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HISTOCHEMISTRY OF THE NEUROSECRETORY CELLS OF THE PARS INTERCEREBRALIS OF THE DRAGONFLY, *ORTHETRUM CHRYSIS* (SELYS) (ANISOPTERA: *LIBELLULIDAE*)

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The neurosecretory cells, A, B and C, of the pars intercerebralis in *O. chrysis* (Selys) differ from one another in their neurosecretory material (NSM). The cerebral NSM is a muco-proteinaceous complex with glycogen entirely absent. Arginine is present in the NSM of all the above neurosecretory cells. However, the NSM of A cells contains a higher concentration of proteins having cystine or cysteine amino acids than that of the C cells; while the NSM of B cells contains virtually only those proteins which are rich in basic amino acids with negligible amounts of cystine or cysteine amino acids.

INTRODUCTION

Some workers are of the opinion that the histologically recognized cell-types represent different phases of activity of one and the same neurosecretory cell (THOMSEN, 1954; NAYAR, 1955; GIRARDIE & GIRARDIE, 1966, 1967; GUNDEVIA & RAMAMURTY, 1972); while others consider that the cell-types are distinct cells differing from one another in the chemical composition of the neurosecretory material produced by them (JOHANSSON, 1958; HIGHNAM, 1961; EWEN, 1962b; HINKS, 1971; PRENTØ, 1972). The neurosecretory cells in dragonflies have been histologically studied and classified (SCHALLER & MEUNIER, 1968; CHARLET, 1972), but there is no account regarding the nature of the neurosecretory cells as to whether they show different phases of activity of a single cell or differ from one another in their NSM. The present study was, therefore, undertaken to decide the nature of the neurosecretory cells in *Orthetrum chrysis*.

MATERIALS AND METHODS

Adult dragonflies were collected from the Nagpur University Campus. The head capsules were punctured and later placed into aqeous Bouin's fluid for 15-18 hours. The cephalic neurosecretory organs were dissected out in 70% ethyl alcohol, dehydrated and embedded in paraffin wax in the usual way. Serial sections were cut at 4 mμ and stained using one of the following techniques:

(1) Bergmann's chromalum - haematoxylin phloxine: CHP (PEARSE, 1968),
(2) Ewen's aldehyde fuchsin: AF (EWEN, 1962a),
(3) Delphin's alcian blue-phloxine: ABP (DELPHIN, 1965),
(4) Paget's aldehyde thionin: ATH (PAGET, 1959), and
(5) Heidenhain's Azan: Azan (GURR, 1962).

The fixatives and staining methods for histochemical detection of carbohydrates, nucleic acids, proteins and lipids are given in Table I together with the results.

OBSERVATIONS

There are two groups of neurosecretory cells on either side of a root of the medial ocellar nerve in the anterodorsal region of the pars intercerebralis medialis. On the basis of the criteria given by NAYAR (1955); JOHANSSON (1958); HIGHNAM (1961); EWEN (1962b) and DELPHIN (1965) the neurosecretory cells are classified into three categories: A, B and C.

