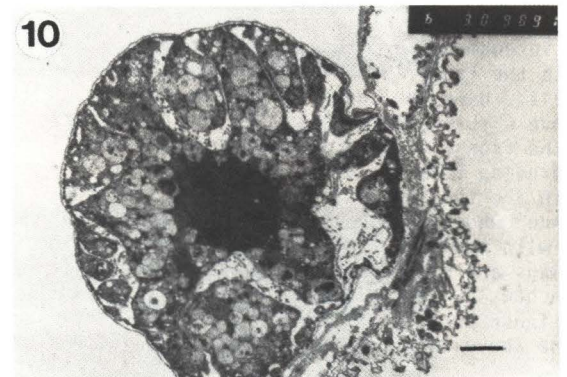
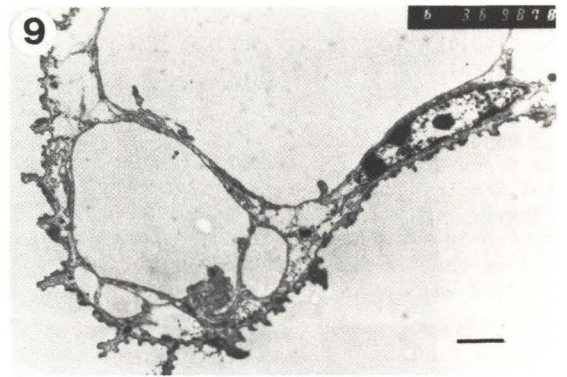
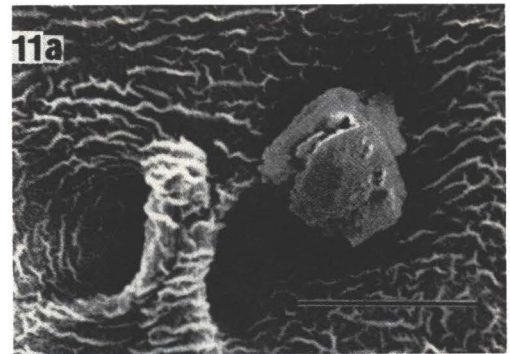


Fig. 10. TEM of axon bundle originating from the protocerebrum and entering the 'floor' of the 'acoustic box'. The individual axons penetrate the various strings. (Bar = 2 μ m).



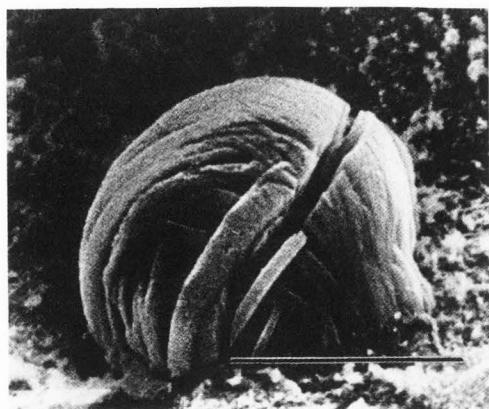


Fig. 12. Gemmule-like accretions on the inner side of the frons containing primarily Ca. Bar = 10 μ m.

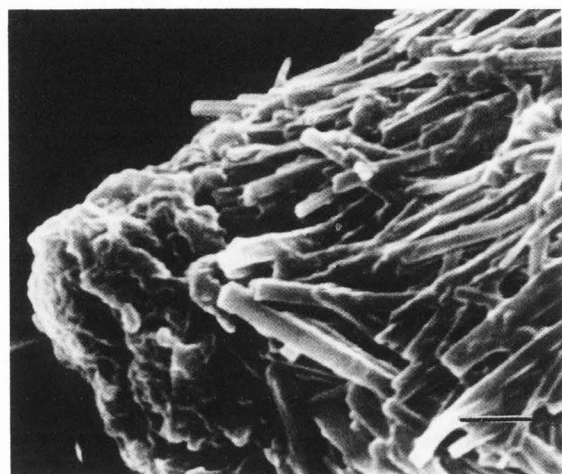


Fig. 13. Side view of the aggregated accretion in the well of a young worker (*V. orientalis*) less than 24 h old. The center of the accretion (left) will develop into a form of a 'mushroom'; the main element is Si. The 'mushroom' is surrounded by 'rods' comprised mainly of Ca. Bar = 3 μ m.

the males, namely, their yellow cuticular stripes lacked the usual lustrous color. Inspection with SEM shows an almost complete absence of pigment granules in the pertinent cuticular region. Instead, there was only a faded yellowish color, which did not seem to be linked with the granules themselves. The ratio of aberrant to normal males on the same comb was about 1:1. There was no evidence of any vital defect nor any impairment of motor activity, or of orientation. In another colony of workers, a very rare aberration was found, consisting of an excess of yellow pigment in such areas of the body usually colored by the brown cuticle (Fig. 17). The ratio of aberrant to normal workers could not be ascertained because the aberrant workers

