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The Role of the Male-produced Pheromone in the Reproduction Behaviour of the Southern Armyworm *Pseudaletia separata* (Wlk.)

A thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Zoology at Massey University.

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Table of Contents.

List of Figures.

Introduction

Detailed courtship patterns have been described for few species of Lepidoptera. Most of the descriptive work in the literature is fragmentary or lacking in experimental or statistical analysis. Brower, Brower, and Cranston (1965) working on wild populations of the queen butterfly Danaus gilippus berenice have statistically analysed the probability of each movement in the sequence and have given a reliable and detailed account of mating in this species. Tinbergen (1958) by the presentation of models of varying size and colouration, and examining the affect of removal of structures suspected to be important in courtship has produced a good experimental account of mating in Satyrus semele the grayling butterfly. Because of the greater difficulties of observing complete mating sequences of nocturnal insects in the wild, most moth studies have been carried out with small caged populations. Again detailed expermental work is rare. The studies of Shorey (1964) on the cabbage looper Trichoplusia ni and Birch (1970) on the angleshades moth Phlogophora meticulosa possess good experimental detail suggesting that chemical cues are much more important in the courtship of moths than in butterflies.

Development of more definitive techniques has allowed the investigation of insect courtship to move from subjective descriptions of the movement sequence to precise studies of the visual, tactile and chemical cues. In particular the availability of gas chromatography and mass spectrometry techniques capable of detecting the very small amounts of material produced by insects, has revolutionised the study of the chemicals, or pheromones, that are used in intra-specific communication.

Pheromones are very widespread amongst the insect orders, and have a variety of functions in addition to sexual attraction. Many families of the Lepidoptera have had pheromones implicated in courtship eg. the Bombycidae Butenandt (1963), the Lymantriidae Jacobsen, Beroza and Jones (1960) the Noctuidae Gaston Fukuto and Shorey (1966) and several others. Female cockroachs produce volatile substances which attract males and induce typical precopulatory behaviour, Barth (1961). Coppel, Cassida and Dauterman (1960) have shown the presence of a potent attractant in virgin females of the sawfly Diprion similis (Hymenoptera). Males of Musca domestica, the common housefly are attracted to the female by a pheromone Rogoff (1964). These examples are a few selected from the immense literature on this subject. Other functions of pheromones are eliciting alarm reactions in bees and ants, trail following in ants and depression of ovary development in worker bees Regnier and Law (1968).

The Noctuidae have attracted considerable attention largely because they are a widespread group of considerable economic importance. Gaston, Shorey and their co-workers have carried out an intensive study of the male attracting pheromone produced by females of Trichoplusia ni. They have discussed the environmental control of mating Shorey (1966), the bioassay of the pheromone Gaston and Shorey (1964), pheromone isolation techniques Gaston Fukuto and Shorey (1966) circadian rhythm of pheromone responsiveness Shorey and Gaston (1964) quantitative aspects of the production and release of the pheromone with respect to age and mating history Shorey and Gaston (1965) Shorey, M^{C}Farland and Gaston (1968) and described the morphology of the female gland Jefferson, Shorey and Gaston (1965) Miller, Jefferson and Thomson (1967). Many other much less detailed studies of the female pheromone of different noctuid species exist.

Very much less is known about the pheromones produced by the males of this family. Because of the difficulty of detecting an overt behavioural response from the female when stimulated by the pheromone of a conspecific male, the function of these pheromones is not at all clear. On the basis of unsophisticated preliminary experiments Shorey (1964) concluded that none of the observed female responses were invoked by any pheromone from the external tufts of brown hair on the males abdomen in T. ni. Electrophysiological evidence from Grant (1970)

demonstrated that the female does detect substances from these hair tufts, though no indication of function has yet been given. In other Lepidopteran families, the function of the male pheromone is clear. N - undecanal the male pheromone of Galleria mellonella is an attractant stimulating the female to move towards the displaying male Roller, Bieman, Bjerke, Norgard and M^{c}Shan (1968). In contrast the pyrolizinone of Danaus gilippus berenice is an arrestant, arresting the flight of the female and inhibiting her from flying away from the male once she has been induced to alight Brower, Brower and Cranston (1965) Pliske and Eisner (1969).

Structurally, the complex organs producing the male pheromone in the Noctuidae are diverse, including the hair pencils of Persectania aversa, the wing glands of Erana graminosa, the giant posterior brush of Plusia chalcites and the tibial hairs of Dasypodia selenophora. The only common feature is that all appear to have evolved from modified scales and the surrounding cells. This great variation in structure and location would seem to indicate that pheromone producing structures have evolved indepenantly many times in response to a powerfull selection pressure. The first accurate description of the noctuid hair pencil was the work of Stobbe (1912). This worker described the hair scales, everting structures, and the hair pouch. His assertion that a gland consisting of a small number of very large cells is the major secretory structure has supplanted the theory of Eltringham (1925) that scales in the bottom of the hair pencil pouch produce the pheromone.

This thesis is a detailed examination of the mating sequence of the noctuid Pseudaletia separata (Wlk.) with particular reference to the chemistry and function of the male pheromone and of the cytology and physiology of the secretory structures.

The Mating Sequence.

Before strict experimental analysis can be carried out, a series of preliminary observations must be made to compile an action sequence for the species. In insects behaviour patterns are totally inherited, the major exceptions being the social species. These inherited patterns may seem very rigid e.g. the form of the cases of the caddis fly larva Mollanna sp is species specific with little individual variation in a natural population. When conditions are altered however, these insects show a adaptability which would not be expected from a chain of unconditioned reflexes Carthy (1965). Because of this adaptability, interpretation of behaviour under laboratory conditions must proceed with caution.

Methods:- The most critical condition for the observation of a nocturnal moth would appear to be lighting conditions. Shorey (1966) found that the response of male Trichoplusia ni was greatly inhibited at light intensities greater than one lux. Compared with the mamalian eye, the visible spectrum of the insects compound eye is shifted towards the ultra violet end. Burkhardt (1964) states "The bee does not react to wavelengths above about 600 m u nor do most other insects investigated unless extreme intensities are applied".

For this study, a light was produced with no emmision of wavelengths below 600 m u (checked with Hitachi recording spectrophotometer). Moths of this species showed definite changes in flight pattern near this light source and its use was discontinued. Other noctuids have also been shown to detect red wavelengths e.g. Birch (1970) found that males of Plusia gamma orientate toward direct light from a red bulb in preference to a receptive female. Distant fluorescent light were used as the light source in this study. By comparison with the accurately measured light intensity of a variac controlled bulb, it was estimated that the experimental light intensity was less than one lux.

It was not found necessary to provide artificial air movement as mating occurred readily in the almost completely enclosed glass cages. In contrast males of Phlogophora meticulosa were unable to orientate to females in still air Birch (1970).

The other experimental conditions were not unusual. Seven to nine pairs of virgin moths from a laboratory culture were placed in a glass observation cage, with an internal volume of 2.8. x 10^4 cm^3, immediately following emergence. A solution of 10% sucrose was continuously available on a wad of cotton wool, suspended from the roof of the cage. Temperatures were in the region of 20°C.

Results:- The observed sequence was not complex. After a period of sexual display the male located the passive female and mated.

The first overt sexual action of the male was the rhythmical extrusion and retraction of the external genitalia while at rest on the side of the cage. The number of males active in this way increased, till about an hour later this display gave way to active flight with the genitalia continously extruded. These males approached females from below and behind, while curving the abdomen upward so that the external genitalia approached the abdomenal tip of the female (see fig 1). Exsertion of the hair pencils was not observed. From this position, which was held for upwards of a second, the male made an upward turning snatch, clasping the tip of females abdomen with the valvae. The whole sequence of coupling from approach to completion rarely required more than two seconds. After coupling the male hung downwards and the pair moved steadily towards the floor. The male appeared to be the active partner in this descent, with the female passively following.

Courtship by the male always appeared to be directed toward females. Only on one ocassion was a displaying male observed to pass close to another male, but it did not orientate itself toward the second male in any way.

Successful mating occurred only with females resting motionless on a vertical surface, or on the lower side of a horizontal surface. The posture of the female was variable. Sometimes the female was observed to assume a "calling" position. The costal margin of the wings was strongly depressed, the thorax and wings forming a triangle while the abdomen was markedly elevated. The posture of other females was indistinguishable from that of resting moth. Most of the observed unsuccessful matings were due to the approached moth walking or flying away

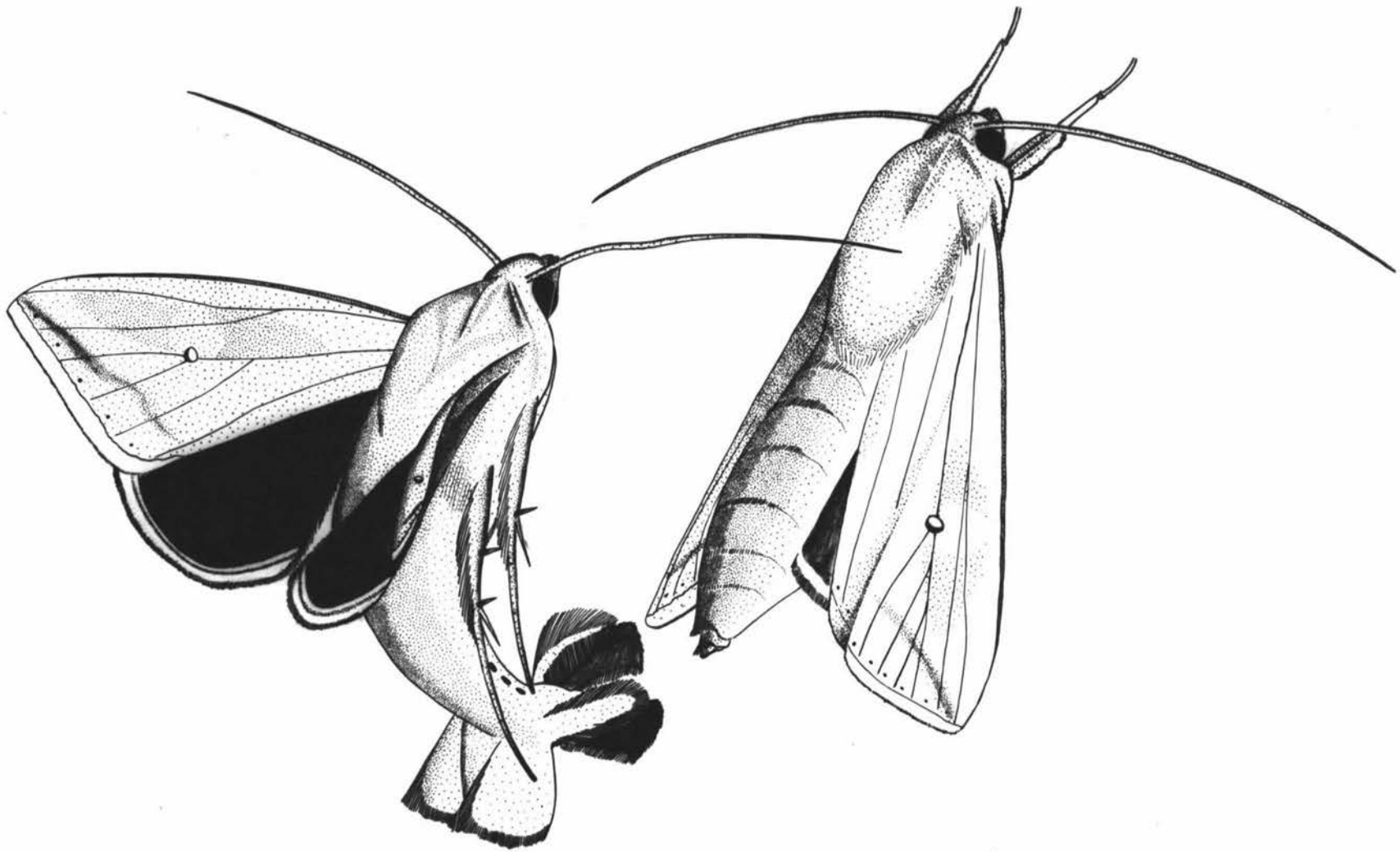


FIG 1 THE MATING APPROACH

before contact was made. One male was observed to clasp another moth as it flew past. The clasped moth beat its wings strongly and the pair separated about a minute after initiation of contact.

The majority of matings were observed to occur between 10:00 p.m. to 11:30 p.m.

Conclusions:-The observed mating sequence of Pseudaletia separata contained no behavioural elements not already described for other species.

Comparison of the mating sequence of Trichoplusia ni Shorey (1964) Grant (1970) and Phlogophora meticulosa Birch (1970) with Pseudaletia separata revealed several differences in the precopulatory behaviour of the males. During the pre-copulatory flight, P. meticulosa and P. separata were observed to evert the external genitalia frequently, an action seen in T. ni only just before copulation. To locate the female, both T. ni and P. meticulosa need to orientate to an airstream while in flight, a requirement not seen in P. separata. The initial contact with the female is tactile in T. ni (antennae and tarsi) and P. meticulosa (antennae only) while P. separata appeared not to touch the female with either. In all three species, the external genitalia are extruded and orientated toward the abdomenal tip of the female. The clasping of the female which follows, occurs while the male hovers beside (T. ni) or below (P. separata) the female, while in P. meticulosa

the male must land before the genitalia are engaged. P. meticulosa and P. separata possess anterior hair pencils, while T. ni has a pair of scent brushs near the external genitalia. These scent brushs appear and are spread briefly just before mating Grant (1970). Similiarly Birch (1970) reports the eversion of the hair pencil following orientation of the external genitalia toward the female. The failure to observe a similiar eversion of the hair pencils of P. separata may be due inadequate experimental conditions. The density of the experimental population may have been too high, or the light intensity may have been too high. The structure is most unlikely to be vestigal, as several individuals from a wild population sampled with a light trap had the hair pencils everted and folded between the thorax and abdomen, or more rarely, hanging loosely. Birch (1970) demonstrated tha hairpencil eversion in P. meticulosa is obligatory for successful copulation. From the description of Grant (1970) it appears that spreading of the brushes does not occur in all successful matings of T. ni. Other species eg. Spilosoma lubricipeda (Arctiidae) and Deilephila elpenor (Sphingidae) mate in the laboratory without coremata or hair pencil oversion Birch (1970). It is thus concluded that in P. separata and some other species, hair pencil eversion is facultative, while in other species eg., P. meticulosa this behavioural element is obligatory.

The "calling" attitude of the Lepidopteran female appears to be adopted in order to disperse a pheromone, a suggestion first

made by Poulton (1928). This attitude is reported from many families eg, Sanninoidea exitiosa (fam. sessidae) Smith (1965) Anagasta kuhniella (fam. Phycitidae) Richard and Thompson (1932) and Protoparce sexta (fam. Sphingidae) Allan and Hodge (1955). The posture of females of T. ni appears to be identical to that of P. separata. The eighth and ninth abdomenal segments usually retracted within the seventh are extruded, Shorey (1964) exposing the modified intersegmental membrane that produces the pheromone. Jefferson Shorey and Gaston (1966). As males of P. meticulosa respond to artificially everted glands at any time, Birch (1970) suggests that eversion of the female gland is the event determining the species specific time of mating.

A mated pair would be very vulnerable to predation due to the difficulty of movement while paired and the conspicuous nature of the behaviour that preceded mating. Brower, Brower and Cranston (1965) suggest that the function of the post nuptial flight in Danaus gilippus berenice is to carry the pair away from where they have been so conspicuously active to a less obvious area. By analogy, a similiar function is suggested for the downward movement of mated pair observed in P. separata.

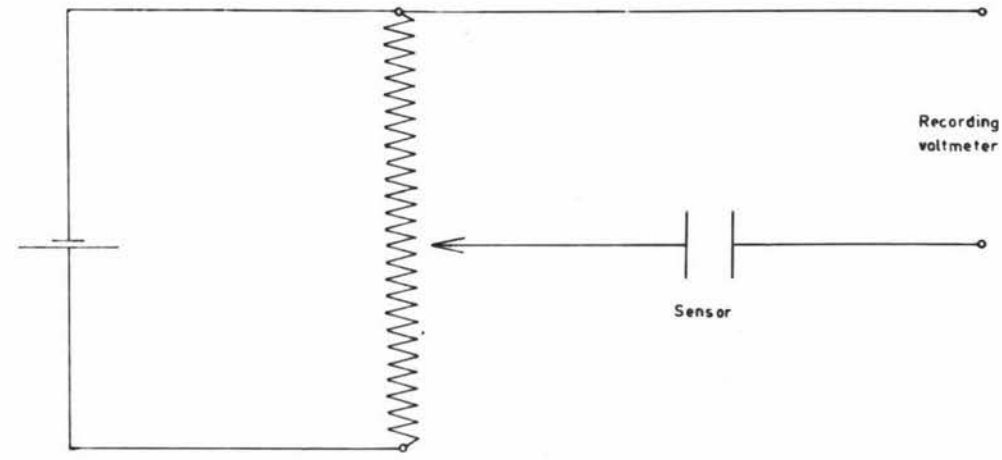
The Development of Flight and Sexual Maturity.

The period of imaginal activity is moderately long in noctuids with several types of activity accuring before death. Callahan and Chapin (1960) have found Pseudaletia unipuncta lives an average of 11 days, with Heliothis zea living 7 days, and Peridroma margaritosa 9 days. Feeding appears to be essential before full ovary development can occur in Pseudaletia separata Quo, Wu, Tsai, and Lui (1964), in contrast to species from other shorter lived groups eg. the Bombycidae, where the eggs are ripe immediately after emergence. Wigglesworth (1965). Except in parthogenetic insects, mating is a pre-requisite for the production of fertile eggs. Several noctuid species have periods of migration of hibernation before oviposition Johnson (1969). It is obvious then that the development of several functions must be accurately synchronised during the few days of life of the imago.

Methods:-Two methods were used to measure the overall activity and the proportion of the activity taken up with mating.

A vibration sensitive instrument was constructed to record automatically all movements made during the life of the imago. The design of the sensor was based on some of the early telephone work Walmsley (1904). Fine carbon rods were supported at their tips on steel edges mounted on the surface of a taut membrane. Moth activity threw this membrane into vibrations which were translated into movements of the carbon rods. As these rods were effectively resistances in the circuit, movement had the affect of varying the circuit resistance and consequently the voltage. (see fig 2) The voltage changes were recorded on a Heathkit Recording Voltmeter. Tests with an amplified all wave generator revealed that the sensor would detect frequencies as low as 16 c.p.s. when the input was fed into an oscilloscope. The less sensitive Heathkit only began recording when the sensor was receiving 50 c.p.s. This is still adequate as noctuids have a wing beat frequency of 30-50 c.p.s. Wigglesworth (1965). Background interference was the major factor limiting amplitude sensitivity. Two female moths were placed in the container under the membrane and supplied with 10% sucrose. Activity was recorded between 6:00 p.m. to 8:00 a.m. The number of peaks recorded each night was taken as the index of activity.

A population of 34 moths in three containers were scored each night for the number of mated pairs. The observations were taken at approximately hourly intervals between 6:00 p.m. and 12:00 p.m., covering the time of mating in this species. Both light phase (14 moths in one container) and dark phase (20 moths in two containers) insects were used and the observation conditions were those of the mating sequence observations.



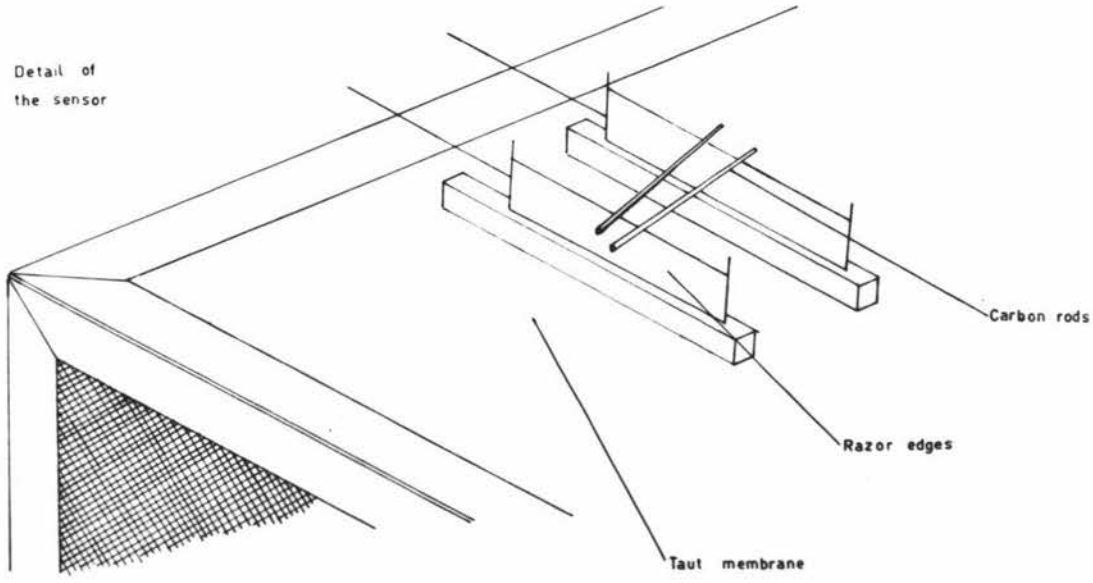


FIG 2 THE CIRCUIT DIAGRAM OF THE VIBRATION SENSOR

Results:-Maximum flight activity appeared to occur twice, before and after the peak of mating.

On the night following emergence, little activity occurred, but the amount of flight was greatly increased during the second and third nights. After a marked drop, activity peaked again on the seventh night (see fig 3).

During the first and second night following emergence, feeding was frequent especially during the early hours, but no overt sexual behaviour could be observed. In all three cages, extensive male genitalia eversion was first observed on the third night, and the first successful mating noted. After the peak, the mating frequency dropped off rapidly (see fig 4). Oviposition occurred one day after mating, the first eggs being laid on the fourth night.

Conclusions:-Comparison of these results with those produced for other noctuid species suggested that development of sexual maturity in P. separata is markedly affected by migration. This species is a very strong migrant with flights of 900 miles recorded by Li, Wong and Woo (1964). The suggestion that migration takes place before mating is supported by the work of Hwang and How (1966). Using a flight mill, they demonstrated that the longest flights took place on the third to fourth days. Later, flights decreased greatly in duration as the ovaries attained full development of the fifth day. An insect tethered in a flight mill is

FIG. 3 THE FLIGHT ACTIVITY OF THE IMAGO

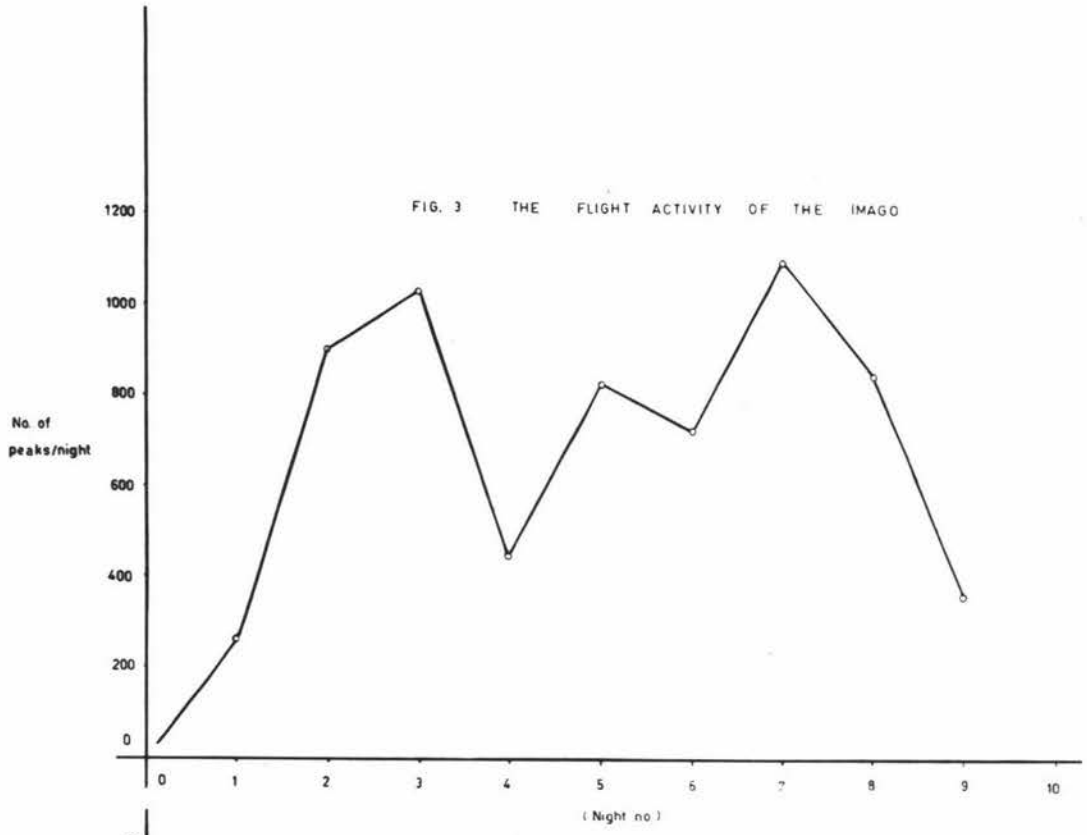
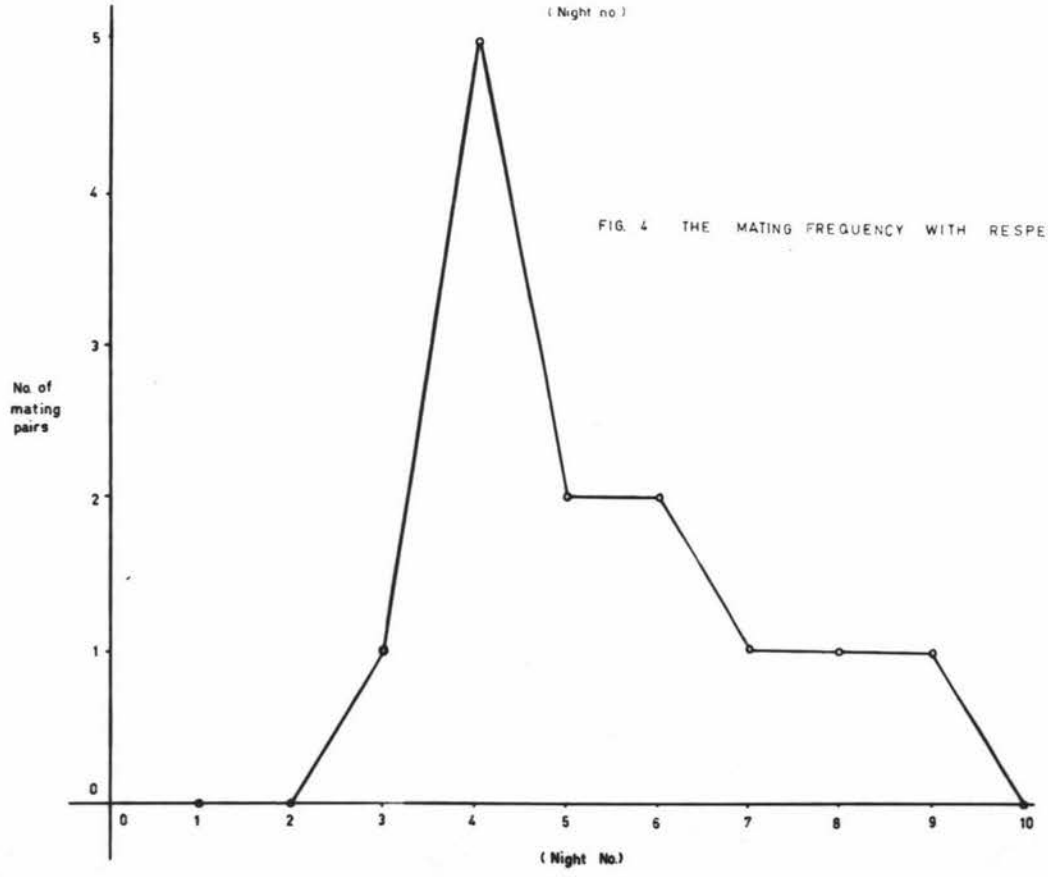


FIG. 4 THE MATING FREQUENCY WITH RESPECT TO TIME



deprived of tarsal contact and because of the tarsal reflex will fly more extensively than in the natural environment. Measurement of freely flying insects in the vibration sensor has allowed a more natural assessment of the New Zealand population of this species. Flight duration of individuals from this population was greatest on the second and third nights with oviposition beginning on the fourth. Other migratory noctuid species similarly exhibit extensive flight soon after emergence eg., Spodoptera exempta Brown and Swaine (1966) and Chorizagrotis auxillaris Koerwitz and Pruess (1964). Mating in P. separata is not initiated till the third night, apparently following migratory flight. In most of the eight species studied by Shorey Morin and Gaston (1968) Shorey M^{c}Farland and Gaston (1968) mating begins from the night of emergence (Prodenia ornithogalli) to 1.5 days later (Rachiphusia ou). With only one exception, none of the species in this study were considered migratory. Spodoptera exigua carries out extensive overwater movements to England Hurst (1963). The American population may be non - migratory or mating may occur before migration in this species. No information is available on the migratory status of Pseudoplusia mcludens (female 0.5 days male 2.5 days) and Autographa californica (male 3.5 days). As only those individuals surviving a rigourous migration are able to mate, initiation of mating on the third day would have a powerful selective affect. Some noctuids have been shown to mate more than once Shorey (1964). Some of the matings recorded after the peak may have been second matings, but no disections were carried out to confirm this.