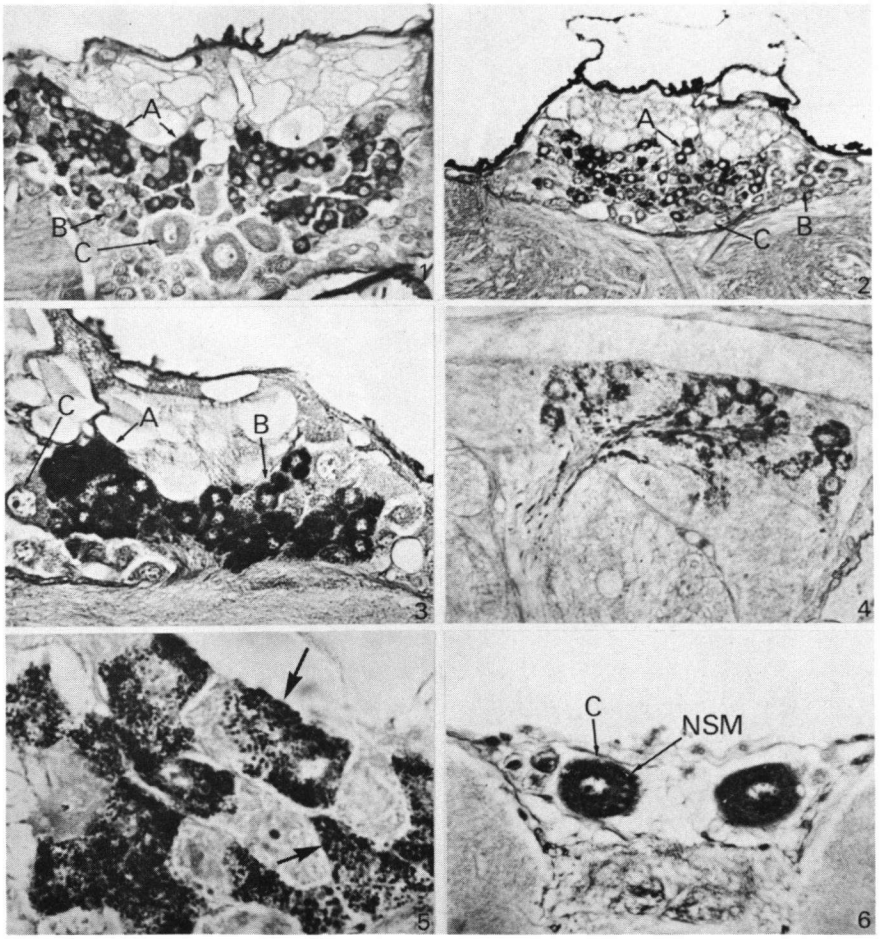
The neurosecretory material of A cells stains blue-black with CHP, dark purple with AF, blue with ABP, purple with ATH and bright red with Azan. The NSM in the cytoplasm of the A cells appears as granules or colloids with CHP, AF, ATH and ABP (Figs. 1-4) and droplets of varying size in Azan stained sections. (Fig. 5)

The NSM of B cells stains reddish with CHP and ABP (phloxinophils), greenish with AF and ATH and faint bluish with Azan. The NSM is clearly evident in the form of granules in the cell bodies and their axons after staining with phloxine.

The C cells differ from the A and B cells in their staining affinities as they show the amphibious type of tinctorial staining reaction with the routine techniques. The NSM of C cells stains blue with CHP, purple (or sometimes brown) with AF and ATH, bluish with ABP and pinkish in Azan stained sections and their cytoplasm stains with the counter stains of these techniques. The C cells are larger in size than the A and B cells (Fig. 6).

HISTOCHEMICAL OBSERVATIONS

The results of the histochemical studies are given in Table I.



Figs. 1-6. Sections through the pars intercerebralis of adult *Orthetrum chrysis*: (1) CHP, 265 ×; – (2) AF, 215 ×; – (3) ABP, 430 ×; – (4) ATH, 430 ×; – (5) Azan, 1350 ×; – (6) CHP, 430 ×.

PAS–Positive substances

The cytoplasm as well as the axonal tracts of the A, B and C cells show the presence of PAS-positive substances (Fig. 7). The alternate sections, treated with Schiff's reagent without prior oxidation, give a negative reaction, suggesting the absence of free aldehyde groups. No PAS-positive material is observed after acetylation but deacetylation restores the material in the cytoplasm, suggesting the presence of 1-, 2 glycol groups.

