CANADIAN THESES ON MICROFICHE

THÈSES CANADIENNES SUR MICROFICHE

NAME OF AUTHOR / NOM DE L'AUTEUR

TITLE OF THESIS / TITRE DE LA THÈSE

UNIVERSITY / UNIVERSITÉ

DEGREE FOR WHICH THESIS WAS PRESENTED / GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE

YEAR THIS DEGREE CONFERRED / ANNÉE D'OBTENTION DE CE GRADE

NAME OF SUPERVISOR / NOM DU DIRECTEUR DE THÈSE

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Kenya East Africa

NL-91 (4-77)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

EDMONTON, ALBERTA

SPRING, 1975

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE PHEROMONE SYSTEMS OF MALES OF [illegible] (WALKER) AND OTHER NOCTUIDS (LEPIDOPTERA) submitted by John Russell Clearwater in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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External Examiner

Date March 10, 1975

ABSTRACT

The general form and scale surface of the paired, male pheromone producing scent organs of six noctuid species (Pseudaletia separata (Walker), Mamestra configurata (Walker), Peridroma saucia (Hubner), Agrotis orthogonia (Walker), Agrotis ypsilon (Walker) and Euxoa scandens (Smith) were examined and an hypothesis was developed to explain the evolution of scale form in this family.

The most complex system, the Stobbe's gland and hair pencil of M. configurata, was investigated in detail (fig. 70). The Stobbe's gland is a small group of 60 to 70 very large cells clustered around a duct formed by a bundle of scales. The hair pencil is a tract of specialized scales linked by a cuticularized lever to the second abdominal sternite. The distal half of each of these scales has a very unusual surface. Longitudinal ridges are drawn into sinusoidal waves, bound together where they approach each other by small blocks of cuticle. A very thin (120 Å) membrane forms the surface between the ridges. These substructures form an evaporative surface for pheromone release.

The major component of the male pheromone of *M. configurata* is 2-phenyl ethanol. Two smaller components were not identified. Benzaldehyde has been identified as the pheromone of *P. separata*.

Both Stobbe's gland and hair pencil begin development on the surface of paired lateral abdominal pouches in the exuvial pharate adult. The trichogen cells of the hair pencil primordium produce rapidly-elongating columns of cytoplasm surrounded by a fine membrane.

Once their full length of 3.5 mm is attained, the primary or cell defined exocuticulature is formed and the scale begins cuticularization. The hair pencil hard parts, the basal sclerite and the lever are formed by local sclerotization of the walls of the neobranch's pouch.

The trichogen cells of the Stobbe's gland rapidly hypertrophy with much of their later increase in size resulting from the formation of an extracellular cavity within each cell. Each mature gland cell extrudes large vesicles into its extracellular cavity.

These vesicles contain phenylethyl-β glycoside in *H. subflavescens* and benzyl-β glycoside in *H. zea* immediate precursors, respectively, of the pheromones 2-phenyl ethanol and benzaldehyde. The Stobbe's gland alone appears to be responsible for the synthesis of these compounds. L-phenylalanine, and more tentatively, cinnamic acid, are incorporated into the glycoside of *H. subflavescens* and may represent the start of the metabolic sequence. Both simple precursors are taken up most rapidly at that time in development when the wing scales begin to be pigmented.

The hydrolysis of this glycoside to the pheromone 2-phenyl ethanol is accomplished by an enzyme produced by the hair pencil cap cells (fig. 70). The enzyme seems to travel down the hairscales to make contact with the glycoside near the transition area (2).

ACKNOWLEDGEMENTS

I thank Prof. B. S. Heming most sincerely. My advisor has freely given me his time and advice on all matters, and has given me a thorough tuition in insect physiology. I am also grateful for generous financial support from Mr. Fred Hunt, N.S.E.R.C. and AMRI grant no. 55-515 to the late Prof. B. Herring. I have been very fortunate in gaining the interest and support of many people from several departments of this university. From the Department of Entomology, Prof. R. H. Gooding has assisted me with investigation of the enzyme of the hair pencil cap cells, Dr. B. K. Mitchell has taught me methods of electrophysiological recording, and Prof. D. A. Craig and Mr. G. Braybrook have been invaluable in producing quality scanning electron micrographs.

From the Department of Pharmacy, Prof. R. A. Locock and Mr. D. Odynski have assisted with chromatography and Mr. C. Ediss and Prof. L. Wiebe have supervised my experiments with radioisotopes. Prof. A. M. Hogg (Chemistry Department) has allowed me to use his recently-developed technique of chemical ionization mass spectrometry using ammonia. Mr. T. Budd and Mr. J. Olekszyk produced the spectra.

Prof. D. D. Cass of the Botany Department has allowed me frequent access to his Nomarski interference microscope.

I thank my supervisory committee, Prof. B. S. Heming, Prof. D. A. Craig and Prof. R. H. Gooding, and Prof. R. F. Ruth (Department of Zoology). Prof. T. K. Shnitka (Department of Pathology) and Dr. R. Hooper (Department of Entomology) served on my Candidacy committee.

Prof. [illegible] of the Department of Entomology, University of Manitoba, Mrs. [illegible] of the Canada Department of Agriculture Research Station, Lethbridge, Alberta, have given me greatly appreciated opportunities of working in their laboratories for short periods.

Mr. H. Philip (Alberta Department of Agriculture, Edmonton), Dr. A. P. Arthur (Agriculture Canada Research Station, Saskatoon, Saskatchewan) and Mr. E. Agnew (Department of Zoology, Victoria University, Wellington, New Zealand) have provided me with live insects.

Dr. D. Bundle (Department of Chemistry) synthesized authentic samples of benzyl-B-glucoside and phenylethyl-B-glucoside.

I thank all my fellow students for their helpful advice and criticism and, in particular, Mr. R. Lee and Mr. M. Jones. The typing of Mrs. S. Hamilton, Miss K. Benschop and Mrs. K. Baert has greatly assisted this project.

AUTOBIOGRAPHICAL NOTE

I was born on the 16th October 1947 in Gore, New Zealand. I have always been interested in living things, the first animals to engage my attention being the great octopi that lived under the wharves a few yards from my home. Fascination with birds and lizards followed, with frequent dissections of road-killed specimens upon my mother's kitchen table.

I began university study at Massey University, Palmerston North in the Botany and Zoology department. People influencing me at this time were Mr. L. Gurr who taught me how to observe animals, and Dr. D. Penny, a plant physiologist, who convinced me that biochemistry could offer penetrating insights into biology. Purchase of "The Principles of Insect Physiology" by Sir V. B. Wigglesworth during my second year of study, helped me select insect pheromones as a subject for experimental study.

Following a summer working on grass grub pheromones and ecology with Mr. R. Henzel and Mr. W. Kane at the Ruakura Agricultural Research Station, I began work with the complete pheromone system of *Pseudaletia separata*. I rapidly realized that not only did the work on the male system proceed more satisfactorily than that on the female, but that this field of study had been almost completely neglected. I began work on my doctoral program at the University of Alberta in August, 1971, and thanks to the excellent facilities and tuition, was able to satisfy longstanding ambitions to work with radioisotope and electrophysiological instruments, while Prof. B. Heming fostered a new interest in development.

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I. INTRODUCTION

Male lepidoptera have long been known to possess scent organs that are used during courtship. A review of the older literature by Mitchell (1914) revealed that all of these systems involve scale tufts, hair pencils provide a common means of transferring the chemical to the female of diurnal species. Three methods of producing airborne scales have been described: (a) fragmentation of elongated scales at preformed breaking points in *Pieris brassicae* (Jasper, 1967) and *Colias eurytheme* (Jasper, Jasper's report) (Linnaeus) and *Colias philodice eriphyle* (Poda) (Sollier, 1972), (b) pinching off of small outpocketings of the epicuticle of the scale in *Danaus gilippus berenice* (Cramer) (Fliske and Salpeter, 1971) and (c) release of whole minute scales in *Anthocharis cardamines* (Clerck) (Vane-Wright, 1972).

In contrast, all nocturnal species so far examined evaporate the scent from a highly dissected scale surface (Birch, 1970b; Grant and Brady, 1973; Weatherston and Percy, 1969). These scents, compounds of low molecular weight, have been isolated from the pheromone systems of several species (Aplin and Birch, 1968, 1970; Brady and Brand, 1972; Clearwater, 1971, 1972).

Stobbe (1912) first described the major components of a complex pheromone system in the Noctuidae. Birch (1970b, 1972) has recently redescribed this system, including details of muscle distribution and function, and of the variations in structure occurring within the family. The system consists of paired scent brushes (hair pencils), scent glands (Stobbe's glands) and storage pockets (fig. 70). The

[illegible] abdominal segment [illegible] modified to form the paired [illegible] and paired eversible [illegible] the brushes and spoolers for the attachment of muscles that operate the brushes.

The Stobbe's gland within the second abdominal segment produces a pheromone precursor (Birch, 1970b). Each gland consists of several cells opening into a scale duct which leads to the brush. In [illegible] (Walker) the secretory cycle of this gland consists of three phases (Clearwater and Sarafis, 1975). Though many pheromones have been isolated from the hair pencils, the nature of the secretions from the Stobbe's gland is unknown. Birch (1970b) has tentatively suggested that a β-glycoside is produced, preceding formation of benzaldehyde, the pheromone of *Lasiorhynchus barbarus* (Hübner).

Formation and storage of a glycoside is a common characteristic of insect endocrine systems (Happ, 1968; Roth and Stay, 1958) and of detoxification mechanisms (Mehendale and Borough, 1972). The defensive glands of *Pseudocerus longicollis* (LeConte) secrete ρ-diphenol glucosides. These substances are hydrolyzed to the free diphenol, and ρ-benzoquinones by enzymes associated with a cuticular organelle (Happ, 1968). Simple precursors (acetate, propionate and malonate) as well as aromatic precursors (tyrosine and phenylalanine) are readily incorporated into these benzoquinones. Meinwald, Koch, Rogers and Eisner (1966) proposed two pathways — one leading from the aromatics to ρ-benzoquinone, and the other leading from the simpler precursors to methyl-ρ-benzoquinone and ethyl-ρ-benzoquinone.

Other beetle pheromones are synthesized from simple precursors. Mitlin and Hedin (1974) have shown that the monoterpene pheromones

produced [illegible] (Coleoptera: Curculionidae) are derived from acetate, mevalonate and glucose. Feeding on the host terpene, myrcene, is a prerequisite for synthesis of ipsdienol and ipsenol in [illegible] (Germar) and [illegible] (Eichhoff) (Scolytidae) (Hughes, 1974).

The fat body has been shown to be an important centre for the formation of glycosides in insects (Hutton, 1962; Smith and Turbet, 1961).

2. MATERIALS AND METHODS

2.1 Experimental Animals

Collections were made of adult noctuids (*Mamestra configurata* (Walker) and *Pseudaletia separata* (Walker)) attracted to lights at the University of Alberta. Field-collected pupae of *M. configurata* were obtained from Sturgeon County and Peace River, Alberta. Laboratory-reared, diapause-free pupae of *M. configurata* were provided by the Canada Department of Agriculture Research Station, Saskatoon, Saskatchewan. A field collection of *Pseudaletia separata* (Walker) pupae from Napier, New Zealand was made and sent to me by Mr. F. Agnew.

Four species of noctuid were selected from a collection of 26 species obtained from Palmerston North, New Zealand in 1970 during research conducted for a Master's degree at Massey University. Dried specimens and methylene blue preparations of *Pseudaletia separata*, *Melanchra alcyone* (Hudson), *Prasa graminosa* (Walker) and *Rhapsa scotosialis* (Walker) were re-examined and reinterpreted.

2.2 Microscopy

2.2.1 Light microscopy

Abdominal sternites of *P. separata*, *M. alcyone* and *E. graminosa* were dissected out, stained in methylene blue, and mounted in DPX. Stobbe's glands were dissected from pharate and teneral adults of *P. separata* and *M. configurata*, washed in Pringle's saline (Cummins, Miller, Smith and Fox, 1965), and examined with a Zeiss Nomarski differential interference Photomicroscope II. Additional glands of

M. configurata were incubated for 15 minutes in 0.1% emulsin (Nutritional Biochemical Corp.) in 0.05 M acetate buffer at pH = 5 before examination.

2.2.2 Electron microscopy

Scales from the hair pencils of field-collected *P. separata*, *P. serratifer*, *M. configurata* and *P. praeclara*, and from 12 hour-old adults of *P. separata* and *M. configurata* were air dried and a layer of carbon and gold was evaporated onto their surfaces. Developing scales from pharate adults of *M. configurata* were fixed in 3% glutaraldehyde (Millonig's 1961 buffer), dehydrated in ethanol and transferred to pure amyl acetate through a series of graded steps in preparation for critical point drying and metal evaporation. All scales were examined with an S-4 Stereoscan scanning electron microscope (Cambridge Instrument Co.).

For transmission electron microscopy, scales and hair pencil caps from one-day-old males of *M. configurata* and *P. separata* were fixed in 3% glutaraldehyde (Millonig's 1961 buffer), post-fixed in similarly buffered 1% OsO_4, dehydrated in ethanol and embedded in Araldite. Gold sections cut on a Sorvall MT-2 ultramicrotome were found to be preferable to silver, as much of the scale tended to break out of the thinner sections. Sections were double stained in aqueous uranyl acetate and Reynolds' (1963) lead citrate before being examined with a Philips EM 200 electron microscope at 60 Kv.

2.3 Physiology

2.3.1 Chemistry of the pheromone from *Mamestra configurata*

The very small concentrations of pheromones present in insects demands the use of micro-analytical methods. Depending on the molecular size and volatility of the substance under investigation, either thin layer chromatography or gas-liquid chromatography may be used to separate the pheromone from the other components of the crude extracts, followed by mass spectrometry of the pure pheromone.

Extraction of the hair pencils of *M. configurata* with methylene chloride was carried out between 2:00 and 4:00 a.m.; about 30—40 hours after eclosion. The time chosen for extraction was less than one hour before the beginning of mating activity, when more than 90% of the males had retracted their hair pencils into their abdominal pouches. Less than 5% of the male moths had completed retraction when examined 10 hours after eclosion. One hundred and twelve males were used to prepare three solutions containing 60—70 hair pencils/ml.

All gas chromatographic analyses of these crude extracts were carried out on glass columns since stainless steel appeared to cause degradation of the major component of the extracts. Two six feet long glass columns were used; 3% OV-17 on Chromosorb G and 3% XE60 on Chromosorb W. The instability of this component also precluded the use of coupled gas-liquid chromatography (G.L.C.)—mass spectrometry so that a direct probe of the whole extract was carried out. Great care was taken to prevent contamination. All syringes were thoroughly rinsed with solvent, and checked with the gas chromatograph at the highest sensitivity used.

2.3.2 Chemistry of the glycosides from the Stobbe's gland

Small numbers of whole clean glands from advanced pharate adults of *M. configurata* and *P. leptastrea* were used for the isolation procedure. A ventral cut was made in the second sternite (SII) and the cut edges reflected laterally so that the duct scales could be grasped with micro-forceps where the duct emerges from the gland (fig. 70). Slight tension freed the gland from the enveloping fat body.

Extraction was carried out in micro-separating flasks made from Pasteur pipettes. For each species 2—10 glands were placed in 10 µl of distilled water in a flask and crushed with the beaded tip of a glass thread. Ten microlitres of chloroform were added and the extract stirred to complete partition of the components between the two layers. Strips of silica gel sheet with fluorescent indicator (Eastman Kodak No. 6060) were spotted with 1 µl of either crude extract, aqueous phase, non-polar phase or standard solutions, and run at room temperatures in n-butanol: acetic acid: water (4:1:1). The strips were examined under UV illumination. Crude extracts and the aqueous layers were analyzed on an AEI M.S. 12 mass spectrometer obtaining chemical ionization with NH_3 reagent gas.

2.3.3 Site of synthesis of the glycoside

The fat body of *M. configurata* was examined as a possible site for the synthesis. The entire fat body was removed from the abdomina of eight male and two female pupae at various developmental stages. Each fat body was homogenized separately in acetate buffer (0.08 M, pH = 5.0) and any glycosides present were hydrolyzed with 0.5% emulsin (Nutritional Biochemicals Corp.). Non-polar substances including the

aglycones of the β-glycosides were collected in a methylene chloride layer, which was analyzed on an OV-17 column at 200°C (Beckman G.C.5).

The ability of the Stobbe's gland of *M. configurata* to take up glycosides from the hemolymph was examined. Benzyl-β-glycoside, a close analogue of the natural precursor in *M. configurata*, was injected into the right pupal wing case. The dosage range was from 100 µg to 1 ng per animal. Male pupae of noctuids were estimated to produce 50—100 µg of glycoside per animal, based on reports of a maximum of 10--20 µg of aromatic pheromone per hair pencil in *A. segregata* and *A. subjuncta* (Clearwater, 1972; Grant, Brady and Brand, 1972). Each of six doses was replicated two or three times. The Stobbe's glands, fat body and dorsal thoracic musculature were removed from mature pupae and the aglycones examined with the same methods.

2.3.4 Incorporation of labelled precursors into phenylethyl-β-glycoside

L-phenylalanine-U-C^{14} (464 mCi/mM) from New England Nuclear, Boston Mass. and cinnamic acid — 2-C^{14} (4.0 mCi/mM) from ICN Isotope and Nuclear Division, Irving, Calif. had a radiochemical purity of 99.2% and 97% respectively (determined by thin layer chromatography and autoradiography).

Healthy pupae of *M. configurata* were selected and the degree of development noted (pigment deposition in the compound eye, sclerotization of the I tibial spine and antennae, and pigment deposition in the wing and dorsal abdomen were used as indices of development) (fig. 37).

A 5 µl syringe (Hamilton Co., Reno, Nev.) was used to inject

the labelled [illegible] phenylalanine (C^{14}) in 1.5% NaCl; 1 or 2 µl of [illegible] cinnamic acid (C^{14}) µl methanol into the right pupal wing case, while the insect was under CO_2 narcosis. The puncture was sealed with hot paraffin. No tyrosinase inhibitors were used. Mortality from the operation was less than 1% for insects injected with phenylalanine, but up to 40% for those injected with cinnamic acid.

Nine of the animals were allowed to eclose, and their hair pencils placed in 15 ml of Bray's solution. Stobbe's glands were dissected from 15 pharate adults, just before adult emergence. Each pair of glands was washed, and crushed separately in 10 µl of distilled water. The supernatant remaining after the cell debris had settled was spotted onto strips of silica gel (Eastman Kodak No. 6061). Twenty glands were chromatographed using n-butanol: acetic acid: water (4:1:1) (BAW) or chloroform: methanol (2:1) (Chl: MeOH) (after Happ, 1968) to distinguish between phenylalanine and phenylethyl-β-glycoside. The extracts from six other glands were chromatographed using benzene: acetic acid: methanol (4:1:2) (Be: A: MeOH) to distinguish between the glycoside and cinnamic acid. Two microlitres of labelled phenylalanine and 1 µl of labelled cinnamic acid were run with the relevant developing systems as standards for comparison with the Stobbe's gland extract. Each chromatogram was cut into strips and scraped into vials, with Cab-O-Sil and 15 ml Bray's solution. All vials were counted with a Picker 200 liquid scintillation counter. There was no measurable quenching according to the isotope channels ratio. Cold standards of each compound were run on silica gel with a fluorescent indicator (Eastman Kodak No. 6060). A Ninhydrin aerosol (Nutritional Biochemicals Corp.) was used to locate phenylalanine.

The remainder of the insect, after removal of the Stobbe's gland, was divided into the fore wings, the head, thorax, abdomen and hind legs and the genitalia, and combusted in a Packard Tricarb Model 306 sample oxidizer before scintillation counting.

2.3.5 Enzyme hydrolysis of the glycoside

Glands dissected from *M. confligurata* and *A. separata* were incubated in a solution of β-glucosidase. One small crystal of emulsin (Nutritional Biochemicals Corp.) was dissolved in 20 µl of 0.08 M acetate buffer (pH = 5.0) in which 6—15 glands had been crushed, and left for 24 hours at room temperature. Controls, lacking only the enzyme, were treated in an identical manner. The reaction was stopped by the addition of 20 or 30 µl's of methylene chloride and the compounds partitioning into the non-polar phase were chromatographed on 3% OV-17 and 5% F.F.A.P. columns. Coupled G.C. mass spectrometry (AEI M.S. 12 with a Varian Aerograph 1400 G.C.) was used to identify a major component of the extract from *A. separata*, while a direct probe was used for that of *M. confligurata*.

2.3.6 Assay of an hydrolytic enzyme from the hair pencil cap cells

The method of Gillespie, Jermyn and Woods (1952) was used to bioassay various tissues for the presence of a β-glycosidase. Adult moths of known age were dissected and the fat body, antennae, and the hair pencil cap homogenized in 20 µl acetate buffer. The presence or absence of the distinct odour of the hair pencil and the position of the hair pencil was noted (either folded in the intersegmental membrane where it develops [fig. 70-A] or in the hair pencil pouch [fig. 70-B]).

[illegible]

[illegible]

[illegible]

The triple system [illegible] was that of [illegible]. As [illegible], that scales [illegible] [illegible] underside of the [illegible] (fig. 1). At rest these scales are [illegible] under a long flap which is largely retracted [illegible] the apparent leading edge of [illegible]. Each strap-like scale expands into a spatulate tip, covered with fine, parallel longitudinal ribs (Rp) (fig. 2). An irregular line of depressions (Dp) is situated equidistant from the ribs and is joined to them by a herringbone pattern of fine ridges (R) (fig. 3). Many other smaller depressions are scattered between the ridges. When viewed from an oblique angle, the longitudinal ribs are seen to be heavily covered with ridges (fig. 4). The strap-like, main portion of the scale contains the same elements, organized with a lesser degree of symmetry (fig. 5).

This description is based on 5 specimens, one of which was examined with the scanning electron microscope.