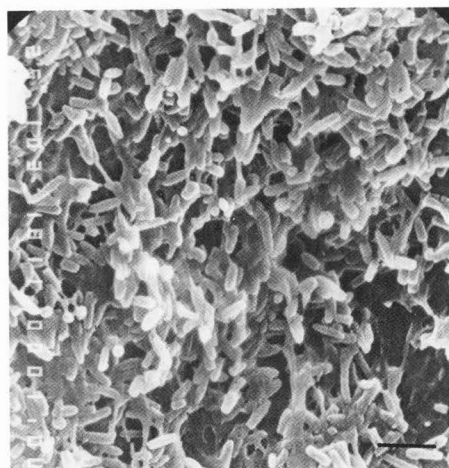


Fig. 14. Elongated-elliptic pigment granules in a queen pupa of *V. orientalis*, with large spaces in between. Relative to that of the adult hornets, note the small diameter of the granules, which are still attached to one another. Bar = 1 μ m.

were very short-lived and could only survive under laboratory conditions. The YGs of aberrant workers appeared normal, but where the yellow and brown stripes met, the aberrants did not show a sharp demarcation between the granules (yellow region) and the brown cuticle (with melanin) as did normal hornets. Instead, there was a mixture of disorganized fibers which terminated in, or intermeshed with the yellow granules.

Photoelectric Properties of the Cuticle

As far as the photoelectric properties were concerned the following was noted: after 10-20 min of illumination, the resistance in the yellow stripes of the cuticle first increased to a maximal value, then decreased to a saturation value. This sequence of events was typical only for the yellow stripes of hornets and wasps in Vespinae but was also encountered in Polistinae. When, after attaining the saturation level, the yellow stripes were subjected to a series of brief (30 sec.), alternate exposures to light and darkness, the variations in resistance were clearly periodic - the resistance decreasing when the light was switched on and increasing when the light was switched off. Irradiation with the light of a He-Ne laser also produced these reversible changes in the resistance. In this case, prolonged initial irradiation was unnecessary and the light of the laser was switched off every 10 sec. rather than every 30 sec. since the longer exposure resulted in irreversible changes.

If a yellow stripe was detached from the body of the hornet (or wasp) and its photoconductive properties measured separately, the properties were invariably seen to be retained as in the intact hornet, except that the

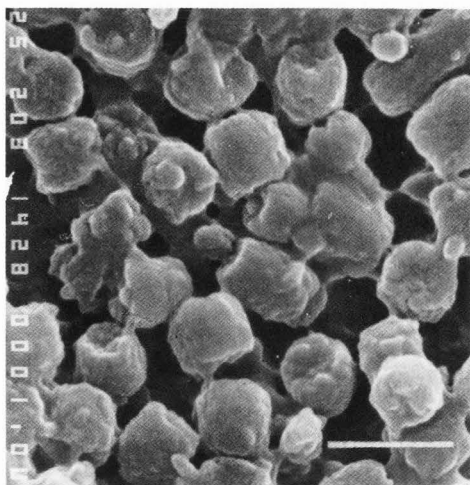


Fig. 15. Pigment granules in an old worker over two months of age whose yellow colour had already faded. Degenerative changes - discernible in the granules - entail external irregularities, a partial breakup and abbreviation of length, although the diameter is larger than usual. Bar = 1 μm.

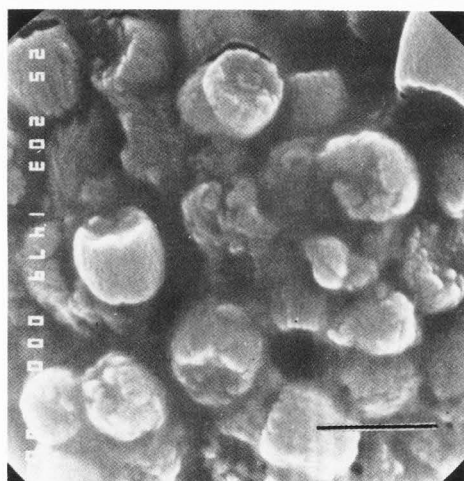


Fig. 16. *V. orientalis* worker which had received colchicine in the diet. The following changes in the pigment granules are discernible compared to the control group: Here they are shorter, with a considerably larger diameter (0.53-0.66 μm), in an advanced stage of breakup displaying raspberry-like grooves. Bar = 1 μm.

initial resistance attained during the saturation period was higher. Measurements taken on the transparent cuticula alone, that is, following removal of the yellow granules together with the base membrane, showed that the resistance is constantly on the rise, both in light and in darkness, meaning that the