The Sensory Modalities Involved in the Location of Mating Partners.

In the mating sequence, it is the male which actively seeks the female. If this search behaviour is not to be random, the male must receive signals of some sort from the female. Brower Brower and Cranston (1965) and Tinbergen (1958) describe the properties and importance of the visual and chemical cues involved in mating in two dayflying species of the Nymphalidae. Using techniques involving the selective removal of each of the sensory systems of the male, Shorey (1964) and Birch (1970) have investigated the type of signal that the male noctuid receives when locating the female.

Methods:-In addition to examining the affect of removing the males sensors, an attempt was made to produce female models functionally competent to induce mating attempts by males.

For the first experiment the effective removal of either the visual or olfactory system was attempted. To measure changes in the mating frequency caused by these changes, the fluorescent dye technique was used. Immediately following emergence all moths allocated to each of the treatments including the control were anaesthetised with carbon dioxide. For the examination of the affect of loss of the olfactory sense, both antennae were removed with fine scissors. As the test was carried out on the fourth night after emergence, the moths were allowed 3.5 days, to recover from the affects of anaesthetisation. The

work of Brady and Smithwick (1968) suggested that this period was more than adequate. During these three nights, the males were fed .1% eosin in 10% sucrose, while kept in cartons separate from the females. On the fourth night, equal numbers of four day old virgin moths were placed in pyramid shaped gauze cages under the following treatments. The control and the antennectomised group were kept under natural night conditions of temperature and light. To remove all light from the third treatment, the pyramid cage was carefully draped with a thick blanket and a double layer of black cloth. The moths remained in these cages for the fourth and fifth nights following emergence, and were dissected the next morning. This experiment was not replicated due to lack of time and material.

The use of models to demonstrate the type of sensory cues required by the male, has several precedents. eg. Rogoff (1964) used "pseudoflies", small knots of black wool, in his demonstration of a pheromone in Musca domestica. More frequently other workers, especially those studying nocturnal Lepidoptera, are able to elicit most of the male responses with only the relevant chemical stimulii eg. Shorey (1964) observed pre-copulatory flight, abdomen curving and genitalia extension in males of T. ni in the presence of a filter paper containing a female extract. Initial attempts to show a similiar orientation towards macerated portions of receptive female failed with P. separata. It was found necessary to construct full scale models of female P. separata with gelatine capsules, cardboard and modelling clay, and painted a colour as close as possible to the natural insect. These models were filled

with macerated portions of four day old virgin females, the capsule perforated and the model pinned to the top edge of a sheet of paper covered with a fine layer of carbon. Three four day old virgin males were placed in a pyramid cage three hours before the test. Maceration of the females and introduction of the sheet into this cage took place at 10:30. The records were preserved with a shellac bath.

Results:- Results from both experiments suggested that visual as well as olfactory cues were important.

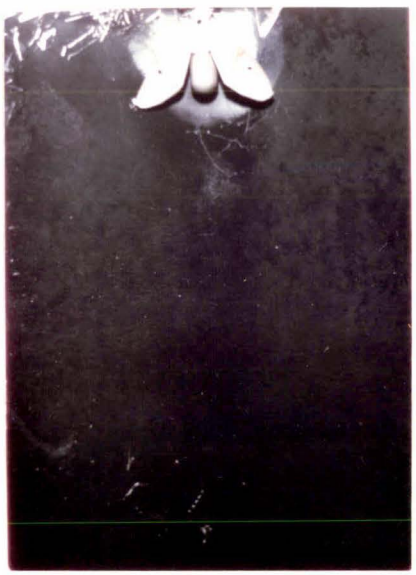
From a control level of 80% mating, removal of the antennae reduced the mating frequency to 32% while only 44% of the moths lacking visual cues mated (see table 1).

The control paper holding amodel lacking in female extract was unscratched below the model, and the head-thorax sheet was similiar. The paper was scuffed slightly near the right wing of the model containing an extract of the posterior abdomen. Examination of the paper below the model containing an extract of the posterior abdomen revealed extensive scuffing strongly suggestive of a mating attempt (see fig 5). The experiment was replicated and again only the model containing extract of posterior abdomen showed scuffs at the rear of the female.

During the preliminary examination of the mating sequence an observation was made that was relevant to these results. Several moths, escaped from earlier experiments, were flying near the glass sides of the observation case. One of the escaped males, with extruded genitalia, was clearly observed

Table 1

Treatment	No. of Males	% Males Dyed	No of Females	% Females Dyed	% Mating
Control	8	62%	8	50%	80%
Antenn-ectomised	9	78%	9	25%	32%
Lightless	11	82%	11	36%	44%



1. The control

2. Head and Thorax Extract

3. Anterior Abdomen Extract

4. Posterior Abdomen Extract

Fig.5. Demonstration of Signals Adequate to Induce Mating Attempts by the Male

to move towards a captive moth on the other side of the glass pane. Such an orientation suggested the use of visual cues.

Conclusions:- In contrast to Phlogophora meticulosa Birch (1970) and Trichoplusia ni Shorey (1964), visual as well as olfactory cues are used by male P. separata to orientate toward receptive females. In both the former species, antennectomy completely abolished mating. While the reduction of mating was great in P. separata it was not total.

The attraction of the male to the model would seem to be sexual rather than to eg. the contents of the gut, as the location of the pheromone gland of the female noctuid is in the last three segments of the abdomen. Jefferson Shorey and Rubin (1968), an observation confirmed for P. separata by Quo Wu Tsai and Lui (1964).

While both visual and olfactory cues are necessary, it is suggested that chemical signals are more important than visual ones. Not only does antennectomy reduce the mating frequency more drastically than light removal, but the models provided were unattractive unless the chemical stimulus was also provided. Birch (1970) suggests that male pheromone producing organs are most likely to develop in species where visual signals are reduced or confusing such as butterfly families exhibiting Mullerian mimcry Brower (1964) or nocturnal moth families. Unlike some species eg. Melanchra mutans where the male and female can readily be told apart by the colour pattern, no reliable colour or form differences were discovered which would allow discrimination between males and females of P. separata. Further, both sexes are polymorphic, ranging in colour from a dusky

charcoal to a plae buff colour (the most common variant which was imitated in model production). Persectania arotis, another species captured in the same area required careful examination before it was distinguishable from P. separata. As the superpostition eye of the noctuids is not noted for its image producing qualities but rather for light gathering Wigglesworth (1965) it is unlikely that the male of P. separata is able to identify a conspecific female visually.

The Biological Function of Benzaldehyde.

In order to conclusively identify a chemical as a naturally occurring pheromone, the specific ethological response must be elicited when the compound is present in physiological quantities. Butenandt and Hecker (1961) demonstrated that synthetic trans 10 cis 12 hexadecadien-1-ol elicited the circling dance from males of Bombyx mori when present at concentrations of 10^{-12} ug/ ml establishing this compound as the pheromone of this species. In contrast the identification of 2-2 dimethyl 3 iso-propylidenecyclopropyl propionate as the pheromone of Periplaneta americana was unacceptable as the synthesised compound was completely inactive in stimulating the male cockroach Jacobsen and Beroza (1965). The apparent lack of overt behavioural responses by female noctuid moths when stimulated by male produced pheromones has made the allocation of specific functions to specific compounds very difficult in this group Grant (1970). Shorey (1964).

Methods:- To examine the response of the female of Pseudaletia separata to benzaldehyde,* an apparatus similiar to that used by Shorey (1964), to demonstrate the anemotactic response of Trichoplusia ni was constructed. A hollow glass tube 60cm x 6cm, with lines dividing it into six equal lengths, and a white reflecting bottom to aid observation was capped with wire gauze ends. The light

* See physiology section.

source was a variac controlled incandescent bulb producing 0.5 lux. The air temperature was 19° - 21°C. The insects were fed on 10% sucrose solution on a cotton wool covered string stretched the whole length of the tube. A centrifugal fan 6.3. metres away produced an air flow down the tube. A glass resevoir with a attached capillary provided a steady supply of synthetic benzaldehyde to the air flow. Ten virgin females were placed in the tube immediately following emergence and experimental observations commenced three nights later. The experiment was carried out twice, and the distributions were noted in two ways. Initially the distribution of the moths with respect to the divisions marked on the tube was noted every half hour from 7:00 p.m. to 10:00 p.m. These readings, taken in the absence of benzaldehyde, served as a control. The benzaldehyde releasor was then incorporated into the described air flow and a further series of readings taken every five minutes between 10:05 and 11:30. The second set of observations, with more precise controls, was carried out with a fresh experimental population under the same conditions. The experiment proceeded for three hours between 9:00 p.m. and 12:00 p.m. with readings taken every five minutes. During the first and last hours benzaldehyde was not introduced and the positions of the moths during these times served as a control. Benzaldehyde was introduced between 10:00 and 10:55.

Results:-The most striking point about the distributions was the tendency for the moths to aggregate in the first and last division of the tube. The moths appeared to exhibit a distinct preference for resting on the gauze rather than the smooth glass walls of the tube. Moths would walk and fly through the middle of the tube, and only infrequently would they stop. The ratio of the sum of the moths in the first and last two compartments to the sum of the moths in the middle compartments was considered to be an index of the activity of the experimental population. (see table II) These ratios were compared with a Chi^2 test with one degree of freedom. For the first set of observations, somparison of (a) and (b) Ratios

$X^2 = 6.56$ $.02 > p > .01$

For the second set.

(a) and (b) $X^2 = 6.30$ $.02 > p > .01$

(b) and (c) $X^2 = 14.09$ $p < .001$

(a) and (c) $X^2 = 2.55$ $.20 > p > .10$

This established that the control distributions did not differ signifigantly but there was a difference between the controls and the test at the 2% level of signifigance. In the presence of benzaldehyde, females are less active. (see fig 6). The average number of moths flying was also recorded, and showed a similiar reduction in activity while benzaldehyde was introduced.

Table II Affect of Benzaldehyde on Females.

Division Number	1	2	3	4	5	6	(Σ End divs)	(Σ Mid divs)
Experiment I (a)	13	7	6	5	3	24	37	21
Control 7:00-10:00								
Experiment I (b)	27	3	6	5	9	47	74	23
Test 10:05-11:30								
Experiment II (a)	66	2	3	7	12	30	96	24
Control 9:10-9:55								
Experiment III (b)	64	1	0	4	0	43	107	13
Test 10:00-10:55								
Experiment III (c)	40	3	3	7	18	49	89	31
Control 11:00-11:55								

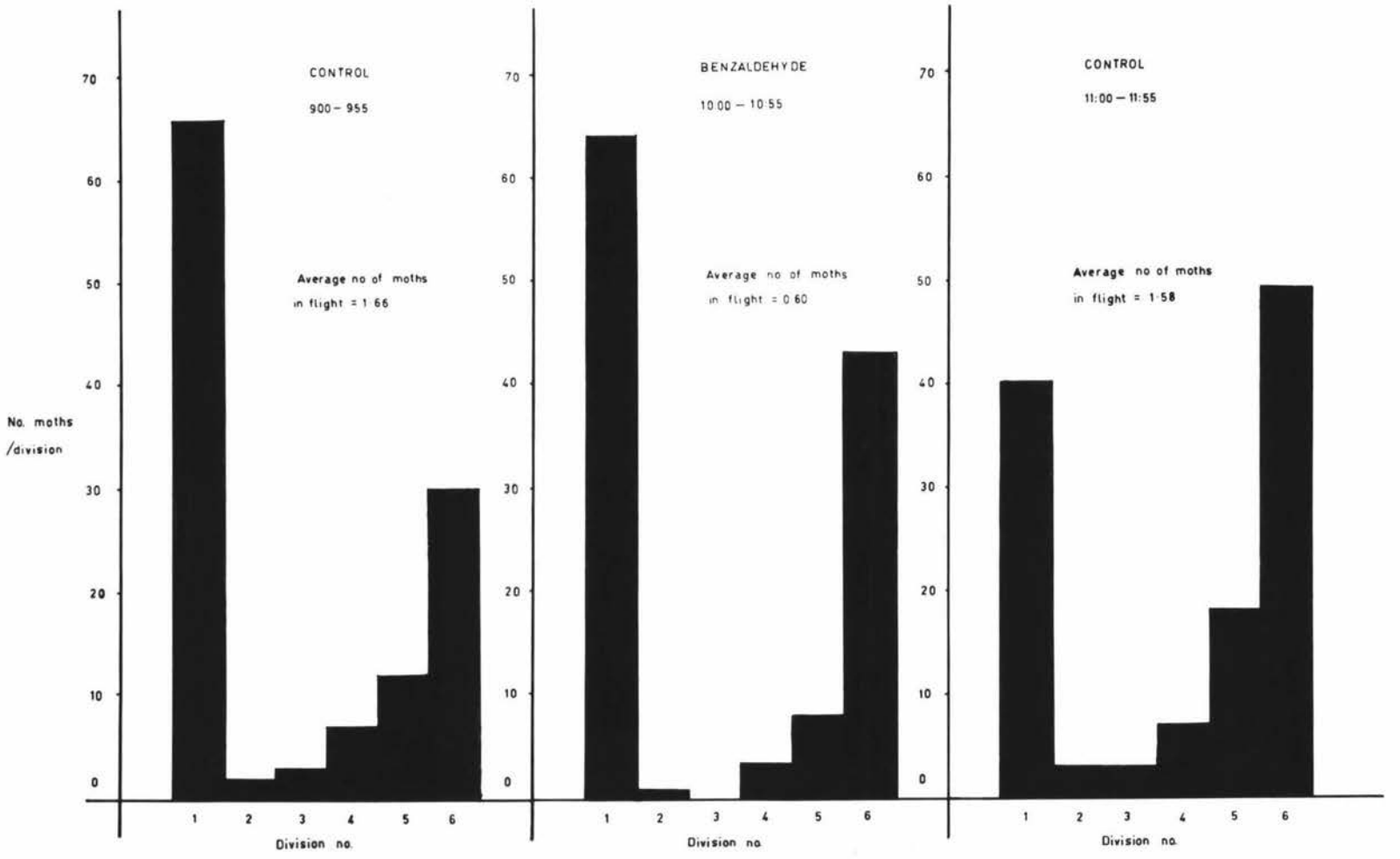


FIG 6 THE AFFECT OF BENZALDEHYDE ON THE ACTIVITY OF THE FEMALE

Conclusions:- The results obtained from this experiment indicate that the effect of the pheromone on the female is to inhibit movement. This hypothesis is in consonance with the earlier observations that males successfully mated only with females that did not move away as the male approached. It is of great interest that antennaeless females of Phlogophora meticulosa typically took flight as soon as a courting male made contact Birch (1970). Possession of a secretion that increases the chance of a successful mating by preventing the escape of the female is clearly highly advantageous.

It is suggested that an approaching male stimulates the female in two ways. The visual stimulus of an approaching object is likely to initiate a general escape reaction. The chemical stimulus that is simultaneously presented is postulated to inhibit this reaction. A similiar interpretation is proposed by Brower Brower and Cranston (1965) for Dananus gilippus berenice. The female queen butterfly avoids the stimulus presented by the pursuing male by initiating an escape flight which is sustained and vigourous. As the male overtakes and hairpencils the female, it slows and descends to the ground. Males deprived of hairpencils actively pursue females and engage in the same aerial manoeuvres, but after having caused their mates to alight, fail to induce them to remain on the ground Pliske and Eisner (1969). The male pheromones of these two species can thus be considered arrestant pheromones according to the terminology of Dethier Browne and Smith (1960). If this hypothesis is

correct, the noctuid system must be considered more advanced as less expenditure of energy on the part of the male and female is required to achieve a successful mating.

Benzaldehyde has previously been reported in insects as a defensive secretion. Aquatic dytiscid beetles secrete this compound possibly as an anti-bacterial agent Gilmour (1965). Benzaldehyde is also the major component of the defensive secretion of the ant Veromessor pergandei Blum et al. (1969). Consequently Aplin and Birch (1968) speculate that this secretion may serve to deter rival males. The fact that the males disseminate the pheromone when approaching a female Aplin and Birch (1968) and not when approached by males while "in copula" render this supposition unlikely. Some Lepidoptera are distasteful to predators eg the Danaid butterflies accumulate poisonous cardiac glycosides. If benzaldehyde is accumulated as a protection against predators, it would not be expected to give great protection as only males would derive benefit.

The experimental situation in which the females were placed was highly unnatural and the moths tended to damage themselves due to collisions with the walls. This may have affected their response. Difficulty was also encountered in obtaining a correct concentration of benzaldehyde in the air flow without knowledge of the behavioural threshold. One trial was abandoned when the females exhibited abnormal behaviour. All were fluttering agitatedly at the far end of the tube. Despite these reservations, it was felt that the results obtained were likely to be applicable to the

the natural situation.

At this point, the relative importance of this signal in the mating behaviour of P. separata should be considered. In the section on the mating sequence, it was concluded that use of the hairpencil was not essential for successful mating of P. separata. This contrasted with a study by Birch (1970) who analysed the courtship interaction of P. meticulosa and observed brush eversion in all except one mating. P. meticulosa is much more dependant on chemical cues as antennectomy completely abolishs mating. The combination of the use of visual cues with the restrictions on female movement inherent in the experimental situation would seem to have resulted in the non-use of the male pheromone under laboratory conditions.

The Structure and Function of the Hair Pencil.

The hairpencil is a simple structure, the major portion of which serves as an erectile surface for the evapouration of pheromone Stobbe (1912). Examination of the morphology of this structure suggests that the muscle sheet covering the insertion plate provides the major mechanism erecting the hair pencil. Considerable reduction of the friction on the hairscales emerging from the pouchs may be effected by an increase in haemocoele pressure dilating the lips of the pouch. A series of experiments were carried out to determine the relative importance of these affects.

Methods:-The morphology of the hair pencil was examined by two methods. The details of the insertion plate were taken from a preparation cleared in KOH and stained with methylene blue. After a series of parafin imbedded sections had proved unsatisfactory, the muscle sheet and insertion plate were place in glutaraldehyde / formaldehyde fixative and after dehydration were imbedded in araldite resin. This technique retained the muscle fibres in the correct spatial relationship to the insertion plate.

Several of the movements of the abdomenal segments were studied by experimental stimulation of the ventral nerve cord between the pherothoracic ganglion and the second abdomenal ganglion. Two types of ringer were used to bathe the preparation. The nerve preparation remained viable when in the simple Ephrussi and Beadle (1936) ringer at least as long as when in the complex buffered ringer based on Cecropia haemolymph. Michejda and Thiers (1963). Clarke

and Harvey (1965). A Palmer students stimulator producing long pulses at 5 c.p.s, strength = 4, was used to stimulate the preparation. To detect minute pressure changes, a fine capillary was sealed at one end, half filled with water and inserted into the abdomen, so that the open tip was situated in the anterior abdomenal segments.

The effects of an artificial increase in haemocoele pressure were studied. Freshly killed males were decapitated and the needle of a water filled syringe was inserted longitudinally through the thorax into the anterior segments of the abdomen. Care was needed not to damage the delicate intersegmental membrane between the thorax and abdomen. Water was carefully forced into the haemocoele.

Results:-Three simple components made up the hair pencil. The hair scales were fine structures, about 3.7 - 4.0 mm in length, covered with fine diagonal sculpturing quite unlike the line and pit sculpturing of the body scales. There were two distinct zones; a distinct shaft most darkly sclerotised at the distal end, and a diffuse lightly sclerotised brush (see fig7.) The hair duct from the Stobbes gland met the hairpencil near the darkly sclerotised portion of the shaft.

The hair scales emerged from a heavily sclerotised sheet of cuticle - the insertion plate. This plate was made up of two regions (see fig 8.) A smooth area continuous with the

Fig.7. The Hair pencil Scales
7 X stereo

Fig.8. The Insertion Plate
X 100 Methylene blue

lever formed the flexible hinge holding the insertion plate at an angle of 50° to the lever when the hairpencil is at rest. The distal portion of the plate was tightly packed with scale sockets. The folding of the cuticle effected by the sockets would add to the flexibility of this plate. The trichogen cells responsible for the production of the hair scales were modified epidermal cells (see fig 9.) The nucleii of these cells were very large and equally developed. A deep sub-scale lumen was transversed by a distinct slender strand of cytoplasm from the point of scale attachment, to the area of the nucleus.

The spatical relationships of the fibres of the muscle sheet were not quite clear. In the region of the hinge the longitudinal orientation of the striated fibres was quite marked. This contrasted with the transverse fibres over the sockets (see fig 10).

Two distinct groups of muscles responded when the nerve cord was stimulated. Due to the contraction of the longitudinal sternals, the sclerites of segment II and III retracted. This retraction did not appear to be accompanied by any signifigant increase in haemocoele pressure, as no change in the level of water in the capillary was observed. The muscle sheet attached to the insertion plate contracted. This contraction bowed the insertion plate, slightly spreading the hair scales and partially unfolding the hinge. The muscle did not appear to exhibit maximum contraction possibly due to the drag affect of the medium on the hydrophobic hair scales.