3.1.2. A multi-component, alar and abdominal system — *Frana graminosa*.

The underside of the strap-like fore-wing of *Frana graminosa* bears a complex wing gland, covered at rest by the greatly expanded humeral lobe of the hind wing (fig. 6). This wing gland differs from that of *F. cuterebrella* in the presence of a tract of short, golden brown scales (Ss) present in addition to the group of overlying elongated scales (Es). Extracts of this wing system contain small amounts of

Plate 1*

Explanation of figures

1. [illegible]. Mar gland.

Underside of wing and thorax, showing the protective flap (FL) extending from the apparent leading edge of the wing (L) to the thorax. A tract of elongated scales (ES) emerge from the wing base. Scanning electron micrograph (SEM).

2. [illegible]. Tip of hair scale.

Spatulate tip of the elongated scales, showing parallel longitudinal ribs (R_1). (SEM).

3. [illegible]. Surface detail of tip.

Herring-bone pattern of ridges (R_2) joining ribs (R_1). Several depressions (D) present, the largest of which form a central line. (SEM).

4. *A. scutellaris*. Oblique view, surface detail of tip.

View showing the ridges (R_2) running up the side of the ribs (R_1). (SEM).

5. *A. scutellaris*. Surface detail of central portion of scale.

Pattern of the strap-like portion of the scale. (SEM).

*All material in plate 1 is air dried.

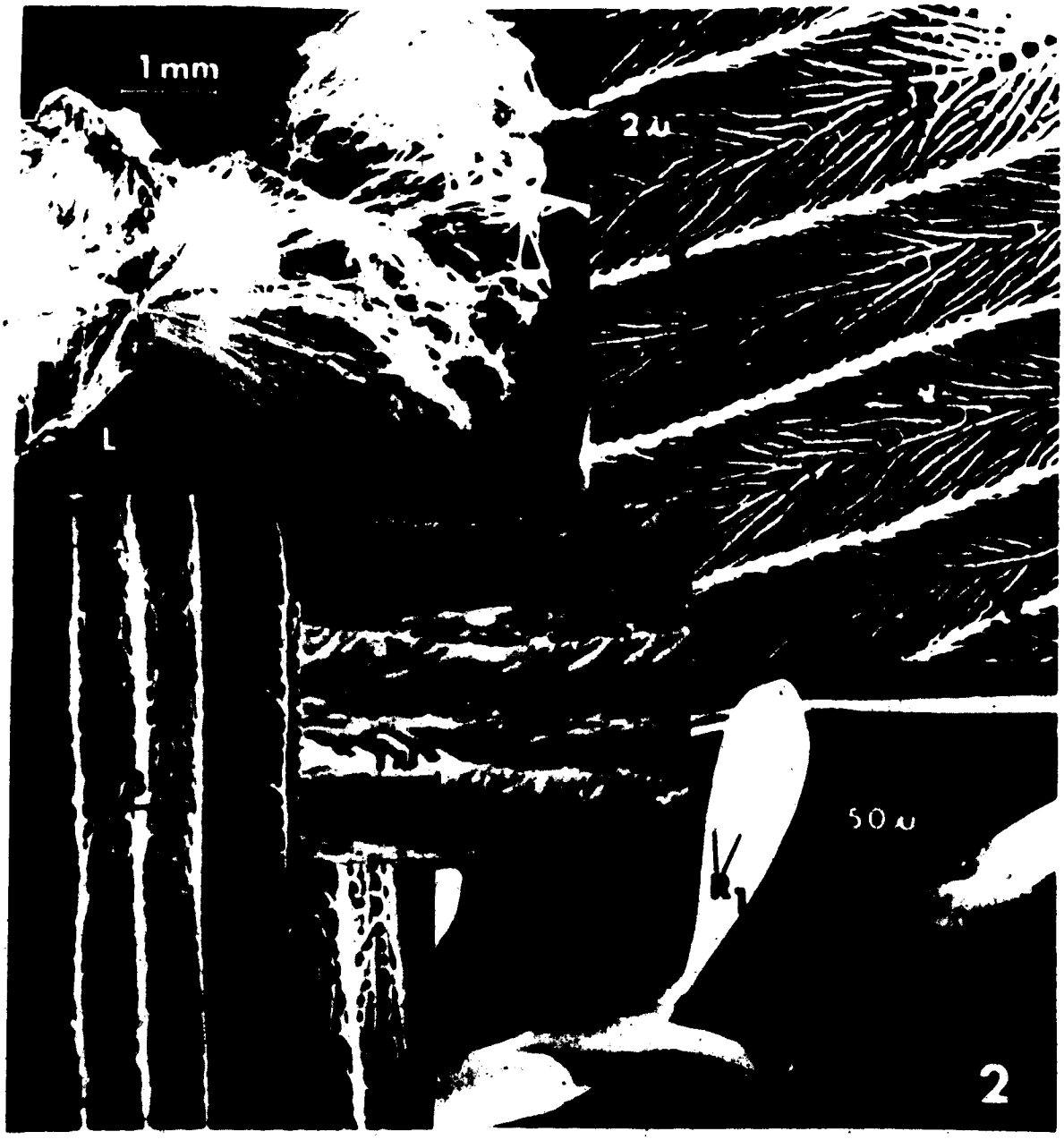

1 mm
2 μ
L
50 μ
K
2

Plate 2*

Explanation of figures

6. *Eremochloa granulata*. Alar gland.

Underside of the fore wing, showing a rectangular tract of short scales (Ss). A narrow tract of elongated scales (Es) form a line along the leading edge of the wing adjacent to the rectangular tract. Normal wing scales are absent in the vicinity of the specialized scales. (SEM).

7. *E. granulata*. Abdominal gland.

Tract of short scales (Ss) in flexible cuticle on the lateral edge of sternite IV. A large tracheole (Tc) runs across the caudal edge of the tract. (Brightfield light micrograph).

8. *E. graminosa*. Surface detail of scales from abdominal gland.

Detail of surface pattern, showing parallel longitudinal ribs (R_1), linked by short ridges (R_2). (SEM).

9. *E. graminosa*. Short scales from alar gland.

Surface pattern of scales from wing tract (Ss). (SEM).

10. *E. graminosa*. Details of surface of short scales in fig. 9.

Dotted line is parallel to the poorly defined longitudinal ribs. (SEM).

11. *E. graminosa*. Long scales from alar gland.

Scale from wing tract (Es), showing spine-like longitudinal ribs (R_1) and central line of variable sized depressions (D). (SEM).

*All material in plate 2 is air dried.


0·3mm
Tc
Ss
7
1 mm
Es

3-methoxy 4-hydroxy benzaldehyde, a probable pheromone (Clearwater, 1971). A third structure that may be implicated in pheromone production is a small tract of smoke grey scales (Ss) enclosed in a shallow, ventral abdominal trough (fig. 7). The surface of these scales is of moderate complexity. A series of irregular, parallel, longitudinal ribs (R_1) are tightly packed together and are joined by short cross ridges (fig. 8). The short scales of the wing gland have a comparable aspect ratio (length: breadth) to the abdominal scales, but have a much simpler surface (figs. 9 and 10). The longitudinal ribs (R_1) are much less distinct, consisting of lines of irregular protrusions. No cross ridges can be seen.

The long scales that comprise the second part of the wing gland are covered with an intricate surface sculpture (fig. 11) comparable to that of *R. scotosialis* (figs. 4 and 5). The longitudinal ribs (R_1) appear to be formed from a series of distally-directed, overlapping spines (but cf. with *M. aleyone* figs. 13—17). The cross ridges (R_2) form a grating pattern rather different from that of *R. scotosialis* but both species possess the central row of depressions.

Only two specimens were available for this study. Three additional specimens were used for the extraction of the pheromone (Clearwater, 1971).

3.1.3 A single-component abdominal system — *Melanchra aleyone*

A modified second abdominal sternite (SII) forms the base of the pheromone system of *Melanchra aleyone* (fig. 12). A tract of long (5 mm) scales (Es) inserts into a sheet of flexible cuticle situated on each side of the anterior apodeme (A) of this sternite. The only

support associated with the scales is a small bar (B) arising from each of these apodemes. The hair like scales are uniformly light brown, and superficial examination reveals no obvious differentiation along their length. The scanning microscope reveals a steady progression in the surface from superficial parallel ribs (R_1 fig. 13) at the scale base, to a deeply dissected tip (figs. 14—16). In more distal portions of the scale, the ribs are transected by oblique channels (fig. 14). These channels deepen, eventually giving the rib the appearance of a series of overlapping spines (cf. *P. maxima*). Fine microribs (R_3) can be seen in the oblique channels of distal areas (fig. 15). The depressions found in the inter-rib space are infrequent near the scale base, but increase in number until they form a very irregular central line. Multiple depressions are common in the mid-portion of the scale. No depressions are found near the tip.

This description is based on a single specimen.

3.1.4. A multi-component abdominal system — Stobbe's gland and hair pencil complex — *Pseudaletia separata*, *Mamestra configurata* and *Rusina verberata*.

The most common system encountered in the noctuid species examined is the hair pencil—Stobbe's gland association (fig. 70). The mechanism of hair pencil eversion, the protective pouch into which the scales are inserted at rest, the short pouch scales, and the adult Stobbe's gland have been described by Birch (1970b). These structures are essentially the same in *Pseudaletia separata*, *Mamestra configurata* and *Rusina verberata*.

Large numbers (> 20) of specimens of each of these species were examined, with the exception of *Rusina verberata* (n = 3).

Plate 3

Explanation of figures

12. *Melanoplus alcyone*. Abdominal gland.

Sternite II. The area of insertion of the large number of elongated scales (Es) is delimited by the dotted line. Anterior apodemes (A) bear small bars (B), the only obvious hard parts supporting these scales. A central convexity (C) covers the second abdominal ganglion. (Direct print from slide preparation).

Figs. 13—17 air dried material

13. *M. alcyone*. Base of hair scale.

Long parallel ribs (R_1) are very rarely linked together (Df). (SEM).

14. *M. alcyone*. Formation of oblique channels on hair scale.

Basal third of scale. Shallow, oblique channels (O) mark the longitudinal ribs (R_1). Depressions and transverse ridges are beginning to appear. (SEM).

15. *M. alcyone*. Formation of microrib in oblique channels.

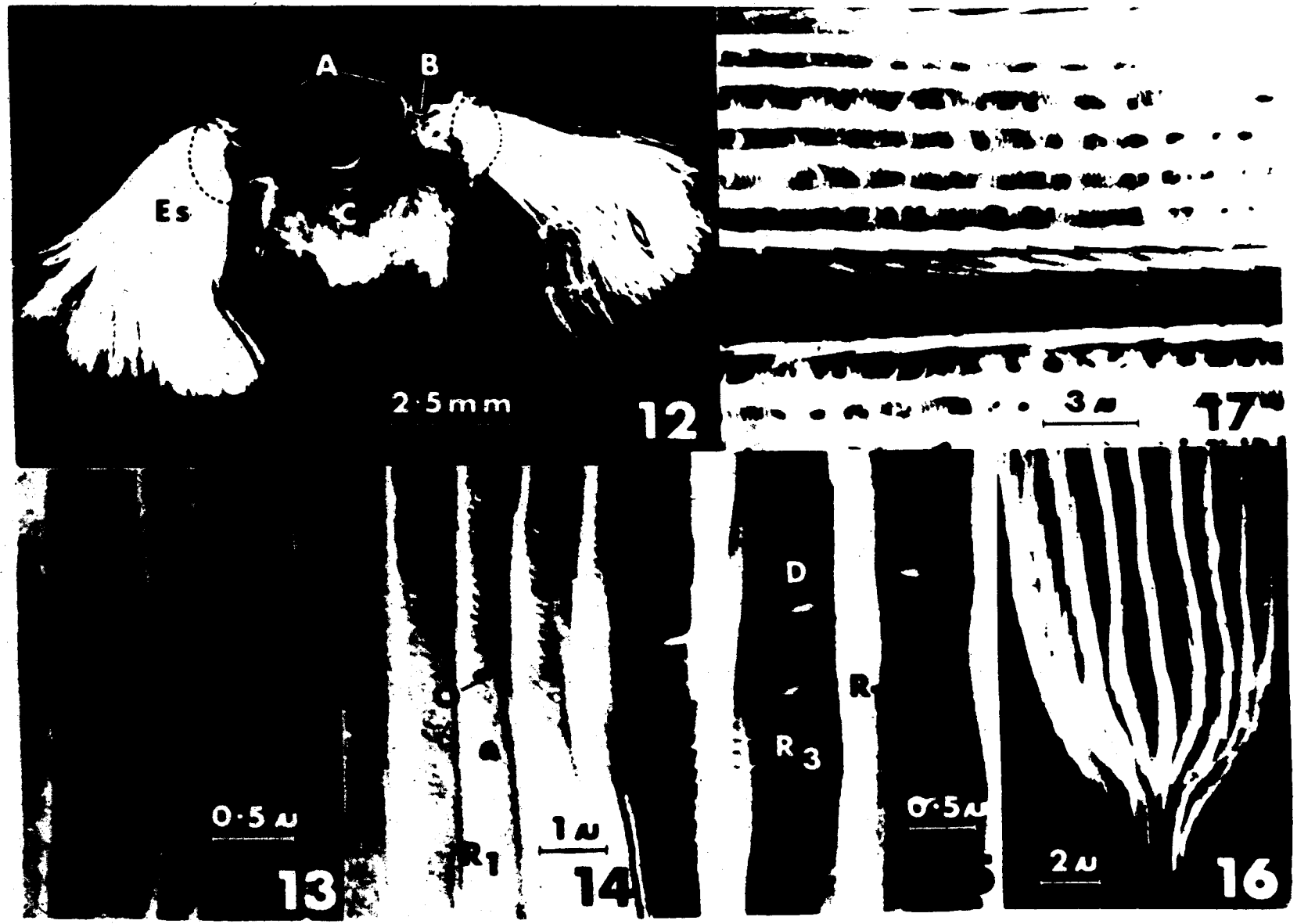
Microribs (R_3) appear in the oblique channels, in the mid-portion of the scale. Depressions (D) are much deeper. (SEM).

16. *M. alcyone*. Hair scale tip.

Deeply dissected ribs of the hair scale tip. (SEM).

17. *M. alcyone*. Hair scale (low magnification).

Low power micrograph showing nine ribs in surface view. (SEM).


A
B
Es
C
2·5mm
12
3 μ
17
D
R
R3
0·5 μ
13
R1
1 μ
14
0·5 μ
2 μ
16

The second abdominal sternite of *A. argentata* (fig. 18) shares several features with that of *M. aleyone*, in particular the well developed, anterior apodemes (A) and a central convexity (C) overlying the second abdominal ganglion. The lateral margin of this sternite is much more sharply defined in *A. argentata*, but lacks the support (B) (fig. 12). A long sclerotized lever (Lv) links the point of insertion of the hair pencil scales to the postero-lateral angle of the sternite. A distinct concave flange (F) makes close contact with the shaft of the hair pencil when at rest.

The Stobbe's gland lies in the fat body under the anterior apodeme and has a duct (Dt) leading to the hair pencil (figs. 19 and 70). The scales emerging from the center of this gland are unremarkable in surface detail or in section (figs. 20 and 21). Smooth flanges (F) are the only structures breaking up an otherwise regular cylinder.

3.1.4.1 The hair pencil cap cells.

The cell at the base of each hair scale is large and appears to be secretory (fig. 23). A three- to four-chambered end apparatus separates the hollow scale lumen from a microvilli-lined cell lumen. The tips of the microvilli cluster around a ductule extending proximally from the end apparatus. The nucleus is large and heterochromatic.

3.1.4.2 Surface structure of the scales.

The hair pencil scales are very complex. Unlike those of *M. aleyone*, these scales are sharply differentiated into three areas: a proximal shaft (Sh), a transition area (T) and an intricate

Plate 4

Explanation of figures

18. *Eusina separata*. Hair pencil and sternite II.

Long apodemes (A) link this sternite to the thorax. Large levers (Lv) link the posterior angle of the sternite to the hair pencil. An expanded central portion, the flange (F), supports the hair pencil shaft at rest. A central convexity (C) covers the second abdominal ganglion. The transition area (T) absorbs methylene blue, thus appearing much darker than the surrounding scale areas (Brightfield light micrograph).

19. *E. separata*. Stobbe's gland.

A group of transparent, bag-like cells with a duct (Dt) leading out from the middle. Living cells in Pringle's saline.

20. *E. separata*. Transverse section of duct scale.

Note flanges (F). Transmission electron micrograph (TEM).

21. *Eusina verlorata*. Surface view of duct scales.

Note flanges (F). (SEM).

22. *E. separata*. Hair pencil regions.

Shaft (Sh) transition area (T) and complex evaporative area (E). (Light micrograph of whole fresh specimen).

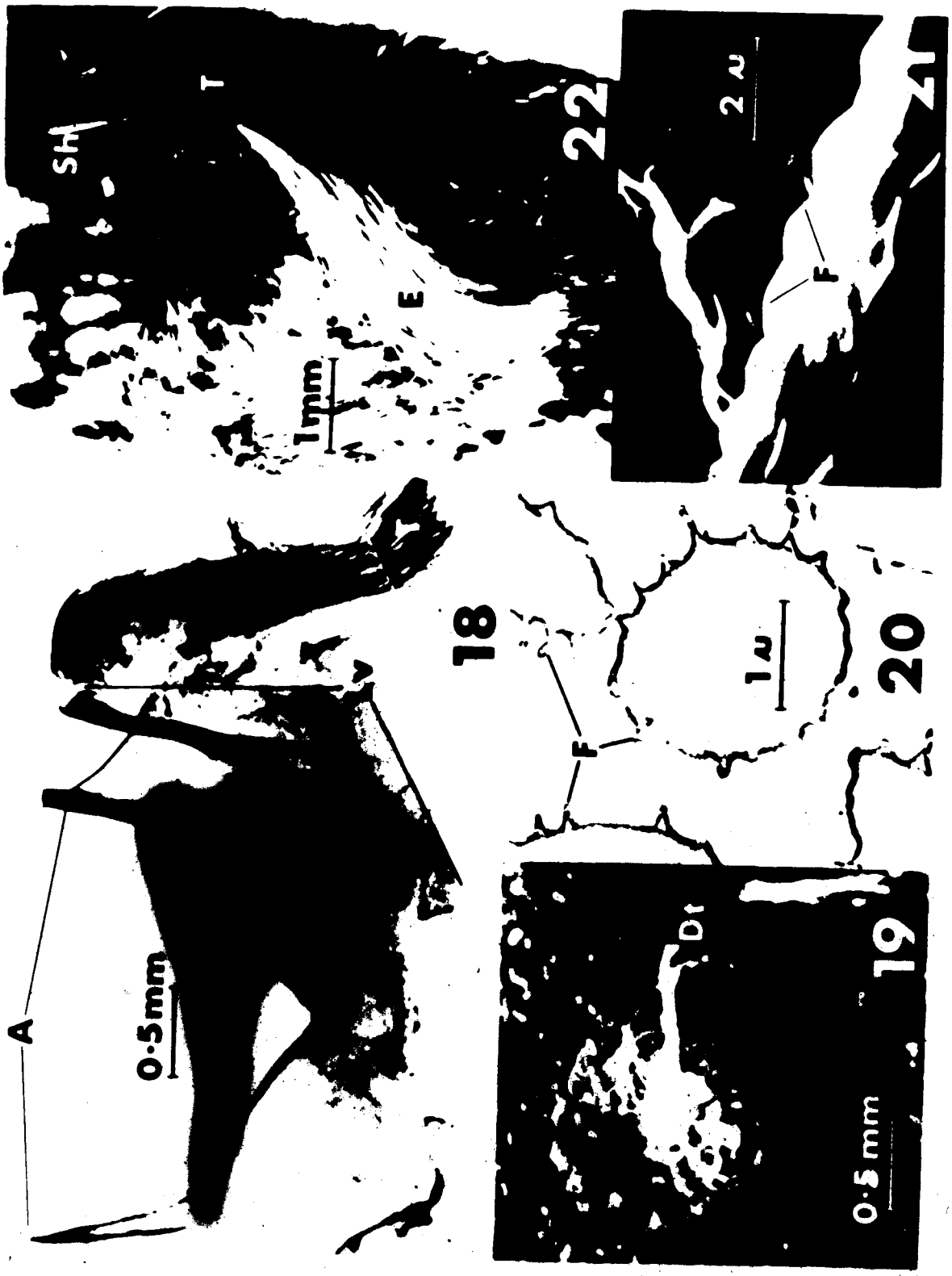
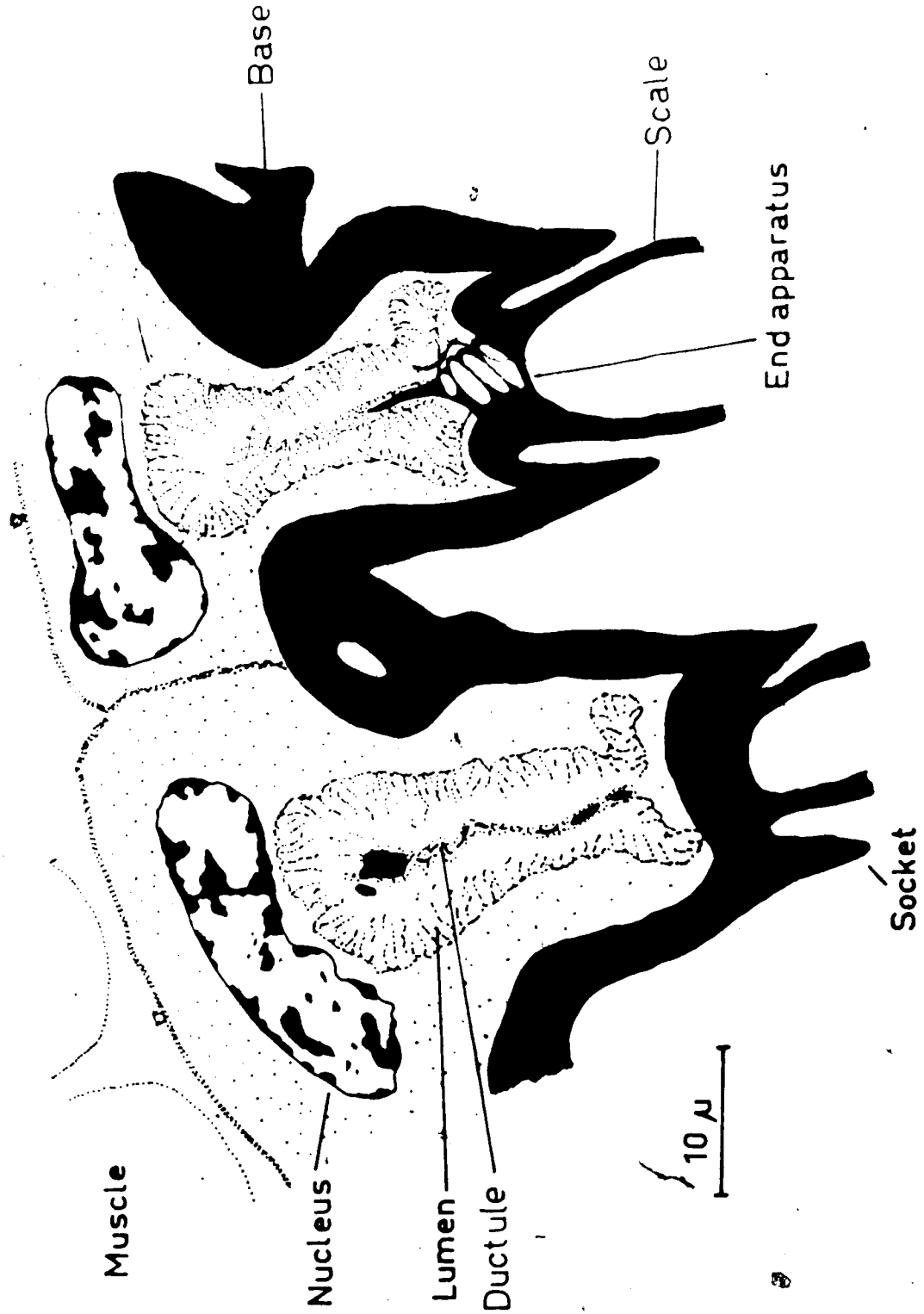

SH
T
E
1mm
22
2 µ
F
21
18
1 µ
F
20
A
0·5mm
Dr
0·5 mm
19

Fig. 25. *Ferdinandea cuprea*. Hair pencil cap cells.