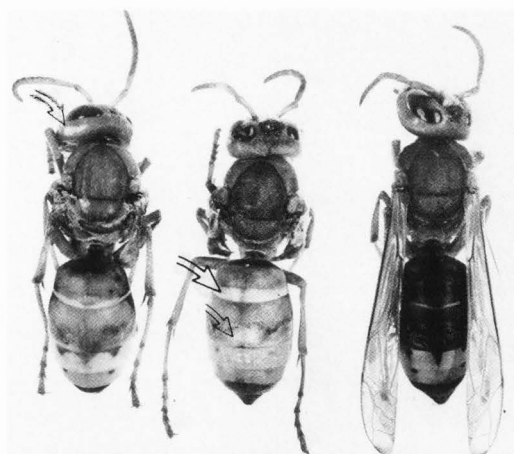


Fig. 17. Macroscopic view from the dorsal aspect: right - control hornet, left - 2 aberrant workers in which the aberration gives rise to the appearance of a yellow color in the usual regions, although in wider strips than normal, as well as in segments usually only covered by brown cuticula (in the abdominal and cephalic regions as indicated by arrows). In addition to the color changes there are also malformations in the alea and pedes.

transparent cuticula per se is not photoconductive. In addition, very high values of resistance were obtained, on the order of 10^9 - 10^{10} ohm, as compared to measurements on whole yellow stripes where the obtained values were on the order of 10^7 - 10^8 ohm, i.e., a 10-100 fold difference in resistance. The resistance values obtained on measuring the photoconductivity of the YGs removed from the cuticle were about the same as in entire yellow stripes (i.e., including the cuticle), attaining up to 10^7 - 10^8 ohm. However, like in measurements of the transparent cuticle alone, the resistance stabilizes at a fixed level and the YGs here show better photoconductive properties than their counterpart intact yellow stripe. Evaluation of the resistance as a function of wavelength for UV, violet, red and IR light as compared to white light was also investigated. We found that the UV light produced practically the same changes in resistance as the white light. The spectral dependence of the photoconductivity in the yellow stripes following prolonged illumination (15-20 min) was similarly investigated. It was found that the maximum of the spectral distribution corresponded to a wavelength of approximately 420 nm. This result gave the activation energy (~ 2.9 eV) necessary to obtain the transition of carriers from the valence band to the conduction band in semiconductors (see Hannay, 1959; Ryvkin, 1964; Kittel, 1968; Watson, 1969).

A mathematical model (Ishay et al., 1980) for the relative changes in resistance in the yellow stripes as a photoconductive process conforms to the general model for a semiconductor with traps. For these materials the electrical conductivity seems to be due to both negative and positive carriers. As a result of releasing an electron, a chemical bond is opened, i.e., a hole is formed (yielding traps). Such holes can be filled by neighboring electrons which in turn will open new holes, etc. This movement of electrons filling neighboring holes contributes to the electrical conductivity in the granule. In our case, when illuminating the yellow stripes of the hornet, a saturation level had to be reached first where all the traps were filled. Only then could additional electrons contribute to conductivity. In darkness the reverse holds true, i.e. carriers move from the conduction band to the valence band, hence, conductivity decreases. Our final model was well supported when we observed the measured data on 8 different hornets.

The photoconductive properties are affected by drugs. Thus, feeding on xanthines improved these properties, as evidenced from the fact that a) the baseline of resistance was retained throughout the measurement; b) the amplitude obtained between the resistance in light and that in the dark did not change significantly after reaching the base level. There was also a marked difference between hornets kept in the dark on xanthine (caffeine) in their regimen, and those living in darkness without caffeine. In the former, the photoconductive properties were marked and quite similar to those in control hornets kept under illumination. In allopurinol-fed hornets there was an abbreviation of the saturation time (to about 10 min), whereas feeding on colchicine provoked a deterioration of the photoconductive properties (Ishay and Shimony, 1982).

Thermoconductivity of the Cuticle

The yellow and brown stripes on the Vespinae cuticle are thermoconductive. The response of the cuticle to temperature changes is fairly stereotypic, entailing the following stages: a) a sharp drop in the resistivity, with a rise of temperature up to values at which hornets naturally become active (around 24°C, vid. Bodenheimer, 1933). A reverse pattern of an increase in the electrical resistance of the cuticle with a decrease in temperature is also found at temperatures down to -35°C. At around 24°C the electric resistivity of the hornet cuticle reaches nadir. b) Within the range of about 27-32°C, a plateau-like phase is obtained in the course of which no further significant change in the resistivity with a rise in temperature took place. These results point to the fact that at the lower temperatures the cuticle behaves like a semiconductive material while at the higher temperatures the cuticle displays quasi-metallic conductance with phase transition (Gutmann and Lyons, 1967). The temperature dependence in darkness and low temperatures

fits the Arrhenius law with activation energies (E_a) = 1.0-2.2 eV. The effect of diet on the electrical resistivity to the direct current of the yellow and brown stripes of the cuticle of hornet workers was measured in the dark within a temperature range of 10-32°C. It was found that diet exerts a significant effect both on the level of the electric resistivity of the cuticle as well as on the shape of the resistivity 'line'. Neither age nor pigment exerted any significant influence. On ordinary diet alone, a hysteresis loop was obtained between the heating and the cooling lines, which pointed to the effect of memory (in the cuticle). Feeding on allopurinol or theophylline resulted in a straight 'line' of resistance, i.e., there was an improvement of the semiconductive properties of the cuticle, although this somehow interfered with the memory effect produced by feeding them on a regular sugar solution diet (Rosenzweig et al., 1985).