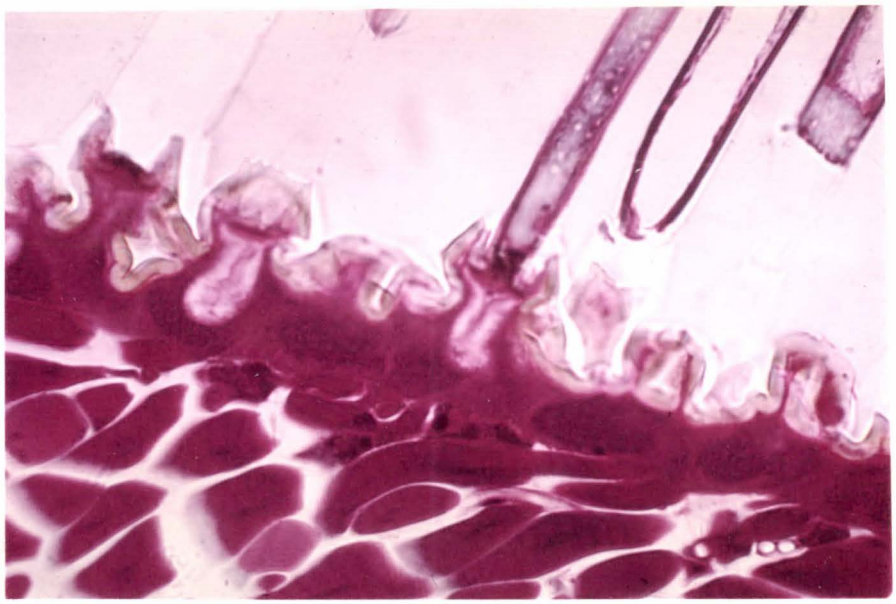


Fig.9. The Cells overlying the Insertion Plate
X 400 Acid fuchsin + Methylene Blue

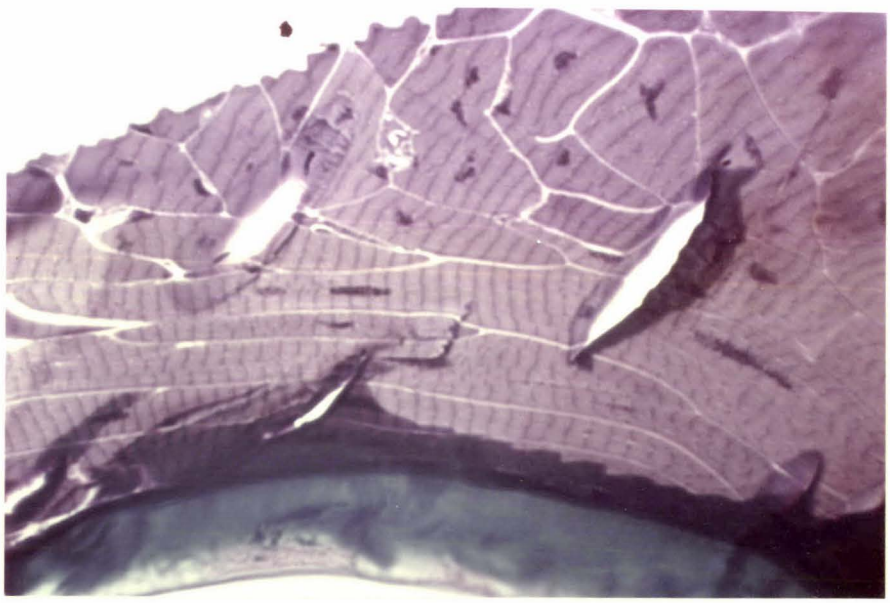


Fig.10. The Muscle Sheet of the Insertion Plate
X 400 Methylene blue

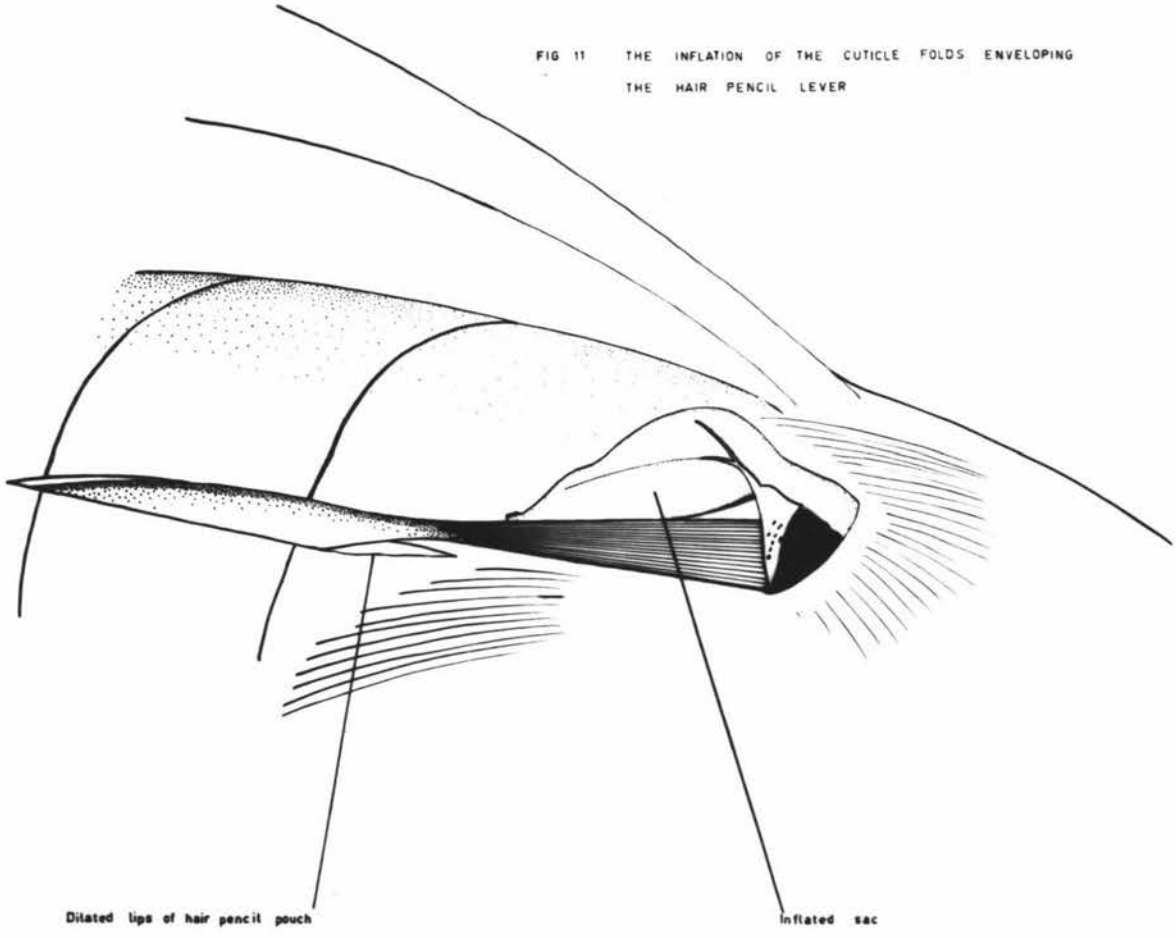
(Compare the longitudinal orientation of these hinge fibres with the transverse orientation of the fibres in the insertion area of Fig.9.)

Artificial increases in the hydrostatic pressure of the haemoncoele produced two effects. The abdomen became distended and the mouth of the pouch dilated exposing the scales of the hairpencil. Further, the membraneous cuticle at the back of the hairpencil became inflated producing a turgid ridge at the back of the hairpencil lever which raised the hair shaft clear of the body (see fig 11).

Conclusions:-The structure and function of the hairpencil are intimately related. In insects, the functional juxtaposition of muscle and elastic cuticle is very common. In the Cicadidae, a tensor muscle works against the elastic resistance of the tympanum to provide sound. Pringle (1954). For flight, contraction of the indirect flight muscles is translated into deformation of the elastic thoracic box, which is then transmitted to the wings. This mechanism allows a high frequency resonant system providing flight precision and power otherwise unobtainable Pringle (1957).

On the basis of the experimental and morphological evidence, there appeared to be two complementary movements in the erection sequence, Contraction of the longitudinally orientated muscle of the hinge moves the axis of the insertion plate from rest to a position parallel with the body axis. This movement erects the shaft of the hairpencil in a manner similiar to the opening of the blade of a pocket knife. Secondly, the expansion of the hairscales occurs as described by Stobbe 1912 for Dichonia aprilina.

FIG 11 THE INFLATION OF THE CUTICLE FOLDS ENVELOPING THE HAIR PENCIL LEVER



> "They have quite strong muscle groups, single strands of which have each end attached to opposite ends of the chitin plate that carries the fan hair. The contraction of these muscles brings the edges closer together. Its centre however is bowed outwards. Through this a far reaching star shaped outburst of the fan hair occurs".

The hypothesis that pressure increases in the haemocoele assists eversion by dilating the pouch lips was not supported by the absence of pressure changes detectable by the capillary. This may reflect a real lack of such changes in the haemocoele, or merely that the punctures caused by introduction of the capillary were allowing haemolymph to escape as contraction occured. Stobbe (1912) noted that the cuticle of the end pocket which holds the hairpencil of Dichonia aprilina was very delicate and suggested that it is everted by blood pressure.

Several males of P. separata captured in the light trap had the hairpencil neatly foled into the ventral cavity situated between the thorax and abdomen, that appears in most Lepidoptera. In this position, the hairpencil was covered with scales, The brush poritions of the two scales did not merely lie in the same cavity but were intimately interwoven. Appreciable tension was necessary to separate them. This folding was also observed in Persectania aversa Noctuids are able to retract the hairpencil into the pouch Stobbe (1912) and Aplin and Birch (1968). Confirmation that P. separata is also able

to retract the hairpencil was obtained by artificially exserting the hairpencils, and observing that they were inside the pouch several days later. No adequate suggestion can be made for the mechanism or function of the observed interweaving.

An interesting comparison can be made between the pheromone evapouration surface of male and female noctuids. Assuming compounds with similiar vapour, the highly dissected surface of the hairbrush would allow a much higher release rate than the surface area of the intersegmental membrane gland of female Trichoplusia ni Jefferson Shorey and Gaston (1967). In Phologophora meticulosa, the hair pencil is erected for one to two seconds, while the female may "call" for very long periods Birch (1970). Pheromone disposal thus differs greatly between the sexes, the male producing high volumes for a short period, while the female releases at lower rates for longer periods.

The Morphology of the Ventral Anterior Abdomenal Segments Associated with the Hair Pencils.

The basic patterns of muscle and sternite form appear to have been modified by the demands of the hair pencil structure. Most early descriptive work has centred on the hair pencil itself, with little of the internal and supporting structures being described. The only detailed work available is that of Stobbe (1912) who has described the muscles affecting the hair pencils and pouchs of Dichonia aprilina.

Methods:-The fixative of Chauthani and Callahan (1966) was used for the dorsal dissection of the anterior abdomen. Muscle detail was taken from a preparation stained with 50% aqueous Grenachers Carmine Alum. Sternites were cleared with KOH and stained with methylene blue.

Results:-The greatest modifications have occured in the second to fourth segments. The first abdomenal segment was typical of most Lepidoptera being represented by membraneous cuticle Imms (1964). A comparison of the second sternite from the mlae and female revealed that the posterior portion of this structure was markedly modified to bear the hair pencils (see fig 12).

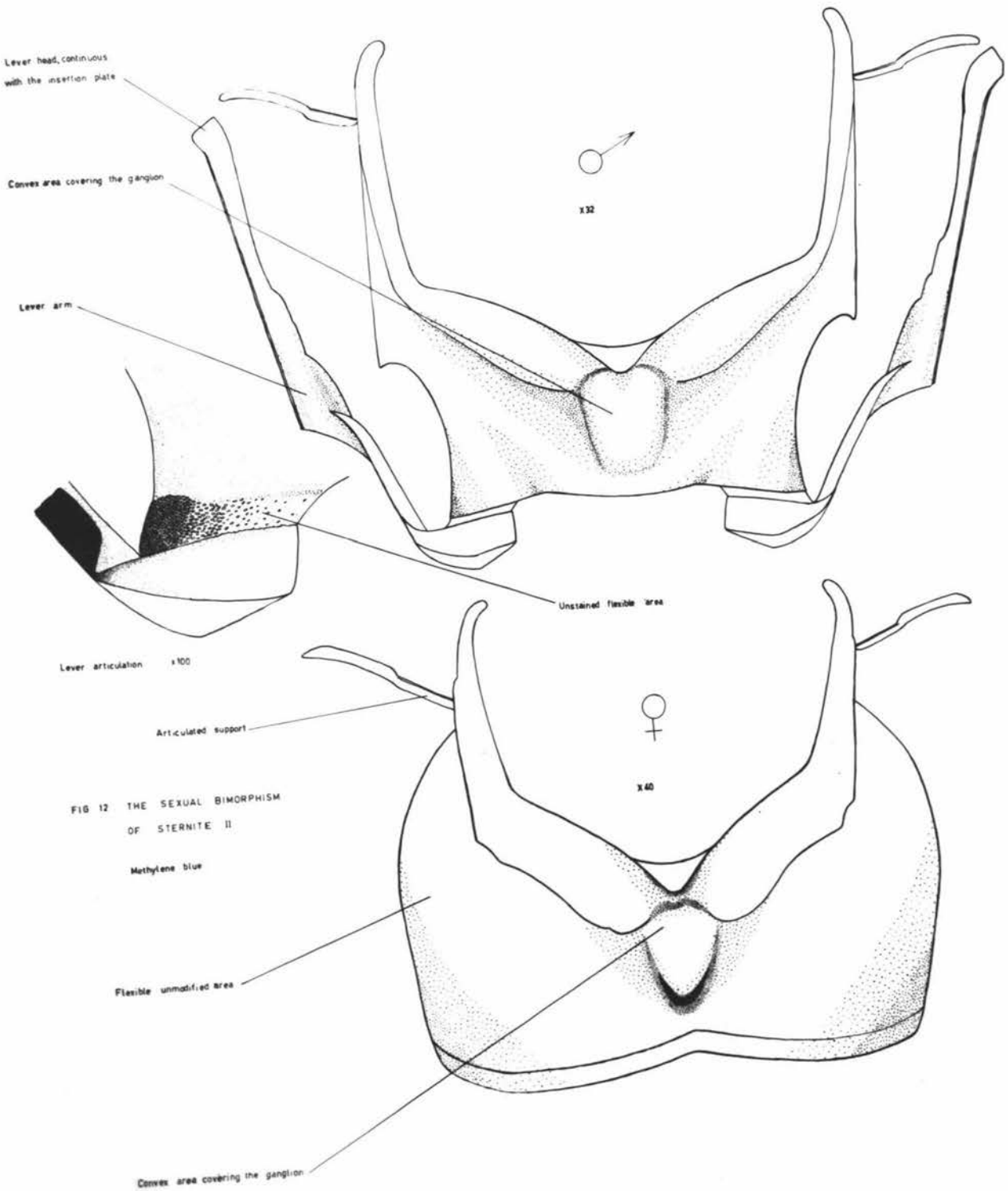


FIG 12 THE SEXUAL BIMORPHISM OF STERNITE II

Methylene blue

Anteriorly both sternites bore a long process with a muscular connection to the metathoracic sternite. Two pairs of muscles rose from the centre, and arched over the tympanum to connect to the thorax. A concavity of the central portion held the second abdomenal ganglion. The lateral margins of the female sternite were extended and flexible, so also the edges of the following sternites. In contrast, those of the male curved inwards and were moderately sclerotised. The liver, present only in males, was attached to the outer posterior portion of this sternite. The flexible cuticle articulating the levers was transparent, and marked by numerous pits which may serve to increase the flexibility of the joint. The liver though mobile was not free being held to the thorax by membraneous cuticle (see fig 13). On part of the outer suface it bore a shallow trough in which the hair scales were held at rest. One of the ventral abdomenal muscles was attached to the base of the lever.

The third and fourth sclerites bore internally a pouch into which the hair pencil was folded. This pouch was a thin bag of flexible cuticle which expanded from a shallow origin in segment III to its greatest volume in the posterior portion of segment IV. The inner pouch wall was lined with a sparse population of flat scales. Externally the pouch opened along a ventral slit for much of the length of segment, III and IV. The margins of this slit are tightly closed by the elasticity of the cuticle.

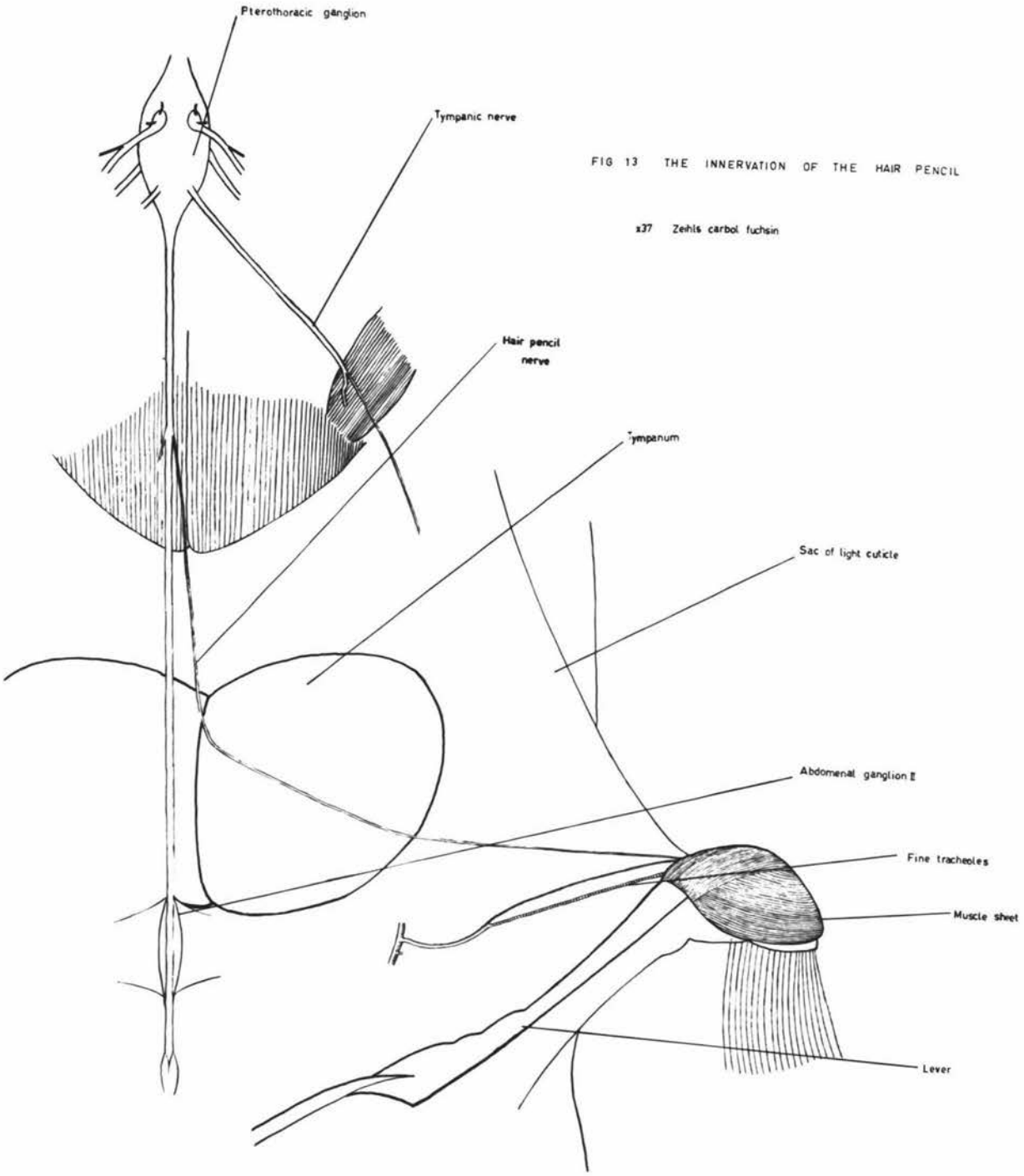


FIG 13 THE INNERVATION OF THE HAIR PENCIL

x37 Zehls carbol fuchsin

The anterior musculature of the male consisted of clearly defined slightly oblique muscle blocks (see fig 14). In contrast, the female possessed a closely spaced series of parallel longitudinal muscles uniformly arranged in comparison with those of the male. The external longitudinal muscles of the sternum consisted of median and lateral groups. In segment IV and those following, these muscles retained a privitive position attached to intersegmental folds. In contrast the median longitudinal muscles of II and III were attached to the antecostae of the following sternite. In this position their function of abdominal retractors would be enhanced. The large lateral longitudinal sternals of segment II were greatly developed in both sexes. A series of oblique sternals were developed only in the anterior segments of the male. The edge of the pouch was rimmed with muscle which on contraction may serve to dilate the edges of the pouch allowing easier withdrawal of the hairpencil.

Conclusions:-Examination of the second sternite of other noctuids confirms the view that observed differences between males and females are due to adaptation for the hairpencil. Males of Persectania aversa which possess hair pencils have a sternite very similiar to that of male P. separata. In contrast this sternite in male Melanchra insignis and M. mutans (which lack hair pencils) resembles that of female P. separata.

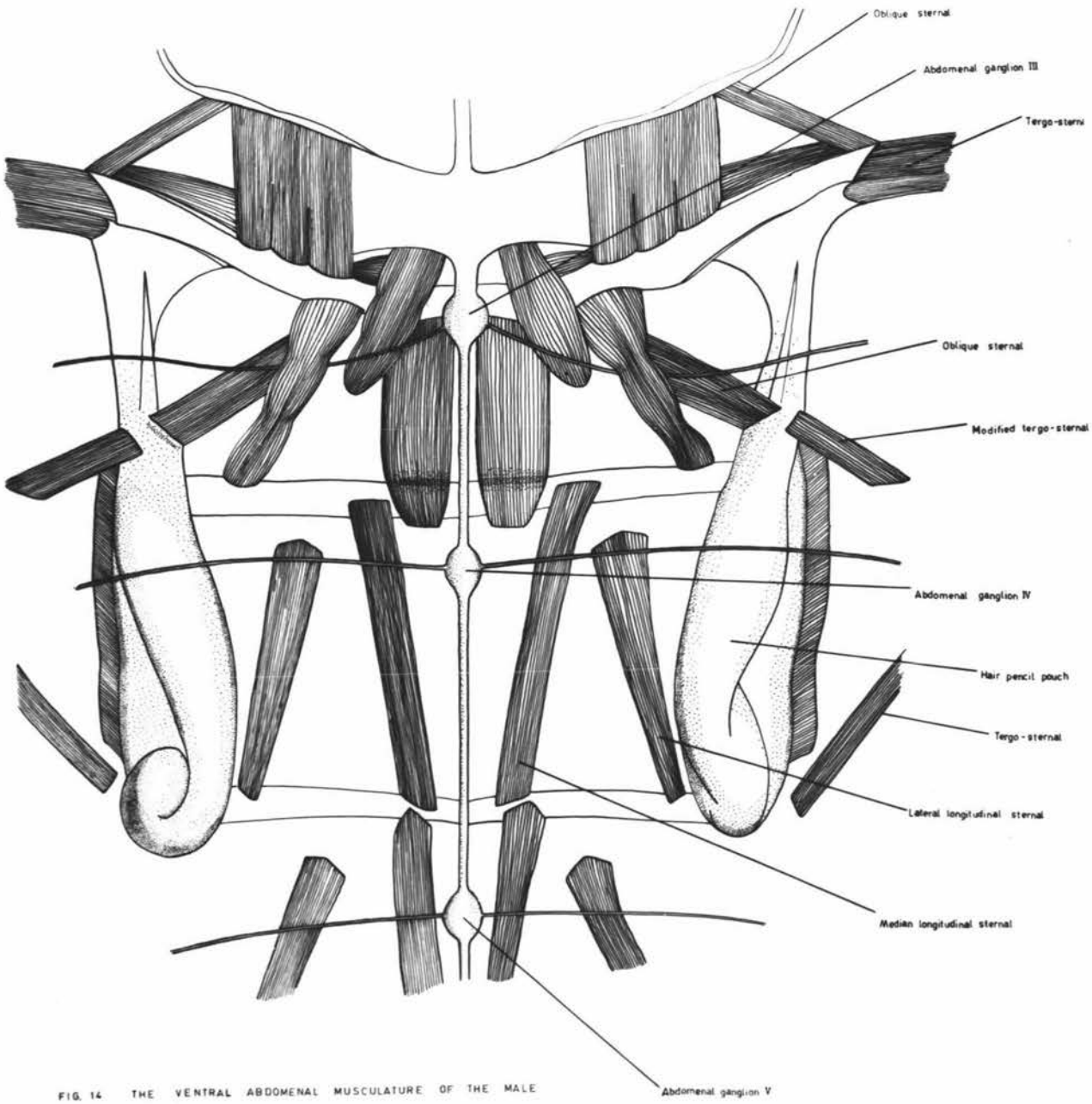


FIG. 14 THE VENTRAL ABDOMENAL MUSCULATURE OF THE MALE

x 35 5% Grenachers Carmine Alum

Eltringham (1925) (Phlogophora meticulosa and Xylophasia monoglypha) and Aplin and Birch (1968) (Leucania conigera) describe a hair pencil pouch similiar to that of P. separata. This shape is not the only one reported. The pouch of D. aprilina consists of a fine channel leading to a voluminous end pocket restricted to segment IV. Stobbe (1912) suggest that this pouch is created by an invagination of a ventral portion of the pleuron together with a part of the neighbouring sternum. The scales lining the inside of the pouch have been implicated by several workers eg. Eltringham (1925) as pheromone secretors. This theory is no longer acceptable as Stobbes gland has been shown to possess this function.

Other workers shed little light on the function of the internal abdominal muscles. In D. aprilina Stobbe (1912) describes a pair of muscle groups attached to the pouch and postulates that contraction of these muscles closes the protective fold more tightly. Similiar muscles do not appear in P. separata.

The Morpholoby and Microstructure of Stobbes Gland.

The major secretory structure of the male pheromone system is a large gland connected to the hair pencil by a hairscale duct. This gland was first described by Stobbe (1912) and assigned the function of producing the hairpencil secretion. This view conflicted with most contemporary workers who considered that the scales lining the inner wall of the pouch were secretory Eltringham (1925). Absence of the pheromone after the excision of this gland confirmed the assigned function Aplin and Birch (1968). Further, the gland is found only in male noctuids possessing hair pencils.

Methods:-Parafin imbedded material was used for the initial examination of the gland while more reliable preparations for detailed cytology were prepared with living material.

Glands dissected from males captured with a light trap were fixed in Bouins, dehydrated, and cleared in terpineol before being imbedded under vacuum in 58^{o} wax. A number of sets of serial sections, cut at 5 u were made, and stained with Ehlrichs haemotoxylin and eosin. Parafin imbedded material is adequate for gross studies, but because of artifacts caused by the type of fixative, imbedding method and temperatures, is no longer considered acceptable for rigourous examination of cellular detail.

Using Nomarski and fluorescent dye techniques, differences in cell organelle densities and distribution of nucleic acids were studied in living material. Males were taken from stock cultures at 0.5 and 1.5 days old, killed, and the glands removed

to Ephrussi and Beadle's (1936) Saline. Several of these were examined immediately with a Nomarski differential interference microscope. This instrument utilises the affects of wave front interference to produce a three dimensional image with materials of different densities. Further glands were placed in a solution of 0.01% Acridine orange in Ephrussi and Beadle's (1936) Saline for five minutes. The preparation was then examined under the illumination of an Olympus HLS ultra-violet source. Attempts were made to dissaggregate the gland cells with EDTA and trypsin solutions. Thought some cells can be removed with EDTA, the delicate cellular structure appeared to be damaged by this technique.