Table I

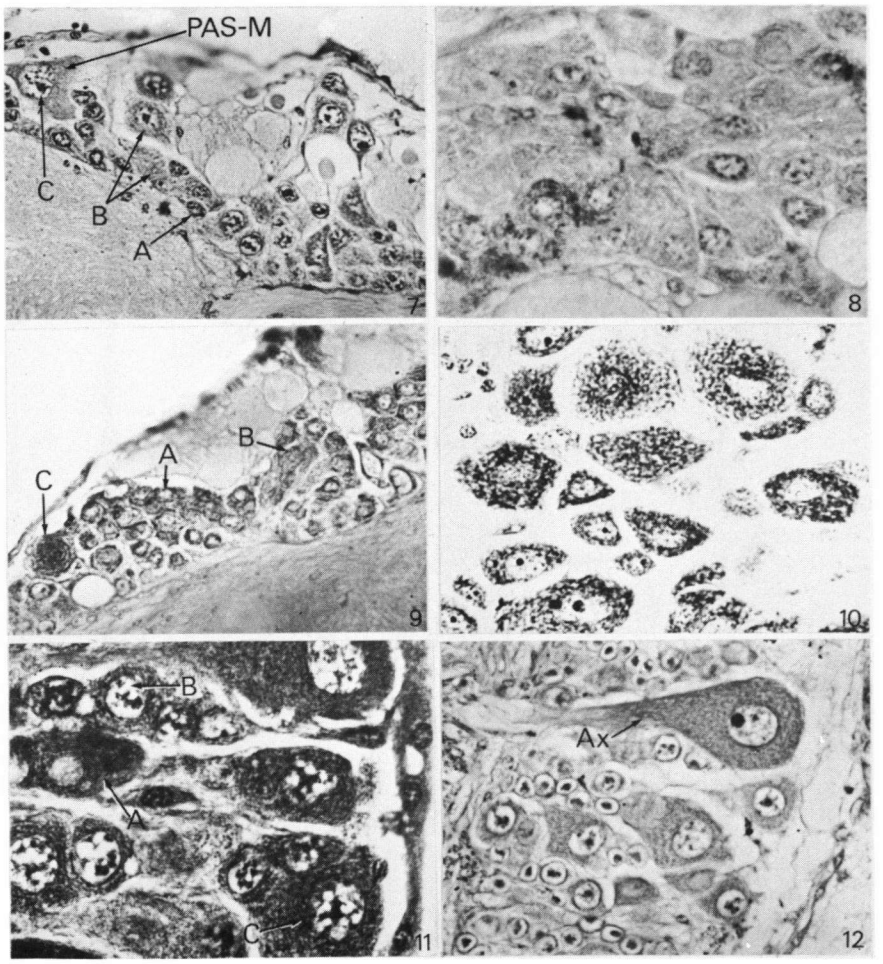
Histochemistry of the neurosecretory cells of the pars-intercerebralis of adult *Orthetrum chrysis*

Staining technique	Fixative	Staining reaction		
		A cells	*B cells*	*C cells*
Periodic Acid-Schiff's (PAS) (Hotchkiss, 1948)[1]	Bouin/Carnoy	+	+	+
Schiff's without PA (Hotchkiss, 1948)[1]	Carnoy	–	–	–
PAS/acetylation (Lillie, 1954)[1]	Carnoy	–	–	–
PAS/deacetylation (Lillie, 1954)[1]	Carnoy	+	±	+
PAS/saliva (GURR, 1962)	Carnoy	+	±	±
PAS/Methanol-chloroform (Bonhag, 1955)[1]	Carnoy	–	–	–
Best carmine (McManus & Mowrey, 1958)[1]	Carnoy/Lison "Gendre fluid"	–	–	–
Bauer Feulgen (McManus & Mowry, 1958)[1]	Carnoy/Lison "Gendre fluid"	–	–	–
Aqueous-thionin (GURR, 1962)	Carnoy	–	–	–
Alcian blue (Aqueous) (Mowry, 1956)[1]	Carnoy	–	–	–
Feulgen/hydrolized (PEARSE, 1968)	Lillie AAF	+ (Nuclei)	+	+
Feulgen/unhydrolized (PEARSE, 1968)	Lillie AAF	–	–	–
Azure B (Flax & Himes, 1952)[2]	Lillie AAF	+	+	+
Azure B/ribonuclease (Flax & Himes, 1952)[2]	Lillie AAF	–	–	–
Toluidine blue (TB) (Brachet, 1953)[2]	Lillie AAF	+	+	+
TB/ribonuclease (Brachet, 1953)[1]	Lillie AAF	–	–	–
TB/perchloric acid (Brachet, 1953)[1]	Lillie AAF	–	–	–
Mercury-bromophenol blue (Hg-BPB) (Mazia et al., 1953)[1]	Carnoy	+	+	+
Hg-BPB/pepsin (Mazia et al., 1953)[1]	Carnoy	–	–	–
Ninhydrin-Schiff's (N-S) (Yasuma & Ichikawa)[1]	Carnoy	±	++	+
Acidified permanganate-AF (P-AF) (Ewen, 1962a)	Carnoy/aqueous Bouin	+++	–	+
Acidified permanganate-methylated-AF (P-M-AF) (PRENTØ, 1972)	Carnoy	+++	–	+
Acidified permanganate-aniline/acetic acid-AF (P-A-AF) (PRENTØ, 1972)	Carnoy	+++	–	+
Performic acid alcian blue (PAAB) (Adams & Sloper, 1956)[1]	Carnoy	+++	+	++
Ferric-ferricyanide (Adams, 1956)[1]	Carnoy	+++	±	+
Neotetrazolium (PEARSE, 1968)	Carnoy	++	±	+
Sakaguchi's reaction (PEARSE, 1968)	Carnoy	+	+	+
Millon reaction (PEARSE, 1968)	Carnoy	–	–	–
Sudan black B (SBB) (Chiffelie & Putt, 1951)[2]	Calcium formol	–	–	–
SBB/pyridine (Baker, 1946)[1]	Weak Bouin's	–	–	–
Acid haematin (AH) (Baker, 1946)[1]	Calcium formol	±	–	–
AH/pyridine (Baker, 1946)[1]	Weak Bouin's	–	–	–
Digitonin reaction for cholesterol (GURR, 1962)	NIL	–	–	–