The thermoelectric (Seebeck) coefficient ($S = \Delta V/\Delta T$) in various cuticular areas of the Oriental hornet (*Vespa orientalis*) fluctuates from 0.3 to 2.4 mV deg⁻¹ within a temperature range of 27-36°C and when the temperature difference between the two measuring electrodes (ΔT) is 0.6- to 8.0°C. The values measured on the brown-colored cuticle suggest an n-type conduction, while those measured on the yellow-colored cuticle point to a p-type conduction. It is suggested that hornets use this phenomenon for temperature detection.

The electric capacitance in the integument of the *Vespa orientalis* was investigated and found to be dependent on various factors. These included: biological factors such as age, region of integument and the predominant pigment in the measured region, as well as physical factors. At 1000 Hz the mean capacitance was about 0.6 nF as opposed to 3.0 nF at 100 Hz. This inverse relationship between frequency and the electric capacitance might point to the presence of polar substances. The capacitance decreases under illumination and increases in the dark. With an increase in temperature there is at times an increase and at other times a decrease in the capacitance, which is inversely correlated to the resistivity.

Discussion

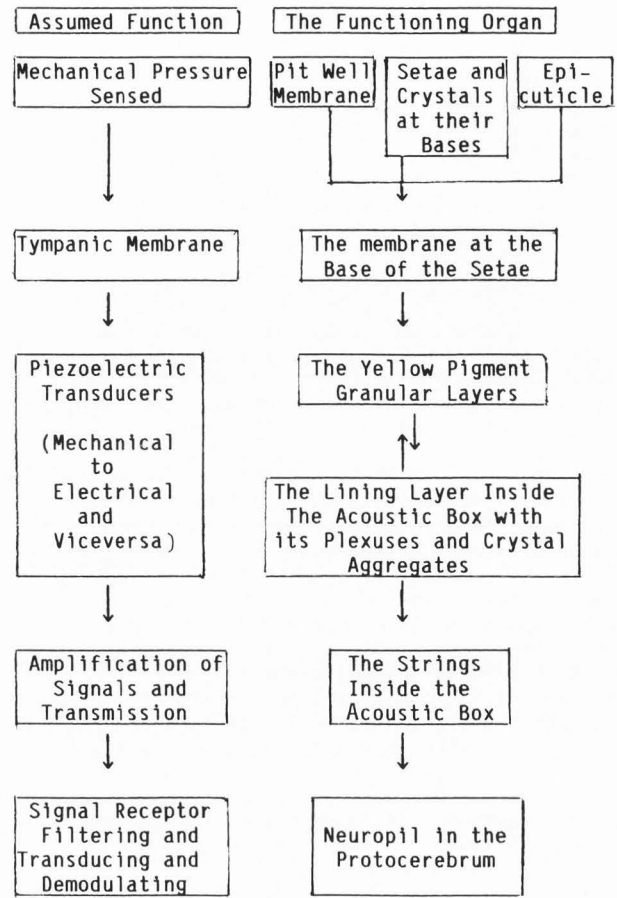
To the best of our knowledge, the structural conglomerate described herein is most likely an organ which is new to science. It is probably a combination of an acoustic and equilibratory organ, similar in some respects to an organ in various vertebrate animals. The 'space' which we have described in the present paper is apparently an 'acoustic box', whereas the strings serve to inwardly transmit sound waves or vibrations (the so-called airborne and solid-borne sounds) as well as data on spatial orientation, all of which are 'forwarded' in the direction of the brain, probably to the areas in the protocerebrum. The 'acoustic box', sealed from all sides, contains strings which are long and

thin in the lower part of the 'box' but which become shorter and thicker towards its upper part near the ocelli. The tegumentary outer plates and their attached structures - the setae, the delicate cuticle at their base, and the layer of YGs and the membrane which seals the deep pit - all act as components of a tympanic membrane.

The Model.

