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ASPECTS OF THE BEHAVIOURAL ECOLOGY OF

STEGOBIUM PANICEUM (L.) (COLEOPTERA; ANOBIIDAE)

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(Coleoptera; Anobiidae)

Jeremy Philip Ward. Ph.D. 1978.

Large numbers of individual live insects were followed through their life-cycle and aspects of their behaviour and ecology were the subjects of observation and experiment.

The pheromone system (discovered by previous authors) was found to be situated in the female abdomen. The mating efficiency of both sexes was found to increase steadily with age after adult eclosion, reaching a peak at 9 days. Female pheromone production/release was significantly reduced after 3 minutes in copula, as was female receptivity to the male. These phenomena are cumulatively induced by mating and are probably the result of the passage of male accessory gland substance.

Eleven main units of male pre-copulatory behaviour are described. An ethogram is given showing the chronological relationships of these units during mating encounters. The use of the male antennae, palps, tarsal-claws and aedeagus during mating and amplexus is related to their morphology and their function in stimulating and being stimulated by the female. Mating experience, while not affecting a male's response to pheromone, does impair his mating efficiency. There is evidence that males learn correct orientation to the female during mating experience.

Oviposition begins within 16 hours after copula and 61% of the eggs are laid within 24 hours. Female age does not affect fecundity, but an increase in age increases the oviposition rate. At least 2·5 minutes in copula are required to stimulate oviposition, but the full-term (mean: 61 minutes) is required for 100% fecundity. Specific tactile stimuli are needed to initiate oviposition. Female dispersal is caused by overcrowding and mating. Mating directly causes an increase in female locomotory activity and tendency to fly. The increase in locomotory activity rises proportionately to the length of time in copula. These changes are probably partly produced by the passage of male accessory gland substance. The adaptive significance of these phenomena is discussed.

An increased density of eggs causes a corresponding significant increase in the mortality of developing insects. The slight preponderance of female numbers also becomes significant at high population densities. Density-dependant control appears to be the result of cannibalism of the eggs by early larvae. A life table is given of the mean percentage survival at different stages in the life-cycle. The largest mortality is at hatching and among early larvae. Thirty-nine percent of larvae did not construct a cocoon. Cocoons have a significant effect on reducing mortality and wing-deformation. Cocoons are shown to be adaptively positioned.

Pheromone.
Mating.
Oviposition.
Dispersal.
Mortality.

D E D I C A T I O N : to Bridget

"The most lasting marriage is the marrying of laughter"

Christopher Fry - 'The dark is light enough.'

A C K N O W L E D G E M E N T S

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I N D E X

Aspects of the behavioural ecology of *Stegobium paniceum* (L.) (Coleoptera; Anobiidae).

INTRODUCTION

Stegobium paniceum (L.) (Coleoptera; Anobiidae) is "virtually cosmopolitan, more characteristically temperate than tropical and can eat almost anything" (Lefkovitch, 1967). As a species *S. paniceum* has been associated with mankind and his stored food for at least 4000 years; evidence of infestation has been found in the grave goods of an XIth dynasty Egyptian tomb (2049 B.C.) and also amongst the objects in the tomb of Tutankhamun (1345 B.C.) (Chaddick and Filce Leek, 1972). Since mankind has been storing food, at least in the Near East, for at least 6000 years (Gabel, 1967) it may be assumed that many thousands of generations of *S. paniceum* have been associated with Man's stored food. Today *S. paniceum* is primarily a pest of retail, rather than wholesale premises and causes loss by spoilage rather than by actual destruction.

As long ago as 1788 Gilbert White was advocating ecological studies of pest species for, as he wrote in 'The natural history and antiquities of Selbourne': "a knowledge of the properties, oeconomy, propagation, and in short the life and conversation of these animals, is a necessary step to lead us to some method of preventing their depradations." It is the purpose of the present study to contribute to our knowledge of the life of *S. paniceum*.

In view of the long association of *S. paniceum* with Man it is assumed that evolutionary changes will have occurred in the species which adapt it to a partially man-made environment. It is therefore concluded that laboratory studies are more applicable to this species than they are to many. With this in mind the present study seeks to provide at least

part of a detailed autecology of *S. paniceum*. To do this, functional analysis of behaviour is combined with morphological and ecological studies and since, as Darwin (1836) says, ".... a number of isolated facts soon become uninteresting, the habit of comparison leads to generalisation", so the present work endeavours to synthesize facts into 'generalisations' about ecological adaptations and thus 'biological significance'.

The experiments recorded in this work in the main consisted of following large numbers of individuals throughout their life; recording and testing at critical stages. The work is therefore divided into three Sections which follow the life-cycle of the insect.

Section A, Part 1, deals with the intra-specific communication system of newly emerged adults, how it changes with age and how it is affected by mating. Section A, Part 2, deals with the behaviour of adults during mating, the signals and structures used and possible adaptation.

Section B deals with the life of the adult after mating: oviposition and dispersal.

Section C deals with the pre-adult insect, population effects and mortality. The life-cycle returns to the start and the thesis ends with the formation of, and development within, the cocoon.

GENERAL INTRODUCTION TO METHODS

The beetles used in this study were initially obtained from several 'Wild' sources found infesting stored fish-food and stored cereal. The initial colonies, having been proven disease free, were kept in 2 lb. sterile 'Kilner' jars half-full of wholemeal flour. The flour was first sterilised by placing it, in a thin layer, in a metal tray which was sealed with aluminium foil and autoclave tape. The tray was then placed in a pre-heated oven for 2 hr at 105°C. This method ensured that the flour was thoroughly heated through and prevented, as far as possible, the loss of moisture and nutrient due to the heating process. After sterilisation the flour was mixed with a 10% proportion of dried yeast to supply any deficiencies caused by sterilisation (Method due to Cox, undated). The flour/yeast mixture was then transferred to sterile Kilner jars and the tops were covered with filter paper to allow a free exchange of gases. The jars were kept in an egg-hatching incubator at an approximate temperature of 25°C and a relative-humidity of 70%.

Initially some difficulty was experienced with mite-infestations, eventually necessitating the destruction of a number of cultures. It was found, however, that by keeping the number of stock cultures in any one place low, and by separating them with 'moats' of oil, infestations were prevented from recurring.

Because of the lack of insect-rearing facilities in the department - notably the absence of controlled temperature and humidity chambers - it was found impossible to employ the synchronous pupal extraction from stock technique used by other authors (Barratt, 1975). A method was therefore devised whereby the last instar larvae were removed from stock cultures by sieving through a 2.4 mm-mesh sieve that retained only the largest larvae, assumed to be in their final instar. Other methods, such as the employment

of a modified Tullgren funnel (Golob et al, 1974), were found to be less satisfactory.

The last-instar larvae were transferred individually to 2 ml screw-top glass vials containing a small quantity (approximately 50 mg) of sterilised flour. The vials were then located in holes in wooden blocks. The blocks, 12 cm by 12 cm, each contained 25 holes arranged in a 5 by 5 hole grid. This grid was lettered A - E and numbered 1 - 5 by columns and rows respectively. Each block was given a serial number. Thus each individual larva could be identified by a code-number relating to the position of its vial. For example: a larva labelled 4C2 could instantly be placed in the 4th block, column C, row 2.

The blocks and their vials were placed in dessicators containing Sodium-hydroxide solution made up according to the method of Madge (1961) to give a relative humidity controlled at 70%. The dessicators were then placed in laboratory incubators set at 28^{o}C, thus providing an environment nearly optimal for most of the growth and development parameters of *Stegobium paniceum* (Lefkovitch, 1967). The incubator photoperiod could not be controlled and the beetles were thus left in continuous darkness except when an experiment was in progress. It is suggested that, since food storage facilities commonly exclude light, continuous dark conditions would not constitute an imbalancing factor in the *S. paniceum* environment. The vials were examined every day and record cards were kept of the state of the insects in each individual vial.

By this method a supply of adult *S. paniceum* was produced whose late larval, pre-pupal and pupal stages could be followed individually and which, since sexing adults is difficult, enabled the sex to be discovered at the pupal stage, when the genital papillae can be easily differentiated (Halstead, 1962). After adult eclosion the course of adult maturation,

mating and oviposition could again be followed with reference to individuals. The age of the adults was determined by considering them to be 0 days old for 24 hr after eclosion, during which period the elytra have not darkened to the same extent as the pronotum (Barratt, 1975). This method enabled the author, in effect, to gain insights into the behaviour and ecology of a population through a study of individuals.

SECTION A:

MATING

SECTION A. : MATING

INTRODUCTION

The information described in this section was gathered during the observation of mating encounters. These encounters were arranged using the apparatus shown in Fig. 1.

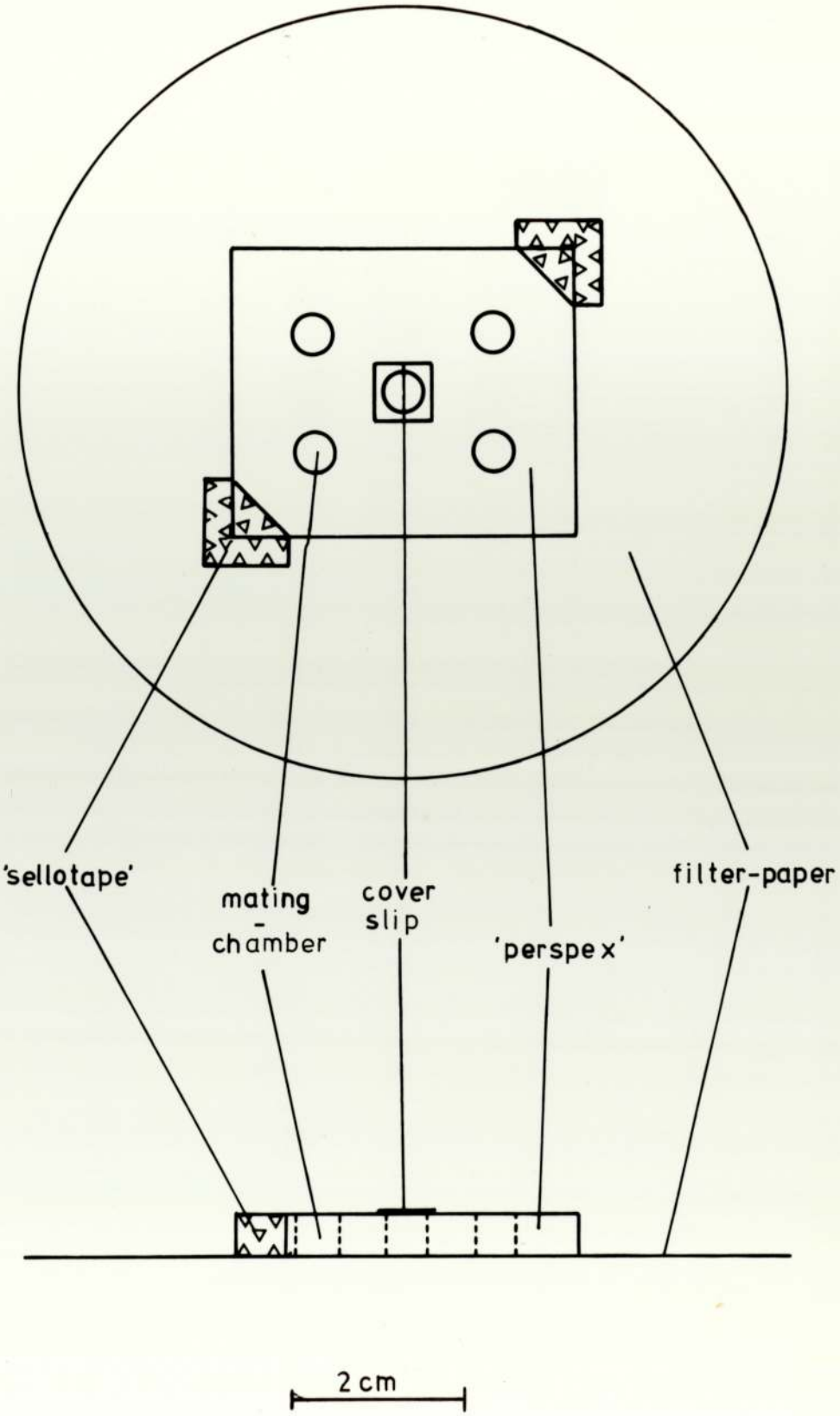
This apparatus was placed on the stage of a binocular microscope, illuminated with overhead light filtered by blue-stained water to reduce heating. Adults of known age (produced according to the method described in the general introduction to Materials and Methods) were introduced into the chambers formed by the holes in the perspex. The female was introduced first and the encounter timed from the introduction of the male, the coverslip being replaced as each individual was placed in the chamber. The insects were manipulated with Size 1 paintbrushes, a separate brush being kept for each sex to avoid possible pheromonal contamination. Clean coverslips and a different chamber were used for each encounter and after five encounters the filter paper was discarded and the perspex washed with alcohol. By duplicating this apparatus larger numbers of encounters could be observed without the need to disturb the insects. At the time of each encounter the ambient temperature, relative humidity and time of day were recorded. Initially a live voice recording of all encounters was made on a portable cassette recorder. The tapes were subsequently transcribed and time intervals were inserted. When the important behavioural units had been identified it was possible to make timings directly from the encounter without the need to record behaviour.

Mating behaviour is described in detail in Section A, Part 2a, but since the behaviour is used to examine aspects of pheromone secretion in

Section A, Part 1, it will be necessary at this point to describe the relevant behavioural units.

Male responsiveness to the female is measured by the time taken for a male to mount a female and evert his aedeagus, thus indicating that copula will occur if the female is receptive. The other behavioural unit, examined for the variability of its timing in Part 1, is that of the interval between the male mounting the female and the pair achieving copula; i.e. when the male dismounts and turns posterior to posterior with the female (see detailed description of mating behaviour, Fig. 3D.).

FIGURE 1 : Plan and elevation of normal mating chambers.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SECTION A. PART 1a. The site of pheromone production.

INTRODUCTION

A pheromone is a chemical substance secreted to the outside of an individual which acts on an animal of the same species (Karlson and Lüscher, 1959). Pheromones have been the subject of much attention in recent years and the literature concerning them is now vast. The biology of the subject is reviewed by Birch (1974), who deals with pheromones in general and their occurence not only in insects but also in many other groups of animal. Butler (1967) deals solely with insects and Shorey (1972) is concerned primarily with the behavioural responses which insects make to pheromones. Jacobson (1972) deals with insect sex-pheromones order by order.

Barratt (1974) discovered a pheromone secreted by female *Stegobium paniceum*. She made bioassays of the response of males to live females and to diethyl-ether extracts of whole dead females. Subsequently the findings were confirmed by Kuwuhara et al (1975). They suggested the empirical formula $C_{13}H_{20}O_3$ for the substance involved. Neither of these two studies however suggested a possible site of pheromone production or release.

Many methods have been used to determine possible positions of pheromone producing organs in the Insecta. A morphological or histological approach would seem to identify glandular tissue associated with an insect known to produce a pheromone. Many instances may be given such as Thornhill (1973) on two species of Mecoptera, Baer et al (1976) on three species of Tortricid Lepidoptera or Schneider and Rudinsky (1969) on a Scolytid beetle. This approach is particularly useful if the pheromone is produced by only one of the sexes, as this allows morphological comparison to be made. Madrid et al (1972) for instance found that the male Ambrosia beetle (*Platypus flavicornis* (F.))

contained in its hind gut different glandular tissue which produced four additional volatile compounds to those found in the female. Some attempt at a histological approach with *S. paniceum* was made by the present author; but this was abandoned in favour of an approach giving more rapid results, thus allowing more time to be spent on other aspects of the work.

By examining the differential attractiveness of excised parts of the anatomy the gross location of a pheromone source may be discovered.. This approach was taken by Ladd (1970) with the Japanese beetle (*Popillia japonica* Newman) and by Henzell et al (1969) with the New Zealand Scarab (*Costelytra zealandica* (White)). Another method is the extraction of different parts of the body such as that carried out on 6 species of *Trogoderma* (Coleoptera; Dermestidae) by Hammack et al (1973) or that of Chang and Curtis (1972) on the New Zealand sugar-cane weevil. Such extracts, when bio-assayed, help to show the position of the pheromone secretor. These approaches were not used in the present study because it was felt that inevitable problems of contamination render them inaccurate in pin-pointing a release location. Some information was obtained, however, by investigating the attractiveness of females after wings or elytra were removed.

Masking of different parts of the insect's anatomy with a non-attractive substance from which the pheromone is not disseminated is sometimes employed. Lacquer was used this way by Stanic et al (1970) on the dermestid beetle *Trogoderma granarium* Everts. In the present study a method similar to this was applied.

METHOD

Melted paraffin wax was used to mask different areas of the female body. Females were used at 6 days of age; this was found to be the optimum age of attractiveness to males in the present study, by Barratt (1974; 1975)

and by Kuwuhara et al (1975). Manipulation of the female presented some difficulty. This was overcome by attaching her to the point of a pair of fine forceps with a small drop of wax. In this way any desired portion of her body could be masked by dipping it into melted wax. The wax was contained in a watch-glass kept warm over a low bunsen flame. Brief contact with warm wax neither killed nor appeared to damage the female.

The present study, Barratt (1974; 1975) and Kuwuhara (1975) determined the optimum male age of response. Only males at or around this age, between 6 and 10 days, were used.

Male response was tested to one group of females without hind wings and to another without elytra. The operation removing wings or elytra was carried out when the female was anaesthetised with CO_2 gas. The females in this experimental group were older than those used in previous tests (9 - 15 days).

Encounters took place in the mating chambers as described in the Introduction to Section A. All encounters were allowed to continue for 5 min (300 sec) and failure of the male to evert his aedeagus within this time was deemed to be a negative result.

RESULTS

In the tables 1 and 2 male response times are presented as the log of the reciprocal of the actual time (in sec) x 1000. Reciprocating the actual time enables a negative response (i.e.: no aedeagal eversion within 300 sec) to be considered as 0, rather than as infinity; this results, however, in a non-random distribution of the figures. In order to correct this, and thus permit parametric tests to be performed, a log transformation was applied to the reciprocated response-times which were multiplied by 1000 in order to get rid of log bars.

It was first determined whether males were attracted to paraffin wax in the absence of females or female extract. Small pieces of wax (2 mm long) were used. In none of 15 encounters did the male show aedeagal eversion. In 15 more encounters a similar lack of response was shown by males to females entirely covered with wax. This demonstrates that the wax is not in itself attractive and furthermore that the pheromone is not disseminated through it.

Next the female dorsal surface was masked with wax and again male response was monitored. In this case the male response was positive in all encounters (Table 1). A t-test was used to compare these response times (treated as above and timing from the start of the encounter to the point when the male mounts the female and everts his aedeagus) with the response times of males to untreated but otherwise similar females. The test showed no significant difference between them ($t = 1{\cdot}31$, $P > 0{\cdot}1$). This indicates that female attractiveness is probably not diminished by covering the dorsal surface.

The ventral surface was similarly treated and male response investigated. Again response was positive in all cases (Table 1) and a comparison with the untreated sample showed no significant difference ($t = 1{\cdot}32$, $P > 0{\cdot}1$); suggesting no reduction in female attractiveness.

The head and thorax were covered next and male response tested. The difference between this and the untreated sample was again insignificant ($t = 1.76$, $P > 0{\cdot}05$) (Table 1). It is therefore likely that masking the head and thorax has no effect on female attractiveness.

The abdomen was then covered and response tested. In some cases males did not respond at all to females so treated and even in cases where there was aedeagal eversion the response time was generally longer (Table 1). The t-test between these response times and that measured under the treatment

of the head and thorax showed a significant difference ($t = 2{\cdot}97$, $P < 0{\cdot}01 > 0{\cdot}001$) However, when compared with the male response times after the treatment of the dorsal and ventral surface, male response to the abdominally treated females was just insignificant ($t = 1{\cdot}92$, $P < 0{\cdot}01 > 0{\cdot}05$; $t = 1{\cdot}99$, $P < 0{\cdot}01 > 0{\cdot}05$, respectively). Finally, comparison of responsiveness to the abdominally treated females with those of the untreated sample also shows a significant difference ($t = 2{\cdot}6$, $P < 0{\cdot}02 > 0{\cdot}01$). In general, however, these results show that there is a diminution in attractiveness to males when the female abdomen is masked with wax.

The possibility that pheromone production takes place either in the wings or elytra was tested by examining male responsiveness to females with these organs removed. Table 2 shows the results of these tests. Older females were used in this experiment; this accounts for the raised response times of males to the untreated sample. The t-test was again used to examine differences between the experimental groups. A slight comparative diminution of male response was noted to females with wings removed; but statistically the difference between this response and that of males to untreated females was insignificant ($P > 0{\cdot}1$). A markedly lowered responsiveness characterised the reaction of males to female without elytra. This reaction, however, when statistically compared to the response of males to untreated females showed no significant difference ($P > 0{\cdot}1$). These experiments appear to show that the removal of neither wings nor elytra results in the loss of attractiveness of females to males. The possibility that attractiveness is diminished, particularly in the case of elytral removal, is not ruled out.

TABLE 1: Variations in male responsiveness to females with different body areas masked with wax.

Male response times expressed as

log [(1/male response time (sec)) x 1000]

Replicate number	Control (no wax)	Wax on dorsal surface	Wax on ventral surface	Wax on head and thorax	Wax on abdomen
1	2·0969	1·8537	1·4440	2·0969	0·7634
2	2·0969	1·4683	1·6990	1·7634	1·2272
3	2·2219	1·1238	2·0457	1·9586	0·0000
4	1·6571	1·4440	1·9206	2·2219	1·9206
5	1·6201	1·6021	1·6776	2·2219	1·0864
6	2·3010	2·1550	2·1550	1·6385	1·6021
7	1·6021	2·0969	1·8241	1·1072	0·5315
8	1·7959	2·2219	2·0000	1·6201	1·6990
9	2·3010	2·3010	0·8573	1·7959	0·7924
10	2·3010	2·0000	1·9206	1·1875	1·2227
11	1·6990	2·3010	1·9586	1·0334	0·0000
12	1·7959	1·3365	1·6990	1·6571	0·7482
13	1·8241	0·9191	0·6532	1·6021	0·8513
14	1·6990	2·1550	2·0969	1·1430	0·0000
15	2·0969	1·1492	1·6021	1·6532	0·5185
16	1·8241	1·4082	1·5855		
17	2·0000	1·2279	2·0457		
Mean:	1·9372	1·6990	1·7168	1·6467	0·8643
Standard deviation:	0·2536	0·4672	0·4156	0·3903	0·6075

TABLE 2: Differences in male response times with 1) hind wings removed, 2) elytra removed compared with the response to untreated females.

Male response times expressed as

log [(1/male response time (sec)) x 1000]

replicate number	control (untreated)	wings removed	elytra removed
1	1·2923	2·3979	0·0000
2	2·3010	1·3979	1·0569
3	2·0000	0·0000	0·8062
4	1·7443	1·3874	1·4683
5	1·3560	0·9085	0·0000
6	1·3010	0·9445	0·8261
7	1·3979	1·2014	1·2430
8	1·3181	0·0000	1·6532
9	0·0000	0·5682	1·2601
10	0·6990	1·5682	1·4683
11	0·0000	1·2601	0·0000
Mean:	1·2190	1·0576	0·8893
Standard deviation:	0·7329	0·6968	0·6267

DISCUSSION

The results outlined above indicate that the pheromone producing organ of female *S. paniceum* is situated either in the abdomen or in the wings (including the elytra). This result is unsurprising when viewed in relation to the distribution of scent glands in other insects.

Modified wing-scales used in pheromone production are possessed by males of many species of Lepidoptera from several families. Instances may be given of the Phycitidae (Grant, 1974), the Danaidae (Urquhart, 1976) and males and females of *Galleria melonella* L. (Khalifa, 1950). Kashef (1956) describes the structure and veination of the hind-wings of *S. paniceum* and fails to report the existence of a glandular area at light-microscope level. In this species the wings are folded under the elytra (modified fore-wings) when at rest and any glandular surface would therefore not only be covered by the folds of the wing but would also be contained within the closed box formed by the junction of the elytra with the abdomen. One would assume, therefore, that in order for a pheromone to be disseminated the wings would have to be regularly unfolded from beneath the elytra and perhaps fluttered as they are in *G. melonella* (Khalifa, 1950). No such behaviour was noted in *S. paniceum* by the present author. In view of this, and the fact that the attractiveness of females with wings removed was not significantly reduced, it is concluded that the hind wings probably do not play a part in pheromone production.

This, of course, does not rule out the possibility that pheromone is produced in the elytra of *S. paniceum*. If this were the case one would anticipate a drop in male responsiveness not only when the abdomen (and thus the major part of the elytra) was covered, but also when the entire dorsal surface was covered. In fact the reduction in male response to females

with their dorsal surface covered was insignificant when compared statistically with the male response to untreated females. In addition the experiment involving elytral removal demonstrates that females without these organs remain attractive to males. It may be proposed, therefore, that the site of pheromone production probably does not lie in the elytra. The reduction in attractiveness of females with elytra removed, while not statistically significant, may nevertheless be important and is discussed below.

It is therefore likely that in *S. paniceum* the site of pheromone production is in the abdomen as it is in many other insect species. Such is the case in many of the Curculionoidea. In these the site of production is internal and connected with the hind gut. This is true of many of the Scolytidae (Carle, 1974; Schneider and Rudinsky, 1969), the ambrosia beetle *Platypus flavicornis* (Hbst) and the sugarcane weevil *Rhabdoscelus obscurus* (Chang and Curtis, 1972). In *S. paniceum*, however, the female faeces proved unattractive to males, contrary to the situation in those beetles which produce pheromone in the gut (Peacock et al, 1973; Tumlinson et al, 1969). This, in itself, suggests that the gut is not the site of pheromone production in *S. paniceum*. Indeed, the presence of a pheromone in faecal material would only make good ecological sense where it is functioning to produce aggregation, as it is in most of the Curculionoidea. In *S. paniceum*, however, the pheromone is an aphrodisiac - directly responsible for the initiation of mating behaviour (Birch, 1974). In such a case the presence of material extraneous to the female and yet secreting a pheromone that causes mating behaviour could result in copula attempts that are not directed towards the receptive female. Such futile attempts might mean the failure of some males to impregnate females. For this reason it seems unlikely that the gut is the source of pheromone in *S. paniceum*.

The possibility remains, therefore, that the pheromone source is

associated with the exoskeleton. The Lepidoptera are the most intensively studied of all orders possessing abdominally located pheromone producers. These organs are generally glandular modifications of the intersegmental membranes and in many cases are capable of being everted to provide more efficient dissemination of the pheromone (Jacobson, 1972). Although there is no evidence for the presence of eversible glands in *S. paniceum* there is still the possibility that intersegmental membranes function as the production site. The latter are known to secrete pheromones in several species of *Trogoderma* (Coleoptera: Dermestidae) (Stannic et al, 1970; Hammack et al, 1973). In other Coleopteran species the gland is associated with *Kheper nigroaeneus* (Tribe, 1975) and *Costelytra zealandica* (White) (Henzell et al, 1970) (Hoyt et al, 1971).

When an insect produces a pheromone from a point source as in the examples above it often exhibits 'calling behaviour'. This takes the form of a behaviour pattern designed to disseminate the pheromone. In *Trogoderma glabrum* (Herbst), for instance, the female assumes a posture where the abdomen is elevated and the ovipositor elements (and thus the pheromone secretors) are exposed (Hammack et al, 1976). *Kheper nigroaeneus* also adopts a calling posture which exposes the secretors located on the 1st abdominal stermite (Tribe, 1975). The only action that could possibly be attributed as calling in *S. paniceum* is the abdominal 'flexing' which often occurs. But since this action is neither sex-specific nor noted in every encounter it is unlikely that this can be considered as a calling activity; indeed a comparison with similar activities in other species makes it much more easily understood as a type of grooming by 'positioning' (Valentine, 1973).

The wax covering experiments with female *S. paniceum* of optimally attractive age demonstrate, by variations in male response, that it is the abdominal region which is most attractive and therefore most likely to contain

the pheromone source. This method does not demonstrate the precise source of the pheromone however. But a comparison of the differences in male response to females with different parts masked can be made. From such comparisons it appears that the entire elytral and ventral surfaces are involved in pheromone dissemination.

Tschinkel et al (1967) have demonstrated that, while the cells secreting the sex pheromone in *Tenebrio molitor* L. are located in the region of the metathoracic sternum and the first two abdominal tergites, the pheromone does not remain in the vicinity of the source and is gradually spread over the whole of the beetle's body. It is postulated that a similar mechanism operates in *S. paniceum*; this would explain the reduction in female attractiveness when the elytra were removed, since it is likely that in an older female a considerable amount of pheromone would have spread into such a large area. The removal of the hind wings had less effect presumably because the nature of the wings is such that pheromone spread does not so easily occur on them. The diffusion of the pheromone over such a large area may provide a clue to the absence of calling behaviour in this species since there would be no need to adopt a specific posture in order to expose the site of pheromone production.

SECTION A. PART 1b. The effect of male and female age on male response.

INTRODUCTION

Some insect species during their life span, show little variation either in volume of pheromone release and synthesis or in their ability to respond to pheromones. Others, however, show a marked maturation in both production and response; reaching a peak at which the insects are in an optimally receptive condition. Concomitant with this maturation there is often a decrease in pheromone production and a diminution of response with the onset of senility (Jacobson, 1972).

The fact that *Stegobium paniceum* has an optimum response age has already been mentioned in the previous chapter. It is the purpose of this chapter to demonstrate this optimum and to discuss the effects of maturation and senility on both female pheromonal production and male response.

In order to make a study of this kind the behavioural response of the male to the pheromone must first be determined. It is usual to quantify either the whole or a part of this response in order to assay the pheromonal activity and any variation caused in it by age. Several behavioural responses have been used as assays of pheromonal activity in the Coleoptera. For instance, a group of activities involving antennal deviation or vibration and increased locomotion have been used as response criteria in *Trogoderma inclusum* Le Conte, *Lasioderma serricorne* (F.), *Anthonomous grandis* Boheman and *T. granarium* by Burkholder and Dicke (1966), Coffelt and Burkholder (1972), Keller et al (1964) and Levinson and Bar Ilan (1970) respectively. Barratt (1974; 1975) was of the opinion that no such clear-cut behavioural responses were shown by male *S. paniceum*; she therefore

used as an assay criterion the time spent by a male in the vicinity of female pheromone extract or females confined in a perforated gelatine capsule. In this study however the response used as a criterion of male response was aedeagal eversion, which may be considered as the initial stage of copula. Similar behaviour was also considered as the positive response to pheromone extract in the studies of Tschinkel (1970) and Henzell et al (1969) on *Tenebrio molitor* and *Costelytra zealandica* respectively. The use of a criterion such as this allows no ambiguity as to the functional nature of the response and its connection with mating.

Female extract and live females contained within a perforated gelatine capsule have already been mentioned as the objects used by Barratt (1974; 1975) to investigate the presence of pheromone in *S. paniceum*. Other authors have used methods involving air passed over crushed females and blown towards males (Henzell et al, 1969) or exposing males to filter papers on which females have rested (Burkholder and Dicke, 1966). It is necessary to use such procedures when establishing the existence of a pheromone in order to eliminate the possibility of other cues (auditory or visual for instance). Since the existence of a pheromone has already been established in *S. paniceum* it is possible to further investigate female attraction and male response by the observation of interactions between live males and females. Not only does this generate quantitative information about maturation but also about mating behaviour as a whole. This is the method used in the present study.

METHOD

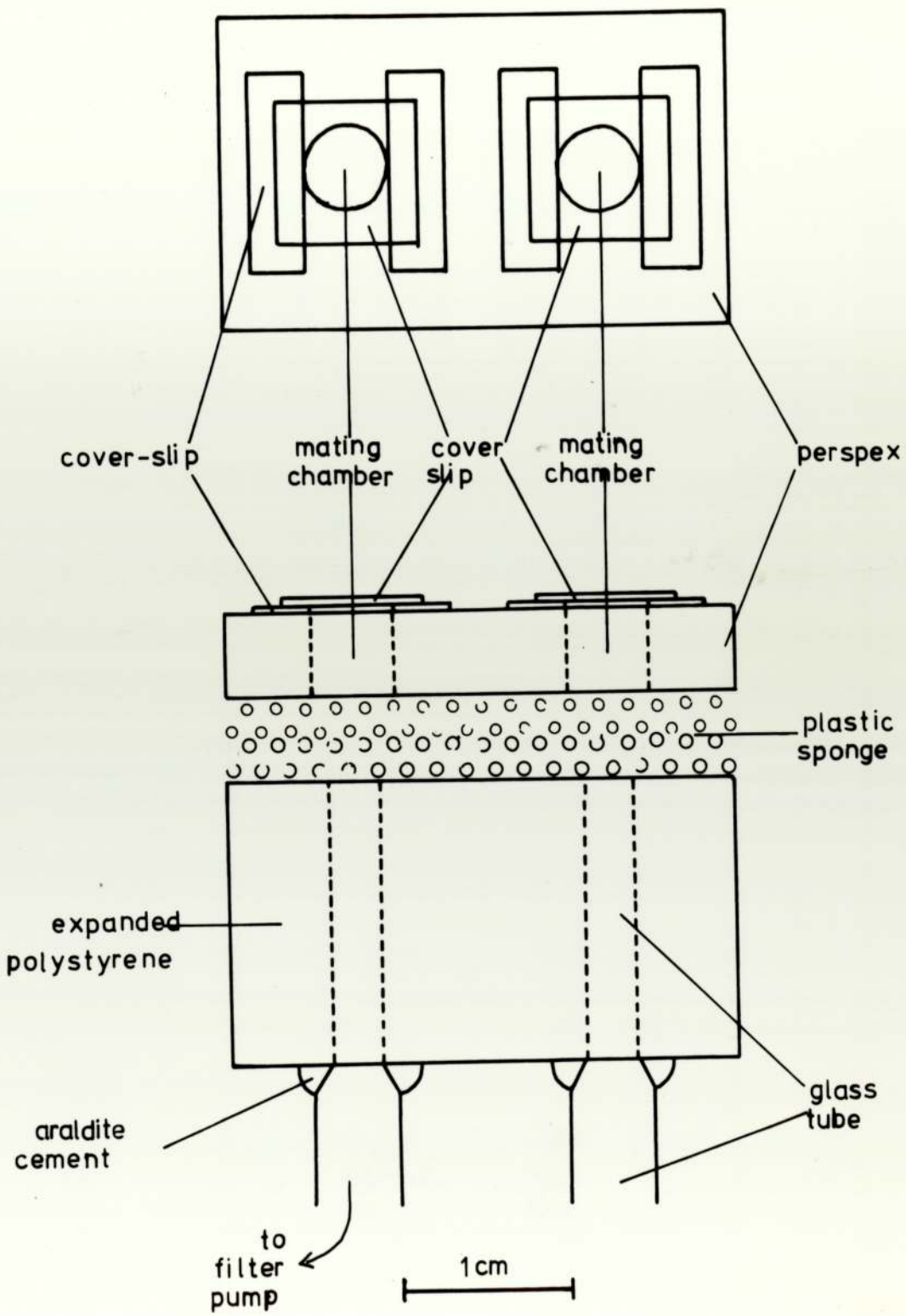
Encounters between males and females were individually examined in the confines of the mating chambers previously described. The encounters were timed and the relevant variables were recorded for males and females of

varying ages. First, the delay from placing the male into the chamber and his mounting of the female and aedeagal eversion. Second, the time from the male mounting and everting until the eventual achievement of copula - signalled by the male, his aedeagus still engaged, rotating 180° with respect to the female (fig. 3D). In addition it was noted that in a number of cases the male mounted the female with incorrect orientation, his aedeagus directed towards the female head. The time taken for the male to correct this position was recorded. When producing the results, where relevant, the period of the male being incorrectly orientated was subtracted from the total time, after mounting, taken by the male in achieving copula.

Males of optimum age were used in encounters with differently aged females and vice versa. For each age group a number of replicate observations were made.

In order to demonstrate that the small volume of the chamber (0.8 ml) did not affect results, by concentrating the pheromone released, a specially constructed piece of apparatus was used. This apparatus (a 'through-flow' mating chamber) is shown in fig. 2. It consists of a piece of perspex, drilled with 0.5 cm holes in the same way as the normal mating chambers. The perspex was glued with 'Araldite' to a slice of plastic 'sponge' and this, in turn, was glued to a block of expanded polystyrene. Glass tubes were inserted through the polystyrene in positions corresponding to the holes in the perspex, the tubes were sealed into the block with 'Araldite'. When the tubes were connected to a filter-pump air was drawn through the holes in the perspex. The plastic sponge functions partly as a 'grid' to prevent the beetles in the chamber from being sucked into the tube, and partly to diffuse the effect of the suction over the entire chamber floor. The holes in the perspex were covered, as in the normal mating chamber, by glass coverslips. But in this apparatus, instead of lying directly on the perspex these were

FIGURE 2 : Plan and elevation of 'through-flow' mating chambers.


cover-slip
mating chamber
cover slip
mating chamber
perspex
plastic sponge
expanded polystyrene
glass tube
araldite cement
to filter pump
1cm

slightly raised by allowing them to rest on small pieces of coverslip glued flat to the perspex. This arrangement allowed air to be drawn into the chamber from above, at the same time producing a gap not large enough either to let the beetles escape or into which they were able to insert legs or antennae. The results of optimum-age encounters in this apparatus were compared with similar encounters in the normal mating chambers.

RESULTS

The results of times taken for males to mount females during the encounters that took place in the through-flow mating chambers are compared with similar encounters in ordinary chambers (Table 3). The results are recorded as the log of the reciprocals of the delay time multiplied by 1000. From these results it is possible to determine whether the small size of the normal chamber leads to a concentration of the pheromone such that the male response is affected. If such a concentration were to build up in the normal chamber it is assumed that a difference would be detected in the male response when this was compared with that during encounters made in the through-flow mating chamber where any pheromone produced by the female was continually drawn away in the air flow. Since statistical comparison of the two sets of replicates shows no difference between them (Table 3) it can be concluded that the small size of the chambers used in normal encounters probably has no effect on male response.

Use can therefore be made of the chambers and the individual-pair encounter technique to investigate the effect of male and female age on male response. Table 4. shows male responses to females aged between 1 and 20 days where ages are counted from the date of adult eclosion. The males used were all aged between 6 and 10 days, the optimum for response as will be

shown below. As previously, male responses are tabulated as the log of the reciprocal multiplied by 1000. Reciprocating the results means that a longer delay appears as a lower number and the graph therefore reflects a rise in responsiveness by an upward trend rather than the reverse. In Table 4. only the means of the replicates are given. A graph was plotted to show how male response varies with female age (graph 1A). From this graph it can be seen that the male response to females of between 1 and 3 days old is low, at 4 days it rises sharply and reaches a peak at 5 and 6 days. After the peak the male response in general becomes slower and probably falls to a more or less constant low. Standard deviations are large, however, analysis of variance (taking the results from the first 16 replicates of each age group - where their total permits) shows that variability between the points is statistically significant. It is assured, therefore, that significant changes do take place in the speed of male response to a female during her lifetime, and these changes probably follow a pattern similar to that shown in graph 1A. A comparison of this graph with graphs of male response to pheromone extracted from different ages of female published by Barratt (1974; 1975) and Kuwuhara et al (1975) shows a striking similarity in general configuration. The implication of this similarity is that by measuring the response times of males to live females a reliable bioassay can be made of the pheromone production or release of those females. It must be born in mind that the present experimental procedure examines the amount of pheromone which is passed to the environment by the female, whereas the pheromone extraction technique examines the total pheromone content of the female. Therefore the delay from the start of the encounter to the male mounting the female and everting his aedeagus is only a reflection of the total pheromone content of the female. To be more accurate, this technique can be said to be a measure of the total attractiveness of a live female to a male.