Section through basal plate of hair pencil of *F. cuprea* showing the cap cells.


Base
Scale
End apparatus
Socket
10 µ
Muscle
Nucleus
Lumen
Ductule

distal sculptured area (fig. 25). The proximal area is smooth darker than the distal and sculptured regions, and takes up methylene blue more readily. The remainder of the scale is usually golden brown.

The surface micro-sculpture of the shaft of *P. separata* consists of parallel ribs (R_1) separated by a smooth surface regularly interrupted by large shallow depressions (D) (fig. 24). The longitudinal ribs (R_1) are barely discernible on the shaft of *M. configurata*, the most prominent features being the shallow depressions (D) (fig. 28). In the complex sculptured area, the surface comprises the same substructures arranged in a different manner. The longitudinal ribs are thrown into sinusoidal waves, bonded together at intervals (BD) so that they enclose a diamond- or hexagonal-shaped surface marked with deep depressions (D) (figs. 26, 27, 30 and 31). These depressions may be serially homologous with those on the shaft. In both species, the bonded areas are marked with small ridges (R_4). A much greater number of deep depressions are observed within the enclosed space of *P. verlenata* compared with that of *M. configurata* (figs. 26, 27, 30 and 31). The two distinct regions are linked by a transition zone that combines the features of both (figs. 25 and 29). The thickness of the longitudinal rib remains constant throughout the length of the scale (figs. 24--31). The scales are markedly constricted at the transition zone (table 1). Despite this, the number of ribs remains constant throughout the length of the hair. The ultrastructure of hair scales of *P. separata* is very similar to that of *M. configurata*.

3.1.4.3 Internal structure of the scales.

Transverse sections of the intricate sculpture area reveal an internal structure strikingly different from that of any

Plate 5*

Explanation of figures

24. [illegible]. Shaft of hair scale.

Note distinct ribs (R_1) and depressions (D). (SEM).

25. [illegible]. Transition zone of hair scale. (SEM).

26. [illegible]. Evaporative surface of hair scale.

Low power micrograph of evaporative surface (SEM).

27. [illegible]. Detail of evaporative surface.

Longitudinal ribs (R_1) are thrown into sinusoidal waves and bonded together at intervals (Bd). Numerous depressions (D) fill the intervening space. Fine ridges (R_4) cover the bonded area. (SEM).

*All material in plate 5 is air dried.

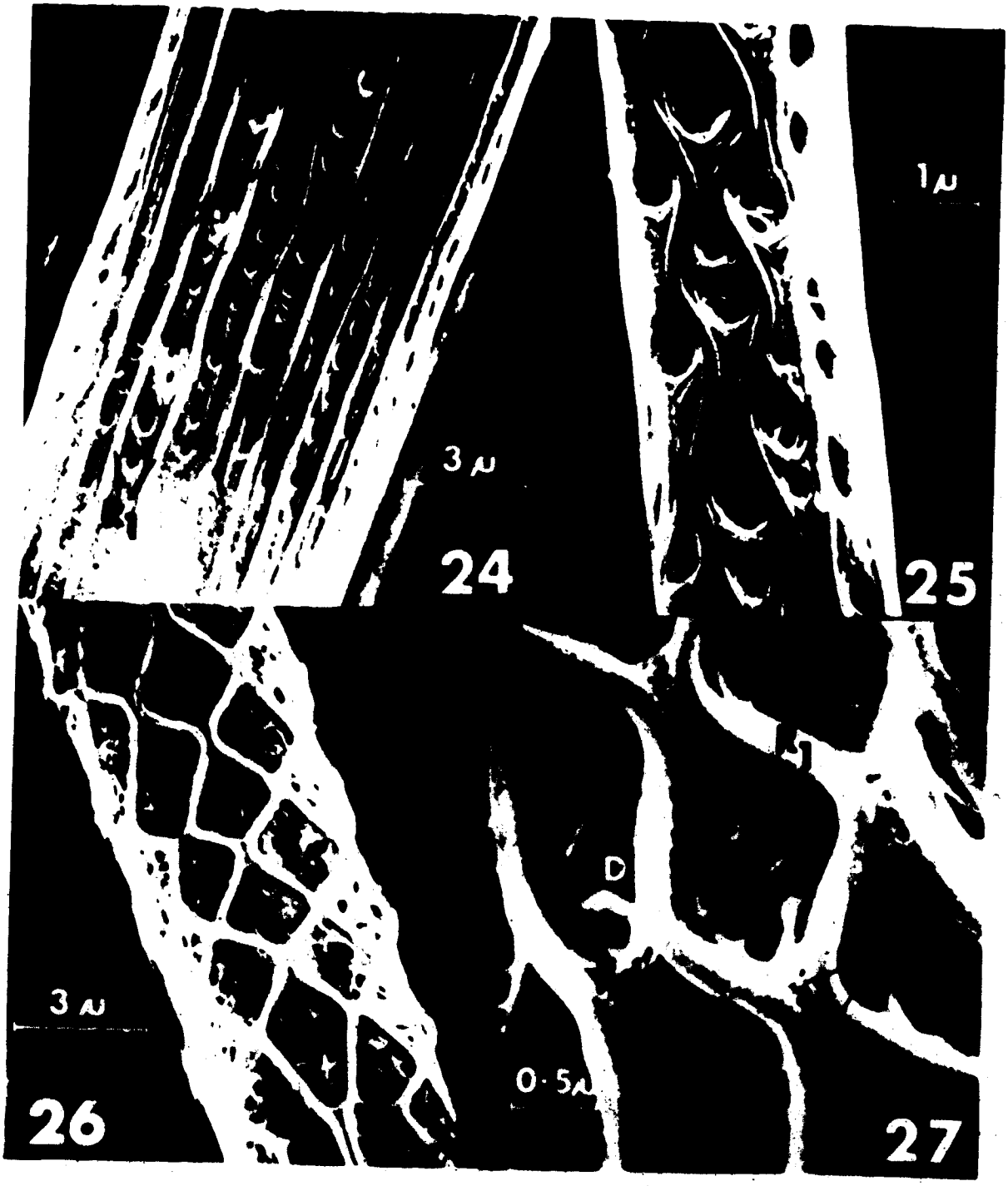

3 μ
24
1 μ
25
D
3 μ
26
0·5 μ
27

Plate 6*

Explanation of figures

28. *Mansonia titillans*. Shaft of hair scale.

Note numerous shallow depressions (D). (SEM).

29. *M. titillans*. Transition zone of hair scale.

Note longitudinal ribs (R_1) thrown into irregular waves. Fine ridges (R_4) link more closely opposed ribs. (SEM).

30. *M. titillans*. Evaporative surface of hair scale. (SEM).

31. *M. titillans*. Detail of evaporative surface.

Longitudinal ribs (R_1) are thrown into sinusoidal waves and bonded together (Bd) at intervals. One to two depressions (D) mark the intervening space. Several ridges (R_4) cover the bonded area. (SEM).

*All material in plate 6 is air dried. The scales were taken from 12-hr males from field collected pupae.

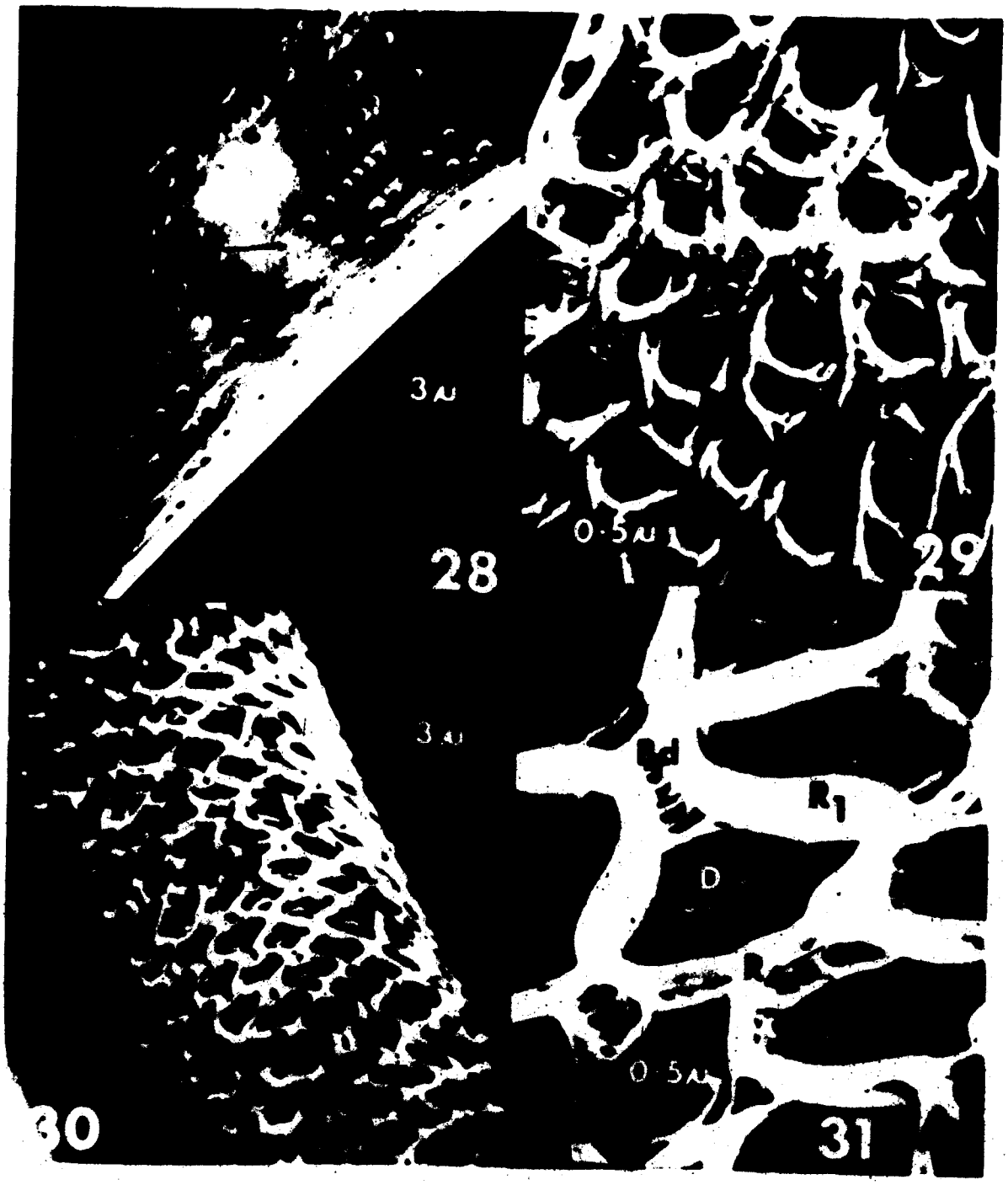

3 AU
0·5 AU
28
29
3 AU
R1
D
0·5 AU
30
31

TABLE I

Diameter of the hair scales

	Mamestra configurata (width in μ)	*Eucosma verberata* (width in μ)	*Phlogophora meticulosa** (width in μ)
Shaft	(a) 9.1 - 12.3	(d) 10.0 - 12.3	13 - 14
Transition Area	(b) 5.3 - 5.4	(e) 3.1 - 3.6	12
Sculptured Area	(c) 10.0 - 12.0	(f) 5.3 - 10.0	16 - 20

These figures are absolute limits of the observed sizes.

(a) n = 3 scales, one moth

(b) n = 3 scales, one moth

(c) n = 5 scales, two moths

(d) n = 3 scales, one moth

(e) n = 7 scales, one moth

(f) n = 11 scales, one moth

*Data from Birch, 1970b.

previously described lepidopterous scale. From a central core of fibres (Fc) a series of pillars (P) radiate to the circumference where they make contact with the surface ribs (R_1) (fig. 32). Because the outer edges of the ribs are curled over, the scanning micrographs suggest wider ribs than appear in section. A very thin membrane (M) (120 Å) linking adjacent ribs (fig. 34) is comparable in thickness, position, and electron density to the cuticular layer of larval cuticle (120—175 Å in *Calpodes ethlius* (Stoll) - Locke, 1966). There are no obvious holes in this membrane. Where ribs approach each other they are linked together with an area of thick cuticle (Bd) (fig. 33).

The transition area shares features of conventional scales and the complex sculptured area (fig. 35). The loose network of fibres underlying the surface is separated from the scale lumen by a distinct boundary (Fb). Large fibre masses (Fm) occupy the position of the pillar fibre in the vicinity of this boundary. Although many ribs are linked together by thick cuticle, the fine membrane (M) constitutes most of the circumference. These ribs are shorter than those of the intricate sculptured area. Ribs seem to be made up of at least three different layers (fig. 36). A central granular core (L_1) is surrounded by a thick, electron dense layer (L_2), with a fine outer layer (L_3) completing the structure.

The shaft of the scale is a simple cylinder in section.

3.2. Development of the Male Pheromone System of *M. configurata*

3.2.1. Timing of developmental events

Adults of *M. configurata* emerge in early summer, 3—4 weeks

Fig. 32. Diagrammatic reconstruction of the evaporative area of the hair scale of *Marcetia configurata*.

A central cylindrical core of fibres (Fc) is surrounded by radially oriented pillar fibres (P). Of the surface features, only the longitudinal ribs (R_1) and the bonded areas (Bd) are heavily cuticularized ▨. The scale is structurally comparable to a rolled up piece of wire mesh. Membrane (M).

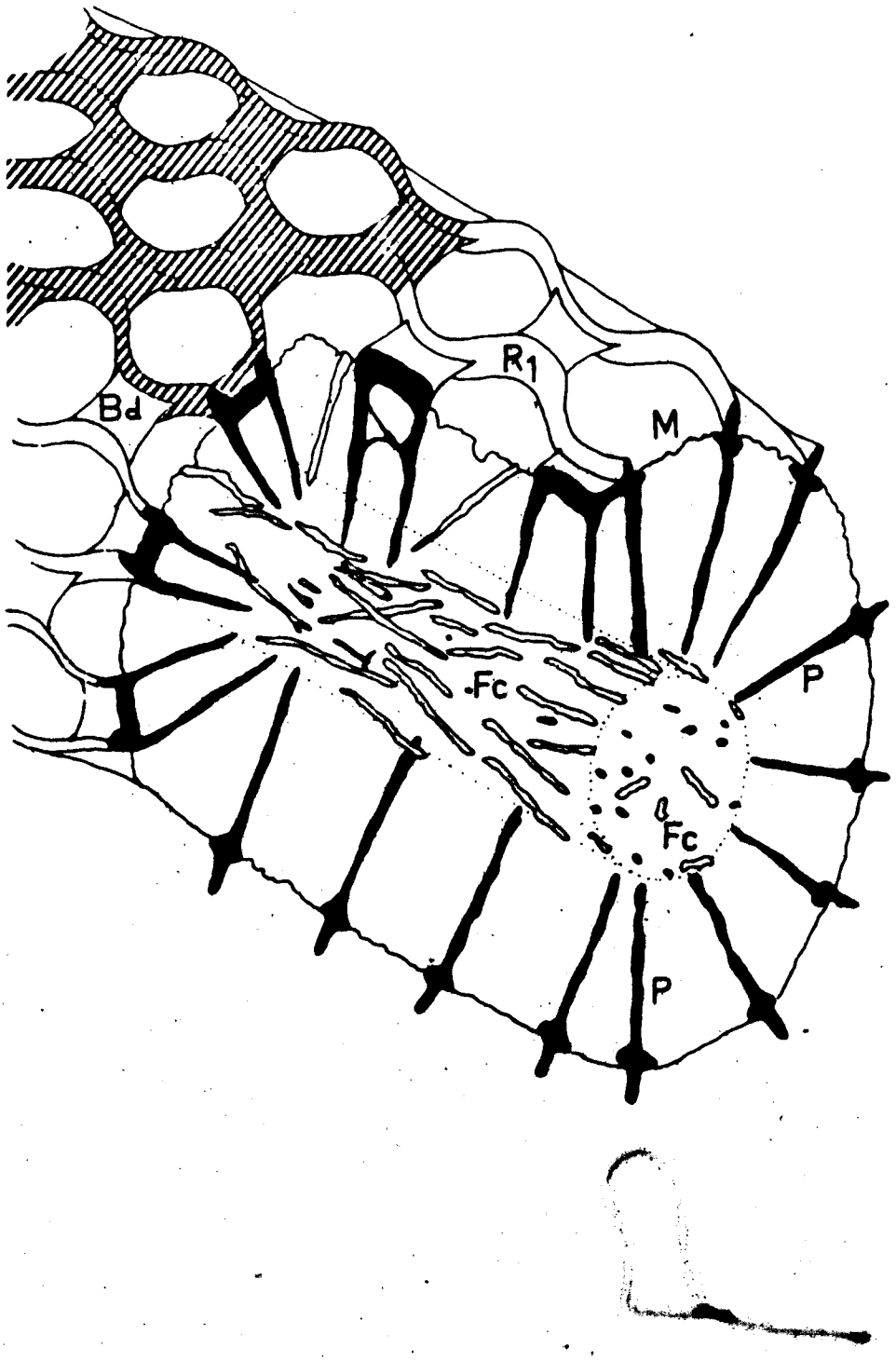

Bd
R1
M
Fc
P
Fc
P

Plate 7*

Explanation of figures

33. *Mansonia configurata*. Transverse section of evaporative area.

A central core of fibres (Fc), surrounded by a radial array of pillar fibres (P) each of which is continuous with the longitudinal ribs (R_1). A block of thick cuticle underlies the bonded area (Bd). (TEM).

34. *M. configurata*. Detail of evaporative area.

Note pillar fibre (P) and very fine surface membrane (M). (TEM).

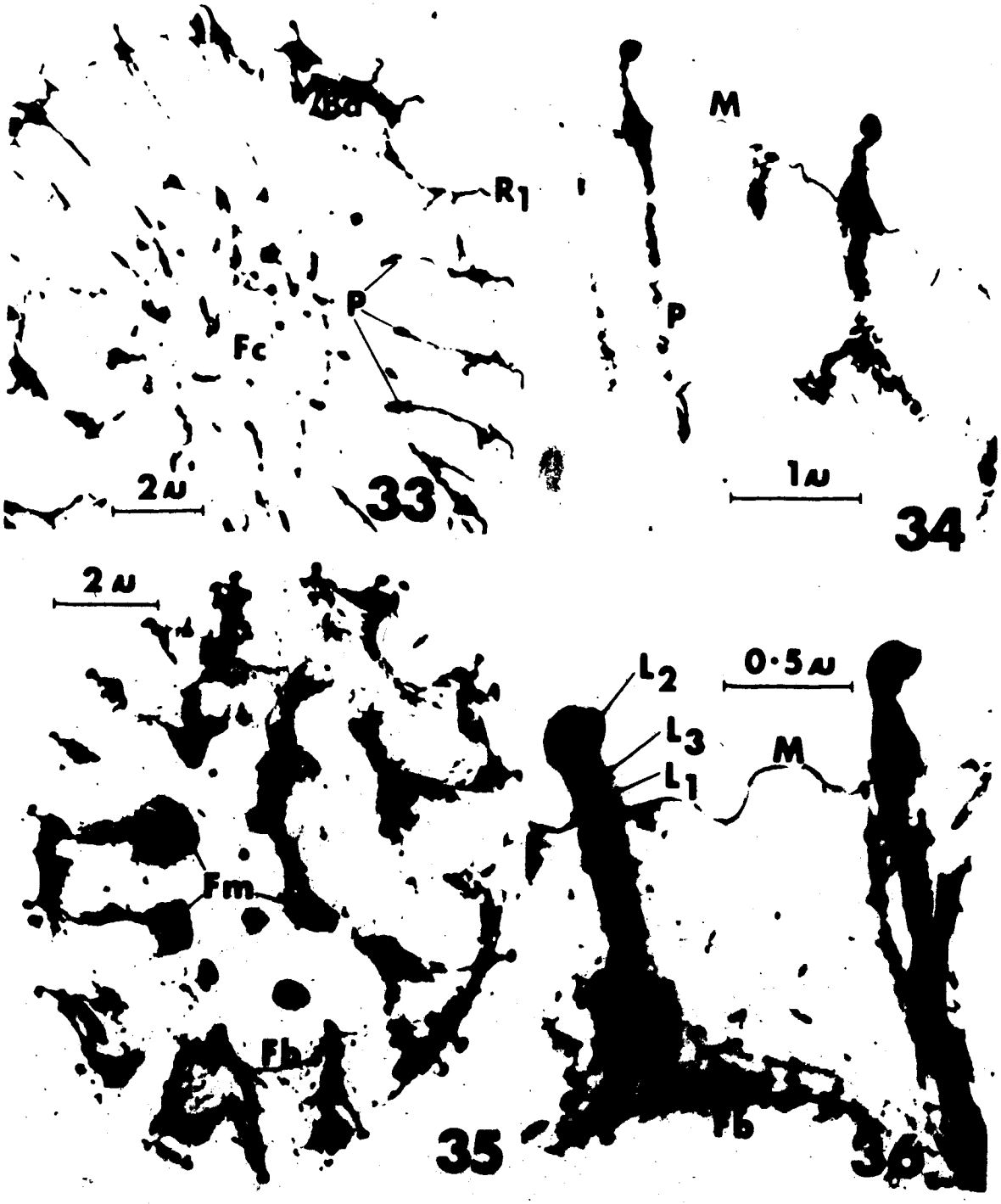
35. *M. configurata*. Transverse section of transition area.

