Studies on a sex pheromone in Stegobium paniceum (L.) (Coleoptera; Anobiidae)

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by

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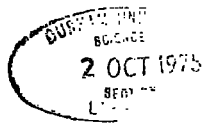

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ABSTRACT

The sex pheromone system of Stegobium paniceum (L.) was studied in the laboratory by means of live female assay experiments as well as extraction of the pheromone from females using solvent and volatilisation techniques. Female beetles were shown to attract only males, which were themselves attractive to neither sex.

Female Stegobium were capable of controlled pheromone emission even when body content levels were high. Newly eclosed females contained small quantities of pheromone which reached maximum levels in 6 to 9-day-old insects. Very little emission occurred until females were 2.5-3.5 days old, after which maximum emission rates were reached rapidly between days 3 and 4, the age at which the first mature oocytes were found in the calyx of female ovaries.

A series of dilutions of solvent pheromone extracts were bioassayed with males and a theoretical minimum threshold level of response extrapolated to 0.0004 female equivalents.

By comparison with a "wild" strain of Stegobium it was suggested that mass rearing over a long period increased male sensitivity to the pheromone but lengthened the period necessary to reach maximum response levels after adult emergence.

Mating slightly reduced female pheromone content, but pheromone emission fluctuated markedly after mating for reasons not fully understood.

The number of eggs laid per female increased from about 1.5 to over 40 if copulation occurred. Male presence during oviposition increased the percentage of females producing eggs but decreased the number of eggs laid per female of those laying. This reflects decreased fertility of females with age.

The antennal sensilla of Stegobium were surveyed by means of light and scanning electron microscopy. The main types present were trichoidea,

basiconica, chaetica and coeloconica, and there was no apparent sexual dimorphism of sensilla numbers or distribution. Male antennectomy completely inhibited sex pheromone response and progressive removal of club segments increasingly diminished male response intensity.

Addendum

For "sex attractant", read "sex pheromone" throughout this thesis.

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I. INTRODUCTION

A. Pheromones: a brief survey of their occurrence and diversity

Insects employ diverse methods of intraspecific communication as do most animals groups, but the use of chemical compounds for this purpose is probably exploited more extensively, and for a greater variety of functions in insects than any other group.

The term "pheromone" is derived from the Greek, pherein (= to transfer) and horman (= to excite) and was first introduced by Karlson and Lüscher (1959). It was criticized by Kirschenblatt (1962) as being etymologically imprecise; he had previously suggested the term "telergone" from the Greek, tele (= afar) and ergon (= action) in 1958 to embrace these chemical messenger substances. Micklem (1959) introduced a third variation, "pherormone" in criticism of Karlson and Lüscher's original term. However, "pheromone" soon became so widely used and accepted that the reasons for changing seemed unimportant.

Karlson and Lüscher (1959) defined pheromones as "substances which are secreted to the outside of an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or developmental process".

Pheromones are conveniently divided into primers and releasers (Wilson, 1963). The former initiate permanent physiological changes in the recipient (eg. caste-differentiation pheromones) and the latter trigger the nervous system to give a temporary, immediate reaction (eg. alarm pheromones in fish).

Most research on pheromones has so far concentrated upon two main groups within the animal kingdom, insects and mammals. However, recent research has suggested that pheromones are present in a far wider range of groups. Amongst the invertebrates, excluding insects, examples of pheromones have been reported in a marine hydrozoan (Miller, 1972) and the periwinkle, Littorina littorea L. (Dinter and Manos, 1972). Within the vertebrates,

rodents have received most attention (Bronson, 1971; Stoddart, 1974) together with primates (Epple, 1974) including man (Comfort, 1971, 1974). Pheromones play an important part in the schooling cohesion of fish, and their alarm reactions (Pfeiffer, 1963, 1974; Thines and Vandenbussche, 1966). A pheromone-mediated fright reaction in Bufo bufo L. tadpoles has been demonstrated and the substance extracted from their skin (Kulzer, 1954). If the pheromone is added to water containing tadpoles, they show a normal fright reaction and descend immediately.

Between insects and mammals, there are many functional homologies in pheromone communication, especially in terms of social structure and organisation. Small mammals are noted for their use in social hierarchy maintenance and territorial marking (Ralls, 1971). Rabbits provide a good example of both these functions where specialised submandibular and anal glands produce the odours (Mykytowyc, 1974). Characteristic group odours are important in communal breeding or resting areas in some mammals (Müller-Schwarze, 1974): urine as well as the secretions from specialised glands are used for this purpose. Individual odours enable recognition between breeding pairs, or in mother-offspring relationships; for example, laboratory rats kill their offspring if their odour is disguised or masked (Meyer, 1964).

In primates, odours are produced in the urine or in genital secretions and non-sweat-producing apocrine glands associated with hair tufts. These pheromones are widely used as sexual releasers in primates, including man, although behaviour patterns have become less obvious because a greater variety of complex signals are incorporated into sexual behaviour. However, pheromone releasers are thought to promote male-female attraction in primates and to synchronise copulation with ovulation.

Insect pheromones perform a wider variety of functions than those recognised in mammals. Classification according to function is probably the most informative and widely accepted system, which is adapted here from that compiled by Butler (1967).

Trail-marking pheromones are normally considered to be terrestrial, but aerial trails are sometimes included in this category. Terrestrial trails are utilised by many Hymenoptera and Isoptera. They are laid by successful foragers returning to the nest and continually reinforced by other workers using the trail to the food source. A mixture of terpenoids is produced by the Nassanoff gland of honey-bee workers, and is used by foragers to secure recruits for food collection from a fruitful source (Butler and Calam, 1969). Army ants co-ordinate emigration by means of trail-marking pheromones (Watkins, 1964).

Alarm pheromones, like trail markers, are used commonly among social insects. Differences in interpretation of the message by the recipients have been shown to be dependent upon the concentration of the pheromone emitted (Blum, 1974). The same pheromone is thought to have the following functions: recruitment stimulation in the event of damage or intrusion to the nest; attraction of workers to prey; recognition marks to label intruders; and disarming stimuli when raiding other colonies. In the latter case, release of the alarm pheromone can destroy cohesion of the host species. This multiplicity of functions has been demonstrated in many hymenopterous species (Regnier and Wilson, 1968; Blum, 1969; Ayre and Blum, 1971).

Caste-differentiation pheromones are primers which influence both the physiology and behaviour of social insects. In the honey-bee, Apis mellifera L., the queen pheromone regulates the worker-queen relationship, the nature of which is best observed when the queen is removed from a nest. General activity of the workers is increased within the colony, workers become agitated and they increase their 'fanning' activity which ventilates the nest. Eventually queen cells are constructed in order to produce a new queen. The presence of the queen inhibits worker oögenesis (Gary, 1970 ·· ·). Olfactory markers, or surface pheromones aid the identification of castes within insect colonies (Wilson, 1965) and are usually absorbed into the wax layer of the cuticle.

Primer pheromones controlling development and sexual maturation are found in some Orthoptera (Loher, 1958). The presence of mature male Schistocerca gregaria Forskal in crowded conditions accelerates maturation of immature individuals of both sexes, thus synchronising breeding within the group.

Aggregation pheromones are usually produced by one sex to assemble insects of both sexes to a feeding or breeding site. They are commonly found in scolytid beetle species where the attractant helps to co-ordinate a mass attack necessary to overcome the resistance of a host tree in which they subsequently breed (Borden, 1974). In scolytids, the initial insect-host relationship triggers pheromone production and release, and it is thought that in some cases, a precursor from the host tree must be ingested before the pheromone can be synthesised (Wood, Browne, Silverstein and Rodin, 1966). Later work has implicated possible hormonal control in initiating pheromone production (Borden, Nair and Slater, 1969).

Finger (Bar Ilan), Stanic and Shulov (1965) demonstrated an aggregating pheromone in the khapra beetle, Trogoderma granarium Everts. The female produced the attractant, resulting in aggregation of both sexes around the odour source. Yinon and Shulov (1967a, b) later found that the male of this species also produces a pheromone to which both sexes are attracted. The function of this rather complex system of attractants is thought to be aggregation of both sexes for mating, although the Trogoderma pheromone has also been found to repel some species of stored-product Coleoptera (Yinon and Shulov, 1969).

The pheromone of the boll weevil Anthonomus grandis Bohemann has both aggregating properties and sex attractant characteristics (Tumlinson, Hardee, Minyard, Thompson, Gast and Hedin, 1968). In the spring and autumn, both sexes are attracted to male infested cotton bolls, and in summer, only females are attracted to males for mating.

This thesis is primarily concerned with a group of attractants known as

sex pheromones. Jacobson (1972) suggests that "A chemical is probably a sex attractant if it brings to it an insect, which then assumes a mating position or attempts to mate with the chemical or with an object on which the chemical has been placed". Sex pheromones are produced by specialised glands in either sex and are distinct from aggregation pheromones in that they attract only the opposite sex. They normally function as a mechanism by which copulation is achieved at a time optimum for maximum reproductive potential and success. Like most of the pheromones described above, sex attractants are often effective at extremely low concentrations. Depending upon the output of the attractant source and prevalent air currents, a responding insect might orientate over a considerable distance. This system is probably essential for sexual encounters between highly mobile insects and/or those occurring at low density.

Aphrodisiacs, which are very close range stimulants, are distinguished from sex pheromones in that the former are produced by either sex only after both sexes have been brought together by chemical or other means. Attraction and copulation, whilst appearing to be a single operation, might thus be the result of two separate chemical processes.

Insect pheromones in general, their occurrence and functional diversity have been reviewed by: Karlson and Butenandt (1959); Karlson (1960); Inoue and Ohno (1961); Jacobson and Beroza (1964); Butler (1967); Regnier and Law (1968); Bruce (1970); Law and Regnier (1971); Callow (1972) and Birch (1974) amongst many others.

Insect sex pheromones as a sub-group have been reviewed by: Butler (1964); Jacobson (1965, 1966, 1972); Moore (1965, 1967); Muto (1968); Shorey, Gaston and Jefferson (1968); Warren (1969); Bosman (1970); De (1970); Eiter (1970) and Beroza (1971). Sex pheromones pertaining specifically to Coleoptera have been reviewed by authors including; Atkins (1968); Burkholder (1970); Silverstein (1970); Tumlinson, Gueldner, Hardee, Thompson, Hedin and Minyard (1970) and Wood (1970).

B. Introduction to the present work on the sex pheromone of Stegobium paniceum (L.)

The initial aim of the present study was to determine whether a pheromone communication system was employed by Stegobium. This had not been reported previously, although a closely related anobiid, the cigarette beetle, Lasioderma serricorne (F.) was shown by Coffelt and Burkholder (1972) to utilise a sex pheromone for facilitating male location of females for mating. A parallel study on Stegobium was therefore considered worthwhile, and indeed, a female-produced sex pheromone, which attracted only males of the species, was discovered.

Further work on Stegobium was directed towards investigating the integration of the attractant with the reproductive biology of the species, and the advantages conferred by such a communication system. The role of the pheromone in the general biology of the species, and the way in which it affected the behaviour of the recipient was the major interest in this research, allowing some speculation upon the importance of the pheromone to the survival of the species under natural conditions.

Factors affecting pheromone communication such as age, mating status and pheromone concentration were studied in order to appreciate the timing of events and general operation of the sex pheromone system, and to build up background knowledge upon which further work could be based. This information was achieved by means of assay experiments using live females and diethyl ether solvent extracts, which were complemented by a volatilisation technique of pheromone collection.

Chemical analysis and identification of the pheromone was not attempted in the present study. Not only would this demand a great deal of time, and specialised knowledge and equipment, but for the purposes of this work, assay of crude extracts of the pheromone was satisfactory, and gave repeatable results.

In order to supplement the basic investigation outlined above, a study

of the timing of female ovary maturation using dissection and histological techniques was carried out. The degree of synchrony of pheromone production and emission with reproductive maturity was thereby investigated. Oviposition was monitored under certain conditions with relation to the importance of multiple mating in this species (Section VII).

A brief survey of male antennal sensilla was undertaken in conjunction with a short series of antennectomy experiments, with the purpose of relating numbers and distribution of possible chemoreceptive sensilla to pheromone perception (Section VIII). Although somewhat superficial, this part of the research was of value in placing Stegobium in the general context of sex pheromone producing insect species.

As exemplified below, in studying laboratory reared insects, it is essential to be aware of any differences which may have developed as a result of laboratory rearing conditions in comparison with "wild" insects, particularly if eventual application of the results to a natural situation is anticipated. In Section V of this thesis, the pheromone content of females of a "wild" strain of Stegobium is compared with that of laboratory reared insects, as is male responsiveness of the two strains.

Stegobium is a stored-products pest of world-wide distribution. Any study relating to a possible non-insecticidal control mechanism for such a species is of considerable economic importance as well as biological interest. However, before control measures can be developed and implemented (involving great expense), a good working knowledge of the general biology and behaviour of the species must be attained.

Millions of dollars have been spent in the United States on the gypsy moth (Porthetria (Lymantria) dispar (L.)) control programme. A sex pheromone in this species was isolated, identified and synthesised (Bierl, Beroza and Collier, 1970), and then used for field testing and attempted disruption of chemical communication between the sexes, with a small degree of success. Only fairly recently, Richerson and Cameron (1973) have demonstrated significant

differences in pheromone emission, diurnal rhythmicity and male responsiveness in "wild" and laboratory reared strains: all preliminary testing had previously employed laboratory reared insects with direct application to "wild" moths. This example demonstrates the need for a good understanding of the biology of an insect pest species before effective control measures can be reliably implemented. It is towards this end that the present research is directed.

II. GENERAL MATERIALS AND METHODS

A. Rearing room and culture techniques

The Stegobium cultures were maintained, and most experimental work performed, in a constant temperature room of dimensions 3.05 x 2.74 x 2.59 m. This room was kept at 30 ± 1°C by a thermostatically controlled electric fan heater, and at 75 ± 2% r.h. by means of a large fan blowing over an open tank of heated water fed from a 10-gallon reservoir. Humidity was controlled by a horse-hair hygrometer which operated the water heater. A continuous record of temperature and humidity was obtained with an appropriately placed Caseda thermohygrograph. A central ceiling light and time switch gave a 12 h photoperiod starting at 09.00 h daily.

No insect cultures other than Stegobium were allowed into the room, although mites and psocids presented a pest problem at times as outlined below.

Although experiments were performed in the presence of an inevitable 'background' of sex pheromone from the stock cultures, repeatable results were obtained in a varied series of experiments, and the interference factor was therefore considered constant.

The laboratory strain of Stegobium paniceum was supplied by the Pest Infestation Control Laboratory, Slough, Bucks. Records of the place of origin are not available since they have been in culture for many years.

A "wild" strain of this species was obtained following a request to the Ministry of Agriculture, Fisheries and Food, Newcastle upon Tyne, from an Infestation Control Laboratory in Wolverhampton. Stegobium had been found infesting a bread bakery, breeding in old dough and bread residues under dough mixers and bread coolers. They were associated with broad horned flour beetles, Gnathocerus cornutus (Fab.) and spider beetles, Gibbium psylloides (Czemp.). The localised conditions outside the machinery were 26°C and 40% r.h. Approximately 50 adults were isolated from the samples and taken into laboratory culture, their treatment being identical to that of the laboratory strain.

Mixing of the two strains was strictly prevented and the "wild" strain was left for about five generations to adjust to the new environmental conditions and to build up numbers to the level necessary for experimentation.

The Stegobium culture medium comprised wheatings (fine milled wheat) mixed with 10% yeast as recommended by the P.I.C.L. The yeastwas provided as a source of vitamin B, which has been shown to accelerate larval growth in Stegobium (Azab, 1943). Baker's yeast was used in Stegobium culture medium since this was easily obtainable in bulk, although brewer's yeast was used in the mass rearing medium of the grain beetle, Oryzaephilus surinamensis (L.), (Arbogast, Roppel and Carthon, 1972); Trogoderma inclusum LeConte and T.variabile Ballion (Brower and Tilton, 1972) and the cigarette beetle, Lasioderma serricorne (Tobin and Smith, 1971). The wheat was sieved through a 1.27 mm mesh, retaining only the finer fraction, which was then sterilised by heating to 60°C for at least two hours. Mites and most other macro-organisms are killed at this temperature, without the nature of the food material being altered. When cooled, 200 g aliquots of wheat, thoroughly mixed with 20 g of finely crumbled yeast were poured into a two litre "kilner" jar and a medical wiper tissue was added to increase the surface area for adult beetles. The open screw top was applied over a 9.0 cm Whatman 29 black filter paper disc and screwed down, allowing ventilation for the culture and easy recognition of mite or other infestation.

In order to make all developmental stages of the beetles available at any time, a system of mass breeding was introduced based on the breeding cycle of the species (Yinon, 1968). As Stegobium develops from egg to adult in approximately 40 days (Lefkovitch, 1967) under these conditions, six sets of four cultures were maintained in series, a new set of four being introduced at weekly intervals. All stages of the beetle were thus available with at most three or four days to wait, the system involving only one or two hours work every week. Adults were selected from a culture in which young beetles were emerging and were removed from the sides of the jar and tissue by means

of an aspirator. About 500 individuals were introduced into a new culture jar which had previously been left to equilibrate in the insectary for at least 24 h.

Mites, identified as Tyrophagus sp. infested the cultures despite the precaution of sterilising the food medium and standing all culture jars at least 10 cm apart in a 1 cm depth of liquid paraffin. They are thought to have entered the insectary, where conditions would be highly favourable to their survival, and fallen from the ceiling and walls on to the cultures. Although they were not directly harmful to the beetles, the mites did eventually present an irritating problem of hygiene and they were finally eliminated by adopting the procedure described below. Chemical acaricides, although physiologically harmless to Coleoptera (Strong, Pieper and Sbur, 1959) were avoided in case they interfered in any way with the beetle olfactory responses during experiments. Once the mites were established in the cultures, adult beetles intended for new cultures were clearly carrying mites with them from infested cultures. By keeping these insects for about 15 min in a small glass tube (which they half filled) it was found that the intense activity of the beetles resulted in mechanical damage and crushing of the mites, with only a small percentage mortality of the beetles.

The mites were eventually eliminated and replaced by a psocopteran which has never been completely eradicated. The above procedure has kept its density to an acceptable level.

B. Rearing experimental insects

For the majority of the experimental work in this study, it was necessary to keep the sexes apart. As Stegobium can only be sexed with accuracy during the pupal stage (Halstead, 1963) pupae were removed from the cultures at the appropriate time. Some larvae construct cocoons against the sides of the glass jar and the development of these individuals could easily be observed. Since the life cycle is short under the imposed conditions, development is fairly closely synchronised, and for maximum yield of pupae,

culture jars could be selected at the optimum time.

The wheat was sieved in small quantities through a 2.54 mm mesh to retain the pupal cocoons. If this was carefully done, few cocoons ruptured and the majority of the loose food material passed through. Pupae which had been shaken out of their cocoons had a very low survival rate as a result of dehydration caused by cuticle damage, so these were not recovered. Insect pupae are extremely vulnerable to mechanical damage as a result of handling (Ryan and Nathanson, 1968) and abnormalities in Stegobium adults often resulted even if the pupae survived handling.

Cocoons, in batches of about 100 were placed in a petri dish under a X10 binocular microscope. Fine forceps were used very carefully to break open the cocoon and lift out the contents. Larvae and adults were put aside and later returned to stock cultures. The sex of the pupae was immediately determined according to the difference in size of the genital papillae (Halstead, 1963). The pupae were then placed in filter paper lined petri dishes, the sexes being kept in separate dishes. When about 200 pupae of each sex had been collected in this way, the lid, with a small piece of dampened filter paper adhering to it, was replaced and the pupae returned to the insectary. During the pre-eclosion period, the lid was slightly moistened each morning to maintain a high humidity, which was fortuitously found to increase the survival rate. Presumably the cocoon provides and maintains a high humidity for the developing pupa, as well as protection, under natural conditions. Excess moisture, however, could not be tolerated by the pupae.

Inspections of the pupae were made at 24 h intervals. Newly eclosed adults whose elytra had darkened to the same extent as the thorax (which darkens earlier) were considered 1 day old and removed from the dishes. Callow adults were thus classified as 0 days old and left for a further 24 h. This classification of insect age proved satisfactory since the 0- and 1-day stages are clearly distinct and conveniently separated by about 24 h. In

this way, unnecessary damage to callow adults was avoided. Being virtually inactive, they were not usually required for experimentation. As greater accuracy was required for some experiments, in these cases 1-day-old adults were removed at 09.00 h and 21.00 h each day. All visibly damaged insects were discarded.

With respect to the above system of insect age classification, it is important to bear in mind that the eclosed adult would normally spend a 4-5 day period in the cocoon. Thus, a 4 to 5-day-old insect (according to this system) would, in a natural situation, be experiencing its first day out of the cocoon.

One-day-old adults were placed in groups of ten in 5 x 1.8 cm tubes. These were previously fitted with a disc of filter paper (Whatman Grade 1) covering the tube base, and a strip approximately 4 x 1 cm standing upright in the tube to increase the substrate area for the insects. Polythene closures in which a large hole had been cut, were replaced over a piece of filter paper, giving good ventilation to the insects, but allowing no means of escape. Tubes were labelled with the date of eclosion and sex of the beetles, and the sexes were stored at opposite sides of the rearing room until required for experimentation.

C. Stegobium life cycle and developmental periods

Stegobium paniceum is an anobiid beetle, 2-3 mm long with a non-feeding adult stage. The species is cosmopolitan and the larvae have been reported infesting wood (Eichler, 1943; Español, 1969); leather (Lengerken, 1922); coffee, tea and tobacco (Chittenden, 1902); drug plants (Armitage and Verdcourt, 1947); calf-starter pellets (Stone, 1949) as well as cereal crops, flour and their products, which they commonly infest.

At 30°C and 75% r.h., developmental periods for Stegobium are as follows (Lefkovitch, 1967): total developmental period (oviposition to adult emergence from the cocoon) 42-3 days, consisting of the egg stage, 7 days; larval instars about 27 days; pupa 4-5 days and pre-adult stage, 4-5 days.

According to Lefkovitch (1967) the temperature and humidity regime

provided in the rearing room during the present study produces;

(i) the most rapid developmental period,

(ii) almost the lowest pre-adult mortality,

(iii) almost the shortest adult life span,and

(iv) a comparatively low mean of about 40 eggs laid per female (maximum of 75 at 22.5°C).

The period spent in the cocoon after adult emergence is not necessary for survival, and is thought to constitute an inactive period of cuticle hardening and gonad maturation.

III. RESPONSE OF MALE STEGOBIUM TO LIVE FEMALES IN ARENA ASSAY EXPERIMENTS

A. Introduction and Methods

If a pheromone system of communication is thought to operate in an insect species, whether from direct observation or evidence from closely related species, it is important to obtain some initial quantitative data using the live insect as a source of attractant. A pheromone was presumed to be present in the mosquito, Culiseta inornata (Williston) from observation of males "easily finding resting females" and "attempting to mate with apparently dead females" (Kliewer, Miura, Husbands and Hurst, 1966). Burkholder and Dicke (1966) reported that female dermestid species were observed to attract males soon after emergence and suggested the possibility of chemical attraction. In both cases these initial observations were followed by the application of more indirect methods of testing.

There are many records of sexual attraction in the field amongst the Coleoptera. Caged females of the tobacco hornworm, Protoparce (Manduca) sexta (Johannson) (Allen, Kinard and Jacobson, 1962); the banded cucumber beetle, Diabrotica balteata (LeConte) (Cuthbert and Reid, 1964) and grass grub beetle, Costelytra zealandica (White) (Kelsey, 1967) attracted large numbers of males to them. Virgin female wireworms, Ctenicera aeripennis destructor (Brown) (Doane, 1961) and wood borers, Xenorhipis brendeli LeConte (Wellso, 1966) in cages attracted males, which moved upwind towards them. Female Japanese beetles, Popillia japonica Newman tethered by a nylon thread were observed to attract very large numbers of males in a short period (Ladd, 1970). There are numerous examples of field attraction within most other insect orders, especially the Lepidoptera.

In the laboratory, chemical attraction was demonstrated directly in the mealworm, Tenebrio molitor L., where both sexes produce pheromones. An air flow was passed over a chamber containing individuals of one sex, and the opposite sex responded by collecting at the outlet (Happ, 1969). The same

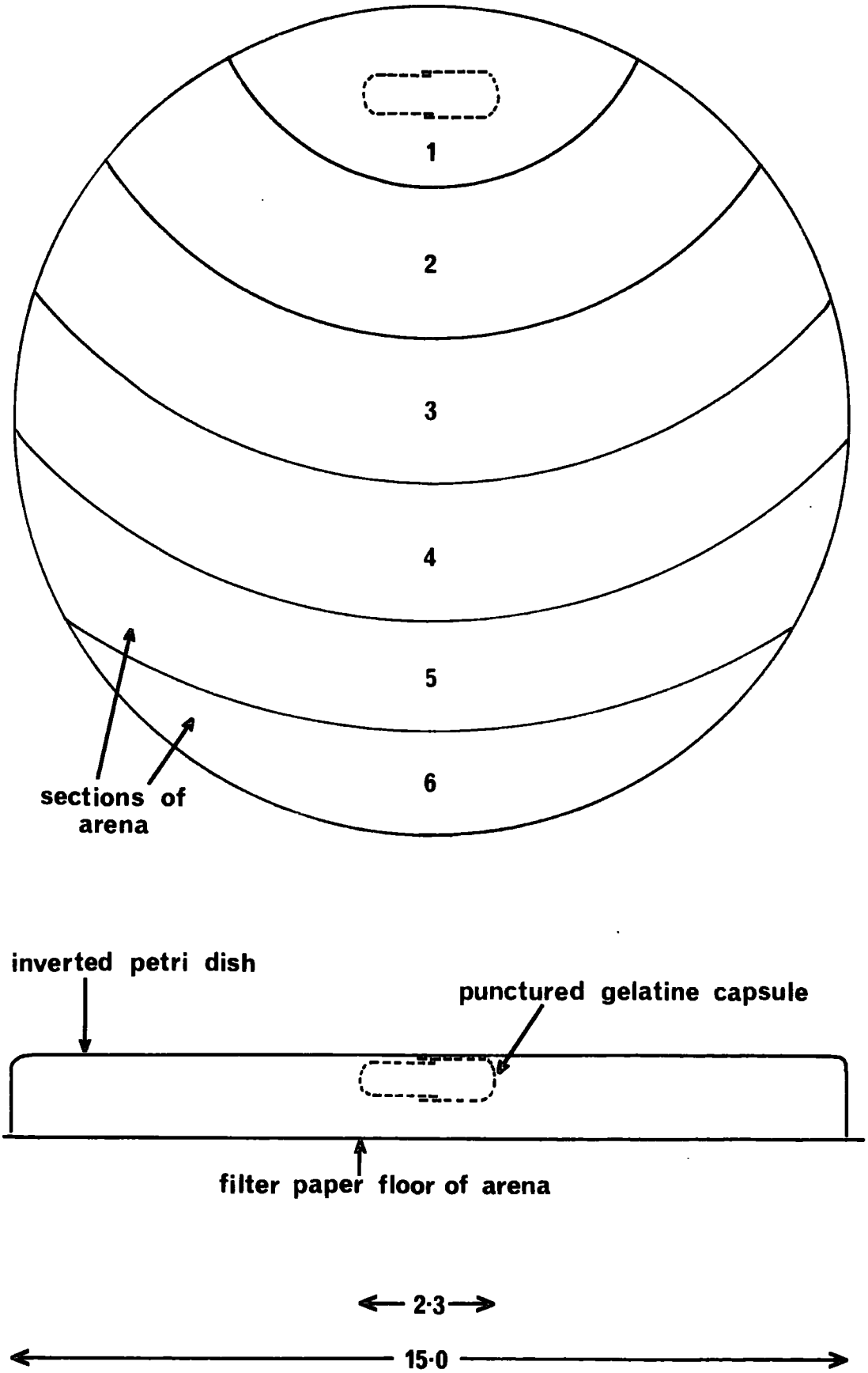
species was bioassayed in a choice chamber, where live insects provided the source of attractant (August, 1971). Crushed females of the grass grub beetle, Costelytra zealandica in one arm of a 'Y-choice' olfactometer attracted only males (Henzell, Lowe, Taylor and Boston, 1969).

The experimental technique described below was designed to detect and demonstrate the presence of a pheromone system of communication operating between the sexes in Stegobium. The experiment also set out to determine the sex(es) producing and/or responding to the attractant and to provide a broad outline of the nature of the pheromone system and its timing in the adult life span of this species.

A closedarena was formed by inverting a 14.0 cm diameter glass petri dish. The rim of the dish was ground on a sheet of glass covered with moistened carborundum powder until all irregularities were removed and beetles were unable to escape from beneath it. A filter paper disc of 15.0 cm diameter formed the floor of the arena providing good traction for test insects. The paper was of the same type and grade as that used in the storage tubes for retaining experimental insects, (see section II,B), so that they would be accustomed to any odour inherent in it. Lines were drawn in pencil on to the arena floor (fig. 1) in such a way that they radiated out from one point on the circumference, concentrically. Similarly, a pencil was used to label the filter paper held by the storage tube closures to condition insects to its odour, if any. The area of the sections and circumference length (since Stegobium tends to follow edges) were kept as equal as possible in the arena. The only section seriously violating this condition was section six which had a greater circumference length. However, this could only result in a bias against the results expected (part B).

A gelatine capsule, size 00, was punctured extensively with a fine mounted needle. The outer half was attached to the inner surface of the petri dish (by means of a small drop of water) at a point approximately 1.5 cm from the periphery, so that when the arena was assembled, the capsule would be

Figure 1. Plan and elevation of the arena used to test the response of 10 males to 4 females confined in the punctured gelatine capsule over a 30-min test period. Diagram to scale.



Actual dimentions in cm.

suspended over the centre of section one (fig. 1). When the water had dried, four females of known age were admitted to the inner capsule portion and the two halves fitted together.

In a typical experiment, ten males of known age were placed in the centre of the arena and the petri dish, complete with females in the capsule, was quickly placed in position on the marked filter paper disc. A stop-watch was started immediately six such arenas had been assembled. A motorised 'Prakti' 35mm camera mounted 1.0 m above bench level photographed the group of arenas periodically. A motorised time-lapse wheel operated a 6-volt solenoid which in turn depressed the camera shutter release once every 60 s during a 30-min period. An angle poise lamp fitted with a 100 watt red light bulb was placed on each side of the group of arenas, giving fairly even lighting. Since some beetle species are relatively insensitive to the red end of the spectrum, eg. Tenebrio molitor(Tschinkel, Willson and Bern, 1967), the red lamps were used to give adequate lighting for photography on Kodak Tri-X film (ASA 400) without providing a strong stimulus which might influence the orientation of the beetles. The film was developed normally and the negatives viewed directly by projection on to a white card 0.5 x 0.3 m. The number of males in each section was recorded for each arena over the entire series of 30 frames.

For a single arena test, a percentage of the possible maximum score for each section (300 males) over the 30 min was calculated, giving a mean percentage value, per section, per test.

All experiments were conducted between 10.00 and 16.00 h.

B. Results

The results were analysed in order to provide information about the following:

(a) occurrence of a chemical attractant mechanism in Stegobium;

(b) nature of the response over the 30-min test period;

(c) gradation of response or distribution of insects within the arena, and

(d) effect of adult age on attractiveness and responsiveness.

(a) Indication of chemical attraction by female Stegobium

A series of exploratory arena assay tests were carried out as described above, with all possible combinations of the two sexes in the arena and capsule respectively. Table 1 shows the percentage responses of the test insects as demonstrated by their representation in section one of the arena.