Table 5 shows the responses of males of different ages to females of optimum age (5 and 6 days - see above). This table is constructed in the same manner as described in Table 4. The results given in Table 5 are plotted as graph 1B. This graph shows a reasonably steady rise in response reaching a peak on about the 9th day after male adult eclosion and thereafter probably falling to a somewhat lower plateau level. Again standard deviations are large, however, an analysis of variance - by the same method as applied above - was made. The results indicate that a very highly significant change in the speed of male response does take place during a male's lifetime. Barratt (1974; 1975) and Kuwuhara et al (1975) have plotted the change in response during the lifetime of a male to a constant titre of a female pheromone extract; these graphs are similar to 1B. It is inferred therefore that graph 1B represents the maturation in the ability of the male to respond to the pheromone produced by females of an optimum age.

It can be seen that graphs 1A and 1B conform to a similar pattern over a similar time span. To test whether this similarity between the set of results for females of different ages and that for males of different ages was significant, Spearman's test was applied. This test examines the significance of the correlation between two sets of ranked data (Bishop, 1966) and is therefore applicable in this case. To ensure that each set of data contained an equal number of means (Tables 4 and 5) it was necessary to sum a few of the means of contiginous female ages and to calculate a new mean. The coefficient derived by using Spearman's test on these two sets of results demonstrates that there is a highly significant degree of correlation between them. It is therefore concluded that the increase of female attractiveness with age and the increase with age in the ability of males to respond to females are correlated.

Next examined was the effect of differences in female age on the time taken by a male (of optimum responsive age) to achieve copula - having

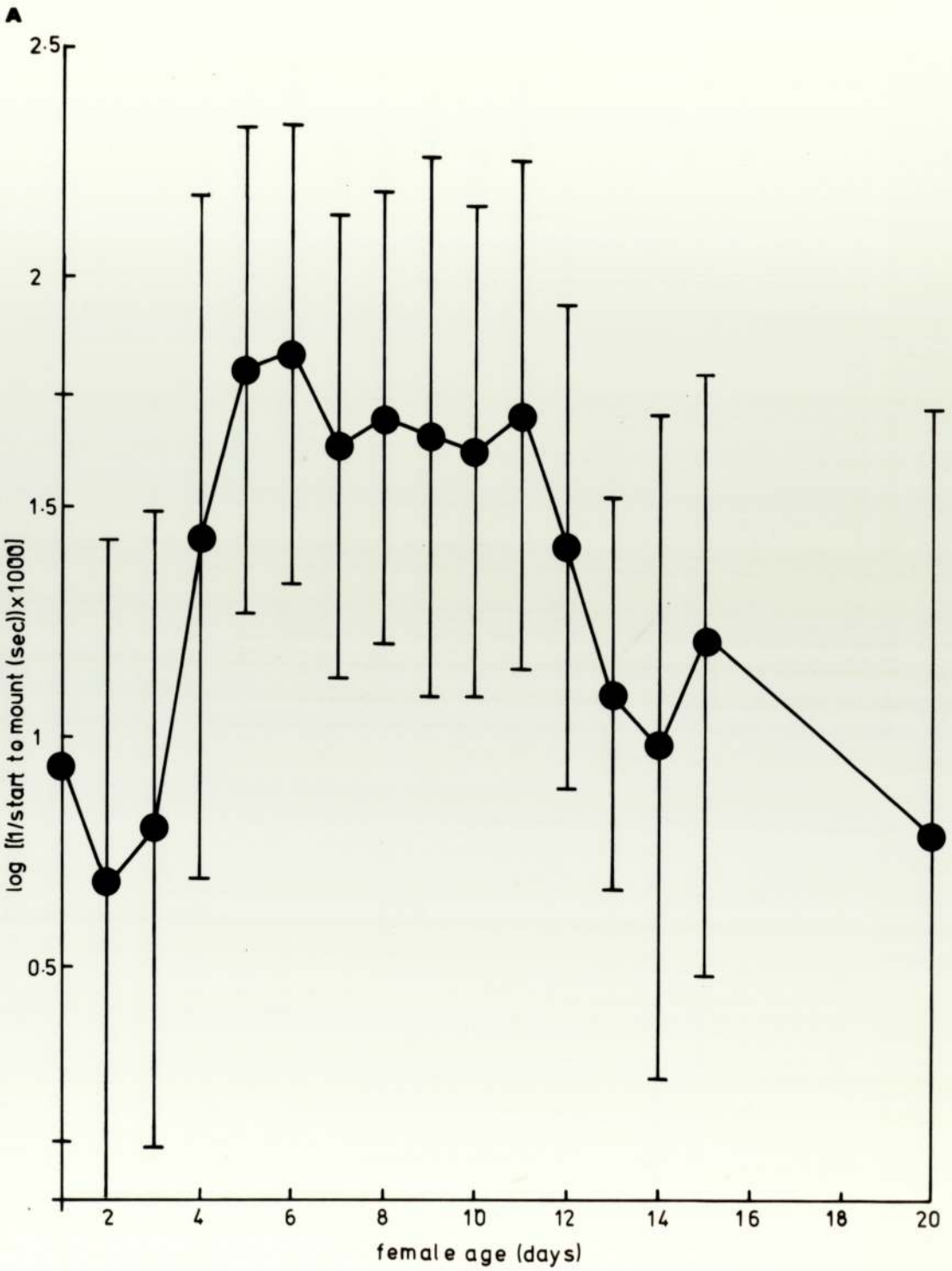
once everted his aedeagus. Table 6 shows the results of these times treated arithmetically in the same manner, and for the same reasons, as the initial male response times described previously. The means shown in Table 6 are plotted on graph 2A. This shows that females of 1 and 2 days old seem unable to achieve copula (since the log reciprocal mount to copula times are consistantly 0). Thereafter, within the next 6 days, a rapid increase in the speed of achieving copula takes place and a peak is reached on the 9th day. Subsequently the speed of achieving copula becomes less rapid and eventually reaches a level. Analysis of variance shows that the increase in the speed of achieving copula is significant. Since it is probable that delays in coupling will increase the chance of disturbance and lessen the likelihood of achieving a successful union. It is concluded that the results indicate a maturation in the ability of the female to achieve copula.

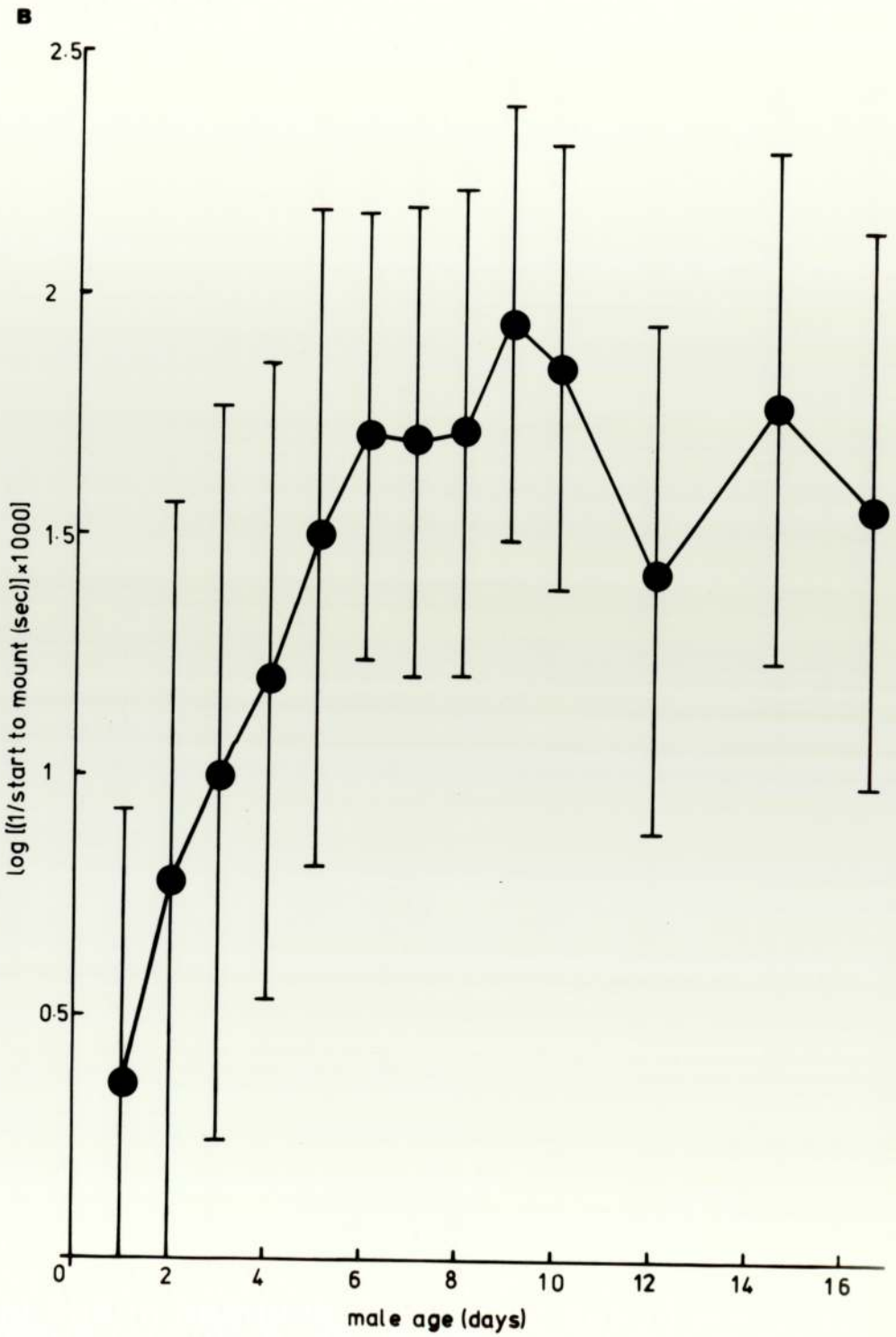
Table 7 deals with similar variables to those displayed in Table 6, this time measured using males of different ages and females of optimum age. Again the means are plotted. Graph 2B shows: that males of 1 day old are unable to achieve copula, that from 2 to 8 days old a steady increase in the rapidity of achieving copula occurred, that a peak occurred at 8 days old and that a steady decline took place after the peak. The analysis of variance demonstrates that these changes are significant. It is probable therefore that this graph shows a maturation in ability which is similar to that found in females.

On inspection the patterns of graphs 2A and 2B appear to be very similar. Treated in the same way as Tables 4 and 5, Tables 6 and 7 were then analysed using the Spearman rank correlation test. The value computed considerably exceeds the tabulated value for $P = 0.01$ and thus shows a very highly significant degree of correlation between the two sets of results. It is probable, therefore, that the maturation in the ability of the female to achieve copula is paralleled by the similar maturation of the male.

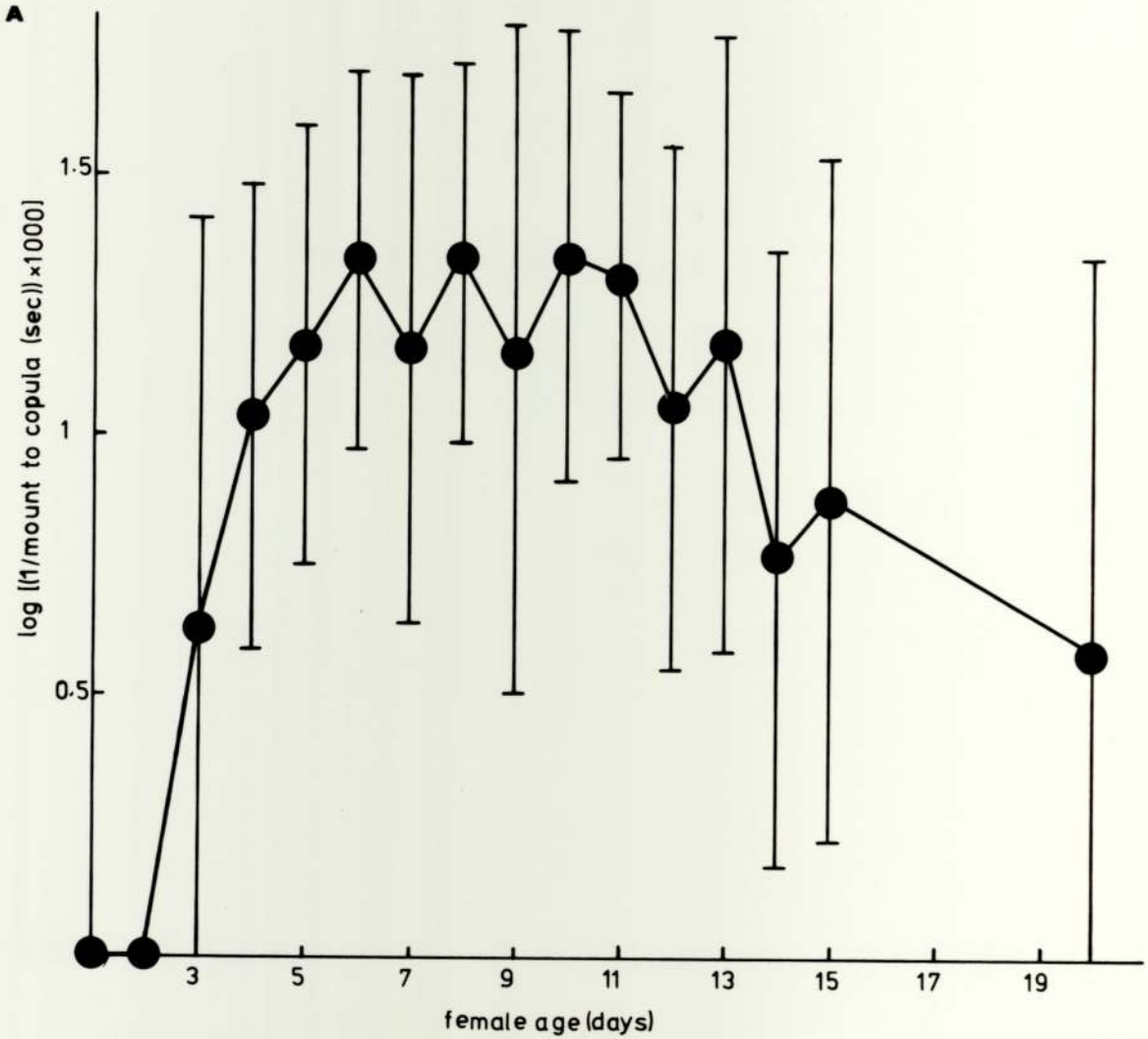
GRAPH 1A and 1B: attraction of females at ages 1-20 days in encounters with males of optimum age expressed as log [(1/male start to mount time (sec)) x 1000].

And ability of males at ages 1-17 days to respond to females at optimum age expressed as log [(1/ male start to mount time (sec)) x 1000].


A
2·5
2
1·5
1
0·5
log [(1/start to mount (sec))x1000]
2
4
6
8
10
12
14
16
18
20
female age (days)


B
2·5
2
1·5
1
0·5
0
log [(1/start to mount (sec)]×1000]
2
4
6
8
10
12
14
16
male age (days)

GRAPH 2A and 2B: ability of females to enter copula at ages 1-20 days when mated with males at optimum age expressed as log [(1/male mount to copula time (sec)) x 1000].
And ability of males to enter copula at ages 1-17 days when mated with females at optimum age expressed as log [(1/male mount to copula time (sec)) x 1000]


A
log [(1/mount to copula (sec)) x1000]
1·5
1
0·5
3
5
7
9
11
13
15
17
19
female age (days)

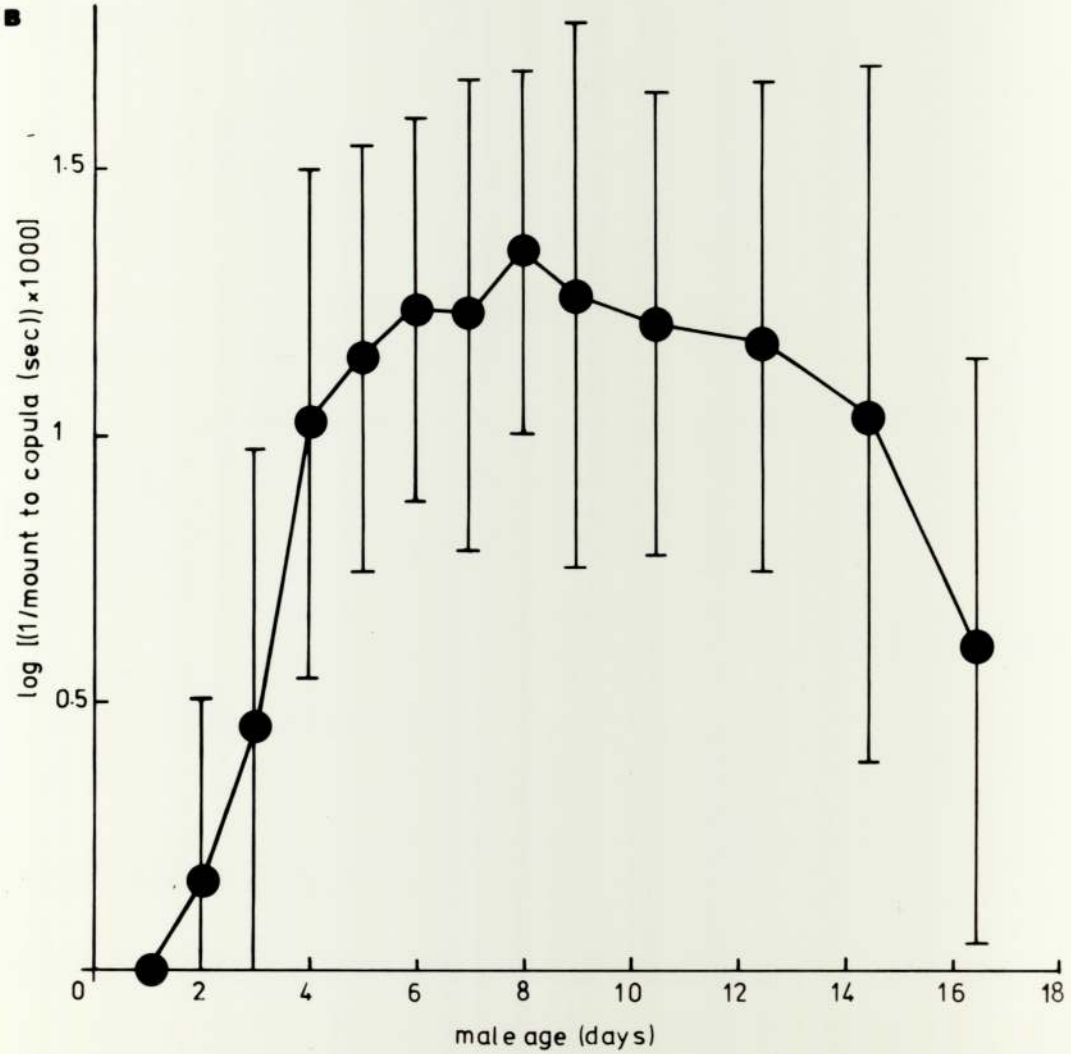

B
log [(1/mount to copula (sec)) x 1000]
1·5
1
0·5
0
2
4
6
8
10
12
14
16
18
male age (days)

TABLE 3: Male response to optimally aged females in through-flow mating chambers compared with the response to similar females in normal mating chambers.

Male response expressed as

log [(1/male response time (sec)) x 1000]

Replicate number	In through-flow mating chamber	In normal mating chamber
1	2·3010	1·2833
2	0·5911	1·2923
3	1·1875	1·9206
4	1·7959	0·7643
5	1·7210	2·0969
6	1·7694	1·5378
7	1·9586	2·0457
8	1·0492	1·2528
9	1·6021	0·8062
10	1·0043	1·6021
11	1·1492	1·9206
12	1·2528	1·2923
13	1·7210	1·0969
14	1·7443	1·6021
15	2·0969	1·2227
Mean:	1·5296	1·4491
Standard deviation:	0·4690	0·4181

t: 0·35

p: $> 0·1$

TABLE 4: Male response (time from start of encounter to male mounting female) for different female ages.

		log [(1/start to mount (sec)) x 1000]	
Female age (days)	number of replicates	mean	standard deviation
1	18	0·9359	0·8108
2	26	0·6840	0·7498
3	16	0·8046	0·6880
4	18	1·4372	0·7425
5	53	1·7984	0·5264
6	57	1·8348	0·4957
7	30	1·6351	0·5029
8	53	1·6977	0·4891
9	25	1·6593	0·5671
10	16	1·6251	0·5321
11	17	1·7051	0·5513
12	15	1·4242	0·5183
13	12	1·1018	0·4265
14	16	0·9850	0·7215
15	17	1·2273	0·7404
20	11	0·7922	0·9272

F: 2·5

P: <0·01> 0·001

TABLE 5: The effect of male age on male response to females of optimum age.

		log [(1/start to mount (sec)) x 1000]	
Male age (days)	number of replicates	mean	standard deviation
1	16	0·3574	0·5686
2	16	0·7810	0·7834
3	16	1·0051	0·7610
4	16	1·1975	0·6578
5	19	1·4938	0·6803
6	42	1·7081	0·4605
7	31	1·6967	0·4863
8	47	1·7164	0·5055
9	16	1·9424	0·4495
10	17	1·8531	0·4592
11 - 13	18	1·4198	0·5256
14 - 15	16	1·7705	0·5269
16 - 17	16	1·5616	0·5751

F: 4·1

P: $< 0·001$

TABLE 6: Time of mounting to copula for males of optimum age with females of different ages.

Female age (days)	Number of replicates	log (1/mount to copula (sec)) x 1000	
		Mean	Standard deviation
1	18	0·0000	0·0000
2	26	0·0000	0·0000
3	16	0·6281	0·7789
4	18	1·0292	0·4409
5	58	1·1696	0·4190
6	54	1·3266	0·359
7	29	1·156	0·5195
8	43	1·3393	0·3586
9	28	1·1407	0·6375
10	19	1·339	0·4296
11	16	1·2977	0·3452
12	15	1·0456	0·4967
13	15	1·1704	0·5855
14	18	0·7586	0·5855
15	19	0·8726	0·648
20	11	0·5823	0·7497
F :		4·6	
P :		0·001	

TABLE 7: Times of mounting to copula for males of different ages with females of optimum age.

Male age (days)	Number of replicates	log (1/mount to copula (sec)) x 1000	
		Mean	Standard deviation
1	16	0·0000	0·0000
2	15	0·1588	0·3441
3	18	0·4564	0·5118
4	16	1·018	0·472
5	48	1·1383	0·397
6	58	1·228	0·3561
7	58	1·2201	0·4374
8	57	1·3341	0·3359
9	47	1·256	0·5052
10 - 11	24	1·2031	0·4285
12 - 13	17	1·1972	0·4565
14 - 15	17	1·0356	0·646
16 - 17	15	0·5954	0·5418
F :		6·21	
P :		0·001	

DISCUSSION

The above results demonstrate that pheromone release and/or production rises steeply after the adult female is three days old and reaches a peak three days later. This result, confirmed by observations on extractable pheromone by Barratt (1974; 1975) and Kuwuhara (1975) is similar to that discovered for many other Coleopteran species. For instance: pheromone production reaches a peak in *Lasioderma serricorne* 4 - 5 days after adult eclosion (Coffelt and Burkholder, 1972), in *Tenebrio molitor* the peak is similarly reached at 4 days (Happ and Wheeler, 1969), *Anthonomous grandis* takes somewhat longer - 4 - 6 days (Hardee et all, 1967) and *Attagenus megatoma* still longer - 6 - 7 days (Burkholder, 1970). Barratt (1975) demonstrates that maximum ovary length in *S. paniceum* is reached at 6 days after eclosion, thus associating it with peak pheromone production. A similar association has been noted by Coffelt and Burkholder (1972) in *L. serricorne* and by Nation (1972) in *Anastrepha suspensa* (Diptera; Tephritidae).

Barratt (1975) demonstrates that emergence of both sexes from the cocoon in *S. paniceum* takes place between the 4th and 5th days after adult eclosion. The coincidence of this timing with the steep rise in attractiveness of the female (graph 1A) is immediately apparent. It appears, therefore, that the sharp rise in pheromone release is not only temporarily connected with ovarian maturation but also with emergence. In this *S. paniceum* behaves in a manner similar to several cocoon forming insect species; instances may be given of *Adoxophyes fasciata* Walsingham (Lepidoptera; Tortricidae) (Nagata et al, 1972) and *Anagusta koehniella* (Zeller) (Lepidoptera; Phycitidae) (Calvert and Corbet, 1973).

The results show that the maturation of the male response parallels the rise in pheromone production by the female. This parallel maturation also occurs in other species such as *Tenebrio molitor* (Happ, 1970),

Attagenus megatoma (Burkholder, 1970) and *Anthonomous grandis* (Hardee et al, 1967). If adult females and males reach maturity at roughly the same age it seems likely that there will be synchronous emergence in order to take advantage of being respectively optimally attractive and responsive at the same time. But in fact the male reaches optimum response age at 9 days whereas the female is optimally attractive at 6 days. This may imply a male pre-adult development which is shorter than that for the female, since this would be compensated by the male's longer adult development time. Thus both sexes would reach a peak at the same time. A plateau phase after reaching maturity is also an advantage in that it provides lee-way if the emergence of a population is not perfectly synchronous.

The differences in the shapes of the graphs plotting male response and female pheromone production is probably significant. While the female shows a sharp and sudden rise in production after the third day the male development is much more smooth and gradual - far more like a classic maturation curve. It is possible therefore that the two phenomena are produced by different types of physiological process. The graph of female pheromone production is most simply explained as the switching on of a pheromone release mechanism at or about the day of emergence from the cocoon; while the form of the graph of male response would be consistent with a gradual maturation of all the physiological processes involved in the perception of pheromone and ability to respond to it.

In other insect species the development of the ability to successfully achieve copula has been less thoroughly studied than pheromonal development. Happ (1970), however, does observe that in *T. molitor* successful matings begin to occur on the 5th day after adult eclosion; this corresponds to the day after the pheromone production peak. Barratt (1975) observes that the oocytes of female *S. paniceum* are immature until the third day after eclosion.

Graph 2A demonstrates that successful copula too is not achieved until the female is three days old; it can therefore be concluded that ovarian maturation causes the release of an hormone which creates the physiological conditions for copula (Engelmann, 1970), such has been demonstrated in *Phaneus* spp. (Copeoptera; Scarabaeidae) (Halffter and Lopez, 1977).

In contrast the male *S. paniceum* can achieve copula at 2 days old; this is similar to the results obtained by Henzell et al (1970) with *Costelytra zealandica*. The speed of achieving copula for females increases more smoothly with age after the 2nd day, as distinct from the development of pheromone release. This is probably a reflection of the maturation of more complex physiological processes; the ability to perceive and respond to signals from the male. For males too the development curve shows a smooth maturation of the ability to successfully achieve copula. In both sexes the plateau phase is well marked, implying an allowance for different emergence dates within a population which is similar to that referred to in connection with the maturation of pheromone release and response.

SECTION A. PART 1c. The effect of mating on female pheromone production and receptivity.

INTRODUCTION

Broadly speaking it is possible to classify once-mated insect females into three types depending on their response to subsequent mating attempts by males. The first type will mate more than once, the second will mate only once and the third is intermediate; mating once and resisting further copulatory attempts until after a refractory period when she will copulate again (Shorey, 1974). Female pheromone production after mating is allied to the type of response the female makes to subsequent mating attempts. Females that immediately accept more copulatory attempts do not show pheromone-content reduction while those that mate once only usually show a pronounced decrease in pheromone production. Intermediate types show pheromone production or release reduced for long or short periods following mating (Shorey, 1974).

The purpose of this section is to examine the mating response of prior-mated female *Stegobium paniceum* and to draw conclusions about the change in pheromone production and receptivity after mating.

METHOD

The responses measured here are the same as those described in the previous section, i.e. delay from start of encounter to male mounting the female and everting aedeagus, and delay from first eversion until full achievement of copula. Two groups of experiments were performed.

In the first group three sets of females were used. All sets were

mated and the first was tested by encounters with males 1 hour after the termination of copula, the second was similarly tested 24 hours after copula and the third tested 3 days after copula.

The second experimental group comprised four sets of females. The first set was mated and each encounter terminated as soon as copula was achieved, i.e. when the male turned posterior to posterior with the female. In the second set the encounter was terminated after the pair had remained in copula for 1 minute. The third set were broken from copula after a delay of 2 minutes, and the fourth set prevented from continuing copula beyond 3 minutes. After copula had been terminated, a delay of 1 hour was allowed before each female was tested by an encounter with a different male.

All encounters took place in normal mating chambers and under conditions similar to those described as applying to experiments in previous sections. The females used were from 5 to 8 days of age and the males from 6 to 10 days of age.

RESULTS AND DISCUSSION

Tables 8-10 show the results of the tests on females allowed to complete copula. The start to mount times will be examined first. As can be seen the differences between the initial male response times and the subsequently tested male response times are in all cases statistically significant. Mating therefore, within 1 hour, significantly reduces pheromone production or pheromone release by females (Table 8). Male response delay-times appear to slightly increase in proportion with delay after mating, but whether this is due to reduction in pheromone production or the usual effect of increased female age is not clear. However, analysis of variance demonstrates that this reduction with delay is not statistically significant.

Table 14 shows a comparison of the results of initial and subsequent tests on a female mated for 3 minutes. Here again the male response delay-times are significantly reduced after mating. When this result is combined with corresponding results from the females allowed to complete copula and an analysis of variance is applied it is discovered that no significant difference exists between them. Such a result requires that the attractiveness-reduction mechanism should operate after an even shorter delay.

Reduction in attractiveness may be brought about by the operation of an inhibition either of a pheromone release mechanism or of the pheromone production process. The situation may or may not be complicated by the presence of a pool of pheromone. Barratt (1975) discovered that after mating the content of bioassayable pheromone in females dropped significantly. Her technique, however, did not show how long after mating the extractions were made; but the delay was probably at least 24 hours. If, as the present experiments show, reduction in female attractiveness occurs almost immediately after mating, any pheromone stored in a pool must be destroyed within 24 hours if the amount of extractable pheromone is to be reduced. It is simpler, however, to explain the facts without the necessity of postulating the existence of a pool. Since female attractiveness appears to be so swiftly reduced it seems more likely that a block occurs in release rather than production and a pool is not built up, and thus the extractable pheromone will also show a decrease. Pheromone production is probably reduced very soon after pheromone release.

The contrast is considerable between the results of subsequent encounters with females mated to completion of copula and for 3 minutes of copula when compared with similar results for females mated for 0, 1 and 2 minutes copula. Here the statistical difference is consistently insignificant. It may be deduced, therefore, that reduction in pheromone release probably occurs either because of the continuation of copula beyond 2 minutes or

because of some discrete event that occurs between the 3rd and 4th minute. A small supplementary experiment was carried out which tested this. Females were mated once and left in copula for 2 minutes; they were then mated again and this time left in copula for only 1 minute; subsequently they were tested again. The results of this experiment, giving the initial and last times only, are set out in Table 15. It can instantly be seen that females treated in this way induce highly variable reactions from males. These range from a complete lack of response to a slight increase in response speed. The difference between the two sets of results is not statistically significant, primarily because of the smaller number of replicates. Biologically, however, it does appear probable that a change is produced in the female not by some discrete action that takes place between the 2nd and 3rd minute of copula but by the effect of being in copula for the space of 3 minutes. This is even more evident when reference is made to the copula ability responses and will be examined below.

Mount to copula delay times are assumed to be indicative of the degree of receptivity of a female, since an optimally receptive female will presumably achieve copula within the shortest space of time and conversely an unreceptive female will not achieve copula at all. These results, as might be expected, parallel the results of response delay times. Combining the results from all groups of females allowed to complete copula it can be seen that a correctly mated female is not usually receptive to subsequent mating attempts; only 1 in 4 females permit re-mating. The results indicate the possibility that barriers to re-insemination are somewhat relaxed after a delay since the proportions increase from 1:15 1 hour after mating, to 1:5·3 after 1 day, to 1:3.6 after 3 days (Tables 8-10).

The females mated for 2 and less minutes show a lack of significant difference between their receptivity before and after copula. It is possible, however, that receptivity begins to be 'switched off' during these 2 minutes

TABLE 8: Start to mount and mount to copula delay times compared at initial mating and after an hour interval following the completion of copula.

Replicate number	Start to mount (sec)		log (1/mount to copula (sec)) x 1000	
	Initial	1 hour after finish copula	Initial	1 hour after finish copula
1	16	80	1·5527	0·0000
2	18	198	1·6571	0·0000
3	205	336	1·5855	0·0000
4	75	363	1·5378	0·0000
5	17	218	1·0569	0·0000
6	42	35	1·6776	0·0000
7	5	177	1·2672	1·0492
8	104	24	1·4564	0·0000
9	59	179	1·3284	0·0000
10	12	91	1·7443	0·0000
11	50	75	1·2068	0·0000
12	191	222	1·6776	0·0000
13	4	135	1·4814	0·0000
14	41	33	1·0086	0·0000
15	61	164	0·9685	0·0000
Mean:	60·18	155·33	1·4138	0·0699
Standard deviation:	62·57	103·66	0·2591	0·2709
t :	2·15		9·82	

TABLE 9: Start to mount and mount to copula delay times compared at initial mating and after an interval of 24 hours following completion of copula.

Replicate number	Start to mount (sec)		log (1/mount to copula (sec)) x 1000	
	Initial	24 hours after copula	Initial	24 hours after copula
1	73	100	1·3463	0·0000
2	22	305	1·5378	0·0000
3	20	58	1·5682	0·0000
4	6	19	1·6385	0·8261
5	7	20	1·3181	0·0000
6	25	388	1·2528	0·0000
7	40	141	1·0864	0·0000
8	72	166	1·3874	1·1248
9	6	25	1·5228	0·0000
10	23	52	1·5527	0·8633
11	84	172	1·0569	0·0000
12	77	143	1·1553	0·4914
13	7	175	1·6776	0·0000
14	185	190	1·5682	0·0000
15	6	12	1·2528	0·0000
16	5	90	1·5855	0·0000
Mean:	41·125	128·5	1·4067	0·2066
Standard deviation:	47:98	106:23	0·2018	0·3874
t :	2·12		7·77	

TABLE 10: Start to mount and mount to copula delay times compared at initial mating and after an interval of 3 days following the completion of copula.

Replicate number	Start to mount (Sec) Initial	3 days after copula	Log (1/mount to copula (sec)) x 1000 Initial	3 days after copula
1	49	18	1·5855	0·0000
2	7	10	1·6571	0·0000
3	16	30	1·3467	0·0000
4	75	87	0·8195	0·0000
5	16	70	1·2601	0·0000
6	6	3	1·4941	0·0000
7	80	210	0·9445	0·0000
8	5	177	1·5855	0·0000
9	10	194	1·6021	1·1303
10	9	66	1·8537	0·0000
11	9	60	1·5682	0·0000
12	16	186	1·5527	0·0000
13	15	112	1·4684	1·1139
14	75	40	1·3979	0·0000
15	5	109	1·1139	0·8129
16	6	77	1·7443	1·2355
17	4	82	1·1492	0·8195
18	73	89	1·8865	0·0000
Mean:	26·44	90	1·4461	0·284
Standard deviation:	28·94	64·27	0·2946	0·4805
t :	2·7		6·18	

TABLE 11: Start to mount and mount to copula delay times compared at initial mating and after an interval of 1 hour following disruption at start of copula.

Replicate number	Start to mount (sec)		Log (1/mount to copula (sec)) x 1000	
	Initial	1 hour after disruption at start of copula	Initial	1 hour after disruption at start of copula
1	11	40	0·5315	0·3979
2	30	27	1·8537	1·7694
3	181	296	2·0457	2·0457
4	10	58	1·2068	1·2601
5	205	110	0·5315	0·8261
6	18	26	2·1550	2·2219
7	13	38	1·7694	1·7959
8	8	4	2·0969	1·5092
9	77	172	0·5315	1·5302
10	21	13	2·3979	2·0000
11	33	13	2·0457	0·9777
12	27	18	2·3010	2·3010
13	75	101	1·6571	1·6021
14	10	30	1·5682	1·7694
15	44	32	0·5441	1·3766
Mean:	50·87	65·2	1·5491	1·5589
Standard deviation:	61·82	78·44	0·6986	0·5289
t:	0·4		0·03	

TABLE 12: Start to mount and mount to copula delay times compared at initial mating and after an interval of 1 hour following disruption of copula after 1 minute.

Replicate number	Start to mount (sec)		Log ((1/mount to copula (sec)) x 1000)	
	Initial	1 hour after disruption following 1 minute of copula	Initial	1 hour after disruption following 1 minute of copula
1	205	197	0·7076	1·0569
2	45	93	0·8751	0·5911
3	94	10	0·9494	1·5682
4	7	13	1·2430	1·4314
5	7	8	1·2014	1·4082
6	68	85	0·9590	1·2279
7	62	25	1·2601	1·7210
8	14	37	1·6201	1·0492
9	68	103	0·8865	0·0000
10	28	38	1·2219	0·6474
11	188	35	1·2219	1·1248
12	42	55	1·7694	1·4948
13	4	18	1·6776	1·5682
14	8	12	1·7447	1·7210
15	28	60	0·9395	1·1038
Mean:	57·9	52·6	1·2185	1·1809
Standard deviation:	62·6	50·62	0·3445	0·4756
t :	0·18		0·17	

TABLE 13: Start to mount and mount to copula delay times compared at initial mating and after an interval of 1 hour following disruption of copula after 2 minutes.

Replicate number	Start to mount (sec)		Log (1/mount to copula (sec)) x 1000	
	Initial	1 hour after disruption following 2 minutes of copula	Initial	1 hour after disruption following 2 minutes of copula
1	25	16	0·7076	0·5911
2	51	58	0·9494	1·2279
3	5	68	1·1553	1·6201
4	10	3	1·0492	0·0000
5	15	28	1·1875	1·0086
6	9	4	1·5855	0·0000
7	265	68	1·0000	1·3010
8	94	85	1·2430	1·7210
9	7	7	1·2014	0·6434
10	188	42	0·9031	1·3979
11	72	123	1·1732	1·3010
12	12	37	1·5081	0·0000
13	16	8	1·0086	1·6776
14	55	28	1·0645	1·2279
15	89	60	1·4564	1·1072
Mean:	60·87	42·33	1·1462	0·9923
Standard deviation:	75·15	34·62	0·2363	0·6045
t :	0·61		0·65	

TABLE 14: Start to mount and mount to copula delay times compared at initial mating and following an interval of 1 hour after the disruption of copula after 3 minutes.

Replicate number	Start to mount (sec)		Log (1/mount to copula (sec)) x 1000	
	Initial	1 hour after disruption following 3 minutes of copula	Initial	1 hour after disruption following 3 minutes of copula
1	18	217	0·7160	0·0000
2	44	44	0·9685	0·0000
3	38	203	1·1875	0·8388
4	5	103	1·1430	0·0000
5	25	37	1·0492	0·0000
6	15	38	1·1553	0·0000
7	9	28	1·3881	0·0000
8	35	55	1·6021	0·0000
9	19	174	1·2672	1·0212
10	108	191	1·2014	1·1931
11	17	20	0·9191	0·0000
12	29	35	1·3560	0·0000
13	38	159	1·4314	0·9590
14	11	82	1·0374	0·0000
15	47	43	1·2148	0·0000
Mean:	30·53	95·27	1·1758	0·2675
Standard deviation:	25·11	72·49	0·2224	0·4642
t :	2·31		4·83	

TABLE 15: Start to mount and mount to copula delay times compared at initial mating (disrupted after 2 minutes in copula) and then disrupted after 1 minute in copula then tested after an interval of 1 hour.