Results:-The gland was found deep within the fat body of the first segment, with a duct leading to the hair pencil of the same side. This duct is formed by a shaft of fine hairscales, each of which is attached to a single hairscale by a basal socket. (see fig 16) An average of 68 cells were present, a number close to the 64 that 6 mitotic divisions of an initial cell would produce. These cells were far from homogenous. Glands dissected from insects soon after their emergence possessed a number of enormous cells each of which could be up to a millimeter in diameter. All cells possessed a single large nucleus which stained heavily with haematoxylin. In the larger cells, this nucleus was more diffuse, and larger in size (see fig 16)

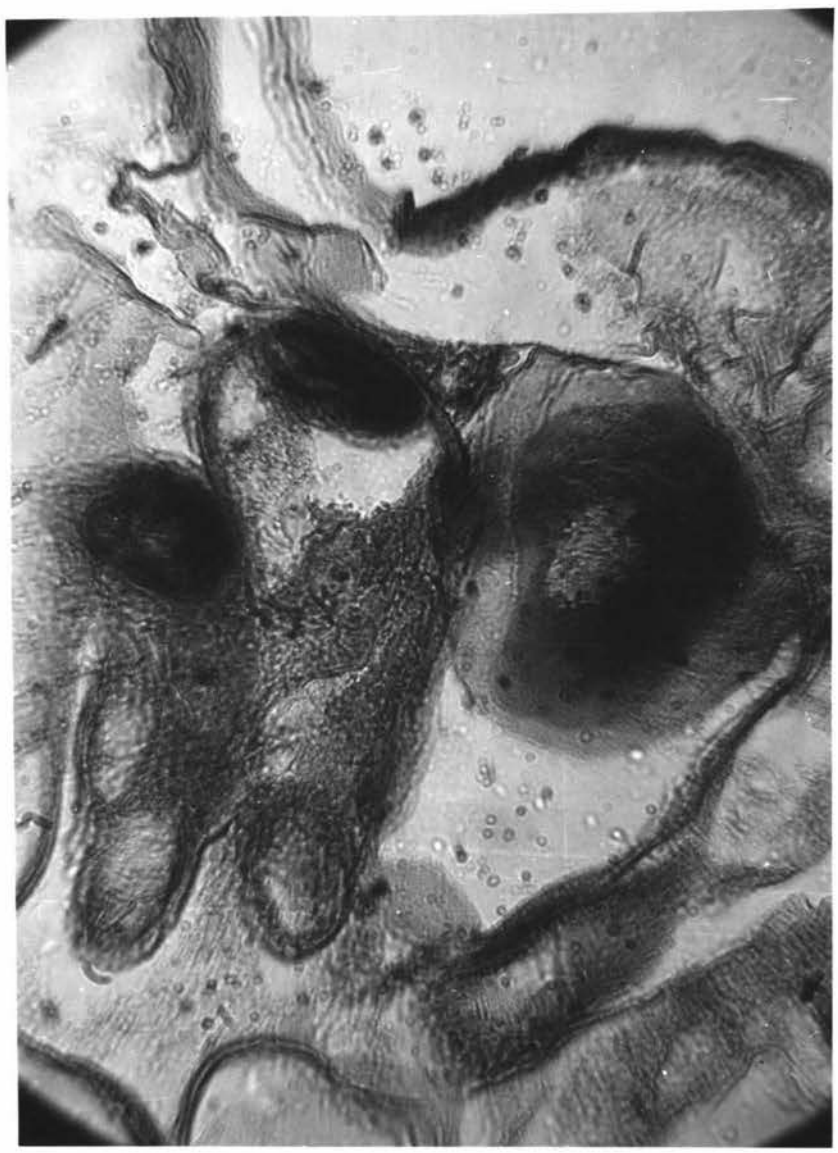


Fig.15. Giant and Normal Cells of the Stobbes Gland (late phase I) X 400. Ehrlichs Haematoxylin and Eosin.

(Compare the shrinkage of these cells with those in Fig.19.)

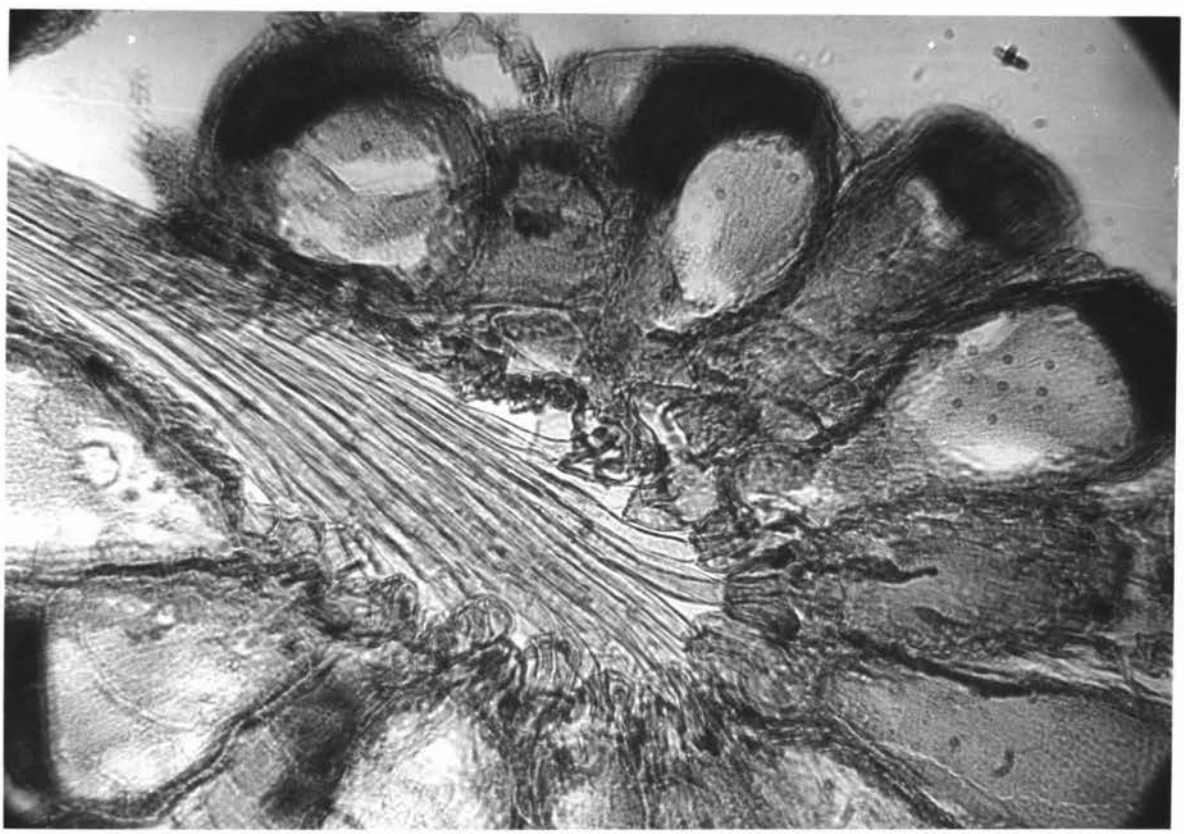


Fig.16. The Spatial Arrangement of the Gland Cells around the Hair Scale Duct (phase II) X 400 Ehlrichs Haematoxylin and Eosin

Comparison of the glands of different ages when stained with acridine orange revealed major differences in the distribution and fluorescence of the stain. In cells 0.5 days old, the cytoplasm was orange red (see fig 17). The colour of the nucleus was obscured by the cytoplasm. The nucleus was a bright yellow green in cells 1.5 days old, while the cytoplasm remained translucent (see fig 18).

The Nomarski instrument revealed considerable structural detail of the subcellular organelles of the giant cells. The cell anc nuclear membranes were clearly visible (see fig 19). At least two major types of cytoplasmic vesicle were observed. These types differed in density suggesting different chemical composition (see fig 20). Though not homogenous in size, one type was usually more than twice the size of the second. The smaller vesicle was frequently found within the larger type. These vesicles were membrane limited as the boundary between vesicles of the same density was sharp. Many moving organelles were observed, some of which appeared to be mitochondria. Others, in shape similar to the cross section of a red blood corpuscle, possessed a long lashing projection and moved actively within the vesicle. Because of the very short time lapse between dissection and examination, it was thought unlikely that these were bacteria resulting from external contamination. As the film required an exposure of several seconds, these organelles could not be photographed. The vesicles themselves appeared to be mobile. The group of very large vesicles in mid right field of fig 19 have changed position during the few minutes elapsing between photographs.

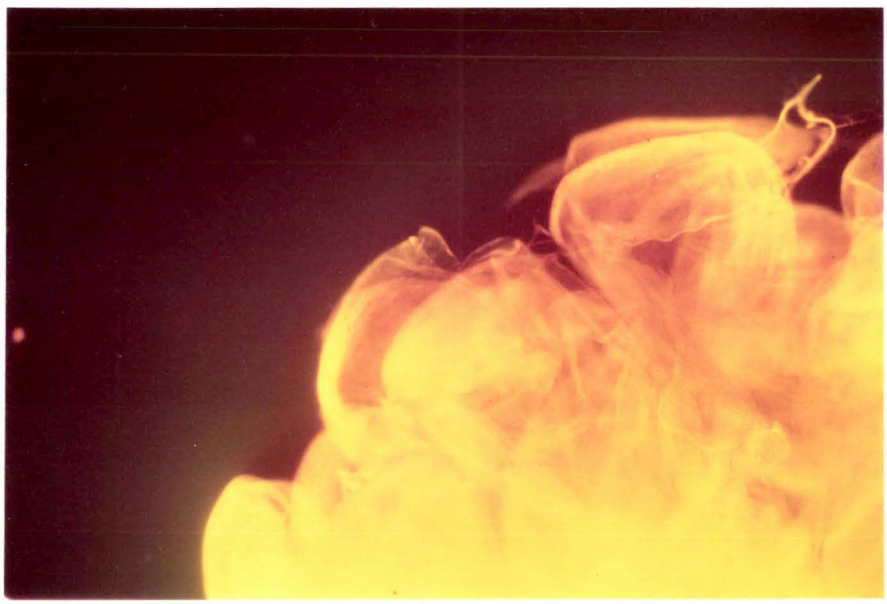


Fig.17. The Cytoplasmic RNA in Cells 0.5 Days Old
X 40 0.01% Acridine Orange

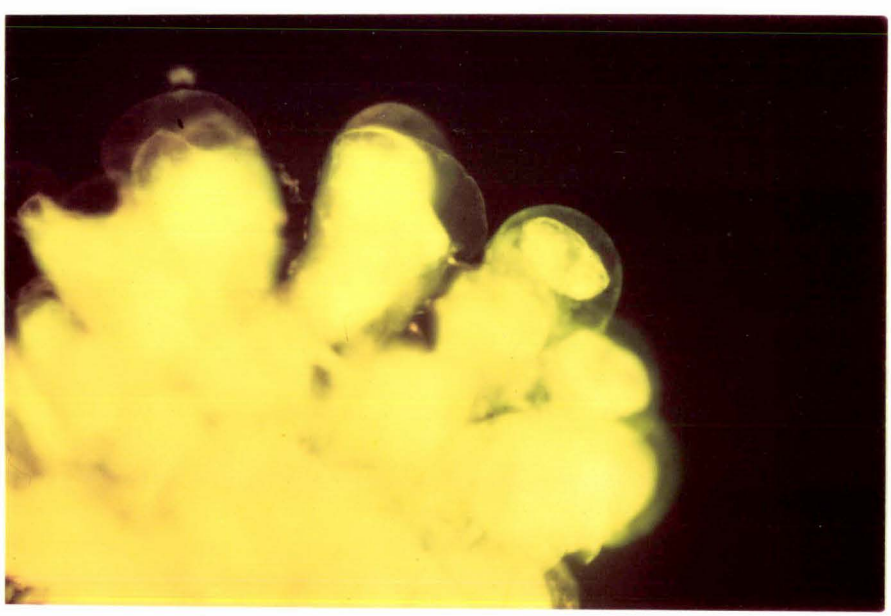


Fig.18. The Nuclear DNA in Cells 1.5 Days Old
X 40 0.01% Acridine Orange

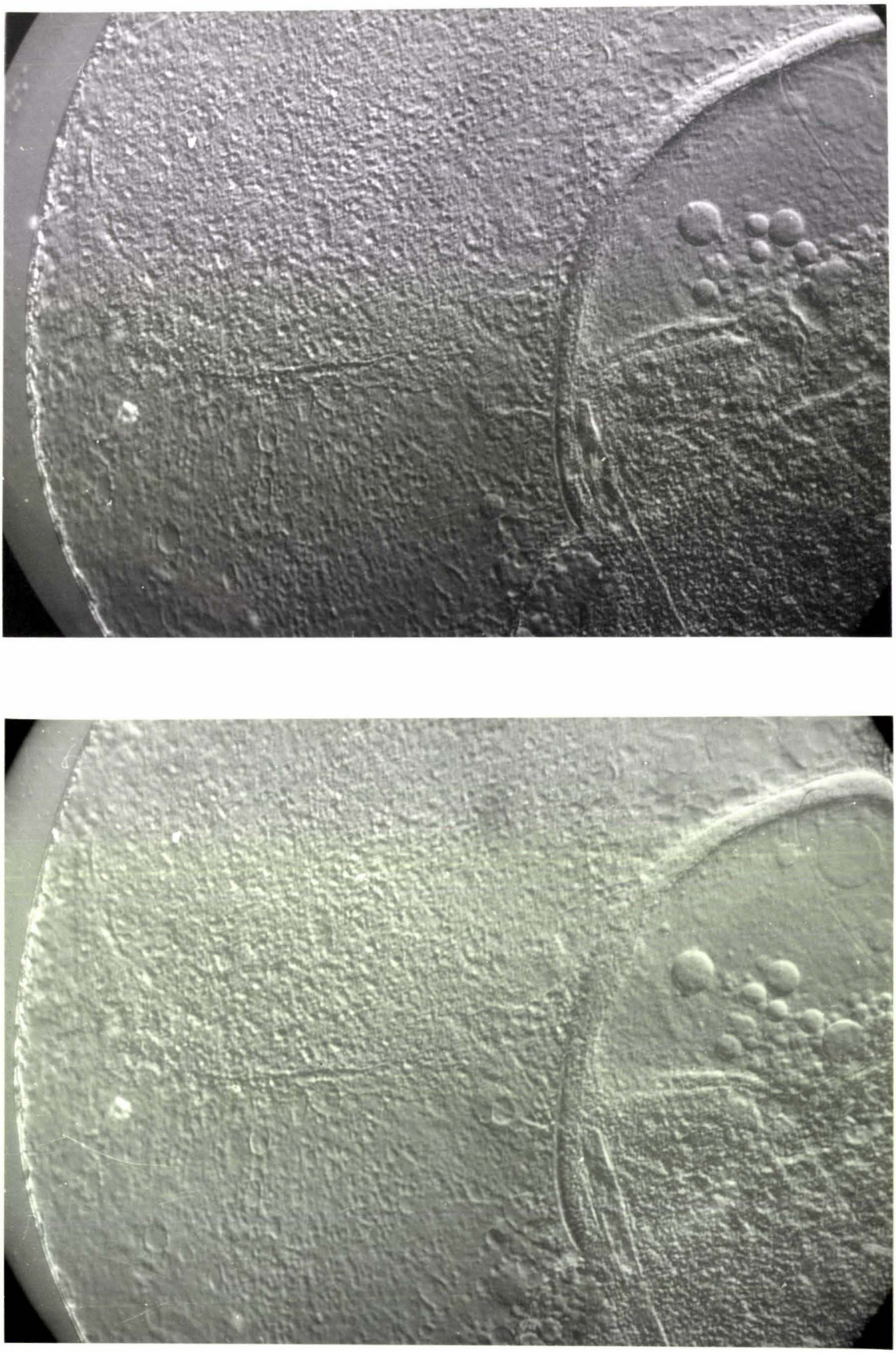


Fig.19. Internal Structure of a Giant Cell.
X 160 Nomarski (early phase I)

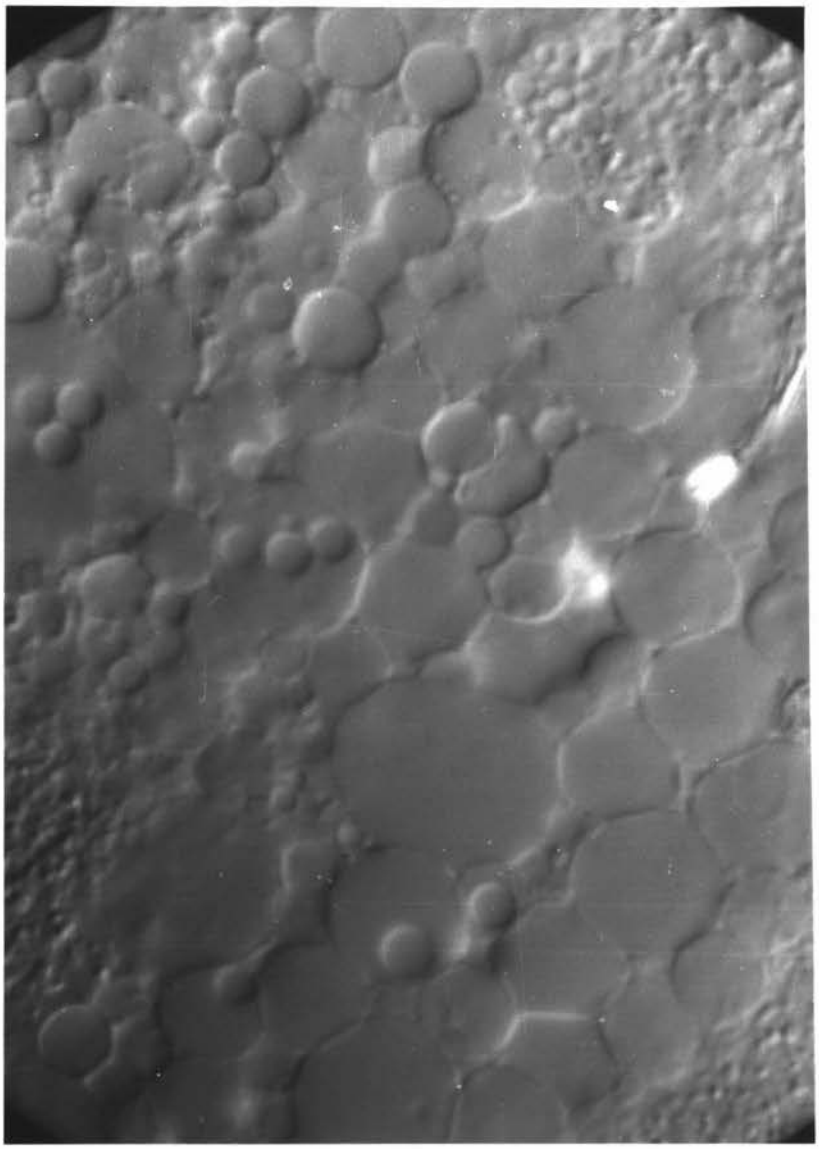


Fig.20. Cytoplasmic Vesicles Giant Cell
(early phase I) X 1000 Nomarski

Conclusions:-Cytological evidence from two sources suggests that extensive synthetic activity is taking place within the cells of glands soon after the emergence of the imago.

Acridine orange (AO) is a metachromatic dye, the fluorescence of which is dependant on the physical properties and chemistry of the necleii acid to which it is bound. The AO - DNA complex consists of DNA metachromatically bound to the phosphorus neighbouring the pourines and pyrimidines. The AO - RNA complex is formed when the AO dimer in solution dissociates into monomers which are intercalated between the bases. Tomita (1967a b). In cells 0.5 days old, the orange-yellow fluorescence of the cytoplasm indicates the presence of substantial amounts of RNA, while in cells 1.5 days old, major amounts of DNA restricted to the nucleus are suggested by the yellow-green fluorescence of this area. Because of the variations in concentrations of cytoplasmic RNA, protein synthesis can be assumed to be proceeding at a higher rate in the younger cells than in those 1.5 days old.

Steinbrecht (1964) working on the pheromone secreting epithelin of the female Bombyx mori demonstrated the presence of lipid vesicles with strong ultraviolet absorbtion at 240 um. As synthetic trans-10- cis-12-hexadecadien-1-ol, the pheromone of this species has an absorption at 230 um. Steinbrecht (1964) considers that these vesicles contain a pheromone precursor. Further Gerok (1950) showed that lipid extracts of B. mori have pheromone activity following reduction with Li Al H_4 This led Regnier and Law (1968) to suggest that

these vesicles contain an acid which is converted to the active pheromone by reduction of the carboxyl group. Because the noctuid P. separata is phylogenetically distant from the bombycid B. mori any suggestion that benzaldehyde or its precursor has a similar origin can only be made with reservations.

The Innervation of the Abdomen and the Hair Pencils.

The nervous system of noctuids does not appear to exhibit great structural diversity in the abdomen. The system of *Pseudaletia separata* resembles that of *Prodenia litura* Mathur (1969) rather than that of *Heliothis zea* Chauthani and Callahan (1967). The nomenclature and homologies of the observed structures are quite controversial, especially in the region of the hair pencil nerve.

Methods:-Ventral and dorsal dissections were carried out on males captured with a light trap. The fixative used was that of Chauthani and Callahan (1966) based on chlorate hydrate, as it was less unpleasant than formalin for long dissections, and did not extract dyes. Stains used were aniline blue, methylene blue, acid fuchsin, carbol fuchsin, and concentrated picric acid. Carbol fuchsin and methylene blue were found to be superior to the other dyes.

Results:-A large pterothoracic ganglion was found to be linked to a chain of five abdomenal ganglia by a ventral nerve cord. The pterothoracic ganglion, formed by the fusion of meso-and meta-thoracic ganglia, was the source of nerve trunks to the wings legs and tympanum. A short distance away a fine nerve originated from the ventral nerve cord and ran parallel to the nerve cord to within a short distance of the first remaining abdomenal ganglion. The fine nerve then ran laterally, eventually connecting with the muscle sheet overlying the insertion plate of the hair pencil

(see fig 13). The first remaining abdomenal ganglion appeared structurally unusual. Dorsally it was continuous with a broad band of lateral muscle inserting on the second abdomenal sternite (see fig 14). The ventral nerve cord ran under this muscle band, forming a local swelling. The seond to fourth ganglia were of typical "button" shape, each releasing a single nerve trunk to the spiracles and dorsal muscles. The major trunks of the first two typical ganglia straddled the pouchs but left no obvious nerve there. Between these ganglia, the ventral nerve cord released very many fine branchs innervating the longitudinal sternals. The last abdomenal ganglion, widely separated from the preceding ones was much larger, appearing to be the major centre for the genitalia.

Conclusions:-The terminology of Mathur (1969) was used to assign the ganglia of P.separata the following nomenclature. The last ganglion is the result of the fusion of VI and VII, while the three ganglia preceding it are III - V. Chauthani and Callahan (1967) consider that the pterothoracic ganglion includes the remnants of the first two abdomenal ganglia. Mathur (1969) has produced evidence indicating that this view is not correct. In Prodenia litura there is a nerve originating from the nerve cord close to the pterothoracic ganglion and innervating the first abdomenal spiracle. The hair pencil nerve may be homologous with this. There also exists in P. litura a ganglionic rudiment similar to that of P. separata.

These two structures would appear to be the remnants of the abdomenal ganglia, I. and III. of the larva. In P. litura, Mathur (1969) places ganglia III to V in segments II to IV and ganglion VI and VII in VI. Chauthani and Callahan (1967) indicate that the first abdomenal ganglion is between segments II and III with the following ganglia arranged similarly. These authors do not commit themselves to any nomenclature. In P. separata each ganglion is in the segment of the same number. This is in line with the views of Snodgrass (1935).

"The nerves from each ganglion however consistently go to the segment in which the ganglion had its origin, thence morphologically a ganglion should be numbered according to the segment it innervates."

In this insect, the major trunk of nerves III to V extend laterally to the spiracles of the segment in which the ganglion is studied. The assertion of Stobbes (1912), that the muscle of the hair pencil is a structure of the first abdomenal segment is supported by its innvervation from a possible remnant of the first ganglion.

The Posterior Abdomenal Brush.

Another structure can be observed on male noctuids that may serve as a pheromone source. This is the posterior abdomenal brush, a large ventral tuft of fine erectile hairs on the eighth segment (see fig 21). Aplin and Birch (1968) suggest that this structure releases a pheromone, but provide no experimental evidence to support this claim. The structure is widespread, but is much less complex than the hair pencil.

Methods:-Conventional sectioning and mounting methods were used. The abdomenal tips of males obtained from stock cultures were fixed in Bouins imbedded in 58° parafin sectioned at 5 /u and stained with Ehrlichs haemotoxylin and eosin. Sternite preparations were produced by dissecting out the relevant portion which was then cleared in KOH, stained methylene blue and mounted in Xam.

Results:-The modifications of the eighth segments appeared to be extensive and affected the intersegmental membranes, cuticle thickness, epidermal cells, distribution, and the associated muscles.

Sternite VIII was attached to the preceding sternite by a large area of loose flexible cuticle - the intersegmental membrane. When the brush was retracted, this area and much of the anterior portion of sternite VIII were reflected under sternite VII (see fig 22). In this condition, no part of the brush was visible externally. The membrane was much

Fig.21. The Posterior Abdominal Brush
X 12 stereo

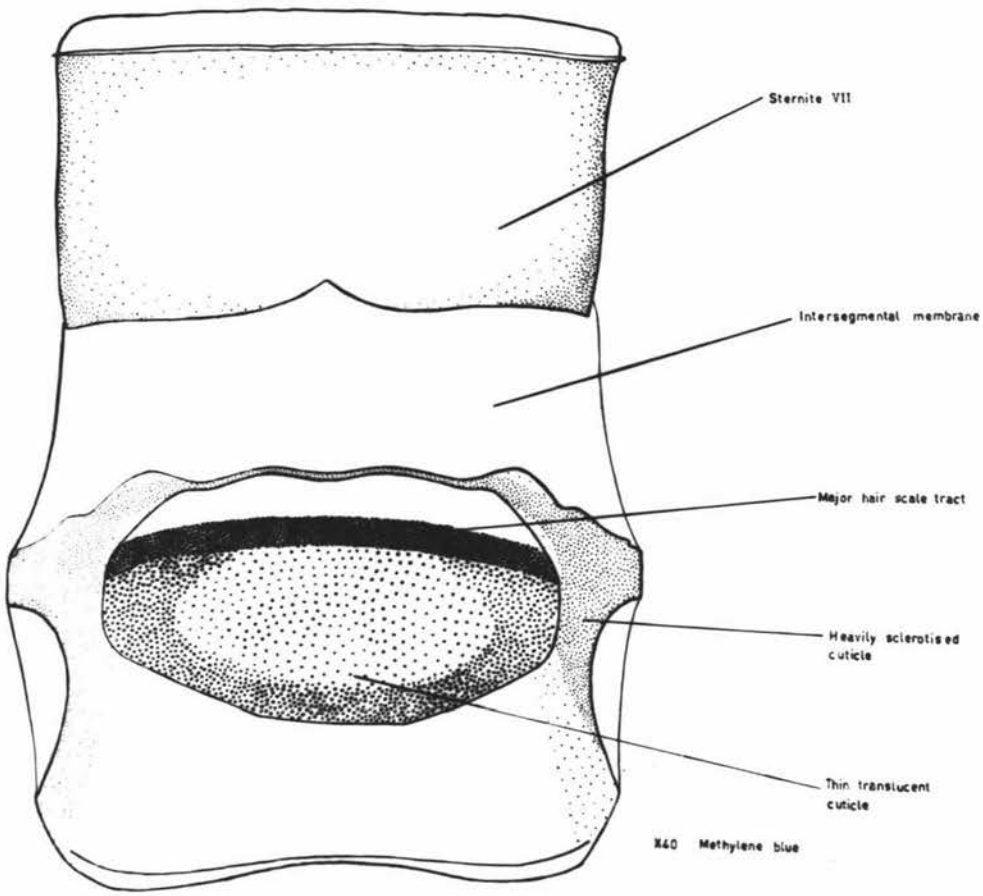
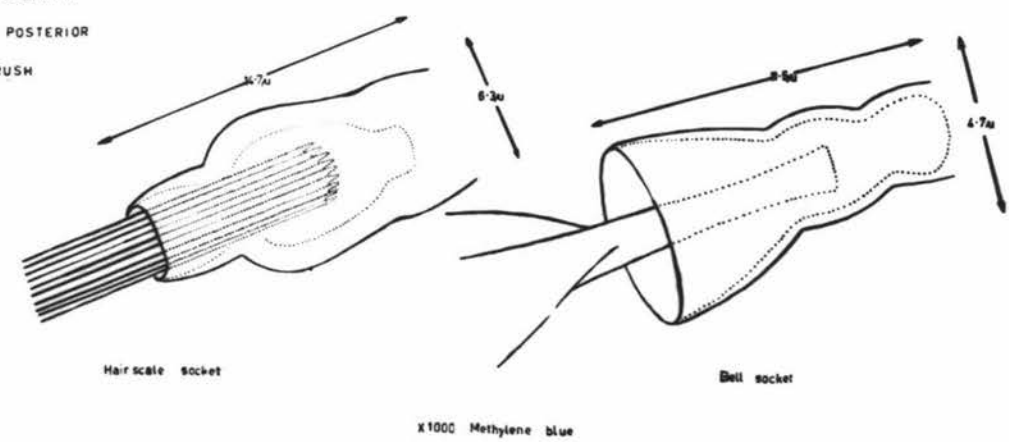


FIG. 24 THE STERNITE BEARING THE POSTERIOR ABDOMENAL BRUSH



more extensive than that between other segments, though in section it appeared morphologically identical to the other membranes.