[1] Reference in PEARSE (1968); – [2] Reference in HUMASON (1962); – The intensity of the reaction is indicated by the following symbols: negative –, nonspecific ±, weak +, intermediate ++, and intense +++.

The intensity of the reaction is not reduced by treatment with saliva. The methanol-chloroform extraction also does not affect the intensity of PAS-positive material. The above findings suggest the absence of glycogen, carbohydrates-lipids, -glycolipids or -phospholipids complexes.

The neurosecretory cells and the axonal tracts give a negative reaction with Best's carmine and Bauer's Feulgen tests, suggesting the absence of glycogen in the neurosecretory material.



Figs. 7-12. Sections through the pars intercerebralis of adult *Orthetrum chrysis*: (7) PAS, 430 ×; – (8) Feulgen, 400 ×; – (9) Azure B, 380 ×; – (10) Toluidine blue, 640 ×; – (11) BPB, 960 ×; – (12) Ninhydrin Schiff, 575 ×.

Aqeous thionin and alcian blue without pre-oxidation with acidic potassium permanganate or performic acid give a negative reaction indicating the absence of acid mucopolysaccharides.

Deoxyribonucleic acid (DNA)

The nuclei of A, B and C neurosecretory cells stain positively with the Feulgen test (Fig. 8). The cytoplasm gives a negative result. The unhydrolysed sections give a negative reaction in the nuclei as well as in the cytoplasm.

Ribonucleic acid (RNA)

The nucleoli and the cytoplasm stain positively with Azure B (Fig. 9) and toluidine blue (Fig. 10). Treatment with ribonuclease followed by the above staining gives negative reactions in nucleoli and cytoplasm. Incubation of sections with perchloric acid gives a negative reaction with toluidine blue. All the above tests confirm the presence of RNA in the nucleoli and the cytoplasm of the neurosecretory cells.

Proteins

The neurosecretory cells of the pars intercerebralis stain positively with mercury bromophenol-blue (Hg-BPB). The NSM in the cell bodies and in their axons stains deeply (Fig. 11). Pre-treatment with pepsin reduces the staining reaction, confirming that the original staining reaction was due to the presence of proteinaceous material.