	Log ((1/start to mount (sec)) x 1000)		Log ((1/mount to copula (sec)) x 1000)	
Replicate number	Initial	after 2 interrupted encounters	Initial	after 2 interrupted encounters
1	0·6021	0·0000	0·8451	0·0000
2	1·2787	0·0000	1·1614	0·0000
3	1·1553	1·2068	1·1523	0·8388
4	1·4440	0·9868	1·1875	0·0000
5	1·6776	1·4200	1·0000	0·0000
6	2·0000	2·0969	1·3284	0·0000
7	0·9590	1·2601	1·2923	0·0000
8	2·0457	1·7447	0·9031	0·0000
Mean:	1·3953	1·0894	1·1088	0·1048
Standard deviation:	0·5016	0·7541	0·1758	0·2966
t :	0·67		5·82	

(the concept of a 'switch-off' is discussed below). This may be deduced from the fact that the proportion of receptive to unreceptive females increases from 0:15 after 0 minutes in copula, to 1:15 after 1 minute in copula to 1:5 after 2 minutes (Tables 10-12). After 3 minutes in copula the receptive to non-receptive female proportions (tested after 1 hour) are 1:3·75 (Table 14). This is less than that in full-term-copula females tested 1 hour after completion of copula, suggesting that receptive behaviour is not fully switched off by 3 minutes in copula. Table 15 demonstrates that it is possible to switch off receptive behaviour by a total of 3 minutes in copula taken in two discrete sessions of 2 minutes and 1 minute respectively. This confirms the earlier suggestion that it is the cumulative effect of time in copula which results in the observed changes.

In order to remove any doubt about the inter-experimental-group variability an analysis of variance was carried out between the combined initial start to mount times of all the groups. Variation was statistically insignificant.

GENERAL DISCUSSION AND DISCUSSION OF THE STIMULUS FOR POST-MATING PHEROMONE REDUCTION.

It can be deduced from the above results that female *S. paniceum* are predominantly monogamous; not permitting further copulation in 4 cases out of 5. It follows that in order to prevent males wasting effort by endeavouring to copulate with non-receptive females it is important that female pheromone production and release should be curtailed. This would have the effect of rendering mated females relatively less attractive than virgin females. Reduction in pheromone activity after mating has been noted in other monogamous Coleopteran species, for instance *Agriotes*

litigiosus (Le Conte) (Elateridae) (Ivashchenko and Adamenko, 1971). The same is true in other insect groups, for instance Barth and Bell (1970) note that in mated *Byrsotria fumigata* (Guerin) (Dictyoptera; Blaberidae) pheromone production is drastically curtailed while in *Adoxyphyes fasciata* the pheromone titre is reduced 120 times after mating (Nagata et al, 1972). Barratt (1975) discovered that the amount of extractable pheromone in female *S. paniceum* was reduced after mating, and the present results, as previously discussed, show that the total attractiveness of females was reduced after mating. It is interesting to note, however, that male response is not completely curtailed and that once having responded a male will endeavour to copulate with a non-receptive female; sometimes these non-productive encounters will continue, in the mating chambers, for up to 40 minutes. Obviously this sort of behaviour is non-adaptive, in that it prevents males from seeking out virgin females. It is therefore postulated that protracted futile mating attempts take place only in the unnatural confines of the mating chambers where no other females are present to distract the male. Further discussion on this subject is contained in Section A, part 2c. The female of *S. paniceum* indicates her lack of receptivity simply by not raising her 6th abdominal tergite - thus preventing the male from inserting his aedeagus (fig. 3c). Other female insects may indicate their unwillingness to mate by a signalling system or by physical repulsion (Engelmann, 1970). It was suggested in the previous chapter that the maturation of pheromone production and female receptivity are related in *S. paniceum* and are in some way connected with ovarian maturation. This is reasonable if all three phenomena are facets of a 'readiness to copulate' syndrome that is initiated by the secretion of a hormone produced in the corpus allatum (Engelmann, 1970; Hartmann et al, 1972). If such is the case it is probable that reduction in female attraction and switching off of female receptivity after mating are similarly connected.

They may also, like the initiation of copula-readiness, be mediated through the endocrine system as a result of some stimulus received during courtship or copula.

Barrass (1976) discusses the inhibitory effect that male courtship, even without copulation, may have on a female insect's later behaviour. In *S. paniceum*, however, it appears that such courtship as there is has little effect on the subsequent readiness of a female to mate, as may be demonstrated by reference to the results obtained with second encounters with females separated after 2 minutes or less in copula. For similar reasons the mechanical stimulus of the mounting can be discounted, although it is this that provides the switch for refractory behaviour in *Lariophagus distinguendus* (Först) (Hymenoptera; Pteromalidae) (Van dem Assem, 1970). In female *Drosophila* spp. receptivity is turned off initially by the mechanical stimulus associated with copula. This however is only a short term effect (lasting for 2 hours) and subsequently a long term change is caused by the sperm (Manning, 1967) and a secretion of the paragonial glands (Merle, 1968). It is these three factors which are the most common switch for refractory behaviour and will be considered in more detail.

The experiments described in this chapter have demonstrated that female receptivity is switched off by the cumulative action of at least 3 minutes of copula. During that three minutes it is possible that the effective stimulus is provided by: the physical presence of the inserted aedeagus, the passage of sperm/spermatophore or the passage of a secretion of the male accessory glands (see Section B2).

If it was the simple mechanical stimulus of the aedeagal insertion into the female that caused refractory behaviour to be switched on, one would deduce that the response would be less variable than in fact it is. In addition the removal of the stimulus should rapidly cause waning of the response.

Therefore the application of the stimulus at two discrete times as in the supplementary experiment should fail to operate the switch. Since such was not the case it is postulated that the insertion of the aedeagus is probably not, of itself, the mechanism involved.

In many insect species it is the passage of the sperm or spermatophore which provides the refractory stimulus; instances may be given of *Nauphoeta cinera* (Olivier) (Dictyoptera) (Roth, 1962) and *Rhodnius prolixus* (Stal.) (Homoptera) (Davey, 1965). In *S. paniceum*, however, the ratio of fertile females to infertile females after 3 minutes in copula is 1·86:1 (see Section B2). Whereas the ratio of unreceptive females to receptive females after 3 minutes copula is 3·75:1. Since these ratios do not coincide it is probable that in this species the passage of sperm is not directly responsible for the initiation of female refractory behaviour.

In *Aedes aegypti* (L.) although the immediate switch-off of receptivity is neural in action and is due to the filling of the bursa copulatrix with seminal fluid (Gwadz and Craig, 1970; Gwadz et al, 1971), the long-term switch-off is achieved by the action of 'matrone', a substance produced by the male accessory glands (Craig, 1967). A similar effect of male accessory gland substance is noted in House-flies (Riemann et al, 1967; Riemann and Thorson, 1969) and the cabbage-root fly (*Hylemya brassica*) (Swailes, 1971). *S. paniceum* males possess 2 pairs of well-marked accessory glands (Section B2) and hitherto no function has been described for them. Since the passage of sperm and the insertion of the aedeagus are not likely to operate as switches to refractory behaviour, it is postulated that this switch is operated by a secretion of the accessory glands. These glands, to date, have not been assigned a function and the above possibility appears worthy of further investigation.

SECTION A. PART 2a. Mating behaviour

INTRODUCTION

"In the life of a sexually reproducing organism there is no single act more important in an evolutionary sense than copulation." (Barth and Lester, 1973). In spite of this it is probable that in less than 0·1% of known insect species have copula and copulatory behaviour been observed and published (Wojcik,1969).

In *Stegobium paniceum* descriptions of the copulatory behaviour have been published by Janisch (1923), Azab (1943), Kashef (1956) and Barratt (1975). These descriptions, however, are somewhat superficial and lack a quantitative analysis. It is the purpose of this section to analyse the pre-copulatory behaviour of the male and female and to present an ethogram for male 'courtship' behaviour.

METHOD

Encounters between virgin male/female pairs at ages around the optimum for responsiveness, attractiveness and copulatory ability were studied individually in the normal mating chambers.

A number of pairs were first observed in order to become acquainted with the general features of behaviour before, during and after copula. Once the behavioural units had been clearly defined it was possible to observe behaviour during an encounter while simultaneously recording a commentary on a portable cassette tape recorder. This recording was subsequently transcribed onto paper and the intervals

between each behavioural unit were timed with a stopwatch. Some 200 encounters were recorded in this way.

RESULTS

Eleven main units were observed in the pre-copulatory behaviour of male *S. paniceum*. These are described in order of occurrence and are labelled A - L as below:

A: Male antennal elevation, extension of fore-legs causing the anterior of the body to be raised (Fig. 3A).

B: Extended male antennae touch female elytra.

C: Male mounts female. He may mount from any direction but most frequently from the rear (see next chapter).

D: Once mounted, with all 3 pairs of legs usually placed on the female elytra, the male begins to palpate the female, usually at the junction of the elytra with the pronotum and on the disk of the pronotum. Both pairs of palps are used with varying intensity of action and the head is usually stretched forward and oscillated from side to side (Fig. 3B). Palpation is occasionally accompanied by a 'chewing' motion of the mandibles.

E: Describes an activity consisting of a swift alternating vibration of the left and right male antennae against the margin of the female pronotum and elytra (Fig. 3B). This action, in general, is temporally associated with D. Both D and E can occur in isolation but this is unusual - observed in less than 10% of encounters.

F: The male extrudes his aedeagus - the complex structure consisting of the penis and accessory organs (see next chapter).

G: The male reverses, backing away from the elytral-pronotal junction. This action is not frequently marked enough to be easily observed; it seems, in general, to be necessary only when the male is considerably smaller than the female.

H: Using his aedeagus the male touches, and pulls at, the 5th female abdominal tergite which is 'hinged' at the anterior margin (Fig. 3C).

J: The male inserts his aedeagus under the female 5th abdominal tergite. Successful insertion appears to occur only if the aedeagus is pushed under the left-hand side of the tergite. After insertion the male right-hand paramere is often still visible.

K: The male dismounts from the female, his aedeagus still inserted. Dismounting is always to the right-hand side of the female. Within seconds of dismounting the male posterior is pressed close against the posterior of the female, his tergite covering hers (Fig. 3D).

L: Describes a period of quiescence in the male which can occur at almost any stage of pre-copula behaviour. During quiescence the male remains completely motionless. Occasionally the antennae are folded under the body, but if they are not a slight vibration may be detected in them.

There are other units which occur in less than 10% of encounters. The most common of these is a scrabbling motion of the male fore and mid tarsi over the elytra of the female. Another series of units are to be noted when the male, usually mounting from the front of the female, begins his pre-copulatory activity facing towards the female posterior. After a number of attempts at achieving copula the male will usually make a 180^{o} turn

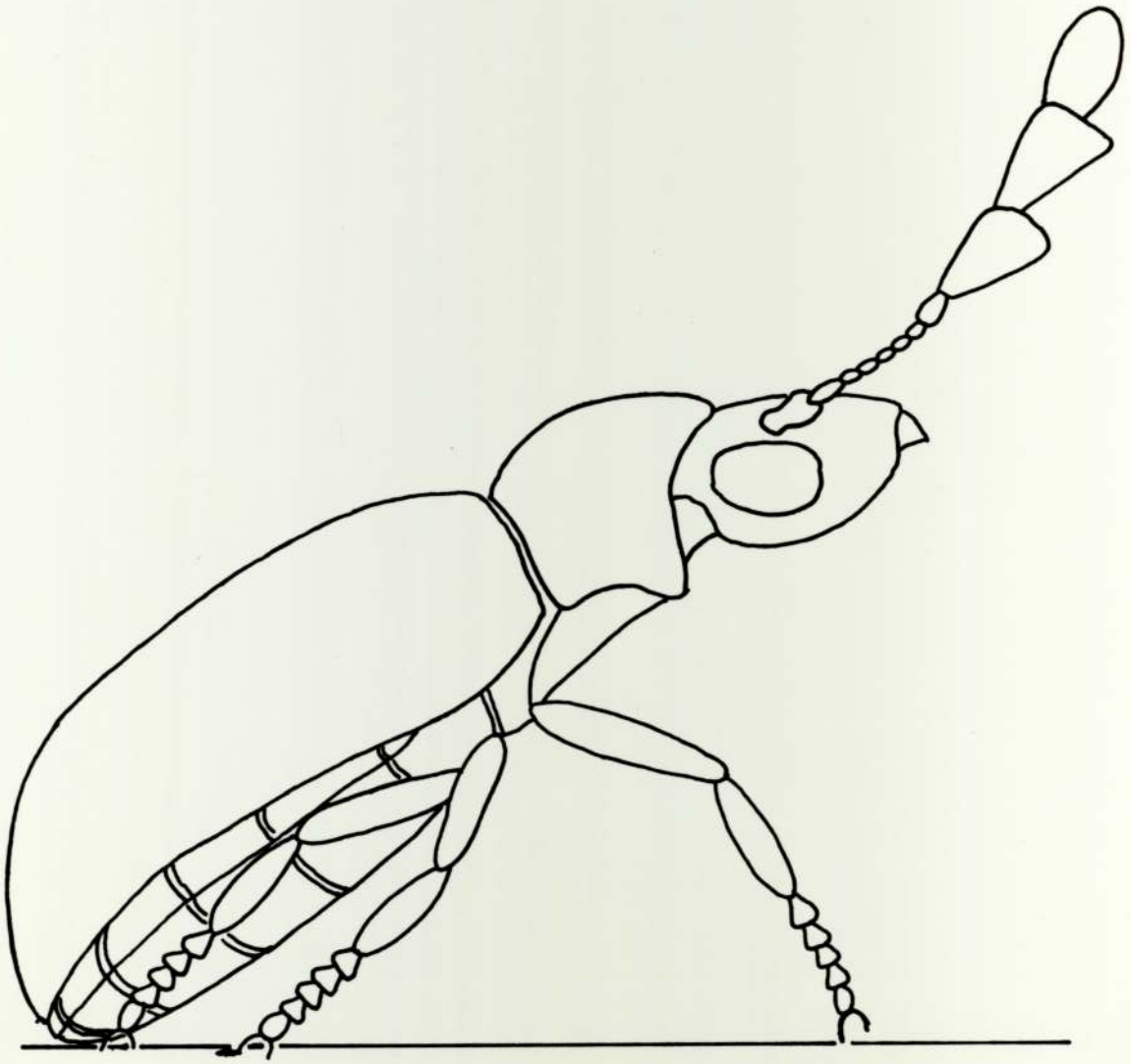
FIGURE 3 : A: Male precopulatory behavioural unit "A".

B: Male precopulatory behavioural units "D" and "E".

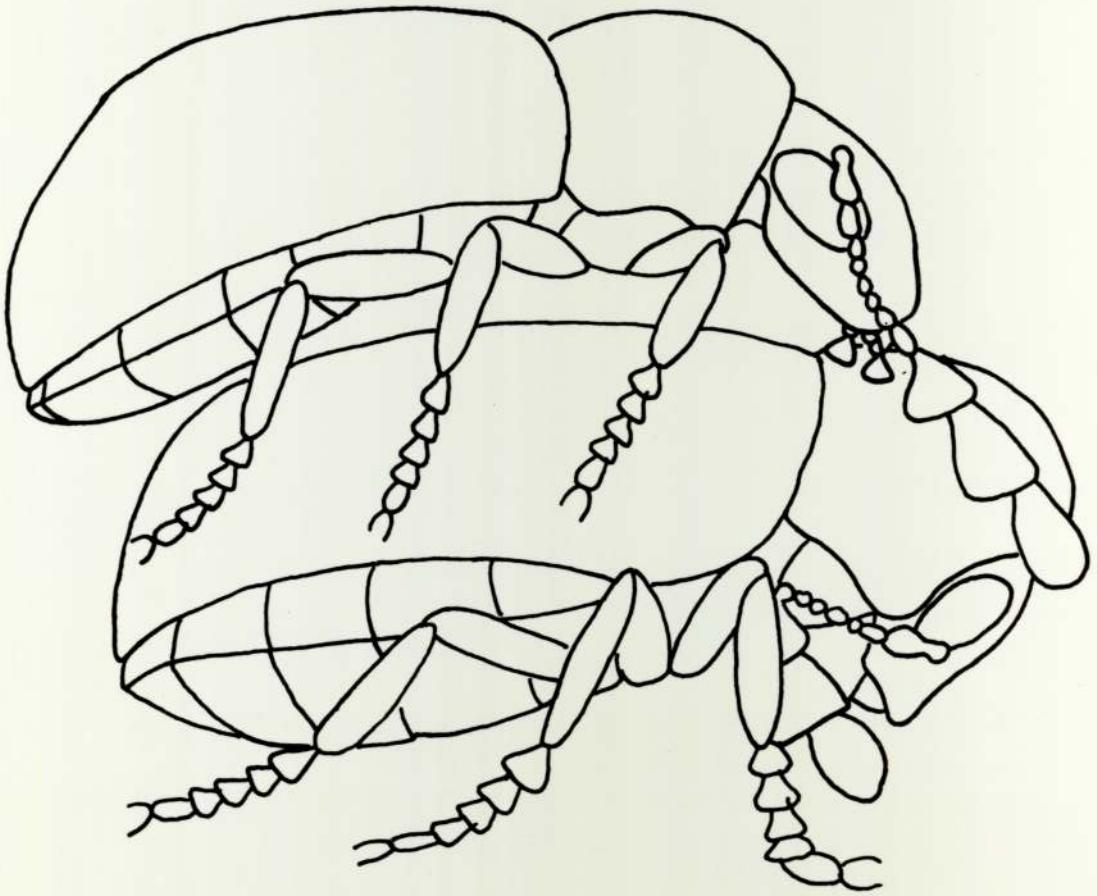
C: Male precopulatory behavioural unit "H".

D: Male precopulatory behavioural unit "K".

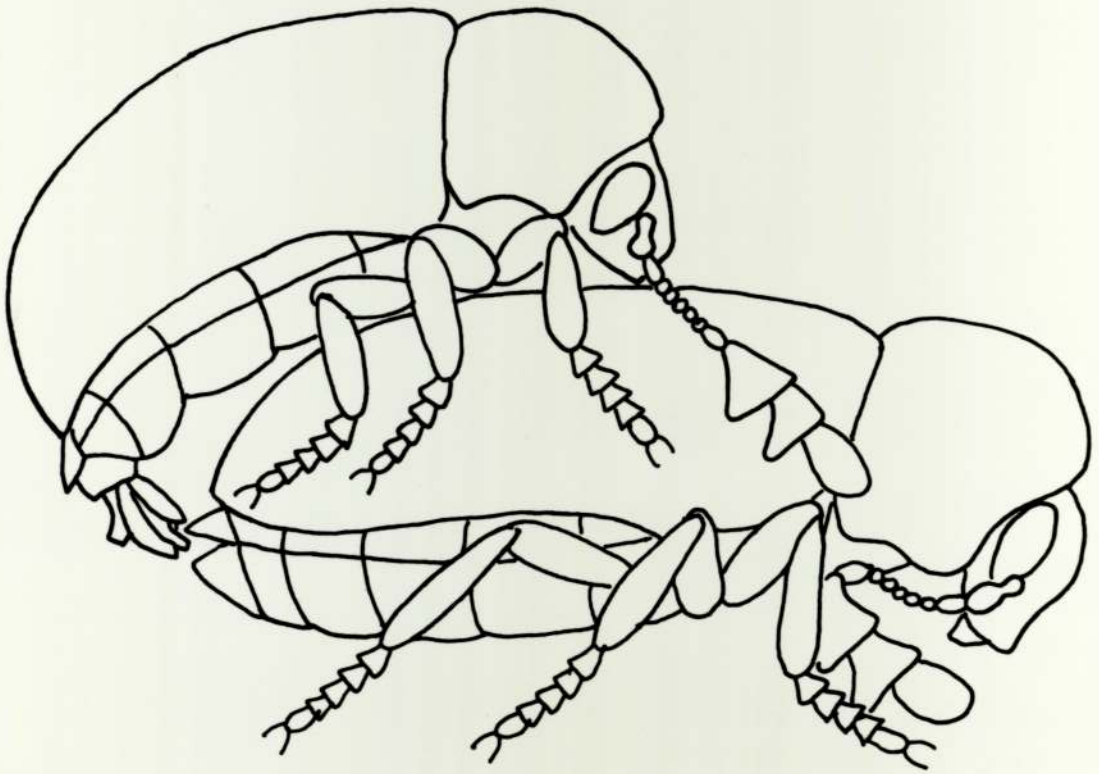
A



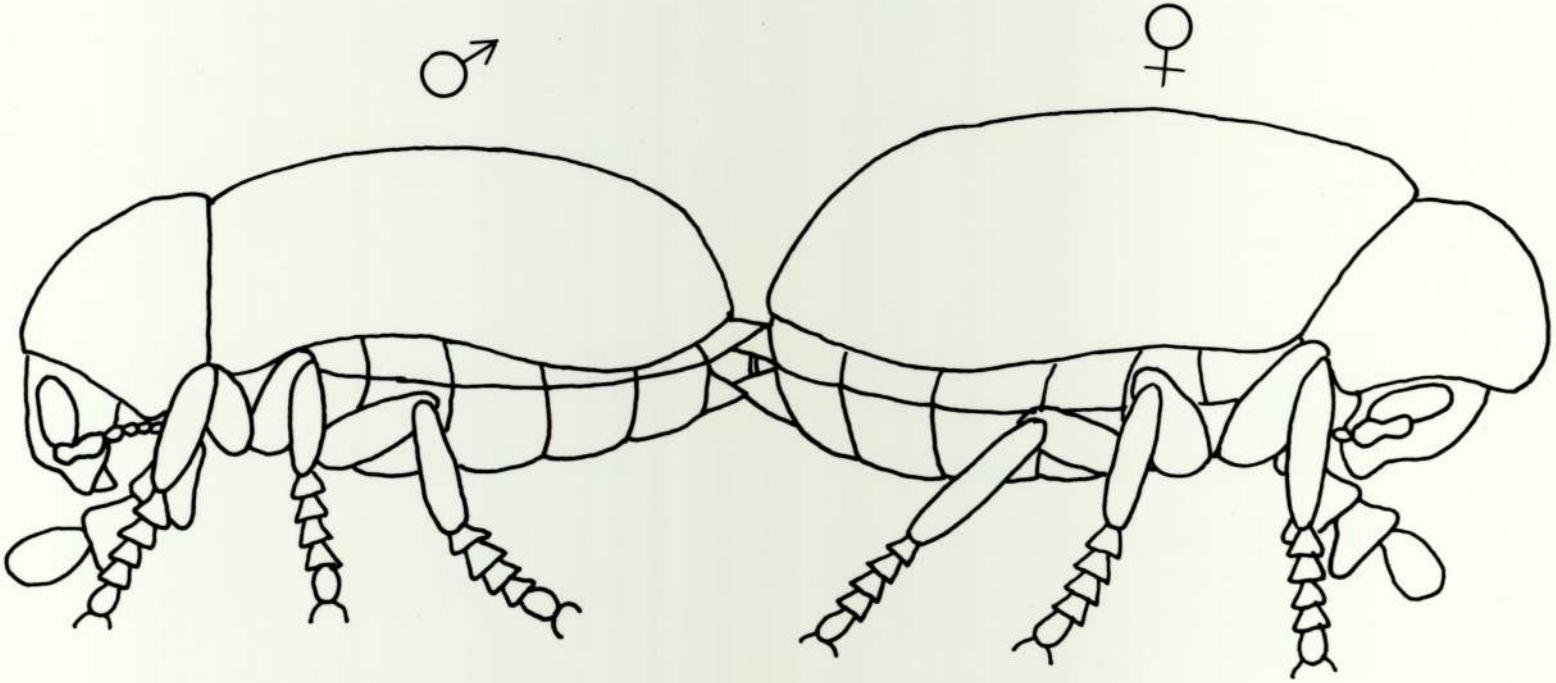
B



c



D



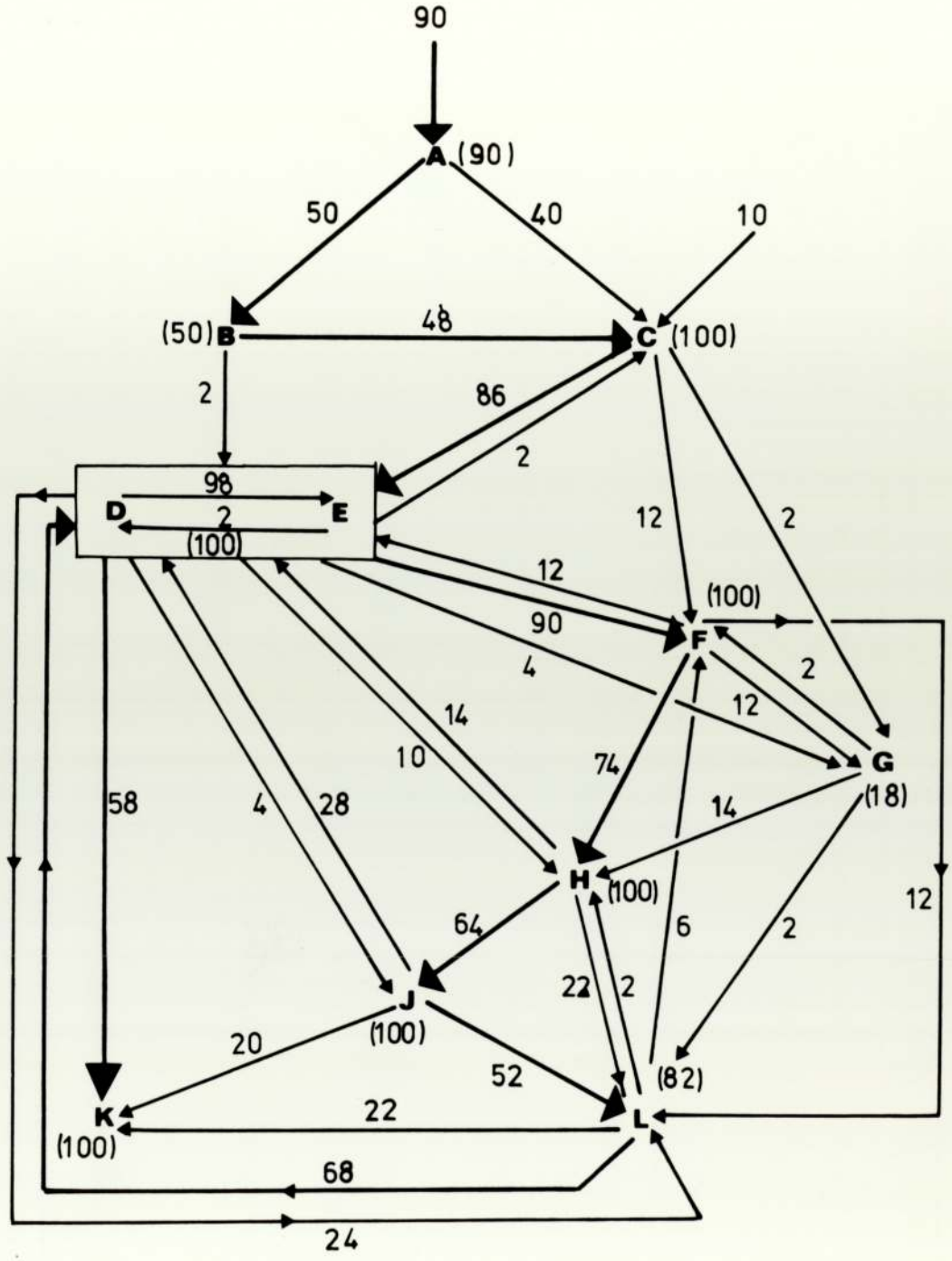
and begin again facing in the opposite direction. This action is discussed in detail in the next chapter.

Other occasional events occur such as: the male falling off the female, the male pumping his abdomen and the male slightly opening his elytra. Using the methods described in Fagen and Goldman (1977) however, it was possible to determine that units A - L constitute an essentially complete catalogue of male pre-copulatory behaviour in *S. paniceum*.

Using the data from 50 encounters leading to copula selected at random, the ethogram shown in Fig. 4 was constructed. This shows a chronological flow diagram of male behaviour units A - L for the 50 encounters analysed. Each unit letter is accompanied by a figure in brackets; this shows the percentage of the 50 males which performed that action. The arrows which connect units are accompanied by figures not in brackets. These indicate the percentage of males which, after performing the unit at the base of the arrow, went on to perform the unit at its point. D and E are considered as 1 unit.

The heavy arrows indicate the course that will most probably be taken by any encounter. After antennal elevation the male will go on to touch the female elytra with his antennae and then to mount her. In a few encounters the male mounted apparently without performing the preliminary actions; this was usually because, having fallen on his back, he climbed onto the female to right himself and subsequently went on to perform copula. After mounting the male will palpate and antennate and then he will evert his aedeagus, going on to touch the female tergite with the everted aedeagus, usually still palpating and antennating. Usually aedeagal insertion occurs next and after this it is probable that the male will become quiescent. After a variable period of quiescence the male will probably recommence palpating and antennating. The cycle of quiescence

FIGURE 4 : An ethogram showing the percentage probabilities of the succession of units in male pre-copulatory behaviour. Figures in brackets next to letters represent percentage of males performing the relevant unit. Unbracketed figures next to letters represent percentage of males pursuing that pathway. Analysis of 50 encounters.


90
A (90)
50
40
10
(50) B
48
C (100)
2
86
2
98
D
2
E
(100)
12
2
12
(100)
90
F
4
2
12
14
10
74
G
(18)
58
4
28
14
H
(100)
12
64
6
2
22
2
J
(100)
20
52
(82)
K
(100)
22
L
68
24

and palpating/antennating activity may occur several times before eventually the male goes on to achieve full copula. The mean number of units in each encounter is 21·86 with a standard deviation of 15·36. Copula lasts about an hour (mean of 50 encounters = 61 minutes, Standard deviation = 18·54).

It can be deduced from the above that the repertoire of externally observable female behaviour is strictly limited. As far as can be seen all that is required of the female is that she should become quiescent when the male mounts, usually folding her antennae under her body (Fig. 3B) and that she should open her 5th abdominal tergite to allow the male to insert his aedeagus (Fig. 3C). The termination of copula, however, is usually initiated by the female who pulls away from the male.

DISCUSSION

Pre-copulatory or courtship behaviour by male insects seems to serve one or more of the following functions: sex recognition, overcoming behavioural barriers to mating or preventing interspecific mating (de Wilde and de Loof, 1973).

Closely related sympatric species, unless they have seasonally separated reproductive periods, may require behavioural cues to provide reproductive isolation mechanisms (Engelmann, 1970). *S. paniceum* has no closely related species - taxonomically it is the only member of its genus. However, being a stored food pest, it is sympatric to many other stored-product Coleoptera of similar size, shape and colour. To remain reproductively distinct from these species, male *S. paniceum* must be capable of identifying con-specific females by the use of cues which may be visual, tactile, olfactory, gustatory, behavioural or a combination of these.

In this chapter we are concerned with the cues given by mating behaviour and it is therefore instructive to compare these, as described here for *S. paniceum*, with descriptions of mating behaviour in other insects. In fact examination of these descriptions shows that behaviour in such widely diverse species as *Lasioderma serricorne* (Tobin and Smith, 1971; Coffelt and Burkholder, 1973), *Anthrenus flavipes* Le Conte, *Trogoderma glabrum* (Herbst), *Tribolium castaneum* (Herbst), *T. confusum* du Val, *Tenebrio obscurus* F., *Oryzaephilus mercator* (Fauvel), *Cryptolestes pusillus* (Schonherr) and *Sitophilus granarius* (L.) (Wojcik, 1969) all possess elements in common with *S. paniceum*, notably antennation and palpation. Since these cues are so commonly found throughout these species it is unlikely that they can have the effect of allowing a female to identify a conspecific male. To test the sensitivity of female *S. paniceum* to tactile stimulation of the elytra several live females were stroked with a soft paint-brush. In the great majority of cases the females responded by raising their 5th abdominal tergite, which, as we have seen, is apparently the way in which the female signals her willingness to mate. In view of this positive response to such a gross stimulus it seems unlikely that during courtship in *S. paniceum* the female receives a stimulus that is characteristic enough to enable her to detect a conspecific. It is still possible, however, that palpation and antennation overcome the resistance of a female to mating.

If mating behaviour, as seems likely, does not provide the barrier to inter-specific insemination it remains to postulate what this barrier can be. Although on the present evidence it is impossible to make any categorical statement, it seems likely that the barrier is provided by pheromone systems peculiar to each separate species. As Engelmann (1970) points out - many sympatric species have a high threshold of response to foreign pheromone.

Although olfactory cues may be used by the male to detect conspecific females at a distance it seems highly likely that the behaviour of the male during courtship is designed to enable him to receive tactile, gustatory and possibly visual cues about the female. It is probable that these cues will enable a male to detect a conspecific, ensure that he is mounted on a female rather than a male, orientate correctly and discover the female state of receptivity. This subject is examined in the next chapter.

SECTION A. PART 2b. Signals and structures used in mating.

INTRODUCTION

Despite extensive morphological and biometrical studies by Kashef (1956) and Monteiro (1957) no detailed description has yet been published of the palps, tarsi and aedeagus of *Stegobium paniceum*. Barratt (1975) has, however, published stereosean photomicrographs of the antennae and their sensilla. This chapter is initially concerned with a description of the male palps, tarsal claws, aedeagus and for the sake of completeness also includes a description of the antennae. The function of these organs and their relation to the female is then linked to an understanding of their involvement in the mating behaviour of the insect as described in the previous chapter. A synthesis of this nature has few parallels in entomological literature and yet seems vital in promoting an understanding of the whole life of the insect.

METHOD

Specimens of male tarsi and female elytral setae were separated from freshly-killed beetles; cleared in xylene and mounted in Deepex. Measurements were made using an eyepiece micrometer. Photomicrographs were taken.

Whole individuals were prepared for stereosean electron microscopy by freeze-drying and sputter-coating with either a silver or aluminium oxide layer (Echlin, 1971). Stereoscan photomicrographs of the antennae (fig. 5A, B), palps (fig. 6A-C) and tarsi (fig. 7G, H) were taken by courtesy of the

Department of Metallurgy, University of Aston.

Stereoscan photomicrographs were also made of the male aedeagus (fig. 8). Male beetles were killed and pressure was applied to the abdomen in order to cause aedeagal eversion. The abdomen was then excised and immediately stuck to a stereoscan-stub with the aedeagus still everted. The specimen was then prepared in the normal way.

Drawings were also made of the everted aedeagus of freshly-killed males examined under a binocular microscope (fig. 9A-D).

In the Section 'A discussion of the morphology and function of the male organs in relation to mating behaviour', evidence is drawn from observations of the orientation of males with respect to females during the normal mating encounters previously described. In addition an analysis is included of the influence of the wax-covering of females on the post-mounting behaviour of males. The nature of this experiment has already been described in Section A1a.

The Antennae.

Fig. 5A shows the appearance of the antennae under the Scanning electron microscope, the concentration of sensory hairs (sensilla) on these organs can clearly be seen (the final joint of the antennal club is missing on this specimen). The parts of the antennae are shown diagrammatically in fig. 5C and are labelled (from left to right): Sc - scape, fn - funicle, cb - club. Fig. 5B shows the second joint of the antennal club. In this photomicrograph the sensilla can more clearly be seen.

Classically 6 forms of externally identifiable sensilla were recognised (Dethier, 1963). In this chapter however, only three forms are discussed: the setiform variety (sensilla trichodea), the peg-like or conic variety (sensilla basiconica) and the bristle-like variety

(sensilla chaetica). The two latter forms have been demonstrated to have a chemoreceptive function in a wide range of insect orders (Hodgson, 1974) as well as in the Coleoptera; for instance in *Tenebrio molitor* (Harbach and Larsen, 1977), two species of the Cerambycidae (Dyer and Seabrook, 1975) and the pine weevil (*Hylobius abietis*) (Mustaparta, 1973). Sensilla chaetica - short, tapering, bristle-like sensilla - appear to be widespread on the antennae and can be seen in fig. 5B. Also present in fig. 5B are peg-like sensilla basiconica which are less widespread and appear to be concentrated in specific areas on the joints of the antennal club. These areas Barratt (1975) has designated as sensory fields. The positions of these fields are indicated by the letters Sf in fig. 5C.

On the evidence of an analysis of these structures and the results of antennectomy experiments, Barratt (1975) concluded that it was the male antennae which played the major role in detecting female pheromone. Since the antennae are thus used by the male it seems possible that secondary sexual differences may occur between the male and female antennae. Sexual dimorphism occurs in the number, type and distribution of antennal sensilla in several coleopteran species; for instance *Tenebrio molitor* (Harback and Larsen, 1977), the pecan weevil *Curculio caryae* (Horn) (Hatfield et al, 1976) and the Chrysomelid *Diabrotica virgifera* L. (Staetz et al, 1976). In Dictyoptera of the genus *Periplaneta* differences in the number of gustatory and olfactory antennal sensilla have been connected with the use to which these are put by the male in mating behaviour (Schafer and Sanchez, 1976; 1976a). In *S. paniceum*, however, Barratt (1975) was unable to detect any marked differences between male and female antennal sensilla; she indicates however the possibility that the male antennal club joints are relatively longer than those of the female. This difference may well be connected with the use of the antennae as pheromonal detectors by the male.

In addition to the chemosensory sensilla the antennae are also invested with large numbers of long, tapering, socketed sensilla trichodea which project beyond the reach of the smaller chemosensory sensilla. Sensilla trichodea are probably mechanoreceptive in function (Rice, 1975) - they can be seen in fig. 5A, B. When viewed under the binocular microscope (fig. 5C) a pair of extremely long sensilla trichodea (St) can often be seen projecting downwards from each joint of the antennal club (cb) and from alternate joints of the antennal funicle (fn). Again there is no readily apparent difference between male and female with respect to the number and distribution of antennal sensilla trichodea.

It has already been shown in the previous chapter that males consistently use their antennae during mating behaviour, beating them against the female elytra and pronotum; it seems quite likely therefore that the stimulus provided by this action is playing a part in causing the successful completion of courtship. In this, as in many other instances of insect mating behaviour (Engelmann, 1970), it is difficult to distinguish between contact chemoreception and tactile stimulus reception. However, although olfactory chemoreceptors are almost certainly present on *S. paniceum* antennae, neither the present study nor that of Barratt (1975) was detailed enough to demonstrate clearly the existence of gustatory sensilla. In view of this and also the fact that the length of the sensilla trichodea would preclude the much shorter gustatory sensilla from coming into contact with the female, it is concluded that, for the male, antennation provides cues which are primarily tactile in nature. What these cues are and what information the female receives from the male during antennation are discussed below.