Tentatively, we speculate that auditory sensation and/or mechanical pressure may be picked up externally and transmitted internally in the following manner: the setae on the frons pick up sound waves or contact pressure or air motion and transmit the resultant impact via the cuticle layer at their base, which acts as a tympanic membrane, to the layer of YGs containing the metallic element K where the mechanical pressure is translated into electric energy due to the piezoelectric properties of the granules (J.S. Ishay, unpublished observations). In this case the YGs probably act as piezoelectric transducers which convert sound waves into alternating electric current (see Mason, 1966; Cope, 1975). As far as electrical conducting elements, their piezoelectric properties and other structures both inside and outside the frons plate are concerned, the 'deep pit' contains granules of Si, K, Ca, Ti and Fe (the presence of Ti and Fe in insect pigments has already been previously described, e.g., Kikkawa et al., 1955). These granules press upon the cuticle, which projects inwardly into the 'acoustic box', and are probably associated with the spatial orientation of the hornet. The lining layer inside the acoustic box with its plexuses containing Ca in an organic matrix converts electric energy into mechanical energy where the latter is amplified and, following filtration, is transmitted as vibrations via the strings (in some of which are aggregations containing Si) to the sensitive areas in the protocerebrum. In our opinion, such conversion of energy types is possible because hornet and wasp cuticle possesses organic semi-conductor properties, found not only in the head region but also in other areas such as the abdomen (see schematic presentation of the model). The cuticle is also endowed with photoelectric properties (Croitoru et al., 1978; Ishay and Croitoru, 1978; Ishay et al., 1980), thermoelectricity (Ishay et al., 1982 b, c; Ishay and Shimony, 1983), thermoelectricity (Seebeck effect - see Shimony and Ishay, 1981b), piezoelectricity (J.S. Ishay, unpublished observations) and, finally, an electrical capacitance (Shimony and Ishay, 1984). Other biological materials are similarly known to possess piezoelectric properties, to wit: a) the bones (Bassett and Becker, 1962; Shamos and Lavine, 1967; Cope, 1975), and b) otoliths (Morris and Kittleman, 1967) in bony fishes where the otoliths contain equal amounts of Ca and Na (although no K was found), and c) the skeleton of various Arthropods (Zilberstein, 1972). (See Schematic Model of the Tympanic Organ, Fig. 18).

Fig. 18: A Schematic Model of the Tympanic Organ



The membrane-ensheathed, cyst-like deep pit in the frontal area, with its secretion aggregates of Si, K, Ca Ti, Fe, largely resembles a statocyst. On general lines, this parallels the situation in decapod Crustaceans (Lang and Yonge, 1935; Schone, 1971) because the deep pit with the metallic aggregation within it is situated between the two antennae, which are homologous to the antennules in crustaceans (Meglitsch, 1972). We contend that the pit can be regarded, at least in its lower portion, as analogous to the area at the base of the antennules in crustaceans which has fused, in the course of evolution, to form a single statocyst at the center of the frons - an ideal site for the organ involved in stabilization and equilibrium (Ishay et al., 1983). But, unlike the situation in crustacean Decapoda where the mineral grain is introduced by the animal from the surroundings available, the Vespinae secrete these minerals both on the inner and the outer side of their cuticles.

Since in the nest of Vespinae various sounds are produced both for purposes of communication as well as in the course of routine nest activity, it seems reasonable that an organ with an acoustic box such as is described above should develop, especially

since hornets and wasps engage in comb building mainly in darkness and since, both under natural as well as experimental (special centrifuge) conditions, the building is directed towards the gravitational force (Ishay, 1975, 1976 a, b; Ishay and Sadeh, 1975, 1977). The building process itself, entailing the formation of hexagonal cells, greatly relies not only on the gravitational sense, but also on the mechanoreceptor one. Thus hornet workers tap with their antennae on each fresh layer of the wall being built, probably to ascertain static conditions such as strength, thickness, and the degree of uniformity (Ishay et al., 1967, 1982a). We believe that the structure discovered by us conforms functionally to a mechanoreceptor organ in which the different length, diameter and spindle-shape of the strings in their various areas enable selective transmission of sensory messages. It is possible that the 'acoustic box' with its strings had developed from a dilatation of a large trachea.

Mechanoreceptors have been described in various insects (Haskell, 1961; Busnell, 1963; Dethier, 1963; Schwartzkopff, 1974), where they are associated with the base of hairs on the joints or occur as an organ within an enlargement of a trachea in a leg, in the abdomen or in the base of the antennae (Johnston's organ). In larvae and adults of Ephemeroptera there are certain organs which are apparently gravity receptors in the center of the head (Gross, 1903; Wodsedalek, 1912). These have been named Palmen's organ and are cuticular secreted nodules at the juncture of four trachae on the mid-dorsal line just behind the eyes.

As for the yellow pigment granules: their general appearance resembles the pterinosomes, as described from the amphibian *Hyla cinerea* (Bagnara and Hadley, 1973), and from fishes (Matsumoto and Obika, 1968). These pterinosomes are spheroid in shape and composed of concentrically arranged membranes, with the pigment (pteridines) enclosed within the inner membranes. In invertebrates, tegumental pigment granules have been observed in the wings of butterflies, in Odonata and in Crustacea (Descimon, 1965; Green and Neef, 1969; Veron et al., 1974). With age there are changes both in the density and the size of the granules; therefore, it appears that development of the granules proceeds in several stages, one of which entails the formation of a new granule and its enlargement by growth within the interspaces between the membranes. Participating in the formation of such granules are the Golgi apparatus and the endoplasmic reticulum (Toda and Fitzpatrick, 1972; Bagnara, 1972). Our findings suggest that the granules develop in synchrony with ontogeny of the insect from the pupal stage to somewhat beyond the eclosion of the imago. Their final dimensions are dependent on, or determined by, the insect caste (worker or queen), or its sex (female or male) where there are, of course, also species-specific differences. The granules gradually