Table 1 Mean percentage response of arena insects to those in the capsule as indicated by those in section one of the arena over the 30-min assay test period.

capsule (4 insects)	arena (10 insects)	replicate tests	mean % in section 1 ± SE
female	male	13	32.15 ± 2.06
-	"	10	16.19 ± 1.48
male	"	10	18.87 ± 1.24
"	female	9	18.84 ± 1.76
female	"	9	19.06 ± 2.36

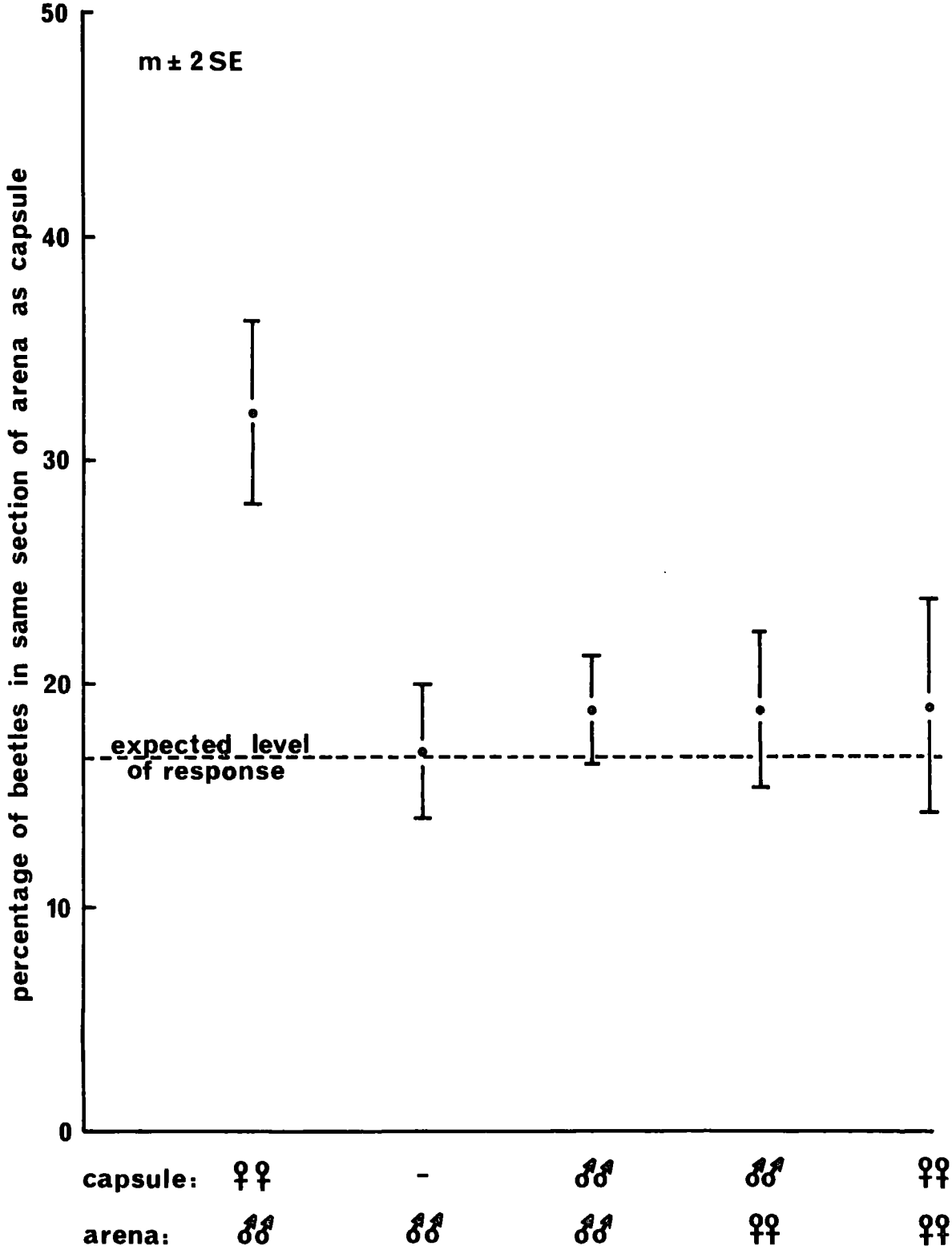
If distribution of test beetles within the arena had been random, 16.7% would be expected in each section. Only in those tests with females in the capsule and males in the arena, were percentages in section one significantly higher than the expected level (fig. 2). Males did not respond to empty gelatine capsules, so this was eliminated as a possible source of attraction. Males were also not attracted to males, and females did not respond to males or females.

Following these results, further arena assay tests were concentrated only on male response to encapsulated female beetles.

(b) Male response intensity over the 30-min test period

Using data obtained for section (d) below, all tests in which both sexes were 5 days old and above were numbered, and 25 chosen for further analysis by

Figure 2. Mean percentage response of insects in the arena (fig. 1) to those in the capsule (sexes as indicated) over the 30-min test period. The 16.7% level of response (indicated by the broken line) would be expected if distribution of insects within the arena was random.


50
40
30
20
10
0
m ± 2SE
percentage of beetles in same section of arena as capsule
expected level of response
capsule:
arena:
-

means of a table of random numbers. A mean value of males present in section one of the arena was calculated for each of the 30-min interval recordings (table 2; fig. 3). Male response was found to be greatest over the first five minutes when almost 50% of males were recorded in section one. Intensity of response gradually declined over the remaining test period, almost reaching the expected level during the last five minutes.

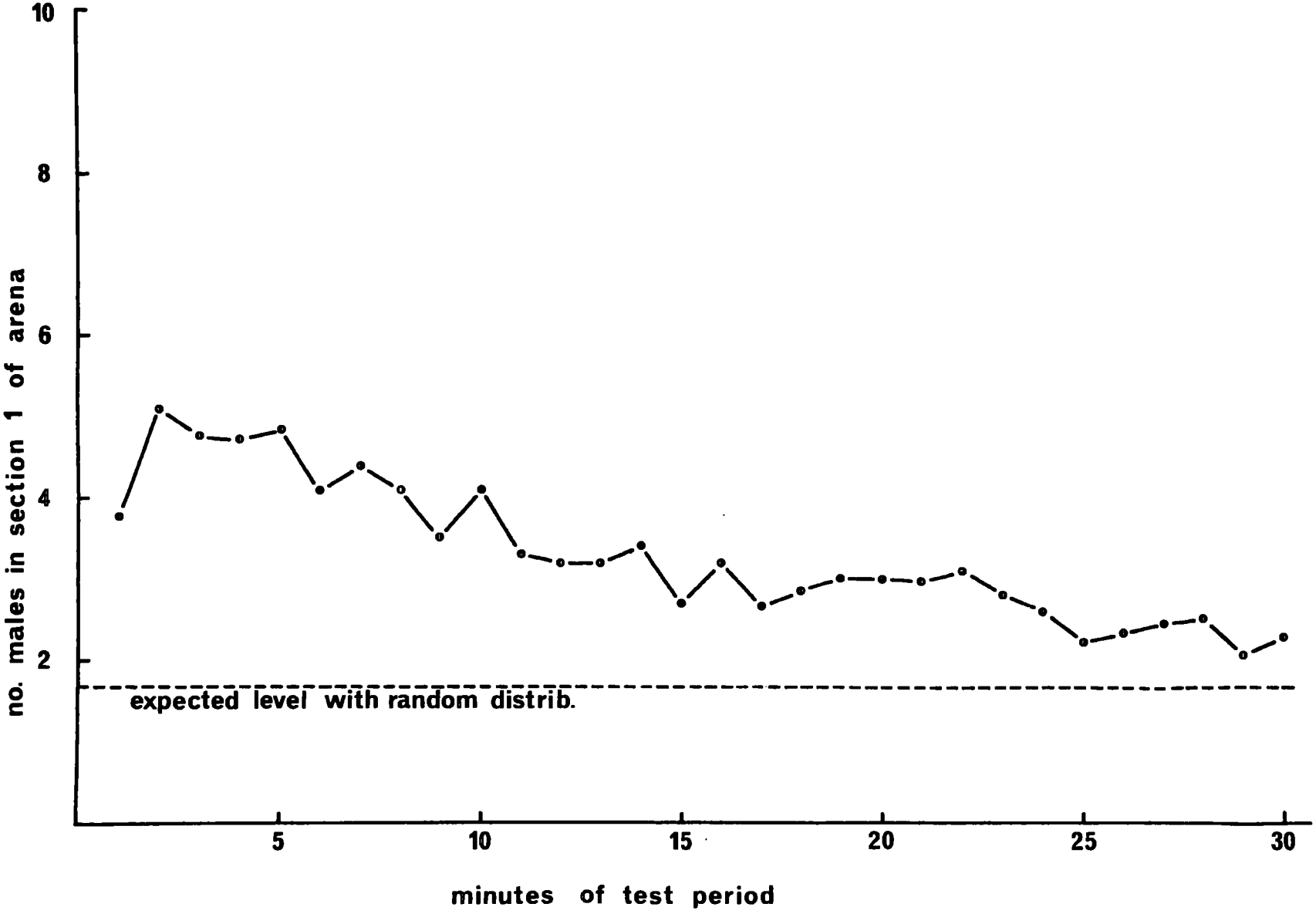
Table 2. Mean number of males in section one of the arena for each minute-interval recording, from a sample of 25 tests.

minute of test	mean no. males in section one	minute of test	mean no. males in section one
1	3.76	16	3.20
2	5.12	17	2.76
3	4.68	18	2.84
4	4.64	19	3.00
5	4.84	20	3.00
6	4.16	21	2.96
7	4.40	22	3.12
8	4.16	23	2.80
9	3.52	24	2.60
10	4.16	25	2.24
11	3.32	26	2.36
12	3.24	27	2.44
13	3.24	28	2.52
14	3.40	29	2.08
15	2.68	30	2.28

(c) Male distribution within the arena

The test arena was divided into six sections in order that any gradation of response from section one through to six would become apparent. Again, from data obtained for (d) below, 30 tests where both males and females were 5 or more days old, were selected using random number tables. The percentage values for each of the arena sections were summed and a mean value calculated

Figure 3. Intensity of male response to live females in the test arena (fig. 1) over the 30-min test period. The mean numbers of males in section one of the arena were calculated from a sample of 25 tests in which both males and females were at least 5 days old.


10
8
6
4
2
no. males in section 1 of arena
expected level with random distrib.
5
10
15
20
25
30
minutes of test period

from the sample of 30 tests (table 3, fig. 4). The expected level of response

Table 3 Mean percentage of males in each of the arena sections for a sample of 30 tests

arena section	mean % males over 30 mins ± SE	range for 2 ½ SE
1	31.12 ± 1.70	26.86 - 35.38
2	15.00 ± 0.48	13.79 - 16.21
3	13.56 ± 0.74	11.72 - 15.40
4	12.22 ± 0.66	10.56 - 13.88
5	13.57 ± 0.84	11.48 - 15.66
6	14.72 ± 0.74	12.88 - 16.56

shown in figure 4 was estimated as follows. If in 99% of cases, 26.86 - 35.38 percent of males were recorded in section one of the arena, then 64.62 - 73.14% must be distributed throughout the remaining five sections. Assuming a null hypothesis, that there was no gradation of male response, then one would expect 12.92 - 14.63% of males in each of sections two to six inclusive. In fact, no significant deviation from the expected level was found, so the null hypothesis could not be rejected. It was thus inferred that, although percentages of males in sections two to six are directly dependent upon that in section one, they do not differ significantly themselves.

(d) Effect of age on female attractiveness and male response

A series of arena tests covering most age range combinations was performed to determine the importance of adult age on the ability of females to attract males and the capability of males to respond. From table 4A, fig. 5B it is evident that females become increasingly attractive to mature males between 3 and 4 days of age, reaching a peak when they are 7-8 days old and gradually becoming less potent thereafter.

Males first respond (table 4B, fig. 5A) when they are about 5 days old, reaching a maximum at 6-7 days and maintaining this level of about 30% positive response throughout the rest of their adult life span.

Figure 4. Mean percentage of males in each of the six sections of the test arena (fig. 1) from a sample of 30 tests in which both males and females were at least 5 days old. The broken line represents the expected level of male response if distribution within sections two to six inclusive was random (13.8%).

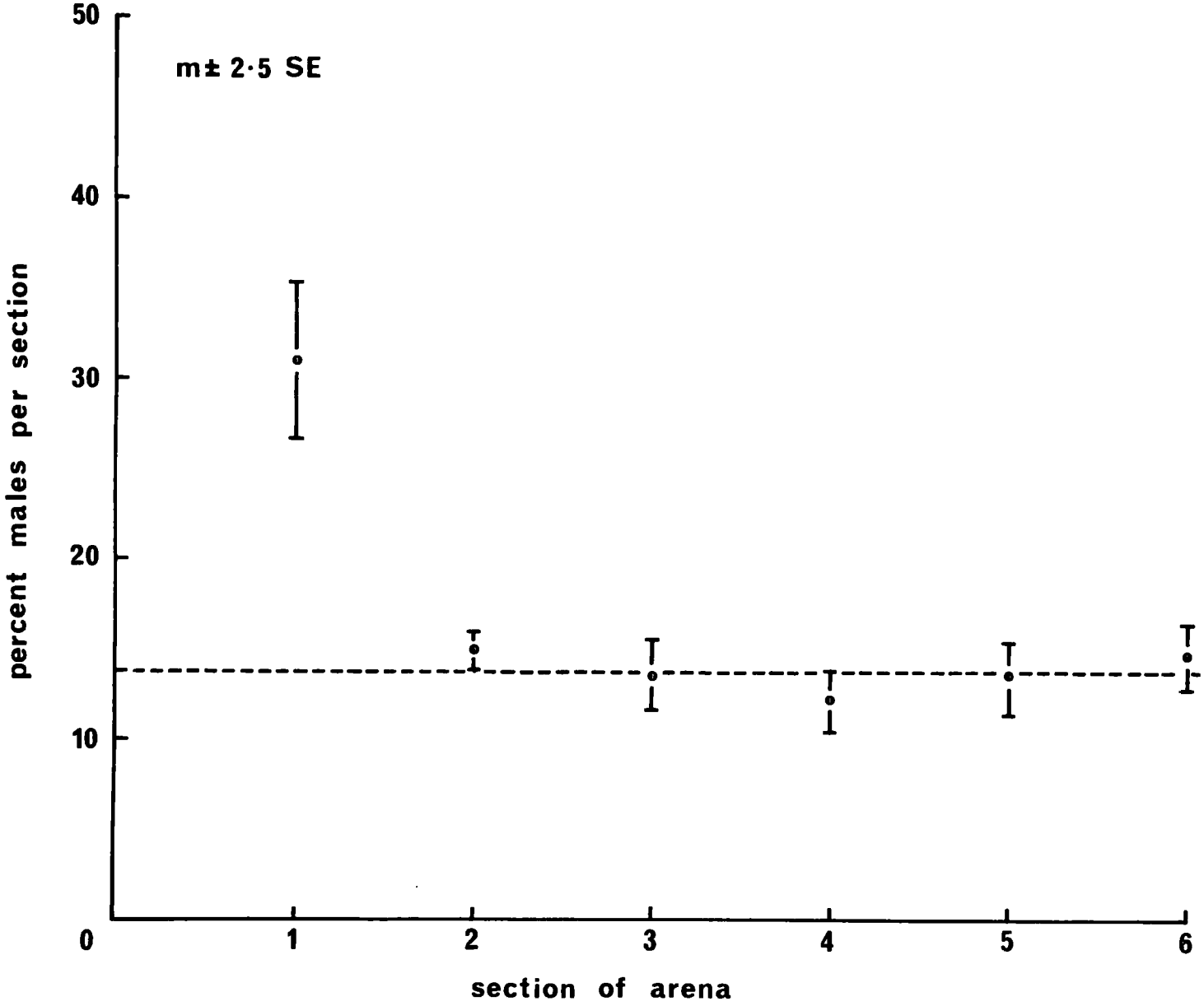

m± 2·5 SE
percent males per section
50
40
30
20
10
0
1
2
3
4
5
6
section of arena

Table 4A. Response of males to females of different age groups, where males were 5 days old or above.

age females (days)	no. replicates with 10 males	mean % in arena section 1 ± SE
1	7	18.1 ± 3.18
2	15	18.8 ± 1.67
3	14	21.5 ± 1.57
4	22	28.2 ± 2.03
5	21	27.6 ± 1.95
6	29	27.9 ± 1.17
7	18	34.4 ± 2.50
8	7	36.3 ± 3.40
9	19	32.2 ± 1.91
10	8	28.3 ± 3.69
11	9	24.0 ± 2.80
12-13	5	27.3 ± 2.74
18	4	15.2 ± 1.51

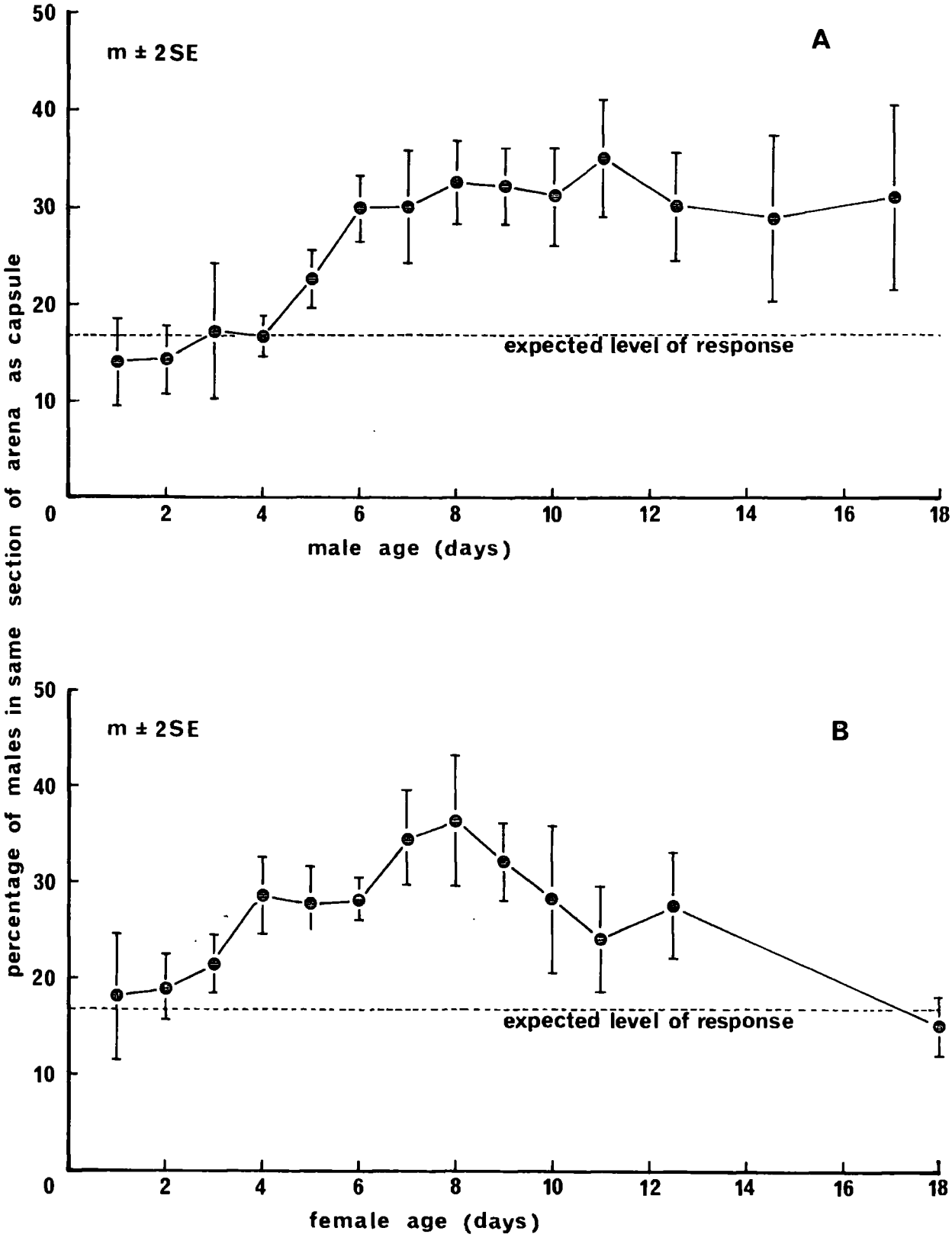
Table 4B. Response of males of different age groups to females of 5 days old and above.

age males (days)	no. replicates with 10 males	mean % in arena section 1 ± SE
1	3	14.3 ± 2.28
2	6	14.4 ± 1.27
3	5	17.0 ± 3.46
4	7	16.4 ± 0.97
5	15	22.6 ± 1.52
6	22	29.7 ± 1.67
7	13	30.0 ± 2.90
8	14	32.5 ± 2.15
9	16	32.1 ± 1.85
10	9	31.0 ± 2.42
11	12	34.9 ± 3.19
12-13	6	29.9 ± 2.69
14-15	8	28.7 ± 4.22
16-18	10	30.7 ± 4.73

Figure 5A. Response of males over their adult life span to live females of at least 5 days old, in arena assay experiments.

Figure 5B. Response of males of at least 5 days old to females between 1 and 18 days old in arena assay experiments.

In figures 5A and B the broken line represents the expected level of response (16.7%) if male distribution in the arena was random over the 30-min test period.


m ± 2SE
A
expected level of response
male age (days)
percentage of males in same section of arena as capsule
m ± 2SE
B
expected level of response
female age (days)

C. Discussion

A demonstration of male attraction to females of a certain age range was achieved using a live insect assay technique. Visual and auditory factors cannot be dismissed, but it seems improbable either that males are able to visually differentiate between males and females, or that they can distinguish between females of different ages. Auditory signals, beyond the human range of sensitivity, produced by females of a certain age class cannot be discounted at this stage.

Assuming that the mechanism of attraction is olfactory (which is verified in the following sections), the relative change in male response intensity during the 30-min test period is interpreted as an intereaction between two factors, namely, adaptation of the male chemosensory apparatus, and gradual diffusion of the pheromone from the capsule and eventual dispersion of the odour throughout the arena.

The sectioning of the assay arena was in some ways unsatisfactory since the divisions could not be absolutely equal in area, circumference length and distance from the capsule. However, distribution of beetles in sections two to six inclusive was shown to be homogeneous such that the arena was ultimately considered to comprise of section one and "the rest". There is, of course, the possibility that real differences in male distribution may have been masked by unequal section sizes.

The age of adult insects was found to influence both female attractiveness and male response. Newly emerged beetles are relatively quiescent for 2-3 days and active pheromone emission or response would not be expected at this stage. Burkholder (1970) found that females of the black carpet beetle, Attagenus megatoma (F.) induced a stronger response when they were 6-7 days old than 2-3 days, when assayed with mature males. The virgin female mealworm, Tenebrio molitor reached a peak in pheromone emission 4-5 days after eclosion, whilst young females were unattractive to males (Happ and Wheeler, 1969). The response of the male Mediterranean flour moth, Anagasta (Ephestia)

kühniella (Zeller) to females increased progressively up to a maximum when they were 5 days old. Females reached a peak of emission when 3-5 day old, but younger, and older moths were less attractive to males (Traynier, 1970).

Pheromone production and response in insects must be linked with gonad maturation to provide the maximum possible reproductive potential and success. Gonad development of Stegobium will be considered in detail in a later section, but it is suggested that this species attains sexual maturity while in the cocoon after eclosion (Azab, 1943) as does its close relative, the cigarette beetle, Lasioderma serricorne (Howe, 1957; Coffelt and Burkholder, 1973). In this case, the timing of pheromone emission and initiation of male responsiveness corresponds well with adult emergence from the cocoon.

IV SOLVENT EXTRACTION OF FEMALE *STEGOBIUM*: FACTORS AFFECTING FEMALE PHEROMONE CONTENT AND MALE RESPONSIVENESS TO EXTRACTS

A. Introduction and Extraction Methods

To confirm the finding that *Stegobium* females attract males by a chemical sex attractant system, it was necessary to isolate and bioassay the substance in the absence of females. This was achieved by means of a solvent in which the pheromone readily dissolved and which was sufficiently volatile to be removed quickly in conditions which would not result in denaturalisation of the pheromone.

A wide range of solvents and extraction techniques have been employed successfully with different insect species. Solvents include benzene (Rodin, Silverstein, Burkholder and Gorman, 1969; Vick, Burkholder and Gorman, 1969; Traynier and Wright, 1972); ethyl alcohol (Cuthbert and Reid, 1964; Lilly and McGinnis, 1965; Tschinkel *et al* 1967); methylene chloride (Berger, 1966; Roelofs and Cardé, 1971; Nagata, Tamaki, Noguchi and Yushima, 1972); hexane (Coffelt and Burkholder, 1972); acetone (Happ and Wheeler, 1969); dichloromethane (Tumlinson, Hardee, Minyard, Thompson, Gast and Hedin, 1968) and most commonly, diethyl ether. This solvent has been used to give surface extracts of whole insects (Bar Ilan, Stanic and Shulov, 1965; Levinson and Bar Ilan, 1967; Carlson, Mayer, Silhacek, James, Beroza and Bierl, 1971) or parts, especially for Lepidoptera, where the abdominal tips are clipped off and extracted (Allen *et al.*, 1962; Jacobson, Lilly and Harding, 1968; Bartell and Shorey, 1969a; Jefferson, Sower and Rubin, 1971) but in some cases, whole insects or parts have been macerated in ether (Shorey and Gaston, 1965; Levinson and Bar Ilan, 1967; Ikan, Bergman, Yinon and Shulov, 1969; Calvert and Corbet, 1973). Another method often preferred is extraction of paper on which pheromone-emitting insects have crawled and the inside of containers in which they have been kept (Cuthbert and Reid, 1964; Burkholder and Dicke, 1966; Adeesan, Rahalkar and Tamhankar, 1969).

The solvent chosen for extracting the sex pheromone from *Stegobium* was

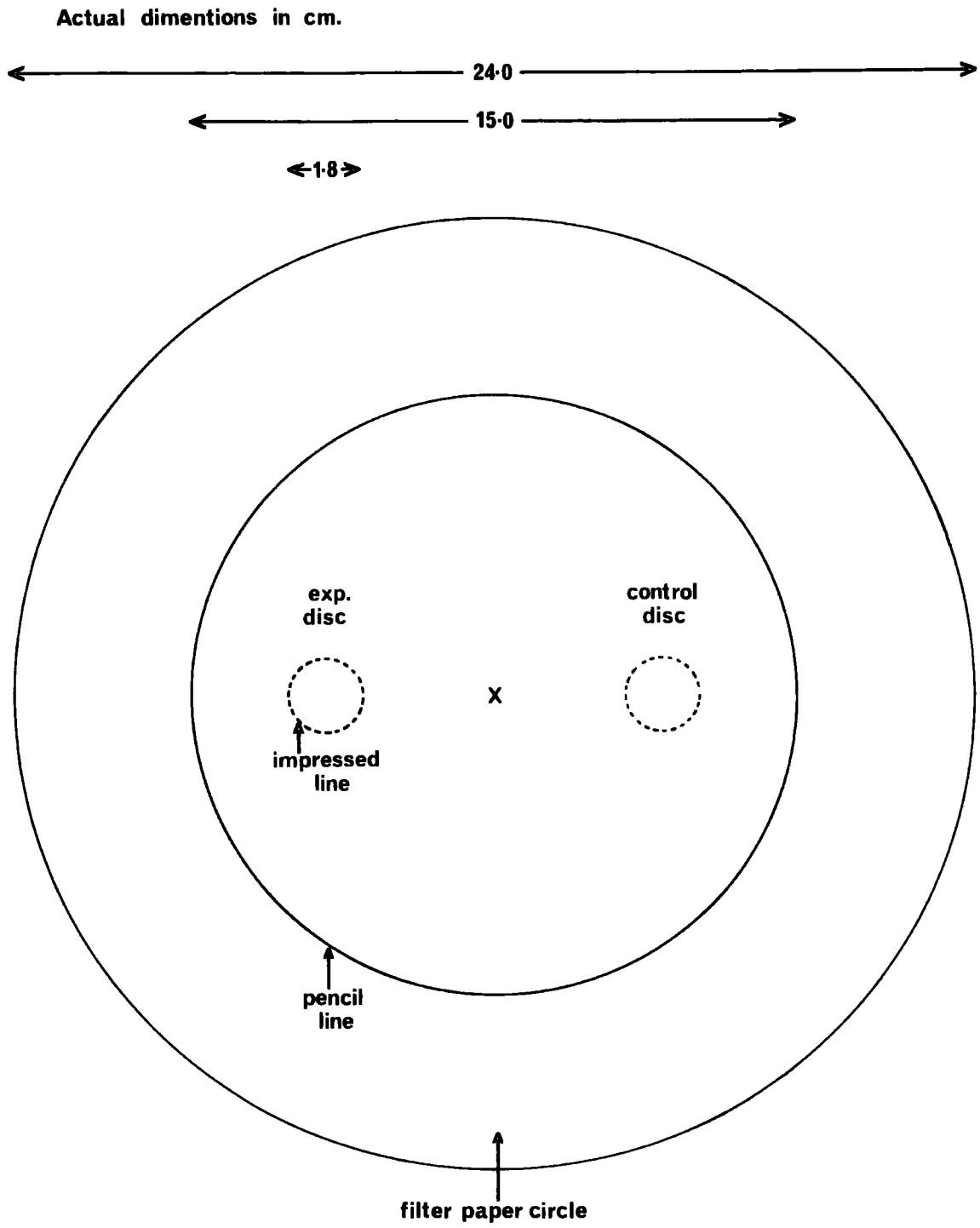
diethyl ether, as this was shown to be equally or more efficient than the others originally considered (Appendix I). A minimum of 50 live females of known age were immersed in diethyl ether at a concentration of 10 females per ml solvent. They were left at room temperature (20 ± 2°C) for exactly 24 h in a glass tube with a tightly fitting closure. The solvent was decanted from the beetles using a pipette, and stored at -10°C until required. No extracts were kept for more than two months although they were known to retain their active properties for much longer.

B. Bioassay technique

A very simple arena was used to bioassay the female pheromone extracts, the design adapted from that employed by Levinson and Bar Ilan (1967) and Adeesan et al (1969). A circle of filter paper 25.0 cm in diameter with a 15.0 cm diameter circle drawn concentrically within it, was impressed with two 1.5 cm discs by pressing the mouth of a glass tube down on to the paper (fig. 6). The impressions were made 2.5 cm in from the circumference of the smaller diameter circle, diametrically opposite each other to mark the positions where extract and control samples would be absorbed. This technique was preferred to the use of separate cut-out discs or anti-bacterial assay discs which might present a barrier to such small insects, which would have to climb the discs in order to locate and respond to an extract.

The arena was left open rather than covered with an inverted petri dish for the following reasons. Firstly, it was hoped to avoid confusion of insects as a result of the closed space becoming saturated with the odour. Secondly, this procedure allowed beetles to be removed quickly following a positive response (Part C). Finally, the modified technique avoided the potential problems which arise from the tendency of this species to climb vertically. Even the use of "Fluon" failed to prevent males from climbing the inverted petri dish and hence confusion might have resulted from males orientating on the dish above the experimental disc, rather than on the disc itself. This would have rendered the evaluation of male response far more

Figure 6. Plan of the arena used to bioassay diethyl ether extracts of female Stegobium. X marks the point of release of males. Diagram to scale.


Actual dimentions in cm.
24·0
15·0
1·8
exp. disc
control disc
X
impressed line
pencil line
filter paper circle

difficult.

Extract samples of 10 µl were applied to the centre of one disc of the arena, using a micropipette, and the same volume of pure diethyl ether was applied to the other as a control. The arena was left for 2 min for the ether to evaporate before it was taken into the insectary for testing. Males were placed in the centre of the arena between the two treated discs, in groups of up to five. The response of an individual beetle is not thought to be influenced by the presence of others, but this is further discussed in part G below. Three minutes were allowed for beetles to respond, but they were removed immediately if a positive response was displayed before this. A positive response was recorded on observing the following behaviour pattern: the male moved on to the disc, circling continuously within it in an excited manner, returning directly to the disc if it moved outside the impressed border. This behaviour, for the purposes of these tests, had to be maintained for at least 10 s. An insect moving outside the 15.0-cm-diameter circle was returned carefully to the centre using fine forceps. If a beetle failed to exhibit the above behaviour sequence within the time allowed, it was considered a negative response, although this in no way implies that the beetle was repelled.

Some insects have been described as displaying very characteristic behaviour in the presence of the appropriate sex pheromone extract. The response of the dermestid beetle, Trogoderma inclusum LeConte was characterised by "...a forward and upward extension of the antennae, and by a zig-zag pattern of approach with intermittent stops, during which the front legs were straightened so that the body was tilted upward at the front..." (Burkholder and Dicke, 1966). Male cigarette beetles, Lasioderma serricorne were described as moving rapidly around the arena, elevating the antennae and extending the pro- and mesothoracic legs (Coffelt and Burkholder, 1972). Female boll weevils, Anthonomus grandis Boheman in response to the male-produced pheromone, display "...very rapid walking, standing as high on their front

legs as possible, heads held high, and antennae high and forward." (Keller, Mitchell, McKibben and Davich, 1964). The male khapra beetle, Trogoderma granarium Everts in the vicinity of the assembling scent, "...lowers its head and frequently touches the floor of the arena with both antennae which are set into vibration..." (Levinson and Bar Ilan, 1970). In the absence of such distinctive behaviour patterns in Stegobium the system for the recognition of the pheromone response outlined above, was considered to be the most suitable for this species. However, certain behavioural manifestations often accompanied a positive response, such as excited antennal movements and a more halting rate of progression.