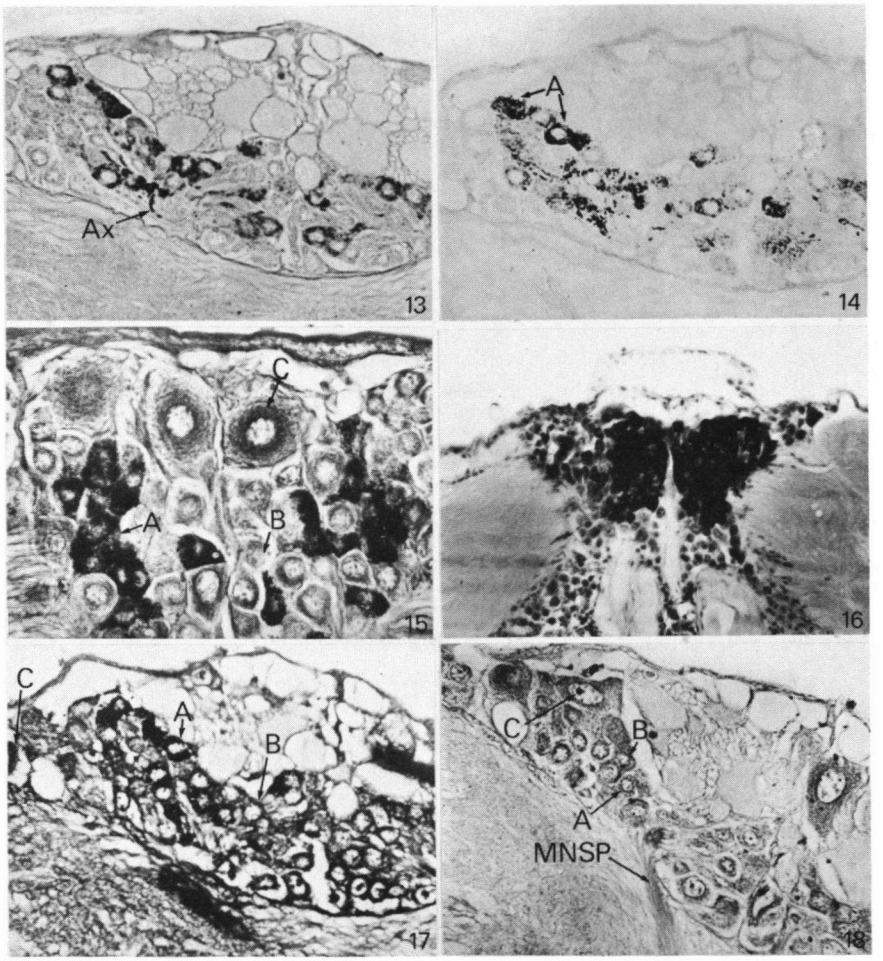
(1) *Basic amino acids*. The Ninhydrin Schiff's method for protein bond – NH group gives a very faint reaction (Fig. 12).

(2) *Cystine or cysteine*. Sections stained with acidified potassium permanganate aldehyde-fuchsin (PAF) were alternately subjected to the following modifications:

(a) The sections stained with P-AF after methylation in hydrochloric acid/methanol (P-M-AF) at 60°C for 15 minutes, demonstrate the presence of stainable purple inclusions in the cytoplasm of the A cells only (Fig. 13). The B cells do not show stainable material. The perikarya of the C cells show a few purple-stained granules.

(b) The sections stained with P-AF after treatment with aniline/acetic acid (P-A-AF) at 40°C for 30 minutes give a similar reaction (Fig. 14) as that observed with the P-M-AF treatment.

(c) A specific staining technique such as performic acid/alcian blue (PAAB) (Fig. 15) gives a strong reaction with A cells and a moderate one with C cells. The B cells also contain a few granules.



Figs. 13-18. Sections through the pars intercerebralis of adult *Orthetrum chrysis*: (13) P-M-AF, 380×; – (14) P-A-AF, 380 ×; – (15) PAAB, 480 ×; – (16) Ferric-Ferricyanide, 190×; – (17) Neotetrazolium salt method, 380 ×; – (18) Sakaguchi, 380 ×.

(d) The specific reactions with the ferric-ferricyanide (Fig. 16) and the neotetrazolium slat (Fig. 17) methods give strong reactions with the A cells, moderate with the C cells and only a few granules are stained in the cytoplasm of the B cells.

(3) *Arginine*. The specific staining reaction with Sakaguchi's naphthol-hypochlorite method (Fig. 18) shows a moderate distribution of arginine in the neurosecretory cells.

(4) *Tyrosine*. Millon's specific reaction for protein containing tyrosine gives a negative reaction in the neurosecretory cells.

Lipids

The neurosecretory cells of the pars intercerebralis do not stain with Sudan black B, acid haematin or Nile blue staining methods, suggesting the absence of lipids, phospholipids and neutral lipids from the cerebral NSM.

Cholesterol

The specific digitonin reaction does not appear in the neurosecretory cells, suggesting the absence of cholesterol.