become shorter and lose their characteristic shape with increasing age of the insect, by their constant exposure to illumination, or to certain drugs. Under the effect of colchicine, for instance, there are degenerative changes of the pigment granules' diameter at a faster rate than that due to aging and its subsequent breakup into subunits. Interestingly, the feeding of caffeine (or purine) together with colchicine prevents the breakdown of the granules without altering the longevity of the insect. Apparently the various xanthines in some way prevent the destruction of the membranous system of the granules by colchicine. The mode of action of xanthines in hornets is as yet unclear. In addition to the phenomena associated with elevated cAMP levels, it is possible that the stereo-electronic structure of the metabolized methylated alkaloid partially resembles that of juvenile hormone (as in the distance between the two methyl groups and the keto group on the pyrimidine ring). It might therefore act to block the receptor to juvenile hormone. The disappearance of the YGs caused by feeding with allopurinol is to be attributed to its inhibitory activity on the enzyme xanthine oxidase; whereby the oxidation of various pterins is inhibited and consequently there is no formation of such products as xanthopterin, isoxanthopterin, and leucopterin, (all of which are dyes). In other words, there is inhibition of the formation of pigment granules from uncolored precursors. These and like pigments were detected in hornets (Ikan and Ishay, 1967). Mutant hornet males lacking the yellow pigment also lack the oxidized derivatives (which are present in the normal males), which suggests that the mutation relates apparently to a deficiency or impairment in the activity of xanthine oxidase (Croitoru et al., 1977). Conversely, the presence of excess yellow pigment stems probably from overactivity of this enzyme at the expense of melanin. Bagnara et al. (1979) in vertebrates and Schliwa and Euteneur (1979) have reported that in invertebrates pigment granules contain several types of pigment like purines, pteridines, melanins, and others.

The yellow pigment granules (organelles) in social wasps deserve to be called xanthosomes because they are located within chromatophores that contain only yellow pigment, apparently of one single type (xanthophores). These pigment granules are apparently fixed more or less in their position, without much degree of freedom, in contrast to those reported in Odonata (Veron et al., 1974), where some pigment bodies migrate during intermediate phases of color change.

The brown stripes of the cuticle, respond similarly to the yellow stripes, to various physical treatments like photo and thermoconductivity, i.e., they are endowed with the same or similar electrical properties, but with one noticeable difference: these properties are not influenced (or are less influenced) by the different drugs used by us in the various

experiments. As we have shown, the drugs tended to influence the integrity, size or shape of the yellow pigments, and the resultant changes were usually correlated with photo- or thermoconductivity.

The fact that there are two types of cuticular interaction with light - in one of which the resistance rises up to a saturation value, i.e., positive photoconductivity, while in the other the cuticle immediately behaves as a photoconductor, i.e., negative photoconductivity - leads to the conclusion that it is essential to verify which energy of the incident light induces the rise in the resistance and which energy gives the transition of carriers when the yellow stripe behaves as a photoconductor. By correlating the increase in resistance with the wavelength of the incident light it was found that UV light is responsible for that increase, whereas the photoconductivity which occurs after the saturation is dependent on violet and blue light. These findings suggest that molecular changes must take place because of the energies involved. A lower energy of the light is responsible for free carriers in the photoconduction process.

We may therefore assume that the electrical conductivity, as well as other properties of the yellow stripes, are due to the contained YG. We believe that the described tympanic organ in the hornet head is 'involved' in geotropic orientation, whereas such yellow pigment as is dispersed throughout the body integument is 'involved' in phototaxis, and enables the conversion from phototaxis to geotaxis in these animals, a phenomenon assumed to occur in all Pterigota (Linsenmair-Ziegler, 1970).

Acknowledgments

We thank Mr. Jacob Delarea for his excellent technical assistance in the electron microscopic study, and Ms. Stella Levi-Weitman for her time and help in preparing the manuscript.