Much of the anterior and mid portion of sternite VIII consisted of thin clear cuticle, rimmed by thick heavily sclerotised sclerotised cuticle (see fig 22). The thick anterior ridge formed the point of insertion of the protractor muscles. The scales had a quite distinct distribution. The anterior portion of the clear area was almost completely devoid of scales. Following, and clearly demarcated from this, was a dense crescent shaped tract of scales (2.62 x .14 mm, 160 x 10 scales). In longitudinal section, this tract appeared as a distinct cup (see fig 23) with the longitudinal axes of the cup scale sockets parallel. Examination of the topography of the sternite revealed that when flattened, the anterior sockets of the crescent tract opened cephalad, while the posterior sockets opened caudad ie. the positions attained when the brush is fully everted. Behind this tract was a sparse irregular distribution of scales.

The scales of the crescent tract appeared to be specialised structures. In comparison with the flat bell-shaped body scales, the tract scales were long and thin (4 x 1000 /u). The surface sculpturing was not changed as the scale hairs retained the 4-5 longitudinal striations and the frequent pitting of the body scales. The scale population of the crescent tract was dominated by the hair scale, though the flat body scale was also present. The sockets into which

body and hair scales insert, though basically similiar, differed in details of size and structure. Those of the hair scales were larger and more strongly sclerotised. The major difference was in the socket mouth, wide and bell shaped in body sacle sockets, and narrow and tubelike in the hair scale sockets (see fig 22). Underlying the hair scale sockets were a group of cells that stained deeply with haematoxylin (see fig 23). Their structure and boundaries were indistinct, but they appeared to be modified trichogen cells. No cells with similiar staining properties were observed under the body scale sockets.

Two functional groups of abdomenal muscles occurred, the long internal muscles (see earlier section) and shorter external groups. Examination of 40 sections showed that the external ventrals complemented the internal ventral functionally in all except posterior brush segments. The origin of the external ventral muscle of sternite VII was transpored to the posterior margin of the sternum in 63% of these sections. Functionally this muscle appeared to be antagonistic to the internal ventrals and to serve as a sternite protractor. A further 12% of the muscles of this segment resembled those of the other abdomenal segments. These sections were from the lateral portion of the posterior brush segment. The remaining 12% lacked musculature.

Conclusions:- Morphological evidence suggests that this structure may disseminate a chemical for sexual communication. The brush is found only in the males and in such a position that extrusion of the external genitalia would cause erection

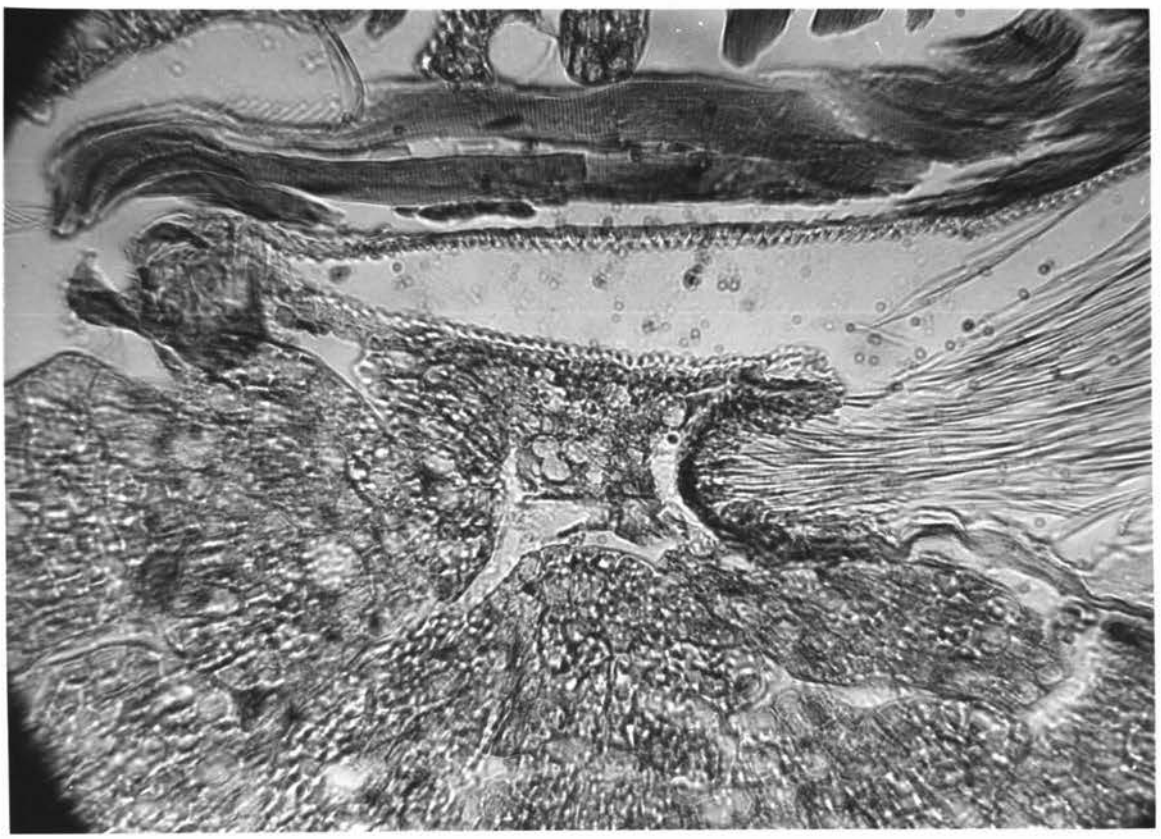


Fig.23. Longitudinal Section of the Posterior Abdominal Brush X 100. Ehrlichs Haematoxylin and Eosin.

of the brush. Many long thin structues provide an ideal physical surface for the evapouration of a chemical - the brush shares this characteristic with the hair pencil whose pheromone function has been established. The position and cup shaped base of the brush greatly resembles the hair pencils of Danaus gilippus berenice Brower Brower and Cranston (1965), though in this danaid the organ originates between the VIII and IX sternite. An interesting morphological similarity is also seen in the neotropical wasp Mischocyttarus drewseni, which applies a secretion to the nest stem which is repellent to foraging ants. The exocrine gland is on the anterior portion of the terminal gastral sternite and consists of a small clear non-sclerotised area bearing a tuft of hair Jeanne (1970). As this hymenopteran is phylogenetically remote from the noctuid P. separata the similarity is very likely to be due to concergent evolution of exocrine structures.

Faced with cells of a similar appearance to those observed underlying the hairscale sockets, Eltringham (1925) postulated that the darkly staining cells underlying "scent scales" in the hair pouch of noctuids were actively secreting a pheromone. As he was shown to be wrong Stobbe (1912) the allocation of a pheromone producing role to the posterior brush cells must be made with considerable reservations. The function of the posterior brush is thus strongly indicated, but in the absence of chemical or biological evidence cannot be considered proven.

Brush erection would be effected by release of the elastic energy in the cuticle. When the brush is retracted, the

flexible cuticle of the hair scale tract is deformed into the cup formation observed in section. The firmly braced margins of this sternite would store this tension as potential energy which would be released when the protractor muscle slides sternite VIII forward, releasing the hairscales from the restraint of the overlying sternite VII. The hair scale sockets appear to maintain a firmer hold of the scale due to the tubelike socket mouth. This structure would be a more effective transmitter of forces than the unmodified body scale socket. As sternite VIII is slid forward, the hair scales would move through an arc of 0^{o}- 180^{o} (depending on the position of the socket in the crescent tract) this erecting the brush.

The Chemical Identification of a Pheromone from Males of *Pseudaletia separata*.

Most of the complex molecules produced in minute amounts by insects for intraspecific communication are indectable by the olfactory sensors of other phyla. The male produced pheromones for sexual communication are an exception, as many can be detected by the human nose. For example, *Hepialus hectus* is reported to smell of pineapple Deegener (1902), *Euploea radamanthus* vanilla *Danaus plexippus* milkweed or red clover Brower Brower and Cranston (1965) and *Lethocerus indicus* of cinnamon Caillot and Boisson (1954). The observation that males of *Pseudaletia separata* have a distinctive almond odour initiated this study of the chemistry of its pheromone.

Methods:-

(A) Pheromone Collection:

An attempt was made to utilise a thermal gredient to collect a relatively pure extract. Yamamoto (1963) used a similar principle in an attempt to collect the pheromone of *Periplaneta americana*. Hair pencils dissected from males were placed in a wire cage at the top of a corked boiling tube. The bottom was imbedded in a crushed ice - salt misture at approximately $-10^{o}C$ and the whole apparatus left at room temperature. As the vapour pressure of a compound is less at lower temperatures, the gradual accumulation of the volatiles in the bottom of the tube was expected. After extraction of twenty hairpencils for a week, the characteristic odour of this structure could not be detected at the bottom of the tube and the technique was abandoned.

As the hairpencil consists largely of modified scale hairs, it was considered likely that a crude extract of the pencil could be analysed by gas chromatography. The epicuticle waxes of the scales were expected to differ markedly in molecular weight and volatility from the pheromone. Complex distillation and thinlayer techniques are normally necessary to obtain an extract pure enough to inject into a gas chromatograph. Gaston Fukuto and Shorey (1966). Of the two solvents used methylene chloride gave a better separation. Hair pencils and Stobbes glands were dissected from males captured in the light trap and placed in 1 ml of solvent. Extracts containing 10 H.P. /ml, 50 H.P./ml, 10 Stobbes glands/ ml (wet dissection) and 5 2.g/ml (dry dissection) were analysed. Two instruments and three columns were used.

(1) F and M 5750 with flame ionisation detector, carrier gas argon, column 6' x ⅛" int. diam. aluminium. Column packing was 3% Cyclohezane dimethanol succinate on 60–80 ## Chromosorb W.

(2) Packard with flame ionisation detector, carrier gas nitrogen, column packing (a) 15% Carbowax 20M on 100–120 ## gaschrom Q. Flowrate 60 ml/min. (b) 12% ethylene glycol succinate (B_2) on 70–80 ## Anakron A. Flow rate = 50 ml/min. Recorder at 35 cm/hr.

(B) Determination of the Mass Spectrum

Two methods were attempted, to obtain a sample of the unknown for mass spectrometry. A 20:1 flow splitter was inserted into the Carbowax 20M system, and the material coming off the

column between 80-95 mm collected. Collection was by a dry-ice cooled micropipette, inserted into the column outlet. The material from four successive runs, with 5 ul on the column each time, was collected and the pipette sealed with a bunsen flame.

A crude sample, containing longer amounts of the unknown with heavy contamination by the solvent, was obtained by differential evapouration of the extract on a hotplate. Methylene chloride boils at 40-41^{o}C and can be removed by heating without the loss of the other volatile components, (benzaldehyde B.P. = 178.1^{o}C).

The mass spectrum was run with an AEI MS902 mass spectrometer, with an ionising energy of 70 e.v. The sample was vapouised externally and admitted to the ion source at 100^{o}C. Mass measurements were made at a resolving power of 12,000.

Results:-

(A) Evidence from G.L.C.

Analysis of the Hair pencil extract showed the presence of a peak, the behaviour of which was consistent with an identification with benzaldehyde. On three different columns, the unknown and standard benzaldehyde possessed similar retention times. (see fig 24)

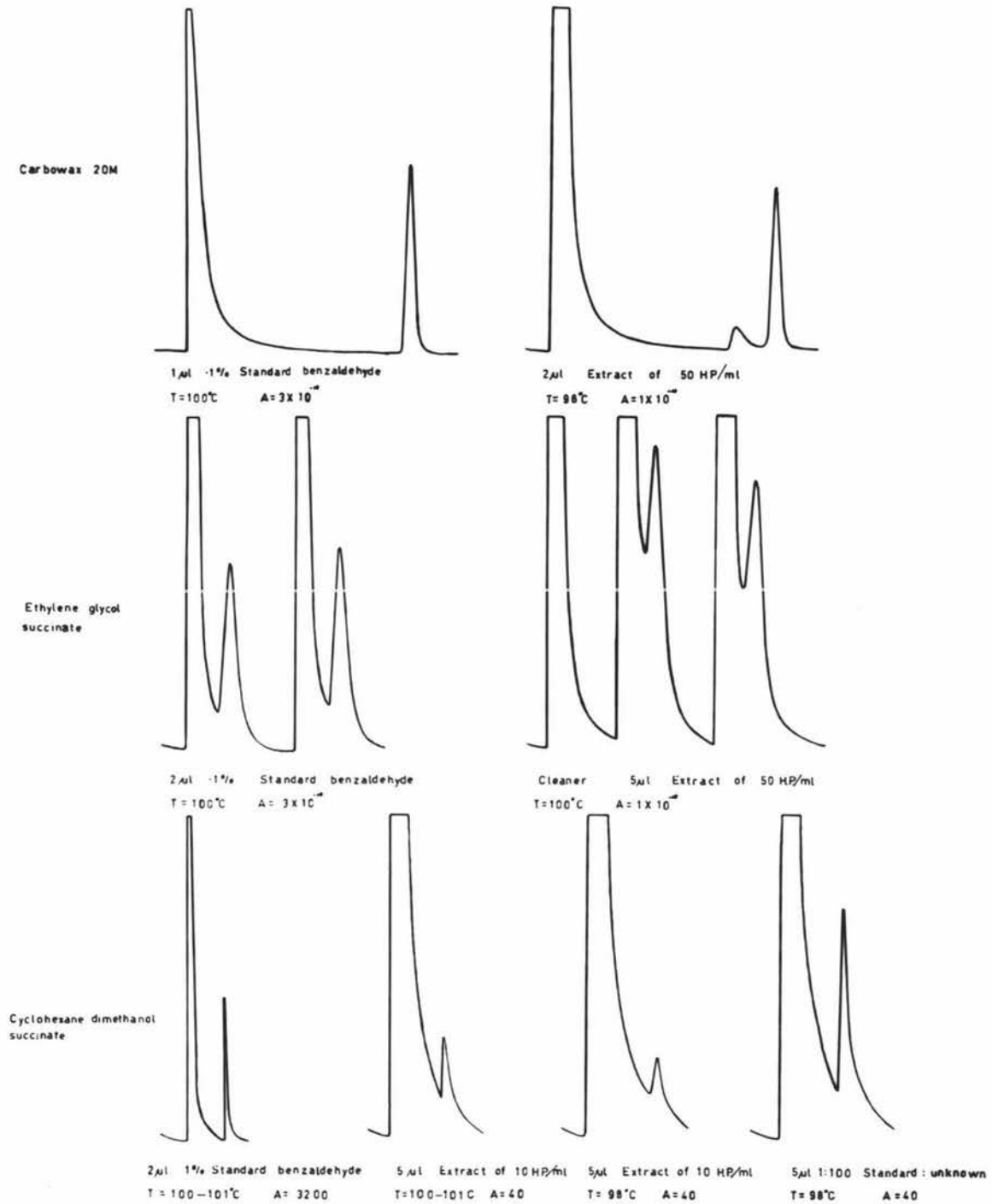


FIG 24 THE IDENTIFICATION OF THE MALE PHEROMONE WITH GLC METHODS

Column	Temperature	Unknown	Standard Benzaldehyde
Carbowax	100°C	91mm 90mm	91mm
"	96°C	92mm 92mm	
Ethylene glycol succinate	100°C	16mm 17mm	18mm 18mm
Cyclo hexane dimethanol succinate	100–101°C*	22mm	15mm
	98°C	27mm 29mm	26mm.

* Unstable temperatures resulted in a considerable disparity between test and standard.

The two ester columns did not achieve as great a separation as the more polar carbowax column. Addition of standard benzaldehyde to an extract which was then run on the cyclo-hexane dimethanol succinate column confirmed the fact that standard and unknown had the same retention time, as complete superimposition of the peaks occurred (See fig 24). In addition to the major peak, a smaller peak was observed, only on the carbowax column, with a retention of 75mm. This peak was not identified.

No noticeable peak was observed in extracts of the Stobbes gland, dissected from males under water. Benzaldehyde is only slightly soluble in water, but it was thought possible that much of the compound could have gone into solution during dissection. A further extract was made from glands dissected dry but again no peak was observed.

(B) Evidence from Mass Spectrometry

Due probably to the minute amounts coming off the column (1 ug) adequate peaks could not be shown in the mass spectrum of the material collected from the G.L.C. column. The dry ice trap was developed for the collection of phytoecdysones, compounds of lower volatility than benzaldehyde. Consequently the efficiency of the trap may not be great enough for this study.

The spectrum from the second method, though exhibiting very heavy solvent peaks from 82-88 also showed large peaks at 77 105 and 106 (see fig 25) that are typical of the disintegration pattern of benzaldehyde M^{c}Collum and Meyerson (1963).

Compound Composition	Table Value	Experimental Value
(C_6H_5) +	77.0391	77.0391
$C_6H_5C = O+$	105.0340	105.0339
$(C_6H_5\ CHO)+$	106.0419	106.0414

The disintegration pattern is initial loss of hydrogen, a typical reaction of aldehydes, followed by the loss of uncharged and hence undetectable carbox monoxide. It was of interest that no peak at 122 (benzoic acid) was observed.

Conclusions:-

The preceding evidence conclusively proves that benzaldehyde is an extractable component in the hair pencil of Pseudaletia separata. This is the second report of benzaldehyde from the Noctuidae, Aplin and Birch (1968) demonstrating its presence in Leucania impura, L. conigera and Phlogophora meticulosa. The only other pheromone apparently possessing arrestant

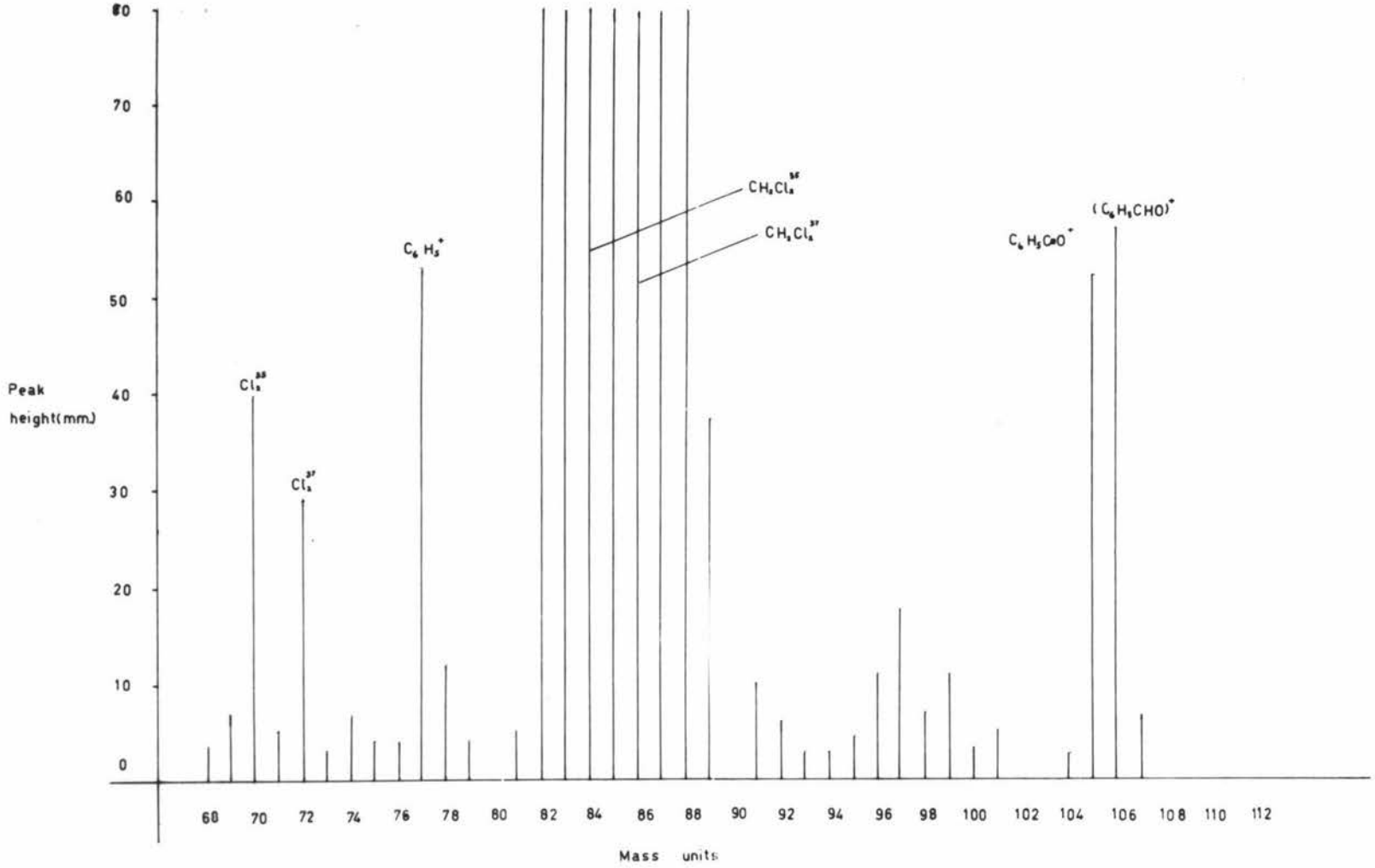
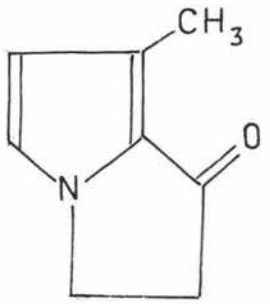


FIG 25 THE IDENTIFICATION OF THE MALE PHEROMONE WITH MASS SPECTROMETRY

properties is that of Danaus gilippus berenice Meinwald Meinwald and Mazzocchi (1969).



2-3 Dihydro 7 methyl 1H. pyrolizin-1-one.

Like benzaldehyde this compound is cyclic and possess a carbonyl group. With molecular weights of 106 and 135 these two compounds are comparable to ant alarm pheromones, rather than the female produced pheromones which have molecular weights above 200. Wilson and Bossert (1963).

There are at least three possible metabolic origins for benzaldehyde in Pseudaletia separata. Insects are unable to synthesise the conjugated benzene ring, consequently phenylalanine is an essential component of the diet Gilmour (1965). Modification of one of the intermediates, from the pattway producing the quinones essential for sclerotin production may produce this pheromone. Alternatively the food ingested during the larval stage may contain molecules that are modified to form benzaldehyde. It is also possible that some other aromatic derivative may be elabourated by the Stobbes gland.

The N-acetyl dopamine quinone that stabilises the procuticle protein in Calliphora, and probably other insects, is produced by the hydroxylation and decarboxylation of phenylalanine, (see fig 26), Gilmour (1965). Deaminated derivatives are also produced by this pathway. The modified epidermal cells (trichogen cells) which produce scales, are capable of secreting sclerotin Wigglesworth (1965). As Stobbes gland cells are highly modified epidermal cells, it is likely that these gland cells possess some of the enzyme complement shown to produce quinones. It is however difficult to suggest where the benzaldehyde producing sequence could link with the quinone pathway. Many carabid and tenebrionid beetles and a few cockroachs and earwigs have developed defense mechanisms based on quinones. Benzaldehyde and o hydroxy benzaldehyde are found in a few species. These quinones are not identical to the tanning quinones (different location of the hydroxy residues) but they do demonstrate how products of an established pathway can be modified for a completely different purpose.

A more attractive suggestion is that benzaldehyde is derived from cinnamic acid in the diet. The vascular plants produce lignin, a water resistant coating on tracheids and vessels. The units of this polymer are derived from phenylalanine, itself the product of the Shikimic acid pathway from carbohydrate Neish (1965) (see fig 27). Not only is cinnamic acid a possible precursor of benzaldehyde but vanillin might be similarly produced from ferulic acid in Erana graminosa.