Bioassay arenas were renewed every 10-15 min to maintain the quantity of extract on the disc at a fairly constant level. Old arenas, in fact, still attracted males two days after use, but to a lesser degree.

Males were never tested more than once a day and if possible, not on consecutive days in case their response was influenced by the previous days exposure to an extract. However, the same males were tested several times during their life span and not used once only as was thought necessary for Trogoderma sp. (Vick, Burkholder and Gorman, 1969).

At least 60 individuals of the same age were tested with any one extract and a percentage response value was calculated from the observed ratio of positive to negative responses. On no occasion was a beetle seen to respond positively to the ether-treated control disc. Similarly, extracts of males excited no response from either sex and females were not attracted to female extracts. Appendix II contains results of all ether extract experiments and controls for this and the following section of this thesis. A series of experiments employing diethyl ether extracts of female Stegobium were designed to investigate factors affecting both pheromone production by females and male response.

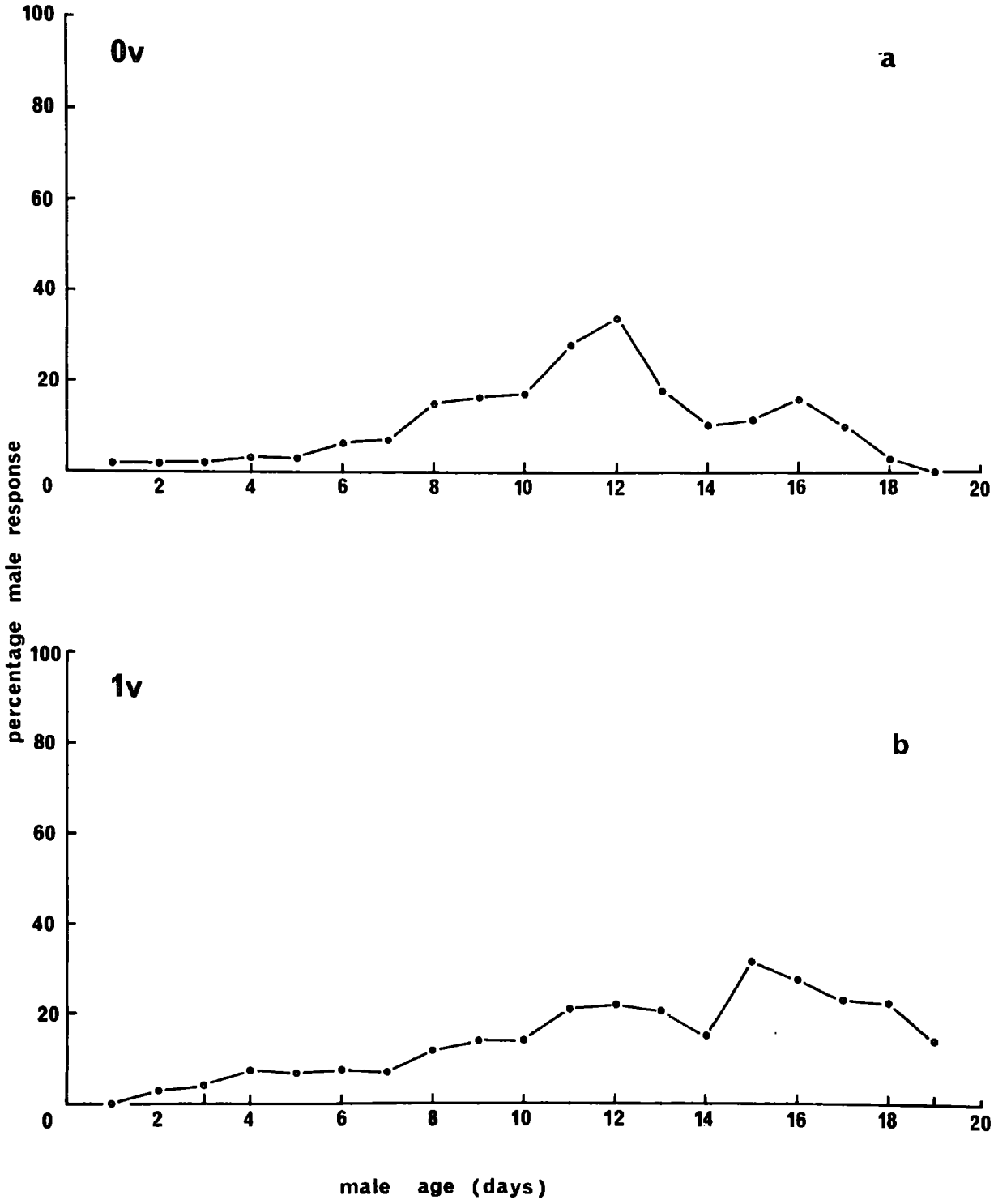
C. Effect of adult age on pheromone content of females and male responsiveness to pheromone ether extracts

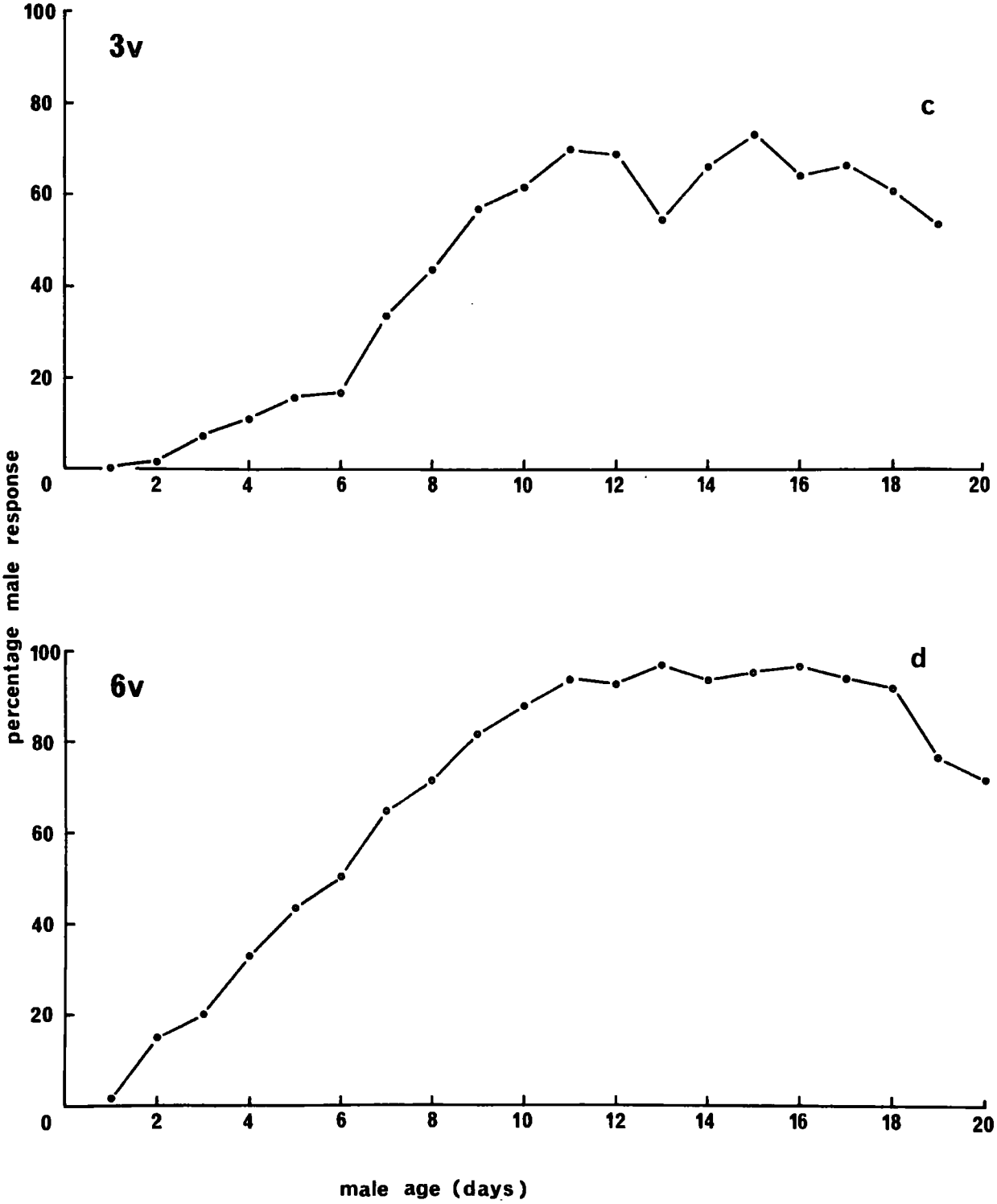
Adult age and its effects upon the pheromone communication system was again considered in order to compare results obtained from bioassay of female extracts with the live female tests (section III), and provide more detailed and precise information than was possible with the latter technique. Much emphasis has been placed upon the age effect in most studies involving pheromone bioassay, since workers have aimed ultimately at revealing the timing of reproductive events, gonad maturation, copulation and the synchronisation of pheromone production to control and co-ordinate this sequence of events.

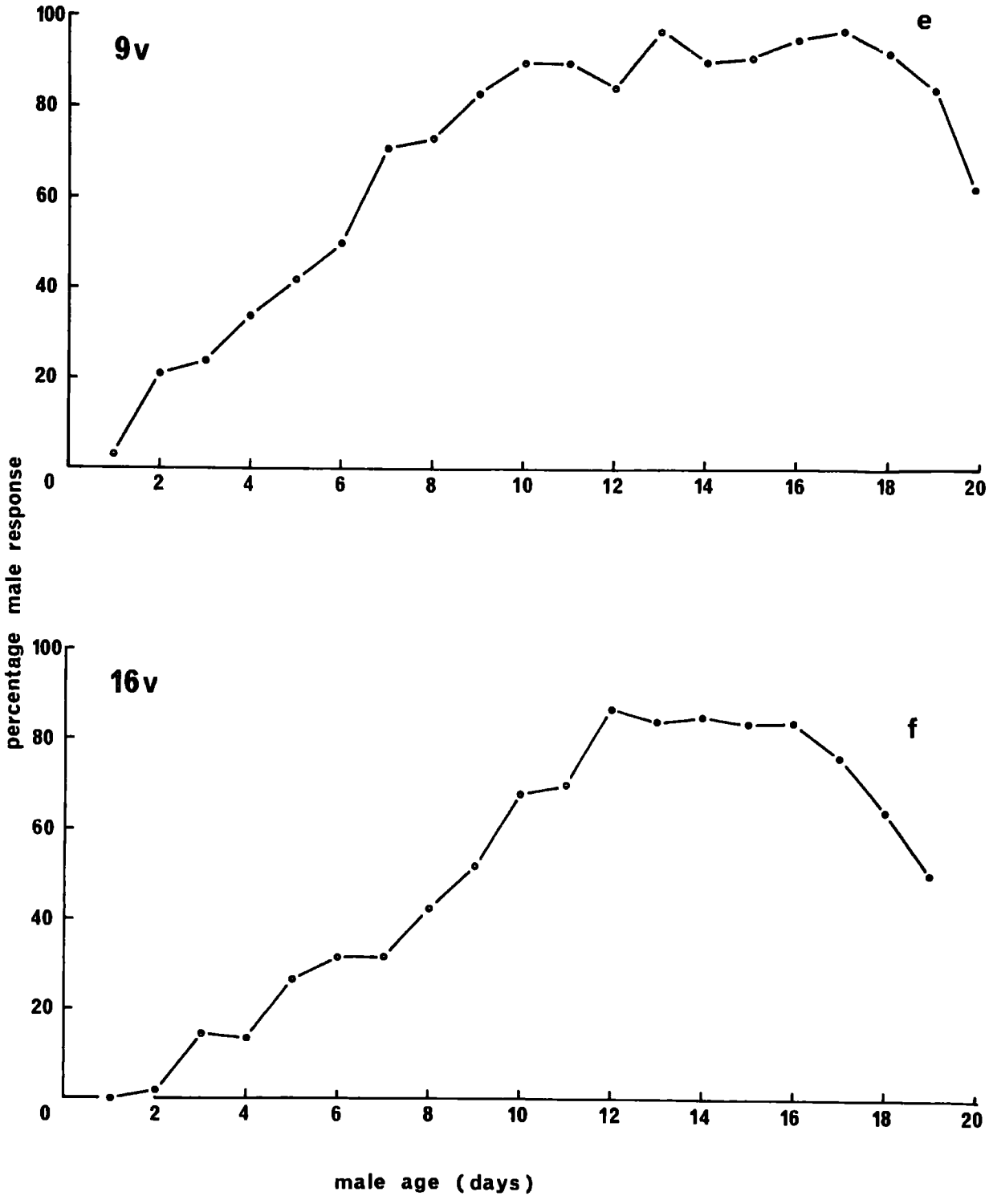
The results presented here will later be discussed in conjunction with those in Section VII, where a detailed investigation of female reproductive development is described.

The age of male Stegobium was found to have a great influence on their ability to respond to pheromone extracts, as might be reasonably expected. Young males were relatively inactive until 3-4 days of age (just before they would normally leave the cocoon) and only a small percentage responded positively to even the more potent extracts. Over the adult life span, however, a general pattern of response intensity emerged, to which males conformed for the majority of female ether extracts assayed. A good example of this pattern is shown in the response of males to an extract of 6-day-old virgin females (fig. 7d). The rate of increase of males responding positively was almost linear for the first 10-11 days, by which time maximum response levels were reached, in this case, about 95%. This initial period will in future be referred to as the "regression phase" of male response. The maximum level reached was maintained for a further 6-7 days giving the "plateau phase". Males of 17-20 days old became senile and their response intensity diminished. Different extracts were found to alter this pattern only quantitatively, the two major phases usually remaining distinct. The

Figure 7a-g Percentage response of male Stegobium over their adult life span to pheromone ether extracts of virgin females of 0, 1, 3, 6, 9, 16 and 23 days old respectively. Each point represents a percentage calculated from at least 60 males.
v = virgin


0v
a
1v
b
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
percentage male response
male age (days)


3v
c
6v
d
percentage male response
male age (days)
0
20
40
60
80
100
2
4
6
8
10
12
14
16
18
20


9v
e
16v
f
percentage male response
male age (days)
0
20
40
60
80
100
2
4
6
8
10
12
14
16
18
20


23v
g
percentage male response
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
male age (days)

slope of the regression phase and magnitude of the plateau phase varied and, in some cases, the point at which the plateau phase was reached was variable, as this and the following sections will illustrate.

Diethyl ether extracts were prepared from virgin females of the following ages: 0, 1, 3, 6, 9, 16 and 23 days. The notation 0v, 1v etc. (v = virgin) was given to these extracts. Each one was bioassayed with virgin males and the percentage male response found for each daily age group, (fig. 7, a-g; Appendix II). The regression phase values (regression b, where y = a-bx) and plateau means were calculated for each extract (table 5).

The 0v and 1v extracts attract very small percentages of males (fig. 7a and b) and the male response pattern described above is barely recognisable. For ease of comparison, however, they have been treated in the same way.

The regression value increases to a maximum with extracts 6v and 9v and gradually decreases thereafter (fig. 8). The plateau phase mean values, on the same figure, almost echo the regressions. In order to provide information which would be of the greatest value to this thesis in its entirety, pairs of female extracts were chosen for statistical comparison (table 6). This was preferred to a comparison of each individual extract to a chosen standard for uniformity (Barratt, 1974). The regressions of these selected pairs were compared by means of the "d" statistic, the significance of which is determined as if it were a normal Student's t-distribution, but with f degrees of freedom (Bailey, 1959). The plateau mean values were compared using a Student's t-distribution. To obtain an overall comparative picture for the entire 1-16 day period of male life span (before senility), a paired "t-test" analysis of the mean differences was computed.

Male response to the 0v/1v and 6v/9v pairs was not significantly different in any respect, suggesting that 0- and 1-day-old females are relatively inactive in terms of pheromone synthesis, although there is undoubtedly some pheromone present at this early stage. A rapid increase in pheromone content

Table 6 Statistical comparisons of virgin male response to pairs of female ether extracts and the response elicited from pairs of male groups by a 6v extract (unless otherwise indicated). See text for details.

relevant section and part of thesis	male response/ diethyl ether extracts compared A	B	Comparison of regression coefficients b_1-b_2	t_f	P	comparison of plateau means $\bar{x}_1-\bar{x}_2$	$t_{(n_1+n_2-2)}$	P	paired mean differences (1-16 days) A-B $\pm$ SE	t_{15}	P
	(females)										
IV:C	0v	1v	+0.47	1.42	NS	-3.31	0.74	NS	-1.44 $\pm$ 1.60	0.90	NS
	1v	3v	-6.39	9.03	<0.001	-43.09	11.45	<0.001	-26.78 $\pm$ 4.60	5.82	<0.001
	3v	6v	-1.85	2.52	0.05-0.02	-28.78	9.46	<0.001	-24.73 $\pm$ 2.38	10.39	<0.001
	6v	9v	-0.13	0.30	NS	+3.80	0.62	NS	+0.08 $\pm$ 0.97	0.08	NS
	9v	16v	+2.44	4.06	<0.01	+6.49	1.11	NS	+16.53 $\pm$ 2.78	5.95	<0.001
	16v	23v	+2.15	3.22	<0.01	+11.08	8.72	<0.001	+9.39 $\pm$ 2.20	4.27	<0.001
IV:D	6m	12m	+3.31	4.97	<0.001	+16.46	3.86	<0.01	+16.07 $\pm$ 2.37	6.78	<0.001
	12m	16m	+2.97	7.23	<0.001	+25.04	5.03	<0.001	+20.24 $\pm$ 2.68	7.55	<0.001
	6v	6m	+0.26	0.43	NS	+4.57	2.53	0.05-0.02	+6.10 $\pm$ 2.62	4.66	<0.001
	16v	16m	+3.97	8.07	<0.001	+35.78	11.58	<0.001	+26.09 $\pm$ 3.54	7.37	<0.001
IV:E	(males)$^{1-6}$										
	v	m$^{1-6}$	+2.00	0.93	NS	+21.69	18.82	<0.001	-	-	-
	v	(ph)$^{1-6}$	+2.05	6.02	NS	+15.22	13.45	<0.001	-	-	-
IV:F	(extract conc'ns) FE										
	1.0	0.1	+0.70	0.68	NS	+1.54	1.46	NS	+7.70 $\pm$ 1.69	4.56	<0.001
	0.1	0.01	+0.34	1.02	NS	+12.55	5.30	<0.001	+8.42 $\pm$ 1.37	6.16	<0.001
	0.01	0.001	+3.29	3.67	<0.01	+23.98	7.40	<0.001	+21.19 $\pm$ 2.81	7.54	<0.001
	0.001	0.0005	+2.02	1.98	0.1-0.05	+39.61	15.75	<0.001	+20.63 $\pm$ 4.74	4.35	<0.001
IV:G	(males) v	v'	+0.21	0.20	NS	+6.18	3.52	<0.01	-0.49 $\pm$ 2.11	0.23	NS
V:B	(strains) ext_1	$ext^w(males_1)$	-0.32	0.77	NS	-9.63	7.40	<0.001	+8.28 $\pm$ 0.90	9.20	<0.001
	$males_1$	$males^w(ext)$	-	-	-	-20.35	10.38	<0.001	+9.64 $\pm$ 2.94	3.28	<0.01
	$males_1$	$males^w(ext^w)$	-	-	-	+27.17	18.28	<0.001	+10.45 $\pm$ 4.84	2.15	0.05
	ext	$ext^w(males^w)$	-	-	-	+16.45	7.88	<0.001	+9.10 $\pm$ 2.32	3.92	<0.01
Appendix V	(females)$_{abd}$ 6v	$6v^{abd}$	-0.27	0.48	NS	-3.03	1.55	NS	+7.46 $\pm$ 1.68	4.44	<0.001

symbols as for table 5 and:

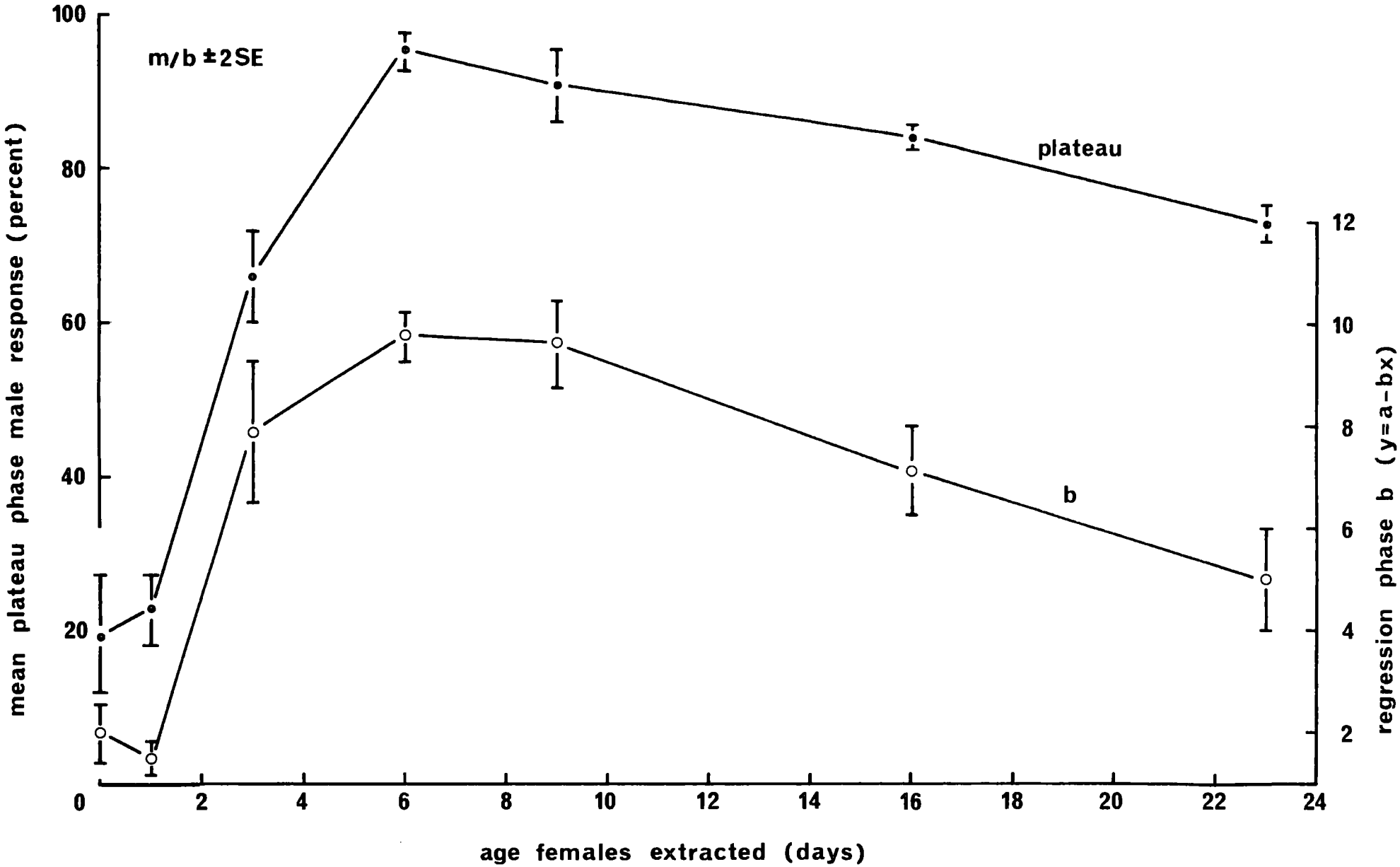
ext = female ether extract

Table 5 Percentage male responses: regressions of male response with respect to age and the mean response at the plateau phase. Complete results for all bioassay tests using diethyl ether extracts (see relevant section for details).

relevant section and part of thesis	females extracted				males tested		(i) regression (y=a+bx) phase male response				(ii) plateau phase male response	
	age (days)	mating status	strain	conc'n (FE)	mating status	strain	b ± SE	a ± SE	r	days (inclusive)	mean ± SE(%)	days (inclusive)
IV:C	0	v	1	0.1	v	1	1.97 ± 0.29	-3.55 ± 1.79	0.924	1-10	19.67 ± 3.80	11-16
	1	v	1	0.1	v	1	1.50 ± 0.16	-0.61 ± 0.97	0.959	1-10	22.98 ± 2.36	11-16
	3	v	1	0.1	v	1	7.89 ± 0.69	-15.10 ± 4.30	0.988	1-10	66.07 ± 2.93	11-16
	6	v	1	0.1	v	1	9.74 ± 0.24	-6.63 ± 1.50	0.998	1-10	94.85 ± 0.82	11-16
	9	v	1	0.1	v	1	9.60 ± 0.42	-3.69 ± 2.58	0.993	1-10	91.05 ± 2.34	11-16
	16	v	1	0.1	v	1	7.16 ± 0.43	-11.10 ± 2.94	0.984	1-11	84.56 ± 0.60	12-16
	23	v	1	0.1	v	1	5.01 ± 0.51	-5.12 ± 3.73	0.953	1-12	73.48 ± 1.12	13-16
IV:D	6	m	1	0.1	v	1	9.47 ± 0.59	-12.20 ± 3.65	0.985	1-10	90.28 ± 1.61	11-16
	12	m	1	0.1	v	1	6.16 ± 0.31	-8.38 ± 2.14	0.988	1-11	73.82 ± 3.95	12-16
	16	m	1	0.1	v	1	3.19 ± 0.27	-7.33 ± 1.96	0.967	1-12	48.78 ± 3.03	13-16
IV:E	6	v	1	0.1	m^{1-6}	1	7.74 ± 2.31	-17.00 ± 21.00	0.889	7-11	73.16 ± 0.81	12-16
	6	v	1	0.1	$v(ph)^{1-6}$	1	7.69 ± 0.77	-26.80 ± 7.85	0.976	7-13	79.63 ± 0.78	14-16
	6	v	1	0.1	$v(ph)^{1-18}$	1	3.14 ± 0.58	-6.63 ± 2.57	0.925	1-7	19.97 ± 0.79	8-16
IV:F	6	v	1	1.0	v	1	10.43 ± 0.98	+1.88 ± 4.96	0.974	1-8	96.39 ± 0.66	9-16
	6	v	1	0.01	v	1	9.39 ± 0.31	-10.70 ± 1.88	0.996	1-10	82.30 ± 2.22	11-16
	6	v	1	0.001	v	1	6.10 ± 0.84	-11.70 ± 5.23	0.931	1-10	58.32 ± 2.36	11-16
	6	v	1	0.0005	v	1	4.08 ± 0.58	-6.35 ± 2.27	0.962	1-6	18.71 ± 0.87	7-16
IV:G	6	v	1	0.1	v^{I}	1	9.53 ± 1.02	-0.99 ± 6.36	0.957	1-10	88.67 ± 1.55	11-16
V:B	6	v	w	0.1	v	1	10.06 ± 0.34	-15.87 ± 2.13	0.995	1-10	85.22 ± 1.01	11-16
	6	v	1	0.1	v	w	-	-	-	-	74.50 ± 1.78	11-16
	6	v	w	0.1	v	w	-	-	-	-	58.05 ± 1.09	11-16
Appendix V	6	$v^{(abd.)}$	1	0.1	v	1	10.01 ± 0.51	-17.27 ± 3.18	0.990	1-10	91.82 ± 1.77	11-16

v = virgin
m = mated
1 = laboratory strain
w = "wild" strain
$(ph)^{1-6}$ = exposed to a 6v pheromone extract for days 1-6 of adult life
$(ph)^{1-18}$ = " " " " " " " " 1-18 " " "
v^{I} = males tested individually
(abd.) = extract of female abdomens only
NS = not statistically significant
FE = female equivalents

Figure 8. Effect of the age of female Stegobium when extracted in ether on the response of males at the regression (b) and plateau phases of their response pattern (about 1-10 and 11-16 days respectively) during arena bioassay experiments.


m/b ±2SE
plateau
b
mean plateau phase male response (percent)
regression phase b (y=a-bx)
age females extracted (days)
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
22
24
12
10
8
6
4
2

is apparent between days 2-3 and the maximum is reached probably between days 6 and 9. Extracts from progressively older females gradually lose their attractive properties in terms of male response, although even in 23-day-old virgin females, a considerable pheromone content is reflected by male response levels reached (plateau mean = 73.5%), despite this being near the mean life span limit for virgin females.

The effect of age upon both male responsiveness and female pheromone content described above is very much in line with results of similar work on other species. Depending upon the adult life span of an insect, males invariably reach their peak response levels in stages over the first part of their adult life. Shorey, Morin and Gaston (1968) found that among eight species of noctuid moths, the time taken to reach 50% of maximum response to the female attractant ranged from 1-3 days. The equivalent period in Stegobium (which is a longer-lived adult) was 5-8 days depending upon the attractant source. Newly emerged mealworms, Tenebrio molitor are unresponsive, but from this time, there is an almost linear daily increase in response up to about 70% on day 5, when a plateau effect is maintained (Happ, 1970). Males of the black carpet beetle, Attagenus megatoma, with a similar life span to Stegobium failed to respond to the pheromone at one day old, but reached a maximum between 6-7 days, very gradually declining thereafter, although even 19 to 20-day-old males reached about 84% response (Burkholder, 1970). Bartell and Shorey (1969a) found that the light brown apple moth, Epiphyas postvittana (Walk) males reached peak response levels as quickly as the second night after emergence and this maximum was maintained until the tenth night.

Levels of pheromone extracted from virgin females throughout their adult life span also compared well with data collected from other species. Females of the cigarette beetle, Lasioderma serricorne, which has a similar life span to Stegobium (Howe, 1957) elicited a response when extracted at only 10-12 h old from less than 20% of males, and over 80% when females were 2 days old. Males reached peak response to extracts from 4 to 5-day-old females.

This was followed by a slight decline, but male response to extracts from females older than 9-10 days was not given (Coffelt and Burkholder, 1972).

Female almond moths, Cadra (= Ephestia) cautella (Walk.) contained detectable amounts of pheromone in pupae which had begun their eclosion process. Levels increased up to a maximum in three hours after eclosion which was maintained until death in virgin females (Kuwahara, Kitamura, Takahashi and Fukami, 1968). Stegobium pupae, or eclosing adults were not extracted but since some, or all Ov females contained some pheromone, synthesis may be in progress at an earlier stage. The female flour moth, Ephestia kühniella has been shown to contain some pheromone before pupal ecdysis (Calvert and Corbet, 1973).

It must be stressed that male response levels to ether extracts of female Stegobium reflect only the pheromone content at the time of extraction and not the rate of synthesis or emission.

D. Effect of mating on female pheromone content

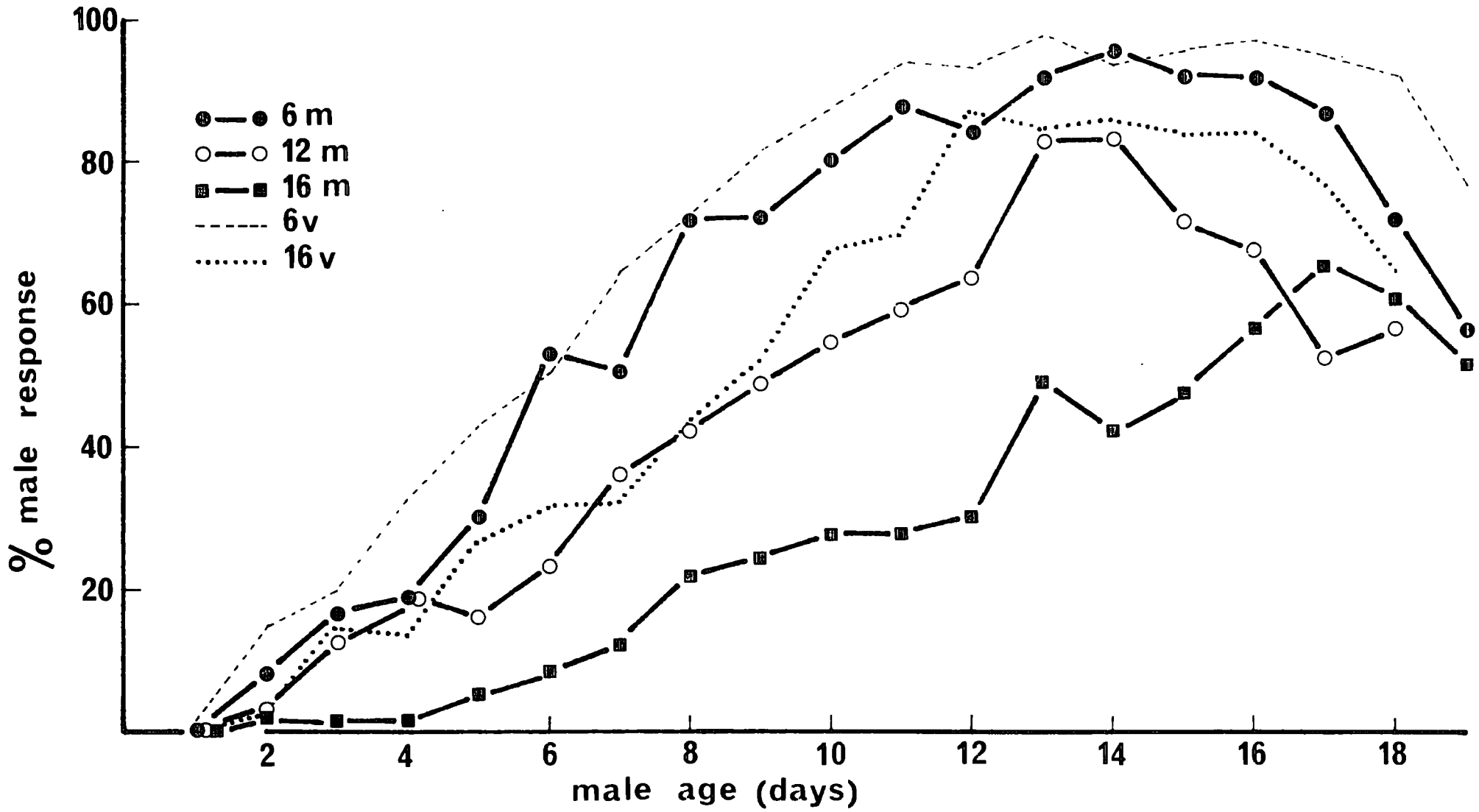
If it is necessary for a female to copulate only once in order to produce a full complement of fertile eggs, it might reasonably be expected that pheromone production would be reduced or terminated following successful mating. Virgin female codling moths, Laspeyresia (Carpocapsa) pomonella (L.) were found to be 42 times more attractive than mated females (Howell and Thorp, 1972). Similarly, Kuwahara et al. (1967) found that pheromone activity in the female almond moth, Cadra (Ephestia)cautella disappears rapidly 4-5 h after the beginning of copulation.