Note large fibre masses (Fm) in positions occupied by pillar fibres and core fibres in the evaporative area. A distinct fibre boundary (Fb) borders the scale lumen. (TEM).

36. *M. configurata*. Detail of transition area.

Note fibre boundary (Fb) and the three layers of the rib. L_1 - central granular core, L_2 - thick dense layer, L_3 - fine outer layer. (TEM).

*All material in plate 7 is from a 24-hr. adult.


Ba
R1
P
Fc
2μ
33
M
P
1μ
34
2μ
Fm
Fb
35
L2
L3
L1
0·5μ
M
Fb
36

after undergoing an obligatory nine-month winter diapause. Consequently, it was difficult to relate the development of the pheromone system to a time schedule. Other, more striking developmental events — development of eye pigment, sclerotization of the integument and wing characteristics — were used as reference (fig. 37). The terminology of Hinton (1961) was adopted. [redacted] diapauses as an exuvial pharate adult. The only portion of the pheromone system that can be recognized at this stage is the pigment in sternite II.

3.2.2 Early development

In the exuvial pharate adult, having little or no eye pigment, a sheet of abdominal epidermis invaginates, on each side of sternite II, to form a deep pouch slightly under the developing anterior apodemes. Two small groups of cells in this sheet differentiate into Stobbe's gland and hair pencil (fig. 38). At this stage, the two groups of hypertrophied cells, each producing elongated scales, appear strikingly similar.

3.2.3 Development of hair scales

The Stobbe's gland and hair pencil primordia arise from columnar epithelial cells (Ep) about 30 μ high with oval nucleus (N_3) 8—10 μ long (figs. 48 and 49). The trichogen cells producing the elongated, hair pencil scales are much larger, each having a round grandular nucleus (N_1) 20—30 μ in diameter and a cell diameter of up to 50 μ in the exuvial pharate adult (fig. 41). Older trichogen cells (fig. 42) are up to 100 μ in diameter. A moderate number of small vesicles (Vs) occur mostly in the intercellular space. Despite their great increase in

Fig. 3. Chronological references for the development of the pheromone system.

Ontogeny of structures serving as references for the development of the pheromone system of *Manduca sexta*. The hemolymph of early pupae is green (top line). Some pupae appear to lose this colouration before the appearance of eye pigment (second line), while others retain it until the eye is dark brown. Mature pupae have black eyes. The first structure to sclerotise is a large spine on the tibia of the prothoracic (I) leg (third line). The sclerotisation of the antennae and hair pencil precedes sclerotisation of the sclerites legs. The wing of the early pharate adult is translucent, later becoming silver (bottom line). Wing pigmentation begins with a single spot.

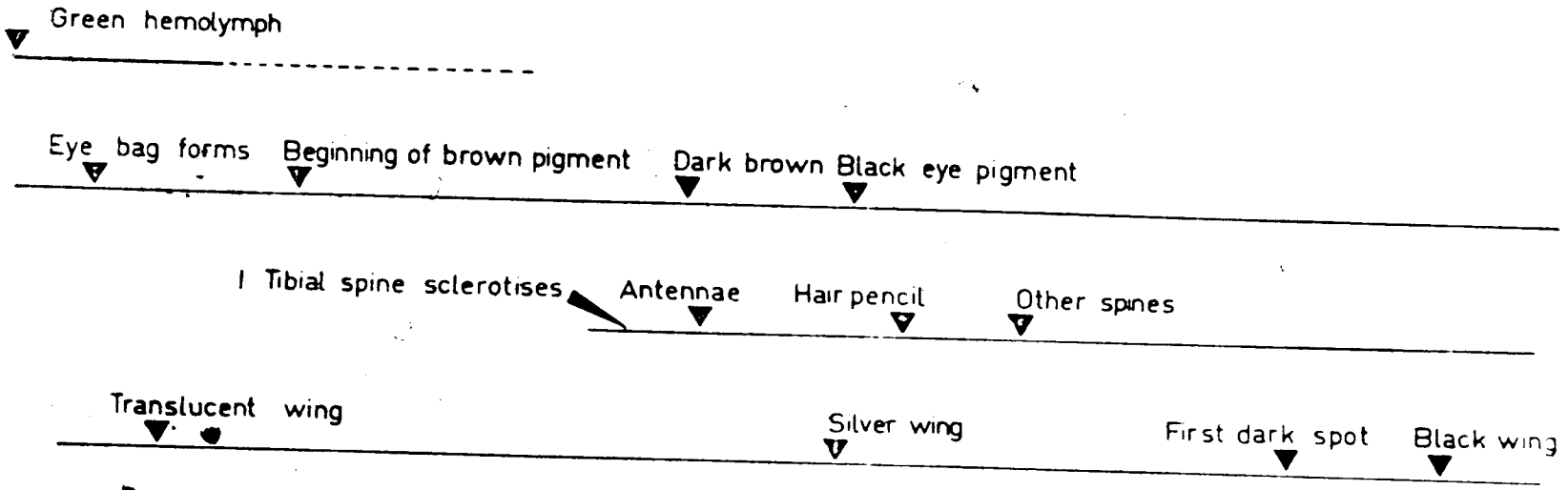

Green hemolymph
Eye bag forms
Beginning of brown pigment
Dark brown Black eye pigment
1 Tibial spine sclerotises
Antennae
Hair pencil
Other spines
Translucent wing
Silver wing
First dark spot
Black wing

Plate 8

Explanation of figure

88. [illegible]. Early development of hair pencil and Stobbe's gland.

Two groups of cells on the surface of a large lateral invagination, producing elongated scales (ls). A small ring of body scales (bs) form on the hair pencil base. Black masses are portions of fat body. Juvenal phalate adult. The eye is a bag of cells with no pigment at all. Glutaraldehyde fixed (Glut), Nomarski interference micrograph (Nom)).


B5
0·11mm
Stobbes
gland
ncll

Plate 9

Explanation of figures

39. *Mansonia configurata*. Developing basal sclerite.

Sockets (S) are interspersed among the crenellations of the basal sclerite (Bsc). Light vertical shadows are hair scales below the focal plane. Darkening lever (Lv) is separated from the basal sclerite (Bsc) by unmodified cuticle, the hinge (H). (Cuticular pharate adult, eye dark with light brown tibial spine and hair pencil lever. Silver hair pencil scales (Nom). Living cells in Pringle's saline (Liv)).

40. *M. configurata*. Extending hair scales.

Note pointed tip and fine surface wrinkles. Scales are easily deformed (lower scale) or burst (upper scale), releasing a mass of featureless cytoplasm (Exuvial pharate adult, eye colourless bag, green hemolymph (Liv) (Nom)).

41. *M. configurata*. Trichogen cells of hair pencil.

Basal portion of a developing hair pencil of the same age as in fig. 40. Flattened trichogen cells (Tr) with large granular nucleus (N_1) and sockets (S) are beginning to form (as in fig. 40).

42. *M. configurata*. Older trichogen cells of hair pencil.

Surface view of older and larger hair pencil trichogen cells (Tr). Small vesicles (Vs) are seen mainly in the extracellular spaces. (Exuvial pharate adult, very small amount of eye pigment in eye bag. Hemolymph still with slight green colour (Liv) (Nom)).


39
40
42
BICEPS
H
LV
10μ
40μ

size, these cells appear structurally similar to the epidermal cells that gave rise to them.

Two distinct types of scales are produced by these cells: a central core of very long ones that develop into the disseminating scales (Ds) and a peripheral ring of normal sized scale rudiments (Bs) that give rise to the sparse covering of normal scales on the flexible cuticle overlying the hair pencil muscle sheet (fig. 38). Elongation of the disseminating scales is very rapid. Most exuvial pharate adults dissected during this study either lacked noticeable hair pencil development, or had scale rudiments 4—5 mm long — almost their imaginal length. The scales are extremely delicate during elongation and appear to consist of a thin membrane covering a column of cytoplasm (fig. 40). When viewed under the dissecting microscope, they are translucent and difficult to see.

The surface microsculpture of hair scales during this phase consists of wrinkled longitudinal ridges (figs. 40 and 43). A slow expansion in width now occurs, and the beginning of hexagonal sculpturing can be seen on scales from pharate adults having light brown eyes (fig. 44). The enclosed spaces are approximately 0.7 μ in diameter and no bonded areas can be discerned. As the scale begins to change in colour from translucent to silver, the surface pattern gains a greater definition (fig. 45). The sinusoidal form of the longitudinal ribs gains symmetry and the bonded areas (Bd) form . The surfaces of the enclosed areas and bonded areas are smooth in contrast to those of the mature scale. This pattern will be called the *primary microsculpture* as it appears to be the pattern initially laid down by the cytoplasm of the trichogen cell. No further changes

Plate 10*

Explanation of figures

43. *Mansonia configurata*. Very young hair scale, with longitudinal ribs.

Note parallel longitudinal ribs (R_1). The wrinkles are unlikely to be an artifact because critical point drying is able to preserve delicate surfaces, and the surface shown in this micrograph corresponds well with the surface of living cells (fig. 40). (Exuvial pharate adult, no eye pigment).

44. *M. configurata*. Older hair scale with sinusoidal wave pattern.

Older hair scale, showing beginning of development of sinusoidal waves (R_1). (Exuvial pharate adult, eye with light brown pigment, no parts sclerotized, hair pencil scales translucent).

45. *M. configurata*. Completed primary pattern.

With the formation of scale cuticle, the pattern gains a much greater definition. (Cuticular pharate adult, medium brown eyes, wings silver, tibial spine sclerotized abdomen light brown).

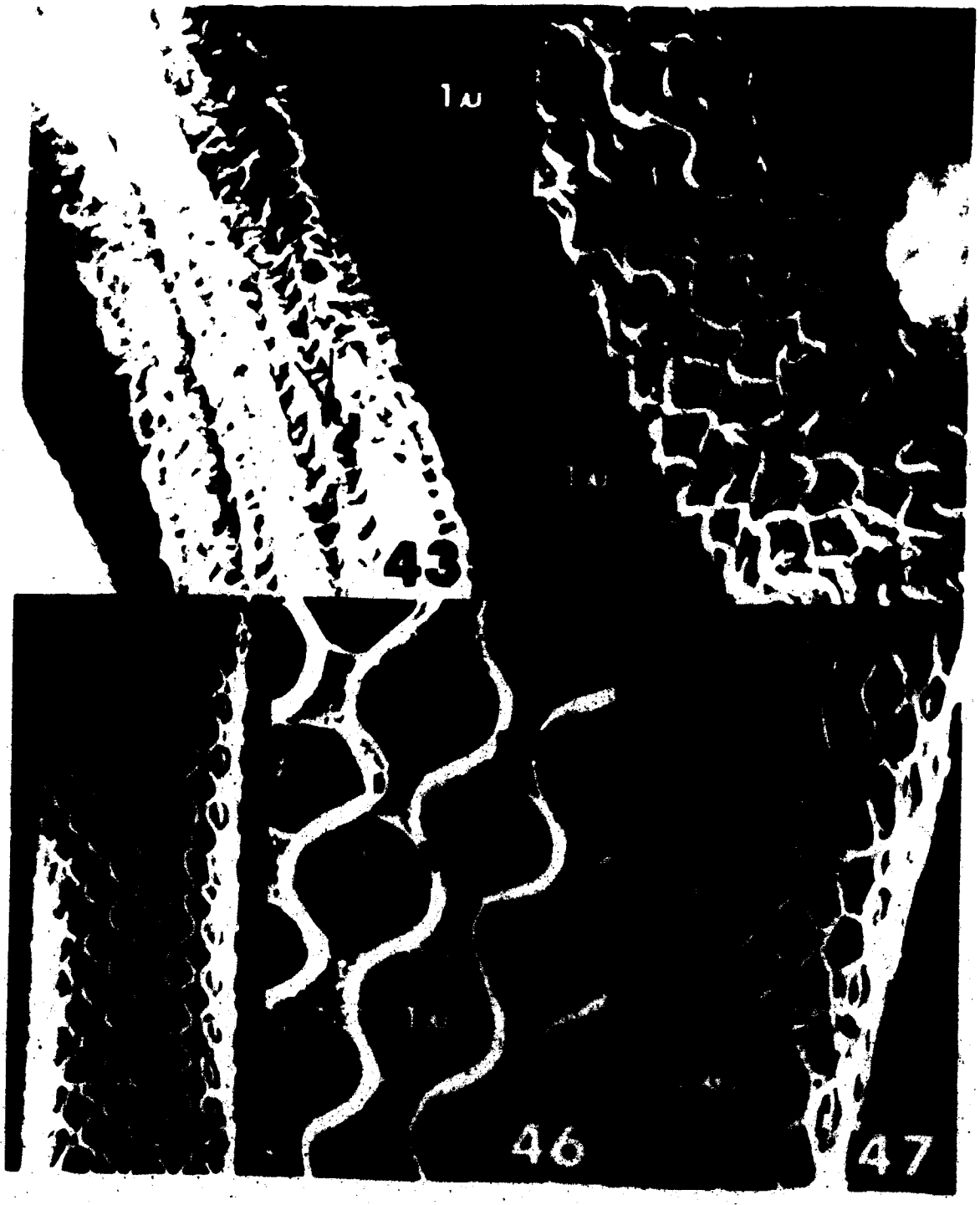
46. *M. configurata*. Detail of primary pattern.

Note the great symmetry of the ribs (R_1) and bonded areas (Bd). All surfaces are very smooth. (As above).

47. *M. configurata*. Hair scale just prior to emergence.

Deep depressions have appeared. (Cuticular pharate adult, ecdysial sutures beginning to break, fully pigmented wings).

*All material in plate 10 is fixed in glutaraldehyde (glut) and critical point dried (crit.pt.) (SEM).


43
46
47

in surface pattern occur throughout the remainder of the pharate adult stage up till 1--2 days before eclosion. Depressions (D) form and the scale assumes the microsculpture of the mature scale (fig. 47). A slight contraction of the scale occurs at this time. This pattern will be called the [illegible] as sections show that it develops in the absence of underlying cytoplasm.

3.2.4 Development of supporting sclerites

Formation of the characteristic hard, sclerotized parts of the system begins long after hair scale extension is complete. The lever (Lv) is merely a local thickening and sclerotization of a strip of the cuticle lining the abdominal invagination (fig. 39). It can first be seen as a light brown streak. Sclerotization of the lever develops first at its tips but progresses rapidly toward the centre. The flange (F) is last to darken. This structure is one of the first body parts to harden after the antennae and I tibial spine.

The cuticle immediately surrounding the hair pencil sockets begins to take on a crenellated appearance at this time. This modification stiffens the area, forming the basal sclerite (Bsc) (fig. 39). The cuticle linking the sclerite to the lever remains unsclerotized and forms a hinge (H). The muscle sheet overlying this sclerite is the last component to appear. The muscle turns from translucent to deep red-brown during deposition of the wing pigment.

3.2.5 Development of the Stobbe's gland

In the exuvial pharate adult, the Stobbe's gland has the appearance of a smaller version of the developing hair pencil -- a small cap

of cells about twice as thick as the surrounding epidermis (figs. 48—51). The developing gland is continuous with the columnar epithelium but its cells are easily distinguished from them; there appear to be no intermediate forms. Both trichogen (Tr) and tormogen (To) cells are present. The trichogen cells are up to 50 μ long, and are pear-shaped, a narrow neck curving down toward the developing scale (figs. 50 and 51). Tormogen cells are smaller with a nuclear (N_2) diameter of 14—16 μ, intermediate between that of the epidermis (10 μ) and trichogen cells (20—25 μ). Each tormogen cell is situated below and slightly to one side of the associated trichogen cell. The whole primordium is cup-shaped, probably the result of expansion in volume of the contained cell bodies as they simultaneously retain a constant area of contact with their basement membrane.

Trichogen nuclei can be separated from the gland primordium by gentle pressure on the coverslip (fig. 52). Isolated nuclei contain a dense granular material (probably chromatin) surrounded by a clear area. Trichogen nuclei from the hair pencil show no change in appearance when treated in the same manner. It is therefore unlikely that osmotic differences between gland nucleus and the Pringle's saline cause the observed change.

The initial development of a very large, extracellular cavity can be seen in glands dissected from pharate adults in which the eyes are fully pigmented, but in which sclerotization of the I tibial spine has not taken place. A long, finger-like protrusion arising near the developing socket extends up toward the nucleus. The rapidly increasing volume of the trichogen cell results from the expansion of this cavity. In pharate adults in which the antennae and tibial spine are sclerotized,

Plate 11

Explanation of figures

48-51. *Manestra configurata*. A series of optical sections through a developing Stobbe's gland.

The series of optical sections move from the outside, to a little past the centre. Scale is identical for all micrographs. (Exuvial pharate adult, eye with a small amount of brown pigment. Green hemolymph. (Liv) (Nom)).

48. Focal plane through outer portion. Epidermal cells with small nuclei (N_3) underlying the trichogen cells with large nuclei (N_1).

49. Focal plane closer to centre. Epidermal cells (Ep) being replaced under the trichogen cells (nucleus N_1) by tormogen cells (nucleus N_2).

50. Centre of gland. Hair scales and sockets clearly visible. Nuclei of tormogen cells (N_2) visible between the pear shaped stalks of the trichogen cells (Tr). Dotted lines indicate size and position of (left to right) epidermal cell, trichogen cell and tormogen cell.

51. Focal plane past centre. Tormogen cell bodies (To) visible. Trichogen cells (Tr) with circular nucleus (N_1) visible on left side of gland.

52. *M. configurata*. Extruded trichogen nuclei.

Note nuclear membrane (Nm) and central mass (chromatin? Ch).

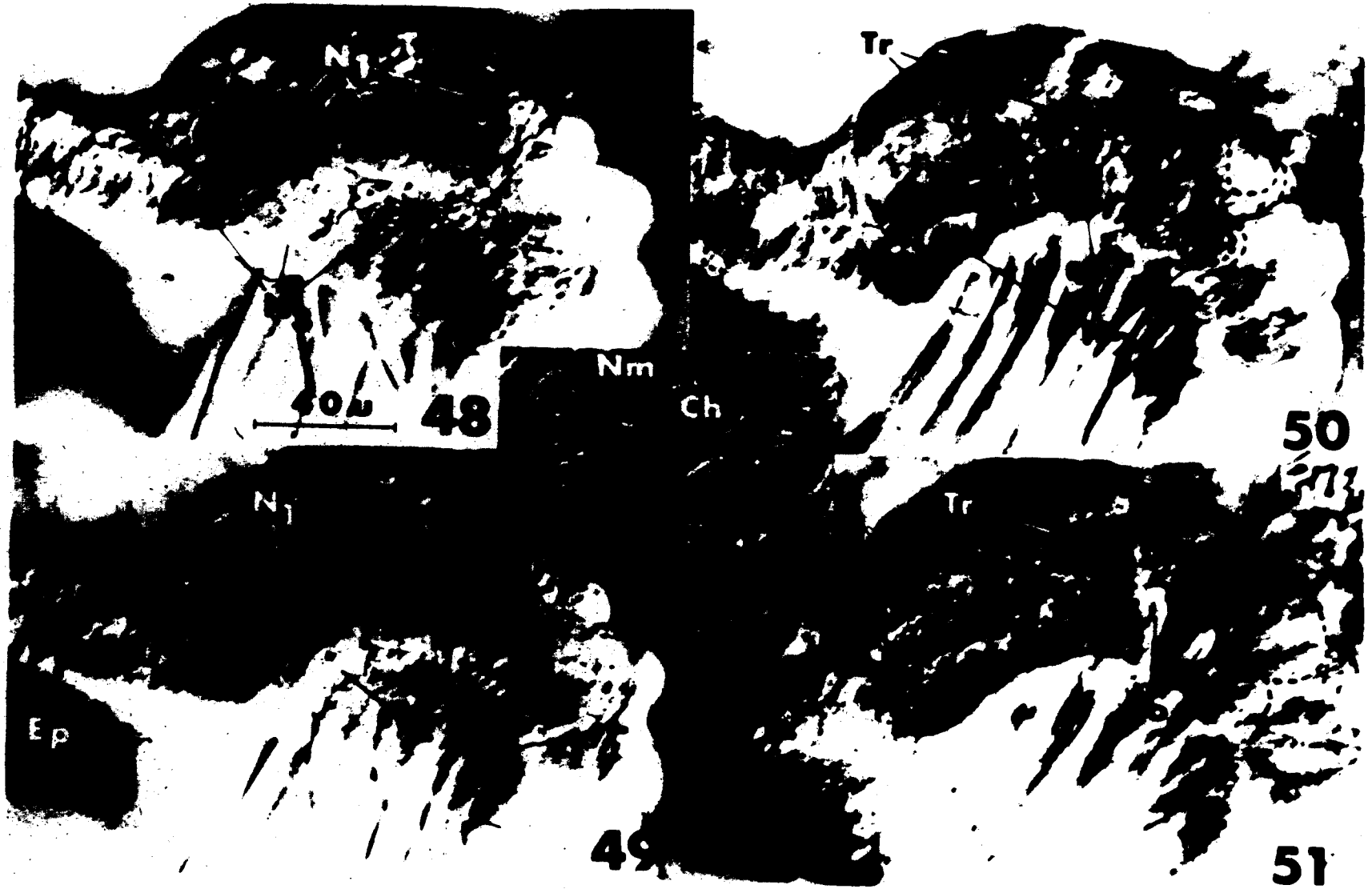

N1
Nm
10μ
48
N1
Ep
49
Tr
Ch
50
Tr
51

Plate 12*

Explanation of figures

53. *Monocystis [illegible]*. Older Stobbe's cell with extracellular cavity.