FIGURE 5 : The Antennae

A : General view (last joint of club missing)

Scale line = 0·1 mm

B : Second joint of club

Scale line = 0·05 mm

C : Diagram of whole antenna

sc = scape

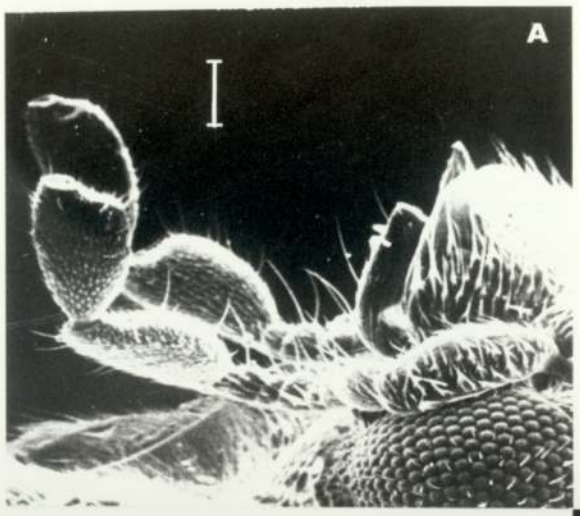
fn = funicle

cb = club

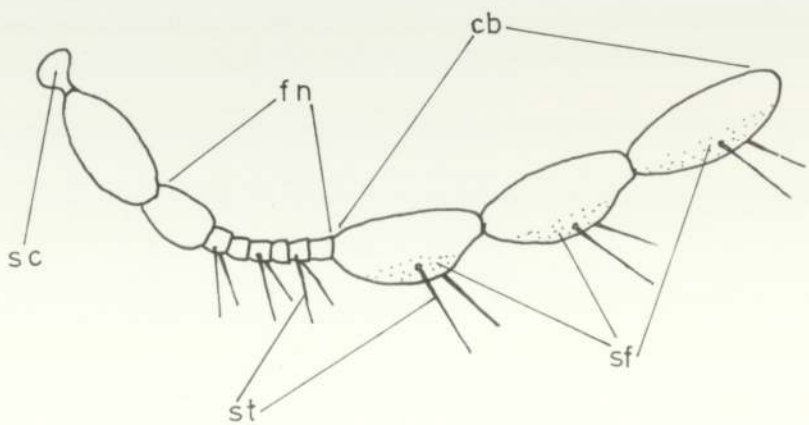
st = sensilla trichodea

sf = sensory field

Scale line = 0·1 mm



C



The Palps.

Fig. 6A shows the left-hand maxilliary palp (mp) and labial palp (lp) of a male *S. paniceum* in situ. As with the antennae sensilla of several types can be seen. Along the edge of the palp, however, only 1 type of sensillum is apparent; these are peg-like structures which are probably sensilla basiconia (Dethier, 1963). As can be seen from fig. 6B these sensilla are arranged in a line with a crown-shaped concentration (cr) at the outermost angle of the palp. This arrangement can more clearly be seen in fig 6C which also demonstrates that these sensilla are isolated in an area devoid of other sensilla.

Fig. 6A-C are each from a different specimen demonstrating the consistency of this arrangement of sensilla; 6B is of a labial palp and 6C of a maxilliary palp - showing that both have similar features. Neither was any difference found between this arrangement of sensilla in the male and the corresponding arrangement in the female.

Basiconic sensilla of the type present on the palps of *S. paniceum* adults may be either olfactory or gustatory in function. In Coleoptera olfactorily sensitive sensilla basiconica tend to have thin walls and a single apical pore (Harbach and Larsen, 1977). Unfortunately this study is not detailed enough to determine which type of sensilla is present on the palps of *S. paniceum*. However, since insect mouthparts are usually associated with contact chemoreception (Dethier, 1963), it is probable that a similar function is to be deduced for the sensilla basiconica of *S. paniceum*.

During mating behaviour the male performs an action described in the previous chapter as 'palpation' - a movement of the tips of the palps across the base of the female elytra and the female pronotal disk (fig. 3B). During this activity the sensilla basiconica described above would be brought

FIGURE 6 : The Palps

A : General view

Scale line = 0·05 mm

B : Labial palp

Scale line = 0·05 mm

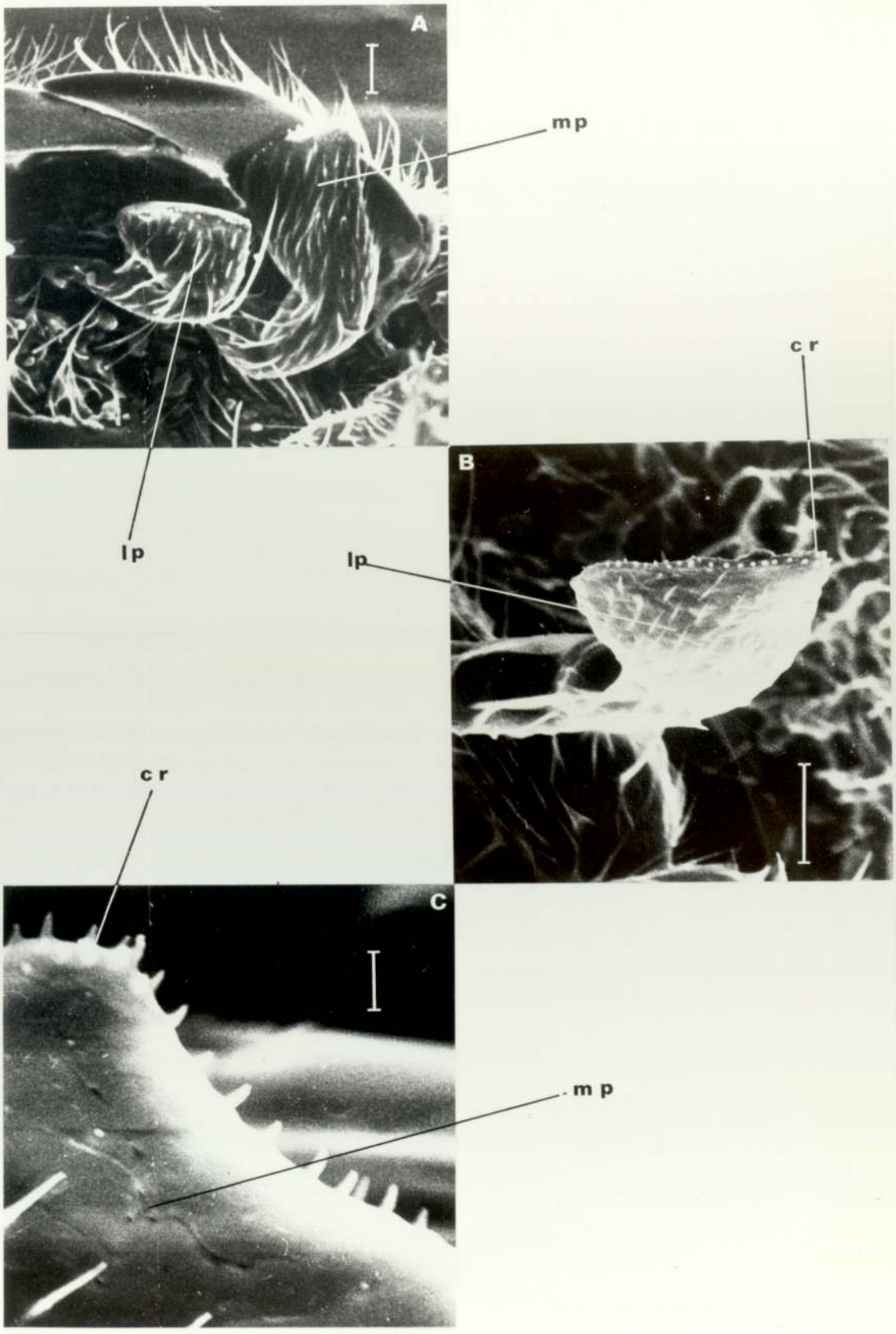
C : Maxilliary palp

Scale line = 0·002 mm

mp = maxilliary palp

lp = labial palp

cr = crown shaped concentration of sensilla basiconica


A
mp
lp
B
cr
lp
cr
C
mp

into contact with the surface of the female, implying that the male is receiving chemosensory stimuli from the female. Since adult *S. paniceum* do not feed (e.g. Lefkovitch, 1967) it seems probable that the sensilla basiconica present on the palps are used by the male primarily during mating. The nature of the stimulus received and whether by this means the male gives a stimulus to the female is discussed below.

The Tarsal Claws

Microscopic examination of male and female claws under high magnification revealed that although the tarsal segments are similar in proportion and detail in the two sexes, the male claw bears a distinct slot-like structure on each of its two elements.

Photomicrographs were taken to confirm this observation: Fig. 7A-C shows pro-, meso- and meta-thoracic tarsal claw elements of different male *S. paniceum*. These should be compared with the corresponding female claws shown in fig. 7D-F. Electron stereoscan photomicrographs show this difference more clearly (fig. 7G, H). This is the first secondary sexual character to be reported in adult *S. paniceum* (Ward and Humphries, 1977).

In animals where there is no parental care of offspring, a secondary sexual character generally plays a significant part in mating behaviour. The claw-slot function is therefore likely to be related in some way to the female elytra with which, as has been shown in the previous chapter, the male tarsi make contact during courtship.

Comparison of figures of the female elytral setae (fig. 7I-K) with those of the male claws at a corresponding magnification shows a close correlation between the diameter of cross-section of the setae and the width of the slots in the male claw. The average width of 23 slots

at their widest point was 2·3 micron. The slots are fairly constant in width for the distal two thirds of their length and constrict proximally to a rounded end. Measurement of 20 setae showed that they taper from a mean basal width of 3·2 micron to a mean tip width of less than 0·5 micron; the mean mid-point width being 2·4 micron. The implication is that the distal halves of the setae may become inserted into the slots.

During amplexus a seta entering the slightly tapering groove will become trapped if the male pulls with his claws in such a way as to distort the seta; this has been demonstrated by the use of a simple model. It is also in accordance with the observation that there is occasionally a delay when the male dismounts from the female during which there are claw movements which give the impression that the claws are being disengaged. The setal-trapping model may also serve to explain the 'scrabbling' movement of the fore- and mid-tarsi mentioned in the previous chapter and also noted by Barratt (1975). Such scrabbling may be an effort on the part of the male to ensure that the claws become properly engaged with the setae.

As described in the previous chapters a male can remain mounted for some time before achieving copula and during this time the female is not necessarily completely quiescent; the male is therefore at risk of being brushed off the female's back. It seems that the simplest explanation of the tarsal-claw slots is therefore to be found in the mechanical action of trapping the female elytral setae and thus improving the security of the male's position. In this respect the action is similar to a mechanism found in some ectoparasites such as fleas (Siphonaptera), bat-flies (Nycteribiidae), bat-bugs (Polyctenidae) and the beaver-beetle (*Platypsyllus*) where the spacing of the elements of the combs (Ctenidia) corresponds to the diameter of the host-hairs (Humphries 1966, 1967). The Ctenidia, like the

FIGURE 7 : The Tarsal claws

A : Male prothoracic tarsal claw

B : Male mesothoracic tarsal claw

C : Male metathoracic tarsal claw

D : Female prothoracic tarsal claw

E : Female mesothoracic tarsal claw

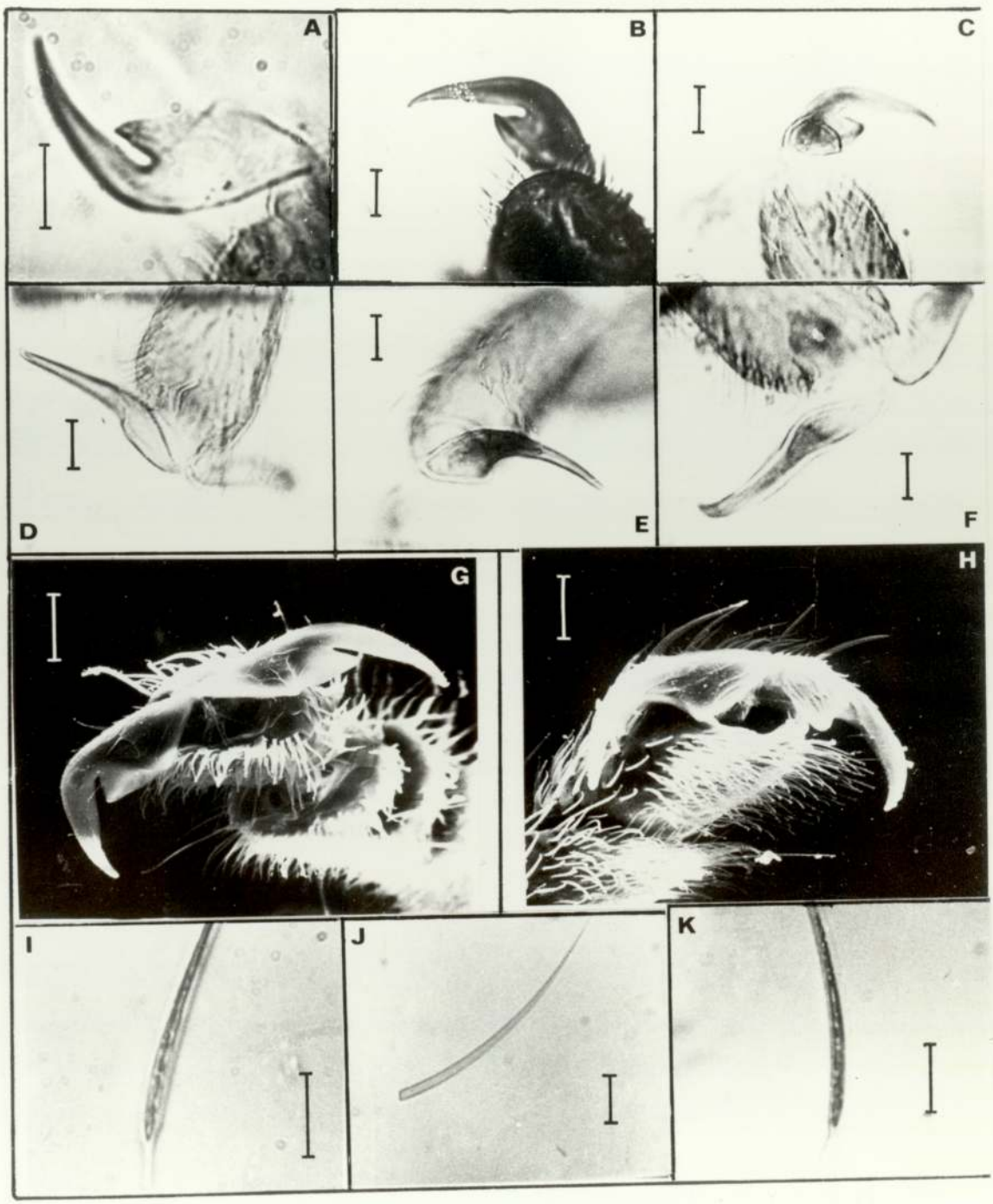
F : Female metathoracic tarsal claw

G : Male prothoracic tarsal claw

H : Female prothoracic tarsal claw

I - K : Female elytral setae

Scale line = 0·01 mm


A
B
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F
G
H
I
J
K

tarsal-claw slots therefore seem to be a mechanism for improving the security of the possessor's position.

The male tarsal-claw slots may have other uses in mating behaviour possibly in helping the male to orientate correctly with respect to the female or in giving signals to the female. These aspects are discussed below.

The Aedeagus

Fig. 8 shows a stereoscan photomicrograph of the intromittent organ of a male *S. paniceum* - the aedeagus. This photomicrograph is taken from the right-hand side of the beetle. The median lobe (c), the flagellum (d) and the left paramere (e) can clearly be seen. The nomenclature adopted here is that used by Sharp and Muir (1912) and Crowson (1967). However, since the terms used by these authors are not adequate to describe all the structures present in *S. paniceum*, I have also employed some of the terms used by Nyholm (1972) to describe parts of the aedeagus of species of the Helodidae. These include the division of the median lobe into dorsal and ventral parameroids (b and c respectively) and the use of the term 'trigonium' to describe the somewhat unusual triangular left valve (f). Fig. 9 illustrates the appearance of the aedeagus of a freshly killed male as seen under a binocular microscope. Fig. 9A-D shows the dorsal, ventral, left- and right-side views respectively. Further structures not noted in fig. 8 are the dorsal parameroid (b), the right paramere (a) and the basal piece (g).

The male genital structures are primarily directed towards placing the sperm in the female spermatheca (Sharp and Muir 1912). The structure most obviously designed for accomplishing this is the flagellum (d) which probably inserts into the base of the female spermathecal duct, thus forming

a direct link between the male testes and the female spermatheca - the presence of a flagellum in *S. paniceum* implies that a spermatophore is probably not employed (see Evans, 1975). The function of the heavily chitimised median lobes (b, c) is generally considered to be concerned with holding the aedeagus rigidly within the female genital opening and with mechanical functions connected with keeping the sperm flow system distended and maintaining and guiding eversion (Evans, 1975).

The functions of the peripheral aedeagal structures are less obvious however. It is generally considered that the two parameres (a and e) are organs used to 'clasp' the female during copula (Imms, 1957). However, in the previous chapter it was noted that in *S. paniceum* the right paramere is seldom engaged when the pair turn back to back and is not therefore engaged in clasping the female. It is possible that the setae with which the parameres are invested may, in fact, be sensilla tricodea and thus function to receive tactile information from the female genitalia.

The structure which I have designated as the 'trigonium' (f) (following Nyholm, 1972) appears to be unusual among the Coleoptera. Its use in the mating behaviour of *S. paniceum* however appears to be reasonably clear. It can be seen from fig. 8 and fig. 9A-D that (f) is a hook-like, chitinised structure projecting ventrally from the aedeagus and thus positioned next to the female surface when the male is mounted in the amplexus position (see A2b- fig. 3C). In this way the trigonium is admirably placed for hooking under the female tergite which opens to reveal the genital opening. This hook-function would account for the 'plucking' motion of the aedeagus which is described in the previous chapter. The trigonium thus hooked in place presumably provides a lever, by the aid of which the median lobe and flagellum can more easily be inserted into the female.

Finally it is interesting to note that the whole aedeagus is

asymmetrical and can be considered as two elements twisted, helically, around each other. This aspect can most easily be appreciated from fig. 9A and fig. 9D. This twisting of the aedeagus seems to explain the reason why male *S. paniceum* always dismount to the right when entering copula, since a 180° clockwise turn would serve to untwist the two elements.

The posterior-to-posterior copula position is noted in several stored-product Coleoptera such as *Cryptolestes pusillus* (Schonherr) (Wojcik, 1969) and in *Lasioderma serricorne* - which also dismounts to the right of the female (Tobin and Smith, 1971). No study, however, has been published that links this behaviour to the structure of the aedeagus in either of these species, so it is impossible to determine whether a similar twisting of the aedeagus is associated with this behaviour. It is possible, however, to hypothesise about the evolution of the posterior-to-posterior position and the apparently related aedeagal twist.

The mean time spent in copula by 6 species of stored-product Coleoptera other than those mentioned above is 4 min. (Wojcik, 1969). In all of these 6 species copula occurs with the male mounted on the dorsum of the female. Compared to this all 3 species of posterior-to-posterior copulators spend a relatively long period in copula: a mean of 60 minutes for both *L. serricorne* (Tobin and Smith, 1971) and *S. paniceum* (see previous chapter) and a mean of 30 minutes for *C. pusillus* (Wojcik, 1969). It seems possible to infer from this that the posterior-to-posterior position imparts the greater degree of pair-bond-security which would be required for a longer copulation time. I therefore propose that the need to attain this more secure position resulted in the evolution of the twisted aedeagus, since a male on making a 180° turn with an initially straight aedeagus would cause it to become twisted. Thus distorted, the aedeagus presumably would present a barrier to the free flow of sperm. But, with the twist initially present,

FIGURES 8 AND 9 :

8 : Stereoscan view of the Aedeagus from the right-hand side.

Scale line = 0·05 mm

9 : Diagramatic views of the Aedeagus from:

A: dorsal side

B: ventral side

C: left side

D: right side

Stippling indicates degree of chitinisation

Scale line = 0·1 mm

a : right paramere

b : dorsal parameroid

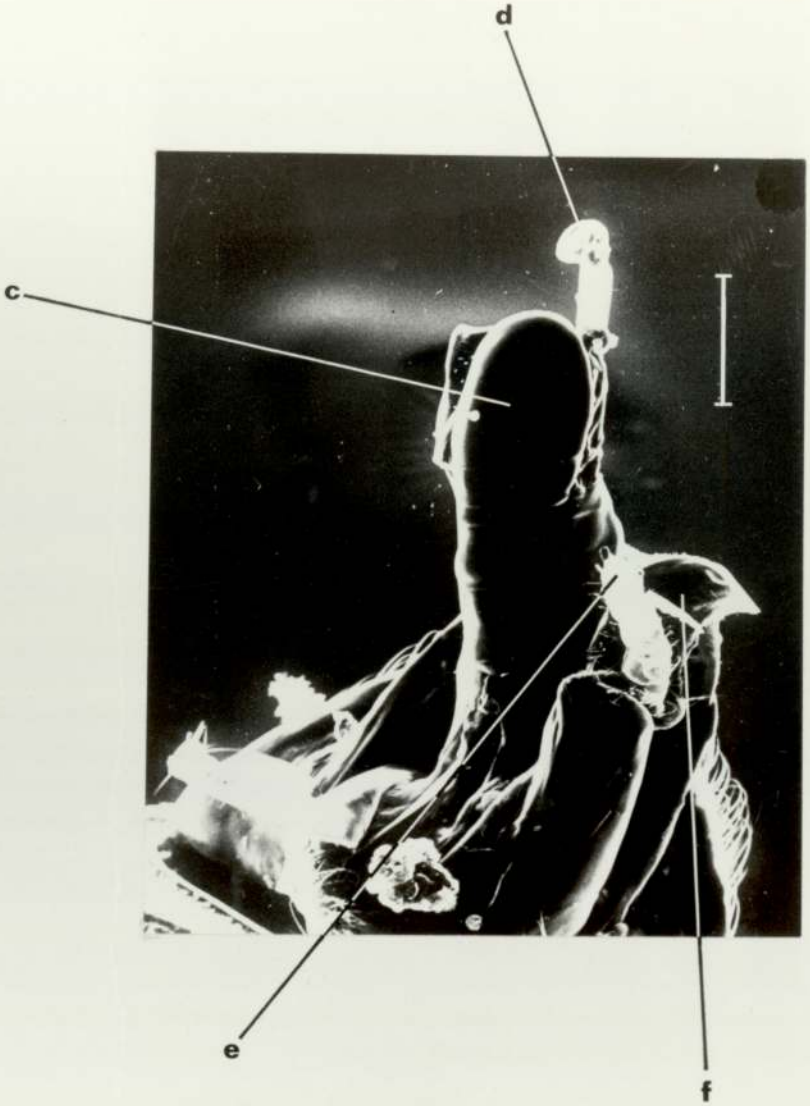
c : ventral parameroid

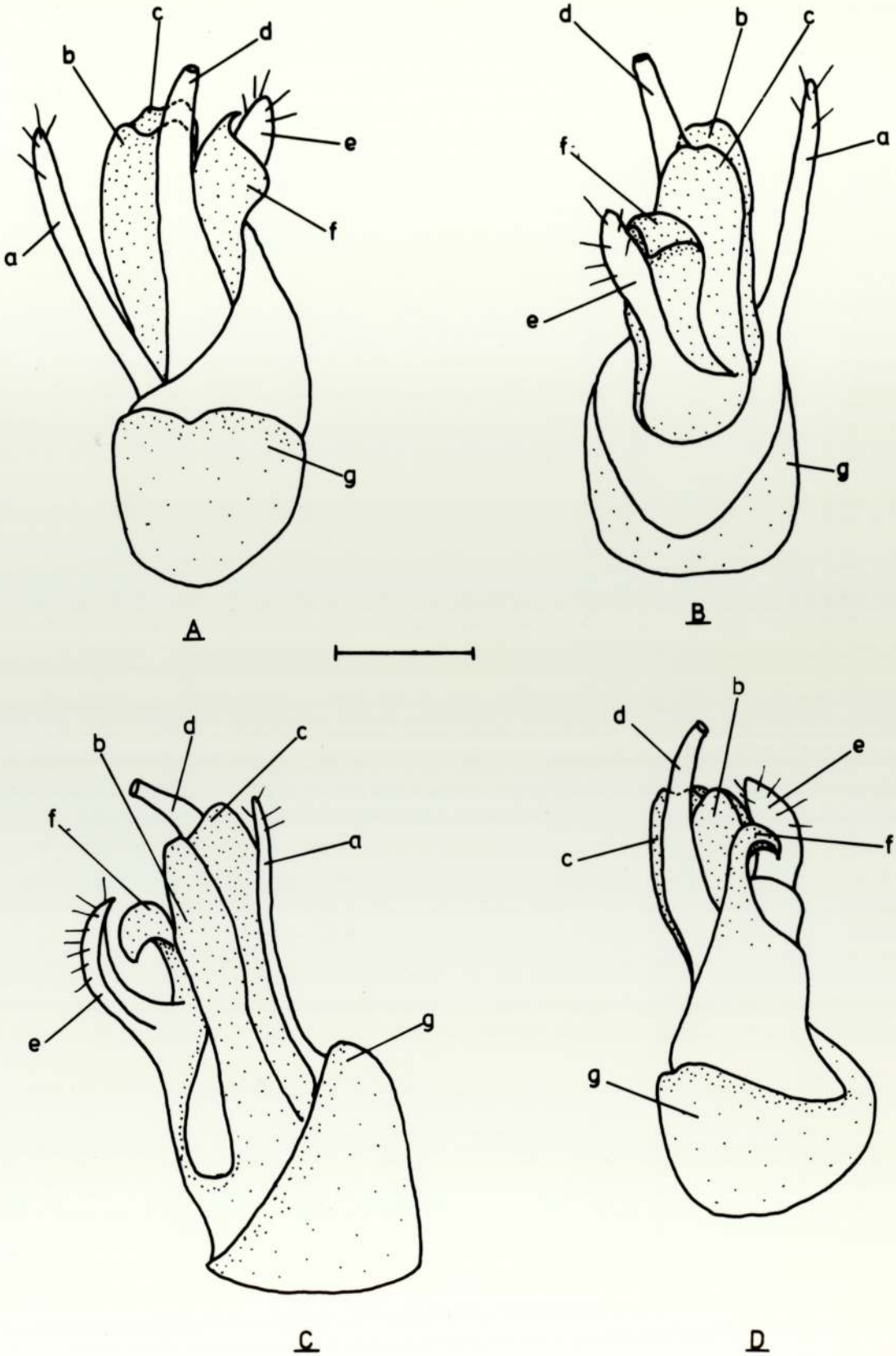
d : flagellum

e : left paramere

f : trigonium

g : basal piece


d
c
e
f


a
b
c
d
e
f
g
A
B
C
D

the 180° turn when accomplished will cause the aedeagus to become untwisted and thus allow the sperm to pass freely.

A DISCUSSION OF THE MORPHOLOGY AND FUNCTION OF THE MALE ORGANS IN RELATION TO MATING BEHAVIOUR

In this discussion the elements of mating behaviour as described in the previous chapter are considered, in turn, in relation to the male organs described above.

The first element is that illustrated in fig. 3A; in this the male raises the anterior of his body and elevates his antennae. Actions similar to this one have been noticed in several pheromone producing Coleoptera such as *Trogoderma* species (Hammack et al, 1973), *Dermestid* species (Burkholder and Dicke, 1966) and *Lasioderma serricorne* (Coffett and Burkholder, 1972). Since these authors have used this activity as a bioassay of pheromone activity it appears probable that antennal elevation is connected with olfaction by the antennal sensilla basiconica and chaetica described above.

Antennal touching (element B in the previous chapter) and antennation (element E) bring the antennal sensilla into contact with the female and for reasons described above are probably primarily tactile in function. Although antennal touching may possibly be connected with pheromonally induced male orientation in the immediate vicinity of the female (see below).

The male then proceeds to mount the female (element C). Table 16 shows an analysis of the directions in which 248 males mounted an equivalent number of females and the orientation (correct or incorrect) which the male assumed once mounted. From this the ability of the males to orientate correctly with respect to females can be appreciated. Incorrect orientation

TABLE 16: Analysis of the directions from which 248 males mounted females.

F = Front

S = Side

R = Rear

r = right

l = left

Male mounts at	No.orientating incorrectly	No. orientating correctly	Combined total	X^2 for each pair	$\frac{(OC - E)^2}{E}$ *
F	23	3	26	7·69	0·09
FSr	10	14	24	0·67	0·18
FSl	2	14	16	9	9·29
Sr	1	27	28	24·14	0·2
Sl	3	31	34	23·06	1·51
RSr	0	7	7	7	15·33
RSl	0	6	6	6	16·86
R	0	93	93	93	155·49
TOP	0	14	14	14	6·66
TOTAL	39	209	248		205·62

χ^2 for P = 0·05; 1^o freedom = 3.84, 8^o freedom = 15·51

* null hypothesis = there is no significant departure for each mounting direction from a mean number of 27·55 males mounting.

appears to occur most frequently when the male mounts the female from her anterior, less often when mounting takes place at the female's sides and not at all when the male mounts from the female's posterior. The hypothesis that a male is likely to turn in either direction along the female long axis after mounting is disproved in all cases except that of a male mounting from the front side right (FSr) and in all cases it is evident that males are capable at least of orientating along the long axis of the female rather than randomly. It appears therefore that a male will probably orientate correctly whichever way he mounts unless it is directly from the anterior of the female in which case the probability is that he will orientate incorrectly. The mechanism of this is discussed later.

Table 16 also shows that there is not an equal probability of the male mounting from any direction: it appears most likely that a male will mount a female from the rear. This disproves the null hypothesis that there is an equal likelihood of the male approaching a female from any direction - given that the female is, in general, stationary. It also implies that the male is able to detect the correct 'end' of the female before mounting. The nature of this mechanism is not clear. It might possibly be visual, and it might be the result of the ability of the male to detect a pheromone gradient in the immediate surroundings of the female. Given that the latter might explain the frequently noticed male antennal 'investigation' of the female prior to mounting (element B) and the fact that visual acuity in insects is poor (Engelmann, 1970), it is probable that the pheromonal gradient system is the most likely explanation.

Previously described experiments such as encounters between normal males and females with wings and elytra removed and elytra covered with wax, as well as observations of the reactions of males to small pieces of cork impregnated with a diethyl-ether extract of female pheromone (Barratt, 1975),

give clues about mating behaviour under abnormal conditions.

Males were able to achieve copula with females lacking elytra and also in 15 out of the 18 encounters between males and females with wax covered dorsal surfaces (see A1a). The number of replicates in the group of encounters between normal males and females without elytra is not large enough to permit analysis; the observation that copula was nevertheless able to occur seems, however, to be of some consequence. This would imply not only that the male is able to find the female genital opening in the absence of cues from the elytra - possibly by the shape of the posterior of the female - but also that the female is able to receive the stimulus that causes her to raise her 5th abdominal tergite. The latter bears out the observation made in the previous chapter that female tergite-raising can be caused by a generalised touching of the female body with a soft paint-brush. In both cases the female seems to be responding to stimuli that stem not from any specific activities of the male (which take place mainly on the elytra) but to an overall tactile impression caused in one case by the male mounting and moving over the female body and in the other by the stroking action of a paint-brush. In the Cerambycidae also, where the males exhibit similar palpation movements as those seen in *S. paniceum*, a similar effect is produced on the females by the similar action of stroking with a soft brush (Michelson, 1962). Thus in *S. paniceum* the stimulus required by a female in order to achieve successful copula appears to be of a simple and generalised nature and probably not as complex as those apparently used by the male in his efforts to achieve copula.

The 18 encounters involving females with wax-covered elytra and pronota were analysed. The time taken to achieve copula for the 15 successful encounters is set out in table 17 where these times are compared with the mount to copula times for 15 encounters in a group otherwise similar but untreated. It can be seen that there is a statistically significant

TABLE 17: Mount to copula delay times (seconds) for encounters between normal male/female pairs and similar encounters between normal males and females with wax-covered dorsal surfaces.

replicate number	(mount to copula) sec for wax covered females	(mount to copula) sec for untreated females
1	47	20
2	78	106
3	98	43
4	135	40
5	260	74
6	50	60
7	155	63
8	315	34
9	142	20
10	240	50
11	217	19
12	45	95
13	28	45
14	100	147
15	130	72
mean	136	59·2
standard deviation	87·26	35·68

t 2·2

P $<0{\cdot}05$

difference between the two groups - implying that the wax covering is hindering the male in his efforts to enter copula. In 7 out of the 18 encounters the male was apparently disorientated, turning the wrong way round even after mounting correctly; this is a ratio of 1 : 2·6 incorrect orientations as compared with a ratio of 1 : 5·4 incorrect orientations in normal encounters (table 16). In addition to this relatively high percentage of incorrect orientations in 6 out of the 18 cases the males dismounted and then remounted during the course of the encounter - a phenomenon never noted in similar encounters between normal males and untreated females.

It is therefore apparent that the wax covering affected the male reception of significant female stimuli rather than vice versa, since females were quiescent as normal and raised their tergites in the customary fashion. It could be that the covering of the elytral setae in some way affected the male tarsal-claw slots. In 2 out of the 18 encounters the male fell off the back of the female. This, while being a high proportion when compared with normal encounters, nevertheless implies that normal tarsal gripping ability is adequate during most encounters (at least in the abnormal surroundings of the mating chambers). It would be interesting to conduct similar experiments on the other Anobiid found to possess these slots - *Nicobium castaneum* (Ol.) (Ward and Humphries, 1977).

It is possible that the slots play a part in detecting the orientation of the male when mounted, possibly by reason of the fact that all the female dorsal setae are backwardly directed. This would explain why the male does not correct his orientation when mounting from the front of the female; if it is assumed that the slots will fit onto a seta as easily if it is directed towards the male as it will if it is directed away from him - which would be the case in an anterior mounting. If this mechanism were in operation it would not only explain why orientation is always along the female's long axis but also

the high percentage of confused orientations when the setae were wax-covered.

The wax covering might also serve to dilute and confuse gustatory and tactile information being received by the male antennae and palps. Contact chemoreception by the palps appears to be of importance in the mating behaviour of several Coleopteran species such as *Trogoderma glabrum* (Greenblatt et al, 1976), Cerambycid species (Michelsen, 1962) and the bean weevil *Acanthoscelides obtectus* Say. (Szentesi, 1973). In the latter species it was found that after palpectomy the male sexual activity decreased significantly whilst not disappearing entirely - a similar result to that of the wax-covering experiments with *S. paniceum*. But in the present experiment the contact chemicals were presumably prevented from reaching the sensilla rather than vice versa. It has been noted above that the antennae may also receive gustatory information during mating as they do in the Dictyopteran *Blatta germanica* (L.) (Nishada et al, 1975). However it was also pointed out above that the reception of gustatory information by the antennae is probably of less importance than the reception of tactile information by those organs. Antennation during mating is widespread in stored product Coleoptera (Wojcik, 1969) and also in the Cerambycidae (Michelsen, 1962); however the use of antennation in receiving a stimulus from the female is not discussed by these two authors. Tactile signals from the antennae are used, however, in detecting the host plant for instance by the clover-head weevil (*Hyper meles* (F.)) (Smith et al, 1976).

In none of their experiments involving the reaction of *S. paniceum* males to filter-paper disks impregnated with a diethyl-ether extract of whole females did Barratt (1974; 1975) or Kuwuhara et al (1975) note antennation, palpation or aedeagal eversion. I too found that, while a male placed in a mating chamber - the floor of which had been impregnated with a diethyl-ether extract of whole females - would exhibit antennal and body elevation (A2a fig. 3A), it would not exhibit antennation, palpation or aedeagal eversion.

I therefore impregnated pieces of cork, of approximately the same size and shape as a female *S. paniceum*, with a similar extract. These models were placed in mating chambers with different males. In these encounters male behaviour was completely different; they approached the cork, mounted it and proceeded to antennate, palpate and evert their aedeagi in a manner similar to that observed as the response of a male to a normal female. From this it is possible to conclude that olfactory stimuli are not, in themselves, sufficient to provoke copulatory attempts. It would appear that a point source of pheromone is required and that the purpose of antennation is to establish that the size and shape of the point source conforms to a species-specific pattern. Palpation probably provides information about the specific chemical nature of the female dorsum. That olfaction alone is insufficient has already been shown, that shape alone is insufficient was demonstrated by encounters between males and female sized and shaped but untreated pieces of cork - the male did not respond to these. Unfortunately experimental encounters between males and pieces of cork in chambers impregnated with extract were inconclusive and thus unable to elucidate the role of contact chemoreception.

SUMMARY

In the chronological summarisation table (overleaf) the events leading up to copula are examined in turn and the most probable mechanisms in use at each point are noted. The nature of these mechanisms, although hypothetical, are nevertheless based on theories strongly supported by the observational evidence outlined above.

CHRONOLOGICAL SUMMARY OF EVENTS IN MATING BEHAVIOUR

Behavioural element (code as in A2a)	Possible stimulus	Possible function	Organ involved	Structure involved
Male antennal elevation (A)	Female olfactory pheromone	detection of female	antennae	sensilla basiconica/chaetica
Male antennae tap female?(B)	Female olfactory pheromone from point source	Male excitation orientation with respect to female	antennae	sensilla basiconica/chaetica
Male mounts female-orientates 'scrabbling'? (C)	Female seta direction	ensure correct orientation once mounted	tarsal claws	claw slots
Male antennation (D)	size and shape of female	Male excitation? to detect that a correctly sized and shaped object has been mounted	antennae	sensilla trichodea
Male palpation (E)	gustatory pheromone on female dorsum	Male excitation? specific recognition	Palps	sensilla basiconica
Female quiescent. female tergite open	Generalised tactile stimuli from male	to allow aedeagal insertion	last visible tergite (5th)	-
Male aedeagal eversion (F)	combination of the above	to ensure passage of sperm	aedeagus	-

(continued)

CHRONOLOGICAL SUMMARY OF EVENTS IN MATING BEHAVIOUR (continued)

Behavioural element (code as in A2a)	Possible stimilus	Possible function	Organ involved	Structure involved
Male aedeagal 'probing' at female genital opening (H)	? female shape ?	to find female genital opening	aedeagus	sensilla trichodea on parameres
Male aedeagal 'plucking' at female tergite (H)	female 6th abdominal tergite	to lever aedeagus into female genital opening	aedeagus	trigonium
Male dismounts to right of female and makes 180^{o} turn	link up between male flagellum and female spermathecal duct	to allow free passage of sperm	aedeagus	helical twisting of aedeagus

SECTION A. PART 2c. The possibility of male response-waning and learning in mating behaviour.

INTRODUCTION

No study of response-waning or trial-and-error learning in *Stegobium paniceum* has been published - although both these phenomena are known to occur in other insect species (Thorpe, 1963). The purpose of this chapter is to examine multiple matings of *S. paniceum* males for evidence of such adaptive changes as a result of experience. Observations concerning the number of times an individual male is capable of mating are also presented for the first time in the literature on *S. paniceum*.

METHOD

Observations were made of encounters between normal males of optimum age (chapter A1b) and normal, virgin females of optimum age (A1b) in the usual mating chambers.

A large number of observations (table 18) were made of encounters between virgin females and males mated at least once on the same day - usually within 4 hours. In addition each of 15 males was mated with successive virgin females until the male failed to achieve copula.

The variables recorded in table 18 are the start-to-mount and mount-to-copula times (corrected for males orientating incorrectly) described in chapter A1b. In addition a variable designated 'seconds per burst' was examined. This was arrived at by dividing the mount-to-copula time by the number of bursts of antennating/palpating activity (see A2a) which the male performs during that time.

RESULTS

Table 18 summarises the results of most of the observations described above.

The first part of table 18 shows the effect of male mating experience on the start-to-mount interval and thus the ability of a male to find a female (see A1b). Statistical comparison of this ability in virgin males with the same ability in males with 1 to 6+ experiences of mating shows that in every case the difference is insignificant. It appears from this that mating probably has no effect on the ability of a male to find a female.