References

- Bagnara JT. (1972). Interrelationship of melanophores, iridophores, and xanthophores, in: Pigmentation: Its genesis and biologic control, V. Riley (ed), Appleton-Century-Craft, NY, 171-180.
- Bagnara JT, Hadley ME. (1973). Chromatophores and color change. Prentice-Hall Inc. Englewood Cliffs, NJ, 202 pp.
- Bagnara JT, Matsumoto J, Ferris W, Frost SK, Turner Jr WA, Tchen TT, Tayler JD. (1979). Common origin of pigment cells. Science 204, 410-415.
- Bassett CAL, Becker RO. (1962). Generation of electric potentials by bone in response to mechanical stress. Science 137, 1063-1064.
- Becker E. (1937). Über das Pteridinpigment bei Insekten und die Färbung und Zeichnung von *Vespa* in besonderen. Z. Morph. Ökol. Tiere 32, 672-751.
- Bodenheimer FS. (1933). Über die Aktivität von *Vespa orientalis* in Jahresverlauf in Palästina. Zool. Anz. 102, 135-140.
- Busnell RG. (ed.) (1963) Acoustic Behavior of Animals. Elsevier, New York, 933 pp.
- Chapman RF. (1969). The Insects Structure and Function. English Universities Press Ltd., London, 819 pp.
- Cope FW. (1975). A review of the application of solid state physics concepts in biological systems. J. Biol. Phys. 3, 1-41.
- Croituru N, Ishay JS, Perna B, Ikan R. (1977). Photoelectric properties on the yellow stripes of the social wasp cuticle. The XI Scientific Meeting of the Association for the Advancement of Science in Israel: p. 69; Dept. Chem. Bar-Ilan Univ., Ramat Gan.
- Croituru N, Ishay JS, Arcan L, Perna B. (1978). Electrical resistance of the yellow stripes of social wasps under illumination. Photochem. Photobiol. 28, 265-270.
- Descimon H. (1965). Ultrastructure et pigmentation des écailles des Lépidoptères. J. Microscopie 4, 130.
- Dethier VG. (1963). The Physiology of Insects Senses. Methuen and Co. Ltd., London, 266 pp.
- Edwards R. (1980). Social Wasps. Their biology and control. Rentokil Limited, East Grinstead, 398 pp.
- Green JP, Neef MR. (1969). Fine structure of the fiddler crab epidermis. Biol. Bull. 137, 401.
- Gross J. (1903). Über das Palmensche Organ der Ephemeren. Zool. Jb (Anal). 19, 91-106.
- Guiglia D. (1972). Les Guêpes Sociales (Hymenoptera: Vespidae) d' Europe Occidentale et Septentrionale. Masson et Cie, Paris, 181 pp.
- Gutmann F, Lyons E. (1967) Organic Semiconductors. John Wiley and Sons, New York, 858 pp.
- Hannay NB. (1959). Semiconductors. Reinhold Pub. Corp. New York, 42 pp.
- Harmsen R. (1966). A quantitative study of the pteridines in *Pieris brassicae* L. during post-embryonic development. J. Insect Physiol. 12, 9-22.
- Haskell PT. (1961). Insect Sounds. H.F. and G. Witherby Ltd., London, 189 pp.

22. Ikan R, Ishay J. (1967). Pteridines and purines of the queens of the oriental hornet *Vespa orientalis*. J. Insect Physiol. 13, 159-162.
23. Ishay J. (1964). Observations sur la biologie de la guêpe Oriental *Vespa orientalis* in Israel. Insectes Sociaux 11, 193-206.
24. Ishay J. (1975). Hornet nest architecture. Nature 253, 41-42.
25. Ishay J. (1976a). Comb building by *Vespa orientalis*. Anim. Behav. 24, 72-76.
26. Ishay J. (1976b). Geotaxis of hornet workers and its improvement during the first days of life as an adult. Insectes Sociaux 23, 243-252.
27. Ishay J, Croitoru N. (1978). Photoelectric properties of the yellow stripes of social wasps. Experientia 34, 340-342.
28. Ishay J, Sadeh D. (1975). Direction finding of hornets under gravitational and centrifugal forces. Science 190, 802-804.
29. Ishay J, Sadeh D. (1977). Geotropism of hornet comb construction under persistent acceleration. Behav. Ecol. and Sociobiol. 2, 119-129.
30. Ishay JS, Shimony (Benshalom) T. (1982). Changes in yellow pigment of the Oriental hornet *Vespa orientalis*, induced by aging, mutations and allopurinol (Hymenoptera Vespidae). Entomol. Gen. 7, 317-325.
31. Ishay JS, Shimony (Benshalom) T. (1983). Electrical resistivity in cuticle of Oriental hornet queen before, during and after hibernation: Evidence for electronic conductance. Physiol. Chem. Phys. 15, 289-310.
32. Ishay JS, Shimony (Benshalom) T. (1986). Tympanic organ in social wasps (Vespinae). Monitore Zoologico Italiano. (in press)
33. Ishay J, Bytinski-Salz H, Shulov A. (1967). Contributions to the bionomics of the Oriental Hornet *Vespa orientalis* in Israel. J. Entomol. 11, 45-106.
34. Ishay JS, Perna B, Hochberg Y, Goldstein (Asanta) M. (1980). Photoelectric properties of the 'yellow stripes' in *Vespa orientalis*: A mathematical model. Bull. Math. Biol. 42, 681-689.
35. Ishay JS, Shimony TB, Schechter OS, Brown MB. (1981). Effects of xanthines and colchicine on the longevity, photoconductive properties and yellow pigment structure of the Oriental hornet *Vespa orientalis*. Toxicology 21, 129-140.
36. Ishay JS, Shimony (Benshalom) T, Lereah Y, Doby T. (1982a). Temperature dependence of electrical resistance of hornet and ant cuticle in low temperature. I. Direct current measurements. Physiol. Chem. Phys. 14, 343-361.
37. Ishay JS, Afek AB, Brown MB. (1982b). Allopurinol and theophylline: Their effect on longevity, fertility and behavior of hornets kept under different light and crowding conditions. Comp. Biochem. Physiol. 73C, 57-65.
38. Ishay JS, Shimony TB, Brown MB. (1982c). Temperature-dependent electrical resistivity of the hornet cuticle: Changes induced by colchicine and purine in the diet. J. Insect Physiol. 22, 21-27.
39. Ishay JS, Shimony (Benshalom) T, Arcan L. (1983). The presence of statocysts and statoliths in social wasps (Hymenoptera, Vespinae). Life Sciences 32, 1711-1719.
40. Keeton WT. (1967). Biological Science. W.W. Norton, New York, 955 pp.
41. Kemper H, Dohring E. (1967). Die sozialen Faltenwespen Mitteleuropas. Verlag Paul Parey, Berlin, 180 pp.
42. Kikkawa H, Ogita Z, Fujito S. (1955). Nature of pigments derived from tyrosine and tryptophan in animals. Science 121, 43-47.
43. Kittel C. (1968). Introduction to Solid State Physics. John Wiley and Sons, New York, 599 pp.
44. Lang D, Yonge CM. (1935). The function of the tegumental glands in the statocyst of *Homarus vulgaris*. J. Mar. Biol. Assoc. U.K. 20, 333-339.
45. Linsenmair-Ziegler C. (1970). Vergleichende Untersuchungen zum photo-geotaktischen Winkeltransponieren pterygoter Insekten. Z. Vergl. Physiologie 68, 229-262.
46. Marcus H. (1956). Über Sinnesorgane bei Articulaten. Z. Wiss. Zool. 159, 225-254.
47. Mason WP. (1966). Crystal Physics of Interaction Processes, Academic Press, New York, 354 pp.
48. Matsumoto J, Obika M. (1968). Morphological and biochemical characterization of goldfish erythrocytes and their pterinosomes. J. Cell. Biol. 39, 233-250.
49. Meglitsch PA. (1972). Invertebrate Zoology. Oxford University Press, Oxford, 961 pp.
50. Morris RW, Kittleman LR. (1967). Piezo-electric property of otoliths. Science 158, 368-370.
51. Rosenzweig E, Fuchs C, Ishay JS. (1985). Electrical resistance of hornet cuticle: Changes induced by xanthines - A statistical model. Physiol. Chem. Phys. 17 (4) 435-449.
52. Ryvkin SM. (1964). Photoelectric Effects in Semiconductors. Consultants Bureau, New York, 186 pp.