A series of 1-day-old male and female Stegobium were kept together in groups of two of each sex per tube for the first 6 days of their adult life. This period was chosen as females were known to have copulated and begun

ovipositing by this time (Section VII). At the end of day 6, the sexes were separated, using body length as an initial guide and checking females by gently extruding the ovipositor. The 150 or so females were divided into three groups. The first group of 50 were immediately extracted in ether as described previously, at a concentration of 10 females per ml solvent to give pheromone extract 6m (m = mated). The second and third groups were kept for a further 6 and 10 days respectively after separation from males and then extracted in ether to give extracts 12m and 16m respectively. Figure 9 shows the percentage response of virgin males to the three mated female extracts, with the 6v and 16v extract responses included for comparison. The regression and plateau phase values are given in table 5 and statistically compared in table 6. The 6m extract elicited a slightly, but significantly lower response from males although the regressions were not different. Male response to the 12m and 16m extracts progressively diminished and there was a significant decrease in all respects of male response when the 16v and 16m extracts were compared statistically.

Mating appeared to reduce the pheromone titre in female extracts, but by no means removed it completely. This does not exclude the possibility that pheromone emission, as opposed to pheromone content, may be diminished following copulation. A further important consideration is that mated females of 16 days old are nearing the end of their life span and are perhaps more comparable to 23v females in terms of age. Mated female sugar beet wireworms, Limonius californicus (Mannerheim) extracted at various intervals following copulation, showed no fall in pheromone content for at least 4 h, but from 8 h onwards it decreased rapidly (Lilly and McGinnis, 1968). Similarly, the male response of the smaller tea tortrix moth, Adoxophyes fasciata Walsingham to extracts of mated females decreased to 23% when extracted 2 days after mating from 54% the day before mating took place (Nagata, et al. 1972). Female cabbage loopers, Trichoplusia ni (Hübner) on the other hand, showed no reduction in pheromone content among females

Figure 9. Response of male Stegobium over their adult life span to ether extracts of 6, 12 and 16-day-old mated females. For comparative purposes male response to 6 and 16-day-old virgin females is included.
v = virgin
m = mated


100
80
60
40
20
% male response
6 m
12 m
16 m
6v
16 v
2
4
6
8
10
12
14
16
18
male age (days)

which had mated (Shorey and Gaston, 1965b); this was explained in terms of the importance of multiple mating in this species. Brady and Smithwick (1968) found that there was no difference in sex attractant content in virgin or mated Plodia interpunctella (Hübner). Stegobium was not found to require more than one copulation in order to produce a full complement of eggs (although fertility was not assessed) but multiple mating was observed (Section VII). Some noctuid moth species showed a slight decline in pheromone titre after mating, including Rachiplusia ou (Guenee); the yellow-striped army worm, Prodenia ornithogalli (Guenee); and the beet army worm, Spodoptera exigua (Hübner) despite all being polygamous (Shorey, McFarland and Gaston, 1968).

The decreased response displayed by virgin males to mated female extracts may result from a combination of factors. In a monogamous species, mating and ensuing oviposition may automatically inhibit pheromone production, or reduce it temporarily in a polygamous species. In Stegobium, a fairly short-lived, non-feeding adult, the mated female life span is reduced to about 60% compared with virgins (Section VII), so aging obviously operates at an earlier stage (simply because food reserves are channelled into egg production) with its respective effect upon pheromone synthesis.

E. Effect of mating and pre-exposure to the pheromone on male response

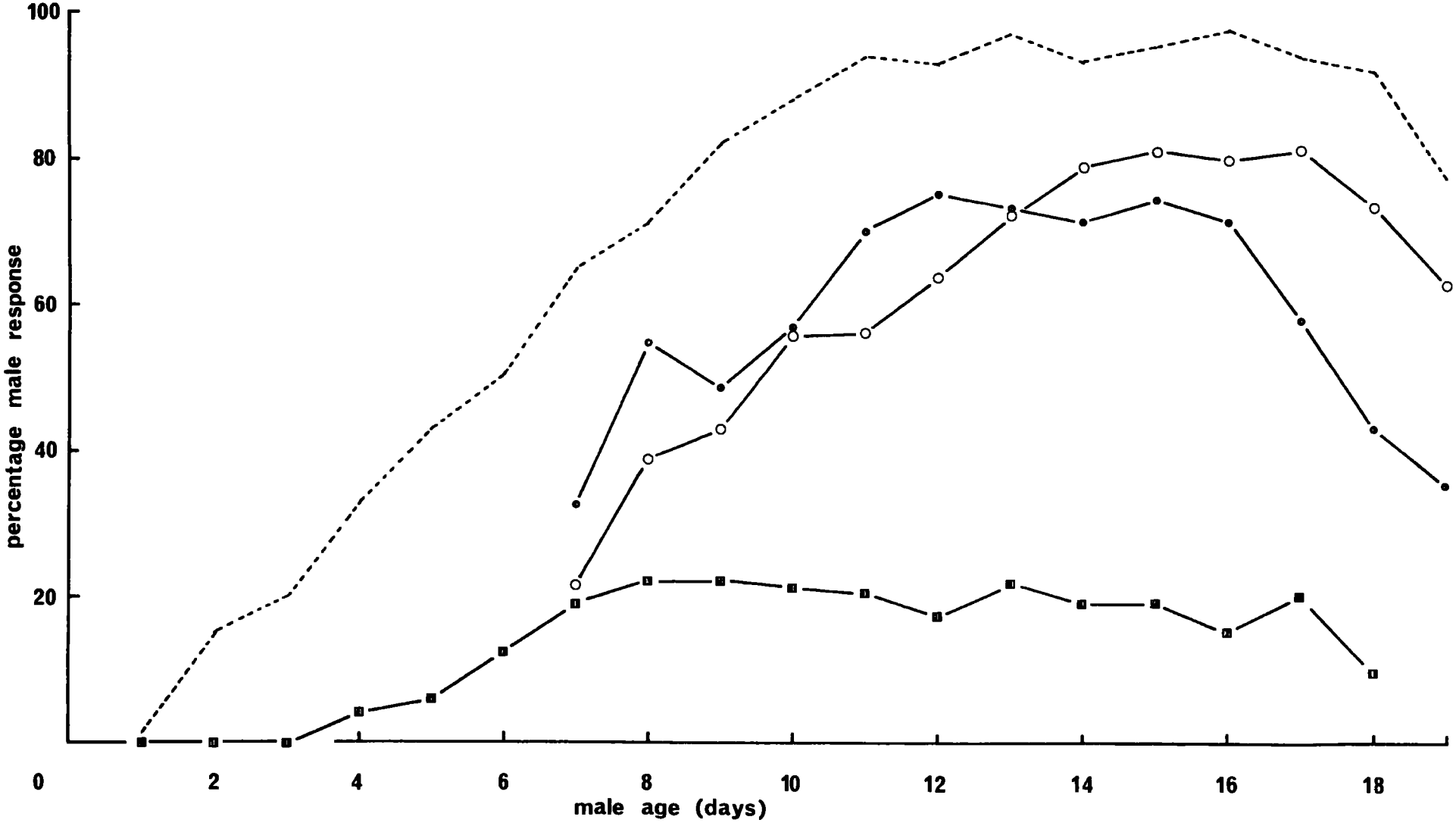
The males from the previous experiment, having experienced 6 days contact and mating with females, were tested for their subsequent response to a 6v extract. A second series of males which remained virgin, were maintained in continual contact with a 6v extract for the first 6 days of their adult life. This was achieved by treating anti-bacterial assay discs with 50 µl of 6v extract, and after allowing the ether to evaporate, one was placed in each tube of 10 experimental males. The discs were replaced with freshly treated ones at 12 h intervals in an attempt to maintain a fairly constant and continuous level of pheromone exposure, comparable with contact with females. At the end of day 6, the males were removed to clean tubes and

the treatment discontinued.

A third series of male beetles was kept in continuous contact with a 6v extract as described above, but the treatment was continued throughout their adult life. This group of males was tested from day 1 onwards for their response to a 6v extract, whereas the former two groups were tested from day 7 onwards. The three male groups were designated δm^{1-6}, δph^{1-6} and δph^{1-18} respectively. Figure 10 shows the response of these treated males to the 6v extract with the normal virgin unexposed male response included for comparison. The mated males, δm^{1-6} responded to a considerably lesser extent than virgin males over the age range tested. The δph^{1-6} displayed a similar reduction in response but the δph^{1-18} hardly reached a response intensity above 20%. The regression and plateau phases were still distinct in the response patterns of these treated males although the plateau phase was reached at a slightly delayed stage. Statistical comparisons were made (table 6) of the response of virgin, unexposed males with both δm^{1-6} and δph^{1-6} although the latter two results were not compared since the original treatments are not comparable. It is evident, however, that exposure to a pheromone extract affected subsequent male response in very much the same way as confinement with females. This suggests that copulation itself probably has little or no effect upon male responsiveness. It is thought that in both cases the diminished response levels are a result of neurophysiological adaptation of the chemoreceptor apparatus (section VIII). Prolonged stimulation "accommodated" the receptors, resulting in an increase in the threshold level for response. In the case of δph^{1-18} only about 20% of males retained a threshold level low enough to enable them to respond positively to the 6v extract during the test periods. When the stimulus was removed (δph^{1-6}) the males recovered to a degree and the threshold level fell. Complete recovery was not attained.

Bartell and Shorey (1969a) found that the response of LBAM (the light brown apple moth), Epiphyas postvittana was reduced from an original 80% to

Figure 10. Response of mated male Stegobium (● ——— ●) and those continuously exposed to a pheromone extract for 6 days (○ —— ○) and 18 days (■ —— ■) to a 6-day-old virgin female extract over the male adult life span. The response of virgin males to a similar pheromone extract is included (broken line).


100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
percentage male response
male age (days)

60% after a 30-s pre-exposure period, and further to 50% after 60 s exposure. Full response was not regained until 24 h later. Stegobium was required to respond only for 10 s in any single test, during which no change in response intensity was observed. Periods of immediate pre-exposure were not attempted but subsequent reduction in response levels would be expected. Male red-banded leaf roller moths, Argyrotaenia velutinana (Walk.) exposed for 10 min before bioassay to 1 µg of RiBLuRe (a synthetic pheromone) failed to respond. If 1 h was allowed between the 10-min exposure period and testing, 7.7% positive response was observed compared with 69.1% in control unexposed males (Bartell and Roelofs, 1973).

F. Effect of extract concentration on male Stegobium response

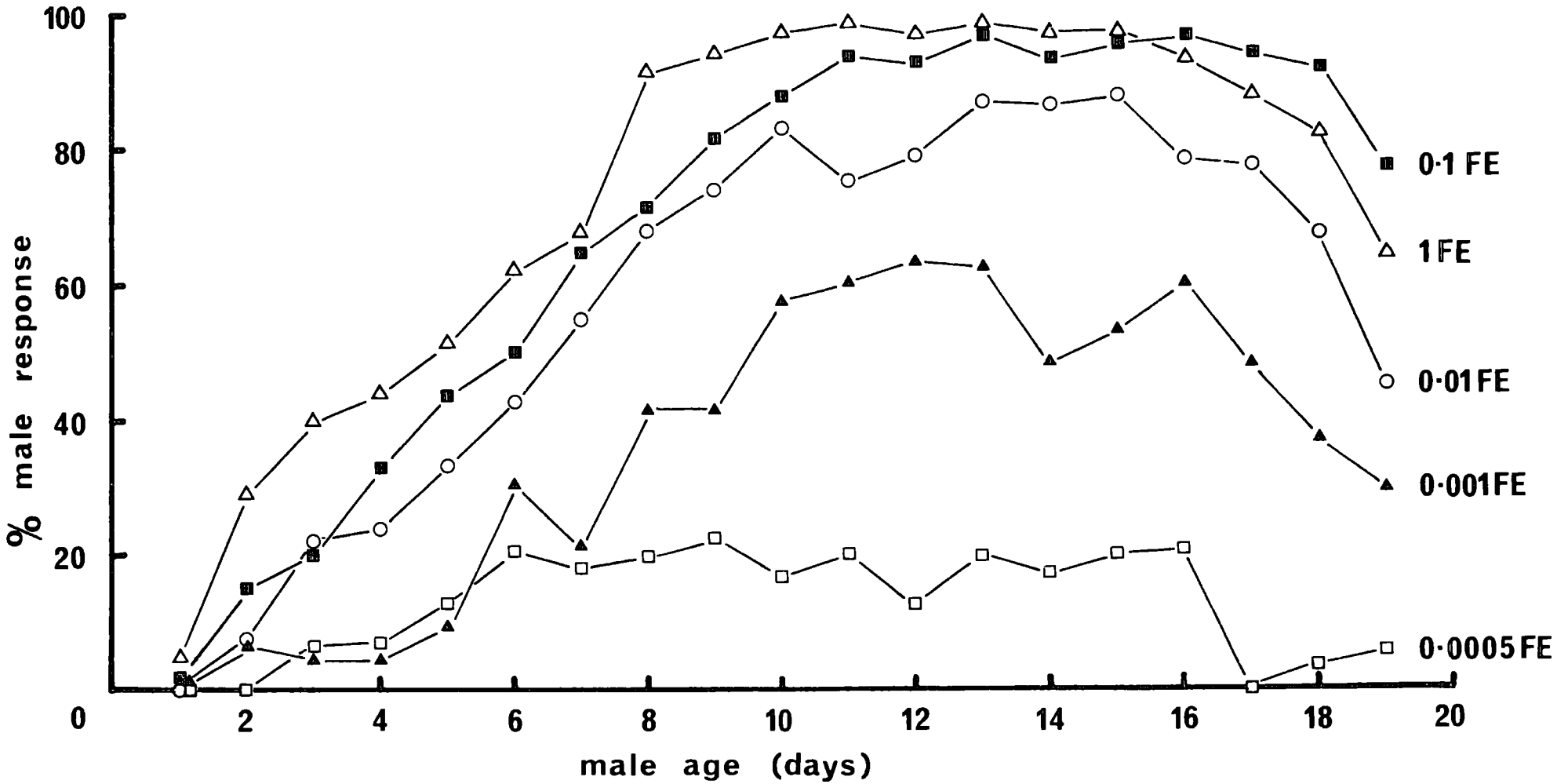
All experiments so far described have involved the use of pheromone extracts at a concentration of 10 females per ml solvent. From the 10 µl extract aliquots used for each test, it can be calculated that 0.1 equivalent part of a female is actually presented to the males on the test disc, or 0.1 FE (Female equivalent).

In order to determine the effect of varying concentrations on male response and the minimum dilution detectable by males, a series of extract concentrations was bioassayed. The 6v extract was selected for dilution since this evoked the strongest response from males. From this extract the following series of concentrations was prepared: 100, 10, 1, 0.1 and 0.05 females per ml ether, or 1, 0.1, 0.01, 0.001 and 0.0005 FE respectively. The most concentrated extract was made up at 100 females per ml but the others were obtained from a serial dilution of the 0.1 FE extract.

Virgin males were used to assay the extracts (fig. 11) and the regressions and plateau means calculated from the male response percentages (table 5).

In response to the 1FE extract, males reached the plateau phase when 9 days old. The slope of the regression was greater, and the plateau phase higher, but not significantly so when compared with the 0.1 FE extract (table 6), but over 1-16 days there was a significant increase in male response of about 8% ($P = <0.0001$) to the more concentrated pheromone extract. The trend with diminishing concentrations was towards a shallower regression and decreased plateau mean, although differences between neighbouring

Figure 11. Effect of the concentration of a 6-day-old virgin female ether extract on male response over their adult life span.
FE = female equivalents (see text).

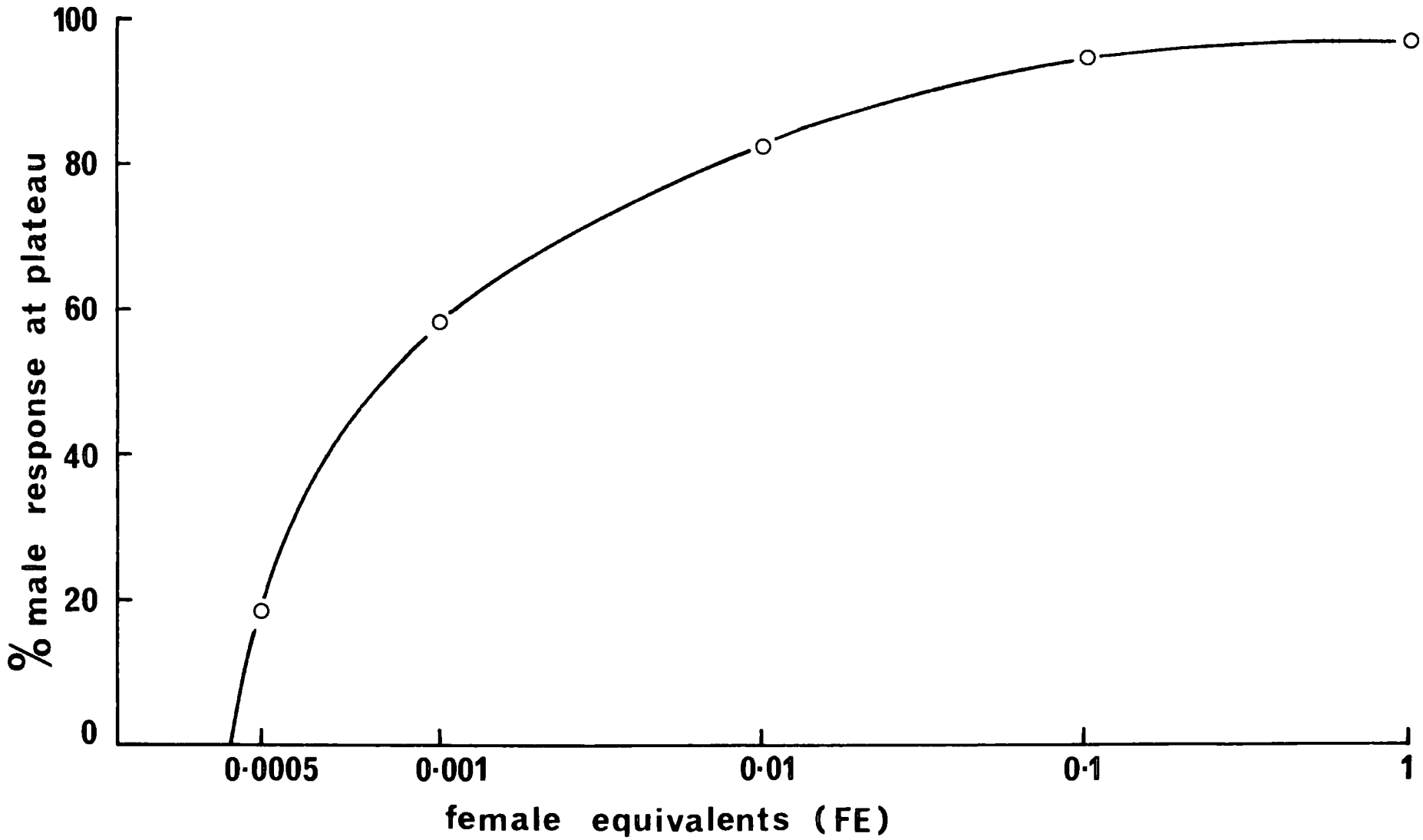

100
80
60
40
20
0
% male response
2
4
6
8
10
12
14
16
18
20
male age (days)
0·1 FE
1 FE
0·01 FE
0·001 FE
0·0005 FE

pairs of regressions were not always significant. However, the intercept values (a) indicate a progressive downward displacement of the regression on the y axis except for the 0.0005 FE extract where the regression is much reduced. This extract, the most dilute, gave a mean plateau phase of male response of only about 19%.

The plateau response means calculated for each dilution were plotted against the concentration (logarithmic scale)producing a curve (fig. 12) which when extrapolated to the 0% response level, intercepted the horizontal axis at about 0.0004 FE. This gives a rough indication of the lower threshold level for male response to diethyl ether extracts of female Stegobium. The curve was also employed, although very approximately, to estimate the equivalent titre of other pheromone extracts. For example, the 23v extract, with a plateau mean of about 73.5% male response contains the equivalent of about 0.006 FE (of a 6v extract) per test aliquot, although it actually contained 0.1 FE of a 23v extract. This represents a reduction in the 23v extract to about 6% of the pheromone titre of the 6v extract. A similar 'calibration' curve was used by Nagata et al. (1972) from a percentage male response/log FE plot. Vick, Drummond and Coffelt (1973) obtained a straight line with this plot over a very small concentration range and showed that a 100-fold concentration change resulted in a 65-70% change in male response.

Dosage/response data of this kind has been treated in several ways by different authors. The original percentage responses have been presented as Figure 12 (August, 1971; Nagata et al, 1972; Vick et al, 1973); percentages converted to a straight line by means of a "probit" transformation (Tschinkel, et al, 1967; Bartell, 1968; Bartell and Shorey, 1969a, b; Burkholder, 1970; Takahashi and Kitamura, 1970; Coffelt and Burkholder, 1970) or a "logit" transformation after Berkson (1953) (Happ and Wheeler, 1969). The fit of these straight line transformations appears to be dependent upon the range of concentrations tested. Burkholder (1970) obtained a good fit of all points over a range of 0.0016 - 0.001 FE, whereas Bartell (1968) assaying a

Figure 12. Effect of pheromone concentration in FE (female equivalents - log scale) on male response at the plateau phase (about 11-16 days). Curve fitted by eye and extrapolated to the x axis.


100
80
60
40
20
0
% male response at plateau
0·0005
0·001
0·01
0·1
1
female equivalents (FE)

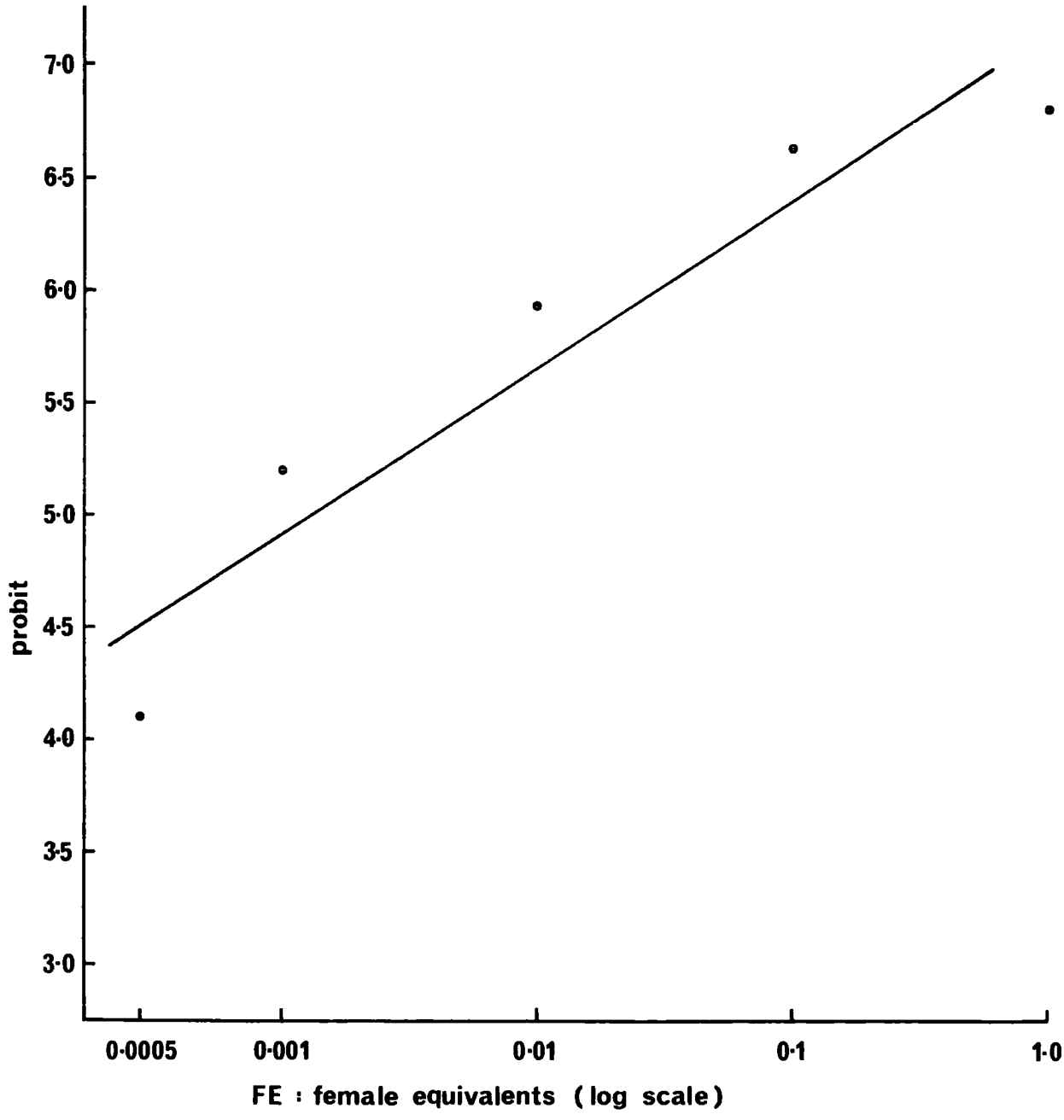
range of 0.01 - 0.00001 FE did not, the highest and lowest concentration values falling below the line. A probit transformation of results obtained for Stegobium (fig. 13) resembles that of the latter author, with the three middle points falling very accurately on a straight line, but the maximum and minimum values falling beneath it. This could have resulted from inaccuracy in the assay technique (the 0.0005 FE concentration was repeated using freshly prepared extract and no significant difference found between the plateau means), or failure of this type of response assay to reach 100%. Probit transformations were designed specifically for toxicity/dosage response experiments, which bear many resemblances to the type of attractant bioassay used here. However, whereas 100% mortality is readily achieved with a drug or toxic substance, 100% positive response to an attractant is not, since a small percentage of individuals are invariably defective, or inferior in some way (see part G).

Threshold response levels recorded for other insect species are not directly comparable since extraction methods, bioassay techniques and response indicators vary widely. Nevertheless, it can clearly be seen that the range of concentrations necessary to initiate a response from males (or females where appropriate) is very wide. An estimated lower response threshold of about 0.0004 FE in Stegobium may be compared with values as low as < 0.00001 FE for Lasioderma serricorne (Coffelt and Burkholder, 1972), 0.000001FE in Adoxophyes fasciata (Nagata et al, 1972) and 0.00001 FE which elicited a 2% response from the male LBAM, Epiphyas postvittana (Bartell and Shorey, 1969a). At the other extreme, the response threshold for the dermestid, Trogoderma granarium to the assembling scent is < 0.4 FE (Yinon and Shulov, 1967b) and about 0.8 FE was extrapolated for Tenebrio molitor (August, 1971).

The threshold response levels for sex attractants are obviously dependent upon the criterion used to recognise a positive response. In LBAM mentioned above, a sequence of behavioural steps consisting of antennal elevation, activation orientation and copulatory movements was distinguished. These

Figure 13. Probit transformation of the curve shown in figure 12.

% response	probit
18.71	4.11
58.32	5.21
82.30	5.93
94.85	6.63
96.39	6.80


7·0
6·5
6·0
5·5
5·0
4·5
4·0
3·5
3·0
probit
0·0005
0·001
0·01
0·1
1·0
FE : female equivalents (log scale)

steps in the process of locating and mating with a female required respectively higher pheromone concentrations in order to promote each successive stage. This is probably true of many species, but individual steps in the behavioural sequence are often more difficult to differentiate.

G. Individual male bioassay of a pheromone extract

Several questions arose from the results presented in the foregoing parts of this section, where the percentage response of 60 or more males was calculated for each daily age group. For instance, in a situation where 95% of males respond positively to a certain extract, why does the remaining 5% fail to do so? As mentioned in part F above, it could be that the same individuals consistently fail to respond because they are inferior or in some way deformed and destined for a shorter than average life span. Alternatively, the residual 5% may, on each day, comprise different males which are for some reason temporarily unresponsive or whose response threshold is slightly raised.

The spread of the regression phase over the first 10 days or so before maximum response is reached possibly presents an even greater anomaly. If males are mature between 4-5 days of age (when they leave the cocoon) one would expect 0% response on day 3, 50% on day 4 and maximum response on day 5. Quite obviously, individual variation will lengthen this period, but 10 days seems excessive.

In an attempt to provide explanations for some of these problems, the daily response of individual male beetles was monitored over the whole of their adult life span.

A group of 50 1-day-old males were placed in separate numbered tubes and individually tested with a 6v pheromone extract each day until death. Males found dead on days 2-4 of the experimental period were replaced with males of the same age. Tests with single males were carried out exactly as with groups of males (part B of this section), allowing 3 min for a response with immediate removal following a positive response. The experiment was repeated three months later with a second set of 50 males from a different culture and generation. The results obtained from the two groups were combined

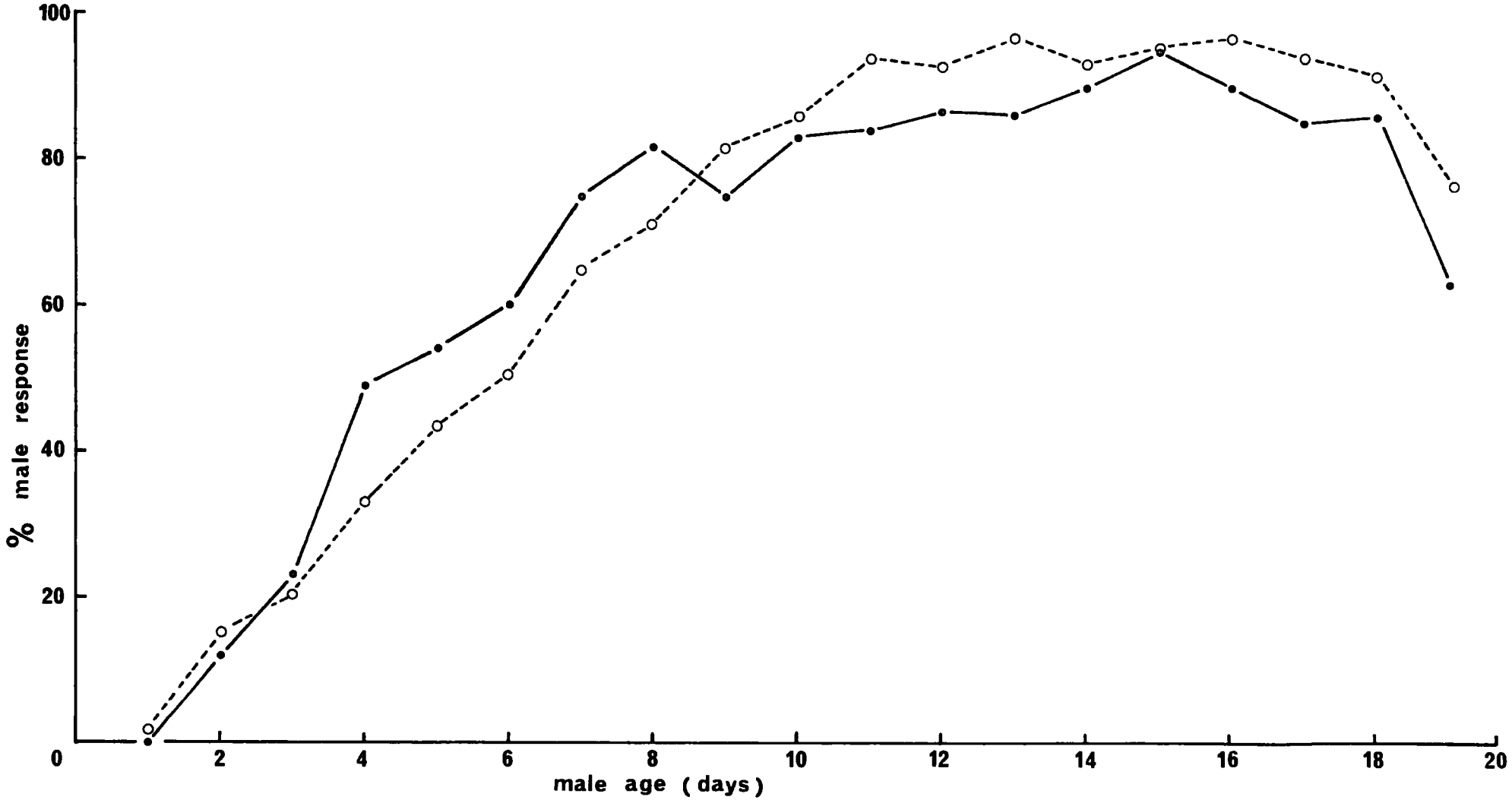
since there were no obvious differences between them.