The second part of table 18 shows the effect of male mating on the mount to copula times and thus the efficiency of the male in achieving copula (see A1b). Statistical comparison was made between this ability in virgin males and the same ability in males with 1 to 6+ mating experiences. The difference was insignificant in all cases except that of a comparison between virgin males and males with 2 mating experiences. However, when a comparison was made between the mean mount-to-copula time for virgin males and the mean mount-to-copula time for the combined total of males with whatever mating experience, the difference proved to be statistically significant ($P = 0{\cdot}02$). In view of this difference and since the mount-to-copula times of males with mating experience are longer than the mount-to-copula times of virgin males, it can be concluded that mating experience decreases the copula-achievement efficiency of males.

The seconds-per-burst index is a reflection of the amount of mating activity performed by a male in a unit time. Part 3 of table 18 demonstrates that male mating, although it raised the index, had no statistically significant effect either on each individual group of mated

males or on all 6 groups collectively.

In addition to these variables the observations permit an analysis of the orientation assumed by males upon mounting. These figures are not included in table 18. In the previous chapter it was noted that the ratio of incorrect to correct orientations for normal encounters was 1 : 5·36. The ratio of incorrect to correct orientations for the sample of virgin male encounters was 1 : 5·6 in this experiment - thus not differing significantly. The ratio of incorrect to correct orientations for all the encounters involving mated males was 1 : 13 and even after the first mating the ratio was 1 : 12·4. X^2 analysis shows these ratios to be significantly different from the ratio of incorrect to correct orientations for virgin males. The inference is that mating improves the ability of males to orientate correctly.

The results from the 15 males mated successively with different virgin females until copula failed to occur may be analysed as follows: 1 male mated 9 times in succession, 2 males mated 8 times, 3 males 6 times, 6 males 5 times and 3 males 4 times. No statistically significant difference in the variables discussed above was noted between the groups of each degree of mating experience. The numbers involved, however, are too small to permit a more exhaustive analysis.

DISCUSSION

The waning of male response to the stimulus of female pheromone has been noted in several Coleoptera - for instance two species of *Trogoderma* : *T. inclusum* (Vick et al, 1973) and *T. granarium* (Rahalkar et al, 1972). In the former species 1 minute of exposure to a pheromone extract was sufficient to cause waning of a bio-assayable response to a pheromone extract

TABLE 18: The effect of male multiple matings on male responses to virgin females.

number of times mated	number of replicates	mean	standard deviation
Start to mount time (sec):			
Virgin	62	33·64	50·47
1	62	32·16	42·32
2	39	34·72	43·41
3	28	28·43	46·65
4	20	35·30	37·10
5	12	32·25	43·05
6+	12	27·50	37·80
Mount to copula time (sec):			
Virgin	62	48·16	32·39
1	62	68·84	51·93
2	39	85·50	55·11
3	28	73·31	47·49
4	20	69·95	40·03
5	12	73·50	62·01
6+	12	56·42	41·46
Seconds per burst			
Virgin	62	12·7	5·1
1	62	14·0	6·1
2	39	14·7	7·0
3	28	15·2	5·0
4	20	15·2	8·8
5	12	11·25	5·6
6+	12	13.9	10·2

for at least 4·5 hours after exposure (Vick et al, 1973). The results above show that in *S. paniceum* however no significant slowing of the male start-to-mount response could be detected; although the males were usually re-tested within 4 hours of being exposed to a female (and thus her pheromone) during the full term of initial copula (mean duration 60 min - see A2a).

Similarly the results show that no statistically significant change after mating took place in the seconds-per-burst index. It was suggested in the previous chapter that antennation and palpation are brought about by the totality of the stimuli received by the male from the female. If such is the case it is logical to assume that the frequency of bursts of such activity will be an indication of the intensity of the male's excitation. The seconds-per-burst index should, if response-waning is occuring, show this by a significant numerical increase - implying that fewer bursts of activity are occuring in the time. Such an increase did occur in all except 1 of the 6 mated groups (see table 18). The increase however was not statistically significant (at the P = 0·05 level). It is possible, therefore, that this demonstrates a degree of response-waning that more rigorous tests might explore more fully.

The results above do however show that a statistically significant lengthening took place in the mount-to-copula times after mating. This implies that prior experience impairs male mating efficiency. This result is similar to that described by Rahalkar et al (1972) in *T. granarium*. They found that in this species prior exposure to the pheromone caused excitation and exhaustion resulting in reduced mating efficiency. By analogy it appears that in *S. paniceum* also waning of response to female pheromone occurs and that the result of this is an impairment of the male mating efficiency. If such is the case this might explain the reason for the continued increase in male mating efficiency up to 4 days after emergence

(see A1b). Such an increase would offset the loss of efficiency resulting from the waning of male response to the presence of female pheromone.

It is known that many insect species do not 'instinctively' make correct choices when mating - the Curculionid *Hypera postica* (Gyll.) for instance does not seem able to differentiate correctly between male and female (Le Cato and Pienkowski, 1970). The adaptive operation of correcting initial mistakes in subsequent trials under the same conditions is referred to as 'trial'and'error' learning and is a well-attested phenomenon in Coleoptera. The T-maze tests on larval *Tenebrio molitor* conducted by Borsellino et al (1970) and Somberg et al (1970) may be cited as an example. Trial-and-error learning is known to occur in the mating behaviour of the Colarado beetle (*Leptinotarsa decemlineata* (Say)) (Hellwig and Lugwig, 1951). Like *S. paniceum* (as shown in the previous chapter) male *L. decemlineata* are initially capable of distinguishing the long-axis of the female body. *L. decemlineata*, however, are at first incapable of distinguishing between the posterior and anterior of the female - only learning the difference by trial and error.

The results shown above indicate that male *S. paniceum* in the laboratory are probably capable of mating 5 or 6 times in quick succession (as a mean result). In all the present observations no male was found to be capable of mating more than 9 times over a period of 5 days. The limitation is presumably imposed by the depletion during copula of the total moisture content of the male body. It was noted that after several matings males appeared unable to fully evert their aedeagi - possibly an indication of loss of hydrostatic pressure in the abdomen. It is doubtful, due to limited life-span and competition, that in the wild a male would be presented with enough mating opportunities to allow this to happen. It seems, therefore, that if trial-and-error learning is to take place in *S. paniceum* little room is allowed for error before a male must arrive at a correct decision.

In fact, as the previous chapter demonstrated, male *S. paniceum* orientate upon mounting in a ratio of 1 : 5·36 incorrect to correct choices. This ratio is significantly better than would be the case if males were incapable of distinguishing female anterior from female posterior. The above results demonstrate however that this ratio is considerably improved after mating experience - to 1 : 13 incorrect to correct choices. Furthermore the ratio after only 1 mating was increased from 1 : 5·6 to 1 : 12·4. Such a reduction in the number of incorrect orientations, even after 1 mating experience, seems to imply that there exists in *S. paniceum* the ability to successfully learn by trial and error. This ability is employed in mating behaviour to increase a male's chances of successful copulation by enabling him to make fewer mistakes in his orientation on mounting a female.

SECTION B:

AFTER MATING

SECTION B. PART 1. Oviposition.

INTRODUCTION

Studies on the oviposition of *Stegobium paniceum* have been carried out by Janisch (1923), Dick (1937), Azab (1943), Kashef (1956), Lefkovitch (1967) and Barratt (1975). These studies produced data on such phenomena as the number of eggs laid per female, the age at which oviposition first begins, the effect of oviposition on life span and the effect of age on total egg yield. Barratt (1975) also speculates on the effect of multiple mating upon fecundity.

All these studies were limited however in that copula was not monitored consistently - the method being to place virgin males in tubes with virgin females, an egg count being made each day. The present study however, using the method of observation of individual matings as described in Section A, enabled the author to gain information about the precise relation of oviposition to copula. Novel data is thus presented concerning such phenomena as the temporal association of copula with oviposition and the amount of time required in copula to enable a female to produce fertile eggs.

Some observations concerning behavioural aspects of oviposition and the morphology of the female ovipositor are also included in this chapter.

METHOD

Male and female pairs were mated in the way described in Section A. Copula was either allowed to continue to its natural conclusion or pairs were forcibly separated after a timed interval. Copula

was timed from the point when the male turned posterior to posterior with the female. Adult males of optimum age (see A1b) and females of different ages were used; the effects of age on oviposition are discussed below. In order to minimise errors due to variations in such factors as temperatures, time and date experimental groups were, as far as possible, randomised. After mating females were generally separated from males and replaced in their individual vials into which 0·05 g of finely sieved wholemeal flour had previously been placed. The vials were examined at daily intervals and their contents sieved in a 0·15 mm mesh sieve (the single eggs are ovoid - 0·25 x 0·15 mm (Kashef, 1956)). Any eggs thus removed from the flour were counted in a glass petri-dish under a binocular microscope. The vials were, as described in the general introduction to methods, kept at 28^{o}C and 70% relative humidity.

The female ovipositor was prepared for stereoscan microscopy in a manner similar to that described in chapter A2b.

RESULTS

1) The effect of age on oviposition.

Table 19 compares the mean total of eggs laid by females mated at different ages. The differences between the means of each group are all statistically insignificant (at the P = 0·05 level). It is therefore probable that age at mating has little or no effect on the eventual fecundity of the female within the age limits examined here.

2) The effect of age on the initial oviposition rate.

Table 20 compares the mean number of eggs laid by two groups of females within 24 hours of copula. It can be seen that the group of females mated on the 4th-6th day after eclosion lay a smaller mean number of eggs

than the group of females mated on the 8th day. This difference is statistically significant at the P = 0·01 level. It is concluded that the initial oviposition rate of females mated between 4 and 6 days after eclosion is slower than that of females mated on the 8th day.

3) The effect of time in copula on the fecundity of females.

The mean time spent in copula by a total of 82 females was 63·06 minutes with a standard deviation of 20·67, an upper limit of 103 minutes and a lower limit of 27 minutes.

Table 21 compares the mean fecundity of two groups of females allowed to complete copula. The first group spent less than the mean time in copula and the second group spent longer than the mean. It can be seen that the group spending less than the mean copula time apparently produced a larger mean number of eggs than that spending longer than mean copula time. This difference however is not statistically significant.

Table 21 also shows the mean number of eggs produced by a group of females separated after 30 minutes in copula. It can be seen that the mean fecundity of this group is apparently lower than the mean combined fecundity of both groups completing copula. This difference is again statistically insignificant.

4) The temporal association of oviposition with copula.

These experiments demonstrated that oviposition begins within 16 hours of completion of copula and that, as can be seen from table 22, by 24 hours 61% of the total egg production is achieved. Table 22 also demonstrates that by 6 days after copula oviposition is complete in all cases.

These results are plotted in graph 3 which clearly shows the exponential nature of the oviposition rate.

5) Time in copula required to initiate oviposition.

Table 23 compares the percentage of egg laying females in

7 groups allowed to remain in copula for different periods. It can be seen that, although 64·7% of females are capable of laying eggs after only 2·5 minutes in copula, only in females allowed to remain in copula until they separated of their own volition was oviposition 100% initiated.

6) Effect of length of copula time on total egg production.

Table 24 shows the mean number of eggs laid by the 13 females which oviposited after remaining in copula for 3 minutes. When this figure is compared with that for females allowed to remain full-term in copula (table 21) a considerable disparity can be seen. This difference is statistically significant - at the P = 0·02 level. It is therefore probable not only that full-term copula is necessary to initiate oviposition but that fecundity is also copula-time critical.

7) Effect of multiple mating on egg production.

A group of females were paired with males and each pair kept together for 3 days. If multiple matings were to occur it is assumed that they would do so during this period, since it was observed during the course of monitored multiple matings that these took place, if at all, within 2 days of the initial mating. Table 25 shows the mean number of eggs laid by females of this group. Comparison of this figure with that of egg production of full-term copula females (table 21) shows that the table 25 group has a higher mean egg production. When examined statistically however the difference between the two means is insignificant.

Table 26 shows the total egg production of a group of females subjected to monitored multiple matings. The mean egg production of this group is larger than the mean egg production of once mated females. The difference is nevertheless again statistically insignificant. The number of replicates in this group is limited by the refractoriness of mated females described in chapter A1c. It is possible that, had the

GRAPH 3 : Mean total number of eggs laid against time after copula in days.

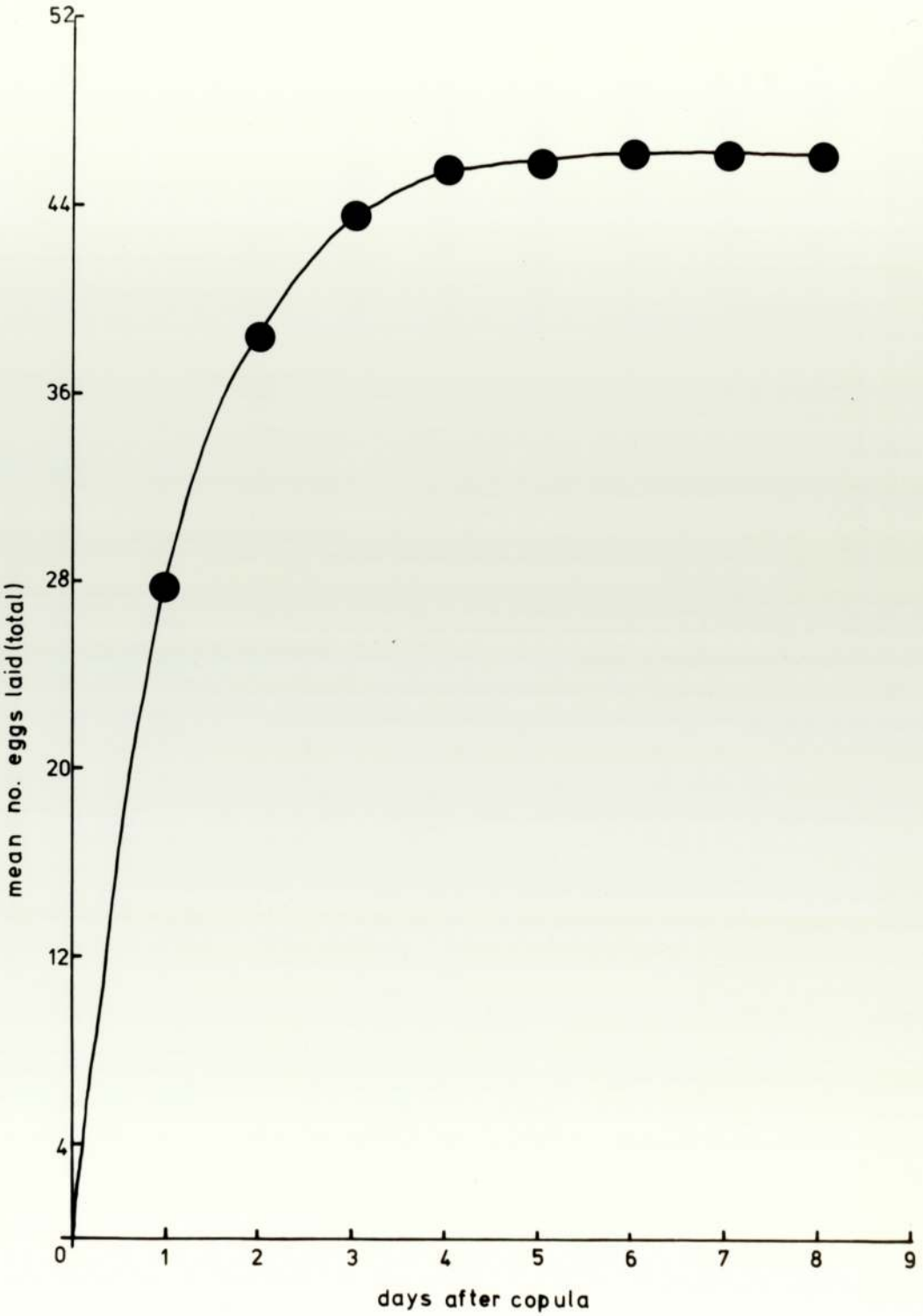

52
44
36
28
20
12
4
0
1
2
3
4
5
6
7
8
9
mean no. eggs laid (total)
days after copula

TABLE 19: The effect of female age at mating on fecundity.

TABLE 20: The effect of female age at mating on oviposition rate.

TABLE 21: The length of time naturally spent in copula related to fecundity.

Table 19

female age (days)	number of replicates	mean total number of eggs laid	standard deviation
4, 5, 6	48	41·08	14·05
8	45	44·11	14·89
>10 <16	25	43·44	17·47

Table 20

female age (days)	number of replicates	mean number of eggs laid (1st day)	standard deviation
4, 5, 6	24	16·29	14·51
8	46	30·56	12·92

Table 21

time in copula	number of replicates	mean number of eggs laid (total)	standard deviation
full term <63 min.	31	45·48	11·99
full term >63 min.	20	39·85	16·30
full term (combined)	51	43·27	13·96
separated at 30 min.	70	35·73	20·01

TABLE 22: Oviposition rate temporally related to copula.

TABLE 23: Percentage initiation of oviposition related to length of time in copula.

TABLE 24: Mean total eggs laid by females given 3 minutes in copula. (where oviposition was initiated only)

Table 22

day after copula	number of replicates	mean total number of eggs laid	standard deviation
1	59	27·76	15·02
2	60	38·50	11·26
3	61	43·62	11·18
4	59	45·51	11·35
5	60	45·88	11·21
6	61	46·34	11·49
7	61	46·34	11·49
8	61	46·34	11·49

Table 23

	Time in copula (minutes)						
	2	2·5	3	4	5	30	full-term
number of replicates	18	17	20	17	17	71	57
number laying eggs	0	11	13	12	15	62	57
percentage laying eggs	0	64·7	65	70·6	88·2	87·3	100

Table 24

number of replicates	mean	standard deviation
13	29	13·13

Table 25

number of replicates	mean	standard deviation
15	48·2	14·61

Table 26

number of replicates	mean	standard deviation
8	50·5	10·31

Table 27

number of females	number of replicates	mean total of eggs per female
3	1	59
5	3	42
7	1	47
10	1	41
15	1	46

(table 26) group been larger, a statistically significant result might have been produced.

From the data described above it is not possible to make a categorical statement about the effect of multiple mating on egg production.

8) The effect of crowding on egg production.

Table 27 shows the results of a pilot experiment to test the effect of placing several females together in the same vial. It appears that a small group of females stimulate an increased mean oviposition in each other whilst a high density of females apparently has no significant effect on mean egg production. The results however are inconclusive.

DISCUSSION

Studies by previous authors have shown several factors in the biology of *S. paniceum* which are of importance to a consideration of its oviposition. For instance the non feeding nature of the adult insect initially established by Kleine (1918). Also the inability of the virgin female to produce more than a very few infertile eggs (Barratt, 1975). Yet another factor, the 71% increased life-span of virgin over mated females, seems to be the result of the interaction of the two former factors (Barratt, 1975); since unreplenished food-reserves would be swiftly exhausted during the process of oviposition.

It is the foregoing factors which make possible an understanding of some of the phenomena revealed in the present study - the failure of age at copula to affect fecundity for instance (table 19). Dissection of adults within the age limits examined in this study (4 - 16 days) reveal that for virgin females internal changes with age are relatively slight. Mated females however show rapid loss of fat reserves and depletion of the

ovaries. These observations illustrate the earlier discovery of slower ageing by virgin females. In the light of the similarity between virgin females of 4 days old and virgin females of 16 days old it is not surprising that the age at which copula first takes place has little effect on the eventual fecundity of the female.

Barratt's (1975) unexplained observations that the percentage of females ovipositing increases with the length of time that a male is kept with a female is probably due to the increased mating efficiency of older females (discussed in chapter A1b). Her observation that females kept longer with males lay fewer eggs is more difficult to explain. She suggests that this is due to a difference produced by the reduced fecundity of later-mating females. As discussed above, however, the present results show that fecundity is not copula-age dependent. The discrepancy, while not statistically significant, may be susceptible of explanation if it is assumed that the continued presence of males inhibits female oviposition. Le Cato and Pienkowski (1972) found this to be the case in *Hypera postica* (Coleoptera, Curculionidae) where males kept with females disturb their oviposition attempts. A similar situation may well pertain in *S. paniceum* since, as has been shown (A1c), males are still attracted to mated females and, once mounted, will persist for some time in attempting copula. It is more than likely that such disturbance will affect the ability of a female to oviposit. In the present experiment in which the male was kept in contact with the female (table 25) the pair were separated only 3 days after initial copula - probably too short a time for male disturbance to become a significant factor.

Table 20 shows that age at copula does have an effect on the oviposition rate in *S. paniceum*. Older-mated females apparently oviposit at a faster rate than younger-mated. For the reasons, discussed above, of the increased longevity of virgin females it is unlikely that the adaptive

significance of this difference in oviposition rate is connected with the need for an older female's egg production to 'catch up' with that of a younger female. In addition the difference cannot be the result of ovarian maturation since this is complete at 5 days (Barratt, 1975).

The phenomenon therefore appears to be the result of a behavioural maturation process. It is interesting that this increase in oviposition rate is temporally synchronised with the maturation of mating efficiency discussed in A1b. However it is not clear whether this synchronicity is caused by a functional connection between the two processes, or whether each is a separate result of more general physiological maturation.

The ecological implications of this difference in oviposition rate may be correlated with the way in which adult females disperse and this is discussed in the next chapter.

Table 21 illustrates the effect on fecundity of the length of time which male/female pairs remained naturally in copula. The difference between the egg production of females mating for longer and shorter times than the mean is small, statistically insignificant and therefore probably of little importance. Table 21 also shows that females from pairs forcibly separated after 30 minutes in copula produced fewer eggs. Again the difference was statistically insignificant between this result and the egg production of females allowed to complete copula naturally. (This is further discussed below.)

Table 22 illustrates the temporal connection between copulation and oviposition. The exponential nature of this relationship is demonstrated by graph 3. The mean total number of eggs laid (46·34) agrees reasonably well with the findings of other authors reviewed by Barratt (1975). As was indicated in the introduction no other author has investigated the precise nature of the interval between initial copula and the start of

oviposition. The present results show this association by combining the data from many encounters - the female age spread at copula being 4 - 16 days. As has already been discussed, female age at copula has no significant effect on fecundity. However the curve in graph 3 represents a mean of the oviposition rate which, as has been shown, is significantly affected by age.

These results therefore refer to females mated once after the age at which they would emerge from the cocoon (see 1b). The rapidly rising exponential ; females begin to lay eggs within 16 hours after mating - the peak at one day, the rapid diminution of oviposition rate and the cessation of egg-laying by the 6th day after copula may therefore be considered as approximating to the natural situation in *S. paniceum*.

Such behaviour, particularly the rapidly achieved peak oviposition rate, is likely to be of ecological importance to a short-lived insect and as such is reflected by similar behaviour in other Coleoptera such as *Lasioderma serricorne* (Lefkovitch and Currie, 1967) and *Diabrotica virgifera* (Branson and Johnson, 1973). Such behaviour is not, however, present throughout the Coleoptera and other species, such as *Callosobruchus chinensis* (Bruchidae), show a constant oviposition rate throughout their life (Nakamura, 1971).

There is some disagreement between the results described here and those of Barratt (1975). In her experiments the initial oviposition rate was slower and the females continued to oviposit for longer than 6 days after the first eggs were laid. The explanation for these discrepancies, if not due to differences in the experimental populations, is possibly to be found in the fact that in Barratt's experiments the adults were kept together from the first day of adult eclosion. This would result in copulation attempts by males at ages when, under natural circumstances, they would still be in cocoons. The presence of sperm in the female before

ovarian maturation was complete may have resulted in the discrepancies observed.

It has already been noted that insemination is necessary in *S. paniceum* in order to produce fertile eggs. Table 23 however establishes the connection between the length of time in copula and the initiation of oviposition. It appears that the full-term of copula is necessary in order to stimulate 100% of females to commence oviposition. Reference to table 24 and comparison of the mean fecundity of females mated for 3 minutes with the corresponding figure for full-term copula females (table 21) reveals that the natural time in copula is also necessary to maximise fecundity.

Although many insects are capable, unlike *S. paniceum*, of producing eggs without mating experience it is more often the case that mating has a distinct effect on oviposition (Engelmann, 1971). The operation of this effect may in general either be in increasing egg maturation rate as it is in the Coleopteran *Acanthoscelicles obtectus* (Huignard, 1969) or in increasing oviposition rate as it is in the Lepidopteran *Zeiraphera diniana* (Benz, 1969).

Barratt (1975) has demonstrated that fully mature oöcytes are present in virgin female *S. paniceum* at 4 - 5 days after adult eclosion. It therefore appears that insemination in this species is not concerned with the enhancement of ovarian maturation. The function of mating in this respect therefore seems to be connected with the direct stimulation of oviposition behaviour.

Chapter A1c was concerned with the effect of mating on female pheromone content and production and the receptivity of the female to further mating attempts. In that chapter it was demonstrated that mating caused the onset of female refractory behaviour. Chapter A1c further proved

that this refractory behaviour was stimulated by a period of at least 3 minutes spent in copula.

The parallel between the observations of chapter A1c and those described in the present chapter are obvious. It was argued in chapter A1c that the mechanism inducing refractory behaviour was probably released by the passage of a secretion from the male accessory glands. The fact that the ratio of laying to non-laying females after 3 minutes (1·86 : 1 - table 23) and the ratio of unreceptive to receptive females (3·75 : 1) do not coincide was adduced as an argument that unreceptive behaviour was not initiated as the result of the passage of sperm. The stimulation of oviposition behaviour appears to be a more complex phenomenon.

Accessory gland secretions have been proved to have a direct effect on oviposition in many insect species such as house-flies (*Musca domestica*) (Riemann and Thorson, 1969) and the Orthopterans *Melanoplus sanguinipes* (Friedel and Gillott, 1976) and *Schistocerca gregaria* (Leahy, 1973). It is probable that in most insects oviposition is influenced by a combination of the presence of sperm and the presence of an accessory gland substance (A.G.S.); the Curulionid *Hypera postica* may be instanced (Le Cato and Pienkowski, 1973). In the Dipterans *Aedes aegypti* and *Culex pipiens fatigans* female eggs are infertile in the absence of male A.G.S. (Adlakha and Pillai, 1975). Similarly in the Coleopterans *Sitophilus granarius* and *S. zeamais* it has been proved that the presence of A.G.S. is necessary to activate the spermatazoa (Khan and Musgrave, 1969).

It is possible that a situation similar to that of the *Sitophilus* spp. is operating in *S. paniceum*. Table 24 demonstrates that a short term in copula results in the production of fewer eggs. Morphological evidence (chapter A2b) suggests that *S. paniceum* is a non-spermatophore forming species. Therefore the connection between length of time in copula

and the number of eggs laid may be explained by inferring a positive correlation between number of sperm passed and number of eggs laid. If female refractory behaviour is stimulated by the presence of A.G.S. the large percentage of refractory females after only 3 minutes in copula suggests that the initial rate of male secretion of A.G.S. at the start of copula is greater than the initial rate of sperm passage. Or alternatively the effective threshold of response to A.G.S. is lower than amount of sperm effective in causing fertilisation. However, if the A.G.S., as in *Sitophilus* spp., is responsible for the activation of the spermatazoa it would be advantageous for the initial secretion rate of this substance to be high since this would maximise the efficiency of the first few sperm passed - beneficial if disruption to the pair occurs during the first few minutes of copula.

The precise relationship between fecundity, sperm-passage and A.G.S. secretion can only be determined by more sophisticated experiments involving the use of sterile males and the injection of A.G.S. into the haemocoel of virgin females. The facilities for performing this type of experiment were not available to the present author. From the observations outlined above, however, it is possible to deduce that the relatively long copula time of *S. paniceum* is necessary in order to initiate fully oviposition behaviour and to maximise fecundity. These deductions in turn explain the need for increased mechanical efficiency of the mechanism used to bind the pair in copula as discussed in chapter A2b.

In some Coleopteran species multiple matings are essential to ensure the continued fecundity of the female. This is generally shown by examination of the effect on oviposition of removing access to males from a group of females. It has been postulated that a decrease in oviposition rate relative to that of females allowed access to males is an

indication of sperm depletion. Instances may be given of work on the Curculionids *Hypera postica* (Drea, 1969) and *Anthonomous grandis* (Villavaso, 1975) and the Dermestid *Dermestes maculatus* (Azab et al, 1972). The present work on *S. paniceum* indicates that in this species, although there is a possibility that multiple insemination results in increased fecundity, the small effect is probably statistically insignificant (tables 25 and 26).

This result is not surprising in view of the other observations concerning oviposition in *S. paniceum*. In contrast to these the three species mentioned above have very different oviposition characteristics: the oviposition period is longer, the rate of egg laying is fairly constant, a larger total of eggs is laid and the females do not show refractory behaviour. It is evident that these characteristics will probably result in sperm depletion and thus allow for re-insemination to occur. In *S. paniceum*, a short-lived species, the eggs are all laid within a short period after mating and because further mating would interfere with the speed of this activity further attempts at copulation are rejected by the female. Sperm depletion is therefore probably not an important factor. It is suggested that while multiple insemination undoubtedly can occur in *S. paniceum* it is not essential in promoting maximum effective fecundity and probably occurs only rarely under natural conditions.

The effect of crowding on oviposition was not studied in detail during the course of the present work. Table 27 does indicate, however, that a degree of physical crowding among adults apparently has no very serious effect on mean fecundity. Still higher densities and more replicates would be necessary to demonstrate whether any real trend occurs. It is possible that the presence of other females has a stimulating effect at lower densities but that this is off-set by the factor of disturbance at high densities.

BEHAVIOURAL AND MORPHOLOGICAL ASPECTS OF OVIPOSITION

INTRODUCTION

Morphological studies of the ovipositor of *S. paniceum* by Azab (1943), Monteiro (1957), Howe (1957) and Barratt (1975) have shown that it consists of a flexible, extensible tube constructed in three sections which, during oviposition, are extruded telescope-fashion presumably by the action of hydrostatic pressure from within the abdomen.

OBSERVATIONS

The terminal segment of the ovipositor is heavily chitinised and invested with setae. This is illustrated in fig. 10 which is the first published stereoscan-photomicrograph of the terminal segment of a *S. paniceum* extruded ovipositor. The heavily chitinised valves (A) surround the egg-passage (C) which presumably expands to allow the passage of an egg. The setae (B) in life are straight, tapering and socketed. The segment D, the function of which is obscure, in life bears a single terminal seta similar to those on the valves.

During the experiments described in the first part of this chapter various observations were made which are relevant to the understanding of the function of the ovipositor.

It has already been noted by Azab (1943, 1954) that female *S. paniceum* will readily oviposit in the interstices of cotton-wool, and this observation was confirmed by the present author.

Females maintained in vials containing flour but stoppered with

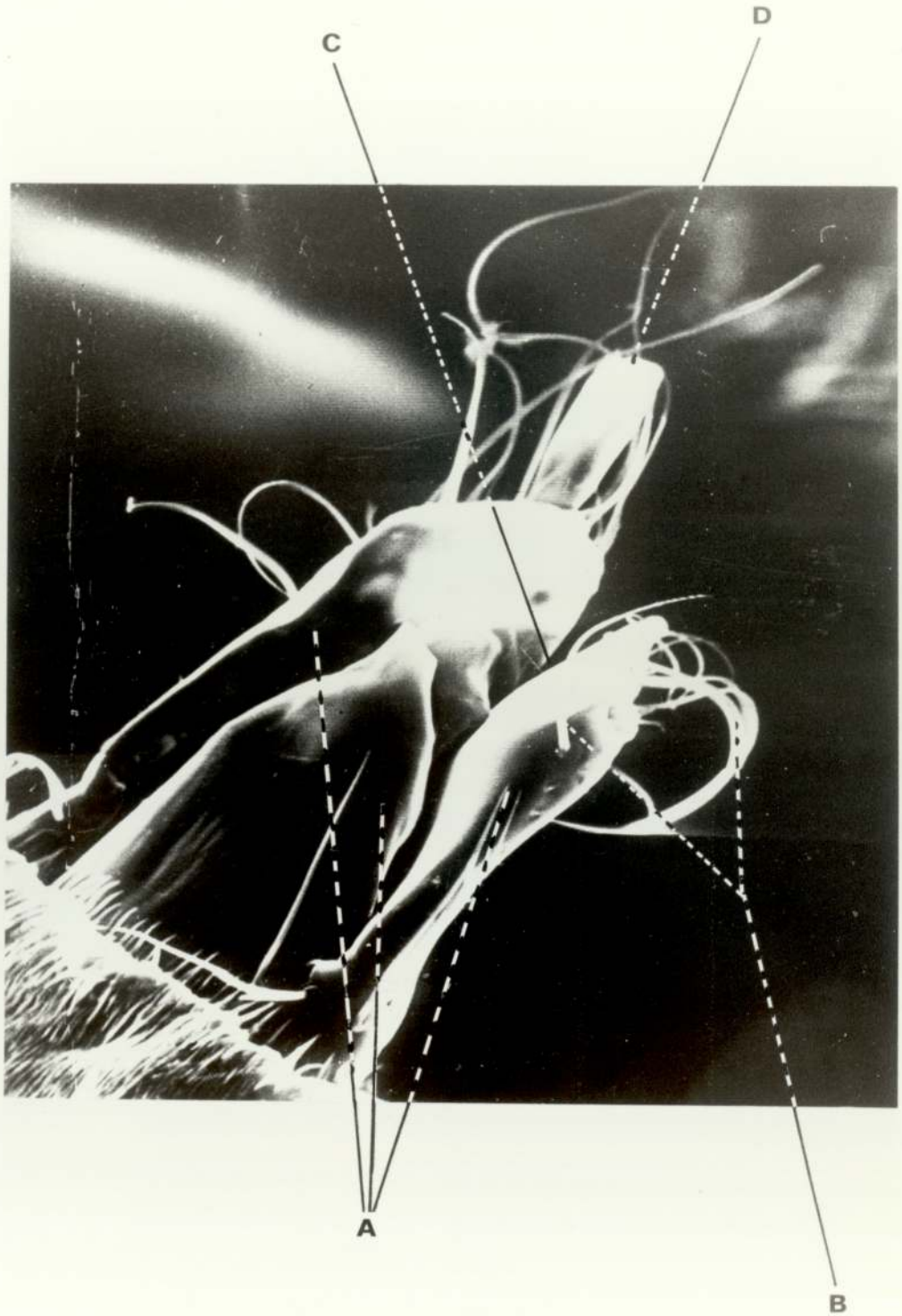
FIGURE 10 : Terminal segment of everted female ovipositor.

A: valves

B: setae

C: egg passage

D: terminal segment


C
D
A
B

cotton-wool laid eggs not only in the flour but also in the cotton-wool.

It was also discovered that females kept in empty plain glass vials with screw-caps would not lay eggs. A group of 15 females thus maintained for 5 days after copula laid no eggs. On the 6th day flour was introduced into the vial and within 24 hours normal oviposition had commenced.

DISCUSSION AND FURTHER OBSERVATIONS

From the observations above some deductions may be made about the external factors important in stimulating the female *S. paniceum* during oviposition.

It is known that in certain insect species oviposition will not commence in the absence of a suitable oviposition site, in many cases a host-plant. From the Coleoptera *Acanthoscelides obtectus* may be instanced; in this species ovarian production will not take place in the absence of a host-plant (Huignard, 1970). In leaf-hoppers of the genus *Oncopsis* also certain highly specific stimuli are required to release oviposition behaviour (Claridge et al, 1977). The above observations on *S. paniceum* indicate that in this species also the presence of a suitable site is essential to stimulate oviposition.

The nature of such a suitable site may be deduced from observations of the behaviour of females in the process of laying eggs. When oviposition is underway the extruded ovipositor is pushed into the substrate, its flexible nature allowing it to be guided to a certain extent. It appears that the female relies for guidance primarily on information about the substrate gathered by her ovipositor; little palpating or antennation of the surface occurs. It is probable that oviposition will only start if the tip of the ovipositor is entirely surrounded by the substrate or is enclosed

within a crevice.

The setae investing the ovipositor tip (fig. 10B) appear to be sensilla trichodea and are therefore probably tactile in function. That the information gathered by the ovipositor is primarily tactile is confirmed by the observation that females will oviposit in cotton-wool even when a food-medium is present. Since, as mentioned above, little palpation or antennation occur during oviposition it may be assumed that the recognition of the site as being of food value is of secondary importance (the palps and antennae being the primary sites of gustatory sensilla). If chemosensory information was being gathered during oviposition it is assumed that all eggs would be deposited in the food-medium since there the larvae would have a better chance of survival. Fletcher and Long (1971) have shown that *Lasioderma serricorne* is also capable of ovipositing on non-food substances; they also show however that a 7-fold increase in fecundity occurs when odours of tobacco or whole wheat flour are used as oviposition stimulants. It is quite likely that the situation is similar in *S. paniceum* and that the presence of a positive chemosensory stimulus is necessary in order to maximise fecundity. Since the young larvae of *S. paniceum* are highly active and can survive for a week without food (British Museum (Natural History), 1962) it is presumably not essential that they are laid directly in the food source.

It is concluded that in order for a female *S. paniceum* to commence oviposition it is necessary that specific tactile stimuli are received from the ovipositor tip. These stimuli ensure that eggs are laid within a narrow, enclosing and thus protective environment. It is also possible that chemosensory stimuli from the presence of a food-medium, although not necessary to initiate oviposition, may nevertheless be important in maximising fecundity.

SECTION B. PART 2. Adult dispersal.

INTRODUCTION

An understanding of the factors affecting the dispersal of stored-product insects is of importance in enabling the detection of low-levels of populations, indicating safer storage conditions and providing a basis for investigating the possibility of localised control (Surtees, 1965).

This chapter is concerned with an investigation of some of these dispersal factors, none of which have yet been studied in the literature of *Stegobium paniceum*. Novel information is provided from laboratory experiments on the locomotion and flight of individual adult *S. paniceum* females before and after mating.

Observations were also made about the dispersal of adults from open culture jars. These observations were found to be complementary to the information obtained by observation of individual locomotory and flight behaviour.