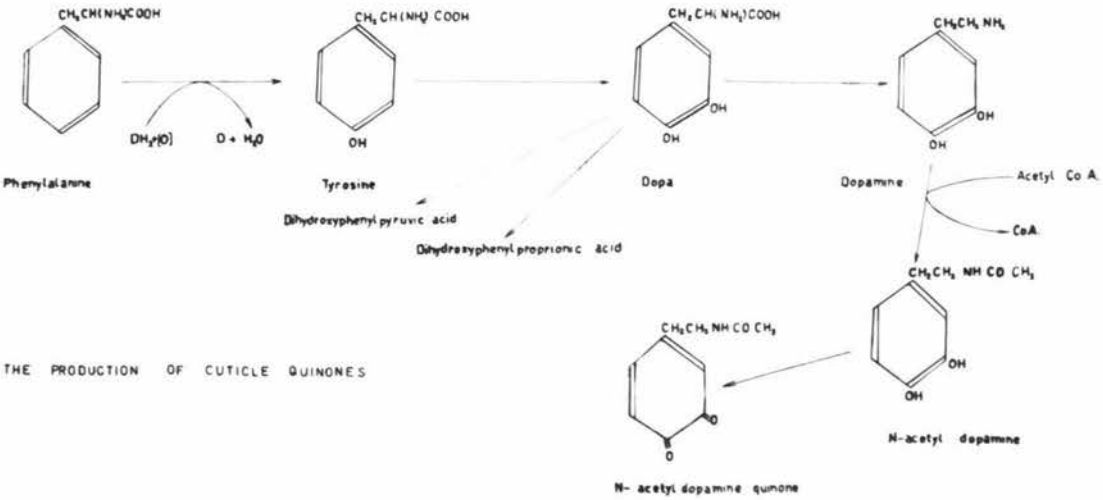


FIG 26 THE PRODUCTION OF CUTICLE QUINONES

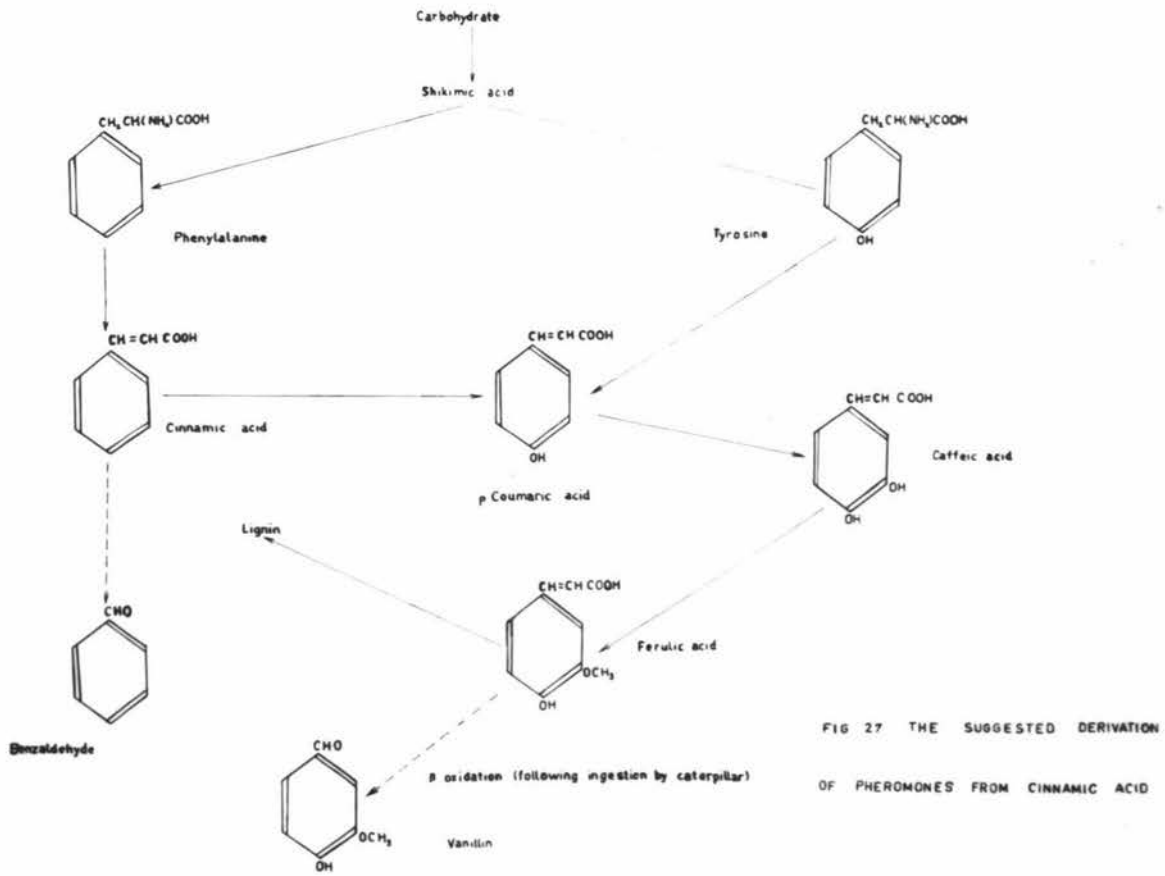


FIG 27 THE SUGGESTED DERIVATION OF PHEROMONES FROM CINNAMIC ACID

In wheat, Triticum vulgare, vanillic acid is produced from ferulic acid by B oxidation Swain (1965) and a similar production of benzoic acid might be likely. Both P hydroxy benzaldehyde and vanillin may be extracted from monocotyledons Neish (1965) by alkaline nitrobenzene oxidation. The suggestion that insects produce a secretion by modifying diet components has several preceedents. Several beetle larva were believed to produce a defensive secretion O-hydroxybenzaldehyde from salicin (the B glucoside) present in the diet of willow leaves. Gilmour (1965) Trans verbenol, one of the male pheromones produced by Ips confusus may be a derivative of the terpene alcohols found in the diet of ponderosa pine Silverstain Rodin and Wood (1966). Johnston Law and Weaver (1965) have produced experimental evidence that precursors of 9 Keto 2 decenoic acid (the worker ovary inhibitor) are fed to the queen of Apis Mellifera by the workers.

The Physiological Cycle of Gland Development and Pheromone Production.

Much work has been done on the physiological cycle of the corpus allatum Legay (1950) Novāk (1954) Lüscher and Engelman (1960) and the variation in the haemolymph concentration of the hormone produced by this gland Wigglesworth (1936, 1940). In this study, the physiological relationship between Stobbes gland and the pheromone present in the hairpencil were studied using the methods of the above workers.

A histological evaluation of the physiological activity of the gland appeared the best approach. Inactive glands are characterised by reduced cytoplasm, a low ratio of cytoplasm to nucleii, indistinct cell boundaries and less basiophilic cytoplasm. In contrast, an active gland has large swollen cells, with basiophilic cytoplasm and a high nucleus to cytoplasm ratio. This ratio is claimed to be a very reliable index of gland activity, its major disadvantage being the time required to make the preparation Novāk (1965). Gland volume alone may also indicate the amount of secretion. This method is considered less reliable as instances are reported where the volume of the corpus allatum increased (presumably by cell division) without corresponding changes in secretion.

Methods:-A previously reported index was used to evaluate the gland volume, and a simple and precise method developed to obtain the number of nucleii.

Many methods of measuring gland volume have been reported. Legay (1950) considered the average gland diameter, while Pflugfelder (1948) prepared models of the glands and measured their water displacement. Because the Stobbes gland is not always spherical, and the model method, though very accurate was considered overly time consuming, the method of Novāk (1954) was used. This worker measured the square root of two linear dimensions.

Workers studying the corpus allatum have cut sections determined the density of the nucleii, and from this calculated the number of the nucleii. Because of the large cell size and small cell number of the Stobbes gland, it was practical to measure the absolute number of the nucleii with a squash preparation.

Male moths from the stock cultures were maintained in cartons and provided with 10% sucrose solutions till they had attained the required age. The average time of emergence was taken to be 12:00 a.m. The gland was removed from the males, placed in Ephrussi and Beadle (1936) ringer, and measured with an Olympus micrometer eyepiece at 40x. Glycerol was initially used as a mountant but plasmolysis was rapid and consequently accurate measurement of the natural volume impossible. In ringer minor shrinkage still occurred. A measurement of 544 decreased to 512 after 15 minutes. Usually measurement was completed within one to two minutes. After measuring the gland was placed in a drop of acetocarmine, squashed, and left for five minutes before counting the nucleii. After staining, the nucleii appeared bright red, while the cytoplasm remained colourless (see fig 28) and accurate counts of the nucleii were easy to obtain. The

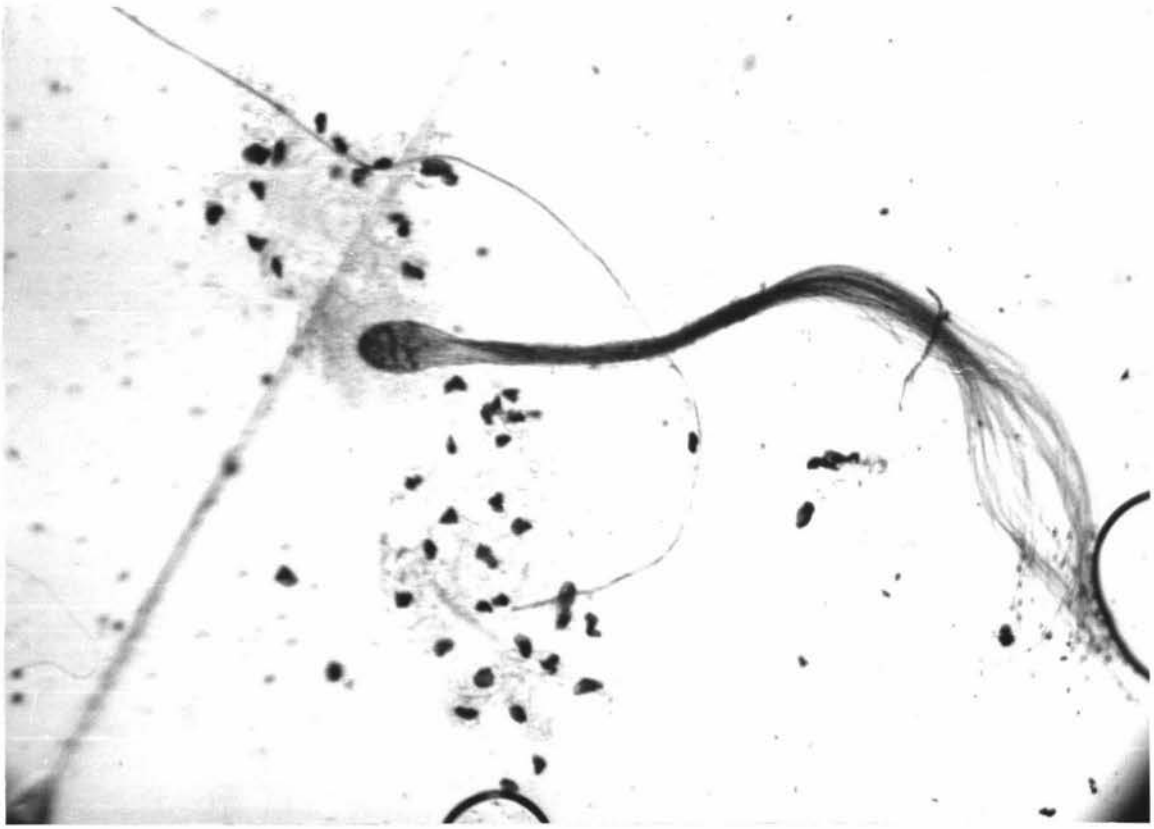


Fig.28. The Use of an Acetocarmine Squash to Determine the Nucleus Number. X 40

enormous translucent cells known as giant cells were also counted.

The G.L.C. methods used to identify benzaldehyde as a pheromone were used to evaluate the variation in benzaldehyde concentration with time. The hairpencils of the moths from which the glands had been dissected were placed in methylene chloride and analysed on a Carbowax 20M column at 110°C.

Results:-The gland appeared to go through three phases (see fig 29). From emergence till 1.0 days, the gland had a volume between 900 to 1100 NI and a cytoplasm / nucleus ratio greater than 12.00 : 1. Giant cells were typical of, and almost completely restricted to this phase. Loss of these giant cells signaled the beginning of the second phase. During this phase, which lasted approximately one day, the gland had a cytoplasm / nucleus ration of 8.00 :1 to 12.00:1, and a volume of 450 - 900 NI. The third and final steep drop in volume occurred between day 2.5 and 3.5. By the end of this decline, the disintegration of the nucleii and cell membranes was almost complete. The gland, now a formless mass, showed no further changes. The frequency distribution of the volume of all the glands measured revealed three distinct groups corresponding to the three phases postulated (see fig 30). Though all glands of the same age were not always in the same phase, the experimental population was reasonsably synchronous .

The development of the maximum benzaldehyde concentrations in the hairpencils occurred on day 2.5, toward the end of the second phase of the Stobbes gland activity. After very low concentrations immediately following emergence, the amount of benzaldehyde increased rapidly to a peak, after which a slow

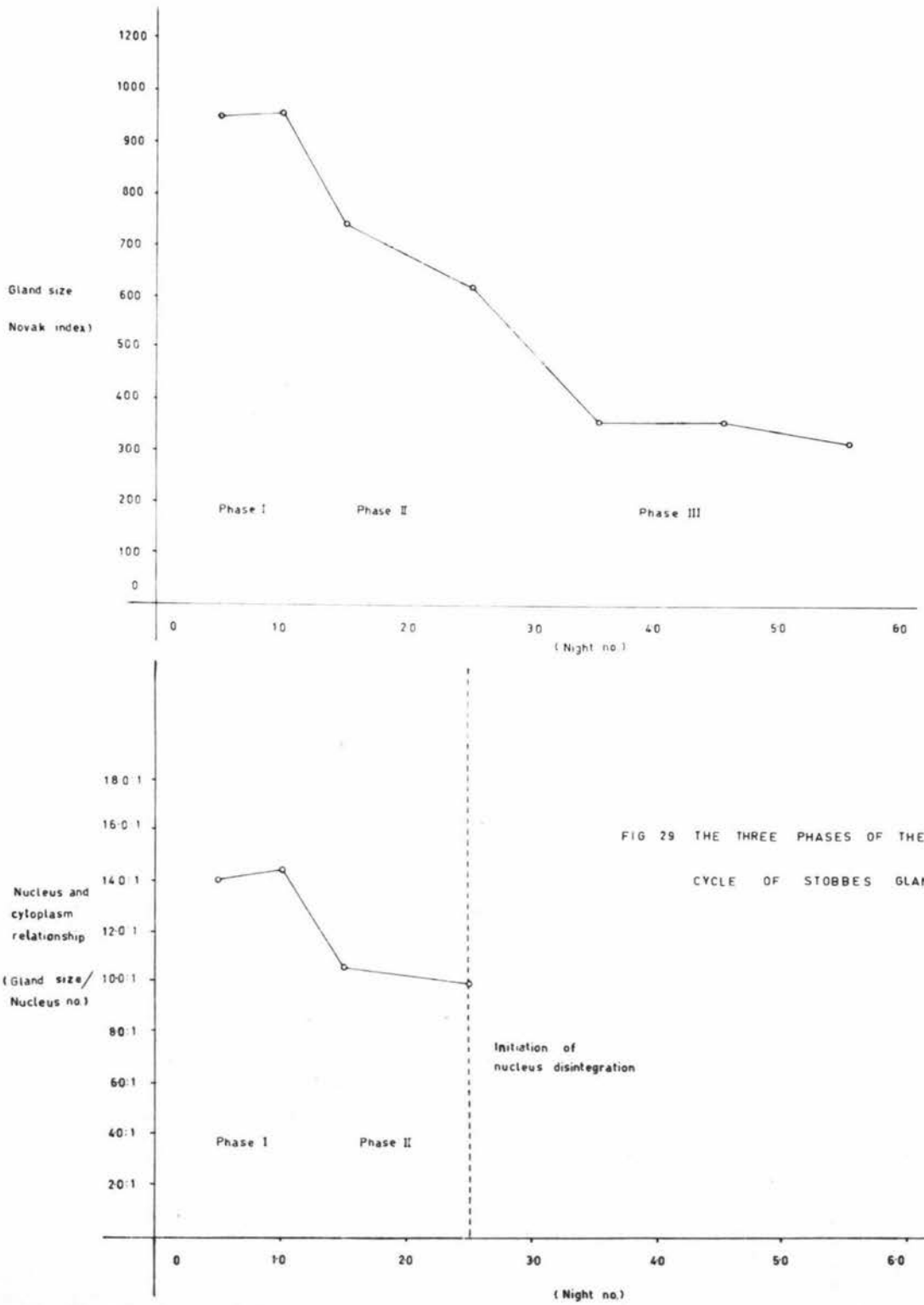
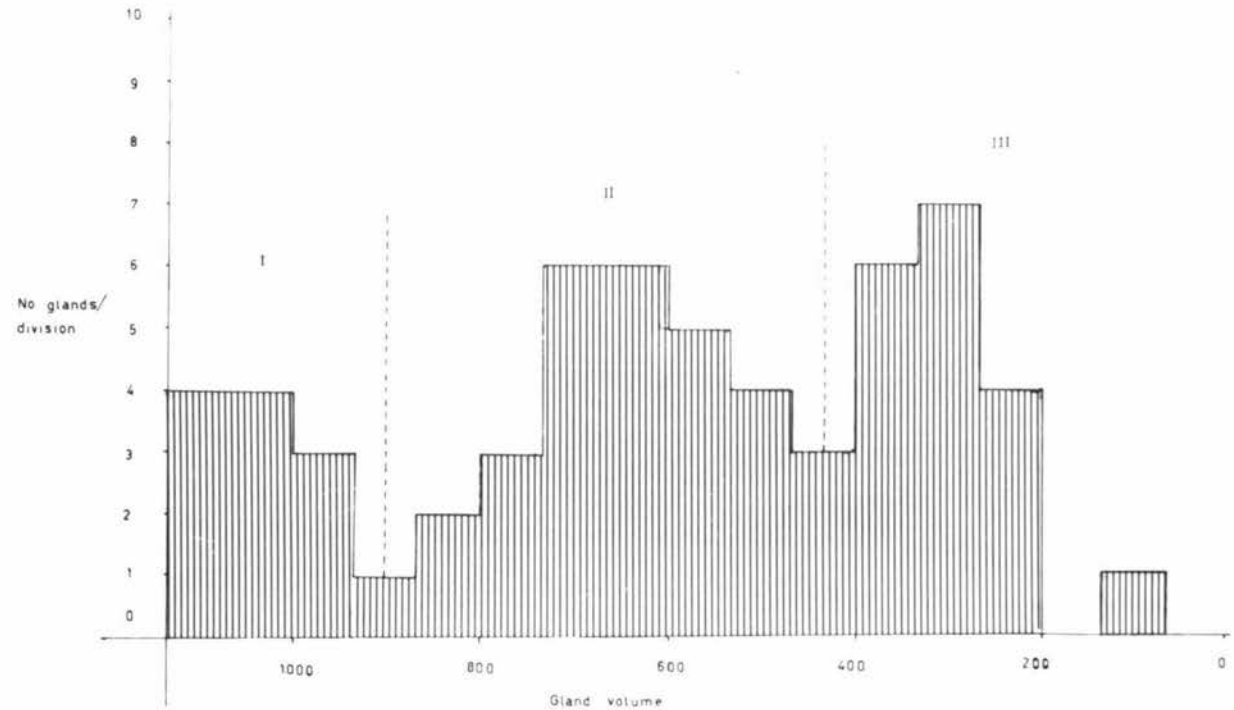


FIG 29 THE THREE PHASES OF THE SECRETORY CYCLE OF STOBBES GLAND



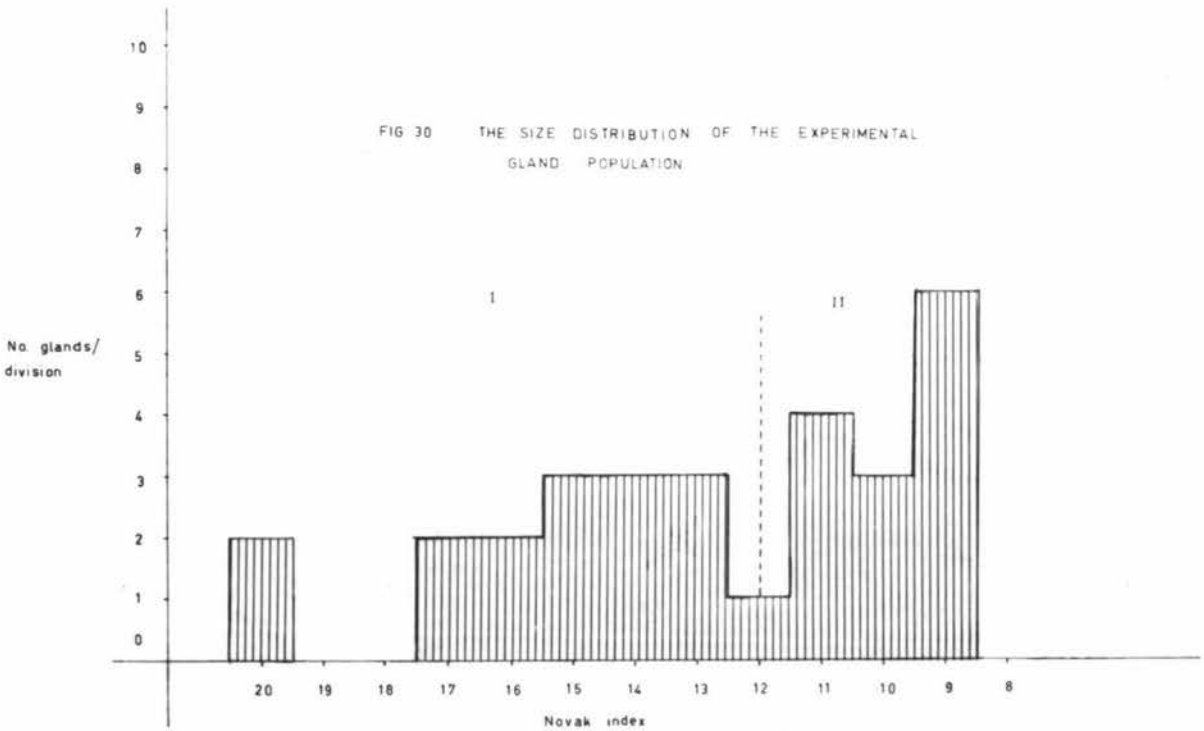


FIG 30 THE SIZE DISTRIBUTION OF THE EXPERIMENTAL GLAND POPULATION

decline was aobserved. Even at the end of imaginal activity, males retained 50% of the pheromone that was observed on day 2.5. (see fig 31).

Conclusions:-The three phases of the Stobbes gland appear to have functional signifigance. It is suggested that the large swollen gland, following emergence represents an active secretory phase. After a period of inactivity (phase II) the glands disintegrate (phase III). This interpretation is supported by the demonstration of the presence of large amounts of RNA in glands 0.5. days old, while the only nucleic acid present in amounts detectable by the fluorescent method in glands 1.5 days old is DNA (see fig 17 and 18).

If benzaldehyde is the male pheromone of this species it would be reasonable to expect a relationship between the mating frequency and the appearance of benzaldehyde in the hairpencils. Examination of fig 31 reveals that the male has a maximum concentration of benzaldehyde on the day before the third night. It is highly signifigant that overt sexual activity in the male is first observed on the third night. The form of the mating frequency and benzaldehyde development graphs is similar, both rapidly reaching a peak followed by a gradual decrease. Shorey and Gaston (1965) demonstrated that females of Trichoplusia ni first attain the maximum of 1 ug of pheromone on the second night following emergence, while the maximum mating frequency occurs approximately 24 hours later Shorey (1964) Shorey M^{c}Farland and Gaston (1968). These workers also report that there is no great decrease in pheromone concentration with increasing age, and suggest that this is due to the fact that T. ni mates more than once during its lifetime. In contrast the

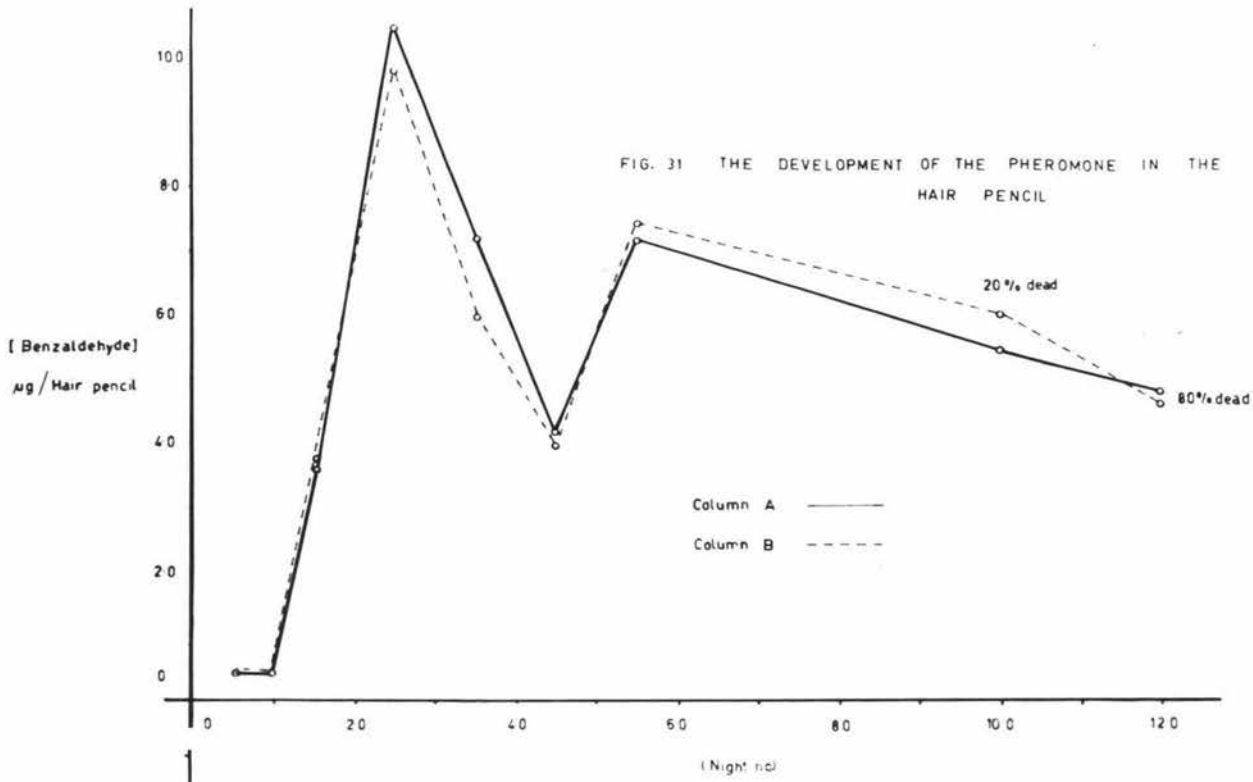


FIG. 31 THE DEVELOPMENT OF THE PHEROMONE IN THE HAIR PENCIL

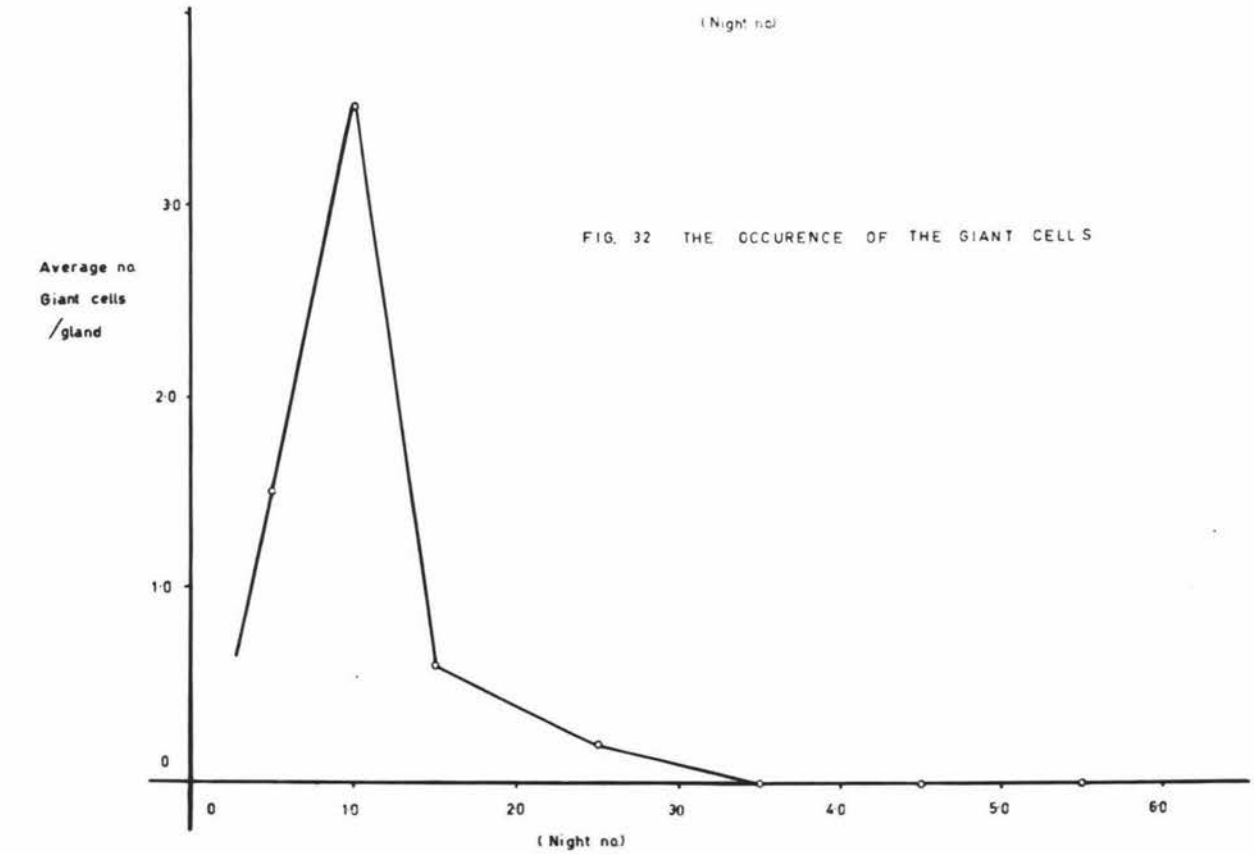


FIG. 32 THE OCCURENCE OF THE GIANT CELLS

quantity of the pheromone decreased rapidly after the peak in Bombyx mori and Porthetria dispar as these two species mate only once and a continuing high pheromone level would have no biological signifigance Collin and Potts (1932) Karlson and Butenandl (1959). Male pheromone production in P. separata resembles that of T. ni.