DISCUSSION

Although the neurosecretory cells have been classified on the basis of their staining peculiarities into A and B cells, it is not yet clear whether they produce different or similar neurosecretory material. NAYAR (1955) believed that the B cells are actually A cells, whose stainable material has been discharged. The same view was proposed earlier by THOMSEN (1954) who claimed to have observed red and blue material in one and the same cell. He thus concluded that the A and B cells represent two different phases of activity of one cell type. DE LERMA (1956); BRANDENBURG (1956); HERLANT-MEEWIS & PAQUET (1956); KOPF (1957); MATSUMOTO (1956) and PASSANO (1954) have supported the view expressed by THOMSEN (1954) and NAYAR (1955).

GIRARDIE & GIRARDIE (1966, 1967), while studying the fine structure of the neurosecretory cells in the brain of *Locusta migratoria*, noticed intermediate stages between type A and B cells and concluded that type A and B cells are opposed secretory phases. In ants, GAWANDE (1968) considered the A and B cells as the active and inactive phases of the same cell. GUNDEVIA & RAMAMURTY (1972) in their studies on *Hydrophilus olivaceus* are of the same opinion. These workers have observed an increase in the number of A cells and a decrease in the number of B cells during the breeding season and concluded that "they are probably one and the same, despite the differences between them in respect of nucleo-cytoplasmic ratio, shape and staining properties. These differences may be resulting merely from their different functional states".

JOHANSSON (1958), on the contrary, considered the A and B cells as different cell types and HIGHNAM (1961) supported this view. EWEN (1962b), in his studies on the neurosecretory cells of *Adelphocoris lineolatus*, writes that "this may be so in the pars-intercerebralis medialis of *A. lineolatus* where both types of cells occur and it is not possible to say that identical cells appear as type

B in all sections. However, this idea would not seem to hold for the B cells of the pars-intercerebralis lateralis and of other ganglia. In these B cells, no blue staining material was observed in any section i.e. no intermediate type of cell was seen and transformation of type A to type B or vice-versa seems unlikely".

HIGHNAM (1961) suggested that A and B are true neurosecretory cells while C are false neurosecretory cells. RAABE & MONJO (1970) have established histochemically the neurosecretory nature of C cells in the phasmid, *Clitumnus extradentatus*. CHALAYE (1967) demonstrated the presence of SH and SS groups in the B cells and recognized them as true neurosecretory cells.

Histochemical observations on neurosecretory cells A, B and C of *Orthetrum chrysis* suggest that the neurosecretory material is a complex of neutral muco-polysaccharides and proteins. The glycogen is entirely absent. The absence of glycogen from A, B and C cells in *O. chrysis* confirms the observations of NAYAR (1955); ARVY & GABE (1962); BANERJEE, DEY, SADHUKAN & CHOUDHURI (1968) and RAMADE (1969a, 1969b). The neurosecretory cells are rich in both RNA and DNA suggesting an equipped machinery for protein synthesis. A high concentration of arginine has been reported in the B cells of *Galleria* and *Ephestia* (REHM, 1955) but BANERJEE et al., (1968) observed the high concentration of arginine in both A and B cells of the grasshopper, *Acrida gigantea*. In *O. chrysis* arginine has been observed in all the neurosecretory cells, A, B and C.

SLOPER (1957); HIGHNAM (1961); ARVY & GABE (1962); PIPA (1962); NAISSE (1966); GABE (1966, 1967, 1972); NOVAK (1966); SCHREINER (1966); TANDAN & DOGRA (1966); RAMADE (1969a, 1969b) and BAUDRY & BAEHR (1970) have accepted that the stained material, whether a hormone or a carrier substance of the former, is histochemically a muco-proteinaceous complex having the cystine or cysteine amino acids. Recently HINKS (1971) and PRENTØ (1972), after conducting various histochemical tests on the neuro-secretory cells of the pars intercerebralis of *Triphaena promuba* and *Schistocerca gregaria* respectively, have concluded that the neurosecretory cells are distinct cell types, each producing chemically different NSM.

In *Orthetrum chrysis* both the A and C cells have a NSM rich in amino acids, cystine or cysteine, although the A cells have a higher concentration of proteins containing cystine or cysteine amino acids than the C cells. In contrast, the B cells either lack these amino acids or they are present in negligible amounts, but they are rich in basic amino acids.

Our present study clearly suggests that the A, B and C cells in the pars intercerebralis region of the brain of the dragonfly, *Orthetrum chrysis* are distinct cell-types producing chemically different NSM.

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