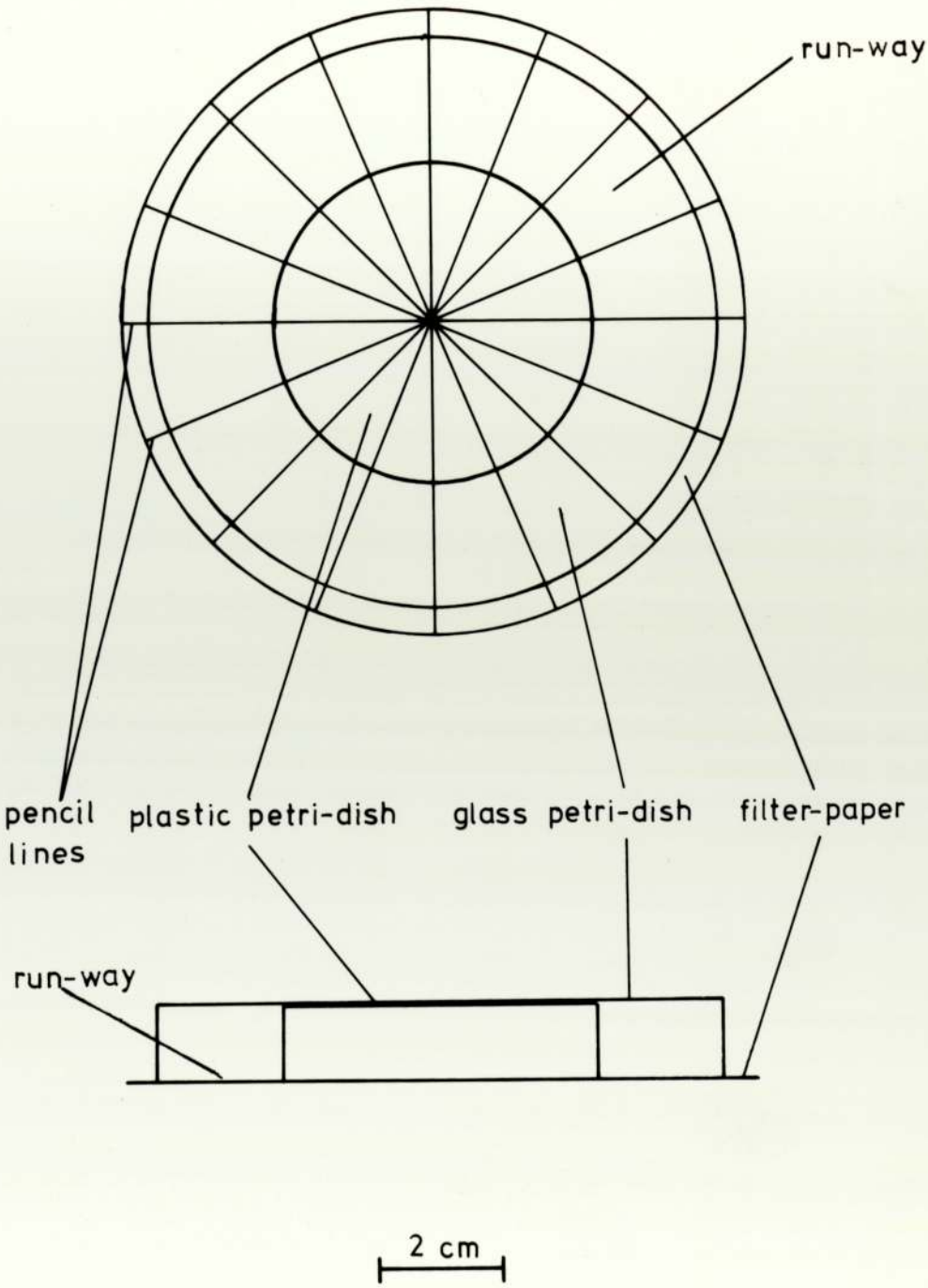
METHODS

Cultures were maintained as previously described (see general introduction to methods).

Virgin females of 5, 6 and 7 days old were used. Mated females of 5, 6 and 7 days old were obtained by means of monitored mating encounters with males of optimum age (A1b) in the normal mating chambers.

Flight and locomotory activity were measured under variable laboratory conditions; but since experimental groups were randomised

FIGURE 11 : Torroidal chamber for testing individual locomotory activity.


run-way
pencil lines
plastic petri-dish
glass petri-dish
filter-paper
run-way
2 cm

it is assumed that the influence of these variables was minimal.

Laboratory illumination was provided by a 60 watt bulb suspended 50 cm above the testing chambers.

Locomotory behaviour was tested in a chamber modified from that described by Barratt (1975). The chamber consists of a torroidal runway 2 cm across divided by 16 lines (fig. 11). Locomotory activity was assessed by a count of the number of lines crossed by the beetle over a period of 5 minutes.

Flight activity was measured by observation of the behaviour of the female on the rim of a glass tube 12 cm in height and 5 cm in diameter. The insect reached the rim by climbing the inner surface of the tube. Five minutes were allowed for each individual to respond.

Dispersal and flight activity from open cultures were examined under laboratory conditions using long-established cultures suspended within a wooden cage 45 cm in height and 30 cm square. This was covered with black gauze. Individuals climbing the suspending cord were monitored by means of a detergent-trap fixed halfway along the length of the cord. Estimation of the relative numbers of males and females normally occurring within a culture was made by random sampling of similarly established cultures.

All dissections were carried out on freshly killed adults in 100% alcohol under a binocular microscope.

RESULTS

1) Locomotory activity.

Virgin females of 5, 6 and 7 days old were tested for locomotory activity. Ten replicates of each age group were used (table 28).

GRAPH 4 : The change in female locomotory activity (number of lines crossed per 5 minutes) with time elapsed after mating.

Female state:

A: virgin

B: immediately after mating

C: 24 hours after mating

D: 4 days after mating

SQUARES : 5/8 days old

CIRCLES : 6/9 days old

TRIANGLES : 7/10 days old.

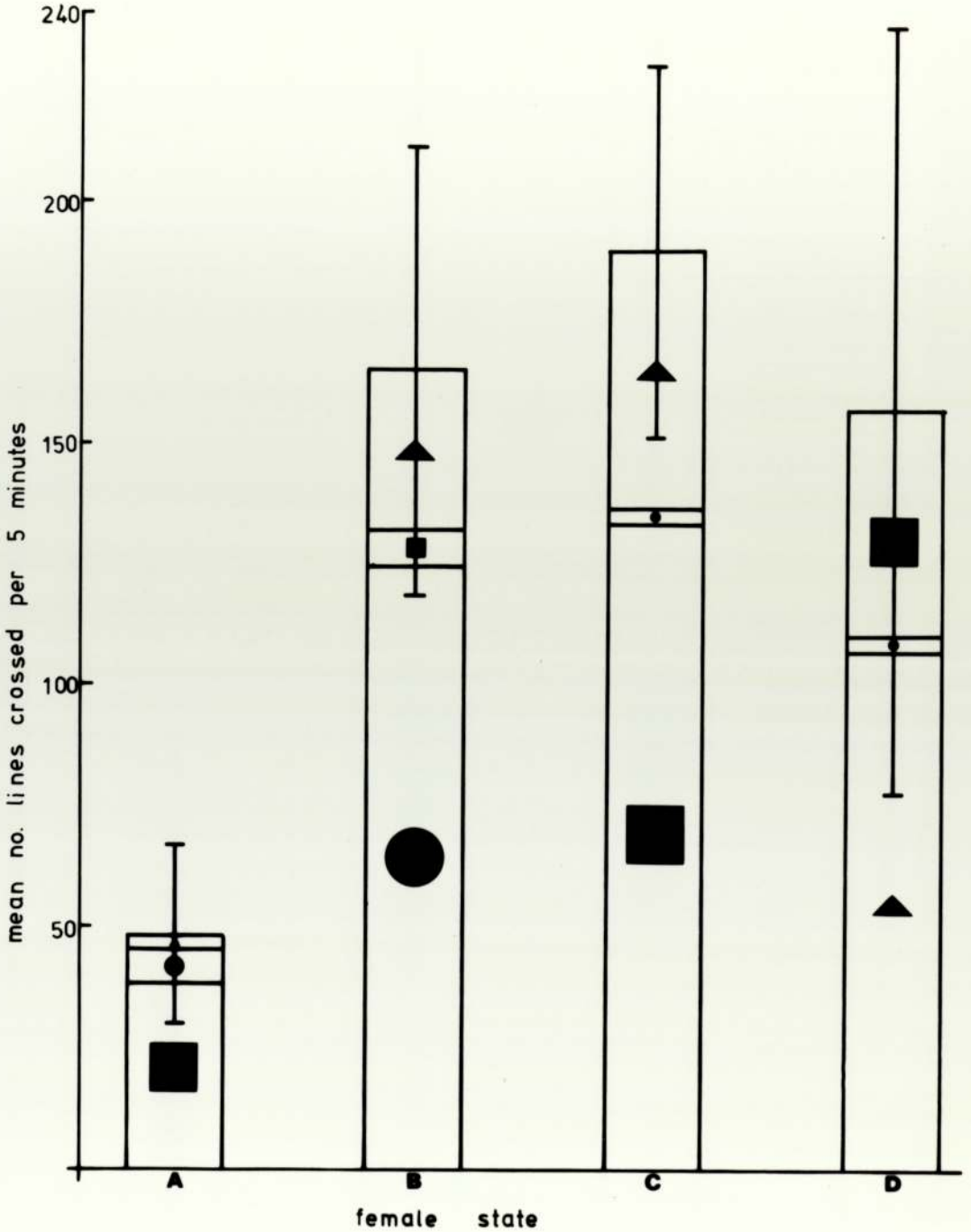

240
200
150
100
50
mean no. lines crossed per 5 minutes
A
B
C
D
female state

TABLE 28: Locomotory activity (number of lines crossed per 5 minutes) of 3 groups of 10 virgin females aged 5, 6 and 7 days.

TABLE 29: Locomotory activity (number of lines crossed per 5 minutes) of 3 groups of 8 females aged 5, 6 and 7 days tested immediately after mating.

Table 28

	Female age (days)		
replicate number	5	6	7
1	37	45	53
2	18	75	27
3	24	93	69
4	27	16	21
5	40	31	59
6	42	34	71
7	66	50	33
8	28	42	71
9	80	15	43
10	27	58	41
mean	38·9	45·9	48·8
standard deviation	19·72	24·62	18·58

Table 29

	Female age (days)		
replicate number	5	6	7
1	199	76	217
2	120	101	128
3	137	203	224
4	123	122	173
5	179	108	116
6	188	88	115
7	96	182	212
8	21	125	145
mean	132·87	125·625	166·25
standard deviation	58·18	44·68	46·47

TABLE 30: Locomotory activity (number of lines crossed per 5 minutes) of 3 groups of 8 females aged 5, 6 and 7 days tested 24 hours after mating.

TABLE 31: Locomotory activity (number of lines crossed per 5 minutes) of 3 groups of 8 females aged 8, 9 and 10 days tested 4 days after mating.

Table 30

	Female age (days)		
replicate number	5	6	7
1	70	81	157
2	125	221	195
3	233	194	196
4	128	61	181
5	66	145	228
6	126	99	225
7	78	174	119
8	251	125	227
mean	134·625	137·5	191
standard deviation	71·24	56·36	38·36

Table 31

	Female age (days)		
replicate number	8	9	10
1	106	203	41
2	91	207	166
3	186	270	32
4	27	46	315
5	92	141	97
6	47	82	93
7	141	224	69
8	200	91	52
mean	111·25	158	108·125
standard deviation	61·39	79·66	93·72

Analysis of variance shows no statistical difference between the locomotory activities of the 3 groups.

Immediately after disengagement from copula females of each age group were tested for locomotory activity (table 29). Analysis of variance again shows no significant difference between the 3 groups.

After 24 hours mated females were tested for locomotory activity (table 30). Again no significant difference was shown between the 3 different age groups.

Four days after mating locomotory activity was tested again (table 31). Again no significant difference could be demonstrated to exist between the 3 age groups.

Since the difference in age within each category caused no significant variation in the ability of the beetles, the ages were combined in order to compare possible differences caused by the time lag after mating. An analysis of variance demonstrated that no significant difference exists between the amount of locomotory activity recorded immediately after, 24 hours after and 4 days after mating.

If, however, a t-test is carried out between the combined means of locomotory activity at whatever time after mating and the combined means for all 3 ages of virgin females (which do not show significant difference) a very highly significant difference (at the $P = 0{\cdot}001$ level) is noted. It is probable, therefore, that mating causes a sharp rise in locomotory activity. This rise is illustrated by graph 4. The standard deviation of the maximum mean in each group is plotted.

2) Flight activity.

The flight activity of the various categories of female previously described is shown in tables 32-35.

"No activity" indicates a state in which a female showed no

tendency towards flight activity (see below). In every case not involving flight activity there was a movement of the female up the walls of the tube. In 51% of the cases the rim was reached but no tendency towards flight activity was shown.

"Pre-flight" behaviour may be described as the activity which precedes flight and involves specific units of behaviour as follows: (1) elevation of anterior portion of body; (2) elevation of antennae, each antenna forming one element of a U shape; (3) side to side movement and upward stretching of the head. All these movements are clearly visible with a low magnification hand lens. The behaviour is similar to that observed in a male when he detects a female pheromone; described in chapter A2a and illustrated in fig. 3A. No flight takes place without these movements occuring first.

The pre-flight behaviour continues for a variable length of time and if flight is going to take place will always be followed by the next category of flight behaviour - "open wings". This describes a simple opening of the elytra allowing the hind wings to unfold. When not followed by flight the wings are re-folded under the elytra by means of a flexing movement of the abdomen. This activity may be repeated several times, each time preceeded by pre-flight behaviour.

"Flight" behaviour occurs only after the elements described above have been performed. It may broadly be divided into two categories: true flight and 'parachuting'. In the latter case the insect makes little progress in a horizontal direction since wing beats apparently do not occur. In both cases however all other elements of flight and pre-flight behaviour are present and for the purpose of the present study no distinction is drawn between them.

The tendency of a particular category of female to fly is assessed as a percentage of a total possible score obtainable if all

GRAPH 5 : The change in female tendency to fly (see text) with time elapsed after mating.

Female state:

A: virgin

B: immediately after mating

C: 24 hours after mating

D: 4 days after mating

SQUARES : 5/8 days old

CIRCLES : 6/9 days old

TRIANGLES : 7/10 days old.

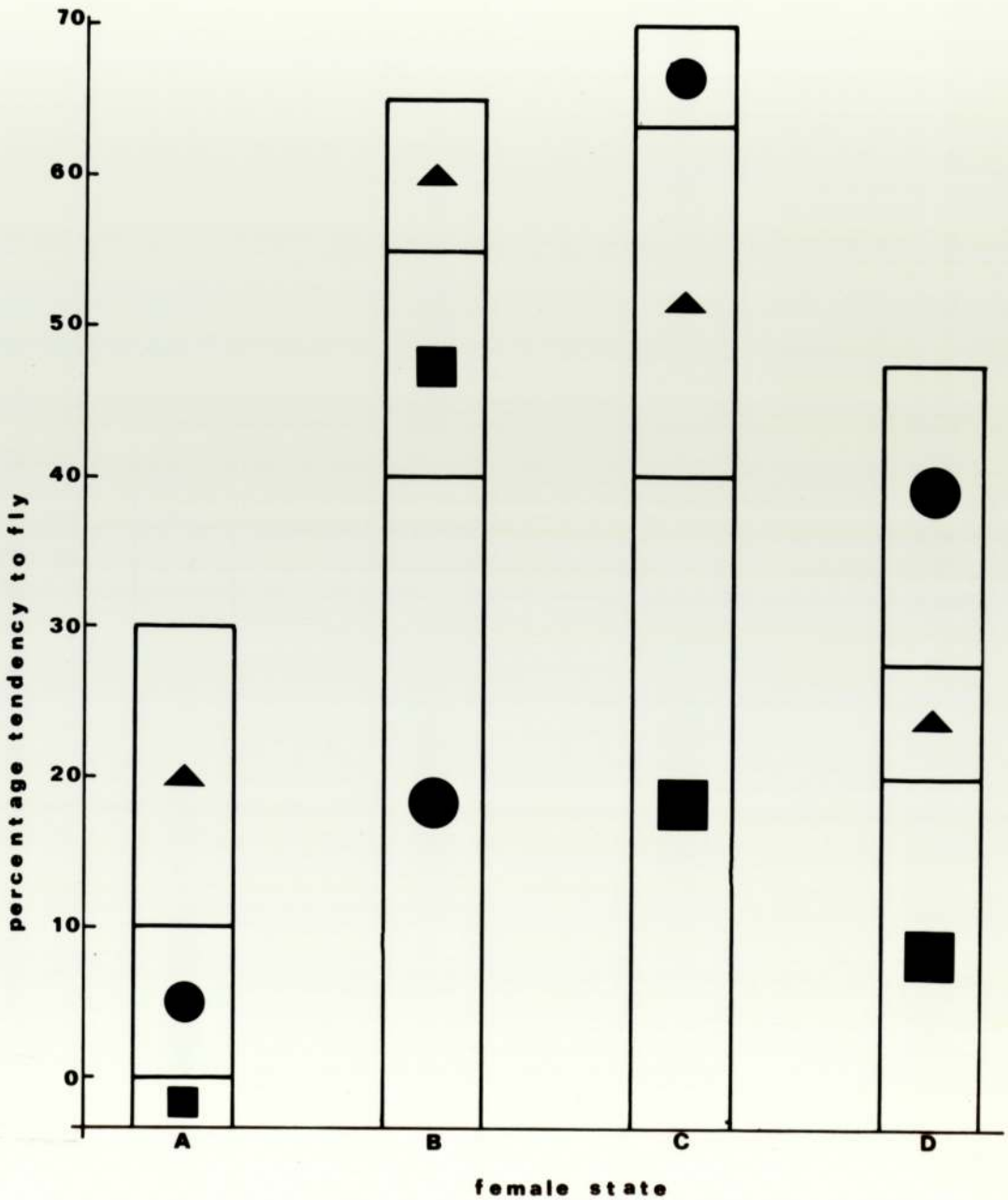

70
60
50
40
30
20
10
0
percentage tendency to fly
A
B
C
D
female state

TABLE 32: Virgin females of 5, 6 and 7 days old tested for their tendency to fly.

TABLE 33: Females aged 5, 6 and 7 days tested immediately after mating for their tendency to fly.

Table 32

	Female age (days)		
Behaviour	5	6	7
No activity	8	7	5
Pre-flight	0	0	1
Open wings	0	1	1
Flight	0	0	1
Number of replicates	8	8	8
Tendency to fly - per cent.	0	10	30

Table 33

	Female age (days)		
Behaviour	5	6	7
No activity	2	3	1
Pre-flight	3	4	3
Open wings	2	1	3
Flight	1	0	1
Number of replicates	8	8	8
Tendency to fly - per cent.	55	40	65

TABLE 34: Females aged 5, 6 and 7 days tested 24 hours after mating for their tendency to fly.

TABLE 35: Females aged 8, 9 and 10 days tested 4 days after mating for their tendency to fly.

Table 3 4

	Female age (days)		
Behaviour	5	6	7
No activity	4	1	2
Pre-flight	1	3	3
Open wings	2	1	1
Flight	1	3	2
Number of replicates	8	8	8
Tendency to fly - per cent.	40	70	60

Table 3 5

	Female age (days)		
Behaviour	8	9	10
No activity	6	3	5
Pre-flight	1	2	2
Open wings	0	2	0
Flight	1	1	1
Number of replicates	8	8	8
Tendency to fly - per cent.	20	47·5	27·5

the replicates in a particular category flew. Scoring is assessed by multiplying the number of replicates by the indices as follows: "no activity" x 0, "pre-flight" x 1·5, "open-wings" x 2, "flight" x 2·5. This makes the total possible score, for 8 replicates, 20 points.

Chi-squared analysis of the numbers of females performing a particular behaviour was made by combining the results of the 3 age groups in the virgin, immediately post-copula, 1 day post-copula and 4 days post-copula categories. A contingency table was constructed (table 36). The analysis was performed according to the method described by Snedecor and Cochran (1967). Using this method a value of 23·1 was obtained for the chi-square. This figure is statistically significant at the P = 0·01 level with 9^{o} freedom. It can therefore be said that the relative numbers of individuals with different flight behaviours are not the same in each category of virgin/mated females.

Graph 5 plots the percentage scores for the tendency to fly.

3) Dispersal from open cultures.

By means of dissection inferences were drawn about the state of dispersing adults. Both males and females were classified into 3 states.

For females, state 1) is described as "virgin/not started ovipositing". This state is characterised by an abdomen entirely filled with eggs and a crop not detectably expended. Whether or not the eggs are fertilised cannot be deduced from this simple examination.

Female state 2) is termed "still ovipositing". The abdomen of females within this category contain fewer eggs than the abdomen of females within state 1). The crop is also somewhat expanded with air. It may be inferred that females in this state have been inseminated and have begun to lay eggs.

Female state 3) - "finished ovipositing" - is recognisable as

that in which there is an absence of eggs in the abdomen and a correspondingly large increase in the size of the crop.

In males similar broadly defined states are recognisable and are classified as follows: 1) abdomen full of fat, crop not expanded; 2) abdomen with some fat, crop somewhat expanded; 3) abdomen containing very little, if any, fat, crop greatly expanded with air. In both sexes an increase in age may be assumed from state 1) to state 3).

The results of dispersal from the cultures described in "methods" are recorded in table 37. This table was used as a contingency table and the value of chi-square was discovered. This value is 4·79 with 2^{o} freedom. The P value is therefore greater than 0·05. The relative numbers of individuals in each state are therefore statistically equivalent between the two sexes. However the relative total numbers of each sex are widely different. Taking an expected value of 83 - half the grand total - the chi-square value calculated gives a P value considerably less than 0·001. The relative numbers of males and females dispersing are thus statistically very different.

Flight activity from open culture A was recorded by opening the jar and recapturing individuals observed flying from its rim. A duration of 30 minutes was allowed for this experiment. Table 38 summarises the results of this experiment; the state of the adults is analysed according to the method given above.

Open culture B was the subject of a slightly different experimental method. An adult climbing to the rim of the culture jar was observed for a timed interval of 5 minutes. During this period all other adults were prevented from reaching the rim. The behaviour of each individual was recorded. It was then placed, alone, in a vial containing finely sieved wholemeal flour. After 1 week this was sieved, any eggs were counted, the adult was killed and dissected.

In this way it was possible to differentiate between virgin females and those which had been inseminated but had not commenced oviposition. The results of this experiment are recorded in table 39 which therefore shows state 1) females as virgin and state 2) females as those still ovipositing.

The numbers of replicates in both tables 38 and 39 are too small to permit chi-square analysis by the contingency table method (Snedicor and Cochran, 1967). It can be demonstrated however that a statistically significant difference exists between the relative numbers of males and females flying from the open culture jars.

To determine the state and sex of a cross-section of individuals in an adult population; 2 long established cultures - similar to those used in the dispersal and flight tests - were randomly sampled. Table 40 shows the results of this sampling from culture X. The individuals were killed and their state determined in the manner previously described.

Culture Y adults were placed individually in vials containing wholemeal flour. These were then treated in a similar manner to that described for the adults of culture B above. The results of this sample are recorded in table 41.

Both table 40 and 41 were combined (table 42) in a contingency table. A chi-squared analysis was performed. The value thus obtained is 6·57 with 2^{o} freedom. This gives a P value of 0·05, thus indicating that the relative numbers within each state-group are not statistically equivalent between the two sexes. Examination of the table shows that this lack of equivalence is produced by the larger number of females in state 3). It is evident however that the total numbers of males and females are not significantly different.

TABLE 36: Contingency table of numbers of females showing flight tendency both before and after mating.

V: virgin. I: immediately after mating.

N.A.: no activity. P.F.: pre-flight activity

O.W.: open wings. F.: flight activity.

TABLE 37: Numbers of adults dispersing from a well-established open culture jar within 24 hours. (For explanation of states see text.)

Table 36

	Behaviour type				
Mating status	N.A.	P.F.	O.W.	F.	total
V	20	1	2	1	24
I	6	10	6	2	24
24	7	7	4	6	24
4	14	5	2	3	24
Total	47	23	14	12	96
			CHI-SQUARE		
V	5·79	1·97	0·64	1·33	9·73
I	2·81	3·14	0·66	0·33	6·94
24	1·92	0·27	0·07	3·00	5·26
4	0·43	0·10	0·64	0·00	1·17
Total	10·95	5·48	2·01	4·66	23·1

Table 37

State of adult	Male number	Female number	Total
1)	13	110	123
2)	6	22	28
3)	0	15	15
Total	19	147	166
	CHI-SQUARE		
1)	0·08	0·01	0·09
2)	2·45	0·31	2·76
3)	1·72	0·22	1·94
Total	4·25	0·54	5·79

TABLE 38: Numbers of adults flying from well-established open culture jar A within 30 minutes. (For explanation of states see text.)

TABLE 39: Numbers of adults showing pre-flight and flight activity from well-established open culture jar B. (For explanation of states see text.)

Table 38

State of adult	Male number	Female number
1)	0	0
2)	1	17
3)	0	3
Total	1	20

Table 39

Behaviour: flight		
State of adult	Male number	Female number
1)	0	0
2)	0	8
3)	1	3
Total	1	11
Behaviour: pre-flight		
1)	0	0
2)	0	0
3)	1	0
Total	1	
Behaviour: Open wings		
1)	0	1
2)	0	6
3)	0	0
Total	0	7

TABLE 40: Random cross-section of adult population in well-established culture jar X. (For explanation of states see text.)

TABLE 41: Random cross-section of adult population in well-established culture jar Y. (For explanation of states see text.)

TABLE 42: Contingency table analysing the numbers of adult replicates from both the culture jars X and Y. (For explanation of states see text.)

Table 40

State of adult	Male number	Female number
1)	5	2
2)	6	6
3)	0	3
Total	11	11

Table 41

State of adult	Male number	Female number
1)	4	4
2)	7	6
3)	0	3
Total	11	13

Table 42

State of adult	Male number	Female number	Total
1)	9	6	15
2)	13	12	25
3)	0	6	6
Total	22	24	46
	CHI-SQUARE		
1)	0·47	0·43	0·90
2)	0·09	0·08	0·17
3)	2·87	2·63	5·50
Total	3·43	3·14	6·57

4) Time in copula and locomotory activity.

Experiments were carried out to discover whether changes in locomotory activity were connected with length of time spent in copula. Groups of male and female pairs were forcibly separated immediately after turning posterior to posterior and at timed intervals therefrom. Locomotory activity was tested after a delay of 1 hour.

Table 43 summarises the results of these experiments. Comparison was made statistically between the mean locomotory activity thus produced and the mean locomotory activity of the combined 5, 6 and 7 day old virgin females. The mean locomotory activity of females subjected to 1·5 minutes and less in copula is not statistically significantly different from the mean locomotory activity of virgin females. However, for a similar comparison between virgin and 2 minute copula females the P value is less than 0·05. For a comparison between virgin females having experience of 2·5+ minutes of copula the P value is less than 0·01. It can therefore be deduced that at least 2 minutes in copula are necessary to significantly affect locomotory activity.

Graph 6 illustrates table 43 with the addition of the locomotory scores for virgin females and females tested immediately after natural completion of copula. The standard deviations are also plotted.

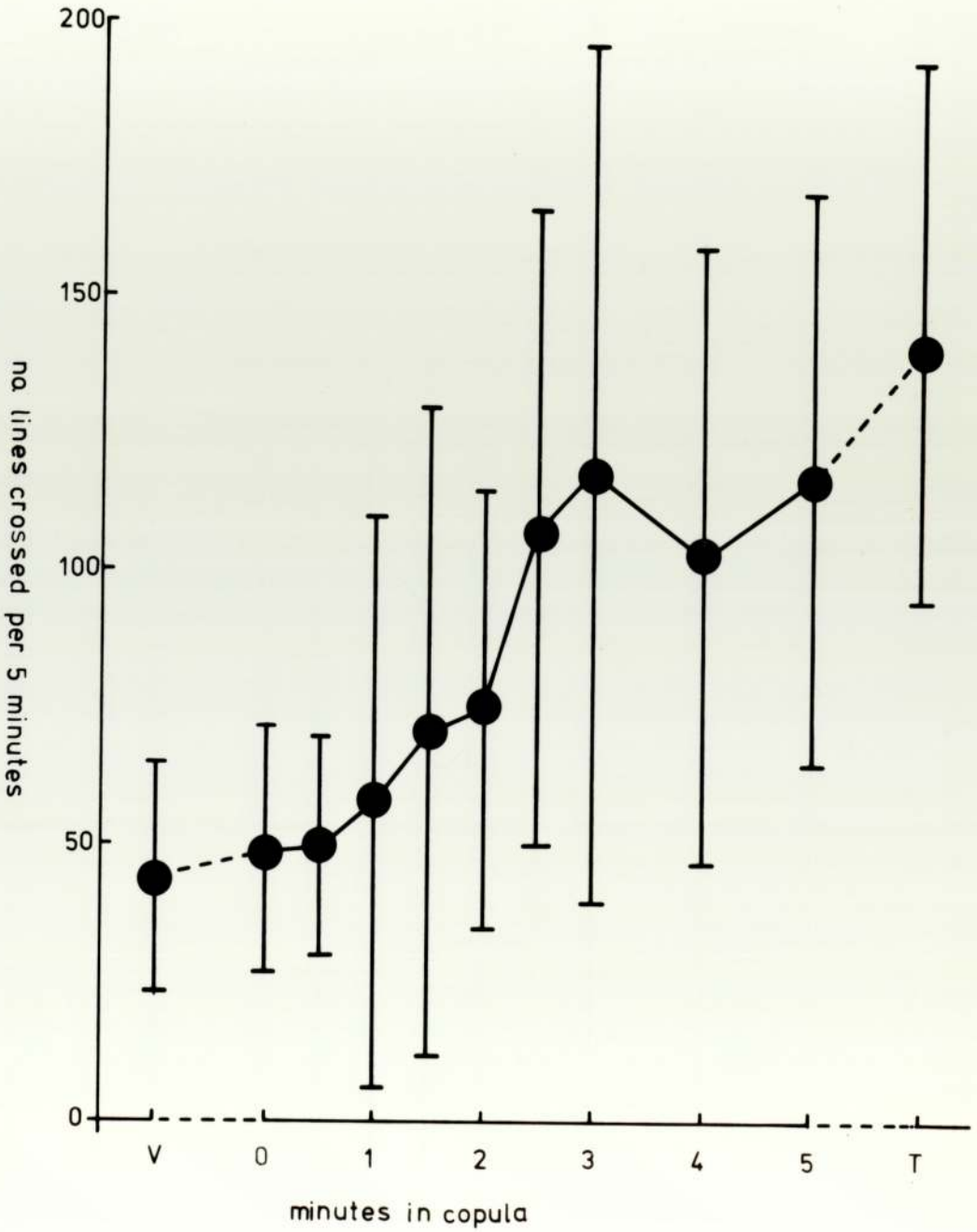
Table 44 compares the locomotory scores of those females in the 2·5 and 3 minutes categories which did and did not oviposit. The locomotory scores of egg laying females are higher than those of non-ovipositing females. Statistical comparison however gives a P value greater than 0·05; the difference is therefore not statistically significant.

Graph 7 is a scatter plot of the replicates in table 42 with

GRAPH 6 : The change in female locomotory activity (number of lines crossed per 5 minutes) against length of time spent in copula (minutes).

V: virgin

T: full-term copula


200
150
100
50
0
no. lines crossed per 5 minutes
V
0
1
2
3
4
5
T
minutes in copula

GRAPH 7 : Scatter diagram showing locomotory activity (number of lines crossed per 5 minutes) of 5 and 6 day old females against time spent in copula (minutes).

TRIANGLES : ovipositing females

CIRCLES : non-ovipositing females

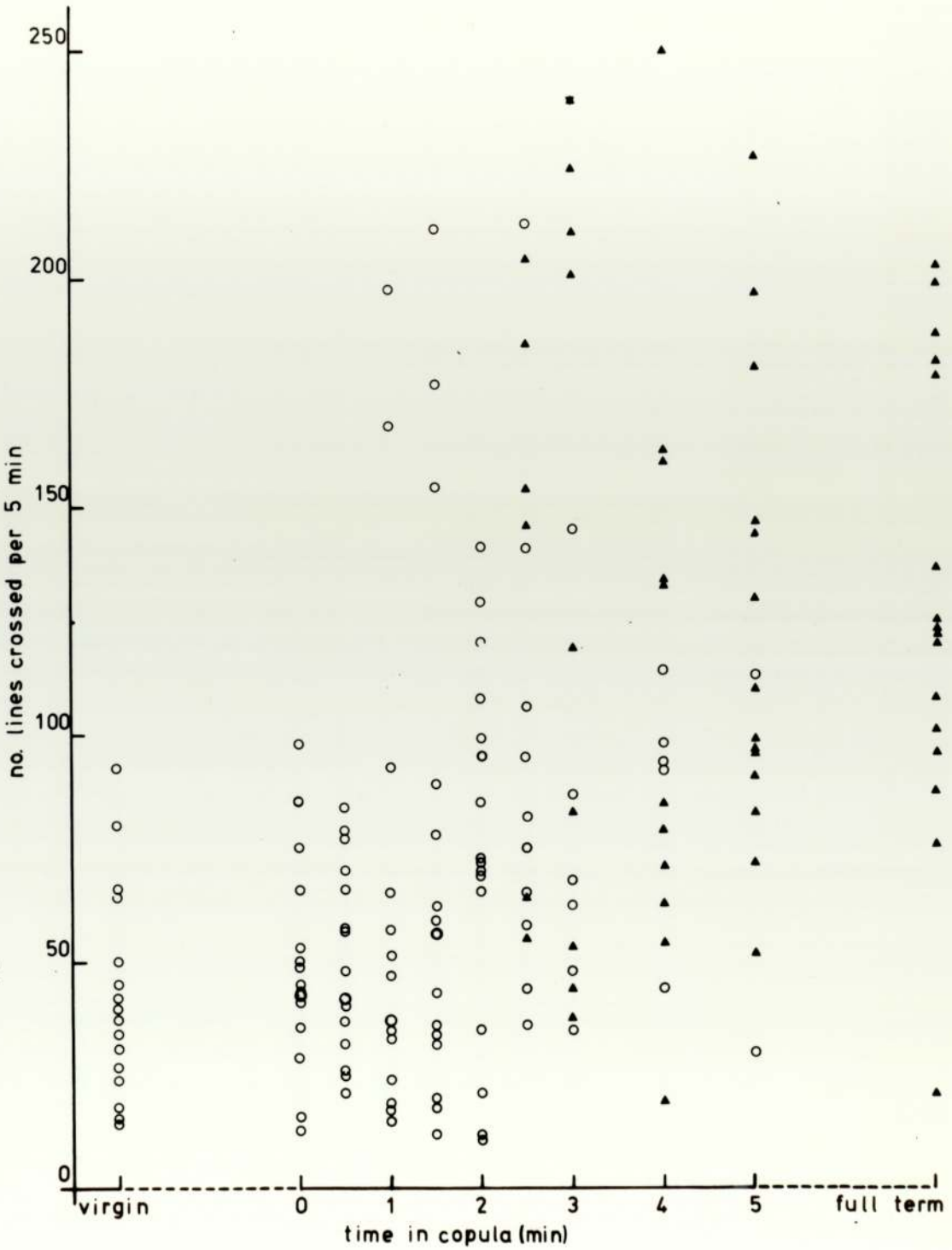

250
200
150
100
50
0
no. lines crossed per 5 min
virgin
0
1
2
3
4
5
full term
time in copula (min)

TABLE 43: Locomotory activity (number of lines crossed per 5 minutes) of females separated after different intervals in copula.

Table 43

	When separated (minutes)								
Replicate number	0	0·5	1·0	1·5	2·0	2.5	3.0	4·0	5·0
1	98	84	198	211	141	212	239	250	226
2	85	79	168	177	129	204	239	163	197
3	75	77	93	154	120	186	224	160	181
4	66	70	65	89	108	154	210	134	147
5	53	66	57	78	99	146	201	133	144
6	50	58	51	62	95	141	145	114	130
7	49	57	47	59	85	109	119	98	113
8	45	48	37	56	73	95	87	94	110
9	44	42	36	56	72	82	83	92	99
10	43	42	35	43	70	75	68	85	97
11	43	40	33	36	69	65	62	79	96
12	41	37	24	34	65	64	53	71	91
13	36	32	19	32	35	58	48	62	83
14	29	26	18	20	21	55	44	54	72
15	16	25	15	18	12	44	38	44	52
16	13	21	37	11	11	36	35	19	30
mean	49	50	58	71	75	108	118	103	117
standard deviation	23	20	52	59	40	58	78	56	52

TABLE 44: Comparison of locomotory scores (number of lines crossed per 5 minutes) for ovipositing and non-ovipositing females after 2·5 and 3 minutes in copula.

TABLE 45: Contingency table comparing the numbers of ovipositing and non-ovipositing females above and below a line dividing high from low locomotory scores (number of lines crossed per 5 minutes).

Circles: non-ovipositing females.

Triangles: ovipositing females.

Table 44

	Locomotory scores			
	2·5 min		3·0 min	
replicates	Ovipositing	Non-ovipositing	Ovipositing	Non-ovipositing
1	55	212	239	145
2	64	141	239	87
3	186	111	224	68
4	204	95	210	62
5	154	82	201	48
6	146	25	119	35
7		65	83	
8		58	53	
9		44	44	
10		36	38	
mean	135	92	145	74
standard deviation	62	53	86	39

Table 45

	Above	Below	Total
Circle	10	109	119
Triangle	27	30	57
Total	37	139	176
		CHI-SQUARED	
Circle	4·01	2·40	6·41
Triangle	18·89	5·01	23·90
Total	22·90	7·41	30·31

the addition of the replicates for virgin and immediately post-completed copula. Open circles indicate the locomotory scores of females which did not oviposit and black triangles the locomotory scores of females which did oviposit.

An analysis was made to demonstrate the possible existence of a connection between the initiation of oviposition and an increase of locomotory activity. An arbitrary division was made parallel to the x axis of graph 7 at the 125 lines crossed per 5 minutes point (i.e. half way up the y axis). A contingency table was drawn up analysing the relative numbers of females ovipositing and not ovipositing above and below this arbitrary line (table 45). It can be seen that the percentage of ovipositing females above this line is 47·37% compared with only 8·4% of non-ovipositing females. Chi-squared analysis of the contingency table produces a value of 30·31, giving a very highly significant value for P of less than 0·001 (with 1^{o} freedom). The relative number of ovipositing/non-ovipositing females can therefore be said to be different above and below the line.

It therefore seems probable that at least part of this significant difference can be accredited to the increase of locomotory activity associated with females in which oviposition was initiated.

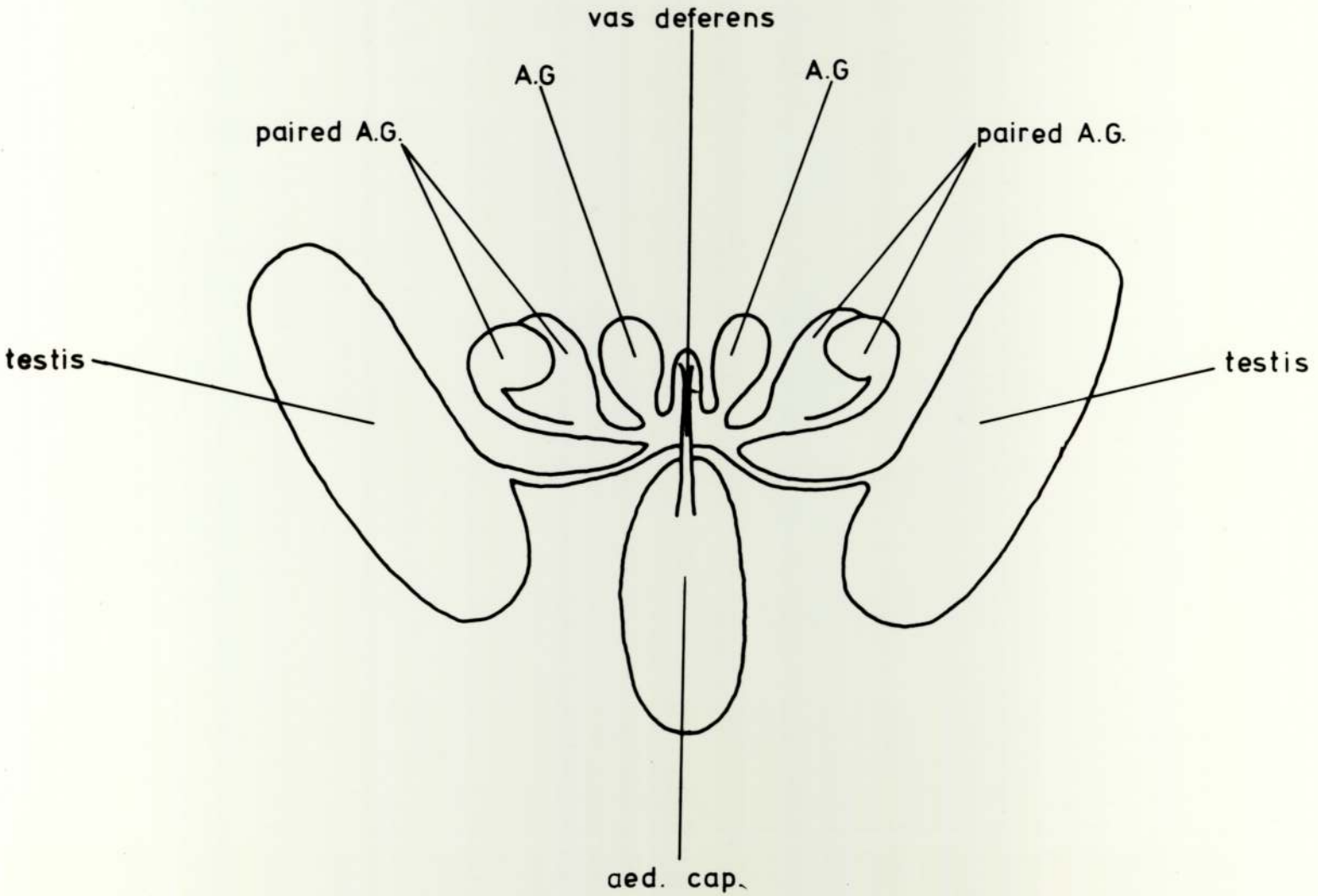
5) The male accessory glands.