Cell from developing Stobbe's gland, showing large extracellular cavity (Ex) forming. Dotted line delimits sub-nuclear face of this cavity. Small vesicles (Vs) are found only in cytoplasm near the nucleus. The nucleus (N_1) is large and irregular.

54—57 *M. [illegible]*. Series of optical sections through a Stobbe's cell nucleus. (Scale is identical in figs. 54—57).

54. The inner face of the wall of the extracellular cavity is shown. A portion of the nucleus (Ar) is surrounded by small vesicles (Vs).

55. A large clear area (Cl) fills the centre of the nucleus with four nuclear arms (Ar) extending into the cytoplasm.

56. The nucleus is constricted, near a body surrounded by short radial arms (Rb).

57. Nucleus (N_1) shows a conventional profile near the centre.

*All material in plate 12 is from a cuticular pharate adult, eyes dark brown, antennae sclerotized (Liv) (Nom).


Vs
50 µ
53
Vs
Vs
56
40 µ
57

this cavity by [illegible] with the cell's base (fig. 54). Ultrastructural detail support the observation that the cavity is extracellular in origin. Electron micrographs of the wall show a distinct basement membrane on the side facing the cavity. Very small cells, rich in ribosomes and microtubules are found inside the cavity attached to the wall clearwater, in preparation. The perinuclear cytoplasm contains numerous small vesicles 2--3 μ in diameter, but these are absent from the lower walls of the cavity (figs. 53--57).

The nuclei in cells of this age require some comment (figs. 54--57). A series of optical sections reveal several unusual structures within the same nucleus. At the mid-section, this nucleus is 100 μ in diameter, roughly oval in outline and regularly granular in contents (fig. 57). A little below this section, the nucleus is still regularly granular but shows a marked constriction with a central body surrounded by short radial arms (Rb) (fig. 56). At lower levels the nucleus is dominated by a large circular clear area (C) surrounded by four arms (Ar) containing irregular granular material (fig. 55). The upper nuclear arm can still be seen in sections showing the inner wall of this extracellular cavity (Ex) (fig. 54). Other nuclei showed considerable variation in number of arms and constrictions.

3.2.6 Maturation of the Stobbe's gland

The expansion of individual cell volumes observed in the maturing gland accentuates the "cupping" of the underlying basement membrane shown in figs. 48--51 until the gland becomes a symmetrical ball of cells (fig. 19 of *P. separata*) with the basement membrane restricted to a finger-like sleeve around the core of sockets and elongated scales of

the duct. Continued expansion in cell volume draws the gland deeper into the fat body of the anterior abdominal segments.

A distinct end apparatus duct can be observed in slightly older cells (fig. 58). Every cell observed at this stage revealed a fine, convoluted thread with infrequent expansions, first appearing at the level of the nucleus, and extending a third of the way into the extracellular cavity. The cell-nuclear face of this cavity, initially convex (fig. 58), becomes strongly concave (fig. 58). The nucleus becomes thinner and slightly crescent shaped, eventually assuming the form of a discus in the mature gland. Until this stage of development is reached the gland does not begin biosynthesis of phenylethyl-β glycoside (cf. 3.4.5 and fig. 66).

A steady expansion in size of the extracellular cavity continues throughout the remainder of the stadium, without additional morphological complexity developing, until 1—2 days before eclosion. Large (20- 40 μ diam.) vesicles (Vl) are then extruded into the extracellular cavity, mostly from the cytoplasm in the vicinity of the nucleus (fig. 59). Many small cytoplasmic vesicles (Vs) are found near a developing major vesicle. Incubation of these cells with emulsin (β glucosidase of plant origin) results in a distinct mottling of the surface of the major vesicles (fig. 60). This may be interpreted as resulting from the degradation of the pheromone precursor contained in the vesicles into the lipophilic pheromone (2-phenyl ethanol, Clearwater, 1975a).

3.3. Chemistry of the Male Pheromone of *M. configurata*

G.L.C. analysis of the hair pencil extract showed three

Plate 13

Explanation of figures

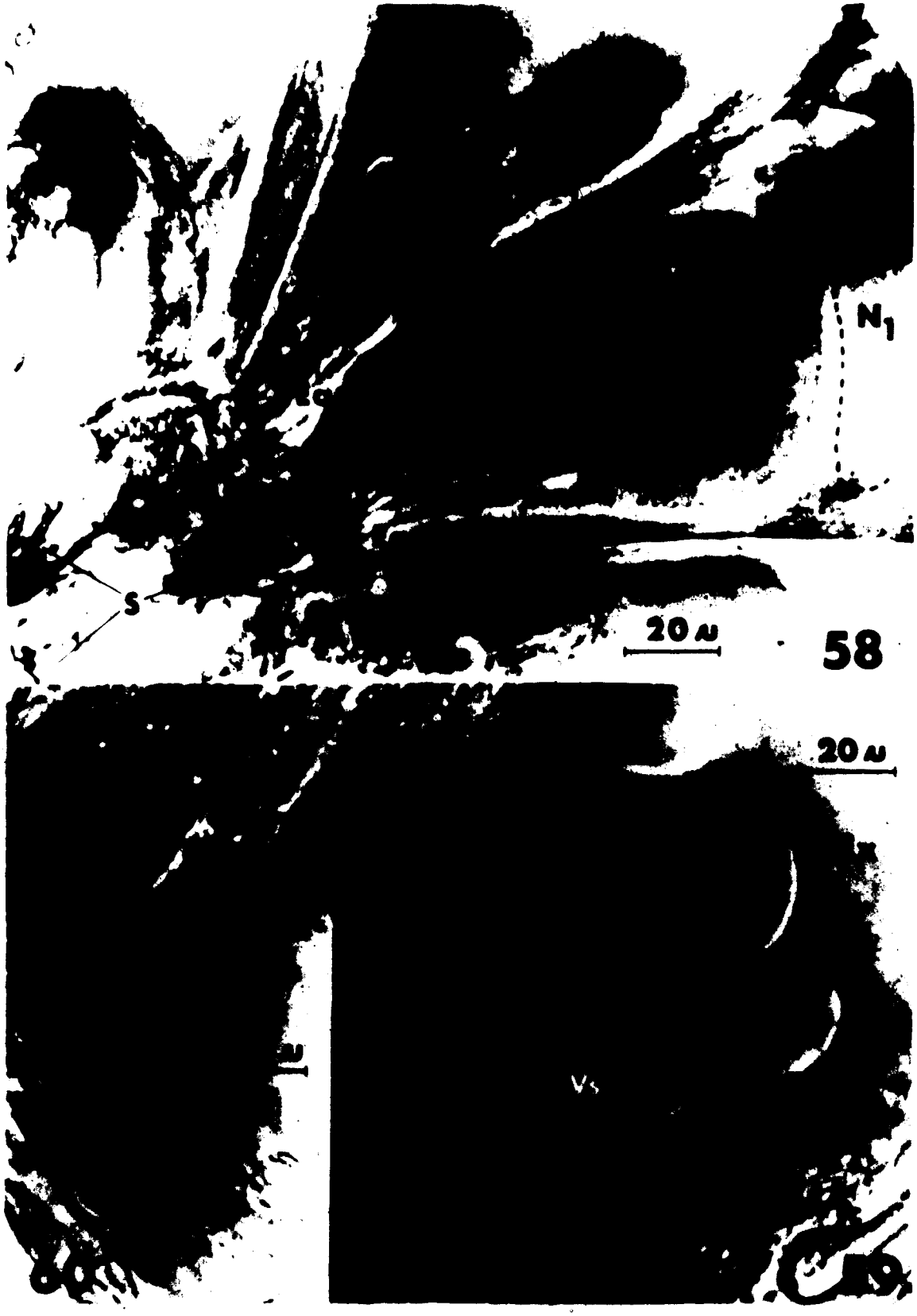
58. *M. [illegible]*. Stobbe's gland cell with end apparatus.

Stobbe's gland cell, with a 2–3 filament end apparatus (Ea) attached to the socket (S) of the duct scale. The nucleus (N_1) is discus-shaped. (Cuticular pharate adult, eyes dark brown, tibial spine, antennae and lever sclerotized, silver hair pencil scales).

59. *M. [illegible]*. Extrusion of large vesicles into the extracellular cavity.

Hypertrophied Stobbe's gland cell. Large vesicles (Vl) are emerging from the sub-nuclear face of the extracellular cavity (Ex). Numerous small vesicles (Vs) are clustered near the point of emergence. A fine tracheole (Tc) is present. (Cuticular pharate adult. Fully pigmented wing, ecdysial sutures beginning to break).

60. *M. [illegible]*. Stobbe's gland cells treated with β-glucosidase. Large vesicles showing surface pitting (arrows). (As above). (Liv) (Nom).


N1
S
20 μ
58
20 μ

peaks, the first of which was very much larger than the two following ones (fig. 61A). The major component of this extract had retention times identical to 2-phenyl ethanol on two columns (table II). When standard 2-phenyl ethanol was added to extracts of the hair pencil, complete superposition of the peaks occurred, confirming the fact that standard and unknown had the same retention time. Complete loss of the major peak on stainless steel columns could also be duplicated with standard 2-phenyl ethanol at dilutions comparable to that of the extract.

The mass spectrum of the extract (fig. 62A) contained peaks of mass 91, 92 and 122. The heights of these peaks were in the same ratio as those of standard 2-phenyl ethanol (fig. 62C). Peaks of mass 104, 105, 128, 130, 132, 133 and 149 were often present, but peak heights held no constant relationship to the peaks of mass 91, 92 and 122. Peaks of mass 84 and 86 were from methylene chloride. No attempt was made to identify the two remaining materials found in trace amounts.

3.4 The Metabolic Pathway Leading to the Pheromone

3.4.1 Chemistry of the glycosides from the Stobbe's gland.

Thin layer chromatographs of crude extracts and aqueous layers from the partition step (see 2.3.2) showed the presence of a single large spot when examined with ultraviolet light. The Rf of the spot from both steps of the extraction procedure in *P. separata* was 0.70 identical with authentic benzyl-β-glucoside. The compound from *M. configurata* had an Rf = 0.71, close to *P. separata* and standard benzyl-β-glucoside while other standards run had greater value, e.g. isopropyl-β-glucoside (Rf = 0.79). The chloroform layer from *P.*

Fig. 61. *Maresina configurata*. Gas-liquid chromatography — evidence for identification of pheromone and hydrolysis product.

A. Hair pencil extract 60HP/ml OV-17 glass 6' x 1/8" T = 125°C. A = 10 x 16 Perkin Elmer 990 G.C. B. OV-17 glass 6' x 1/8° T = 110°C. A = 1 x 10^3 Beckman G.C.S. (i) Standard phenyl ethanol; (ii) 15 glands in 20 µl buffered (pH = 5.0) emulsin after 24 hours at room temperature, 30 µl $MeCl_2$ extraction; (iii) 4 glands in 20 µl buffer, 20 µl $MeCl_2$ extraction.

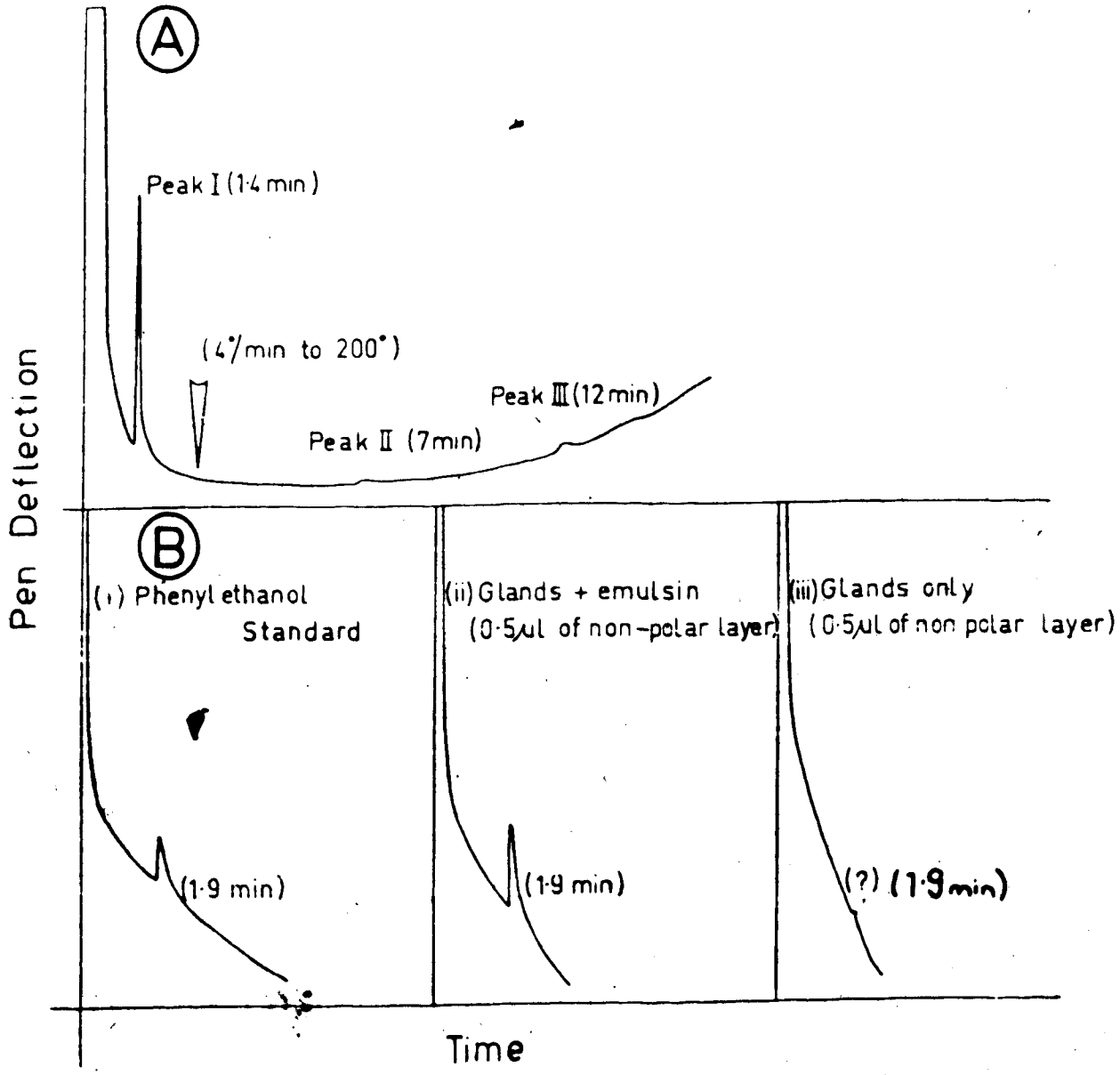

A
Peak I (1·4 min)
(4°/min to 200°)
Peak III (12 min)
Peak II (7 min)
Pen Deflection
B
(i) Phenyl ethanol
Standard
(1·9 min)
(ii) Glands + emulsin
(0·5 µl of non-polar layer)
(1·9 min)
(iii) Glands only
(0·5 µl of non polar layer)
(?) (1·9 min)
Time

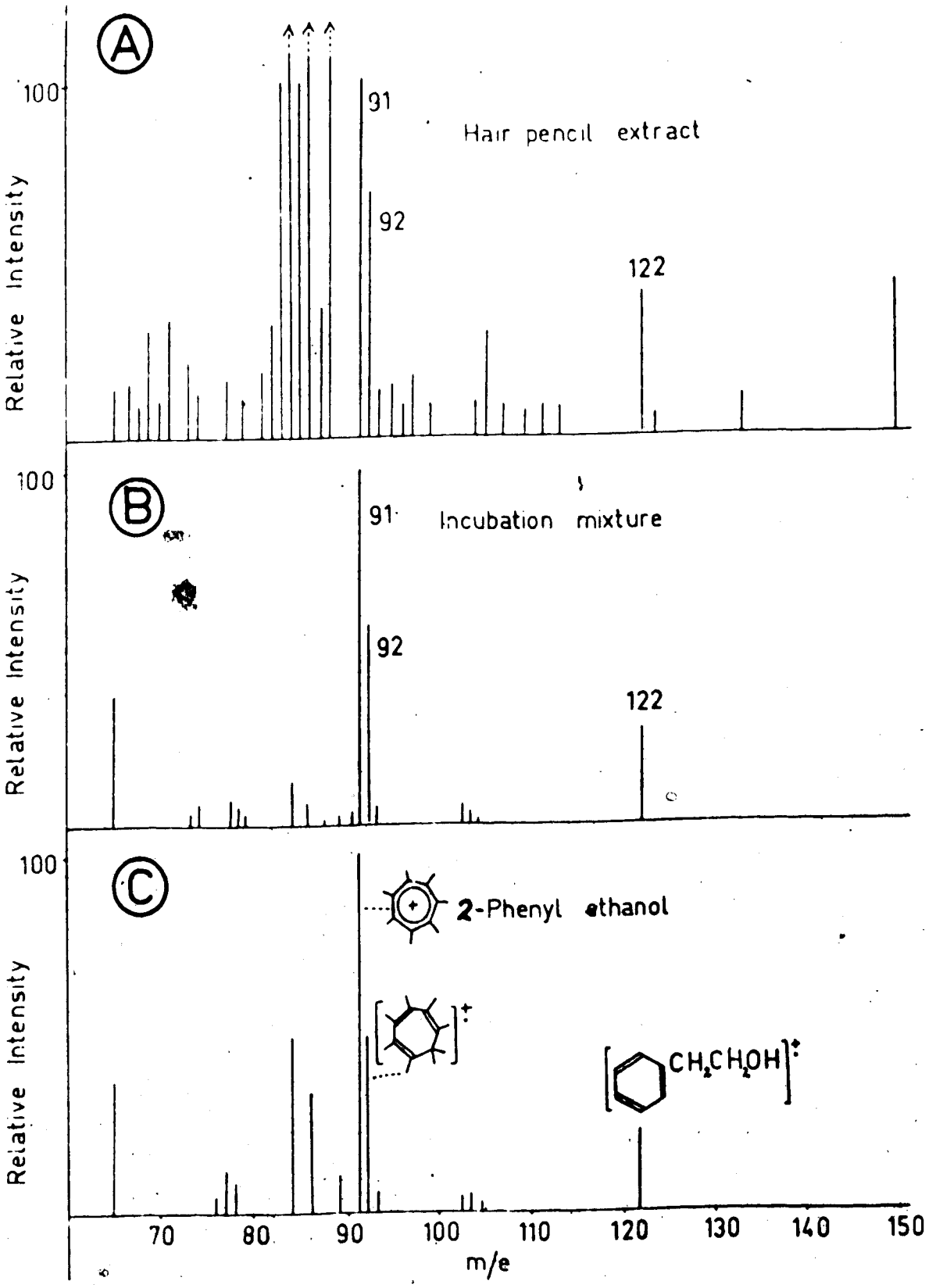
TABLE II

Gas-liquid chromatography of the pheromone of *Mamestra configurata*

Material	Retention (mins)	Column	Temp. °C
Hair pencil extract	1.9	OV-17	110
Standard 2-phenyl ethanol	1.9	OV-17	110
Hair pencil extract	4.4	XE60	170
Standard 2-phenyl ethanol	4.4	XE60	170

Fig. 62. *Manestra configurata*. Mass spectra of pheromone and hydrolysis product.

A. Crude extract of 60 hair pencils/ml methylene chloride. B. Hydrolysis product from 15 Stobbe's glands/20 µl buffered (pH = 5.0) emulsin after 24 hours at room temperature. C. Standard 2-phenyl ethanol in methylene chloride. AEI M.S.9.


A
100
Relative Intensity
91
92
122
Hair pencil extract
B
100
Relative Intensity
91
92
122
Incubation mixture
C
100
Relative Intensity
2-Phenyl ethanol
CH2CH2OH
70
80
90
100
110
120
130
140
150
m/e

separata also showed a spot (Rf = 0.71) that turned pale lemon yellow on exposure to light for 24 hours.

Mass spectra from both crude extracts and aqueous layers from both species were free of significant contamination (fig. 63). The parent peak of the compound from *M. configurata* had a mass of 302 ($M + NH_4^+$), corresponding to a molecular weight of 284 (fig. 63A). Both spectra show a significant peak at 180, which corresponds to the unsubstituted hexose fragment of the molecule ($M + NH_4^+ - ROH$) (Hogg and Nagabhushan, 1972). The spectra of standard benzyl-β-glucoside showed three major peaks at 180, 270 and 288, corresponding to those from the spectrum of *P. separata* (fig. 63B). Neither benzaldehyde nor 2-phenyl ethanol could be extracted from these glands.

3.4.2 Site of synthesis of the glycoside

The fat body of male and female *M. configurata* does not contain measurable amounts of phenylethyl-β-glycoside. Uptake of glycoside by pupal tissues is unlikely. The only aglycone detected in significant amounts from the Stobbe's gland was the 2-phenyl ethanol derived from the natural precursor. A trace of benzyl alcohol, derived from the injected analogue, was found in only one replicate. Nothing measurable was found in the non-polar fraction from fat body or dorsal muscles.