Two facts suggest that the product of the Stobbes gland is likely to be a precursor rather than the pheromone itself. There is a considerable temporal gap between the secretory phase of the gland and the maximum pheromone concentration. Also no detectable amounts of benzaldehyde are present in the gland. (see previous section).

The male pheromones of the Noctuidae appear to be present in much greater amounts than comparable female produced pheromones. As the hairpencil is a paired organ, a male P. separata has 20 ug of benzaldehyde available. Aplin and Birch (1968) give the ccomparable value of 10 ug for Leucania impura. For comparison, female T. ni secrete 1 ug Gaston and Shorey (1965) and 1 ug is also the value given for Bombyx mori Schneider (1966).

The major source of error in this experiment would be underestimation of the amount of benzaldehyde, due to losses by evapouration following diesection. Losses due to formation of benzoic acid are unlikely, as the mass spectrum of similar extracts did not reveal the presence of this compound.

Table III Activity Cycle of Stobbes Gland

Day 0.5

Moth No.	Wt (gm)	Stobbes G. (1)	Stobbes G. (b)	Novak Index	Nucleus No	Nuc/Cyt.	Large Cell/No	HP. position.
1	.1777	924 u	850 u	886	66	13.42:1	0	In pouch
		1018 u	1095 u	1056	76	13.89:1	0	In pouch
2	.1490	1146 u	1012 u	1077	67	16.07:1	3	Folded
		1146 u	1067 u	1106	68	16.26:1	0	Folded
3	.1585	1017 u	933 u	974	67	14.54:1	6	In pouch
		843 u	821 u	832	65	12.80:1	0	In pouch
4	.1746	1159 u	843 u	988	67	14.74:1	3	In pouch
		707 u	643 u	674	55	12.25:1	0	In pouch
5								
Mean	.1650			949	66	14.25:1	1.5	

Day 1.0

Moth No.	Wt (gm)	Stobbes G (1)	Stobbes G (b)	Novak Index	Nucleus No	Nuc/Cyt.	Large Cell No	HP position
1	.1476	1133 u	963 u	1045	68	15.36:1	8	Folded
		*					6	Folded
2	.1339	1646 u	624 u	1013	72	14.08:1	3	In pouch
		858 u	658 u	751	58	12.95:1	1	In pouch
3	.1619	886 u	682 u	777	69	11.27:1	0	In pouch
		1557 u	1280 u	1412	71	19.88:1	3	In pouch
4	.1621	890 u	765 u	825	77	10.72:1	1	In pouch
		630 u	835 u	725	75	9.67:1	2	In pouch
5	.1535	1029 u	1135 u	1081	55	19:65:1	6	In pouch
		1140 u	931 u	1030	61	16.89:1	5	In pouch
Mean	.1518			962	67	14.50:1	3.5	

* Lost in dissection.

Day 1.5

Moth No.	Wt (gm)	Stobbes G (l)	Stobbes G (b)	Novak Index	Nucleus No	Nuc/Cyt.	Large Cell No	HP position
1	.1437	1213 u	942 u	1069	64	16.70:1	4	folded
		920 u	1014 u	966	67	14.42:1	2	folded
2	.1385	751 u	674 u	711	80	8.89:1	0	In pouch
		700 u	668 u	684	76	9.00:1	0	In pouch
3	.1432	666 u	541 u	600	66	9.09:1	1	In pouch
		672 u	648 u	660	66	10.00:1	0	In pouch
4								
5	.1436	670 u	678 u	674	76	8.87:1	0	In pouch
		614 u	717 u	664	73	9.10:1	0	In pouch
Mean	.1423			754	71	10.76:1	.6	

Day 2.5

Moth No.	Wt (gm)	Stobbes G (1)	Stobbes G (b)	Novak Index	Nucleus No	Nuc/Cyt.	Large Cell No	HP position
1	.1486	593 u	582 u	587	35	–	0	In pouch
		661 u	612 u	636	38	–	1	In pouch
2	.1262	518 u	751 u	634	59 (v. clear)	10.75:1	0	In pouch
		590 u	522 u	555	61 (v. clear)	9.10:1	0	In pouch
3	.1244	544 u	653 u	596	20*	–	0	In pouch
		486 u	877 u	653	10*	–	0	In pouch
4	.1223	852 u	648 u	743	68 (v. clear)	10.93:1	0	In pouch
		550 u	636 u	591	60 (v. clear)	9.85:1	1	In pouch
5	.1476	648 u	529 u	585	26*	–	0	In pouch
		614 u	672 u	642	23*	–	0	In pouch
Mean	.1338			622	40	10.16	.2	

* Portions of disintegrating Nucleus Visible.

Day 3.5

Moth No.	Wt (gm)	Stobbes G (1)	Stobbes G (b)	Novak Index	Nucleus No.	Nuc/Cyt.	Large Cell No	HP position
1	.1211	345 ⋀	312 ⋀	328		–	0	In pouch
		88 ⋀	118 ⋀	102		–	0	In pouch
2	.1197	520 ⋀	445 ⋀	481		–	0	In pouch
		280 ⋀	347 ⋀	312	7 +	–	0	In pouch
3	.1447	447 ⋀	357 ⋀	399		–	0	In pouch
		437 ⋀	432 ⋀	434	3 +	–	0	In pouch
4	.1364	492 ⋀	385 ⋀	435		–	0	In pouch
		265 ⋀	203 ⋀	232		–	0	In pouch
5	.1041	488 ⋀	454 ⋀	471		–	0	In pouch
		608 ⋀	402 ⋀	494		–	0	In pouch
Mean	.1252			369			0	

Day 4.5

Moth No	Wt (gm)	Stobbes G (l)	Stobbes G (b)	Novak Index	Nucleus No	Nuc/Cyt.	Large Cell No	HP position
1	.1423	381 u	398 u	389		-	0	In pouch
		454 u	304 u	372		-	0	In pouch
2	.1415	372 u	276 u	320		-	0	In pouch
		* -	-	-	-	-	-	In pouch
3	.1166	319 u	332 u	325		-	0	In pouch
		* -	-	-	-	-	-	In pouch
4	.1164	300 u	306 u	303		-	0	In pouch
		520 u	449 u	483		-	0	In pouch
5								
Mean	.1292			365	0		0	

Day 5.5

Moth No.	Wt (gm)	Stobbes G (1)	Stobbes G (b)	Novak Index	Nucleus No	Nuc/Cyt.	Large Cell No	HP position
1	.1354	475 u	341 u	402		–	0	In pouch
		347 u	196 u	261		–	0	In pouch
2	.1494	356 u	317 u	380		–	0	In pouch
		392 u	277 u	330		–	0	In pouch
3	.1583	383 u	298 u	338		–	0	In pouch
		*						
4	.1403	264 u	158 u	204		–	0	In pouch
		266 u	213 u	238		–	0	In pouch
5	.1457	443 u	305 u	368		–	0	In pouch
		343 u	288 u	314		–	0	In pouch
Mean	.1458			315	0		0	

* Lost in dissection.

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Comparitive Biochemistry and Morphology of the Male Pheromone System in the Noctuidae.

A noctuid phylogeny based on variations in the chemistry and structure of this system would be at variance with the classification normally accepted. The male pheromone system of this family is highly variable, several different structures being found on the anterior and posterior abdomen, tibia or on the wing. Though many of these type of structures have been described in the literature Dickens (1936) Barth (1937052) there does not seem to have been any attempt to place the Nocutid organs in any sort of evolutionary sequence.

Methods:-Noctuid species available at Massey University and at Pohangina Reserve were captured with a light trap, and the structure of the secretory organ, chemistry of the pheromone and degree of pectination of the antennae measured.

A dissecting microscope was used to investigate the surface morphology and gorss internal anatomy of the secretory organ. The sternite modifications were noted on preparations cleared with KOH and stained with methylene blue.

The antennae of several specimens of most species were removed with fine scissors and kept flexible in Chauthani and Callahan (1966) fixative, till measured. The width of the 20^{th} segment was measured with an Olympus Micrometer eye piece at 20X, and the length with an eyepiece insert at 7X.

The G.L.C. methods described earlier were used to evaluate the chemistry of the compounds. A Carbowax 20M at 100° C was used for the detection of benzaldehyde and a 10% SE 30 column at 210°C for vanillin. The use of only two columns for all extracts meant that not all the major volatile components would have been detected.

Results:- The secretory structures were found on many species and varied greatly in position and type.

A common type was the hair pencil, found in eight species, (see table IV). In all cases the structure was identical, except in details, with that described for P. separata (see fig 7.) The colour of the hair scales was a light straw colour except for the distal portion in Persectania aversa, which was a light smoke grey. It was of interest that the Stobbes gland of Melanchra coeleno was a lemon yellow, a colour not observed in any other species.

Melanchra alcyone possessed a structure with clear affinities to the hair pencil (see fig 32). Sternite II had the moderately sclerotised lateral edges of a pencil bearing sternite, but lacked a lever. The numerour hair scales were inserted into a sheet of light flexible cuticle. The dense scale tract formed a tight cloak around the ventral anterior abdomenal segments at rest (see fig 33). The external positioning of this structure was in marked contrast to the hairpencils held in internal pouchs. No odour was detectable and no Stobbes gland could be found.

The Distribution of Pheromone

Table IV
Disseminating structures amongst the Noctuidae

ecies	Hair Pencil	Stobben Gland	Posterior Brush	Other	Comments
nchra ans	-	-	+	-	
nchra gnis	-	-	+	-	
nchra striga	+	+		-	... large and straw coloured.
nchra yone	+	-	+	+	post. brush in two tracts. ... completely external. Three pairs of abdominal tufts.
nchra ena	-		+	-	
nchra nana	+			-	
nchra orata	-		+	-	Post brush v. large (♂ ♀).
nchra leno	+	-	+	-	Stobbes gland small and yellow in colour
nchra causta	-	-	-	-	
nchra laca	+	+	+	-	
nchra ta	-	-	+	-	
nchra isita	-	-	+	-	

Species	Hair Pencil	Stobbes Gland	Posterior Brush	Other	Comments
rsectania aversa	+	+	+	–	H.P. a smoky grey in colour
rsectania eropastis	+		+	–	
rsectania rotis	–	–	+	–	
ucania mivittata	–	–	+	–	
seudatetia eparata	+	+	+	–	
Aletia derata	–		+	–	
rana raminosa	–	–	+	+	A trough with a small abdomenal tract and a large wing gland.
lusia halcites	–		+	+	Long hairs in lateral grooves Post. brush v. large.
hapsa cotosialis	–		+	+	Costal area of forewing holds a hairscale tract.
grotis psilon	–	–	–	–	
grotis nnominata	–	–	–	–	
raphiphora ompta	–		–	–	
eliothis unctigera	–		–	–	

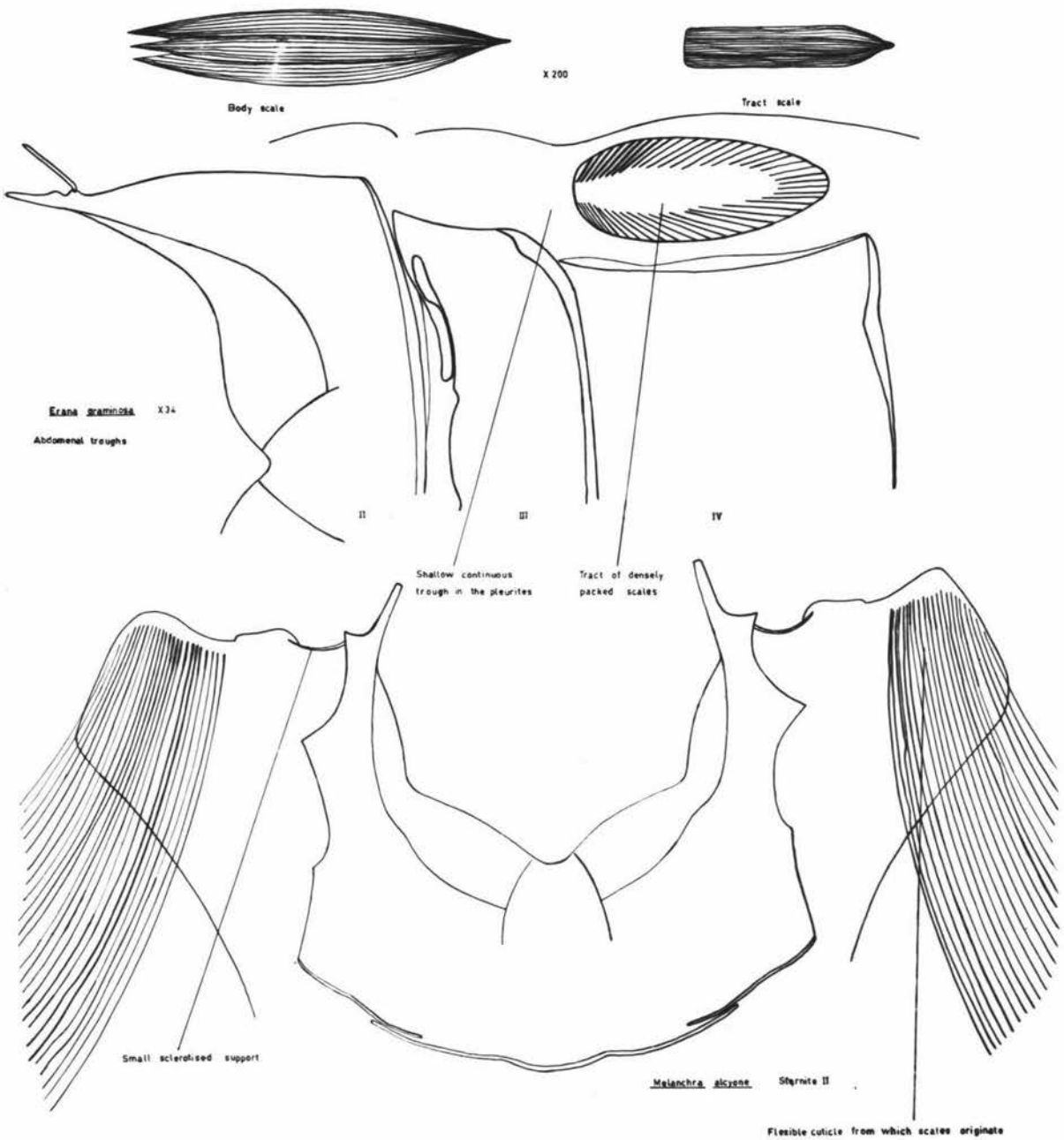


FIG. 33 SIMPLER FORMS OF SCALE TRACTS

Fig.34. The Pheromone Releasors of Melanchra alcyone and Erana gramihosa. X 7 stereo

A even simpler structure was found in the same area of Erana graminosa (see fig 32). A pair of very shallow antro-lateral troughs extending from segment II to IV had a small tract of short smoke grey scales. The remainder of the trough was devoid of scales.

The major pheromone secreting structure in this species was in the wing gland (see fig 33). The androconia of the underside of the forewing had become modified in two directions. The two tracts of long fine hair scales served as the pheromone disseminator, while the dense tract of short scales appeared to be a secretory area. This area was morphologically very similar to the trough hair tract. The very large humeral lobe of the lower wing completely covered the androconia, forming a barrier to the loss of the pheromone. A wing gland was also found in Rhapsa scotosialis.

The most widely distributed structure was the posterior abdomenal brush found in all but one species of the subfamilies Melanchrinae and Plusiinae. The structures of the former subfamily were very similar to that of P. separata though Melanchra decorata and M. alcyone possessed very large brushs 3-4 mm in diameter and divided into two tracts. The most complex brush was that of Plusia chalcites (see fig 34). The two enormous brushs were supplemented by a pair of small black brushs beside the external genitalia.

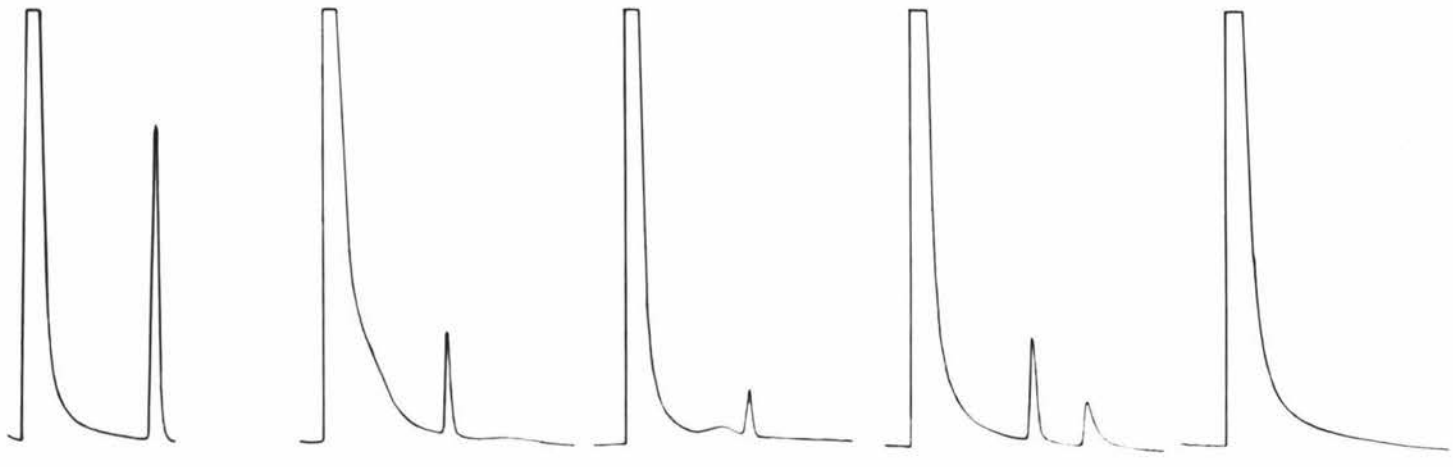
Amongst the brown hair scales on the tibia of the mesotharacic leg of Dasypodia selenophora was an evertible tract of white hair scales. This tract was in a position almost identical to

Fig.35. The Abdominal Brush of Plusia chalcites
X 7 stereo

a similar tract in the puiriri moth Charagia virescens. The hepialid structure appeared to be a pheromone secretor, as Deegener (1902) has assigned the tibial gland in Hepialus hectus, the function of attracting the female. By analogy it is suggested that the structure of D. selenophora may be assigned a similar function.

Only the chemistry of the hair pencil and wing gland secretions were investigated. Benzaldehyde was detected in the hair pencils of Pseudaletia separata, Persectania aversa, P. steropastis, Melanchra ustistriga, M. lignana and M. omoplaca, while vanillin, 3 methoxy 4 hydroxy benzaldehyde was found in the wing glands of Erana graminosa (see fig 35). With the exception of M. lignana and P. aversa other small peaks were also present. P. steropastis was noted to have a sweet odour in addition to the odour of almonds. M. coeleno possessed an acrid smell that was not chemically identified.

The antennae of the male of each species was examined in relation to the presence or absence of pheromone. All species possessing a hair pencil had filiform antennae (see table V and fig 36). M. alcyone and E. graminosa were included in this group. Of the species lacking pheromone producing structures, half had highly pectinate antennace, while half had filiform antennae comparable to the first group. Only Rhapsa scotosialis (s. fam. Plusiinae) with pectinate antennae and a wing gland, did not fit these divisions.



1 Standard Benzaldehyde 1 µl of 50 µg/ml

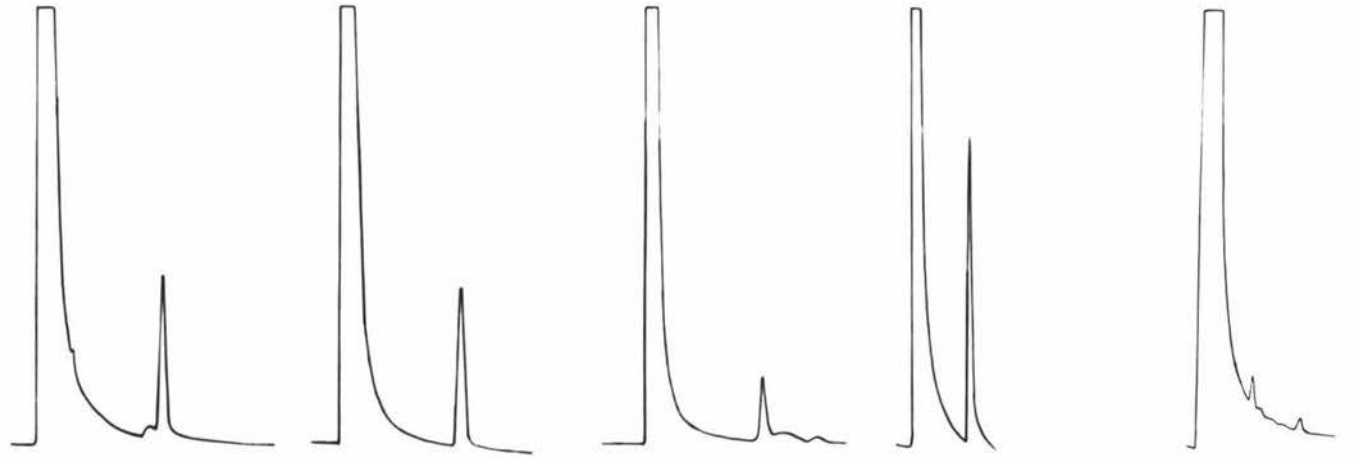
2 Melanchra lignana 5 µl of 4 HP/ml

3 Melanchra omoplaca 5 µl of 2HP/ml

4 Melanchra ustistriga 5 µl of 12HP/ml

5 Melanchra coeleno 5 µl of 2HP/ml

FIG. 36 THE CHEMISTRY OF THE MALE PHEROMONES OF THE NOCTUIDAE



6 Pseudaletia separata 5 µl of 8HP/ml

7 Persectania aversa 1 µl of 30HP/ml

8 Persectania steropastis 5 µl of 2HP/ml

9 Standard Vanillin 1 µl of 1000 µg/ml

10 Erana graminosa 5 µl of 6WG/ml

1-8 Carbowax 20M A = 1×10^{-7} T = 110° C

9-10 10% SE 30 A = 1×10^{-7} T = 210° C

Table V. Antennae Morphology

Species	Sex	No.	Width (u)	Length (mm.)	Pectination w/l	Relative Pect Factor Male/Female
Pseudaletia separata	Male	16	173.8	10.79	16.10	
	Female	24	144.8	11.15	12.99	1.24
Melanchra exquista	Male	1	304.7	8.67	35.15	
Melanchra lata.	Male	1	133.0	8.00	16.63	
Melanchra omoplaca	Male	1	184.5	8.93	20.65	
	Female	1	150.2	9.20	16.32	1.27
Melanchra ustistriga	Male	25	341.0	11.41	29.88	
	Female	9	176.0	11.58	15.20	1.97
Melanchra coeleno	Male	3	164.4	9.33	17.62	
	Female	2	139.5	9.07	15.38	1.15
Melanchra insignis	Male	21	578.9	10.55	54.87	
	Female	12	156.3	9.88	15.81	3.47
Melanchra mutans	Male	31	554.9	10.05	55.25	
	Female	17	184.0	10.20	18.04	3.06
Melanchra lignana	Male	20	183.2	10.40	17.62	
	Female	16	154.7	9.95	15.52	1.14
Melanchra alcyone	Male	1	201.7	11.87	16.99	
Melanchra paracausta.	Male	4	888.4	9.17	96.87	
Persectania aversa	Male	21	278.9	9.86	28.28	
	Female	15	162.8	9.52	17.02	1.66
Persectania steropastis	Male	1	197.4	11.60	17.02	
Persectania arotis	Male	4	229.6	10.86	21.13	
	Female	4	168.5	10.70	15.74	1.34