Fig. 12 is a diagram of the internal genital apparatus of male *S. paniceum*. The testes, the 3 pairs of accessory glands (A.G.) and their relationship to the aedeagal capsule (aed. cap.) are illustrated. This is the first diagram of these organs to be published in the literature on *S. paniceum*.

FIGURE 12 : Male internal genital organs

A.G. : accessory glands

aed.cap. : aedeagal capsule.


vas deferens
A.G
A.G
paired A.G.
paired A.G.
testis
testis
aed. cap.

DISCUSSION

The relationships between mating and certain subsequent changes in the female *S. paniceum* have already been discussed in previous chapters. Chapter A1c showed that there is a positive correlation between mating, female pheromone reduction and female refractory behaviour. In chapter B1 a connection was established between mating and the initiation of oviposition. The present chapter is concerned with yet another effect of mating; one which has had no previous discussion in the literature on *S. paniceum*.

Section 1) of the results examines the way in which mating affects locomotory activity and section 2) examines the effect of mating on flight activity. Both these activities were found to increase significantly after mating and the importance of this increase in the life of the insect is discussed below. To understand this importance it is necessary to view the increase of activity in the context of the facts about dispersal and flight from open cultures examined in section 3) of the results.

Firstly it must be pointed out that in discussing dispersal the prime concern is with females. Section 3) of the results demonstrates that significantly fewer males take part in dispersal, although there are probably equal numbers of them within the population (table 42). With reference to 11 species of diurnal Lepidoptera Scott (1975) notes that males tend to remain in sites favourable for mating whilst females tend to disperse. As Johnson (1969) points out: "it is the females that place the eggs into new habitats, and they are therefore of greater significance than the males in the study of migration (i.e. adaptive dispersal)."

The dispersal of insects may be considered to be either

accidental and inadvertent or adaptive - in which case it may correctly be termed migration. In many species the degree to which dispersal is adaptive is difficult to gauge and its value as a regular means of ensuring survival has to be assessed quantitatively in relation to the ecology of the species (Johnson, 1969). In the ecological respect the examination of the movement of a population from an open culture jar is not comparable to the behaviour of individuals kept in isolation; except insofar as the behaviour of individuals superimposes itself onto the behaviour of the population as a whole. The phenomena with which this chapter is concerned are therefore of two contrasting types. The first is an effect of the experience of an individual with another individual (i.e. mating); the second is an effect of the interaction of the whole population. It may be inferred that neither phenomenon should be considered in isolation but that each may modify the other.

Thus certain comparisons between the two phenomena may produce apparent contradictions. It can be seen from table 37 that the vast majority of females dispersing from an open culture are in state 1); the virgin or inseminated by not ovipositing state. This is in contrast with that which one would be led to expect from an examination of Section 1) of the results. From this, since oviposition begins within 16 hours of mating (B1), it would be deduced that the majority of females moving any distance could be expected to be in state 2).

It is probable that the apparently similar reaction - an increase in activity - is produced in the two cases above by two different causal phenomena - one individual and the other the result of population interaction. To examine the latter case: in *Tribolium* spp. for instance (Coleoptera, Tenebrionidae), Ziegler (1976) has demonstrated that dispersal from the culture is, in its initial stages, a density dependant phenomenon.

The age of the cultures of *S. paniceum* used in the present experiment resulted in considerable crowding; it is probable therefore that any dispersal by egg-bearing females away from this situation would be ecologically adaptive and thus a true migration. Since Johnson (1969) demonstrates that for the majority of insect species migration is performed by pre-reproductive females, it is not surprising that the greater majority of migrating *S. paniceum* females were in state 1).

The increase of locomotory activity caused by mating on individual females, however, probably serves a different adaptive purpose. It has already been shown (chapter B1) that oviposition takes place shortly after mating and that particular stimuli are necessary to initiate oviposition. It would be reasonable therefore to expect an increase in overall female activity in order that she should ensure discovery of the correct stimuli and thus a suitable oviposition site. Such changes in behaviour resulting from mating are known in other insect species, for instance the coleopteran *Melolontha melolontha* L. (Scarabidae) (Stengel and Schubert, 1972) and the heteropteran *Dysdercus superstitiosus* (F.) (Gatehouse and Hall, 1976).

The component in table 37 of females in state 2) probably represents those individuals which, having mated and begun oviposition, are dispersing from the population randomly as a result of their increased overall activity. This dispersal could be adaptive; for instance Shapiro (1971) has shown that sexual interactions within dense populations of *Pieris protodice* (Lepidoptera) lead to the emigration of gravid females which colonise new habitats. Or it could merely be random; Kehat and Wyndham (1973) for instance, demonstrate that in *Nysius vinitor* (Heteroptera) flight responses are migratory in immature females but dispersive in mature females.

The pattern of flight in female *S. paniceum* is somewhat different. Although, as results in section 2) demonstrate, there is an increased percentage tendency to fly immediately after mating, it is not until 24 hours after mating that the number of individuals actually flying becomes significant (cf: tables 33 and 34). It is postulated that the reasons for this are predominantly physiological. These may be connected with certain changes which take place in the insect as the eggs are laid. During oviposition there is an extension of the abdomen which results in some increase in the total body volume. In order to maintain the pressure of the haemolymph under these conditions it is a common phenomenon for insects to swallow air into the crop and midgut caecae; the expansion thus created will increase as the eggs are laid so that haemolymph pressure is maintained (Chapman, 1971). The air-sac thus formed may well have other functions. For instance an insect with air-sacs has a lower specific gravity than a similarly sized insect without them and they may therefore make flight easier (Imms, 1957). In the Australian coleopteran *Heteromyx obesus* the crop becomes distended with air just before swarming flight begins (Morgan, 1977); this phenomenon is apparently associated with the pre-flight behaviour of individuals of that species. There is an apparent similarity between this phenomenon in *H. obesus* and the similar distension of the crop in female *S. paniceum* as they begin to oviposit. It seems probable that in *S. paniceum* mating stimulates in females the tendency to fly by increasing the occurrence of pre-flight behaviour, but that actual flight is prevented from occurring until the correct physiological change - distension of the crop as the eggs are laid - has come about. This observation is confirmed by the fact that the majority of females flying from an open culture are in state 2) (tables 38 and 39).

Tables 40 - 42 demonstrate that normal adult populations of *S. paniceum* are composed of males and females in approximately equal numbers. The significant difference between numbers of males and females in state 3) (table 42) is probably a reflection of the method used to define these states; the gradation between male state 2) and state 3) being insufficiently clear to allow 100% correct identification. In fact, as would be expected, the age range probably follows a normal distribution pattern. Dispersal from this population follows a pattern in which changes in individuals are superimposed upon the effects of the interactions of the whole population.

In addition to the pattern formed by the interaction of the two aspects discussed above, it is important to take into account the individual's reaction to external stimuli. Burges and Jarrett (1976) for instance have demonstrated that in 5 species of noctuid and tortricid moth the basic pattern of flight is modified by an individual's responses to external physical stimuli. External stimuli, such as the presence of a suitable site and the smell of food, are necessary to initiate oviposition in *S. paniceum* (B1). It is therefore probable that certain external stimuli will also be involved in such activities as the initiation of flight activity. Such has been found to be the case in *Aphis fabae* (Hemiptera) (Binns, 1977). It will be remembered from results' section 2) that in *S. paniceum* the tendency to fly was tested by allowing the female to climb to the rim of a glass tube. This method was the result of observations which showed that no attempt at flight behaviour took place while the insect was crawling on a flat substrate. Flight attempts in *S. paniceum* appear to be stimulated by the signals received by an individual when it has succeeded in surmounting an object. Pre-flight behaviour takes place only when the object has been surmounted; and the functional

nature of this behaviour may be connected with the reception of stimuli necessary for correct orientation before the flight can begin.

From all the foregoing observations it is possible to produce a synthesis of the ecological consequences of the factors involved in dispersal by migration and by flight. Individually newly eclosed virgin adult females have a low level of locomotory activity and a very low tendency to fly. Older females however tend to show a somewhat increased activity and a greater tendency to fly. In view of the probable existence of synchronised emergence in *S. paniceum*, discussed in A1b and further to be discussed in C2, it would make ecological sense for the female to remain close to the site of emergence since there she would have an increased chance of being inseminated. However it would also make good ecological sense for an older and still unfertilised female to show more activity and thus increase her chances of finding a male - since without insemination the few eggs she may lay will be infertile (B1). This age-dependent increase in activity and tendency to fly could well be the result of the maturation process discussed in relation to mating efficiency (A1b) and oviposition rate (B1). If the adaptive significance of this process is that it enables an older virgin female to fly, and thus increase her chance of finding a male, an ecological reason for the process becomes more apparent.

Once a female is mated her locomotory activity increases significantly and so does her tendency to fly - but not her actual flight activity. This increase in activity is ecologically sound if, as is suggested above, it is directed towards the location of a suitable oviposition site. It is suggested that the corresponding increase in the tendency to fly is, initially, merely the result of the same process initiated by mating and, at this stage, has no intrinsic adaptive significance.

It has been noted by several authors including Azab (1943) and Kashef (1956) that female *S. paniceum* show a tendency to lay their eggs in groups of from 5 to sometimes 14; the eggs being cemented together by a mucilaginous secretion. This observation was confirmed by the present author. It is likely that the eggs, thus laid in groups, will, during the first 24 hours after mating, be placed near the site where mating took place - if this site is also suitable for oviposition. On the day after mating however, when locomotory activity, the tendency to fly and actual flight are at their peak, it is likely that the female will climb an obstacle and thus receive the stimuli necessary to begin a dispersal flight. This flight may enable a female to oviposit in a previously uncolonised food resource. Of course it is likely that the female will not succeed in reaching a suitable oviposition site, in which case - in adaptive terms - it is valuable that she has already oviposited. The significance of clustering eggs is here apparent; such behaviour would ensure that when any eggs, speculatively placed after such a dispersal, reached maturity, the emerging adults would be more likely to be in the vicinity of members of the opposite sex and thus in a position to copulate at once. A secondary infestation may thus be started.

Superimposed on this pattern of individual behaviour are the effects caused by a large adult population. It is probable that when competition for resources is small, at the beginning of an infestation, the level of locomotory activity in virgin females will be low - as it is in the individuals examined. From a comparison of the open culture experiments with the experiments on individuals however, it is apparent that a large population will cause an increase of activity in virgin females, under suitable circumstances resulting in their migration

away from the culture or initial infestation. It is also possible that a dense population may cause the migration of inseminated but non-ovipositing females as a result of increased competition for suitable oviposition sites. The ecological significance of thus restricting the number of ovipositing females within a population is probably connected with density-dependent reduction in percentage maturation from egg to image which is demonstrated and discussed in detail in the next chapter (C1).

These considerations have obvious implications for pest management. It can be said of a *S. paniceum* infestation that:

1) any flying insects captured are, in all probability, females which have already oviposited and may have started secondary infestations.
2) the majority of insects discovered crawling away from a source of infestation are likely to be migrating females which have not yet oviposited. In this case the indication is that the infestation is well established.

It only remains to consider the question of length of time spent in copula and its effect on the increase in locomotory activity. In some ways this increase is a more precise tool for the study of the effect of time in copula than either refractoriness or oviposition-induction, the latter two being either/or effects whereas the former appears to be more evenly graduated (see graph 6). There is a discontinuity however which, as in refractoriness and oviposition, appears to occur at the 2·5 minute point, although in fact the 2 minute group is significantly different from the virgin group. The problem with examining locomotory activity is the wide range of variation between individuals which is more marked in mated than virgin females. There is a possibility that there is a genetic element to this variation.

Such has been found in other Coleoptera, for instance *Tribolium* spp., in which Ogden (1970) successfully selected for active and inactive lives.

There is also the question of what precise event during mating produced this increase in activity. The connection between locomotory activity and fertilisation is illustrated in graph 7 and table 45 shows a positive correlation between increase in locomotory activity and oviposition. The correlation is not exact however, as can be seen from the fact that several females show greatly increased activity - well beyond the range of variation in the virgin female group - without a concommittent initiation of oviposition. On the other hand all except 1 of the ovipositing females fall above the lower limit of the range of variation of the full-term copula females - indeed several exceed the upper limit of the range. Table 44 also demonstrates that the locomotory activity of non-ovipositing females is not significantly different from the locomotory activity of ovipositing females within the 2·5 and 3 minutes in copula groups examined - even when the corresponding categories from each copula-length group are combined.

This again leads us to suppose that more than one factor is involved: the passage of sperm which is necessary to fertilise the eggs and the passage of another substance which is probably produced in the accessory glands illustrated in fig. 12. Examination of graph 7 shows that this substance probably begins to pass from the male into the female at around the first minute after mating, since it is then that the first individuals with locomotory activity beyond the virgin-range begin to appear. However the volume of this substance does not reach a value sufficient to have a significant effect until the 2nd minute of copula, and oviposition is not initiated until 2·5 minutes of copula have passed.

It therefore seems probable that the A.G.S. alone is capable of initiating the increase of locomotory activity. But whether oviposition-initiation and the onset of refractory behaviour are products of A.G.S. alone or sperm alone or a combination of the two cannot be deduced from the present experiments.

SECTION C:

PRE-ADULT DEVELOPMENT

SECTION C. PART 1. Population density and its effects on early developmental mortality and sex ratio.

INTRODUCTION

The developmental mortality of *Stegobium paniceum* at different temperatures and humidities has been studied by Lefkovitch (1967). However, the effect of population density on mortality in this species has not been previously studied. This chapter examines the way in which percentage mortality (from egg to imago) is affected by the population density within a fixed volume of food at a constant temperature and humidity.

Observations are included of the adult sex-ratio and percentage mortality at the early larval stage.

METHOD

Eggs collected and counted as previously described (B1) were transferred to 3 ml glass vials containing 0·75 g of finely sieved wholemeal flour. The vials were closed with perforated plastic caps and then stored in dessicators at a constant temperature of 28°C and a relative humidity of 70%. Approximately 80 days later, when the development cycle was complete and most adults were dead (Lefkovitch, 1967), the vials were examined. The adults were counted and sexed.

Eggs hatching and early larval mortality were studied by placing a known number of eggs into glass petri-dishes which were then covered with transparent 'cling-film'. The dishes were maintained at 28°C and 70% relative humidity. Eggs and hatched larvae were counted at different intervals.

RESULTS

1) Population density and percentage mortality.

Graph 8 is a scatter plot of percentage survival to adult as a function of the number of eggs initially placed in the vial. A downward trend of percentage survival with increasing population density can be appreciated from this graph.

Table 46 analyses graph 8 and demonstrates that the percentage survival to adult in the 0 - 50 initial population category is, in nearly all cases, statistically significantly higher than the percentage survival in categories with initially larger populations.

It is inferred from these results that high population densities have an adverse effect on percentage survival to adult.

2) Early larval mortality.

The observations recorded in tables 47, 49 and 50 are not exhaustive. They do, however, provide some indication of the degree of early larval mortality. For 70% relative humidity and 27·5^{o}C Lefkovitch (1967) gives the duration of the egg stage in *S. paniceum* as 8 days. The present figures appear to show that, in the experiments under consideration, hatching begins at around the 6th day after oviposition. Table 47 demonstrates that at 8 days and more, statistically, the mean percentage hatch does not differ between any of the groups.

Comparison of the mean percentage hatches in table 47 with the mean percentage maturation figures of table 46 shows that these are not significantly different when analysed statistically. This seems to imply that the highest percentage mortality occurs during the early larval period.

Table 49 shows the results of re-counting eggs and larvae after an interval of 24 hours. First, however, a percentage retrieval factor

was determined by re-sieving and re-counting eggs. Thus the degree of error produced by the counting method could be assessed; this is shown to be a mean of 7·57% (table 48).

Inspection of table 49 demonstrates that a decline in the number of unhatched eggs over 24 hours is not matched by a corresponding rise in the number of larvae. These disparities are in excess of 7·57% and are probably not, therefore, the result of counting error.

Table 50 further demonstrates that for egg/larvae groups of between 8 and 13 days old there is a disparity between the actual total egg/larvae number and the expected total egg/larvae number. This disparity was examined statistically. A counting error of 10% of the original total egg number was subtracted from that number and this was taken to be the value of the total unhatched eggs + larvae to be expected when the dishes were examined (table 50 column 5). However a chi-squared comparison of this expected value with the actual total of unhatched eggs + larvae (table 50 column 4) showed a highly significant p value of less than 0·01. It therefore appears from tables 49 and 50 that a significant number of eggs and/or larvae are disappearing.

3) Sex ratio.

Table 51 examines the sex ratio of adults reaching maturity within 104 vials into which variable numbers of eggs had initially been placed. The mean number of females from each vial is just statistically greater than the mean number of males at a significant level of $p = 0{\cdot}05$.

Table 52 compares the sex ratio above and below an original density of 40 eggs per vial (chosen because there are an equal number of replicates above and below this figure). Statistical comparison of the means in table 52 reveals that above the 40 egg density point the numbers of males and females reaching maturity are significantly different at the $p = 0{\cdot}05$ level.

GRAPH 8 : Scatter diagram of percentage survival to adult against number of eggs originally placed in a constant volume of food.

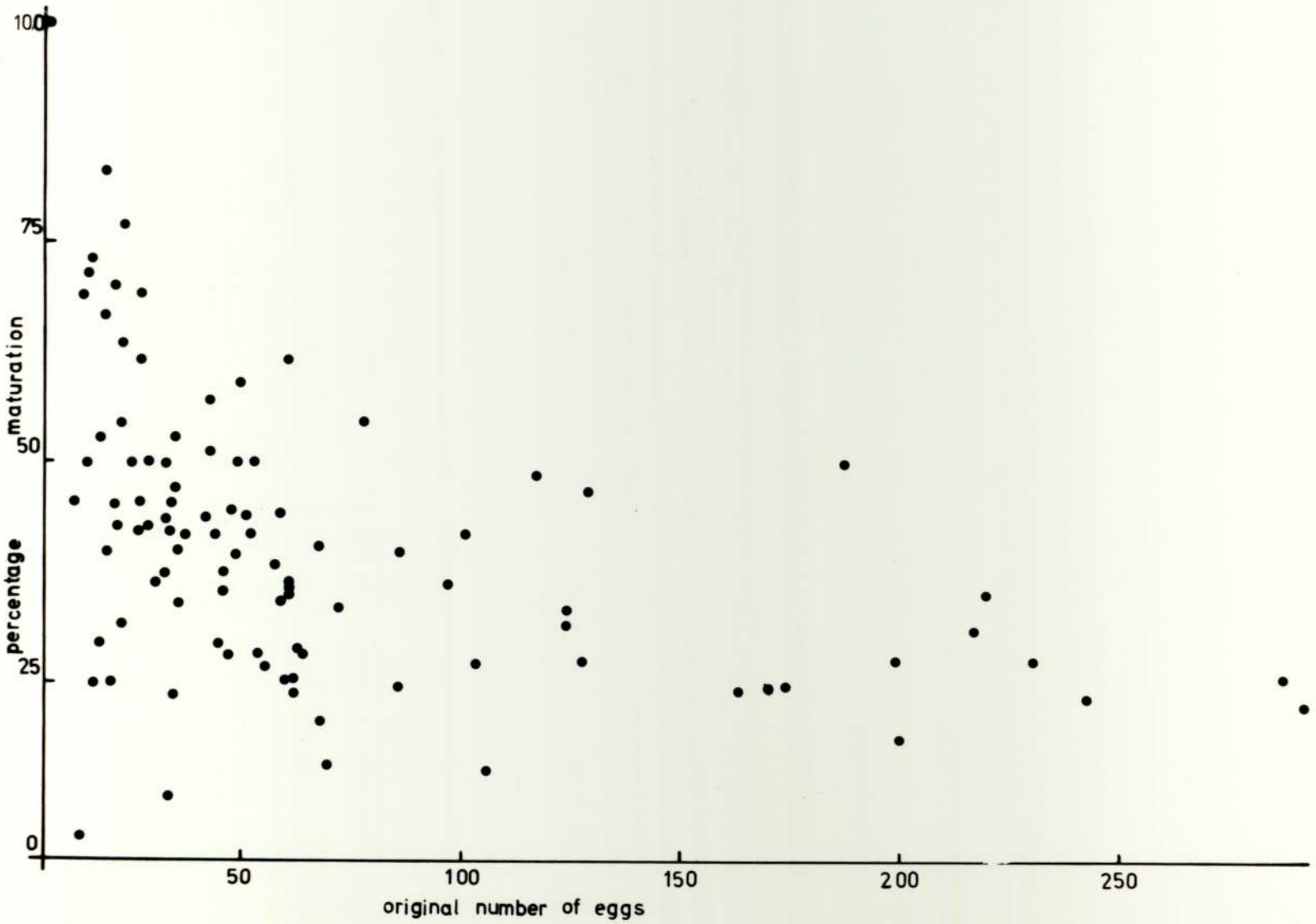

100
75
50
25
0
percentage maturation
50
100
150
200
250
original number of eggs

TABLE 46: Analysis of graph 8: percentage maturing in 5 groups of vials categorised by the number of eggs originally placed in them.

TABLE 47: The difference in percentage hatch at different time intervals after oviposition in days.

Table 46

			Percentage maturing		
Category	Number of eggs	Total eggs in category	Number of replicates	Mean	Standard deviation
A	0-50	2639	55	48	18·5
B	50-100	928	27	34	10·7
C	100-150	273	8	34	11
D	150-200	143	6	24	3·3
E	200-300	171	6	28	5·8

t - test

Between categories	t value	Degree of freedom	p value
A - B	2·71	80	0·01
A - C	1·7	61	0·1
A - D	3·0	59	0·01
A - E	2·4	59	0·02

Table 47

Days after oviposition	Number of replicates	Mean percentage hatch	Standard deviation
6	1	14·44	-
8	2	56·51	41·68
10	3	50·74	25·2
11	5	33·59	19·14
12	3	25·97	14·44
13	4	51·39	13·42
15	4	44·06	13·19
18	2	38·23	2·5

TABLE 48: Calculating a percentage retrieval factor (mean result - column 4) by counting the same group of eggs twice.

TABLE 49: Variation in numbers of eggs and larvae retrieved at two successive counts on 5 groups at an interval of 24 hours.

Table 48

Replicate number	First count	Second count	Percentage not retrieved
1	49	41	16·33
2	56	53	5·36
3	56	52	7·14
4	24	22	8·33
5	40	38	5·00
6	128	119	7·03
7	246	228	7·32
8	74	70	5·40
9	193	180	6·73
10	212	197	7·07
mean			7·57

Table 49

	First count			Second count		
original egg number	days after oviposition	number of eggs	number of larvae	days after oviposition	number of eggs	number of larvae
125	13	7	67	14	2	64
193	12	22	70	13	13	66
115	11	53	33	12	44	37
222	11	153	21	12	123	21
144	10	74	32	11	50	34

TABLE 50: Comparison of the total number of eggs + larvae in 7 groups with an expected value calculated by subtracting 10% of the original number from that number.

TABLE 51: Relative mean numbers of males and females reaching maturity from 104 vials.

TABLE 52: The mean numbers of males and females maturing in a group of 52 vials with an original number of less than 40 eggs compared with the mean numbers of males and females maturing in a group of 52 vials with an original number of more than 40 eggs.

Table 50

Original egg number	egg number	Larvae number	Eggs + larvae	Expected value	chi-squared
160	42	107	149	144	-
169	57	86	143	152	0·53
52	8	28	36	47	2·57
65	6	34	40	59	6·12
60	8	42	50	54	0·3
10	3	6	9	9	0
107	1	92	93	96	0·09
total					9·61

Table 51

	Male	Female
Number of replicates:	104	104
Mean number maturing:	7·34	10·15
Standard deviation	5·28	8·65

Table 52

	Female			Male		
	Number of replicates	Mean	Standard deviation	Number of replicates	Mean	Standard deviation
below	52	5·54	3·08	52	4·48	2·56
above	52	14·77	9·91	52	10·19	5·76

Statistical analysis of the difference between male and female percentage maturity below this 40 egg level however reveals that this is not significantly different (p is greater than 0·1).

From these results it may be implied that the product of a relatively high population density is a sex ratio where there is a preponderance of females.

DISCUSSION

The effects of population density on an insect population are complex. In studying these effects it is important to realise the difficulties of separating the direct effects of density from the indirect effects that are concurrent with increased numbers per unit area (Peters and Barbosa, 1977). Thus population studies on the flour beetle *Tribolium confusum*, for instance, have shown the importance of such phenomena as depletion of the food resource and poisoning caused by the build up of excretory products (Chauvin, 1967). The growth of micro-organisms has also been shown to be a density-dependent factor in insect populations (del Solar and Godoy, 1971).

The present experiments, however, as distinct from those commonly employed in the study of *T. confusum* populations, used only eggs and thus examined the effect of crowding within a single generation. Examination of the vials at the end of this generation showed no evidence of micro-organism infestation, excessive build up of frass or complete depletion of the food resource. The latter is not surprising since the mean weight of adult *S. paniceum* bred at 27·5°C and 70% relative humidity is only 1·63 mg (Lefkovitch, 1967); the initial weight of flour in the vial thus being equivalent to approximately the weight of 460 adults.

In view of the above it seems probable that another mechanism of reducing maturation percentage at high population densities must be sought. For instance intra-specific larval competition for space has been shown to be the sole means by which the scolytid *Ips typographus* survives overcrowding (Ogibin, 1973). This seems, however, to be a difficult phenomenon to demonstrate in *S. paniceum*; although it may be of importance in cocoon construction which is discussed in the next chapter (C2).

Tables 47-50 of the results, however, point to a more obvious population-density control mechanism in the early larval stage; the possibility of cannibalism. Although most insect cultures do not have cannibalism (Peters and Barbosa, 1977), it has been shown that in some species population size is effectively controlled by cannibalism. Examples may be given to *T. confusum* (Young, 1970) or *Anagasta kühniella* (Lepidoptera, Phycitidae) (White and Huffaker, 1969).

Larvae of *S. paniceum* on emergence from the egg consume the remains of the Shell (Bosley, 1976) and in this way obtain the symbiotic yeast cells (Pant and Fraenkel, 1954) which are situated in the surface of the egg (Jurzitza, 1972). It is easy to imagine that this mechanism, once activated, may result in the larva eating any egg with which it comes into contact; thus causing the destruction of an unhatched larva. The young larvae are active and it is possible that, since the petri-dishes used in the present experiment were not supplied with food material, a relatively large number of unhatched eggs were eaten. This would explain the significant variation between the actual eggs + larvae total and the expected eggs + larvae total (table 50).

The adaptive significance of this mechanism, apart from reducing competition within a limited food resource and/or a high population density may also be that it causes the elimination of later-deposited or slower-

developing eggs and thus helps to produce a population which will develop synchronously. The importance of synchronicity to mating efficiency has been discussed in several previous chapters (for instance A1b, A2b).

In the light of the above discussion of cannibalism it is clear that for a female to deposit a high density of eggs in one place is non-adaptive. The function of the increase in locomotory and flight behaviour discussed in the previous chapter (B2) is now more obvious. Such increases will presumably lead to a wider scattering of eggs which thus lessens the chance of early larval cannibalism.

It is fairly common for an insect species to have a slight preponderance of females (Gaaboub, 1971). This would make adaptive sense in a species where the females are monogamous but the males are polygamous. *S. paniceum* is such a species and it seems to possess this female preponderance (table 51). However, the possible existance of a change in the sex ratio induced by a high population density, such as that demonstrated in table 52, is unusual.

Density-dependant effects on the sex-ratio are not unknown in the insects; commonly, however, they are the result of overcrowding at the adult stage, for instance as the result of increased mating competition in hymenopterous species where unmated females produce offspring parthenogenetically (Vicktorov and Kochetova, 1971).

In other species however differences in the sex ratio may be accounted for by the differential mortality rates of the sexes. For instance the number of early larval males in a culture of the Japanese beetle (*Popillia japonica*) exceed the number of early larval females. The relatively higher male mortality rate however gives, by the time of adult emergency, a sex ratio favouring females (Goonewardene et al, 1973). Again, in the hymenopteran *Neodipron sertifer* the mortality rate of the

male eggs and early larvae is higher than that of the female (Lyons and Sullivan, 1974).

The present results indicate that in *S. paniceum* the greatest percentage mortality occurs in the early larval and egg stages, probably by the mechanism of larval cannibalism (see results 2). It seems likely, therefore, that sexual differences in this early mortality result in a slight preponderance of females. This preponderance becomes more significant as the population density, and thus the opportunity for cannibalism, increases. Adult interference in this mortality syndrome was not examined in the present group of experiments. Such interference, however, is likely to be minimal since, at least in laboratory cultures, the surface dwelling adults do not come into contact with the medium-enclosed larvae.

The adaptive significance of the population-density/sex-ratio relationship is not clear. It may be, however, that greater numbers of females will increase the pro-migratory stimulation discussed in the previous chapter (B2). Under normal circumstances such migrations will relieve the population pressure and increase the chance of colonisation of a previously unexploited food resource.

SECTION C. PART 2. Late larval, prepupal and pupal mortality and cocoon building.

INTRODUCTION

By means of the tube-block method, described in the general introduction to methods, it was possible to record the development of individual *Stegobium paniceum* from late larva to adult. Information about the later stages in the life-cycle was thus gathered in greater detail than by any previous author.

Using this data the present chapter continues the observations recorded in chapter C1 by presenting novel information about percentage mortality in the later stages of the life-cycle. Conclusions about sexual differences in percentage mortality at the pupal stage are drawn from the data. Information is also presented concerning percentage cocoon building, possible sexual differences in cocoon building and adult wing deformity, the effect of cocoons on mortality and wing deformity, and the positioning of cocoons.

METHOD

As described in the general introduction to methods, large larvae selected from stock cultures were transferred to individual vials, raised under conditions constant at 28^{o}C and 70% relative humidity, and examined daily. 10 batches of from 125-300 individuals were examined in this way.

Cocoons were always constructed against the wall of the vial. This enabled the pupae to be sexed without the necessity of removing them from their cocoons.

The distribution of cocoons in a culture jar was examined by placing a large number of eggs on the surface of the food in a 2 lb. 'Kilner' jar half full of wholemeal flour kept at 28°C and 70% relative humidity. When larval development was complete the depth at which cocoons were formed against the side of the jar was measured.

RESULTS

1) Relative numbers of males and females.

Table 53 shows the numbers of male and female pupae in each of the 10 batches. Statistical comparison of the mean number of females with the mean number of males gives a t-value of 3·5 and thus a highly significant p value of less than 0·01. It can therefore be said that the mean number of female pupae in a batch is significantly greater than the mean number of male pupae.

2) Mortality at late-larval, prepupal and pupal stages.

A small number of the large larvae transferred from culture to vial died within 48 hours, presumably as a direct result of this disturbance. This number was subtracted from the total number of larvae transferred. All subsequent percentage calculations are therefore based on the original number in the batch minus the number dying within 48 hours of transference. This initial mortality varied, among the 10 batches, between 0 and 8·9% with a mean of 3·65% and a standard deviation of 3·28%.

Table 54 shows the percentage mortality in each of the 10 batches at the late larval (after transference but before pupation), at the prepupal and at the pupal stage.

Graph 9 illustrates these mortalities by a histogram showing the percentage of an original 100 eggs surviving at each stage of the life cycle.

GRAPH 9 : Histogram showing percentage survival out of an original 100 eggs at different stages in the life-cycle.

A: original eggs

B: hatching

C: early larvae

D: prepupal

E: pupal

F: adult

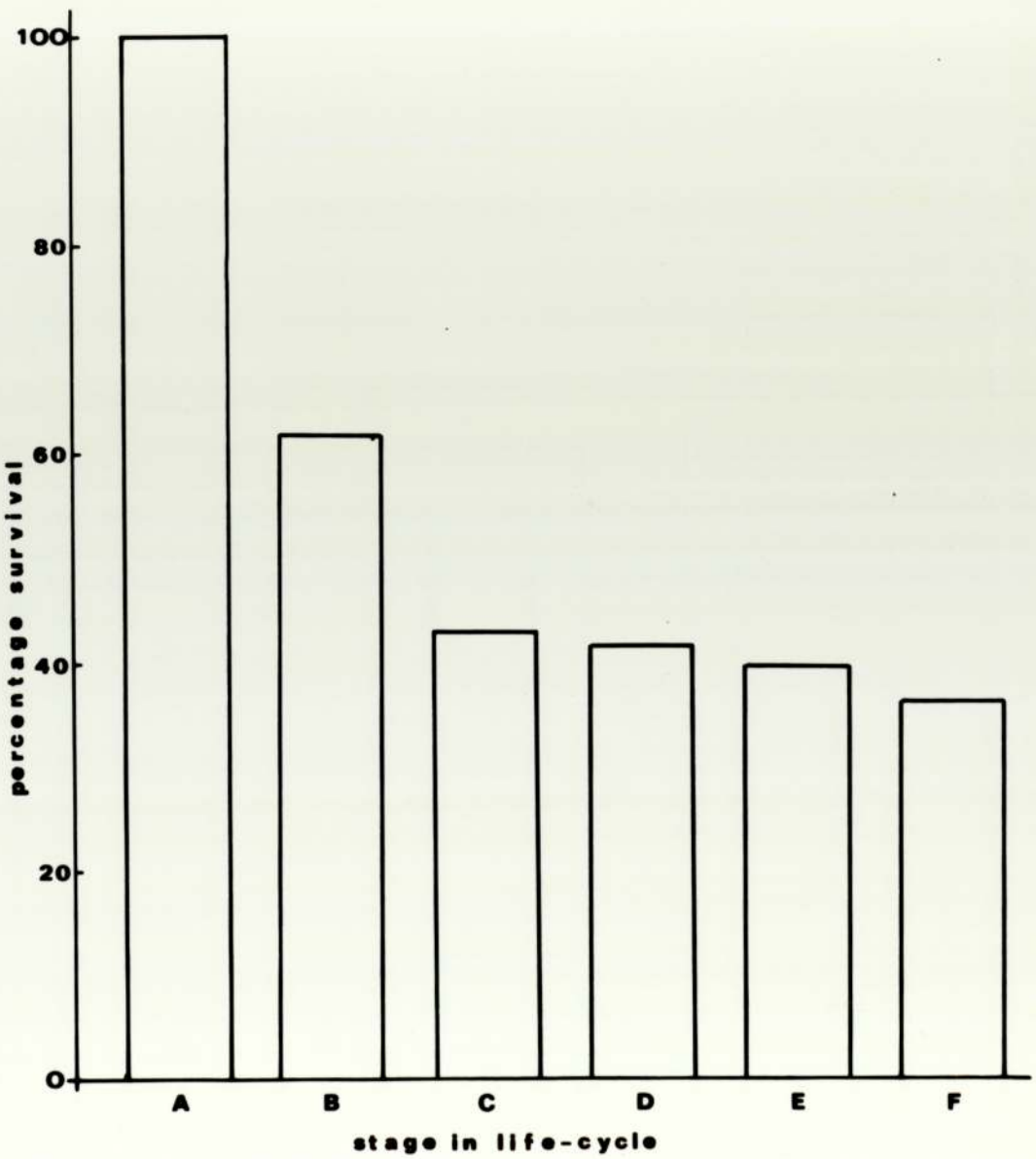

100
80
60
40
20
0
percentage survival
A
B
C
D
E
F
stage in life-cycle

TABLE 53: Number of male and female pupae in each of the 10 replicate batches.

TABLE 54: Percentage mortalities at 3 stages in the life-cycle for each of the 10 replicate batches.

Table 53

Batch	Number of females	Number of males
1	87	22
2	96	32
3	110	58
4	107	79
5	79	74
6	131	75
7	160	43
8	217	49
9	208	64
10	161	34
mean	135·6	53
total	1356	530
S.D.	49·1	20·07

Table 54

	Late larval		Pre-pupal		Pupal	
Batch	% age	Transformed	% age	Transformed	% age	Transformed
1	1·69	7·49	4·31	11·97	11·71	20
2	2	8·13	9·52	17·95	16·54	23·97
3	4·84	12·66	2·82	9·63	7·56	16
4	2·44	8·91	2·0	8·13	11·22	19·55
5	7·32	15·68	17·89	25·03	12·82	20·96
6	2·26	8·72	1·85	7·92	3·3	10·47
7	1·01	5·74	1·7	7·49	5·88	14·06
8	1·03	5·74	5·54	13·56	9·16	17·66
9	2·37	8·9	4·17	11·83	5·8	13·94
10	2·36	8·9	4·35	11·97	2·52	9·1
mean	2·73	9·09	5·41	12·55	8·65	16·57
S.D.		3·02		5·4		4·74

To make this cover the entire cycle, with the exception of the middle larval instars, the relevant percentage hatch has been added - 62% at 70% relative humidity and 27·5°C (Lefkovitch, 1967) (Column B - graph 9). The early larval survival, the mean of the 8+ day old groups in table 47, recorded in the last chapter (C1) has also been included in graph 9 (Column C). It can clearly be seen that the highest percentage mortalities are those at hatching and the early larval stage. The figure in graph 9 thus arrived at as percentage survival at the adult stage (F) (36%) comes easily within the range of percentage survival actually observed and recorded in chapter C1 (table 46).

In order to compare statistically the mean percentage mortalities at the different stages recorded in table 54, it was necessary to perform an angular transformation on these percentages (Fisher and Yates, 1963). The means of these transformed values (table 54) were compared. The value of t between late larval and prepupal, and between pre-pupal and pupal means was 1·25 in both cases. The consequent p value is greater than 0·1 (18^{o} freedom). The difference between these two pairs of means is therefore statistically insignificant. However a comparison of the mean late larval mortality with the mean pupal mortality gives a t value of 3·53 and thus a p value of less than 0·01 (18^{o} freedom). Late larval mortality rate is therefore significantly lower than pupal mortality.

It was observed that the great majority of mortalities occurred at the pre-pupal and pupal stages due to failure on the part of the insect to successfully complete its moult - either from pre-pupa or from pupa to adult.

3) Comparison of male and female pupal mortalities.

Before the pupal stage it is not possible to sex *S. paniceum* on the basis of external characters (Halstead, 1962). Direct comparison of male and female mortality before this stage is therefore not possible.