3.4.3 Incorporation of labelled precursors into the glycoside

L-phenylalanine is a good precursor of phenylethyl-β-glycoside with up to 1% of the label incorporated. The relative migration of cold phenylalanine, and phenylethyl-β-glucoside corresponded with the

Fig. 63. *Mamestra configurata* and *Pseudaletia separata*. Mass spectra of the glycosides.

A. *M. configurata*. Aqueous layer, 2 glands in 10 µl distilled water. B. *P. separata*. Aqueous layer, 10 glands in 10 µl distilled water. C. Standard benzyl-β-glucoside. (Note differences in source temperature affecting ratio of fragments to parent peaks). AEI M.S. 12 with NH_3 reagent gas.

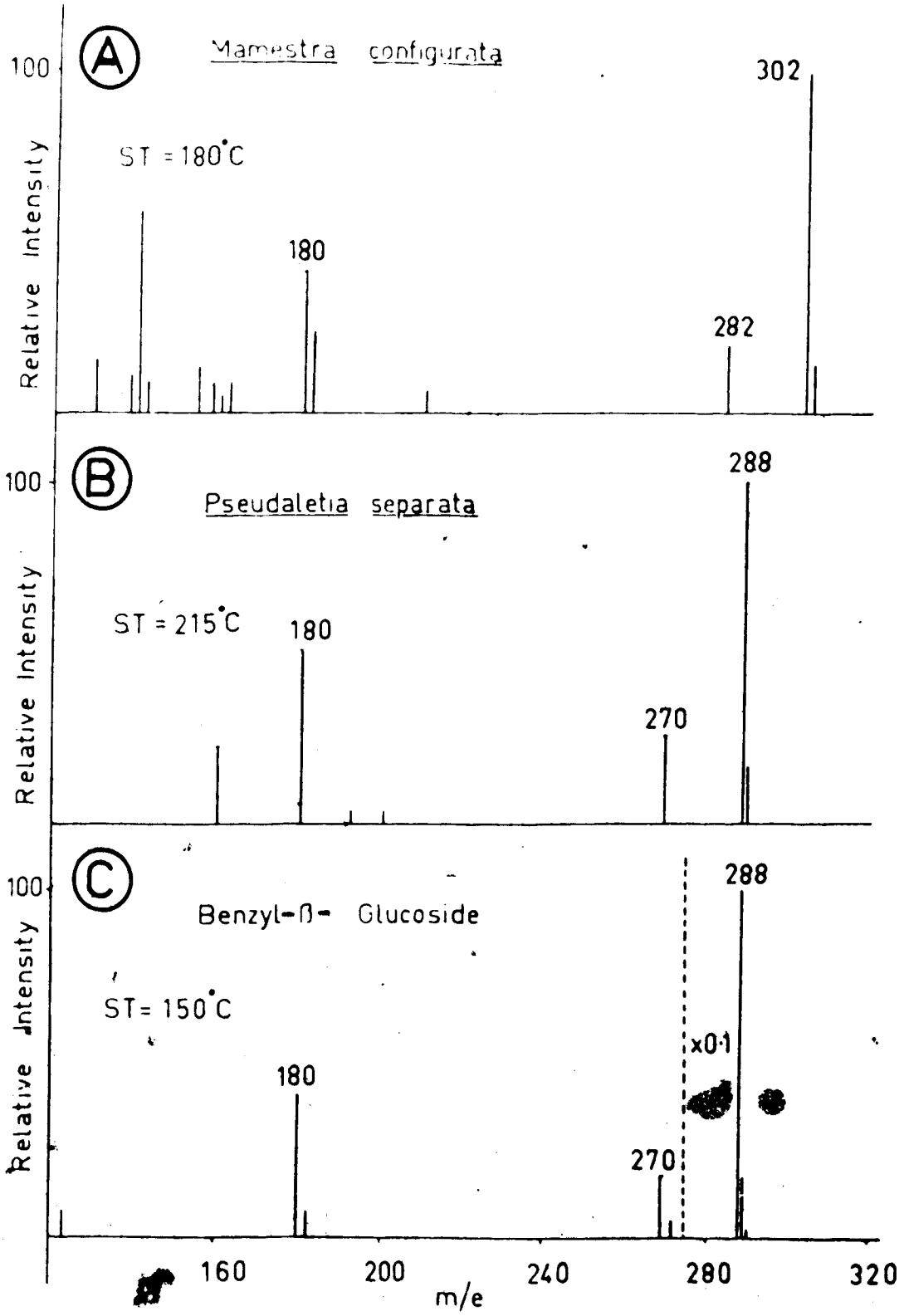

A
Mamestra configurata
ST = 180°C
100
Relative Intensity
180
282
302
B
Pseudaletia separata
ST = 215°C
100
Relative Intensity
180
270
288
C
Benzyl-ß- Glucoside
ST = 150°C
100
Relative Intensity
180
270
x0·1
288
160
200
240
280
320
m/e

Fig. 64. *Pseudobaeria separata*. Hydrolysis product of the glycoside.

A. (i) Standard benzaldehyde and benzyl alcohol;

(ii) methylene chloride layer of 6 glands in 10 µl buffered (pH = 5.0) emulsin after 24 hours at room temperature. 5% FFAP 6' x 1/8" stainless steel. T = 134°C. A = 5 x 10^3. B. Mass spectrum of peak II AEI M.S. 12 with coupled Varian Aerograph. 1400 G.C.

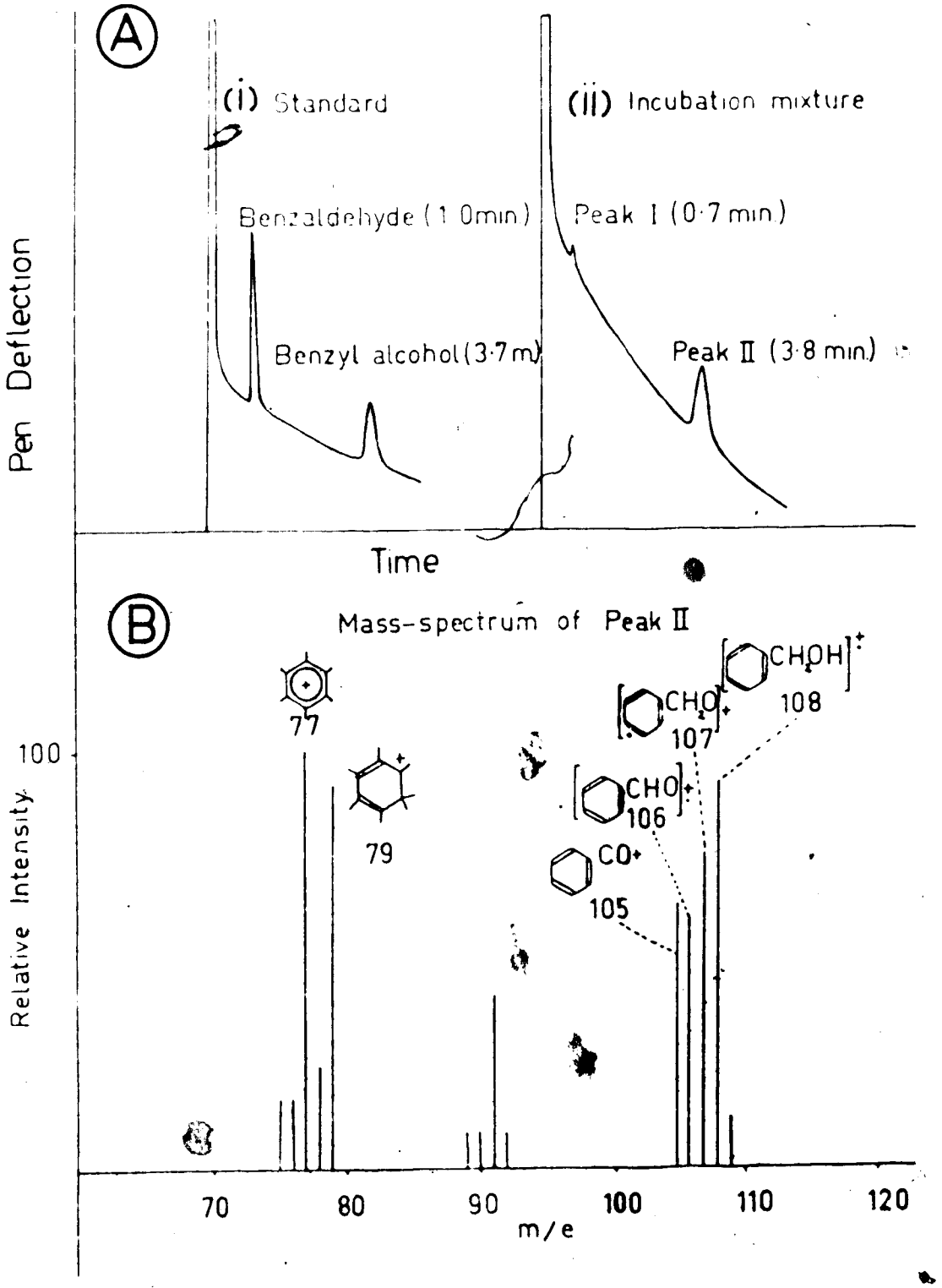

A
(i) Standard
(ii) Incubation mixture
Benzaldehyde (1·0min)
Peak I (0·7 min.)
Benzyl alcohol (3·7m)
Peak II (3·8 min.)
Pen Deflection
Time
B
Mass-spectrum of Peak II
77
79
CHO 106
CO 105
CH2O 107
CH2OH 108
100
Relative Intensity
70
80
90
100
110
120
m/e

Fig. 6. Radioautograph of paper chromatograph of labelled products from the Stobbe's gland.

A—C. Radioactivity from strips of a chromatogram measured by liquid scintillation counting, with a cold chromatogram below for comparison.

A. 0.2 μCi phenylalanine-U-C^{14} (std.  and labelled products from two Stobbe's glands  using n-butanol; acetic acid, water, 4:1:1. Lower chromatogram of l-phenylalanine + phenylethyl-β-glycoside developed in same solvent system at origin from contaminants in std. phenylalanine.

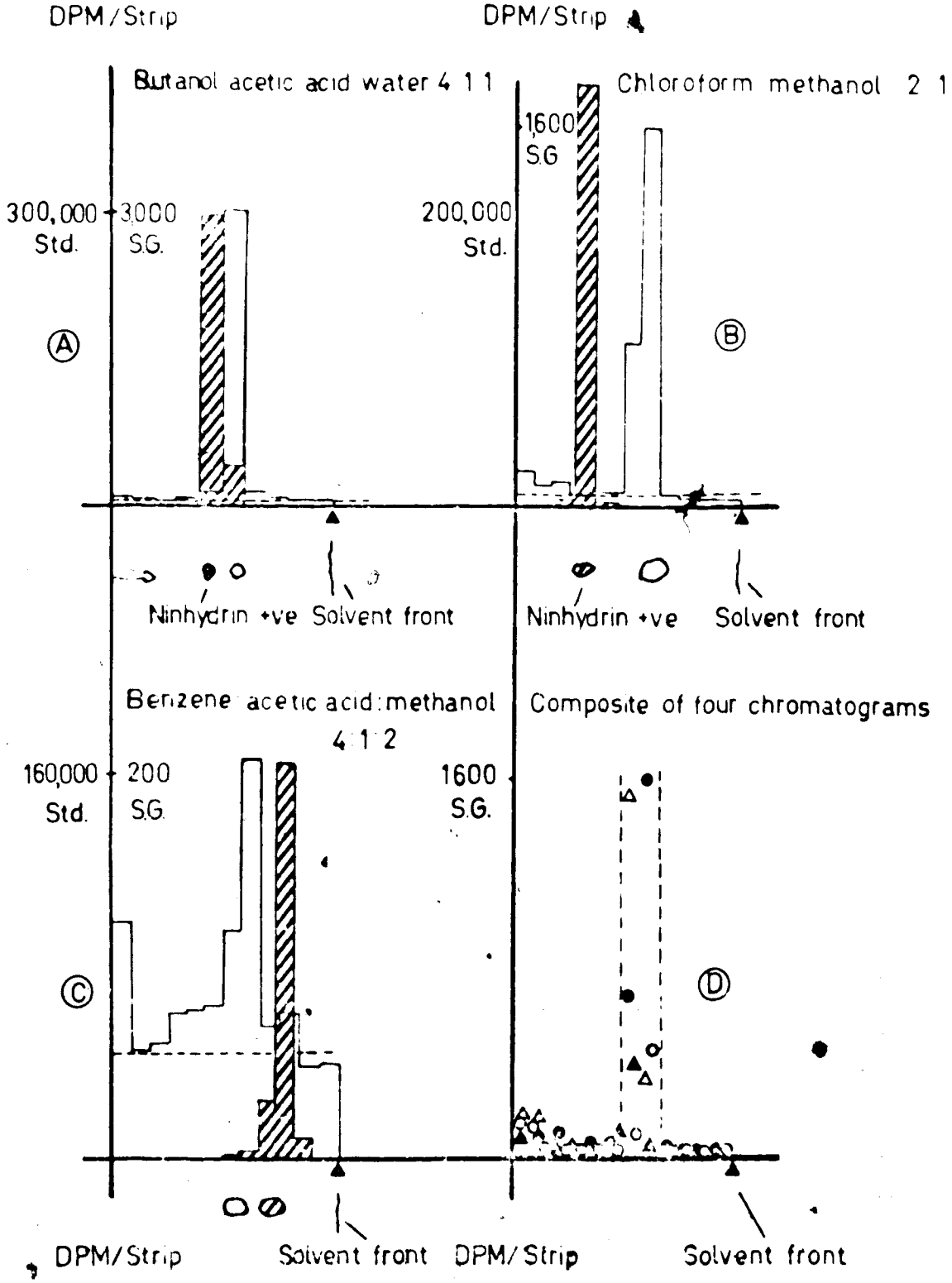
B. 0.2 μCi phenylalanine-U-C^{14}  + labelled products from two Stobbe's glands  chromatographed using chloroform: methanol 2:1. Lower chromatogram of l-phenylalanine and phenylethyl-β-glycoside using same solvent system.

C. 0.25 μCi cinnamic acid-2-C^{14}  and labelled products from two Stobbe's glands  , chromatographed using benzene: acetic acid: methanol, 4:1:2. Lower chromatogram of cinnamic acid and phenylethyl-β-glycoside, using the same solvent system.

(All 10 replicates of A and B and all 3 replicates of C had significant radioactivity in the indicated areas).

D. Composite of four chromatograms of type B, showing the spread of radioactivity observed.

(A,B,C Background--------).


DPM/Strip
Butanol acetic acid water 4 1 1
300,000 Std.
3000 S.G.
Ⓐ
Ninhydrin +ve
Solvent front
DPM/Strip
Chloroform methanol 2 1
1600 S.G
200,000 Std.
Ⓑ
Ninhydrin +ve
Solvent front
Benzene acetic acid:methanol 4:1:2
160,000 Std.
200 S.G.
Ⓒ
DPM/Strip
Solvent front
Composite of four chromatograms
1600 S.G.
Ⓓ
DPM/Strip
Solvent front

radioactivity found in chromatograms of a standard dose (0.2 μ C) of L-phenylalanine and the major labelled product of the Stobbe's gland respectively (fig. 65A and B). In n-butanol: acetic acid: water, the spots are small (average diameter - 8 mm) but only separated by a short distance (fig. 65A). This system was highly reproducible, all replicates showing most labelling in the same 1 cm strip. Greater separation was achieved with chloroform: methanol, but the spots were larger (average diameter = 15 mm) (fig. 65B). In this system, a larger area of the chromatogram contains a significant number of counts (fig. 65D).

The chromatograms of the Stobbe's gland extract were very clean. Only one large peak of activity was seen. Apart from a little activity at the origin and the single peak, all other strips were not significantly different from the background. In particular, there was no activity in the area of phenylalanine migration, suggesting that there is little free phenylalanine in the mature Stobbe's gland. This result agrees well with the observation that few other soluble substances are present with the glycoside in the Stobbe's gland (Clearwater, 1975a).

Uptake of phenylalanine is not uniform throughout development (fig. 66). Young pharate adults, from the beginning of development to the stage in which eye pigmentation, and antennae and I tibial spine sclerotization is complete, consistently incorporate very little activity. Most activity is found in pharate adults beginning to incorporate pigments into the wing scale (stage III). At this stage, the Stobbe's gland has hypertrophied and developed an end-apparatus (see 3.2.6). Older pharate adults show an erratic decrease in ability to incorporate this precursor.

Fig. 66. [illegible]. Ability of different stages to incorporate radioactive precursors.

Stage of development at time of injection.

Stage I. Deposition of pigment in the compound eyes.

Stage II. Sclerotization of the large tibial spine on the developing prothoracic leg and antennae.

Stage III. Deposition of pigment in scales of the wing and thorax.

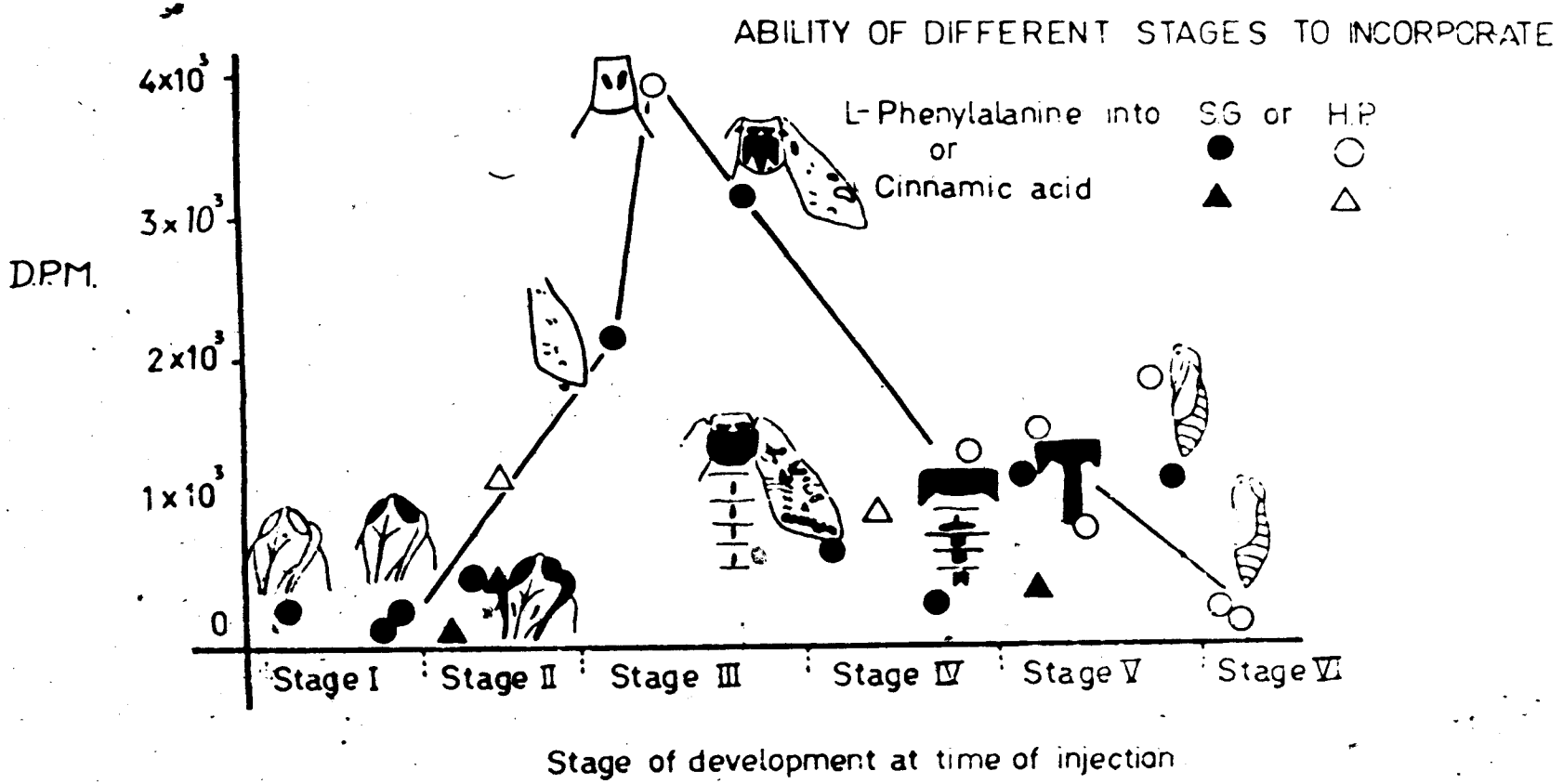
Stage IV. Deposition of pigment in scales on dorsal abdomen.

Stage V. Completion of pigmentation.

Stage VI. Formation of ecdysial sutures.

Stobbe's gland - S.G.

Hair pencil - H.P.


ABILITY OF DIFFERENT STAGES TO INCORPORATE
L-Phenylalanine into S.G or H.P
or
Cinnamic acid
D.P.M.
4x10³
3x10³
2x10³
1x10³
0
Stage I
Stage II
Stage III
Stage IV
Stage V
Stage VI
Stage of development at time of injection

The Stobbe's gland and the hair pencil incorporate comparable amounts of phenylalanine (fig. 66). The hair pencil contains 2-phenyl ethanol (M.W. = 122) plus two other compounds in trace amounts while the Stobbe's gland contains only phenylethyl-β-glycoside (M.W. = 284) (Clearwater, 1975a). If the labelled carbon is uniformly spread throughout the glycoside molecule, the hair pencil would receive less than half of the activity when the precursor is hydrolyzed to release the pheromone. In fact the hair pencil has slightly more activity than the Stobbe's gland, suggesting that all the radioactivity is from the aglycone.