Species	Sex	No.	Width (u)	Length (mm.)	Pectination w/l	Relative Pect Factor Male / Female
Erana graminosa	Male	2	180.2	10.33	17.45	
Leucania semivittata	Male	4	217.6	9.63	22.60	1.23
	Female	3	171.7	9.37	18.32	
Aletia moderata	Male	3	260.5	10.09	23.90	1.31
	Female	3	186.0	10.22	18.20	
Agrotis ypsilon	Male	17	624.8	12.86	48.59	3.34
	Female	5	179.4	12.32	14.56	
Agrotis innominata	Male	3	555.4	11.15	49.82	
Graphiphora compta	Male	2	199.5	9.27	21.52	
Heliothis punctigera	Male	1	137.3	8.70	15.61	
Rhapsa scotosialis	Male	5	1648.0	9.28	177.60	11.63
	Female	1	120.1	7.87	15.26	
Plusia chalcites	Male	1	103.0	11.99	8.59	

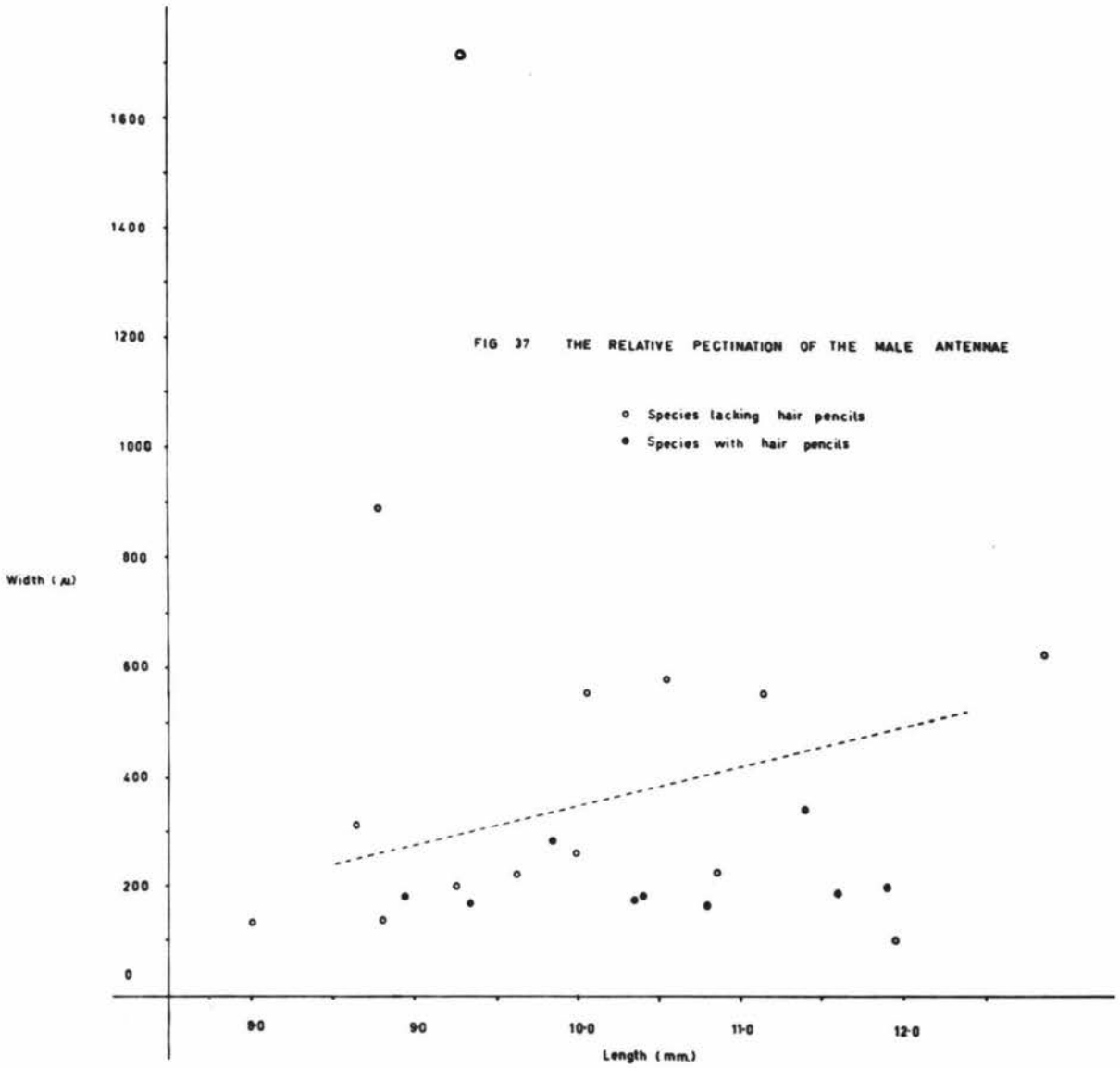


FIG 37 THE RELATIVE PECTINATION OF THE MALE ANTENNAE

Ichneutica ceraunias
Pectinate antennae

Pseudaletia separata
Filiform antennae

Fig.38. The Antennae of Two Noctuid Species
X 7 stereo

Conclusions:-The structural variety of the pheromone system suggests that several convergent evolutionary lines are present, while the wide distribution of benzaldehyde and its derivative and the universal use of modified scales indicates a common origin.

The simplest pheromone producers reported are androconia, slender brush tipped scales on the upper wing surface of Satyrus semele (fam. Nymphalidae). The completely exposed androconia play an important role in the courtship of this species Tinbergen (1958). Metabolically it would be more economic to produce a protected structure that would not be continuously losing its pheromone by evapouration. If the hair tract in the abdomenal trough of E. graminosa is a secretory area, it illustrates the postulated modification admirably. The air enclosed by the trough would become saturated with any released compound, preventing further evapouration till opened. The great development of the wing gland in this species may have allowed the trough to retain a primitive morphology. The posterior brush of the Melanchrinae is protected by retraction into the intersegmental fold, and the wing gland by the close apposition of the humeral lobe. Several Danaid species (Nymphalidae) protect the pheromone secretors by retracting them into the intersegmental fold. Deepening of such a trough might have produced the hair pouches of P. separata.

As the rate of evapouration of any compound is proportional to the exposed surface area, the development of high release rates requires a further modification. The long hair scales found in all the structures with a chemically defined pheromone

would provide such a surface. These elongated scales may have developed by the lengthening of androconia. In this case M. alcyone may provide an illustration. (As most pheromones have high vapour pressures Wilson and Bossert (1963) an unprotected dense tract could lose pheromone at a high rate. Consequently this structure of M. alcyone may have a non - pheromonal function e.g., as a visual cue).

Dichonia aprilina, described by Stobbe (1912) may illustrate a further step in the evolutionary sequence - an efficient secretory structure, the Stobbes gland, separate from the releasing area. Like M. alcyone, D. aprilina lacks a lever. The acquistion of this lever and the insertion plate would brace the segment allowing a more effective eversion of the large hair scales. The fact that eight species possess this structure suggests that it is an efficient device.

The presence of benzaldehyde in the hairpencil of several species of noctuid makes the species specificity of the signal questionable. In addition ot the moths in this study Aplin and Birch (1968) have detected benzaldehyde in the hairpencil of Leucania impura L. conigera, Phlogophora meticulosa and possibly L. pallens making a total of nine to ten species. The detection of additional peaks during the chromatographic examination of the extracts suggests that some olfactory discrimination is possible. Benzaldehyde may have been an original secretion to which has been added potential new pheromones as the speciating populations diverged. The production of vanillin indicates that the benzaldehyde molecule

itself may have been modified in the course of the separation of Erana graminosa from the basic Noctuid stock. Pheromone chemistry can constitute a complete ethological isolating mechanism e.g. the elegant study of Roelofs and Comeau (1969) showed that males of the sibling species Bryotopha similis B_1 and B_2 react specifically to the cis and trans forms of a tetradecenyl acetate secreted only by conspecific females. More frequently males respond to pheromones from females of closely related species Schneider (1962). Present thought indicates that though not absolute, pheromones are important components of a complex of mechansisms effecting ethological isolation Wilson and Bossert (1963). For example the noctuids in this study are partially isolated by season of emergence and food plant. The biggest sample of P. separata is captured in May and June, while September and October see the maximum activity of M. ustistriga and P. aversa Spitzer (1970), P. separata feeds on graminaceous crops, P. steropastis on native flax, while the larva of M. ustistriga is largely arboreal, and M. omoplaca feeds on plantain Gaskin (1967). These habitats are varied and spatially separate.

Specialisation of the Noctuid family with one group emhasising the development of female produced pheromone while another group emphasises the male pheromone may be developing. The Saturnidae, Bombycidae, Lymantriidae and Psychidae are four

families where powerful female produced pheromones have been isolated or strongly indicated, Schneider (1962) Butenandt (1963) Jacobsen, Beroza and Jones (1960) and Sharma and Hussein (1955). Males with highly pectinate antennae are typical of all these families. By analogy it is suggested that the pectinate antennae of Melanchra mutans, M. insignis M. paracausta, Agrotis ypsilon A. innominata and Rhapsa scotosialis indicate possession of an important female pheromone. In contrast the eight species shown to have hairpencils without exception possess filiform antennae. In support of this theory it must be noted that M. paracausta the species possessing the most pectinate antennae of the Melanchrinae lacks even the simple posterior abdomenal brush, indicating that this species has moved furthest along the suggested lines of lack of male structures and development of a powerful female pheromone.

The Maintainance of the Culture.

To obtain a supply of adults of known age and mating status, a culture was maintained in a glasshouse.

Imagos obtained initially from Rukuhia Hamilton were allowed to oviposit on maize. Pseudaletia separata produces 800 to 900 eggs per female Quo Wu Tsai and Lui (1964). The site favoured is the inner leaf whorl, the first instar emerging from this area a week later. These caterpillars were fed on several food types. P. separata is polyphagous, feeding on many of the Graminae Gaskin (1967). Frozen chopped maize, and large maize leaves from a commerical crop were acceptable, though due to the high fibre content of the large leaves smaller instars had difficulty in eating this food. This food source was inconvenient, as the material only remained edible for one day before becoming too dry. Two commercial strains of maize Golden Cross Bantam and Sweet Carmel Cross were grown in potting mix. The young plants were highly acceptable, and remained edible for several weeks till completely consumed. Grass clumps were used as a supplementary food source for the last instar which consumed the major portion of the food (80% in Pseudaletia unipuncta, Davis and Sathewait (1916)). P. separata produces a brown pupa in earthern cells. Large trays filled with ½" of dry sieved soil were placed under the maize pots and appeared to provide a suitable substrate as only a few specimens pupated in the potting mix of the maize seedlings. Vermiculite (an expanded mica potting material) was unsatisfactory in an initial attempt to culture this species,

as very few specimens pupated normally in this substrate. Pupae were not harvested until most of the population had passed the delicate prepupal stage. They were assessed for viability and sexed, before being placed 20 to a waxed paper carton of damp vermiculite. Sex determination was on the basis of the incipient genital appertures (see fig 37). While sexing, it was noted that most rotated the abdomen tip when gently squeezed. Pupae failing to show this movement were discarded. Two species of parasite were found in the initial collection. An ichneumonid Amblyteles rodatorius and a tachinid Cerosomya sp caused a loss of 6% of the 186 pupae of the initial collection. No serious microbial diseases were encountered during culture. As moths emerged they are held till needed in waxed paper cartons with a crushed paper towel and fed on 10% sucrose solutions.

Caterpillars of this species show an interesting phase polymorphism if environmental conditions dictate. Crowding and starving alter the colouration, behaviour, rate of development, and resistance to starvation Iwao (1967). This phenomenon was observed in this study, but little attempt was made to differentiate between dark phase and light insects during the experiments.

Temperature control was attempted. Cooling was achieved by a combination of the effects of two large fans expelling air, and a water cooled blanket over the glass-house roof. Hot water pipes controlled by a thermostat gave the heating. Control was adequate with temperatures of $20^{o}C \pm 80^{o}C$ being achieved.

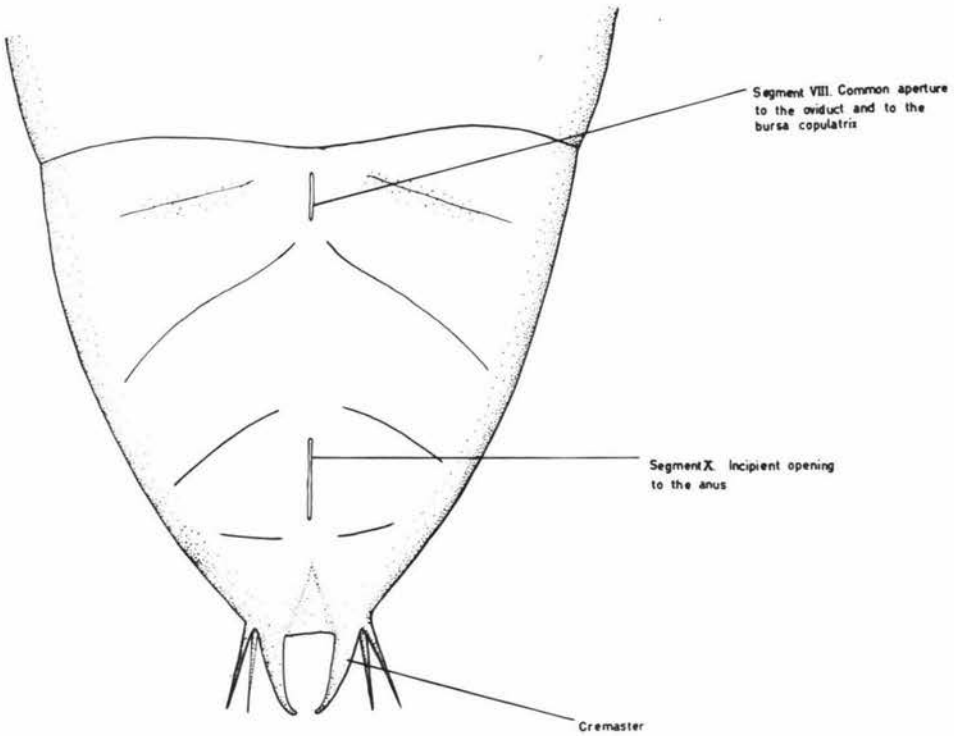
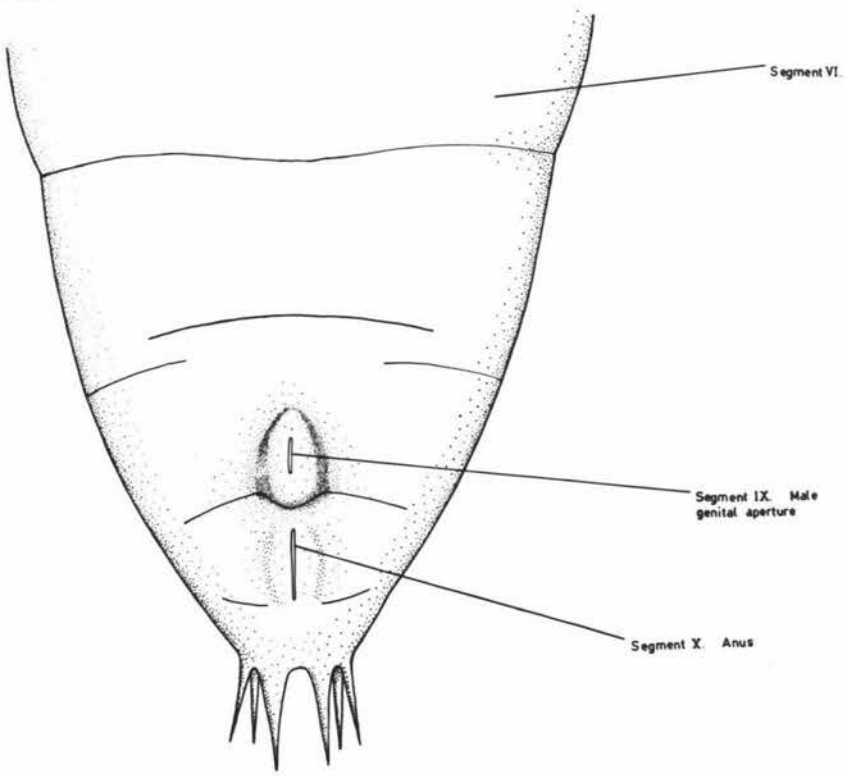


FIG. 3 INCIPIENT GENITAL APERTURES OF THE PUPAE



Determination of Mating Frequencies by Use of Fluorescent Dyes.

Vail, Howland and Henneberry (1966) working on the noctuid Trichoplusia ni (S. fam. Plusiinae) have developed a very useful technique for determining the mating frequency of experimental populations of moths. The males are fed fluorescent dyes in aqueous sucrose solution. These compounds are concentrated in the ductus ejaculatoris simplex where the spermatophore is elabourated Callahan and Chapin (1960). When mating occurs the dyed spermatophore is transferred to the bursa copulatrix of the female where it is visible as a coloured mass on dissection.

Methods:- The above technique was adapted to investigate the mating frequencies of Pseudaletia separata (s. fam Melanchrinæ). Eight different dyes were evaluated under two different conditions. Cartons were prepared with paper towels, and a wad of cotton wool placed in the lid. Two freshly emerged males from a stock culture were placed in each carton and fed a solution of 0.1% dye in 10% sucrose solution placed on the cotton wad, for two nights. Primulin, Pyromin B, Thionin, Methyl Violet, Neutral Red, Auramine and Eosin Y were fed in this initial experiment. On the day preceding the third night, the dye and sucrose solutions were replaced with solutions of 10% sucrose only and two virgin females of the same age introduced. Following the fifth night, the moths were killed and dissected.

This experiment was then replicated under conditions closely resembling those planned for the experiments on mating frequency. The glass observation cages replaced the cartons, in this evaluation. Rhodamine B solutions were fed to six males and Eosin Y to five males for two days before the introduction of virgin females of the same age. A similar period was allowed for mating before the moths were dissected.

Results:-As in T. ni the male of P. separata appeared to accumulate the dye in the ductus ejaculatoris simplex. Dye accumulation in the female appeated to be largely in the accessory gland resevoirs. Six of the dyes evaluated were transferred to the female to a varying extent. (see table VI). Considerable variation in specificity, and dye brightness rendered most of the dyes unsuitable for use.

Conclusions:-The appearance of dye in the acessory glands resevoirs was unexpected, but did not detract from its use as a mating marker. Female Lepidoptera secrete proteolytic enzymes in the bursa copulatrix, breaking down the spermatophore in order to allow the gametes to escape Davey (1964). It appears that the two or three days that the spermatophore might remain in the female before dissection were adequate to allow this breakdown. A dilute solution of Eosin Y was left on a dissected female for two days. Dyeing was non-specific, most being taken up by the fat body. This would indicate that the transport of the dye from the corpus bursae to the accessory gland resevoir took place inside the reproductive system and was not released into the haemocoele and selectively absorbed by the tissue of the resevoir. The function of the accessory

Table VI - Genital Dye Effectiveness

Dye	No Male dyed / No Male in expt.	Colour	No Female dyed / No Female in expt.	Colour	Specificity
Thionin	1/2	Light blue.	0/2	-	+ve
Methyl Violet	0/2	-	0/2	-	-
Eosin Y	3/3	Bright red to orange	2/3	Red to orange pink	+ve
Primulin	1/2	Lemon yellow	1/2	Lemon yellow	+ve
Pyronin B.	2/2	Bright lime green	1/2	faint lime green	+ve
Auramine^{o}	2/2	red. bright lime green	1/2	v. faint khaki	+ve
Neutral Red	2/2	faint pink to bright scarlet	1/2	bright red.	-ve - near testis in male and ant. abd in female as well as gentilia
Rhodamine B.	4/6	Bright scarlet	2/6	pink to bright scarlet	+ve
Eosin Y^{+}	4/4	bright rose pink	4/5	orange brown to brown	+ve

+ one male. lost.

glands is to produce egg cement Wigglesworth (1965). The above observation suggest that spermatophore material may be re-used as a component of the egg cement.

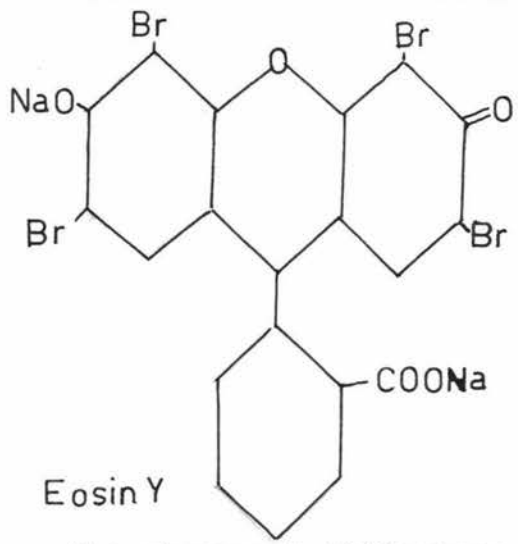
Very many factors determined the effectiveness of the dye.

The primary factor was the degree of acceptance of the dye by the male. Males were observed to feed less readily on sucrose solutions containing Rhodamine B, than on control sucrose. On the control, feeding was continuous, the proboscis remained still and intermittent movement of the antennae occurred. On the dye plus sucrose soaked cotton wad, males continually tapped with the proboscis and moved around. Eosin appeared the most acceptable. Cartons containing moths fed this dye were more stained with voided dye than any other. This greater acceptablility was also reflected in the high percentage of males dyed under these conditions.

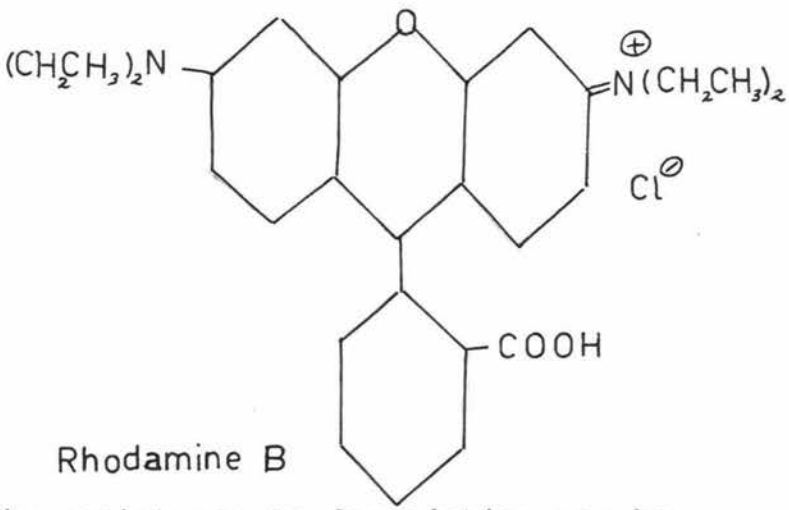
The concentration of dye in the ductus ejaculatoris simplex is a useful feature of this technique. Only neutral red is non-specific. The male insect commits a considerable portion of the body resources (40% in Ephippiger sp Busnel and Dumortier (1955)) to the production of the muco-polysaccharide and proteins of the spermatophore. This may cause the diversion of ingested food (which contains dye under the experimental conditions) to the area of spermatophore production.

Dye brightness and degradation resistance are also vital properties. In this respect Rhodamine B was excellent, in one instance, the red colour was visible through the intersegmental membranes of a female. Eosin was not so good

as the fresh dye was less colourful, and appeared to breakdown in the female, releasing products rangings in colour from salmon pink to orange browns. In their structure, Eosin Y and Rhodamine B are markedly similar.



Eosin Y



Rhodamine B

The observed difference in resistance to degradation may be due to the different side chains. Red colours generally seem to be the best, as they stand out well against the lemon yellow of the fat body. Chi Lung Ho (1964) reports that the scrotum of the testis of P. separata is crimson, but the frequent dissections in this study revealed a testis at most slightly orange yellow. There is thus little possibility of confusion. Primulin, a lemon yellow dye, was in contrast very difficult to differentiate from the fat body. As the dyes are fluorescent, doubtful cases were examined under ultraviolet light. For example Rhodamine B fluroesces orange, while the fat body, was orange green.

In these trials it was assumed that mating frequency was not affected in any quantitative way by the presence of the dye, but no attempt was made to test the validity of this assumption.

From these results, it was concluded that eosin Y was the most satisfactory dye for use in experiments with this specis.

Abstract

The majority of studies on the mating behaviour of noctuids have emphasised the role of the female produced pheromone. In contrast this study of Pseudaletia separata examines in detail the function of the male pheromone.

The mating sequence was not complex. Utilising chemical and to a lesser extent visual cues, the male located the female, and approached from below and behind. Under laboratory conditions mating took place without further preliminary, but considerable evidence is presented indicating that a pheromone may be released before the males external genitalia makes contact with the female. A major component of this pheromone was identified as benzaldehyde. This compound functions as an arrestant, preventing the general escape reaction of the female elicited by the stimulus of an approaching object.

A marked functional specialisation of the organs concerned with producing this signal was observed. A fine pencil of highly modified scales provided a highly dissected evapouration surface allowing high release rates. This structure was thought to be spread just before contact is made with the female. Another group of scales and the underlying cells have become modified for a secretory function. These gland cells, because of their large size

and high content of RNA were thought to be metabolically active, producing a pheromone precursor immediately following emergence.

The sequence of events leading to the full development of the males reproduction system was quite complex. Cytological evidence suggested that production of the pheromone precursor was restricted to the first day following emergence. However, the development of high concentrations of pheromone and the initiation of mating did not occur until 2 - 3 days after emergence. This unusual delay appears to be due to a preceding period of migratory flight.

A second structure, the posterior abdomenal brush, is described and a pheromone producing function suggested. Though not conclusive, morphological evidence supports this supposition.

This pheromone system is not unique to P. separata. Hair pencils containing benzaldehyde are found in several species, and vanillin (a benzaldehyde derivative) was found in the wing gland of another noctuid. Though several other volatile components were present, it was thought that the chemical stimulus presented by males of this group was unlikely to be species specific. Other factors such as niche and season of emergence would make

an important contribution to the ethological isolation of these species. While this group has developed a complex system in the male, the development of highly pectinate antennae in other species suggests the existance of a group developing an important female produced pheromone.

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