For each male, a complete log of its daily response was recorded, including details of death or a moribund state (Appendix III). The daily percentage response (Appendix II), regression and plateau phase values were calculated for the whole group (table 5) and compared with previous results obtained for the 6v extract where males were tested in groups (fig. 14, table 6). Only the plateau means were significantly different; the males tested individually reaching 88.7% which is about 6.2% lower than the group tested males ($P = <0.01$). This difference may be a result of testing every day, which was previously avoided when possible. If this is the case, it is the plateau phase during which the majority of males are responding, which would be most affected. If testing males in groups produced higher response levels as a result of the stimulation of otherwise non-responders, or of an aggregation tendency, this would be more consistent throughout the regression and plateau phases than was actually found.

Occasions on which males failed to respond, which was the main consideration of the present part of this section, were expressed as a percentage of the number of days for which they lived (excluding a moribund state) and thus had the opportunity to respond (table 7). It is clear that males with a shorter life span (4-10 days) fail to respond for a greater proportion of their life than longer-lived males. This might be because shorter-lived individuals, which formed the minority of the whole group, were in some way inferior and thus less likely to be responsive to the sex pheromone. Alternatively, all males might pass through a period of low responsiveness at the beginning of their adult life. Most individuals tended not to respond over the initial 2-4 days, and this clearly constituted a greater proportion of the life span of shorter, than longer-lived males.

In order to examine these problems, the males with a 19-day life span (the largest single group) were analysed over the early part of their adult life to compare their response failure with that of short-lived males as

Figure 14. Response of male Stegobium over their adult life span to a 6-day-old virgin female extract when tested normally (in groups of 4-5 males) (o----o) and individually (•——•).


100
80
60
40
20
0
% male response
2
4
6
8
10
12
14
16
18
20
male age (days)

follows:

19-day group							short-lived group						
% negative	response	over	1-5	days	versus	males	with	a	5-day	life	span		
"	"	"	"	1-6	"	"	"	"	"	6	"	"	"
"	"	"	"	1-7	"	"	"	"	"	7	"	"	"
"	"	"	"	1-8	"	"	"	"	"	8	"	"	"
"	"	"	"	1-9	"	"	"	"	"	9	"	"	"

These percentages are shown in table 7 opposite their corresponding group of short-lived males. It is apparent that males which eventually lived for 19 days failed to respond on fewer occasions than the shorter-lived groups of individuals. Since the sample sizes of the latter were small, life span classes were formed (table 8) to provide reasonable numbers in each group, whilst excluding as few individuals as possible (in this case only 3). For each of the age classes, a mean value of response failure was calculated (table 8) as was a mean value for the first day when a positive response was recorded. There was an apparent positive relationship between life span and the day of first positive response, although none of the latter values was significantly different (table 8). However, using these values as an "allowance" of a period of days when a negative response is expected from males, a percentage of the life span when males would be expected not to respond to the pheromone can be calculated (table 8). This was taken from the middle age group of each life span class. The difference between the observed and expected response failure therefore indicates the response behaviour of males over the remainder of their adult life, after the first positive response has been recorded. This difference was highly significant for the 5 to 9-day life span class which suggests that short-lived males fail to respond consistently, even when they have previously given a positive response. Males which never responded (of which there were four, all in the 5 to 9-day life span class) were omitted from these calculations since

Table 7 Failure of individual males to respond to a 6v extract, expressed as a percentage of their adult life span. The percentage of occasions of negative response of the 27 males living 19 days in all, up to 5, 6, 7, 8, and 9 days is also included (see text).

life span (days)	n	mean % life span when -ve response given	mean % of 5-9 day periods when -ve response given by males living 19 days
1			
2			
3			
4	2	62.5	
5	2	100	68.4
6	6	66.7	61.5
7	2	78.6	56.6
8	3	70.8	50.9
9	1	77.8	47.3
10	2	65.0	
11			
12	1	50.0	
13	1	35.7	
14			
15	4	18.3	
16	7	27.8	
17	12	26.5	
18	12	37.0	
19	27	31.1	
20	5	38.0	
21	7	27.9	
22	3	17.9	
23	1	34.8	
24	1	50.0	
25			
26	1	100	

Table 8. Mean percent negative response of males (from table 7) grouped into life span classes. An "allowance" was calculated from the mean day when a positive response was first given for each life span class to give an expected percentage of the life span when males would fail to respond to a 6v extract. Males which never responded were omitted from these calculations (see text).

life span class (days)	n	mean % life span when -ve response given ± SE	mean day when first +ve response given (range ± 2 SE)	% life span when -ve response expected*	observed -expected (%)	t_{n-1}	P
5-9	10	64.7 ± 3.0	3.4 (2.5-4.3)	48.5	+16.2	5.4	<0.001
$5\text{-}9^{19}$	27	56.9 ± 3.8	4.6 (3.9-5.3)	65.0	-8.1	2.1	0.05-0.02
10-14	4	53.9 ± 7.3	4.0 (2.6-5.4)	33.3	+20.6	2.8	NS
15-19	62	30.0 ± 1.7	4.4 (3.9-4.9)	25.9	+4.1	2.4	0.02-0.01
20-24	17	30.2 ± 4.4	4.9 (3.7-6.1)	22.3	+7.9	1.8	NS

* calculated for middle of age class, ie. 7, 12, 17 and 22 days respectively

$5\text{-}9^{19}$: age class over which males with 19-day life span were calculated

the expected response failure was dependent upon the value of the first day of positive response. Males living 19 days responded negatively on fewer occasions than would be expected over the 5-9 day period, although this difference was not highly significant. The 15 to 19-day life span class were observed to respond negatively only about 4% more than would be expected. This suggests that after the initial 4.4 days, most males respond positively to the sex pheromone for most of the time. Males living 20-24 days were also "good responders".

The daily response records were further analysed to determine whether the way a male responded on day n influenced its subsequent response on day (n + 1). They were also examined in order to detect any increased tendency towards negative response on the day prior to the death of an individual. The first four days were ignored and during the remaining period it was calculated that a positive response was repeated on about 87% of occasions (table 9) but a negative response was only repeated on about 51% of occasions. Once

Table 9 Analysis of consecutive-day responses of individually tested males and their response to a 6v extract on the day prior to death

response	response on day (n+1) after +ve on day n (%)	response on day (n+1) after -ve on day n (%)	response on day prior to death (%)
+ve	86.67	49.17	61.0
-ve	13.33	50.83	39.0

a male had responded positively, it was more likely than not, to continue doing so on future occasions. On the day preceding death, however, 39% of males failed to respond, despite their previous record.

Individual male testing enabled possible explanations for anomalies arising from the group bioassay technique to be put forward. The regression phase, prior to the maximum response level, is lengthened because:
(a) the mean day of first positive response of all males was 4.43 ± 0.22 but

the range was 2-14 days.

(b) mortality of males up to about 10 days of age involved individuals which failed to respond to the attractant source on a greater proportion of occasions than longer-lived males. A small percentage of short-lived individuals would be expected in any insect population for a variety of reasons but handling during experimentation may have damaged some beetles and increased this proportion.

(c) Some individuals never respond (5% in this set of males). In the main, these are short-lived, but this group does include a small percentage (1%) of very long-lived males. This individual was checked after death and found not to be a wrongly-sexed female, so it can only be presumed that a non-lethal, morphological or genetic deficiency was responsible. This beetle, being the longest-lived male of the entire group suggests the possibility of a reduced metabolic rate which might in turn have influenced its ability to respond to the sex pheromone.

The plateau phase does not reach 100% response partly as a result of (c) above and because:

(d) senility often resulted in response failure prior to death even in "good responders". Most males died at 19 days of age but large numbers died between days 16 and 22.

(e) adaptation may occur from continual testing resulting, perhaps in failure to respond on some occasions.

H. Discussion

Extraction of live females in diethyl ether at a known concentration is a convenient and fairly precise method of obtaining a source of sex pheromone for bioassay. The purity of the extract is, of course, unknown and compounds other than the sex attractant are almost certainly ether-soluble and therefore extracted along with the pheromone. Furthermore, it is not known what proportion of the total pheromone present in a female is removed by the solvent. Levinson and Bar Ilan (1967) calculated that 0.4% of the body weight of a

female Trogoderma granarium was removed by surface extraction (ie. the technique used for Stegobium), whereas 4.9% was removed from a total body extract which involved macerating females and extraction in a soxhlet assembly for 88 h. Body extracts were found by these workers to contain 5-10 times more attractant material than surface extracts. The findings of Levinson and Bar Ilan (1967) would probably apply equally to Stegobium.

The effect of age upon the pheromone content of females, and male response was shown to adhere to essentially the same pattern as that obtained when live females were assayed (section III). The latter technique was far less sensitive than ether extract bioassay since males were required to respond for only 10 s to an extract, but 30 min in the case of live female assay tests. Moreover, since pheromone content does not necessarily reflect emission by the female, differences in male response might be expected in these two experimental situations.

Individual male testing, although ideal for detailed analysis of male response was too lengthy for general use. However, the pattern of male response displayed throughout the tests was better understood following the sample of individual test sequences.

V SEX PHEROMONE PRODUCTION AND MALE RESPONSE OF A "WILD" STRAIN OF STEGOBIUM IN COMPARISON WITH THE LABORATORY STRAIN

A. Introduction and Methods

Study of the sex pheromone of an insect pest species has potential significance in the context of devising new biological control measures as has been amply demonstrated by Hardee, Lindig and Davich (1971) with 'grandlure' for the boll weevil, Anthonomus grandis, RiBLuRe for the red-banded leafroller, Argyrotaenia velutinana (Walk.) (Roelofs, Glass, Arn and Comeau, 1970) and utilisation of several other synthetic sex pheromones.

It cannot be assumed that results obtained from a laboratory stock of insects, which has been in culture for many years, are representative of pheromone communication in wild populations. Laboratory insects are subjected to altered selective pressures, some being removed and others inadvertently introduced. For example, Fletcher, Claborn, Turner and Lopez (1968) reared the screw-worm fly Cochliomyia hominivorax (Coquerel) in darkness in order to reduce mortality of insects at high density. It was later shown that, under these conditions, sex recognition in this species changed from partial reliance upon visual stimuli to an increased dependence upon chemical means of communication.

A lesser degree of dependence upon the sex pheromone of insects reared in dense population conditions might be expected since chance meetings between the sexes are frequent. Work in this field has produced varied results. Sower, Hagstrum and Long (1973) found no significant difference between either female pheromone content or male response of "wild" and laboratory almond moths, (Cadra (Ephestia) cautella). However, the extractable pheromone content of cultured summerfruit tortrix moths, Adoxophyes orana Fisher von Röslerstamm was 10-100 times lower than that extracted from a "wild" strain (Minks, 1971) and it was suggested that the sex pheromone had lost its selective advantage. Conversely, in the Californian red scale (Homoptera), Aonidiella aurantii (Maskell), laboratory rearing was thought to have influenced the attractiveness of laboratory females, since

laboratory males found the latter more attractive than did "wild" strain males (Tashiro, Beavers and Moreno, 1969).

A "wild" strain of Stegobium was brought into the laboratory and reared as described in section II. In order to compare pheromone production and male response of this population, which was kept in laboratory culture for only about five generations, with the laboratory culture which has been reared at high densities for thousands of generations, a pheromone extract was prepared from 6-day old "wild" strain females and assayed in the same way as previously described for laboratory females.

B. Results

The response of laboratory males to a "wild" female extract was slightly less than to the laboratory female extract (fig. 15; table 5). The reduction in response was about 8% ($P = <0.001$) over the male life span (table 6). However, the characteristic pattern of male response, with clear regression and plateau phases, was maintained.

"Wild" males deviated from the above response pattern (fig. 16) in that the regression was very steep over the first 3 days of male age (reaching almost 40% male response) but was then interrupted by a small plateau encompassing the following 3 days. A further increase in male response between days 6-10 brought male response levels up to a typical plateau phase.

The response of "wild" males to "wild" female extract was similarly high over the first 3 days, reaching almost 40% positive response on day 3, which is three times that attained by laboratory males of the same age. However, the plateau phase mean (58%) was far below that which the same extract elicited from laboratory males (85%) as shown in figure 17.

Since "wild" strain males consistently deviated from the almost linear regression phase displayed by laboratory males under most circumstances, a value for the regression has not been calculated. The plateau phase and paired mean differences over 1-16 days of male age has been calculated (table 6).

Figure 15. Response of laboratory strain male Stegobium over their adult life span to a 6-day-old virgin female extract of both the "wild" (• ——— •) and laboratory strains (------).

Figure 16. Response of "wild" and laboratory strain male Stegobium over their adult life span when tested with a pheromone extract of 6-day-old laboratory strain virgin females.

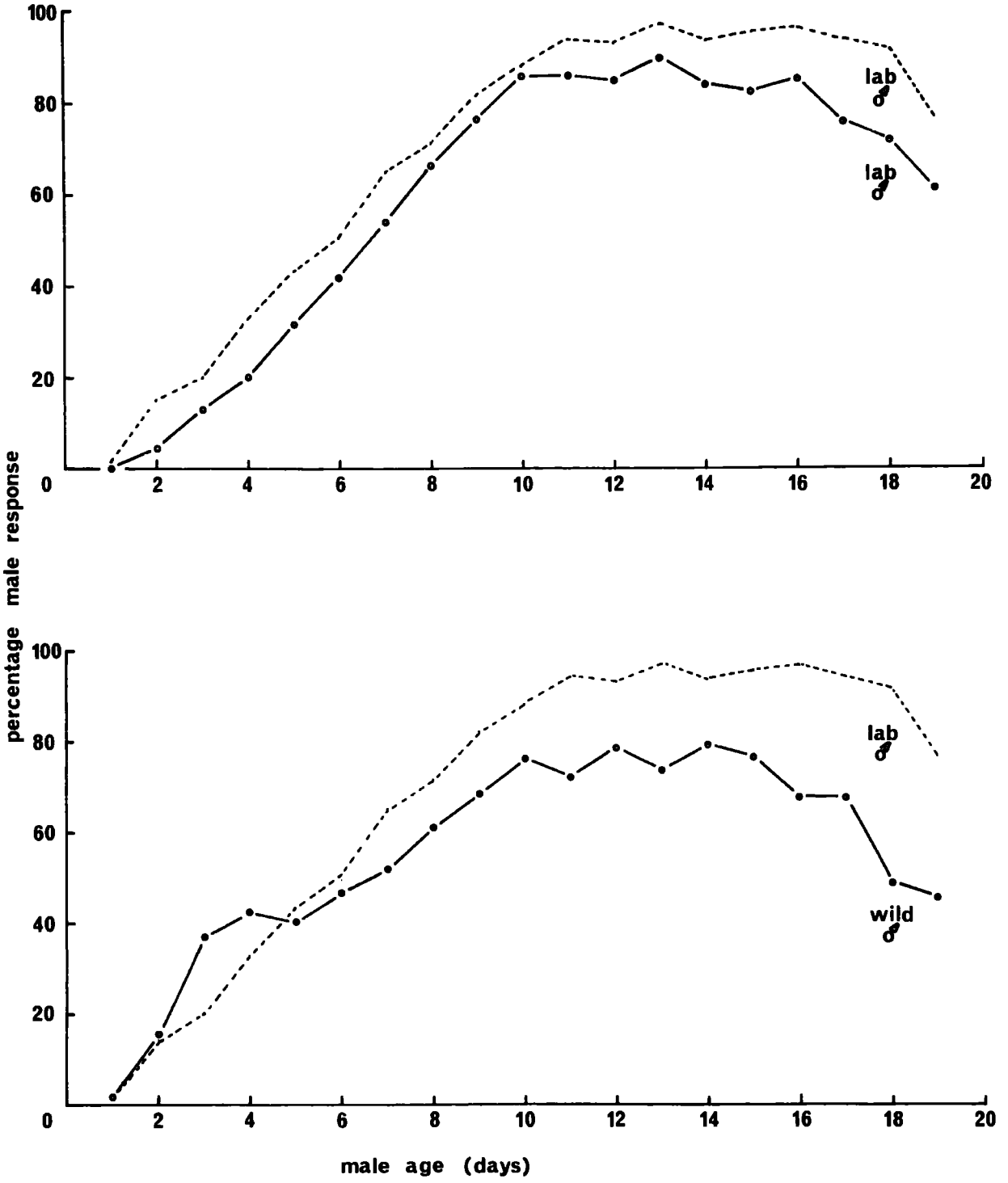
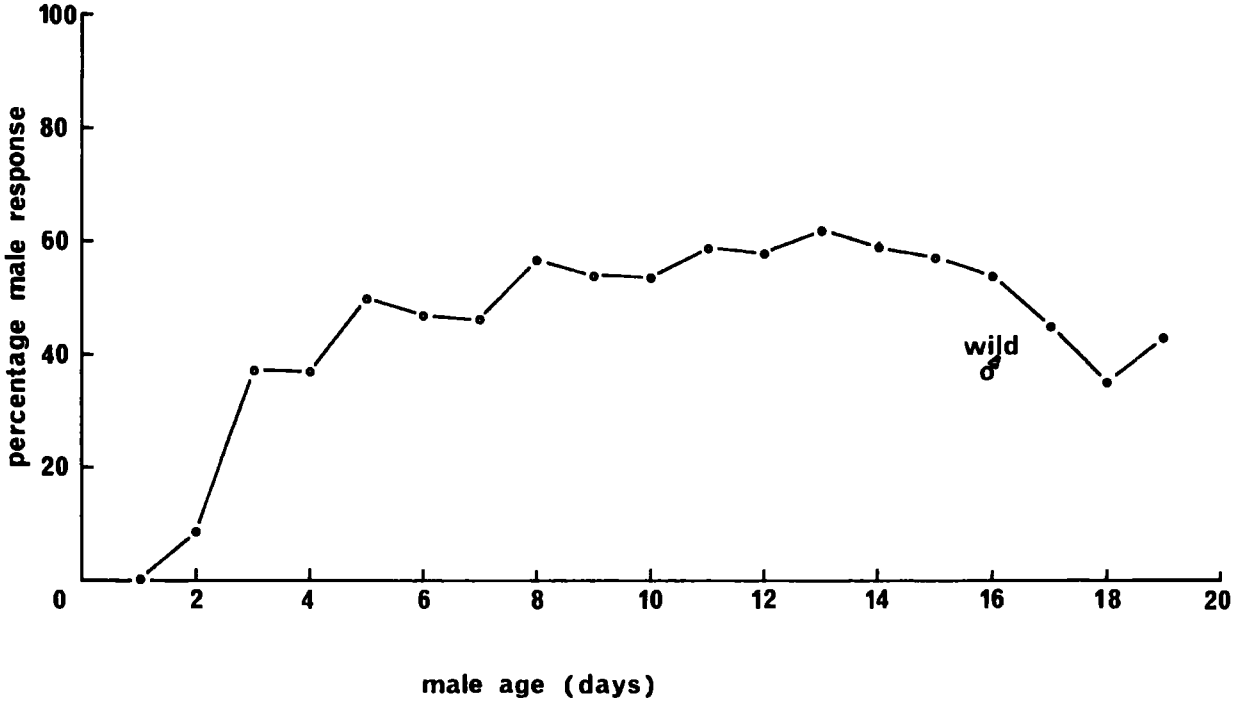

percentage male response
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
lab ♂
lab ♂
lab ♂
wild ♂
male age (days)

Figure 17. Response of "wild" strain male Stegobium over their adult life span to a pheromone extract of 6-day-old "wild" strain virgin females.


100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
percentage male response
male age (days)
wild
♂

C. Discussion

Laboratory males display a slight reduction in response to a "wild" female extract as compared with that prepared from laboratory females. This suggests that "wild" females produce either reduced quantities of the attractant, or perhaps a slightly different chemical configuration of the compound reducing its attractive properties to laboratory males. However, since the response of "wild" males is greater to the laboratory than "wild" female extract, the possibility of a quantitative difference in pheromone production between the two strains appears more probable.

In the situation where both strains of males are tested against the laboratory female extract (fig. 16) the results imply a trend towards increased sensitivity in laboratory males except over the initial 3-4 days when "wild" males display a very rapid rate of increase in response which appears to be characteristic of this strain. The same phenomenon may be observed in their response to a "wild" female extract (fig. 17). Stegobium males are reproductively mature at 3-4 days of age but fertility declines rapidly during their short adult life span (Azab, 1943), and early breeding is an obvious selective advantage. In laboratory insects, where hazards such as predation and disease are largely removed, the relative advantage of early breeding is reduced because aging males with lower mobility and fertility are still able to fertilise females.

Assuming that "wild" males spend approximately the same period in the cocoon following adult eclosion as laboratory males, the former have very nearly reached their maximum response potential at emergence on day 4-5 as compared with a period of 9-10 days in laboratory males. This again stresses the changes in selective pressures which might be operative when laboratory rearing of insects is undertaken, and the danger of relating data directly to a "wild" population.

Rather than the sex pheromone communication system becoming superfluous to successful breeding in laboratory cultures, it must be assumed from these

results that both pheromone production by females and male responsiveness has become increasingly important to the species. Females producing a more potent attractant might have competed more successfully for males since they could be located above the general pheromone "background" which must be continually present. Similarly, males with a more acute chemosensory receptor apparatus, able to orientate towards a single mature female without confusion from the ambient pheromone levels, are likely to fertilise more females. Responsiveness of individuals is variable as concluded from part G, section IV, and heritable as found in the house fly, Musca domestica (L.) (Cowan and Rogoff, 1968) and could thus constitute a selective character.

It cannot be assumed that any "wild" population brought into the laboratory would give the same result as described above. "Wild" populations have been shown to fluctuate in female sex attractiveness for no apparent reason. Bobb (1964) described this phenomenon in Neodiprion pratti pratti (Dyar) where a rapid population decline following a major outbreak was attributed to a great reduction in female attractiveness.

A pest species such as Stegobium is largely confined to interior, heated premises, at least in temperate climates (such as the bakery from which the "wild" strain originated) and might be subject to forming small isolated demes. Restricted gene flow might result in localised races, particularly if conditions favour rapid population turnover.

The results would suggest that in a control scheme, a sex pheromone bait from laboratory females would be more effective in attracting "wild" males than would pheromone from their own strain. Although field trials would be necessary to confirm this assumption, it is thought reasonable to consider laboratory insects as representative of "wild" beetles in that the sex pheromone communication system has become slightly exaggerated following many generations of laboratory rearing.

VI COLLECTION OF PHEROMONE EMITTED BY FEMALE *STEGOBIUM* BY ADSORPTION FROM AN AIR STREAM

A. Introduction

Solvent extraction of *Stegobium* females and bioassay of the extracts with males provided a good comparative indication of extractable pheromone content of beetles of different ages (section IV). It was considered important to complement this study with information on the rates of emission of volatile pheromone by females over their adult life span, with particular emphasis on pheromone emission during the first few days following adult eclosion while the female is still in the cocoon. Bioassay of 0-, 1- and 3-day-old females (section IV) indicated the presence of pheromone in females at this stage but clearly could not reflect emission rates. Shorey *et al* (1968) working with several species of noctuid moths suggested that high pheromone concentration in the female does not necessarily imply that the system is ready for use.

In order to measure pheromone emission rates in females, the following technique was developed. An air stream of known speed was passed over a standard number of live females confined in a small vessel, and then through a filter paper disc which adsorbed material from the volatile effluent. This method will henceforth be called "air extraction".

The principle of adsorbing volatile pheromone from an air stream has been used successfully by Burkholder and Dicke (1966) for *Attagenus megatoma*, the carpet beetle, and *Trogoderma granarium*. However, most techniques designed for volatilisation of pheromones have incorporated either a cold trap to condense the volatile material, such as dry-ice (Vité, Gara and Kliefoth, 1963; Yamamoto, 1963; Findlay and MacDonald, 1966; Dahm, Finn and Röller, 1970); a dry-ice/alcohol mixture (Tunstall, 1965; Fletcher, O'Grady, Claborn and Graham, 1966); a dry-ice/methanol mixture (Happ and Wheeler, 1969); liquid oxygen (Kettlewell, 1961) or a fat or other high adsorption surface such as retroperitoneal tallow and mesenteric lard (Röller, Biemann, Bjerke,

Norgard and McShan, 1968); carbowax-coated silica gel (Tumlinson et al, 1968); activated charcoal (Keller et al, 1964).

B. Methods

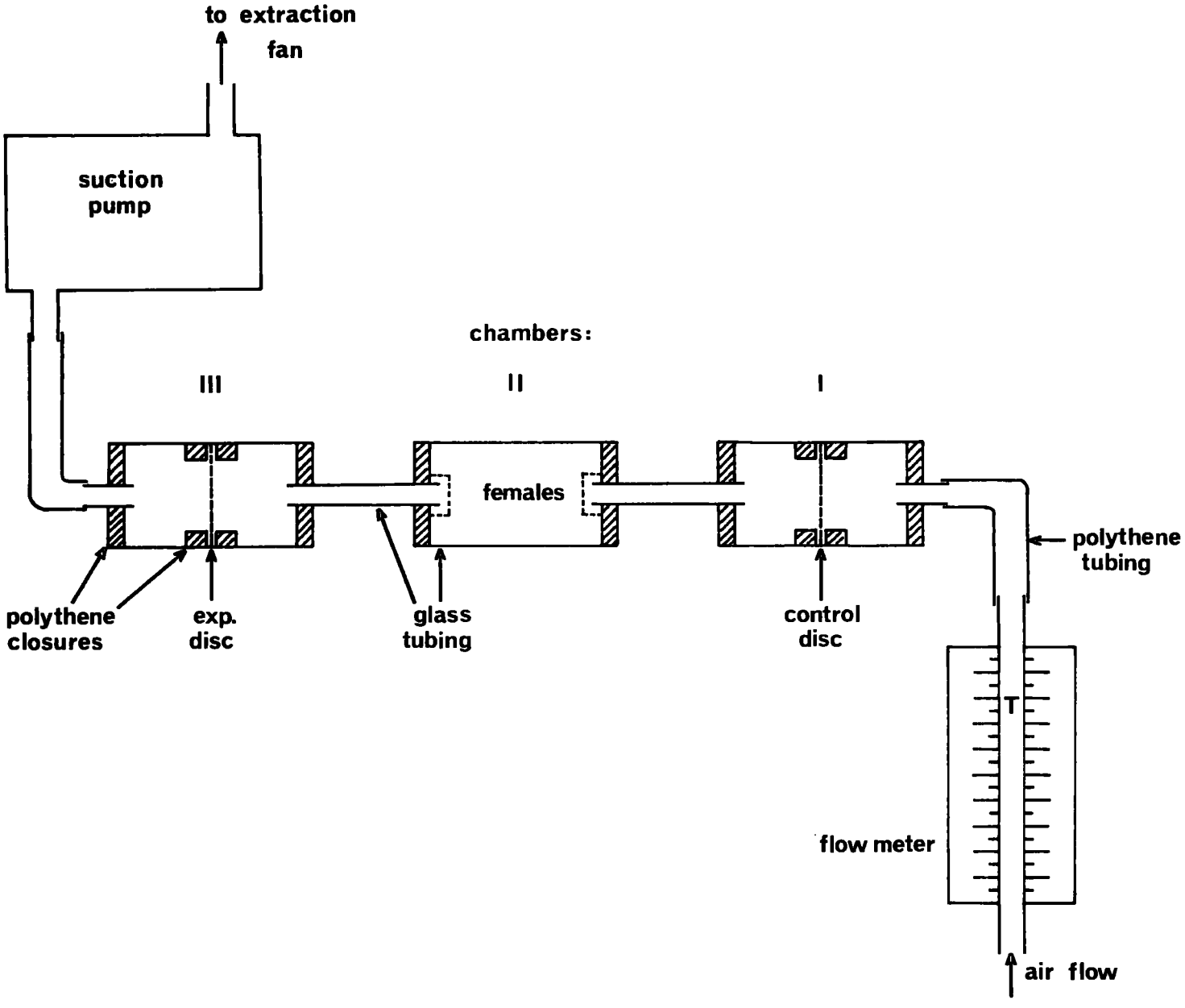
The apparatus assembled for air extraction of live female Stegobium comprised a suction pump which drew air through a flowmeter and three consecutive chambers (I-III) as shown in figure 18. The chambers were 4 cm lengths of 17 mm diameter glass tubing (approximate volume, 10.5 cm^3), which were connected in sequence with 5 cm lengths of 5 mm diameter tubing. Chambers I and III each contained a 17 mm diameter circular disc cut from filter paper (Whatman grade 1) which was held in position across the aperture of the tube, half way along its length by two polythene closures, from which the solid top had been largely removed and which fitted tightly inside the tube, one on either side of the disc.

The central chamber (II) contained the experimental females which were confined by a single thickness of medical wiper tissue held over the open ends of the connecting tubing.

An airflow of 1 ± 0.1 l/min was passed over 25 females for one hour. Preliminary experiments revealed that these levels yield pheromone impregnated discs which elicit a response of at least 50% from 10-day-old males.

Collection of volatile material from females as described above was not carried out in the rearing insectary, but in a small room in another part of the building. This was maintained at 24°C and approximately 43% r.h. and housed no insects or other animals. Although not a good simulation of insectary conditions, this room was completely free from Stegobium pheromone contamination, which was further ensured by directing the outflow from the suction pump by polythene tubing to an extraction fan. The insects were kept in the rearing insectary at all times other than during preparation of air extract discs. Immediately a collection period was finished, both filter paper discs (from chambers I and III) were transferred to separate pre-cooled, dry, well-stoppered tubes and stored at -15°C until bioassay. This was never

Figure 18. Diagram of the apparatus used to collect pheromone from live female Stegobium. An air flow was maintained by the suction pump at 1.0 ± 0.1 l/min and monitored by the flow meter. The control and experimental discs were Whatman Grade 1 filter paper. The broken lines in chamber II represent a single thickness of medical wiper tissue used to confine the females. Diagram not to scale.


to extraction fan
suction pump
chambers:
III
II
I
females
polythene tubing
polythene closures
exp. disc
glass tubing
control disc
T
flow meter
air flow

more than one week after collection and much less than this when possible. Preliminary experiments gave no indication of appreciable loss of attractiveness of the discs over the bioassay period, although once tested, a single disc was not stored for retesting at a later date.

All glassware and polythene closures were soaked in 5% "Decon" for 24 h after each air extraction, and after thorough rinsing, dried and heated for one hour at 80°C. It had been discovered earlier that the polythene closures in particular adsorb and retain the sex attractant despite washing with hot water and a regular liquid detergent.

A series of exploratory experiments performed during the development of the air extraction technique had indicated a source of contamination. During a test-run of the apparatus without females in chamber II, males responded to the disc from chamber III, but not to that from chamber I. This excluded the possibility of contamination from the room. All the components of the assembly were soaked individually in a small quantity of diethyl ether and left over night. Process of elimination following bioassay of each extract implicated the polythene closures as the major source of contamination. They were found to be saturated with pheromone, eliciting over 70% positive response from males. The closures were soaked in 5% "Decon" for 24 h and extracted in ether for a second time. Subsequent bioassay of this extract revealed no remaining pheromone activity.