The radioactive pheromone extracted from the hair pencil is less accurately measured, as, unlike the Stobbe's gland, other metabolic products or unreacted phenylalanine were not separated from the pheromone. The remnant of activity found in insects injected just before emergence (stage VI) is possibly due to contamination. At this stage, the Stobbe's gland is extruding large vesicles of pheromone precursor into the extracellular cavity (fig. 59) and further incorporation of phenylalanine would be unlikely.

Combustion analysis revealed that most of the phenylalanine remained within the insect with less than 3% of the dose present in the meconium. Loss of available phenylalanine through conversion to melanin (via tyrosine) did not appear to be significant. The forewings of this species are black (melanin) with a little green and silver, and were used as an assay of the amount of phenylalanine lost to melanin. The amount of activity incorporated by pharate adults injected just before pigment deposition (stage II) and when pigmentation of the wings was complete (stage IV) was the same — approximately

2×10^3 DPM/wing.

Small amounts of cinnamic acid-2-C^{14} are incorporated into the glycoside and hair pencil (fig. 65C). The smaller incorporation is most likely due to the much lower specific activity of cinnamic acid compared with phenylalanine. All replicates showed activity close to phenylethyl-ß-glycoside, but the amount was only twice background in one case. The correlation between stage of development and ability to sequestre precursor, observed for phenylalanine, also holds for cinnamic acid (fig. 66).

3.5 Release of the Pheromone from the Glycoside

3.5.1 *In vitro* hydrolysis of the glycoside

At least two compounds could be extracted by methylene chloride from the incubation medium containing glands from *P. separata* (fig. 64A ii). The first peak had a comparable retention time to benzaldehyde, but they were not identical. Both peak I and benzaldehyde appeared as separate peaks when the standard solution was mixed with the test. In contrast, peak II showed complete superimposition with benzyl alcohol. The mass spectrum of peak II corresponded with benzyl alcohol (fig. 64B).

The single detectable peak obtained from the same experiment with *M. configurata* had the same retention time as 2-phenyl ethanol (fig. 61B ii). Barely discernible amounts could be extracted from the control. The mass spectrum was relatively free from contaminants and provided a useful comparison with the hair pencil extracts. This preparation was used to establish the accurate masses of the main peaks (table III).

TABLE III

Accurate masses of the hydrolysis product from Stobbe's gland in *Myrestra configurata*

Nominal Mass	Accurate Mass	Calculated Mass
122	122.0737	122.0732
92	92.0617	92.0626
91	91.0548	91.0548

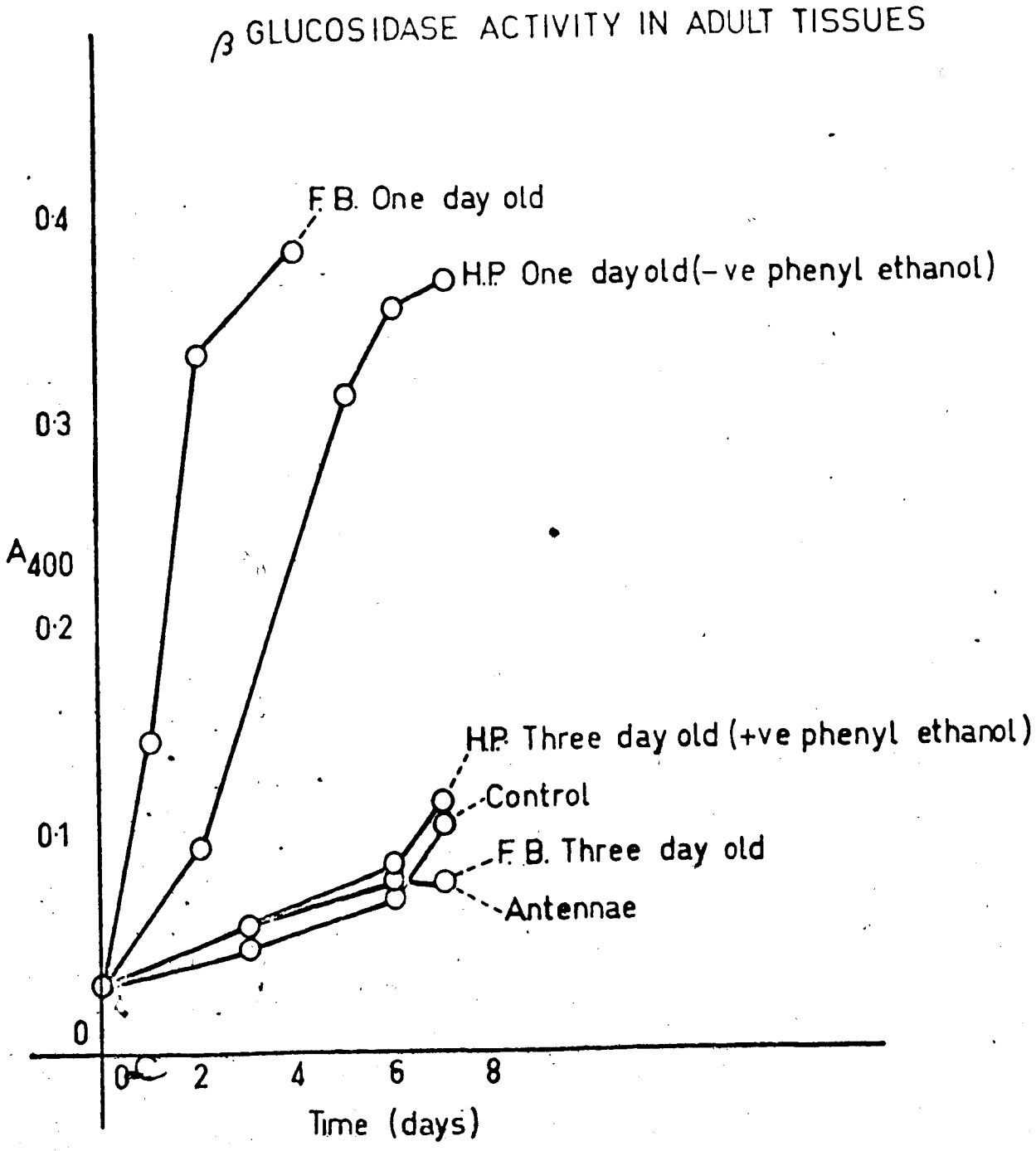
(Direct Probe on AEI M.S. 9)

3.5.2 Assay of an hydrolytic enzyme from the hair pencil cap cells

Beta-glucosidase activity is present in the fat body and in the hair pencil cap (fig. 67). The reaction is slow at room temperature but can be demonstrated with material from a single insect, and is readily reproducible. Activity is found in one-day-old adults, but is lost by the third day. In a newly-emerged male, the brush portions of the hair pencil are intermeshed, and held on the intersegmental membrane between sternites II and III where they develop (fig. 70A). Hair pencils at this stage lack the distinctive scent that can be discerned on hair pencils that have been tucked into the abdominal pouch. It is significant that enzyme activity can be found in the cap of hair pencils that lack the scent, but not in mature animals that possess the scent (fig. 67).

Fig. 67. [illegible]. β-glucosidase activity in adult tissues.

Tissue homogenates (hair-pencil caps (HP), fat body (FB), etc.) from a single insect were incubated with 5 ml of substrate for up to seven days at room temperature and the reaction followed with a spectro-photometer. Spontaneous hydrolysis of the substrate occurred if incubated for more than seven days.


β GLUCOSIDASE ACTIVITY IN ADULT TISSUES
F.B. One day old
H.P. One day old (-ve phenyl ethanol)
H.P. Three day old (+ve phenyl ethanol)
Control
F.B. Three day old
Antennae
A400
0·4
0·3
0·2
0·1
0
0
2
4
6
8
Time (days)

4. DISCUSSION

4.1 Evolution of Male Pheromone Systems in Noctuidae

4.1.1 Functional considerations

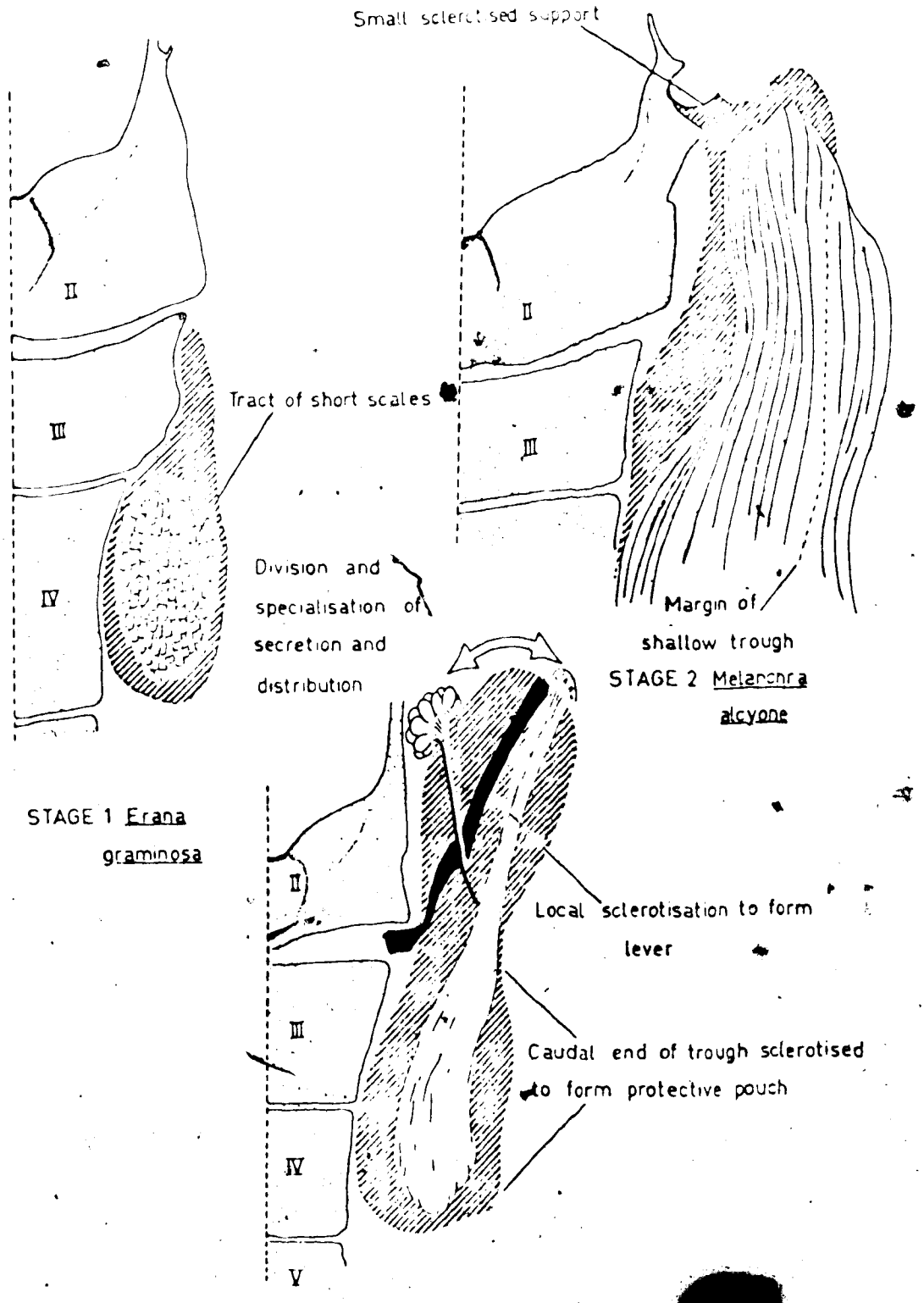
The pheromone system of the moths studied provides insight into processes of evolutionary specialization (fig. 68). The development of a complex wing gland in *P. praeruptaria* seems to have allowed the abdominal system to retain a primitive structure (fig. 68 - Stage 1). The aspect ratio and surface sculpture of the pouch scales are comparable to body scales. It is likely that the ancestral scent-producing scale tract combined the functions of scent production and release. The protected position of the scales inside the pouch would have diminished pheromone losses, but the restricted scale surface area would have been unlikely to have allowed high release rates for any but the most volatile compounds. Elongation of the scale and development of complex microsculpture would have greatly increased the efficiency of a system depending on physical diffusion (fig. 68 - Stage 2). The development of pheromone dust or transfer scales in butterflies is a parallel adaptation effecting a rapid transfer of chemical (Vane-Wright, 1972). An adaptation able to counteract the effect of the wings of the hovering male moths, which must draw off an appreciable amount of the released compound, would have considerable selective value.* Before effective strut support of these scales could have been developed, a change from the diffuse scale insertion of *M. alcyone* (fig. 12) to the restricted area of the basal sclerite

*For a discussion of noctuid mating behavior see Birch (1970a).

Fig. 68. Evolution of hair scale systems in male Noctuidae.

Diagram illustrating possible evolutionary path leading to the complex Stobbe's gland hair pencil system. The system has originated in a shallow, membranous, pleural trough (///). The trough and scales are short in *Feltia jaculifera* (stage 1). Lengthening of both components would have increased system efficiency; the longer scales providing increased evaporation surface, and the longer trough giving a degree of protection from pheromone loss while at rest (*Melanchra adjuncta* stage 2).

Specialization of the trough occurs, with the caudad portion sclerotizing to form the protective pouch, while most of the cephalad portion remains membranous. Local sclerotization of the cuticle forms the lever. Based on the observed developmental events, it is suggested that the original group of pheromone secreting and releasing cells, split to form two specialized groups (white arrow).



STAGE 3 Mamestra configurata

[illegible]

[illegible] scales [illegible] are covered with long, parallel ridges [illegible] apart. The only sculptural element between the ridges are fan-like group[illegible] of short delicate crests. Distinct transverse striae are seen only in [illegible] (Meyrick). Kristensen (1970) believed that these families exhibit scale structure similar to groups ancestral to the rest of the order. McColl (1969) considered that the involvement of scales in pheromone release is a very ancient one. The basal area of the hair scales examined in this study so strongly resemble these primitive micropterygid scales that a direct development may have occurred during their evolution.

Further evidence for the ancient nature of these systems comes from the startlingly similar microsculpture of pheromone scales from individuals of species in different families and from different areas of the body. The hair pencil scale of *Manduca sexta* (Linnaeus) (Sphingidae) is almost identical to that of *M. sleperi* (cf. fig. 2 Grant and Eaton, 1973). The wing scale of *A. scorpialis* is similar to the abdominal tip scales of *Trichoplusia ni* (Hübner) (Grant and Brady, 1973). Either these scales appeared early in the evolution of the

[illegible]

[illegible]

The [illegible] [illegible] [illegible] sculptured and [illegible] differ little only in detail from the [illegible] [illegible]. These systems [illegible] more recent, and appear to have been acquired only by [illegible] [illegible] that the most primitive subfamily of the trifine Noctuidae, the [illegible], lack this system and probably diverged before the Stobbe's gland and hair pencil had evolved. The mutation leading to the development of the distinct hexagonal sculpture may be similar to the one that causes occasional defects in basal areas (fig. 15). Hexagonally-braced structures are extremely strong and light (known as geodetic construction in aircraft manufacture). Development of such a scale would leave the way free for the evolution of extremely long and efficient pheromone release scales.

4.1.3 Possible common origin of secretory and disseminatory components

A marked feature of the more complex systems is the separation and specialization of scales for either pheromone secretion or dissemination. Early development in *M. configurata* strongly suggests that the Stobbe's gland and hair pencil have a common origin evolutionarily as well as developmentally. Both structures develop on the same fold of epidermis and apart from size and number of cells they can be difficult to distinguish in early stages. It is possible that the β-glycoside of the Stobbe's gland and the β-glycosidase of the hair pencil cap cells

[illegible] have been accompanied by [illegible] to synthesize either the enzyme or the pheromone precursor.

The progressive reduction in degree of development and surface complexity of the discriminating scale surface from *E. graminosa* and *M. aleyona* to *E. exigua* is paralleled by a loss of surface detail in scales associated with secretory cells. The small scales of the scent patch on the wing gland of *E. graminosa* possess low ridges and small protrusions (figs. 9 and 10), while the scales leading from Stobbe's gland are quite smooth (figs. 20 and 21).

4.1.4 Are these structures vestigial?

A question that must always be faced in a discussion of this type is whether a particular structure is vestigial rather than close to the ancestral type. Several species of noctuid with vestigial pheromone systems have been collected in Britain and described by Birch (1972). The Stobbe's gland is always the first component to disappear, followed by the hair pencil scales and the protective pocket. Several species retain only the lever, while *Euplexia lucipara* (Linnaeus) exhibits the highest degree of reduction with only two small processes arising from the posterior angles of sternite II persisting. In general, vestigial systems are characterized by retention of the hard parts, while supposedly primitive species (*E. graminosa* and *M. aleyona*) display simple but possibly functional systems. It is also reassuring that these species were collected only in New Zealand, geographically isolated islands that appear to have assured the

survival of many endopterygote primitive species from other orders.

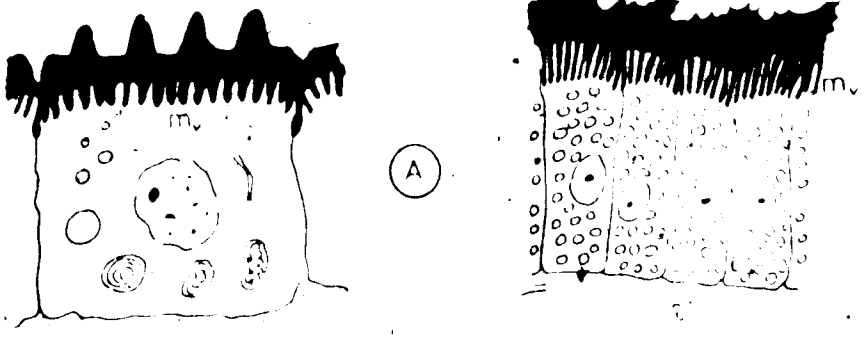
4.1.3 Comparative gland structure

A functional classification of insect exocrine glands can be erected on structural (Noirot and Quennedy, 1974) or chemical grounds. Strikingly similar structures have evolved in widely separated species to produce chemically comparable products. Relatively inert materials such as the long chain, unsaturated compounds that function as long range attractants in lepidoptera (e.g. *Hyalophora cecropia* (Hübner), Berger, 1966; *Bombyx mori* (Linnaeus), Butenandt et al., 1959) are produced in cells having the secretory surface (microvilli) in extended intimate contact with much of the cell cytoplasm (fig. 69A). More toxic compounds such as anisomorphal (the defensive secretion of *Oecanthus fultoni* (Stoll), Happ, et al., 1966) and the short chain fatty acid (component of the sexual pheromone of the mecopteran *Panorpa nuptialis* (Klug), Crossley and Waterhouse, 1968) are produced by cells with microvilli restricted to a small area and cuticle lined ducts, which may prevent diffusion back into the cytoplasm (fig. 69A).

The most toxic compounds are aromatics — aldehydes and quinones. Tenebrionids and noctuids, quite unrelated taxonomically, produce similar chemicals and share (a) glycosides as precursors, (b) extracellular reaction compartments and (c) very long duct systems. The more toxic the secretion, the greater is the distance between cytoplasm and final product (fig. 69C).

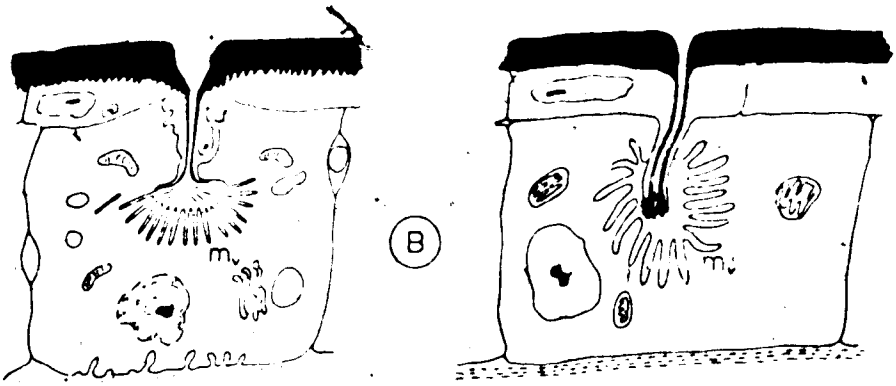
Fig. 69. Similarities between cell structure and secretion.

Diagram illustrating similarities between cell structure and secretion. A. Cells producing relatively inert substances have a long unmodified border of microvilli (Mv) (Jefferson et al., 1966, after Waku and Sumimoto, 1969). B. Microvilli (Mv) are restricted to a region near a cuticular duct, in cells producing moderately toxic compounds. *Eupolitrema nautili* has evolved a specialized tubule carrying cell (tu) (after Crossley and Waterhouse, 1969; Happ et al., 1966). C. Happ (1968) demonstrated that the secretory product in *Pleodes longicollis* appeared as a glycoside. This inert material moves across the extensive border of microvilli (Mv) while the toxic quinone is produced on a restricted cuticular reaction site. Male noctuids have convergently acquired the essential features of this scheme.