Table 55 illustrates a comparison between male and female pupal mortality for each of the 10 batches. It can be seen that the mean percentage male mortality is higher than the mean percentage female mortality. In order to compare these means statistically an angular transformation was again performed on the results. Statistical comparison of the means thus produced yields a t value of 0·93 - p greater than 0·1 (18^{o} freedom). Statistically, therefore, the difference between the male and female mean pupal mortalities is insignificant.

4) The percentage mortality of insects with and without cocoons.

Three categories of cocoon building behaviour of larval *S. paniceum* were recognised for the purpose of this study. The first category (c.p.) describes that in which the larva constructs a perfect cocoon in which it is totally enclosed. Cocoons are built from a combination of food material and frass, held together by a meshwork of bodily secretion. In all cases observed, cocoons were so constructed that the finished product employed the wall of the glass vial as a part of its structure. The second category (c.) describes cocoons that were left unfinished by the larva at the onset of the prepupal stage. In most cases the insect was well enclosed by the cocoon with only one end of the structure absent. Larvae of the third category (no c.) failed to construct a cocoon, although often some bonding of the food and frass by the secretion was apparent.

It is important to note that, in the great majority of cases, failure to construct or complete a cocoon was not the result of an insufficient time interval between transference to the vial and the prepupal stage. The formation of a complete cocoon could be accomplished within 2 or, at the most, 4 days and, in general, larvar were in the vials for at least a week before the prepupal stage.

Table 56 shows the relative percentage of individuals which did or did not construct cocoons in each of the 10 batches. Examination of the

TABLE 55: Male and female percentage mortalities at the pupal stage for each of the 10 replicate batches.

TABLE 56: Percentage construction/ non-construction of cocoons in each of the 10 replicate batches.

c.p. : perfect cocoons

c. : imperfect cocoons

no c. : no cocoon constructed

Table 55

	Female		Male	
Batch	Percentage	Transformed	Percentage	Transformed
1	6·9	15·23	27·3	31·5
2	14·6	22·46	25	30
3	7·3	15·68	8·6	17·05
4	9·3	17·76	15·2	22·95
5	10·1	18·53	16·2	23·73
6	3·	9·97	4·	11.54
7	8·7	17·15	7·	15·34
8	9·7	18·15	8·2	16·64
9	5·8	13·94	6·3	14·54
10	2·5	9·1	2·9	9·8
mean	7·79	15·8	12·07	19·31
S.D.		4·02		7·43

Table 56

	c.p.		c.		No c.	
Batch	% age	Transformed	% age	Transformed	% age	Transformed
1	21·7	27·76	23·2	28·79	14·6	22·46
2	23·8	28·86	19·3	26·06	57·3	49·20
3	59·1	50·24	16·1	23·66	24·7	29·80
4	52·2	46·26	13·2	21·3	37·6	37·82
5	21·5	27·62	30·2	33·34	48·3	44·03
6	55·2	47·98	23·5	29·00	21·3	27.49
7	13·1	21·22	24·6	29·73	62·3	52·12
8	50·7	45·4	9·9	18·24	39·4	38·88
9	69·5	56·48	2·4	8·91	28·1	32·01
10	13·7	21·72	30·3	33·40	56·4	48·68
mean	38·0	37·35	19·28	25·24	39·0	38·25
S.D.		13·13		7·55		10·17

mean percentages shows that roughly equal numbers are in the c.p. and no.c categories, while the percentage in the c. category is much lower. It can also be seen that, within a single batch, if a high percentage of individuals construct perfect cocoons, then a relatively low percentage will construct imperfect cocoons; and to some extent *vice-versa*.

Angular transformation of these results permits a statistical analysis. This shows that the only significant difference between the means is that between the mean percentage of non-cocoon forming and imperfect cocoon forming larvae (t is 2·3, p is less than 0·05 with 18^{o} freedom).

Table 57 shows percentage mortalities, within each batch, of groups in the three cocoon categories described above. It can clearly be seen that the mean percentage mortalities of cocoon-forming insects are far lower than that of insects that do not form cocoons. A statistical comparison of the means, after angular transformation, gives t values of 3·74 and 3·89 between the mean percentage mortalities of non-cocoon forming insects and, respectively, insects forming perfect and imperfect cocoons. The resultant p values of less than 0·01 (18^{o} freedom) show that a highly significant difference exists between these means. Therefore the percentage mortality of non-cocoon-forming insects is significantly greater than the percentage mortality of either perfect of imperfect cocoon-forming insects. Comparison of the transformed mean percentage mortality of perfect and imperfect cocoon-formers gives a t value of 0·07 and consequently a p value greater than 0·9 (18^{o} freedom); there is no significant difference between these two means. Therefore failure to construct a perfect cocoon does not have a significant effect on percentage mortality.

5) Comparison of male and female cocoon construction.

Table 58 shows the percentage of females forming or not forming

TABLE 57: Percentage mortality for each cocoon category in each of the 10 replicate batches.

c.p. : perfect cocoons

c. : imperfect cocoons

no c. : no cocoon constructed

TABLE 58: Percentage female construction/ non-construction of cocoons in each of the 10 replicate batches.

c.p. : perfect cocoons

c. : imperfect cocoons

no c. : no concoon constructed

Table 57

	c.p.		c.		no c.	
Batch	% age	Transformed	% age	Transformed	% age	Transformed
1	11·6	19·91	6·5	14·77	44·8	42·02
2	8·6	17·05	17·2	24·5	38·4	38·29
3	4·5	12·25	13·3	21·39	32·6	34·82
4	10·9	19·28	3·7	11·09	24·7	29·8
5	18·2	25·25	11·3	19·64	54·5	47·58
6	2·5	9·1	3·8	11·24	23·4	28·93
7	0	0	1·4	6·8	13	21·13
8	5·4	13·44	0	0	31·3	34·02
9	0	0	5·7	13·81	21·7	27·76
10	5·4	13·44	3·1	10·14	14·3	22·22
mean	6·71	12·97	6·6	13·34	29·87	32·66
S.D.		8·22		7·24		8·41

Table 58

Female cocoon construction

	c.p.		c.		no c.	
Batch	% age	Transformed	% age	Transformed	% age	Transformed
1	37·9	38·00	46·0	42·71	16·1	23·66
2	25·0	30·00	21·9	27·90	53·1	46·78
3	69·1	56·23	14·5	22·38	16·4	23·89
4	61·7	51·77	10·3	18·72	28·0	31·95
5	31·6	34·20	26·6	31·05	41·8	40·28
6	57·2	49·14	23·7	29·13	19·1	25·91
7	17·5	24·73	36·9	37·41	45·6	42·48
8	58·1	49·66	10·6	19·00	31·3	34·02
9	77·4	61·61	1·9	7·92	20·7	27·06
10	15·5	23·18	31·7	34·27	52·8	46·61
mean	45·7	41·85	22·4	27·05	32·5	34·26
S.D.		13·60		10·26		9·19

cocoons from each of the 10 batches - labelling (c.p., c., no c.) as described above. The mean percentage of females in each cocoon category can be seen to approximate to the mean percentages in table 56, i.e. a smaller percentage of female insects construct imperfect cocoons than construct either perfect or no cocoons. However, there is a higher percentage of females constructing perfect cocoons than there is of females not constructing cocoons at all.

To give a statistical analysis of the percentage means, angular transformation was performed. This enabled t values to be discovered; that between the mean percentage of c.p. and c. being 1·94, between c.p. and no c. being 1·03 and between c. and no c. being 1·17. All these t values give p values greater than 0·05 (18^{o} freedom); there is therefore no statistically significant difference between any of these means.

Table 59 is the equivalent of table 58, but for male percentage cocoon construction. Again the highest mean percentage of males construct perfect cocoons with cocoon non constructors next and the lowest place taken by imperfect cocoon constructors.

Comparison of angular transformed mean percentages revealed t values as follows: between c.p. and c., 2·37; between c.p. and no c., 0·62; between c. and no c., 2·1. Inspection of the corresponding p values shows that a statistically lower percentage of males construct imperfect cocoons than either fail to construct, or construct perfect cocoons ($p = 0·05$, 18^{o} freedom). The number of males not constructing cocoons at all, although somewhat lower, is not significantly lower than the number of males constructing perfect cocoons. Males are therefore equally likely not to construct a cocoon as to construct a perfect one; but they are significantly less likely to construct an imperfect cocoon.

Table 60 is a contingency table comparing the mean percentage in each cocoon-forming category for females and males. The value of

TABLE 59: Percentage male construction/ non-construction of cocoons in each of the 10 replicate batches.

c.p. : perfect cocoons

c. : imperfect cocoons

no c. : no cocoon constructed.

TABLE 60: Contingency table comparing male and female percentages in each of the 3 cocoon categories.

c.p. : perfect cocoons

c. : imperfect cocoons

no c. : no cocoon constructed.

Table 59

Male cocoon construction

	C.P.		C.		No C.	
Batch	% age	Transformed	% age	Transformed	% age	Transformed
1	45·4	42·36	27·3	31·50	27·3	31·50
2	34·4	35·91	21·9	27·90	43·7	41·38
3	58·6	49·95	20·7	27·06	20·7	27·06
4	51·9	46·09	17·7	24·88	30·4	33·46
5	25·7	30·46	28·4	32·20	45·9	42·65
6	62·7	52·36	21·3	27·49	16·0	23·58
7	25·6	30·4	23·3	28·86	51·1	45·63
8	44·9	42·07	12·2	20·44	42·9	40·92
9	68·7	55·98	3·1	10·14	28·2	32·08
10	11·8	20·09	32·3	34·64	55·9	48·39
mean	43·0	40·57	20·8	26·51	36·2	36·67
S.D.		11·29		6·99		8·27

Table 60

	Female	Male	Total
C.P.	45·7	43·0	88·7
C.	22·4	20·8	43·2
No C.	32·5	36·2	68·7
Total	100·6	100·0	200·6
	CHI-SQUARED		
	Female	Male	Total
C.P.	0·03	0·03	0·06
C.	0·02	0·02	0·04
No C.	0·1	0·12	0·22
Total	0·15	0·17	0·32

chi-squared thus produced (0·32) gives a value for p of 0·9 (2^{o} freedom). It is therefore probable that males and females do not differ in the mean percentage of their number which construct perfect, imperfect or no cocoons.

6) Effect of cocoon on adult wing deformity.

By far the most common and obvious external defect of adult *S. paniceum* is deformity of the elytra. This varies from a slight deformation of one or both elytra preventing them from closing completely, to an extensive blistering and twisting of both elytra that stops the hind wings from being folded under them. It was observed that these deformities were, in general, caused by failure to slough off completely the very fine pupal exuviae.

Table 61 shows the percentage of adults from each cocoon category having deformed wings in each of the 9 batches. It can be seen that by far the highest percentage deformity occurs amongst adults not in cocoons, with adults in perfect cocoons next and adults in imperfect cocoons last.

Angular transformation and statistical comparison of the means gives t values as follows: between c.p. and c., 1·18; between c.p. and no c., 2·28; between c. and no c., 2·26. The corresponding p values of 0·3, 0·05 and 0·05 (16^{o} freedom) show a statistically significant difference between the mean percentage wing-deformity of insects not from cocoons and the mean percentage deformity of insects from both perfect and imperfect cocoons. The difference in percentage deformity between perfect amd imperfect cocoon formers is not significant. An insect within a cocoon is therefore significantly less susceptible to wing-deformity than an insect not in a cocoon.

7) The difference between male and female wing deformity.

Table 62 records the percentage male and female wing-deformities for each of the 9 batches. The similarity of the two mean percentages

TABLE 61: Percentage wing-deformity in each of the 3 cocoon categories for the 9 replicate batches.

c.p. : perfect cocoons

c. : imperfect cocoons

no c. : no cocoon constructed.

TABLE 62: Percentage wing-deformity of males and females in each of the 9 replicate batches.

Table 61

	C.P.		C.		No C.	
Batch	% age	Transformed	% age	Transformed	% age	Transformed
1	15·1	22·87	21·4	27·56	17·9	25·03
2	11·4	19·73	10·0	18·43	20·7	27·06
3	9·4	17·85	3·8	11·24	16·4	23·89
4	4·8	12·66	0·0	0·00	17·1	24·43
5	11·8	20·09	5·8	13·94	18·6	25·55
6	7·7	16.11	4.1	11.68	7.7	16.11
7	10·6	19·00	10·3	18·72	14·4	22·30
8	8·5	16·95	0·0	0·00	12·3	20·53
9	6·9	15·23	4·7	12·52	11·4	19·73
mean	9·6	17·83	6·7	12·68	15·2	22·74
S.D.		3·01		8·78		3·43

Table 62

	Female		Male	
Batch	Percentage	Transformed	Percentage	Transformed
1	21·9	27·90	20·8	27·13
2	15·7	23·34	20·7	27·06
3	14·4	22·30	10·4	18·81
4	7·0	15·34	17·7	24·88
5	13·4	21·47	11·1	19·46
6	9·6	18·05	12·5	20·70
7	13·8	21·81	11·1	19·46
8	10·7	19·09	8·3	16·74
9	10·2	18·63	6·1	14·30
mean	13·0	20·88	13·2	20·95
S.D.		3·63		4·50

can be seen.

The two means were compared by means of an angular transformation of the results. A t value of 0·009 is thus calculated, giving a p value considerably greater than 0·9 (16^{o} freedom). Therefore there is no sexual difference in percentage wing-deformity of adult *S. paniceum*.

8) The distribution of cocoons in a culture jar.

Table 63 shows the depth at which 51 cocoons were formed in a culture jar containing wholemeal flour to a depth of 5·2 cm. The frequency of cocoons in 0·5 cm intervals is shown as a histogram (graph 10).

The normally distributed nature of the cocoon frequency can be seen from graph 10, as can the fact that cocoons are concentrated in the top half of the culture, yet not at the surface.

DISCUSSION

The huge discrepancy between numbers of males and females in each batch is probably not a reflection of the relative numbers of males and females within a culture. It was demonstrated in chapter C1 that approximately equal numbers of males and females hatch from any group of eggs - with a slight preponderance of females. This slight female preponderance is by no means large enough to explain the size of the discrepancy noted in the present data.

It is therefore suggested that the difference in male and female numbers recorded here is the result of a sampling error. This error is probably the result of the method used to select larvae in their final instar. The criterion used was one of size; it was assumed that the largest larvae were in their final stage. However, Lefkovitch (1967) has shown that newly emerged female adults are significantly heavier than newly emerged males. It is likely, therefore, that female larvae are

GRAPH 10 : Histogram showing the number of cocoons formed against the walls of a culture jar at 0·5 cm intervals of depth.

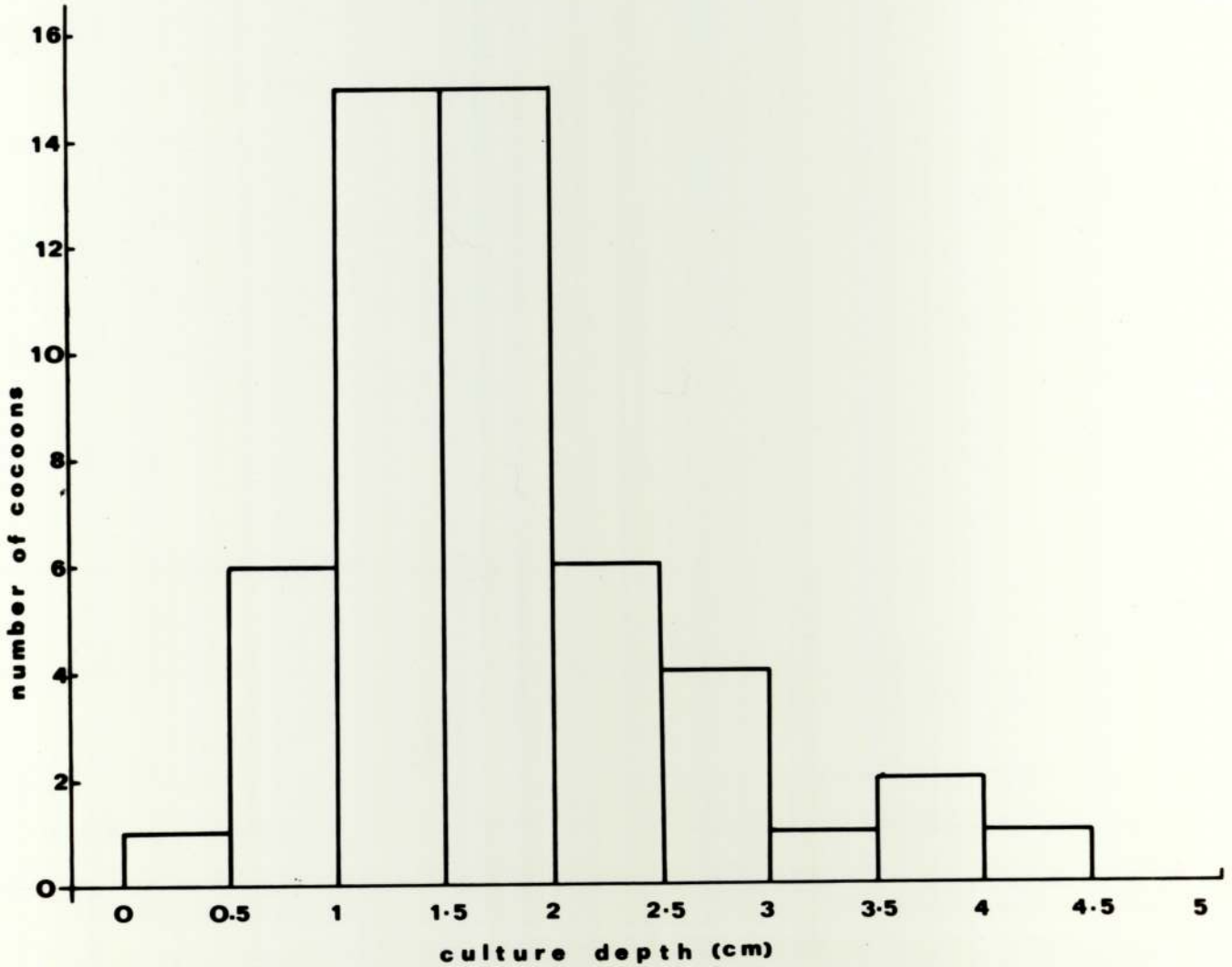

16
14
12
10
8
6
4
2
0
number of cocoons
0
0·5
1
1·5
2
2·5
3
3·5
4
4·5
5
culture depth (cm)

TABLE 63: Depth (cm) at which 51 cocoons were constructed against the side of a culture jar containing a total depth of 5·2 cm of whole-meal flour.

Table 63

cm

3·3	2·9	2·5	1·8	1·8	2·0	2·5	2·2	1·1	1·4
0·9	1·6	1·9	1·3	1·1	0·4	1·7	3·9	4·5	1·3
1·0	1·7	1·7	2·9	2·3	1·2	1·1	0·7	2·6	1·4
2·1	3·7	1·9	2·0	1·9	1·8	1·2	0·9	1·1	2·0
1·6	1·1	1·0	0·7	1·4	1·2	2·3	1·1	1·4	1·7
2·3									

n = 51

mean = 1·79

S.D. = 0·84

total depth = 5·2 cm.

heavier and therefore larger than male larvae; a selection proceedure based on size is therefore liable to favour female larvae. To permit comparison of results, percentages are therefore used throughout this study.

Lefkovitch (1967) estimates the larval-pupal mortality of *S. paniceum* at 19·4% for 27·5°C and 70% relative humidity. This is lower than that estimated from the present data (25·9% - graph 9), although this latter does not take into account possible mortality in the middle instar larvae. This difference may be the result of factors such as crowding - particularly with reference to the figures from chapter C1.

Lefkovitch's (1967) figures do not show how mortality at each stage contributed to the total percentage mortality; the present figures are therefore valuable in this respect. Reference to graph 9 will show that by far the greatest percentage mortality occurs at hatching and in the early larval stage. Such results are by no means unusual; life-tables of several coleopteran species have demonstrated the preponderance of mortality in the egg and early larval stages, particularly amongst species in the wild (e.g. Dempster, 1960; Parnell, 1966). The biggest causes of these early natural mortalities, predation and parasitism, are probably paralleled in laboratory cultures of *S. paniceum* by cannibalism - as they are in *Tribolium* spp. (Park et al, 1965).

If mortality at these early stages is primarily the result of extrinsic factors, such as cannibalism, it seems that mortality at the later stages is the result of intrinsic, physiological failure. It was mentioned in the results that the great majority of deaths at the prepupal and pupal stages occur during moulting, with failure of ecdysis. This seems unsurprising in view of the immense physiological changes which occur at these times and the consequent vulnerability of the insect to

the slightest bodily malfunction.

The preponderance of female adults surviving from any group of eggs has already been discussed. This preponderance may be the result of a sexual difference in percentage mortality. Evidence for a difference of this kind was collected for *S. paniceum* at the pupal stage, the only stage at which they can be sexed easily (Halstead, 1962) (results 3). These results show that there is no statistical evidence of a significant sexual difference in mortality at the pupal stage. Indeed there is an absence of any sexual differences in behaviour or deformity at the stages examined here (see tables 58, 59 and 60, and table 62 respectively). If sexual differences occur, therefore, they must do so at an earlier stage in the life cycle, possibly when the insects are young larvae.

"The pupa of most insects is an immobile and hence vulnerable stage and a majority of insects pupate in a cell or cocoon which affords them some protection." (Chapman, 1971). In fact cocoon construction is rare in Coleoptera, although many do create a pupal chamber by lining the walls of the larval burrow with 'faecal cement' (Evans, 1975).

Lefkovitch (1967) noted that not all *S. paniceum* larvae construct a cocoon; he did not however report what proportion of a population failed to construct. The present results show that a surprisingly high proportion (39% - table 56) of a population do not construct a cocoon. This seems anomalous in the light of a comparison of mortality figures of cocoon-forming and non-cocoon-forming insects. The apparent selective disadvantage of failure to construct a cocoon is obvious from the significantly increased percentage mortality which results (table 57), and similarly from the higher percentage wing-deformity apparent in those adults which did not form cocoons as larvae. It must be concluded, therefore, either that building and/or being in a cocoon itself confers some disadvantage, or that the absence of a cocoon is genetically linked with some positively desirable trait. Neither possibility could be pursued further

in the present study.

The way in which the cocoon reduces percentage mortality is not clear. From observational data, however, it is possible to infer that one of its beneficial functions in a laboratory situation is purely mechanical. It was noted during the course of the experiments that individuals in cocoons moulted more rapidly and efficiently, using the walls of the cocoon as support when removing the exuvium. Cocoonless individuals had no such support and often failed to remove the exuvium completely. This failure in removal often resulted in deformity or death. The primary importance of this mechanical function would also explain the absence of mortality difference between perfect and imperfect cocoon formers; since the imperfect cocoon would probably be as efficient in this as the perfect cocoon.

The position of a pupation site is often of great importance to an insect, particularly to a species in which the adult inhabits an environment different to that of its larve (Evans, 1975). It has already been observed that, whereas the larvae of *S. paniceum* spend their time within a food medium, the adults live on or above the surface. It is reasonable, therefore, that the situation of a pupation site should take this into account. Pupation site selectivity has been demonstrated in some Coleoptera- for instance *Tribolium castaneum* and *Tribolium confusum* (King and Dawson, 1973).

Graph 10 shows that cocoon aggregation in *S. paniceum* takes place in the top half of the culture medium. The mechanism by which larvae arrive at this point is not clear; it may be due to feeding movements in the early stages and therefore unconnected with cocoon formation. Positive selection of a pupation site is therefore only demonstrated by the present study insofar that selection of a stable surface against which to form the cocoon is shown by cocoon position in the vials.

The position in which cocoons are aggregated does, however, display adaptive advantages. Being near the interface of the larval and adult environments gives the emerging adult less far to travel. At the same time the cocoon position beneath the surface prevents disturbance by the adult, provides protection against surface-dwelling parasites and predators and renders the cocoon less susceptible to fluctuations in temperature and humidity.

SUMMARY

A life table is given showing the mean percentage survival of *S. paniceum* at stages in its life-cycle. This table demonstrates that by far the largest percentage mortality occurs at hatching and the early larval stage.

No significant difference is detectable between male and female percentage mortality at the pupal stage.

39% of larval *S. paniceum* were shown not to construct a cocoon. The percentage mortality of cocoonless *S. paniceum* was shown to be significantly higher than that of those which did construct a cocoon.

There was no significant difference between male and female numbers constructing and not constructing cocoons, and male and female wing-deformities.

Adults not from cocoons had a higher percentage of wing-deformities than adults from cocoons.

It is suggested that these differences in mortality and deformity between cocooned and cocoonless *S. paniceum* are partly the result of the mechanical advantage conferred by a cocoon during moulting.

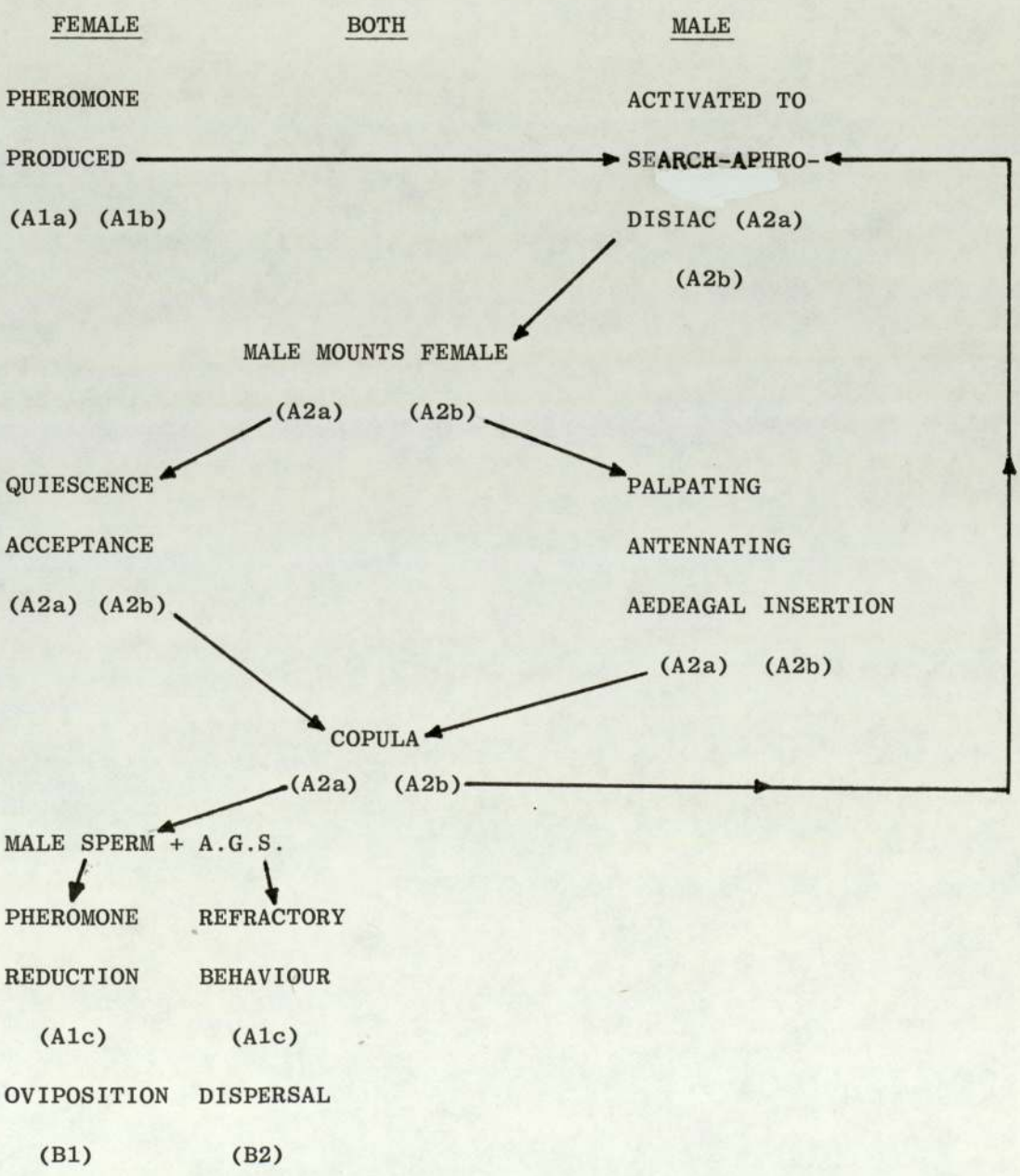
Larvae were shown to aggregate their cocoons near, but not at, the surface of the medium. The adaptive significance of this positioning is discussed.

CONCLUSIONS

Throughout this thesis discussions and conclusions are placed at the end of each chapter. The purpose of this section is to help the reader by drawing together some of the more important conclusions of the thesis. It is hoped that the use of diagrams will serve to integrate these conclusions and thus present an holistic picture of aspects of the behavioural ecology of *S. paniceum*.

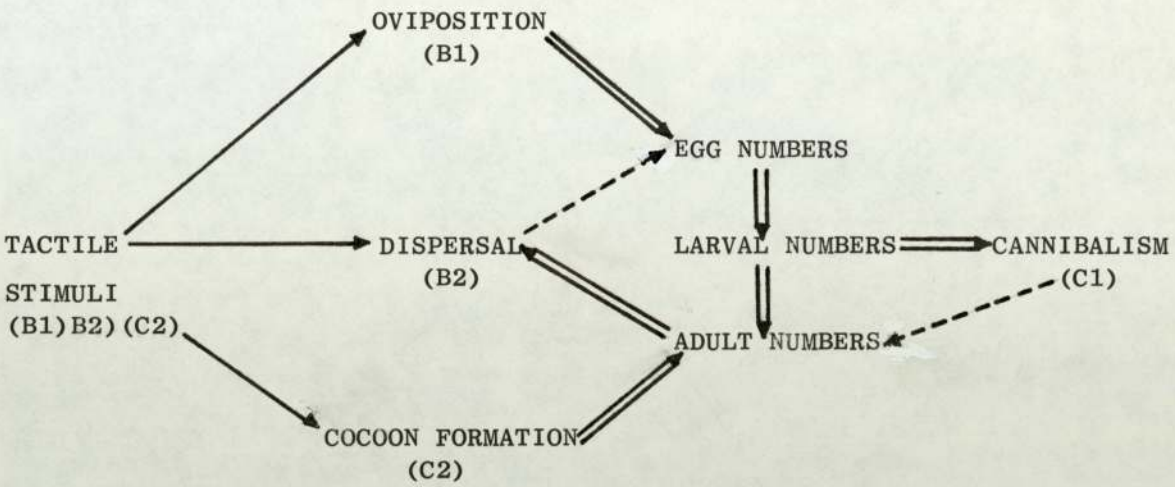
The letters in brackets refer to relevant chapters.

A: Mating.



B: Population effects.

This figure shows the ecological interaction of some of the phenomena discussed in the thesis.



═══ : proportional effects.

- - - - - : inversely proportional effects.

SIGNIFICANCE FOR PEST MANAGEMENT.

A: Pheromonal control.

1) Pheromone levels at the surface of an infestation will be high because: (a) emergence from the cocoon is synchronous (A1b, A1c, C1),

(b) eggs are laid in groups at oviposition (B1, B2).

2) The pheromone is an aphrodisiac and is more effective in stimulating mating behaviour than in assisting the male to find the female (A2a, A2b).

Therefore attempts at the control of mating by external pheromone sources will probably be unsuccesful. Firstly, because of 1), no external pheromone source will compete with the concentration of pheromone at the surface of an infestation. Secondly, because of 2), any increase produced in the overall pheromone level will result in increasing male sexual activity without attracting them to the external source.

B: Other methods.

Chapters B1 and B2 indicate that fertile females are the most active category and that dispersal is stimulated by crowding. The use of traps, possibly sticky-traps, near the source of an infestation will therefore provide a useful indication of the size of an infestation.

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APPENDIX: WARD, J.P., HUMPHRIES, D.A. (1977)
A secondary sexual character in
Stegobium paniceum (L.) (Coleoptera;
Anobiidae) and its probable function.
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SHORT COMMUNICATION

A SECONDARY SEXUAL CHARACTER IN ADULT *STEGOBIUM PANICEUM* (L.) (COLEOPTERA: ANOBIIDAE) AND ITS PROBABLE FUNCTION

(*First received* 3 *February* 1977, *and in final form* 11 *March* 1977)

INTRODUCTION

In a review of the secondary sexual characters of stored product beetles Halstead (1963) gives only the differences in pupal genital papillae as criteria for differentiating between male and female *Stegobium paniceum* (L.) (the bread, biscuit or drug-store beetle). No clear-cut external sexual difference has been reported in the adult, despite several studies of its biology (Klein, 1918; Azab, 1943, 1954; Lefkovitch, 1967; Barratt, 1974, 1975). Extensive biometrical studies by Kashef (1955) and Monteiro (1957) aimed at identifying a reliable sexual difference merely showed great variability in size of both sexes, with females usually, but not always, larger. In no external feature was any difference found in size or proportion that would permit accurate sexing of individuals.

THE SEXUAL DIFFERENCE IN THE TARSAL CLAWS

Microscopic examination of male and female claws under high magnification revealed that although the tarsal segments are similar in proportion and detail in the two sexes, the male claw bears a distinct slot-like structure on each of its two elements.

Photomicrographs were taken to confirm this observation: Fig. 1 a–c shows pro-, meso- and meta-thoracic tarsal claw elements of different male *S. paniceum*. These should be compared with the corresponding female claws shown in Fig. 1 d–f. Electron stereoscan photomicrographs show this difference more clearly (Fig. 1 g, h).

The male tarsal claws of four other common stored product beetles have been examined and none found to possess this secondary sexual difference. Of the Anobiidae *Lasioderma serricorne* (F.), *Anobium punctatum* (de Geer), *Xestobium rufivillosum* (de Geer), *Ernobius mollis* (L.), *Oligomerus ptilinoides* (Woll.) and *Nicobium castaneum* (Ol.) have been examined but only the last has been found to possess the male tarsal claw slots.

FUNCTION OF THE CLAW SLOTS

In animals where there is no parental care of offspring, a secondary sexual character generally plays a significant part in mating behaviour. The claw slot function is therefore likely to be related in some way to the female elytra with which the male tarsi make contact during the initial amplexus stage of mating (Kashef, 1955).

Comparison of figures of the female elytral setae (Fig. 1 i–k) with those of the male claws at a corresponding magnification shows a close correlation between the diameter of cross-section of the setae and the width of the slots in the male claw. The average width of 23 slots at their widest point was 2.3 μm. The slots are fairly constant in width for the distal two thirds of their length and proximally constrict to a rounded end. Measurements of 20 setae showed that they taper from an average basal width of 3.2 μm to an average tip width of <0.5 μm; the average midpoint width being 2.4 μm. The implication is that the distal halves of the setae may become inserted into the slots.

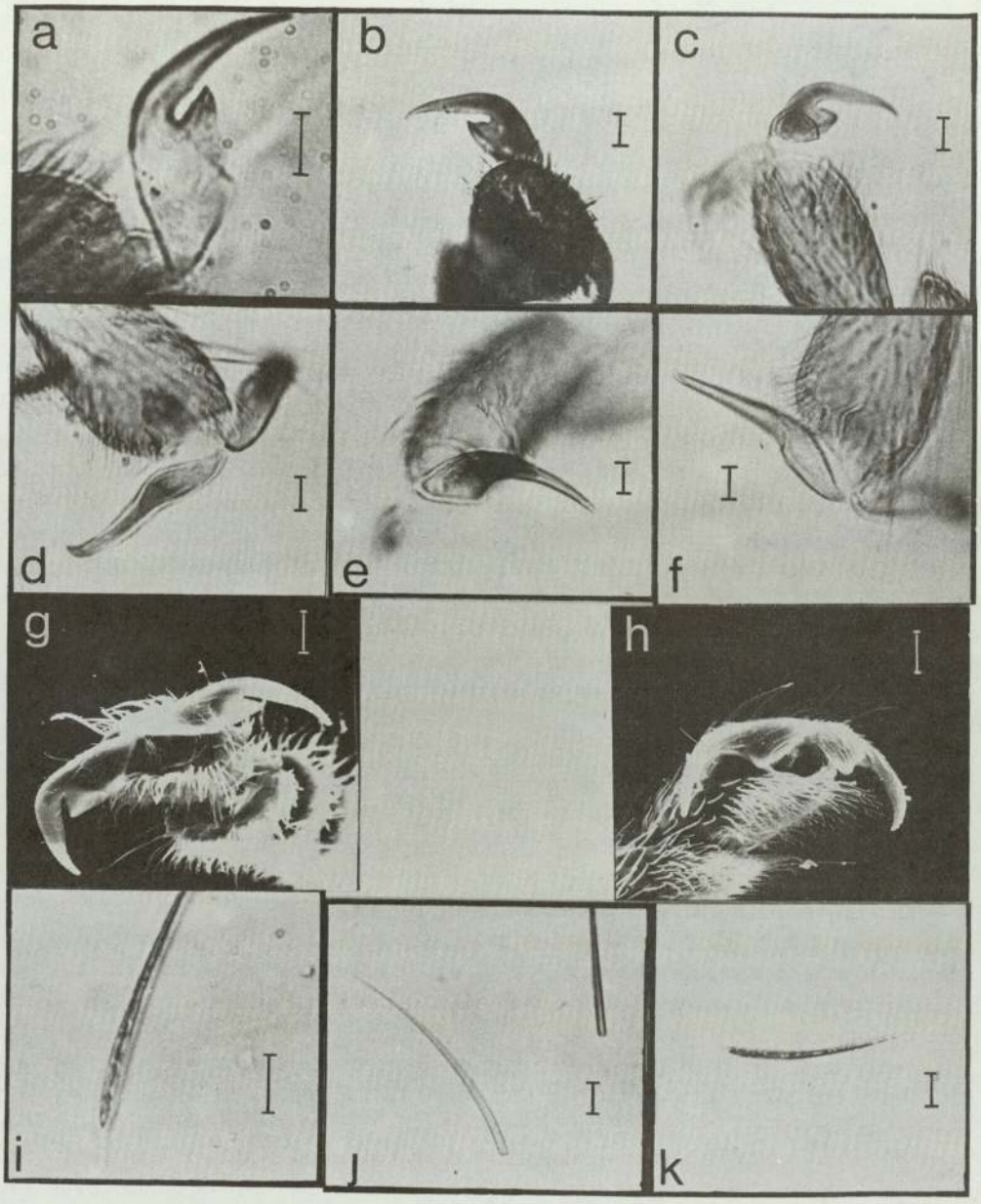

FIG. 1. *Stegobium paniceum*: (a) male prothoracic tarsal claw, (b) male mesothoracic tarsal claw, (c) male metathoracic tarsal claw, (d) female prothoracic tarsal claw, (e) female mesothoracic tarsal claw, (f) female metathoracic tarsal claw, (g) male prothoracic tarsal claw, (h) female prothoracic tarsal claw, (i–k) female elytral setae. All scale lines = 10 μm.

During amplexus a seta entering the slightly tapering groove will become trapped if the male pulls with its claws in such a way as to distort the seta; this has been demonstrated by the use of a simple model. It is also in accordance with our observation that when a male dismounts from the female there is a distinct delay during which movements of the male tarsi occur, giving the impression that the claws are being disengaged.

We observed that the male remains mounted for some seconds and sometimes minutes before achieving copula. During this period the female was not always stationary and the male is at risk of being brushed off. The tarsal claw slots, in trapping the female elytral setae, would improve the security of the male's position. This simple mechanical action is proposed as the most likely explanation of their function.

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