Female pupae were collected in very great numbers so that at the peak of the eclosion period, a large proportion of adults would be eclosing synchronously. Adults were removed immediately after eclosion while the adult cuticle was still completely pale (0 days old). At least 50 females were collected as they eclosed between 08.00 h and 09.00 h on the morning on which a series of air extractions was to begin. This allowed dying females from the group of 25 to be replaced with others of exactly the same age during the course of the experiments. It was presumed that replacement insects would emit pheromone at the same rate as the remainder of the group. Although it

occupies only 1-2 h of each day, the air extraction process might influence pheromone emission by increasing the evaporation rate from females. This might deplete their reserves to a greater extent than those of females not previously exposed to an air stream. Shorey and Gaston (1965b) forced Trichoplusia ni females to emit pheromone in an air flow for 30 min. It was calculated that 24% of the total pheromone content of the females should theoretically have been released as a result of this treatment, but subsequent solvent extraction of the pheromone gland indicated no detectable loss. These workers suggested that rapid synthesis of pheromone, or conversion of pheromone precursors to pheromone might replace loss as it occurred. A comparable test was not performed on Stegobium but replacement of females was considered to be a lesser source of error than allowing the test females to decrease in number.

Twenty-five 0-day-old females were air extracted for one hour beginning at 09.00 h and subsequently at 12-h intervals up to day six and at 24-h intervals thereafter.

The effect of mating upon female pheromone emission was investigated by means of the following experiment. A group of 50 females were kept with males (in groups of two of each sex) for their first 6 days of adult life, by which time copulation was assumed to have occurred. The females were separated from the males at 08.30 h on day six and air-extracted at 09.00 h. Subsequent extractions were carried out every 24 h until less than 25 females remained alive.

Individual females of a second group were placed with a male on day five from 09.00-12.00 h. Only females observed to copulate for a period of at least 20 min were considered mated. These females were also air-extracted on day six at 09.00 h and at 24-h intervals thereafter.

In order to provide additional information about the efficiency of the air extraction technique, three short tests were carried out. A group of twenty-five 10-day-old females were air-extracted for one hour in a modified apparatus with two additional chambers (numbered IV and V). Secondly a two-

hour extraction of twenty-five 8-day-old females and thirdly, a one-hour extraction of fifty 10-day-old females were also performed.

The males used for bioassay of the air extraction discs were predominantly 10 days old, but 11 to 13-day-old males were used when necessary to make up numbers. Practical difficulties were involved in sexing pupae in sufficient numbers to supply males of a particular age on a series of days during the bioassay test period. A minimum of 100 males was used to test each pair of discs, the procedure being exactly as for ether extract bioassay, with the filter paper discs from chambers III and I replacing the 10 μl sample of female ether extract and pure ether control discs respectively.

Table 10 shows the complete results of the bioassay experiments for the air extraction experiments.

C. Results and Discussion

(a) Virgin females

Figure 19 shows the percentage response of 10 to 13-day-old males to air extract discs prepared from a group of 25 virgin females over their adult life span. A very rapid increase in female pheromone emission was indicated by male response to 2 to 3-day-old female air extracts, rising from zero to almost 60% male response. A fairly constant level of male response was elicited by air extracts from 4 to 17-day-old females, the mean level reaching about 76%. Air extracts from older females became less attractive to males. Less than 25 females survived beyond 24 days of age.

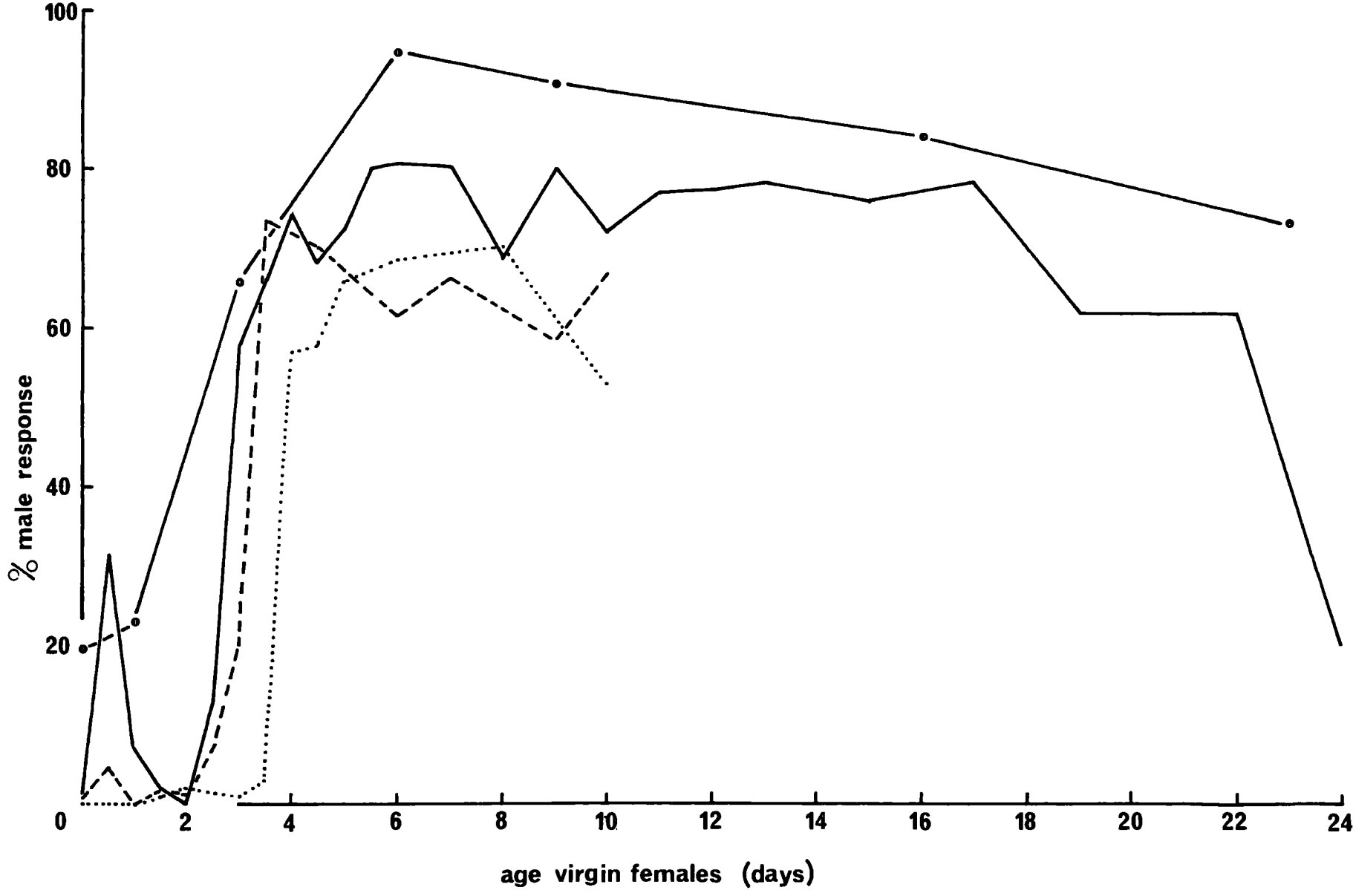
The small premature peak in male response to the air extract of 12-hour-old females was questioned and in case of experimental error or contamination, the experiment was repeated with two further groups of females monitored only over the first part of their adult life (0-10 days). Figure 19 includes these tests, one of which showed a 5% male response to the extracted pheromone output of 12-hour-old females, but the other showed none. The possibility of 12-hour-old females releasing small quantities of pheromone cannot be discounted. In most other respects, the two duplicate experiments confirmed the trend

Table 10. Percentage response of males to air extract discs prepared from females over their adult life span.

Air Extracts:											
sex	: ♀	♀	♀	♀	♀	♀	♀		♀		
mating status:	v	v	v	$m^{(1-6)}$	$m^{(5)}$	v	v		v		
no. extracted:	25	25	25	25	25	25	50		25		
extr'n period:	1h	1h	1h	1h	1h	2h	1h		1h		
other details:								III	IV	V	
age: 0	1.6	0.9	0.0								
½	31.6	4.9	0.0								
1	7.0	0.0	0.0								
1½	2.0	1.9	1.0								
2	0.0	1.0	2.0								
2½	12.6	7.7	-								
3	57.8	20.3	1.0								
3½	66.0	73.6	3.1								
4	74.3	74.0	56.9								
4½	68.3	69.9	58.0								
5	72.4	66.7	66.0								
5½	80.2	-	-								
6	80.6	61.6	68.5	1.6	2.0						
7	80.3	66.3	-	50.0	64.4						
8	68.7	-	69.6	3.8	8.0	84.4					
9	80.0	58.6	-	69.3	55.0						
10	72.2	66.9	53.2	46.4	49.5		57.0				
11	77.2			46.1	47.0						
12	77.6			16.1	49.0						
13	78.6			40.5	-						
14	-			49.5	50.0						
15	76.2			47.3	-			68.3	53.8	62.3	
16	-			-	55.0						
17	78.4			1.9	30.8						
18	-										
19	61.9										
20	-										
21	-										
22	62.1										
23	-										
24	20.5										

$m^{(1-6)}$: males kept with females for days 1-6 of adult life ; III, IV & V: chambers in series
$m^{(5)}$: males copulated with females on day 5 (see text)

Figure 19. Pheromone emission from virgin female Stegobium as assessed by the response of 10 to 13-day-old males to air extracts (see text). Three groups of 25 females were air extracted; one group over their entire adult life span (solid line) and a further two groups, from 0-10 days inclusive (broken and dotted lines). For comparison, the plateau response level of males to diethyl ether extracts of virgin females of certain age groups is included (•——— •).


100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
22
24
% male response
age virgin females (days)

outlined above, with a consistent high percentage of male response to air extracts from 4-day-old females and above, preceded by a very rapid increase in female emission. Actual timing and response levels varied slightly (table 11).

Table 11 Separation of the main phases of Stegobium female pheromone emission over the adult life span in the three sets of 25 virgin females studied by the air extraction technique.

female group	little or no pheromone emission	rapid increase in emission rate	maximum emission rate reached	plateau	% male response at plateau m ± SE
1	days 0-2	days 2-3	day 4	days 4-17	76.1 ± 0.31
2	" 0-2	" $2\frac{1}{2}$-$3\frac{1}{2}$	" $3\frac{1}{2}$	" $3\frac{1}{2}$-10*	67.2 ± 0.72
3	" 0-3	" 3-4	" 5	" 5-10*	62.0 ± 1.25

*female groups 2 and 3 only air extracted up to 10 days of age

A group of 25 females is probably not large enough to eliminate all effects of individual variation which might account for these differences. Sower, Shorey and Gaston (1972) found that individual pheromone release rates of Trichoplusia ni varied from less than 1 to more than 21 ng/min.

Leaving aside for the moment the unconfirmed male response to 12-hour-old females, the above results may be compared with those from the diethyl ether extracts of Stegobium. It should be stressed that male response values cannot be compared directly, due to the different methods of pheromone collection employed. However, it is possible to deduce from these two sets of results, an overall picture of the relationship between female pheromone content and emission. It is apparent that the rate of pheromone release from live females was far more rapid than the rate of increase in pheromone content (fig. 19). Although not confirmed, it is thought unlikely that ether extractable pheromone decreases between days 1 and 3. It can be assumed, therefore, that synthesis and storage of sex attractant in the female built up to a maximum over days 0-6 although synthesis may well begin prior to

eclosion. However, maximum pheromone output was reached between days 2 and 4, the main phase of this increase occurring over a 12 to 24 h period.

Pheromone production (or at least synthesis of precursor substances) might occur during the pupal stage as has been demonstrated by Steinbrecht (1964) in the silkworm, Bombyx mori L. However, neither the smaller tea tortrix moth, Adoxophyes fasciata (Nagata et al, 1972) nor the noctuid moth Trichoplusia ni (Shorey and Gaston, 1965) showed any pheromone activity in solvent extracts of pupae.

As measured by the air extract method, pheromone emission in Stegobium females increased rapidly, the maximum coinciding with the period immediately prior to emergence from the cocoon on day 4-5 (fig. 19). Pheromone content of females also increased over this period, but maximum pheromone content seems delayed by about two days after the attainment of maximum release rate. Once the latter reached a fairly constant level, further synthesis in excess of emission probably resulted in the fairly stable level of pheromone content observed, replacing the attractant as it is lost during emission.

In Lepidoptera, pheromone release can be more easily monitored since a characteristic calling attitude is assumed whereby the pheromone secreting gland, or reservoir is everted. Lawrence and Bartell (1972) found that in the light brown apple moth, Epiphyas postvittana, pheromone content, as shown by male response to solvent extracts, increased as calling became less frequent. Females of this moth also demonstrated a delay in maximum content levels, which were reached a few days after peak calling intensities were displayed. Two hours calling resulted in a reduction of pheromone content to about 20% which was thought to explain the reduced response levels elicited by extracts of moths after calling.

In 17 to 24-day-old Stegobium females, the rate of sex pheromone emission fell quite rapidly (fig. 19) which might account for the surprisingly high male response levels obtained with ether extracts of 23-day-old females.

Returning to the possibility of a burst of pheromone release in 12-hour-old

females as implicated in figure 19, the mechanism of such a phenomenon might be related to the structural properties of the cuticle prior to tanning. Before this process is complete (which is dependent upon the neuroendocrine system and not merely air-exposure) it is conceivable that some pheromone, known to be present at this time, "leaks" through the soft protein of the integument. In Stegobium, tanning, as reflected by cuticle darkening, is usually at an advanced stage by day 1, but incomplete at 12 h of adult age after eclosion. Exposure to an airflow might enhance this escape of pheromone as females normally occupy the cocoon at this stage. For the same reason, there would appear to be no selective disadvantage in this premature release of pheromone, since females are unlikely to attract males as a result.

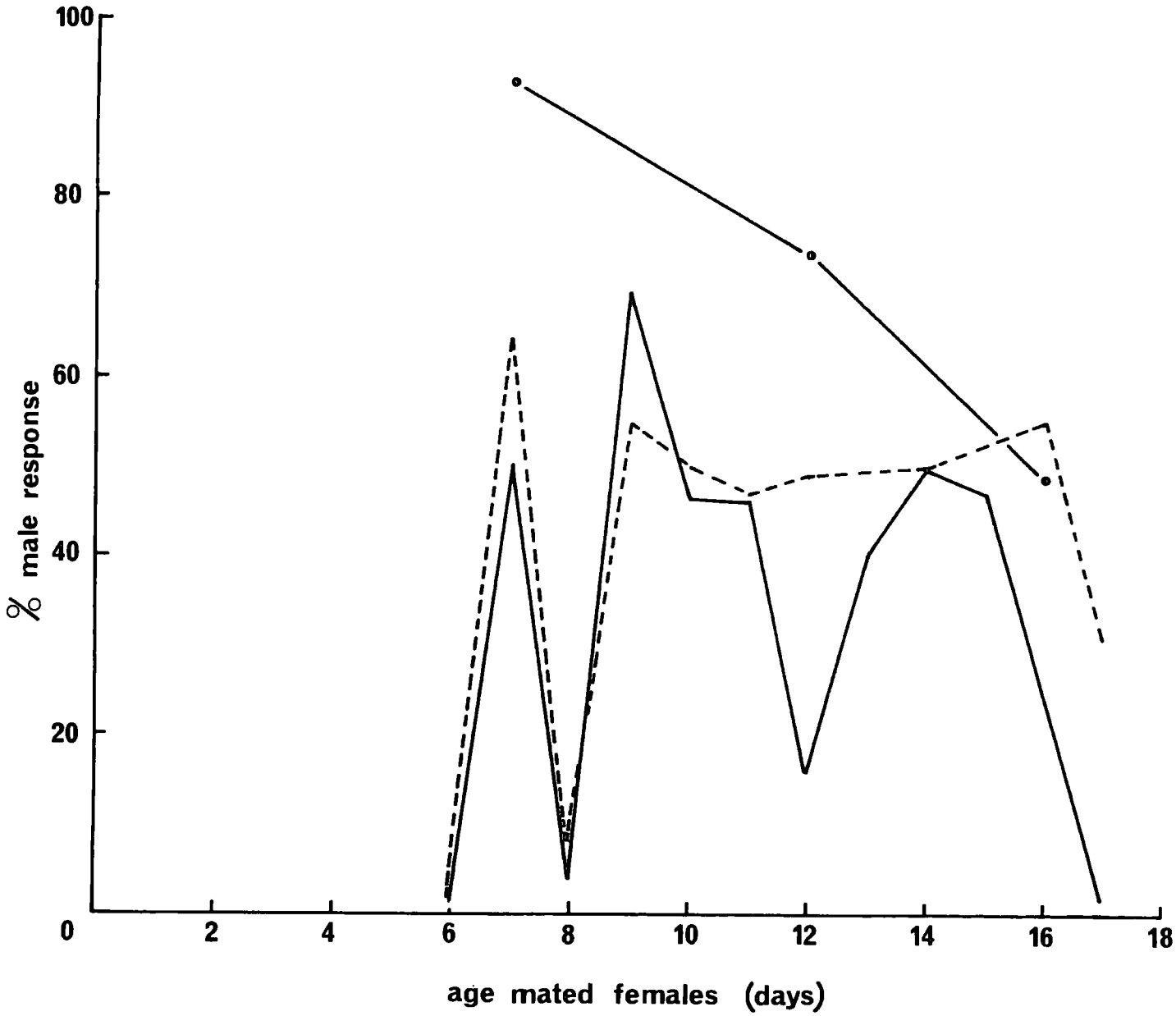
(b) Mated females

The response of males to air extracts from live females monitored after mating was very irregular but remarkably consistent between material from the two groups of females bioassayed. It can be seen from figure 20 that in both cases, pheromone emission on day 6 was negligible, and probably was so on the preceding days while the females were still in contact with males. However, on following days, release rates oscillated between the levels expected for virgin females and almost zero pheromone release. Mated females died between 15 and 17 days of age and thiswas preceded by a period of more erratic pheromone emission and less synchrony between the two groups of females (fig. 20).

Pheromone content over this period as assessed by only three ether extracts of 6-, 12- and 16-day-old females (section IV) showed a fairly steady decline as reflected by male response plateau means to the ether extracts, which fell from about 90-50% (also plotted on figure 20).

The above results will be discussed in greater detail in the final discussion (section IX, part C(c)) in conjunction with the results of the oviposition studies (section VII). In brief, it is thought that pheromone emission is probably inhibited by egg-laying, and the exceptional degree of

Figure 20. Pheromone emission from mated female Stegobium as assessed by the response of 10 to 13-day-old males to air extracts. Two groups of 25 mated females were air extracted on days 6-17 inclusive. The solid line represents male response to female air extracts, prepared from females which had been kept with males from days 1-6, and the broken line represents females which were observed to copulate on day 5. For comparison, the plateau response level of males to diethyl ether extracts of mated females of 6, 12 and 16 days old is included (• ——— •).


100
80
60
40
20
0
% male response
2
4
6
8
10
12
14
16
18
age mated females (days)

synchrony between the individuals and groups results from females being placed together in single large groups after separation from males. It has been demonstrated by the air extraction technique, that control of pheromone release is possible despite the presence of high levels of pheromone in the body.

Several other studies provide examples of mating interrupting pheromone release. Calling intensity of the Indian meal moth, Plodia interpunctella, was greatly reduced following copulation (Brady and Smithwick, 1968) and mated female codling moths, Laspeyresia pomonella, in traps retained almost no attraction to males (Howell and Thorp, 1972). The mealworm, Tenebrio molitor, demonstrated synchrony of female pheromone emission following mating, the drop in release correlating with the onset of oviposition (Happ and Wheeler, 1969).

(c) Efficiency of the air extraction technique

Table 10 includes the results of the three tests designed to give some indication of the efficiency of the air extract method of pheromone collection. On addition of a further two chambers to the sequence (IV and V) the resulting discs from these chambers were only slightly less attractive than the disc from chamber III, and there was no indication of gradation of attractiveness. It would appear that an equilibrium is set up whereby the pheromone is adsorbed on to the filter paper disc, to become revolatilised in the airflow, this process being repeated in each chamber. When a normal three-chambered system was left extracting for two hours instead of one, male response to the disc from chamber III increased to about 84%, compared with about 69% male response to comparable air extract discs from a 1 hour extraction (table 10). This might suggest that the equilibrium process described above is progressive and cumulative.

Fifty females were extracted in an airflow for one hour, and male response to the resulting disc was found to be no higher than if only 25 females had been present (table 10). The quantity of pheromone deposited on the

filter paper disc might thus be more directly affected by time, than the number of females in the central chamber.

VII REPRODUCTIVE DEVELOPMENT IN FEMALE *STEGOBIUM*: MATURATION, COPULATION AND OVIPOSITION

A. Introduction

The role of the sex pheromone is to bring the sexes together at a time appropriate for successful copulation, and thus a close temporal relationship between the peak of female attractiveness and ovary maturation might be anticipated. Such synchronisation has been demonstrated in most species studied in this context. This may be illustrated by reference to a few examples. Female *Lasioderma serricorne* become receptive to males, the ovaries mature and maximum pheromone production achieved, all between 4-6 days of age (Coffelt and Burkholder, 1973). *Epiphyas postvittana* females reached their highest level of mature eggs per ovariole at 4 days of age, coincident with maximum pheromone output (Lawrence and Bartell, 1972). Happ, Schroeder and Wang (1970) found in *Tenebrio molitor*, that oocyte length and emission of the sex attractant increased in parallel over the first 7-10 days after emergence. In a study of seven species of noctuid moths, Shorey, McFarland and Gaston (1968) demonstrated close synchrony of sex pheromone production, ovary maturation and mating receptivity within the first 1-2 days after emergence.

It was considered an important part of this study to resolve these aspects of the reproductive cycle in *Stegobium*, although only a brief investigation was possible. Detailed work on many aspects of development, fecundity and oviposition in *Stegobium* was completed by Azab (1943). Since rearing conditions cannot be exactly duplicated, and precison in timing of events was required from this study, Azab's oviposition experiments were repeated in the present work, with some modifications, and indeed slight differences in the results were found.

The female reproductive system of *Stegobium* has been described, and stages of development detailed by Metcalfe (1932), Kashef (1956) and Monteiro (1957) but between these works there are some discrepancies which will

hopefully be resolved below. External genitalia of both sexes have been described in detail by Mukerji (1954).

B. The female reproductive system of Stegobium

As in most Coleoptera of the sub-order Polyphaga, the ovaries of Stegobium are telotrophic: the nurse cells are confined to the germarium located at the apex of each ovariole. Long nutritive cords connect the nurse cells to the oocytes during their early stages of development. Telotrophic ovary structure has been described by Schlottman and Bonhag (1956), Imms (1957), Bonhag (1958) and Wigglesworth (1972) amongst many other authors.

Figure 21 shows the female reproductive system of Stegobium, drawn from a freshly killed specimen. The structural arrangement shown here is, for the most part, in agreement with that depicted by Metcalfe (1932), Kashef (1956) and Monteiro (1957), although the abdominal nerve mass (fig. 21) was included by Kashef (1956) as an accessory gland of the reproductive system, situated at the junction of the two lateral oviducts. Indeed, this structure is deceptive, since no anterior nervous connection can be seen when the reproductive system is simply removed from the abdominal cavity. The nerves ramifying from its base are extremely fine except for a pair of nerves (omitted by Kashef) which supply the muscle attached to the base of the ovipositor. The muscle is inserted on the chitinous rod which is used to evert the ovipositor during oviposition. The bursa copulatrix is large and muscular, which is characteristic of the Anobiidae (Surtees, 1961) and it gives rise at its base to a fine duct from which two blind-ending sacs of irregular and variable shape, the spermatheca and the spermathecal gland, branch (fig. 21).

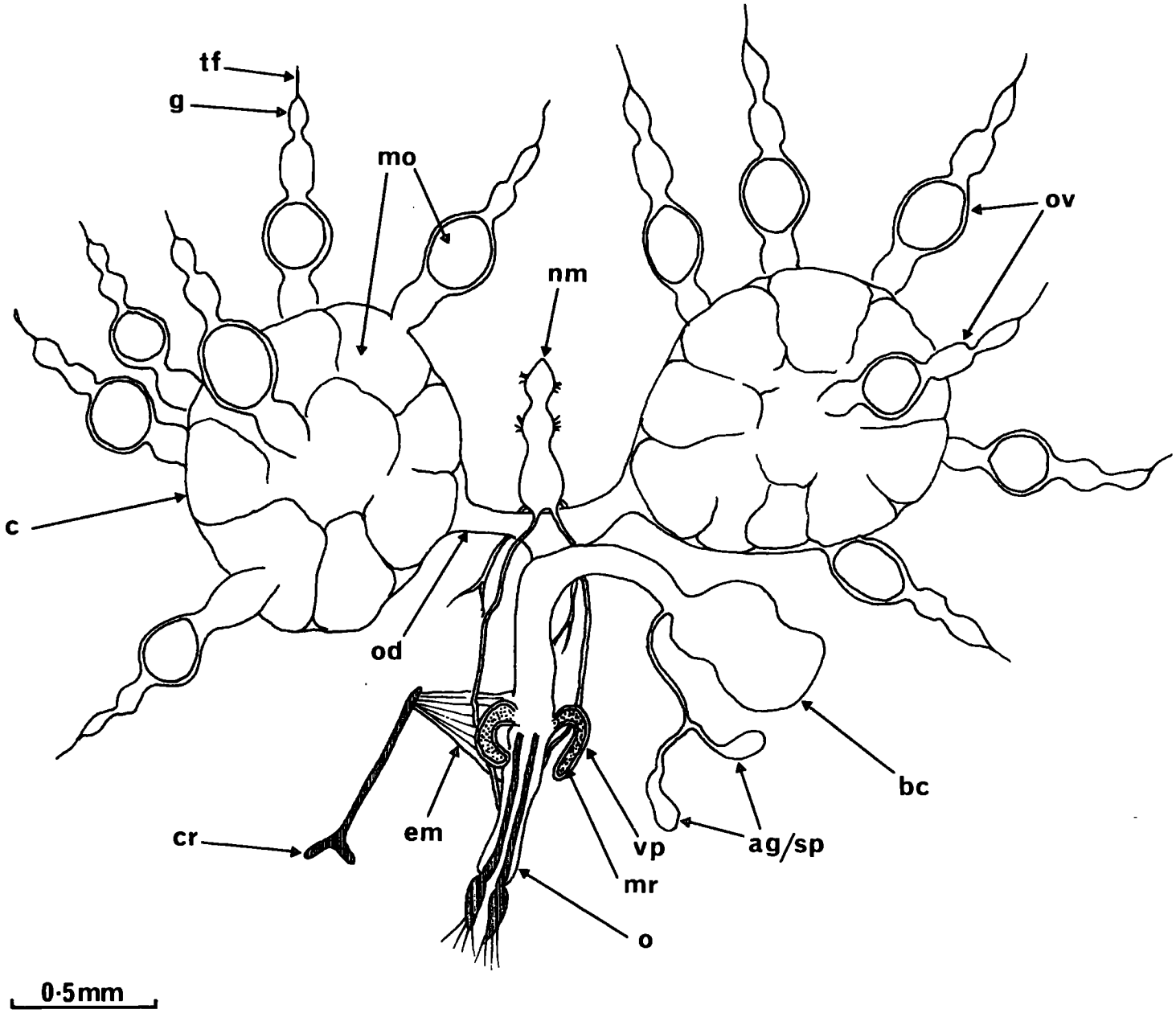
The vaginal pouch contains the mycetome reservoir, or symbiotic yeasts which are deposited on to the surface of the eggs as they are laid, and transferred to the young larva as it eats the shell at emergence (Breitsprecher, 1928).

C. Ovary development

In order to study the timing of maturation of the female reproductive system in Stegobium, both dissection of females, and histological sectioning

Figure 21. The reproductive system of female Stegobium drawn from a freshly killed and dissected 8-day-old virgin female.

ag = accessory gland
bc = bursa copulatrix
c = calyx
cr = chitinous rod
em = ovipositor evertor muscle
g = germarium
mo = mature oocyte
mr = mycetome reservoir
nm = abdominal nerve mass
o = ovipositor
od = oviduct
ov = ovariole
sp = spermatheca
tf = terminal filament
vp = vaginal pouch


tf
g
mo
nm
ov
c
od
bc
cr
em
vp
ag/sp
mr
o
0·5mm

were performed with females at various stages of ovarian development.

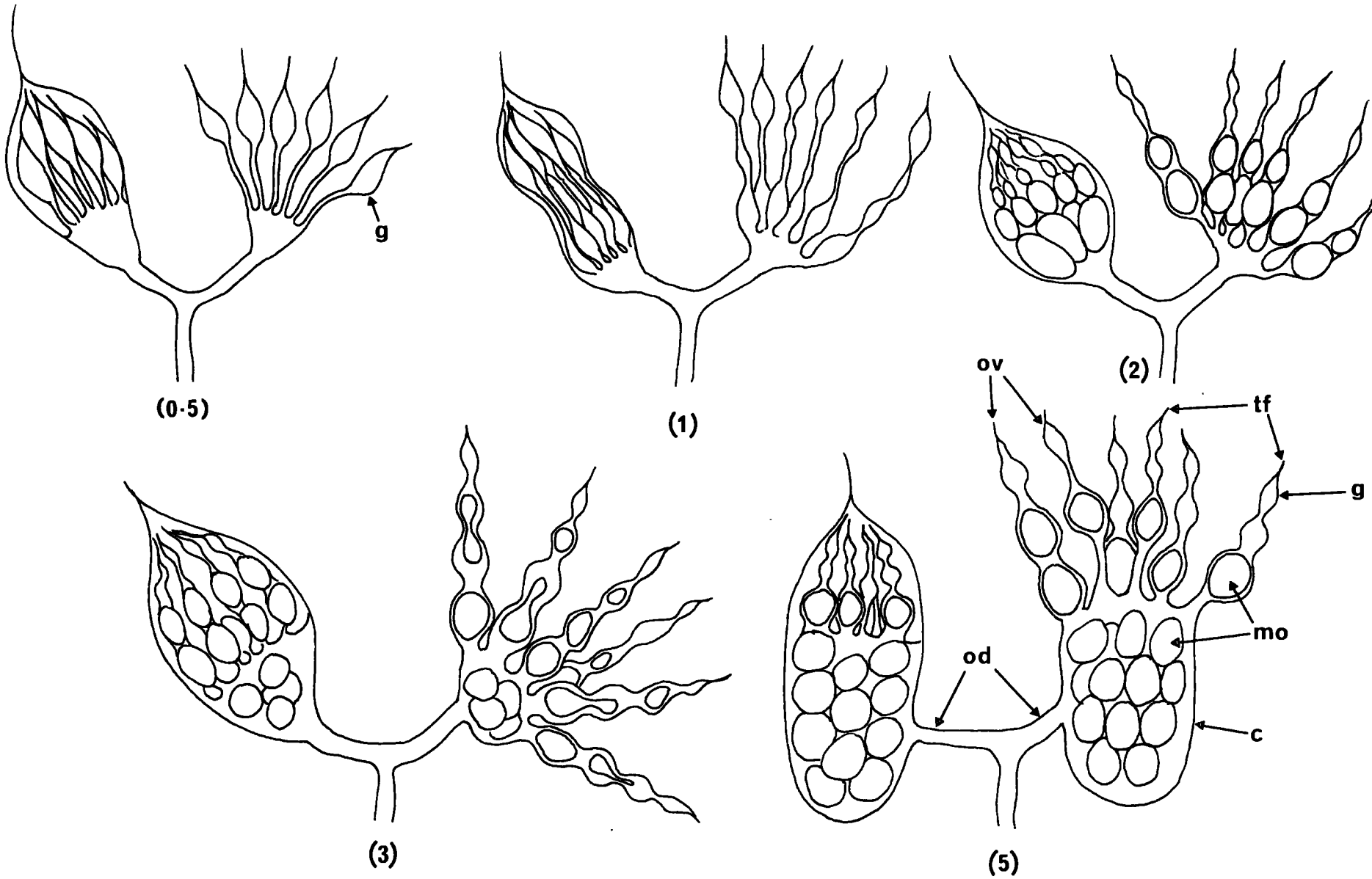
(a) Dissection of ovaries

Females of known age were killed and stored in 70% alcohol until dissection. The following technique proved the most successful for removing the entire reproductive system from Stegobium. This species being rather small, the procedure was best carried out beneath a X10 binocular microscope. With the aid of fine needles and forceps, the head and thorax were pulled away, bringing with them the wings and elytra and the anterior part of the alimentary canal. The remaining abdomen was placed on a slide in a drop of water. The ventral abdominal cuticle was carefully removed by peeling away each sternal sclerite in turn, exposing the internal abdominal tissues. The water was removed and the specimen bathed in methylene blue for 5 min to ease identification of parts of the reproductive system, the latter taking up the stain more rapidly than the fat body. The stain was removed by means of filter paper and replaced with water. Fat body was teased away from the ovaries and other parts of the system, which were usually clearly recognisable at this stage. The ovipositor was severed from the terminal abdominal cuticle, and the reproductive system pulled clear in its entirety from the remains of the abdomen. Measurements of certain parts of the ovary were made by means of an eyepiece graticule in the microscope, and the units converted to millimetres using a calibration slide. Figure 22 shows the major stages through which ovary maturation passes, as seen during the dissections.