53. Schliwa M, Euteneuer U. (1979). Hybrid pigment organelles in an invertebrate. *Cell Tissue Res.* 196, 541-543.
54. Schone H. (1971). Gravity receptors and gravity orientation in Crustacea, in: *Gravity and the Organism*, S.A. Gordon, M.J. Cohen (eds), The University of Chicago Press, Chicago, 223-235.
55. Schwartzkopff J. (1974). Mechanoreception. In: *The Physiology of Insecta*, M. Rockstein (ed.) Vol. II, Academic Press, London, 273-352.
56. Shamos MH, Lavine LS. (1967). Piezo-electricity as a fundamental property of biological tissues. *Nature* 213, 267-269.
57. Shimony TB, Ishay JS. (1981a). Pigment granules in the tegumental yellow strips of social wasps: A scanning electron microscope study. *Z. Mikrosk. Anat. Forsch.* 95, 310-319.
58. Shimony TB, Ishay JS. (1981b). Thermoelectric (Seebeck) effect of the cuticle of social wasps. *J. Theor. Biol.* 92, 497-503.
59. Shimony TB, Ishay JS. (1984). Electrical capacitance in hornet integument. Frequency, light and temperature dependence; Possible p-n junction effects. *Physiol. Chem and Physics* 16, 333-349.
60. Spradbery JP. (1973) *Wasps*. Sidgwick and Jackson, London, 408 pp.
61. Toda K, Fitzpatrick TB. (1972). Ultra-structural and biochemical studies of the formation of melanosomes in the embryonic chick retinal pigment epithelium, in: *Pigmentation: Its genesis and biological control*. V. Riley (ed), Appleton-Century-Crofts, NY, 125-144.
62. Veron JEN, O'Farrell AF, Dixon B. (1974). The fine structure of Odonata chromatophores. *Tissue and Cell* 6, 613-626.
63. Watson HA. (1969). *Electrical conduction in Semiconductors. Devices and Their Circuit Applications*. McGraw Hill Inc., New York, 4, 62-94 .
64. Wodsedalek JE. (1912). Palmen's organ and its function in nymphs of the Ephemeridae, *Heptagenia interpunctata* (Say) and *Ecdyurus maculipennis* (Walsh). *Biol. Bull.* 22, 253-272.
65. Ziegler I, Harmsen R. (1969). The biology of pteridines in insects. *Adv. Insect Physiol.* 6, 139-203.
66. Zilberstein RM. (1972). Piezoelectric activity in invertebrate exoskeletons. *Nature* 235, 174-175.

Editor's Note: All of the reviewers' concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.