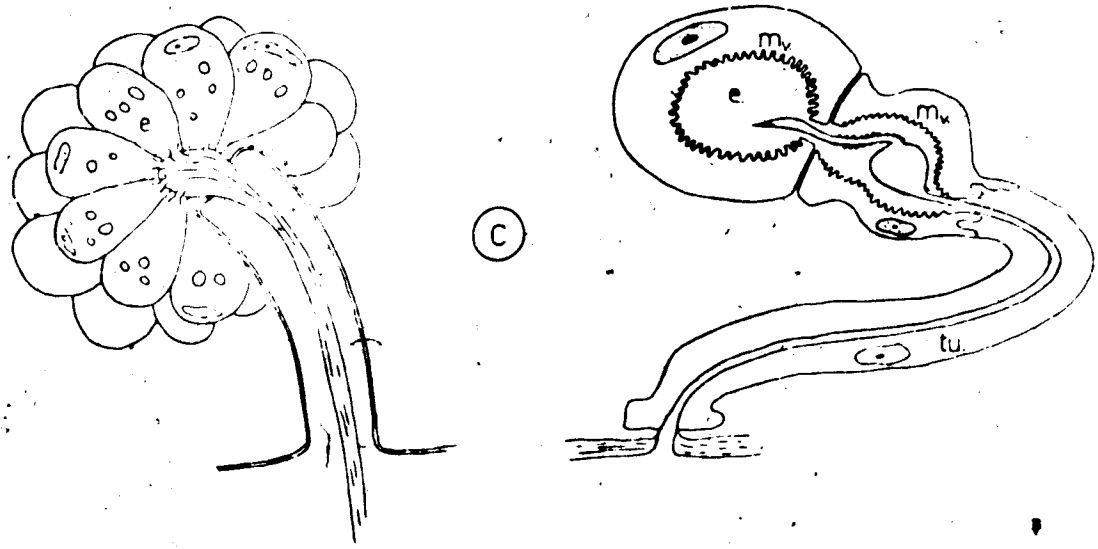
Bombyx mori (Lepidoptera) Trichoplusia ni (Lepidoptera)

(trans-10-cis-12-hexadecadien-1-ol) (cis-7-dodecen-1-yl acetate)



Harpobittacus australis (Mecoptera) Anisomorpha buprestoides (Phasmida)

(short-chain fatty acid) (terpene)



Mamestra configurata (Lepidoptera) Eleodes longicollis (Coleoptera)

(phenyl ethanol) (p-benzoquinones)

4.2 Production of the Pheromone by the

Stobbe's Gland-Hair Pencil Complex

4.2.1 Are there any other organs involved?

The Stobbe's gland and the hair pencil appear to be the only organs contributing to the development of the male pheromone of *M. configurata*. The fat body was an attractive candidate for glycoside biosynthesis, but the presence of measurable quantities of the specific glycoside, and the inability of the Stobbe's gland to absorb a glycoside that differed only in the length of the carbon chain linking the sugar with the aromatic ring, from the normal precursor, renders this hypothesis unlikely.

L-phenylalanine is a normal constituent of insect hemolymph (Gilmour, 1965) and the uptake of this amino acid by the Stobbe's gland from the surrounding hemolymph probably initiates the synthesis of the pheromone of *M. configurata*. The lack of free phenylalanine in the Stobbe's gland suggests that the rate of uptake of the amino acid may be a limiting factor of metabolism.

4.2.2 Synthesis of the glycoside by the Stobbe's gland

Comparison of my results with earlier work allows some conclusions to be drawn about the secretory mechanism of Noctuidae. The perinuclear cytoplasm appears to be the major area concerned with production of the pheromone precursor. In *Phlogophora meticulosa* (Linnaeus) Birch (1970b) observed that microvilli are restricted to the face of the central cavity underlying the nucleus. Concentrated in the cytoplasm surrounding the nucleus are large numbers of vesicles (Vs) in *M. configurata*. Two types of large vesicles are

[illegible] and Sarafis, 1973) in addition to the [illegible], but these are not present in [illegible]. An electron micrograph of [illegible] also failed to reveal [illegible] in the central cavity of Stobbe's gland. Unpublished results, the apparent lack of [illegible] may be due to lack of [illegible] gland contents [illegible] glands in Birch's (1970b) study, rather than to fundamental differences between [illegible] and the other two species.

It seems likely that the glycoside is synthesized in the perinuclear cytoplasm of the Stobbe's gland and is extruded into the extracellular cavity in the form of large vesicles just before eclosion. In *L. separata*, the gland begins to decrease greatly in size one day after emergence (Clearwater and Sarafis, 1973) while the free aromatic on the hair pencil begins to rapidly increase on the following day (Clearwater, 1972), signalling the transfer of the glycoside from the central cavity of Stobbe's gland to the hair pencil. The gland only releases precursor once, as blocking the opening of the gland after the discharge has no effect on the subsequent production of scent in [illegible] (Birch, 1970b).

4.2.3 Interaction between the enzyme and the glycoside

The glandular cells at the hair pencil base of noctuids have been previously noted (Ford, 1955). The presence of a β-glucosidase in the hair pencil cap cells of young adult *M. configurata*, and the absence of this enzyme in the cap area in older insects that have developed their scent, suggests that the β-glucosidase is produced in the cap cells, and moves down the lumen of the hair scales (fig. 70).

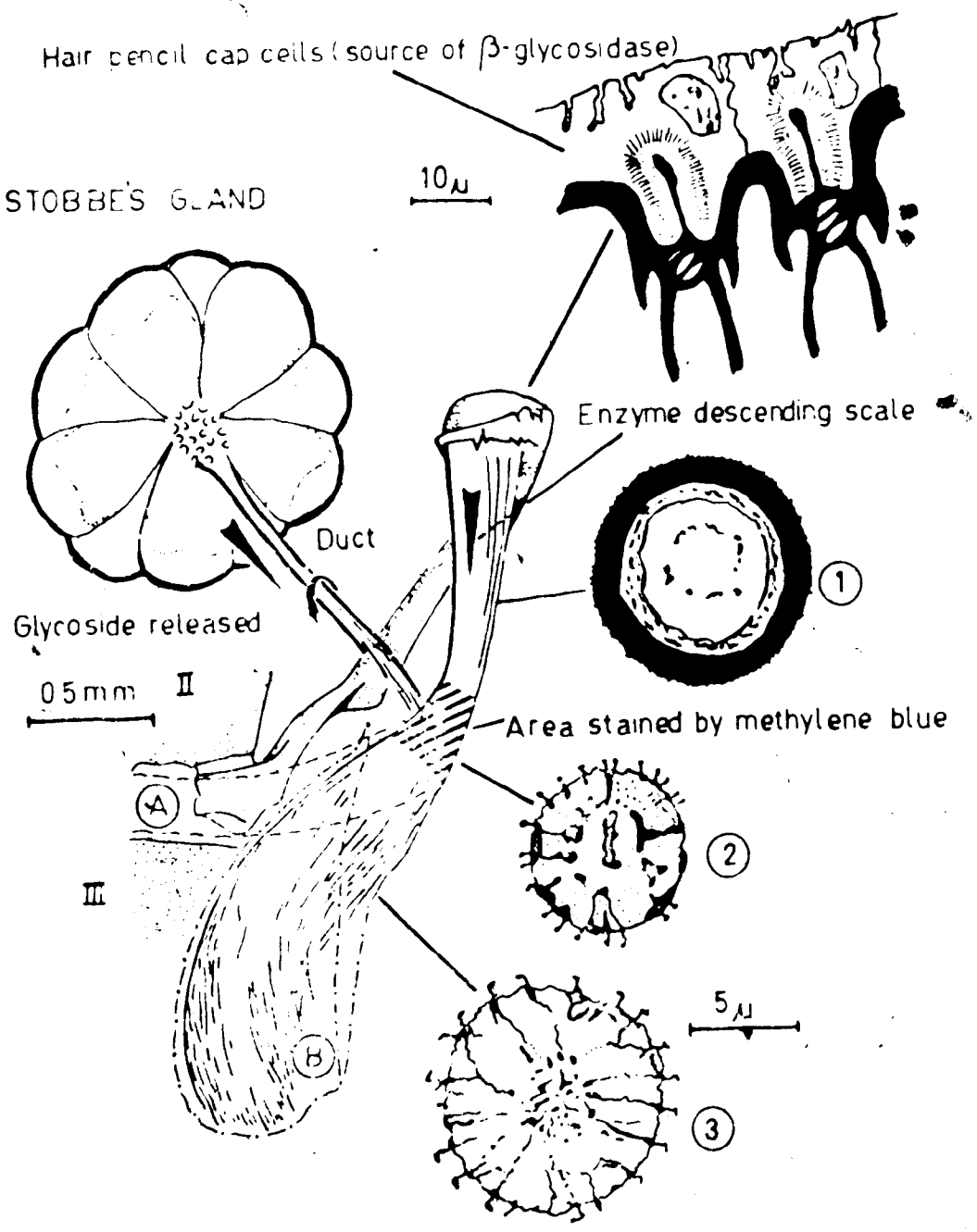
Fig. 70. [illegible]. Flow diagram. Interaction of glycoside and enzyme, producing the pheromone.

The enzyme is produced by the cap cells and moves down the scales from the shaft (1), to the transition area (2), which differs in its surface properties (only area able to strongly absorb methylene blue) from the evaporative area (3).

The distal evaporation area of the scales are initially held between sternites II and III, (A); but in the mature animal, are held in a deep lateral pouch, (B).

The glycoside moves from the Stobbe's gland down the duct scales which contact the transition area (2).

Storage pocket


Hair pencil cap cells (source of β-glycosidase)
STOBBE'S GLAND
10μ
Enzyme descending scale
Duct
Glycoside released
0.5mm
II
Area stained by methylene blue
A
III
B
1
2
3
5μ

[illegible] the hair pencil [illegible] that [illegible] the scale tuft [illegible] the tip of [illegible] hair scale (fig. 70), suggesting that the pore tubules in this area differ from that of the remainder of the scale (Lawrence, 1970, fig. 18). In all species examined, the tips of the hair scales from the scent gland make contact with the transition area. It is possible that the glycoside is absorbed through the surface of the transition area, into the lumen of the hair scale where it reacts with the enzyme. The intricate sculptured surface of the brush is hydrofuge and very thin (120 Å) (fig. 70) and would present a minor barrier to the passage of the pheromone, non-polar 2-phenyl ethanol.

Noctuid males are usually able to mate more than once. *Trichoplusia ni* is able to mate an average of 3.3 times (Shorey, 1964). Birch (1970a) demonstrated that *I. metaurea* males were able to recharge the hair pencil at least twice, but not more than three times. 2-phenyl ethanol and benzaldehyde are very volatile, and all of the pheromone on the scale surface is likely to be lost each time the hair pencil is spread. If all the glycoside is rapidly hydrolyzed as soon as it comes in contact with the enzyme, the pheromone would be available for only the first mating. The enzyme found in the cap cells hydrolyzes glycosides rather slowly (fig. 67) and may have hydrolyzed only a portion of the pheromone precursor by the time of the first mating (third night in *P. separata*, Clearwater, 1972). Continued slow hydrolysis after the hair pencil is replaced into the pouch would recharge them for a second or third mating. In *P. separata* there

[illegible] production [illegible] the glycosides [illegible]

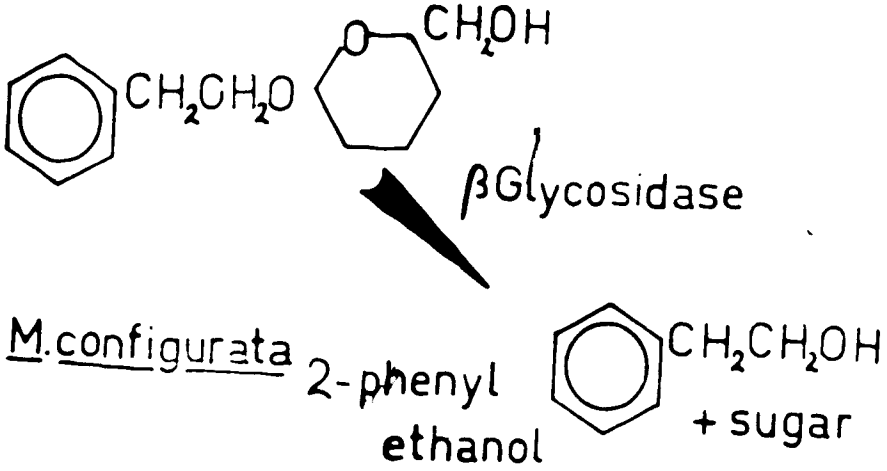
4.2.4 [illegible] at the glycoside [illegible]

The release of phenyl ethanol from phenylethyl-β-glycoside may be simply accomplished by an enzyme with hydrolytic properties similar to emulsin (fig. 71). Formation of benzaldehyde is possibly more complex, and probably arises from the benzyl alcohol released by hydrolysis of benzyl-β-glycoside. Both Aplin and Birch (1970) and Grant, Brady and Brand (1972) have identified benzyl alcohol in male noctuid [illegible] Grant et al. (1972) showed that male *H. subflexa* carry large [illegible].31 μg/brush amounts of benzyl alcohol and little (3.40 μg/brush) benzaldehyde immediately following eclosion, while on the second day the proportion of the two compounds is reversed. The following pathway is proposed for the two species of *Heliothis* (f[illegible]71). Alternatively, a direct conversion to benzaldehyde may be [illegible]sible. Birch (per[illegible] comm.) has used emulsin to produce benzaldehyde [illegible] from a preparation of glands from *Leucania impura*.

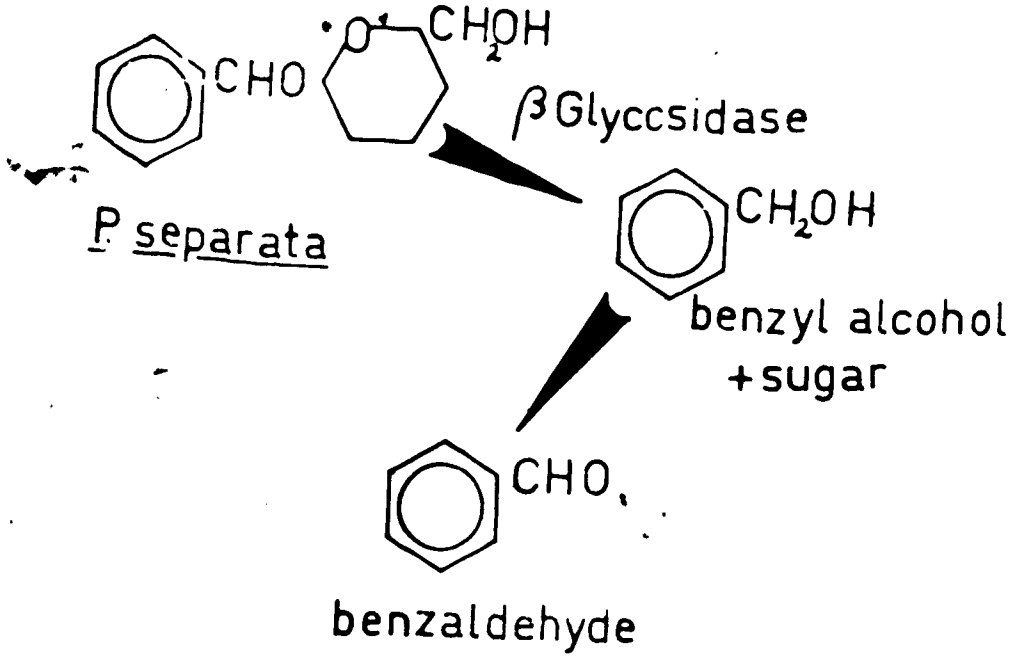
4.2.5 Metabolic pathway to the glycosides

The noctuid hair pencil—Stobbe's gland complex appears to have arisen only once (Birch, 1972). The very great similarity of the pheromones and pheromone precursors of *H. separata* and *M. configurata* allows the supposition that they share a similar metabolic pathway (fig. 72). If the critical step is the introduction of an hydroxyl group by hydration of the side chain double bond, only a slight change in enzyme specificity would result in the production of

Fig. 71. *Myrmica rubra* and *Formica exsecta*. Hypothetical metabolic pathway — glycoside to pheromone.



M. configurata 2-phenyl ethanol

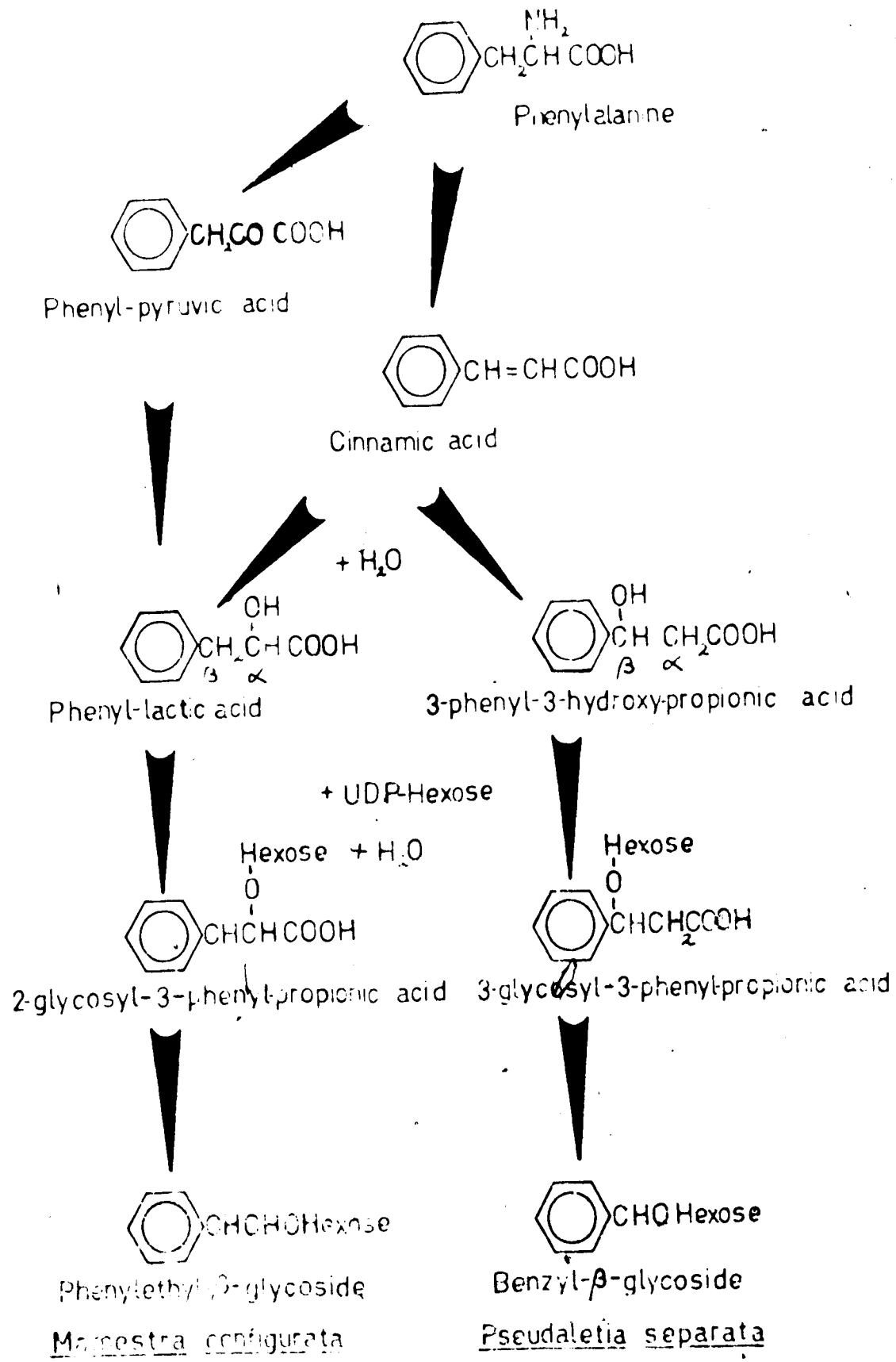


P separata

benzaldehyde

Fig. 72. *Hedeoma pulegioides* and *Lycopus americanus*. Hypothetical metabolic pathway -- phenylalanine to glycoside.

Evidence for involvement of phenylalanine, cinnamic acid, phenylethyl-β-glycoside and benzyl-β-glycoside only.


NH2
CH2CH COOH
Phenylalanine
CH2CO COOH
Phenyl-pyruvic acid
CH=CH COOH
Cinnamic acid
+ H2O
OH
CH2CH COOH
β α
Phenyl-lactic acid
OH
CH CH2COOH
β α
3-phenyl-3-hydroxy-propionic acid
+ UDP-Hexose
Hexose + H2O
Hexose
O
CHCH COOH
2-glycosyl-3-phenyl-propionic acid
O
CHCH2COOH
3-glycosyl-3-phenyl-propionic acid
CHOHHexose
Phenylethyl-O-glycoside
Mancostra configurata
CHO Hexose
Benzyl-β-glycoside
Pseudaletia separata

either the precursor of *T. [illegible]* or of *L. expansa*. A comparable mechanism, however, has been established in the production of unusual unsaturated fatty acids in bacteria (Morris, Matsumura and Bloch, 1968). Dehydration of a hydroxy decanoyl thioester to either 2,3 or 3,4 decenoates, depends on the presence of a removable regulator on the specific hydrase. Condensation of the 2- or 3-hydroxy acid with CoA before may predate the species specific glycoside.

4.2.6 Evolutionary considerations

In common with the defensive secretions of *Eleodes longicollis* (Leconte) and *Cryptochile sericea* (Herbst) (Coleoptera: Tenebrionidae) (Happ, 1968) the male pheromones of *T. [illegible]* and *L. expansa* appear to be stored as glycosides. Like the benzoquinones of the two beetles, benzaldehyde and 2-phenyl ethanol are toxic to cell metabolism. Storage as a glycoside appears to have circumvented the problems of insolubility and toxicity.

The two glycosides from the two noctuids are very similar, possibly differing only by a single C_1 unit in the side chain linking the phenol and the sugar groups. (The sugars of these glycosides were not identified, though both are unsubstituted hexoses). Aplin and Birch (1970) identified both benzaldehyde and 2-phenyl ethanol from *Polia nebulosa* (Hüfnagel) and *Mamestra persicariae* (Linnaeus). The presence of both compounds in a single species suggests that only slight modifications of the metabolic pathway or slightly different precursors may be necessary for their production. Benzaldehyde is an extremely common pheromone, being found in *Leucania impura* (Hübner), *L. pallens* (Linnaeus), *Polia nebulosa* (Hüfnagel), *Mamestra persicariae*

(Hawworth from North America (Grant et al., 1972), [illegible] (Walker), [illegible] Meyrick), [illegible] (Walker), [illegible] and [illegible] (Meyrick) from New Zealand (Clearwater, 1971). Selection may be favouring alteration of the pheromone chemistry toward other aromatics such as 2-phenyl ethanol, or 3-methoxy-4-hydroxy benzaldehyde tentatively identified from [illegible] (Clearwater, 1971).

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