The technique described above was adapted from that used for many insect species, including Tenebrio molitor, by Kamm and Richter (1972) who, instead of opening up the abdomen, made lateral incisions to free the last two abdominal segments which were then pulled posteriorly bringing the ovaries with them. This is not possible with Stegobium, which is too small for this procedure. Even with Tenebrio, the above authors found it necessary to cut the abdomen longitudinally in the case of mature females, swollen with eggs.

For each female dissected, four values were recorded: mean length of the

Figure 22. Diagrammatic representation of the developmental stages of ovary maturation in female Stegobium of 0.5, 1, 2, 3 and 5 days old. Diagram not to scale, abbreviations as in figure 21.


g
(0·5)
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od
c
(3)
(5)

two ovaries; mean length of the six ovarioles on one side; mean length of terminal oocytes on the same side and the total number of eggs in both calyces (since in mature females, eggs invariably errupted from the calyces during dissection).

Ten females of the following ages were dissected, and the above measurements made; 0, 0.5, 1, 2, 3, 4 and 5 days. A mean value for the 10 females was found for each of the measurements (table 12, fig. 23). Development of the female reproductive system clearly begins during the pupal

Table 12 Growth and development of the reproductive system of female Stegobium as determined by dissection and measurement of parts of the ovary from females of 7 age groups.

female age (days)	length ovary m ± SE (mm)	length ovariole m ± SE (mm)	length terminal oocyte m ± SE (mm)	no. eggs in calyces m ± SE
0	0.71 ± 0.02	0.50 ± 0.02	0.13 ± 0.01	0
0.5	0.86 ± 0.02	0.71 ± 0.02	0.18 ± 0.01	0
1	1.12 ± 0.03	0.90 ± 0.02	0.25 ± 0.01	0
2	1.31 ± 0.03	1.09 ± 0.04	0.35 ± 0.004	0
3	1.80 ± 0.03	1.44 ± 0.04	0.36 ± 0.002	5.7 ± 0.67
4	2.02 ± 0.03	1.44 ± 0.05	0.36 ± 0.002	20.7 ± 1.40
5	2.08 ± 0.02	0.97 ± 0.02		29.4 ± 2.50

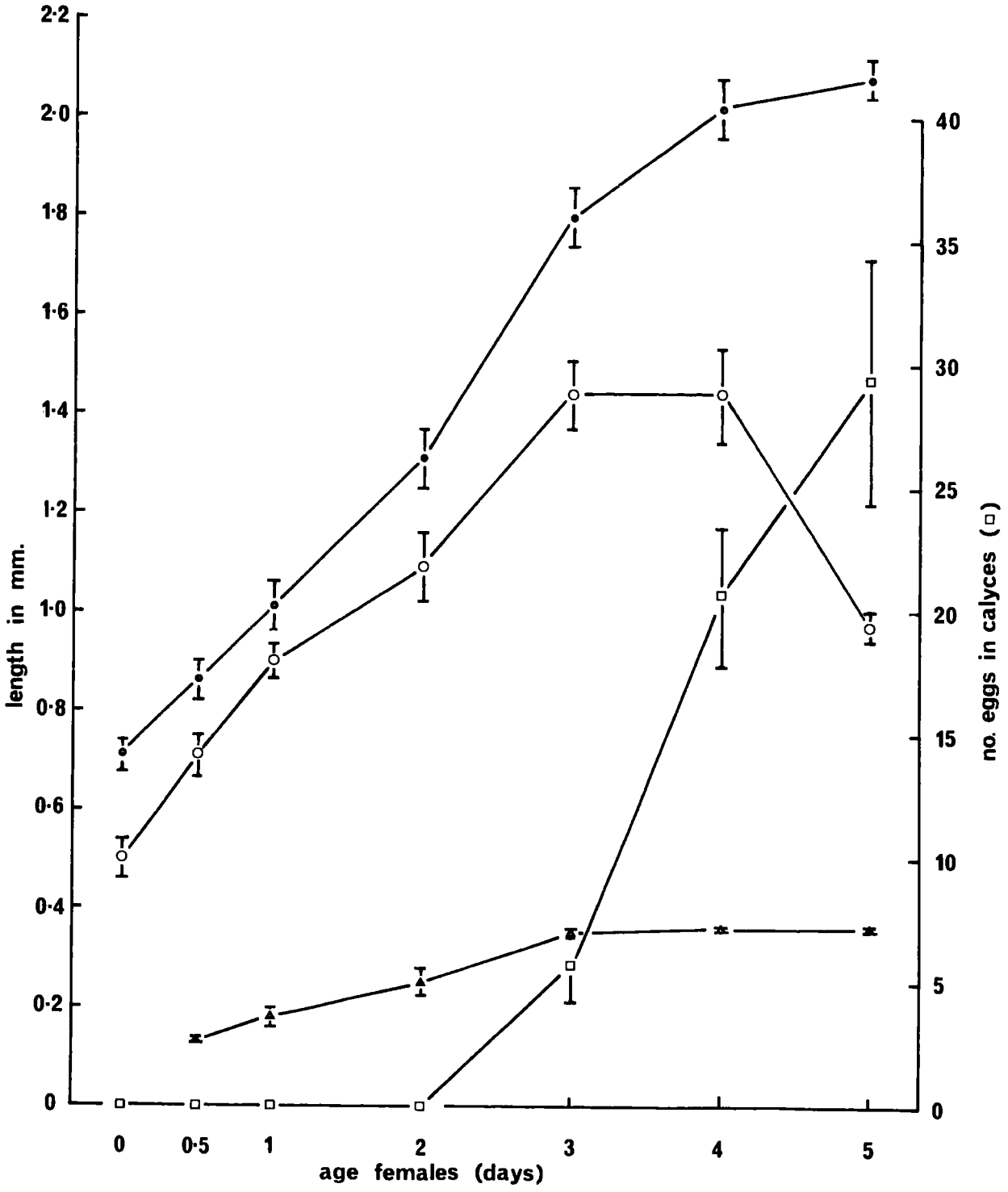
stage, since the ovary was found to have reached 0.7 mm in length at eclosion. Maximum ovary length was reached by about 5 days of age, or perhaps a little later, but maximum ovariole length was attained by day 3, declining in length thereafter. This reflects movement of mature eggs into the calyx, which was first observed on day 3, by which time the terminal oocyte had reached maximum size andwas fully developed and chorionated.

It would be expected, from these results, that a female could be capable of copulation and oviposition of fertile eggs from day 3 onwards.

Figure 23. Relative increase in length of certain parts of the female Stegobium reproductive system from ages 0 (eclosion) to 5 days. Ten females of each age group were dissected and measured, and a mean value was found for:

- ●——● entire ovary length
- ○——○ ovariole length
- ▲——▲ length of terminal oocyte (most developed)
- □——□ number of mature oocytes in both calyces

The confidence limits represent 2 standard errors.


2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
length in mm.
40
35
30
25
20
15
10
5
0
no. eggs in calyces (□)
0
0·5
1
2
3
4
5
age females (days)

(b) Histology of ovaries

Female beetles of known age were killed and fixed in Bouin's fixative before sectioning. The abdomens were removed from the rest of the body, embedded and serially sectioned at 10 μm in wax. Sections were stained in haematoxylin and eosin, and permanent preparations made. A series of females from 1 to 8 days of age were processed in this way.

Figures 24-28 inclusive are micrographs of selected sections through the abdomens of females of 1, 2, 3, 4 and 8 days old respectively. In most cases, the section is taken from the posterior part of the ovary in order to show the most advanced stages of oocyte development. The first section (1-day-old female) has passed through the germarium of each ovariole, no true follicles being present at this stage. Some very small developing oocytes are present in one of the germaria which has been sectioned near the base (not clear in the micrograph). The section of the 2-day-old female shows that vitellogenesis has begun and the highly basophilic follicle cells have passed through the columnar, to the cuboidal phase of their development. The granular appearance of the oocytes indicates yolk deposition. By the third day, some mature chorionated oocytes are present (fig. 26), and the follicular epithelium has become squamous, suggesting massive increase in the diameter of the oocyte. The section through a 4-day-old female (fig. 27) shows an increase in the number of mature, chorionated eggs, and expansion in the size of the calyx to accommodate these (unfortunately, chorionated oocytes tend to collapse during histological preparation). This particular section (fig. 27) has passed through the vaginal pouch. By 8 days of age, the abdomens of virgin females are full of tightly packed mature eggs. The abdomen has been sectioned, slightly obliquely, through the calyx, (fig. 28).

These observations confirm the dissection studies in that mature eggs are present in the calyx from day 3, and rapidly increase in number in 4-day-old females. The oocyte and follicle development is largely in agreement with that described for Lasioderma serricorne (Coffelt and Burkholder, 1973) and

Figure 24. Micrograph of a T.S. through the abdomen of a 1-day-old virgin female Stegobium. Only the germaria are present in this section, very little follicle development being evident.

bc = bursa copulatrix
c = cuticle
ch = chorion
do = developing oocyte
e = elytron
f = follicle
g = gut
gr = germarium
mo = mature oocyte
mt = Malpighian tubule
n = nucleus
nc = nerve cord
s = symbiotic yeasts
vp = vaginal pouch
y = yolk

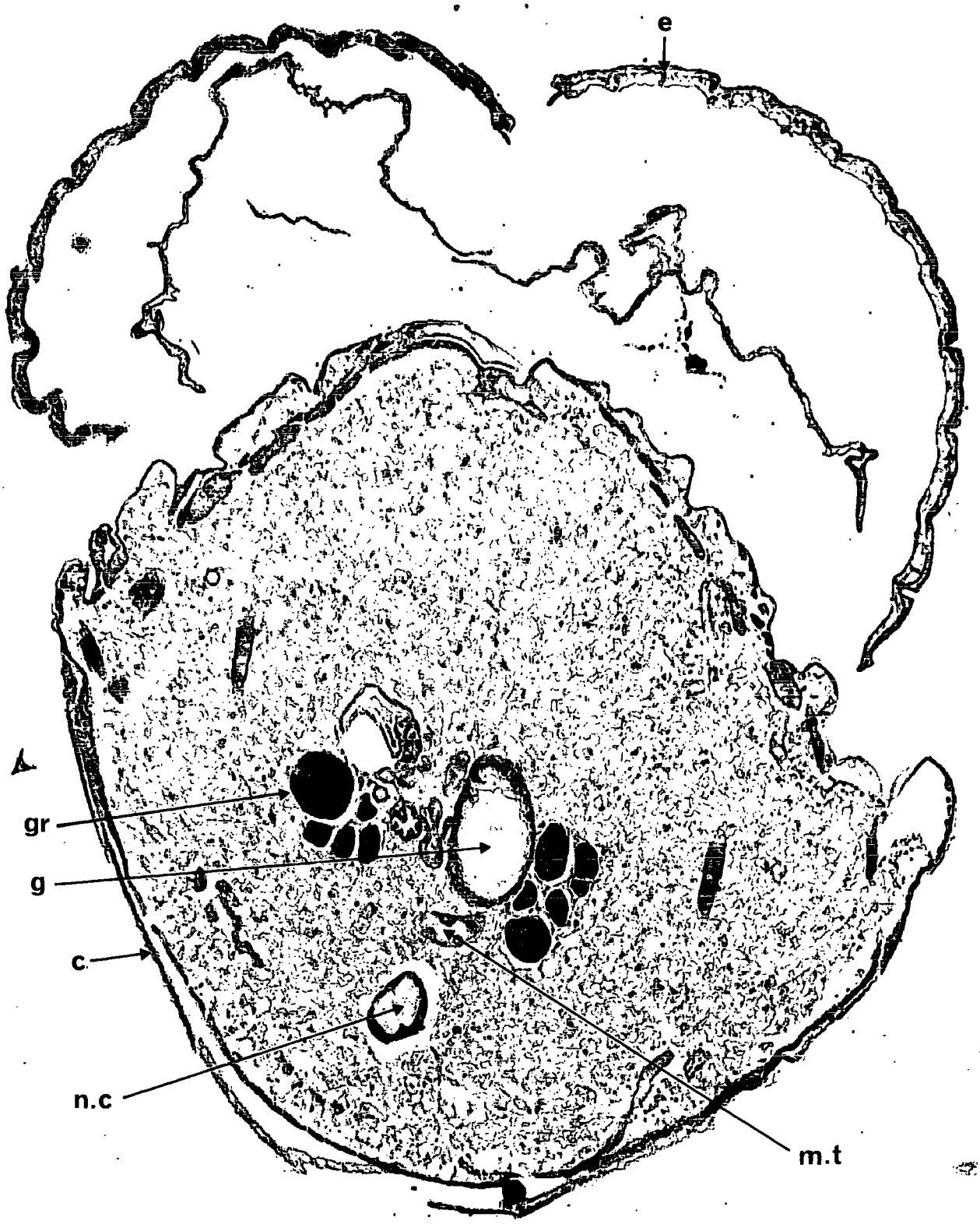

e
gr
g
c
n.c
m.t

Figure 25. Micrograph of a T.S. through the abdomen of a 2-day-old virgin female Stegobium. The cells of the follicular epithelium have become cuboidal and yolk deposition is evident from the granular appearance of the developing oocytes. Abbreviations as for figure 24.

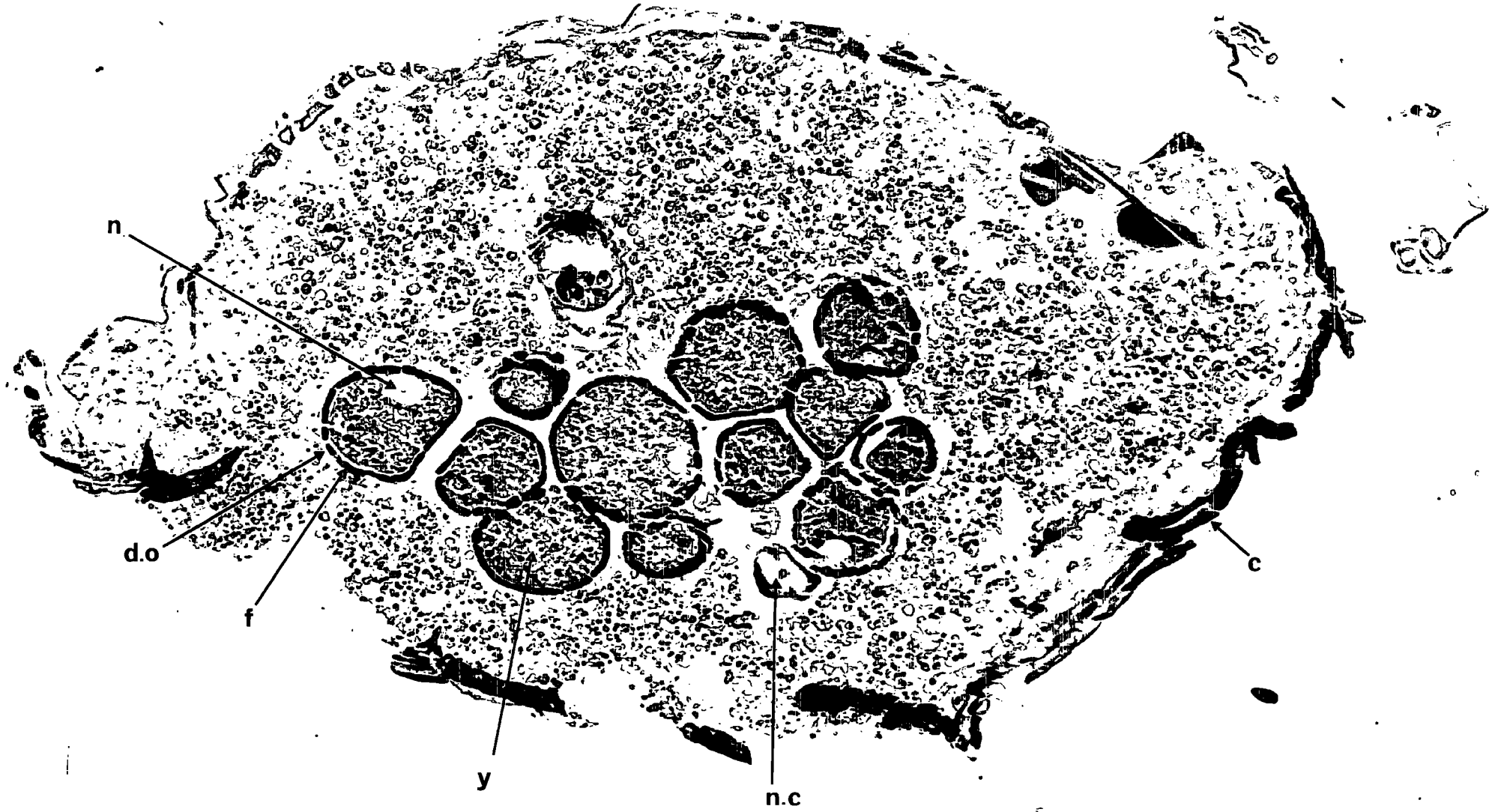

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Figure 26. Micrograph of a T.S. through the abdomen of a 3-day-old female Stegobium. The follicular epithelium has become squamous and the developing oocytes now occupy a much greater proportion of the abdominal cavity. Some mature, chorionated oocytes are present at this stage. Abbreviations as for figure 24.

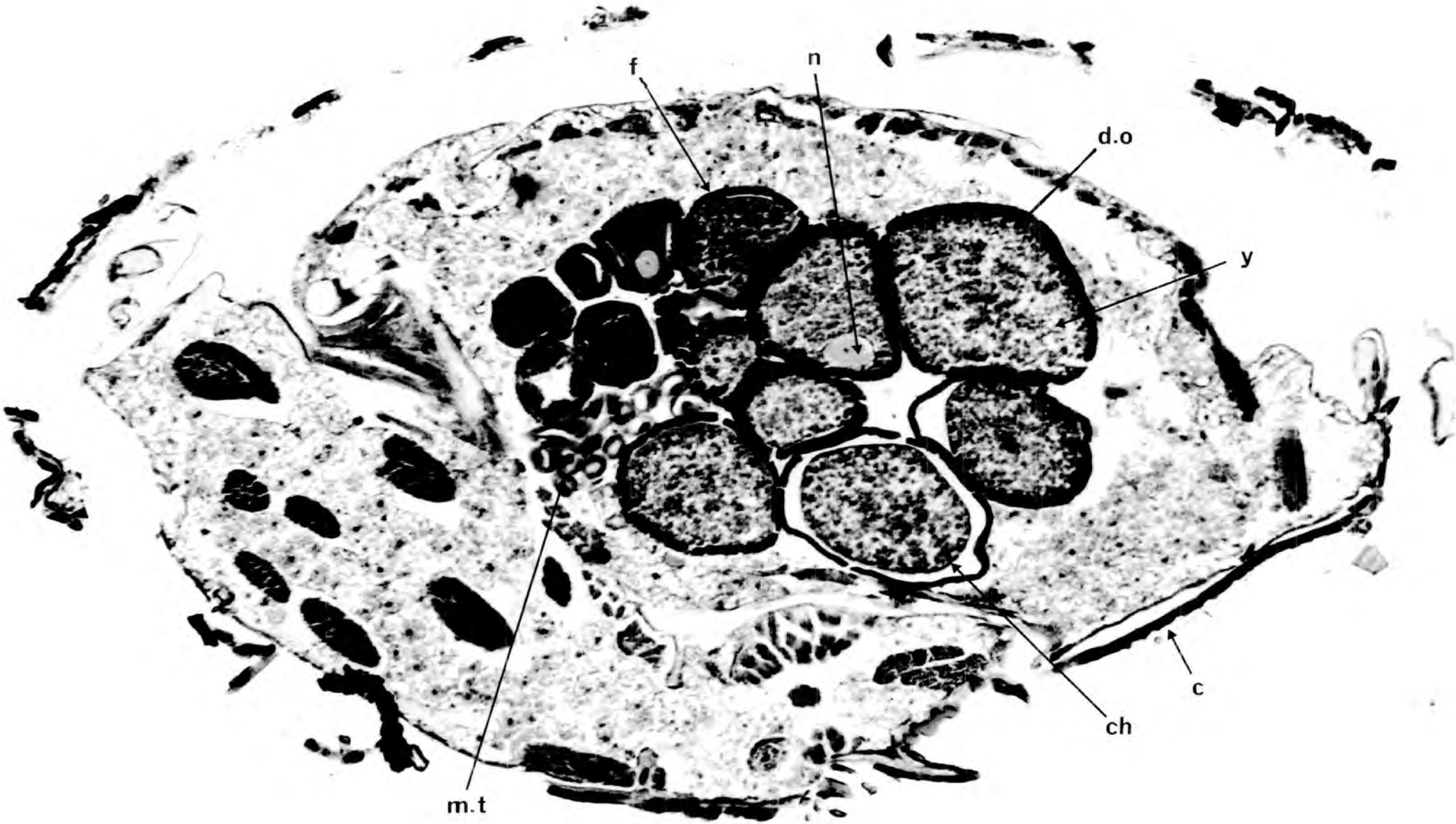
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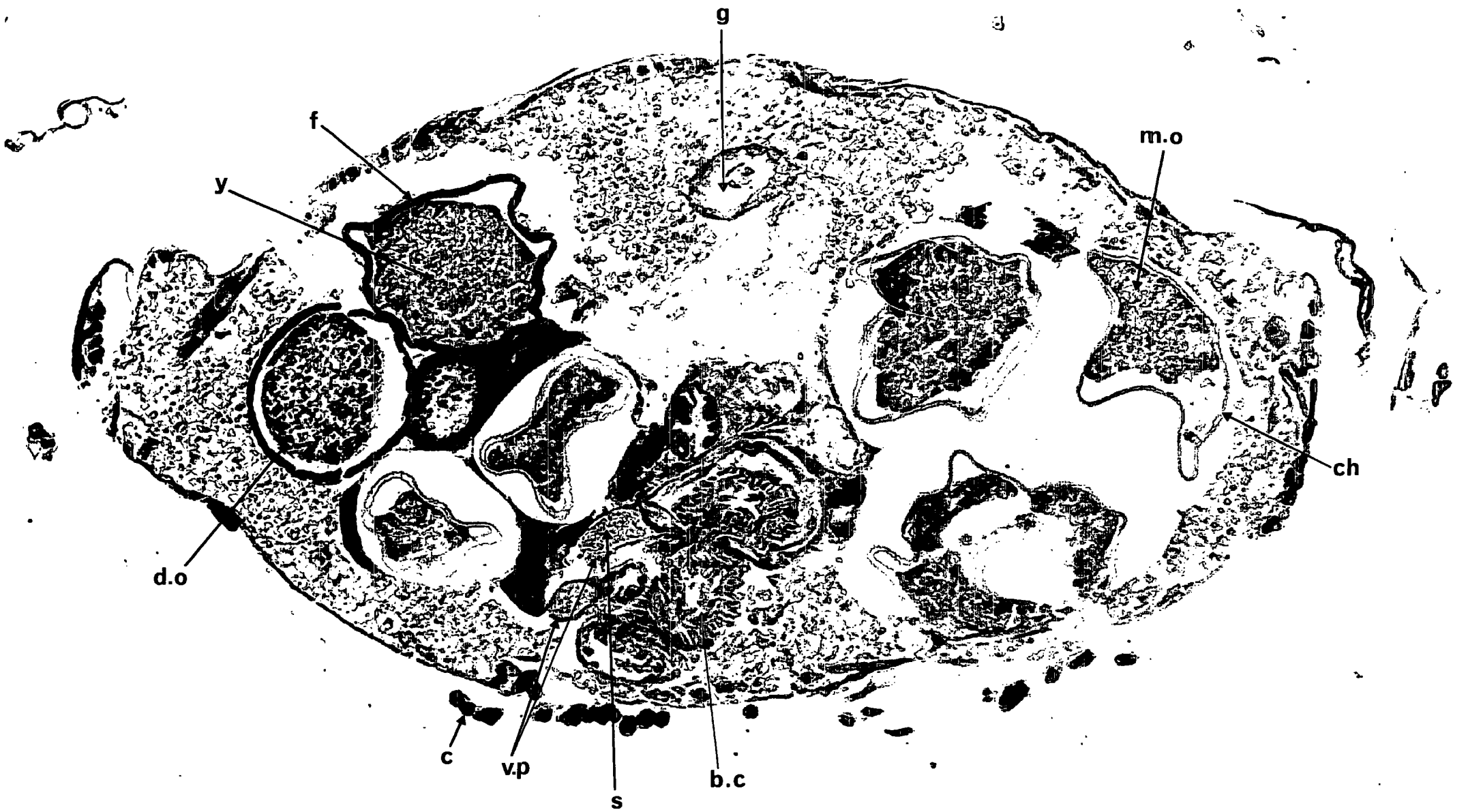
Figure 27. Micrograph of a T.S. through the abdomen of a 4-day-old virgin female Stegobium. Five mature, chorionated oocytes can be seen in this section. The vaginal pouches packed with symbiotic yeasts are present and the muscular bursa copulatrix can be seen. Abbreviations as for figure 24.


g
f
y
m.o
ch
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v.p
s
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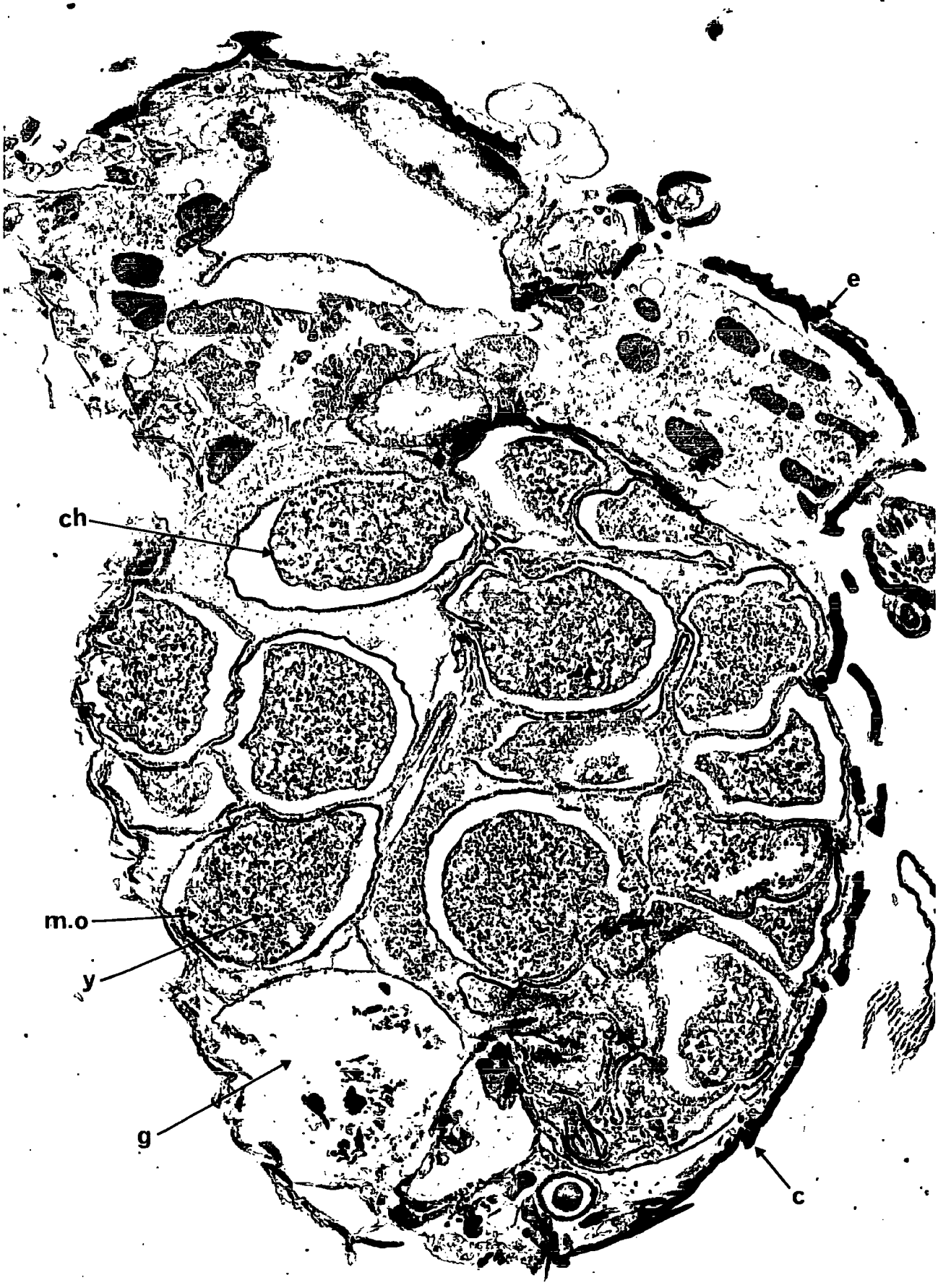
Figure 28. Micrograph of a T.S. through the abdomen of an 8-day-old virgin female Stegobium. The abdomen is tightly packed with mature, chorionated oocytes. Abbreviations as for figure 24.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y
g
c

Tenebrio molitor (Schlottman and Bonhag, 1956).

D. Copulation

(a) Copulatory behaviour in Stegobium

Copulation was observed in several pairs of beetles and behavioural details recorded.

The male approaches the female from the rear, stroking her elytra with the antennae and mouthparts. A receptive female usually stops moving, presumably in response to this stimulus, although males often attempt to mount while the female is walking, usually with little success. The male climbs on to the back of the female, usually supporting the posterior pair of legs on the substratum. Antennal stroking by the male continues, with the anterior pair of legs also being used. The male attempts genital contact after a few seconds, extruding the aedeagus: these attempts alternate with renewed bouts of stroking the thorax and elytra of the female until successful coupling is achieved. At this point, the male turns through 180° and the two beetles assume a back-end to back-end position. The pair may remain in contact for up to 2 h, usually motionless, until the female, who is usually the partner to break contact, walks away. The male is pulled along behind until he is finally detached.

A male encountering a pair "in copulo" has frequently been observed to mount the female and successfully disconnect the pair. The second male may or may not then copulate with the female, depending upon her receptiveness.

Copulatory behaviour has been described for Lasioderma serricorne (Tobin and Smith, 1971; Coffelt and Burkholder, 1973) which also assumes the back-to-back position. The Tenebrio molitor male remains on the back of the female throughout copulation (August, 1971) and Trogoderma granarium assumes a final position at 90° to the female (Karnavar, 1972).

(b) Timing of sperm transfer during copulation

Copulation in Stegobium has been described by several authors, including Janisch (1923), Azab (1943) and Kashef (1956), much as outlined above.

However, these workers all suggest that sperm transfer occurs during the initial period before the back-to-back position is assumed, being completed in only a few seconds. The following experiment was designed to indicate the time during which transference of the spermatophore occurs by interrupting copulation at varying intervals after initial contactwas made.

Four groups of 5 pairs of virgin beetles (one of each sex) 6-7 days old, were placed beneath inverted petri dishes, on filter paper, and observed until copulation occurred.

Group 1 pairs were separated at the moment when the male was turning into the back-to-back position.

Group 2 pairs were interrupted 1 min after they had turned back-to-back.

Group 3 pairs were interrupted 5 min after they had turned back-to-back.

Group 4 pairs were left to complete copulation.

The females from these groups were retained in individual tubes with a very small quantity of wheatings as an oviposition medium, which is essential for this species. The filter paper in the closure of the tube (page 13) was also utilised by females as a substratum suitable for oviposition. Each morning, the entire contents of the tube were checked with the aid of a low-power microscope for eggs, which were destroyed with forceps as they were counted. A daily record of egg laying was thus kept for each female until death. Table 13 shows the results of these egg-counts.

The sample sizes are too small for statistical analysis of these results, but it is suggested that the spermatophore is not transferred to the female until the back-to-back position has been assumed and maintained for at least 1 min, preferably longer, for successful copulation.

The eggs produced by female 5 in group 1 were probably infertile as indicated by the time when they were laid. This phenomenon will be discussed further in part E below.

Table 13 Daily oviposition records of individual females which were previously separated from the male at certain stages during copulation (see text).

female group	no.	no. days after copulation 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	total no. eggs	mean no. eggs per female
	1																0	
	2																0	
1	3																0	1.2
	4																0	
	5												3		2	1	6	
	1		3	9	28	6											46	
	2		23	9													32	
2	3																0	18.2
	4		9	4													13	
	5																0	
	1	36															36	
	2	12		2	2	4	1										21	
3	3	9	1	7		2		1									20	31.8
	4	35	1														36	
	5	35	11														46	
	1		3	6	1												10	
	2	28															28	
4	3	23	1	5	3												32	26.8
	4	31	2	1	1												35	
	5	27	1		1												29	

Table 14 Daily oviposition records of female Stegobium kept in isolation over their adult life span.

female	no. eggs laid per day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	total
1																		3	3	2	1	d				9
2																	1	1	d							2
3																					d					0
4																		d								0
5																									d	0
6																							d			0
7																								d		0
8																			d							0
9																						d				0
10																			2	3	4	1	d			10
11																								d		0
12																				4		d				4
13																								d		0
14																							d			0
15																					d					0
16																						1	d			1
17												1						2	1		d					4
18																				d						0
19													3	2			1		d							6
20																					d					0
21																			d							0
22																				d						0
23																				d						0
24																					1		d			1

d = day when female found dead

Table 15 Daily oviposition records of female Stegobium kept with a male of the same age over their adult life span.

female	no. eggs laid per day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	total
1						5	3	1*											d					9
2					27	15	4	4	9	9	3						d							71
3					5	6	4	2*	1	7	5					d								30
4					38			*			*						d							38
5					2	15	7	6	13*	2							d							45
6					1*	14	2	1*	4		3									d				25
7				*																		d		0
8				14	14		5	5	5	2				1				d						47
9						2	13	1	9	7	3		d											35
10				1	22	28	5	3	2	12	2			1			d							76
11					17	25	5	1	1		*					d								49
12				4	12	6	4	2	15	14	2	*	4	5			d							68
13																							d	0
14					28	8	6	2		9*		1	4	3	1	d								57
15					18	5	3	2	3								d							32
16					27	10	4	3						d										44
17								1	3	19	9							d						32
18					18					11			1d											30
19						3	4			4										d				11
20					27	9	7	6	16				d											65

d = day when female found dead
* = pair found copulating

Table 15 continued

female	no. eggs laid per day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	total
21						18	6	5	9	2			1	8	*							d		49
22				7	28			1	15		4*		3	2		d								60
23				15	21	8	6	2	7	6*								d						65
24						12	25	13	15	9*			6	5			d							85
25				*	24	13	7	6					2			d								52
26																						d		0
27					12	13	5	8	7	3	1					d								49
28						4	5	2	1	2				d										14
29				10	9	10	5	4	10	6	7					d								61
30					24	14	9	5	3	8	4						d							67
31					13	3	1						1						d					18
32				21	18	6	4	2*	12	4			d											67
33					***	19	3	3	6	2	d													33
34					31	1	1		17					1					d					51
35				3	23	3	2	1	1	d														33

E. Oviposition

(a) Virgin and mated females

Two groups of 1-day-old female Stegobium were kept in individual tubes with a small quantity of wheatings, as described in D(b) above. A male of the same age was placed with each female of (what will henceforth be called) the mated group, whilst the virgin group were left in isolation. All tubes were examined each morning for eggs, and again the eggs were destroyed as they were counted, except those produced by virgin females. The day on which each female was found dead was also recorded.

The daily oviposition records for the virgin and mated groups are shown in tables 14 and 15 respectively. Table 16 provides an analysis of the results. All eggs produced by virgin females, were infertile, and far fewer

Table 16 Oviposition records and adult life span of virgin and mated female Stegobium from the individual egg-counts in tables 14 and 15 respectively.

females	mean no. eggs per female ± SE range in ( )	mean day of death of female ± SE range in ( )
virgin	1.54 ± 0.57 (0-10)	21.54 ± 0.41 (18-25)
mated	41.67 ± 3.30 (0-85)	15.31 ± 0.47 (4-18)

in number than was recorded for mated females.

Egg-laying has previously been monitored for Stegobium under various conditions of temperature and humidity. Janisch (1923) obtained a mean of 50 eggs/female, but environmental conditions were not given. Dick (1937) recorded 56.8 eggs/female at 27°C and 73% r.h. and Azab (1943) calculated a mean of 46.6 eggs/female at 30°C and 75% r.h. Kashef (1956) at 24°C and 45% r.h. obtained 58.9 eggs/female. Lefkovitch (1967) found that 22.5°C and 65% r.h. was optimum for maximum egg production for Stegobium females, which produced about 75 eggs/female.

The oviposition results reported by Dick (1937) are of interest in that females always produced about 50% of their eggs on the first day of laying (which was in each case, day 6) and this was followed by a sharp drop in oviposition, and then a small peak 2-3 days later. This consistency cannot be explained, and the patterns of oviposition recorded by the other workers mentioned above, were much more in line with my own results.

The life span of mated females (table 16) was reduced to about 71% of that of virgin females, or shortened by roughly 6 days. This is to be expected since non-feeding adult females channel their food reserves into egg production. Virgin females presumably do not deplete their food store so rapidly and thus survive considerably longer. A more extreme example of this phenomenon was shown by Karnavar (1972) in Trogoderma granarium in which mated females live 4-7 days, virgins live 20-25 days. Mated Trogoderma angustum Solier females lived 9 days at 35°C and virgins, 13 days (Philipp, 1968).

It is relatively common for virgin females to produce infertile eggs before they die in non-parthenogenetic species. Virgin female Tenebrio molitor and Tribolium confusum (Duv.) were shown to produce small numbers of infertile eggs steadily throughout their life span (Dick, 1937). The same author, however, stated that Stegobium virgin females fail to lay eggs. Virgin female Bruchus quadrimaculatus Fab., (Larson and Fisher, 1924); Calandra granaria L., (Back and Cotton, 1926) and the anthribid beetle Aracerus fasiculatus de Geer (Taher el Sayed, 1935) all produce a small number of infertile eggs.

(b) Effect of multiple mating on oviposition

During the previous oviposition experiments, copulation was observed in some pairs of beetles after oviposition had begun. The significance of such multiple mating was investigated with particular interest in connection with the unexplained results obtained from the air extract assays of mated females (Section VI, B(b)).

Oviposition was monitored, as previously at daily intervals, but females

were kept with males of the same age for 3, 4, 5, 6 or 9 days before permanent separation, the pairs having been established on day 1. A further group was set up with 1-day-old females and 6-day-old males. This group was separated at the end of day 3 as the first group above.

Figure 29, table 17 shows the number of eggs produced by females under these conditions and the percentage of females laying. Although the

Table 17 Effect of the presence of male Stegobium for varying periods on female oviposition.

no.days male kept with female	n	percent of females producing eggs	mean no. eggs per female ± SE (of those laying)	comparison of means $t_{(n_1+n_2-2)}$	P
3	33	24.3	22.1 ± 4.3		
3'	30	60.0	56.8 ± 5.3	5.12	<0.001
4	26	53.8	57.5 ± 5.4	5.16*	<0.001
5	33	54.5	48.4 ± 4.4	1.30	NS
6	33	75.8	49.7 ± 2.7	0.25	NS
9	31	90.3	42.4 ± 3.3	1.70	0.1-0.05
life	36	91.7	41.7 ± 3.8	0.15	NS

3' 6-day-old males placed with 1-day-old females

* comparison between 3 and 4-day groups

standard errors of the mean number of eggs/female are large, the results would suggest that the longer males are kept with females:

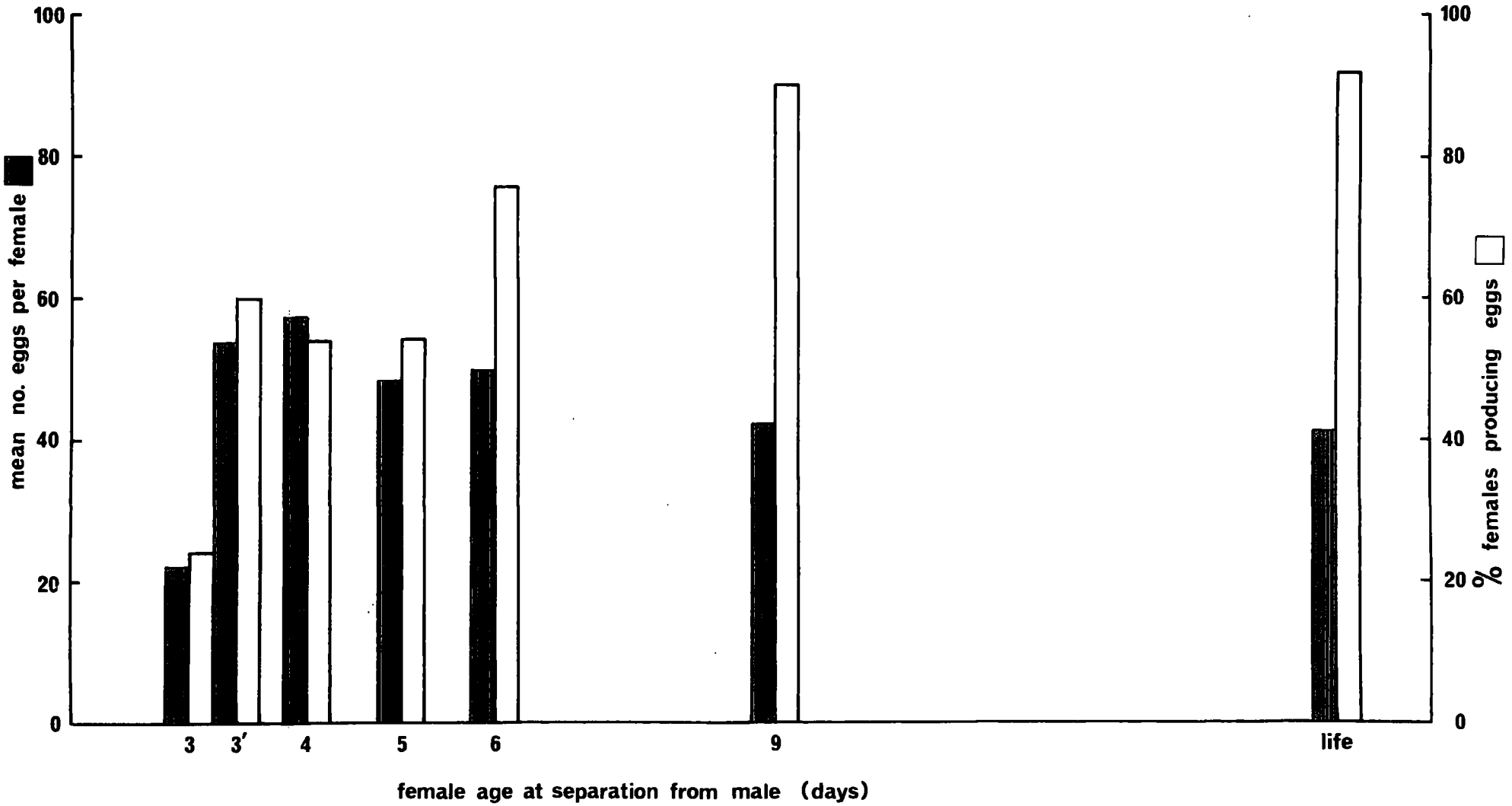
(i) the greater the percentage of females which produce eggs but

(ii) the smaller the number of eggs laid/female.

The latter may reflect reduced fertility amongst females copulating at a later stage in their life cycle. For example, of the 46% of females separated from males after 4 days which failed to lay eggs, the majority would eventually have become fertilised, but they would have produced fewer eggs.

Since a much higher percentage of females separated from older males after 3 days (3' group) produced a very high yield of eggs per female

Figure 29. The effect of male presence on female oviposition. Both the percentage of females which produced eggs and the mean number produced per female (of those laying) were estimated, according to the length of time males were kept with females. Both sexes were 1 day old when placed together, except for the 3' group, where the males were 6 daysold when placed with 1-day-old females.


100
80
60
40
20
0
mean no. eggs per female
100
80
60
40
20
0
% females producing eggs
3
3′
4
5
6
9
life
female age at separation from male (days)

(table 17, fig. 29), it is assumed that males up to 3 days old (or over 70% of them) are immature, fail to stimulate females to mate or are unresponsive to the sex pheromone at this stage.

There was no difference statistically between egg yields of females separated from males at 9 days and those from unseparated pairs. However, the former group produced fewer eggs than females isolated from males at 5 and 6 days. Azab (1943) showed that young female Stegobium produced more eggs and took longer to lay them than older females and that fecundity decreased with reduction of fat reserves. Figure 30, table 18 confirms the trend that the earlier oviposition begins, the more eggs are produced by females of pairs left together throughout the oviposition period (data from table 15).

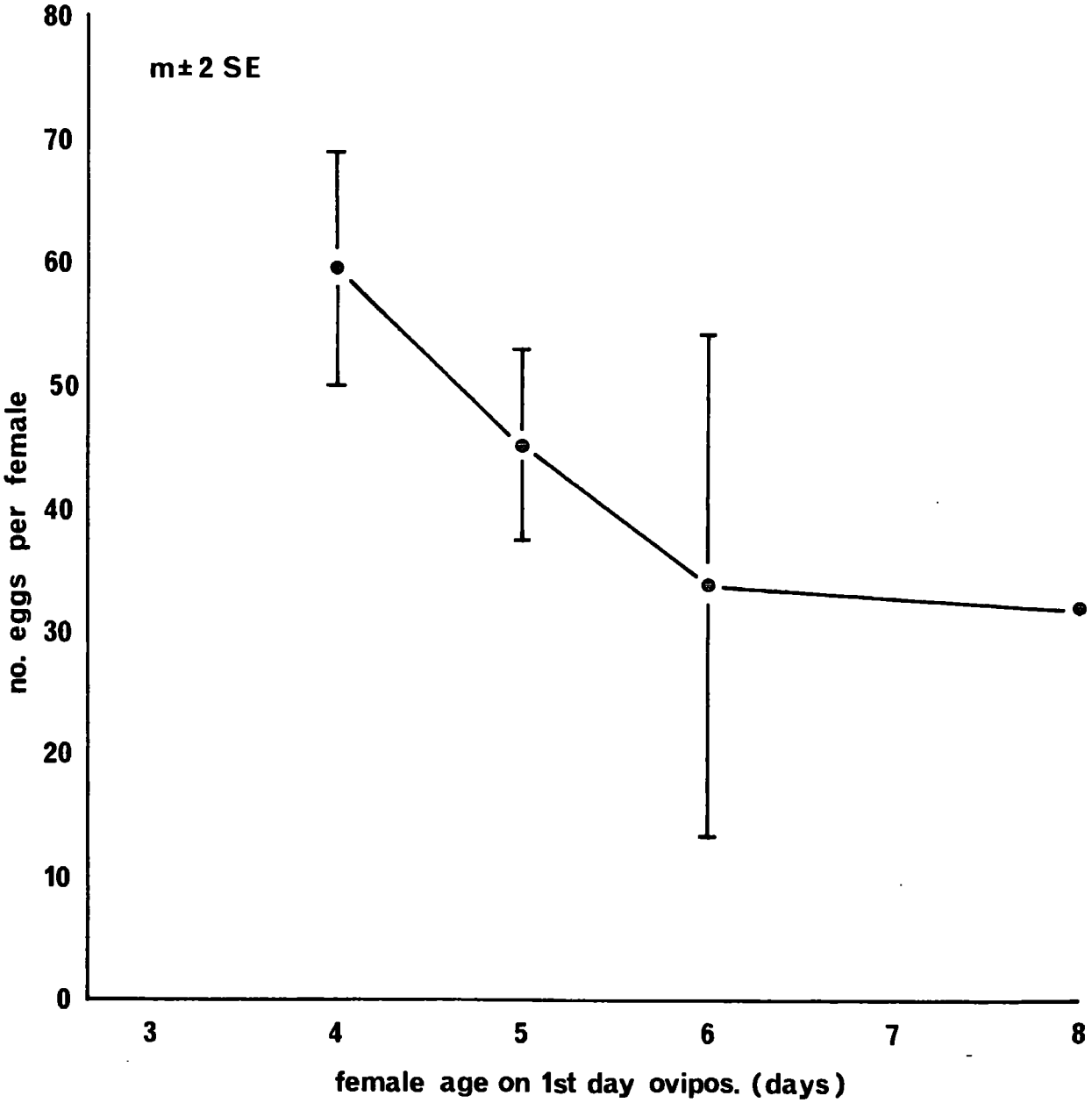
Table 18 Relationship between female age on the first day of oviposition and the mean number of eggs produced per female.

age female on first day oviposition	n	mean no. eggs per female ± SE	comparison of means $t_{(n_1+n_2-2)}$	P
4	8	59.6 ± 4.8	2.34	0.05-0.02
5	16	45.2 ± 3.9	1.05	NS
6	7	33.7 ± 10.2		
8	1	32.0		

In a comparable study, Kashef (1956) separated males from females on the third day of oviposition (which would approximate to day 6 in the scheme presented here) and he found that separated females produced fewer eggs (47.9 per female) than females from pairs left together (58.9 per female). Although there was no significant difference between these values, the result was completely contradictory to my own.

While recording daily egg counts of pairs of beetles kept together permanently (table 15) a record was kept of couples found copulating. Of

Figure 30. The relationship between the first day of oviposition of female Stegobium and the total number of eggs produced over the oviposition period.


m ± 2 SE
80
70
60
50
40
30
20
10
0
no. eggs per female
3
4
5
6
7
8
female age on 1st day ovipos. (days)

the 32 females which produced eggs, a second copulation was observed in 13 pairs (41%). The day on which this was recorded ranged from 5-15 inclusive with a mean of day 10. In 8 of the 13 cases (62%) the second copulation was followed by a further small batch of eggs. Figure 31 shows the daily oviposition records of the 13 females which were observed to copulate more than once. As the observation period extended for only 1-2 h per day, the 13 females are likely to be only a sample of those which did repeat copulation.

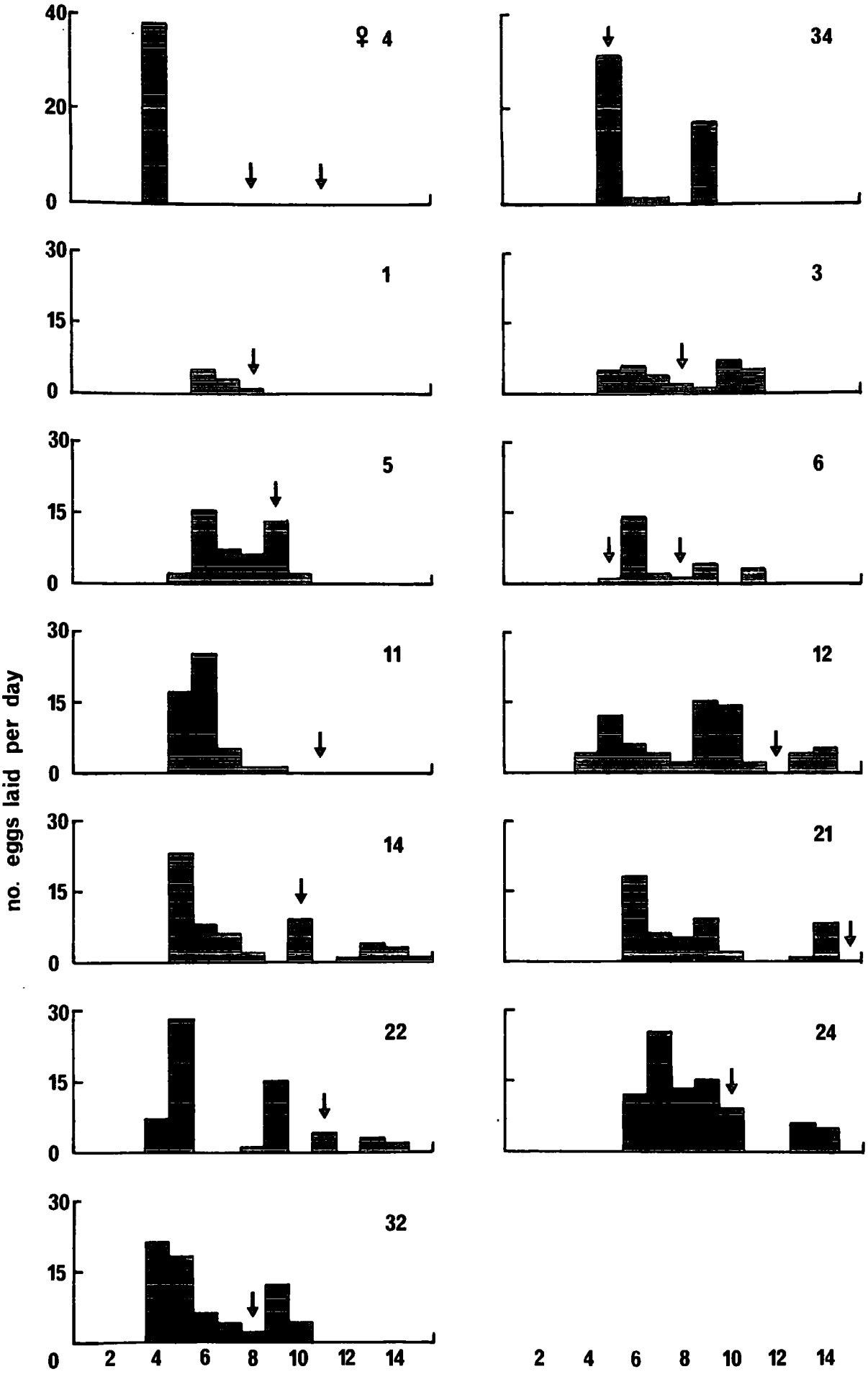
F. Discussion

The purpose of the study presented in this section was to monitor precisely female maturation, reproductive behaviour and oviposition under certain conditions in order to provide a background against which the foregoing details of the sex pheromone system become meaningful and some of the anomalies clarified. In some respects this has been successful but it is evident that much more work on this particular aspect of the study is necessary. Such work would include investigation of egg fertility and its relation to multiple mating, sex pheromone release by individual females and a parallel monitor of oviposition.

Dissection and histological work has shown that at 30°C and 75% r.h. mature oocytes are present in the calyx at 3-4 days, corresponding with the initiation of sex pheromone release. By 4-5 days, both systems are fully operative and synchronisation is apparent.

Multiple mating is not normally considered characteristic of short-lived species (Dick, 1937; Wigglesworth, 1972) and does not appear to increase the number of eggs laid per female in Stegobium. It must be stressed, however, that the proportion of fertile eggs may be influenced by further copulation. Trogoderma angustum females require several copulations to produce a full egg complement and high fertility, (Philipp, 1968). This species has a non-feeding adult with a life span similar to Stegobium. Karnavar (1972) found that Trogoderma granarium females mated 3-4 times, the

Figure 31. Daily oviposition records of 13 females which were seen to copulate after oviposition had begun and were therefore presumed to be mating for a second (or third) time. The numbers refer to those in table 15.


♀ 4
34
1
3
5
6
11
12
14
21
22
24
32
no. eggs laid per day
age of females (days)

second mating occurring 12-24 h after the first batch of eggs were deposited. Twice the number of eggs were produced after the second mating. In Drosophila melanogaster Meigen the fertility of females increased with the length of time females were associated with males, as did total egg laying (Chiang and Hodson, 1950). Trogoderma inclusum females pass through a refractory period of at least 2 days before remating, before which about 75% of their eggs are laid (Vick, Burkholder and Smittle, 1972). A similar situation may be present in Stegobium, whereby the first large batch of eggs is laid, but a second copulation maintains maximum fertility of successive batches.

VIII DISTRIBUTION OF SENSILLA ON THE ANTENNAE OF MALE *STEGOBIUM* AND THE EFFECTS OF ANTENNECTOMY ON MALE RESPONSE TO THE PHEROMONE

A. Introduction

The subject of insect olfaction has received renewed interest in recent years, possibly inspired by work on pheromones and demonstration of the ability of some insects to respond to odours at extremely low molecular concentrations. *Bombyx mori* has a theoretical response threshold of 200 bombykol-induced impulses per second elicited by 200 molecules of bombykol (the silk moth sex pheromone) (Kaissling and Preisner, 1970).

Electroantennogram recording from whole antennae or single sensilla has provided a valuable insight into threshold response levels, habituation and the electrophysiological functions of the insect chemoreceptor apparatus.

At the molecular level, there is still disagreement between two main schools of thought about the nature of olfaction. The stereochemical theory (Amoore, 1952) suggests that molecular configuration determines odour quality, whereas the vibrational theory (Wright, 1954) designates more importance to molecular vibration. An appraisal of both theories is given by Amoore (1971).

It is thought that when a pheromone molecule makes contact with the appropriate antennal sensillum, it combines with a receptor protein to trigger an enzymatic reaction producing a small chemical "transmitter" molecule which depolarises the dendritic membrane (Riddiford, 1971). Once this has occurred, the molecule must be metabolised quickly to prevent either continued stimulation, or blockage of the receptor, both of which might give the insect false information. Experiments with bombykol have suggested that by the activity of two different enzyme systems, the pheromone molecule is transformed into fatty esters and fatty acids (Kosang, 1971).

The principal sites of chemoreceptors in insects are the antennae, maxillae and labial palps, legs and ovipositor. The latter four areas are largely associated with contact chemoreception (Dethier, 1963). In most recorded cases, sex pheromone receptors are located on the antennae, and if

the segments are differentiated, then they are usually found on the flagellum or club segments (Schneider, 1964).

The antennae of Stegobium comprise 11 segments of which the distal three are expanded into a loose club (fig. 32a).

A superficial study of sex pheromone perception in Stegobium is given below. The location of sensilla thought to be concerned with sex pheromone perception has been studied by means of ablation experiments, and the types and distribution patterns of sensilla are described.

B. Characteristics, distribution and abundance of antennal sensilla studied by light and scanning electron microscopy.

(a) Methods

For light microscopy studies, antennae were removed from individual beetles of known sex, cleared in xylene and mounted in D.P.X. mountant. An oil immersion lens was necessary to distinguish the types of sensilla present, and to measure their length. A calibrated eye piece graticule was used for measurements, and a grid eyepiece aided the mapping and estimation of numbers of sensilla types on the antennae. For this technique, antennae were mounted in D.P.X. between two coverslips so that both sides of a single antenna could be viewed using an oil immersion lens. Only three antennae were mapped in all (two male and one female) since practical difficulties at these high magnifications rendered this an extremely slow and highly inaccurate method. Sensilla along the edges of the antennal segments were probably counted twice and the areas of the "sensory fields" (part (b)(ii) below) were always viewed at a poor angle because of the shape of the antenna and its aspect when mounted. Figure 32d shows the view of a sensory field as it appears when mounted for light microscopy.

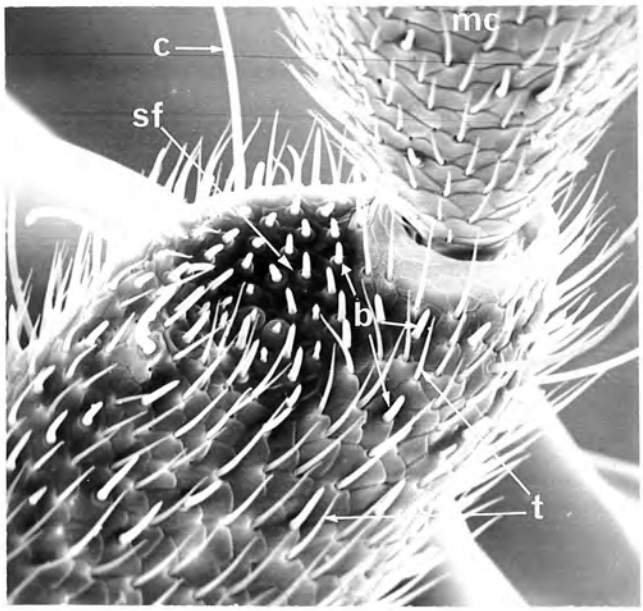
A photographic method would be essential to increase the accuracy of sensilla counts, ideally involving more extensive use of a scanning electron microscope.

The material for SEM was prepared by the Electon Microscopy Unit, School of Chemistry of the University of Newcastle-upon-Tyne. Specimens were

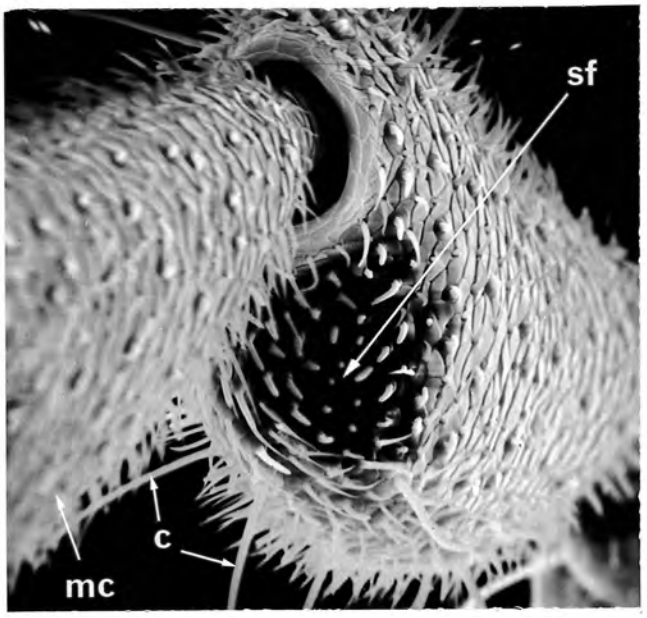
Figures 32-35. Scanning electron micrographs of Stegobium antennae.

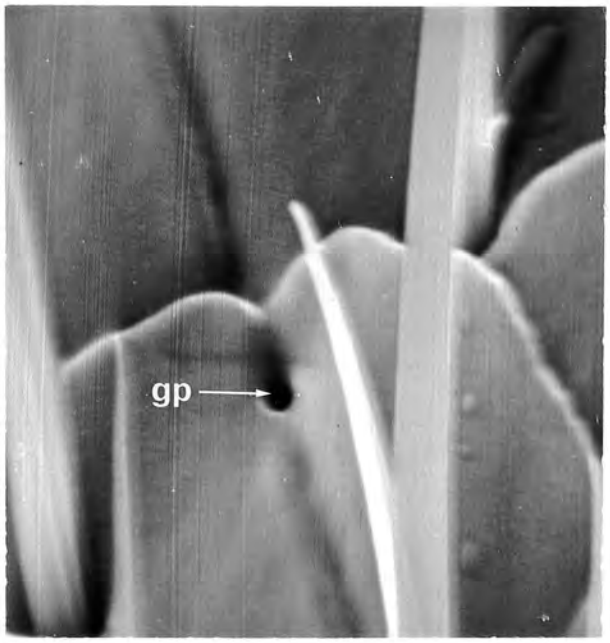
b = sensillum basiconicum
c = " chaeticum
ce = compound eye
co = sensillum coeloconicum
dc = distal club segment
f = funicle segment
gp = hypodermal gland pore
mc = middle club segment
pc = proximal " "
s = scape
sf = sensory field
t = sensillum trichoideum

a



b





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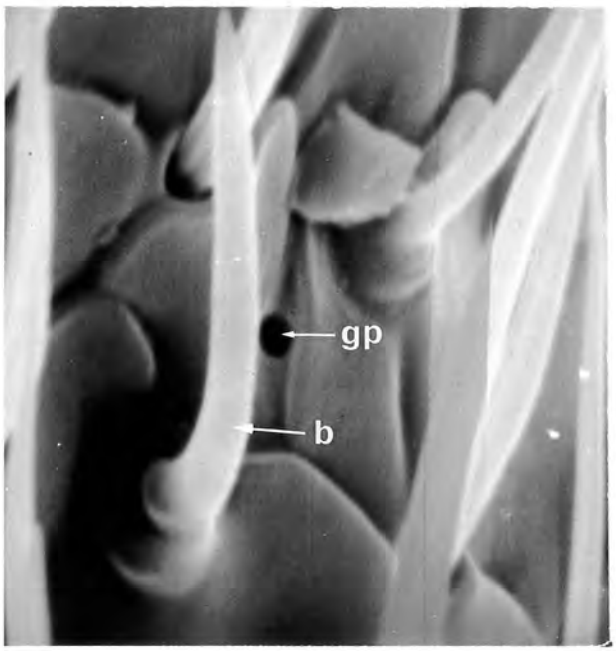


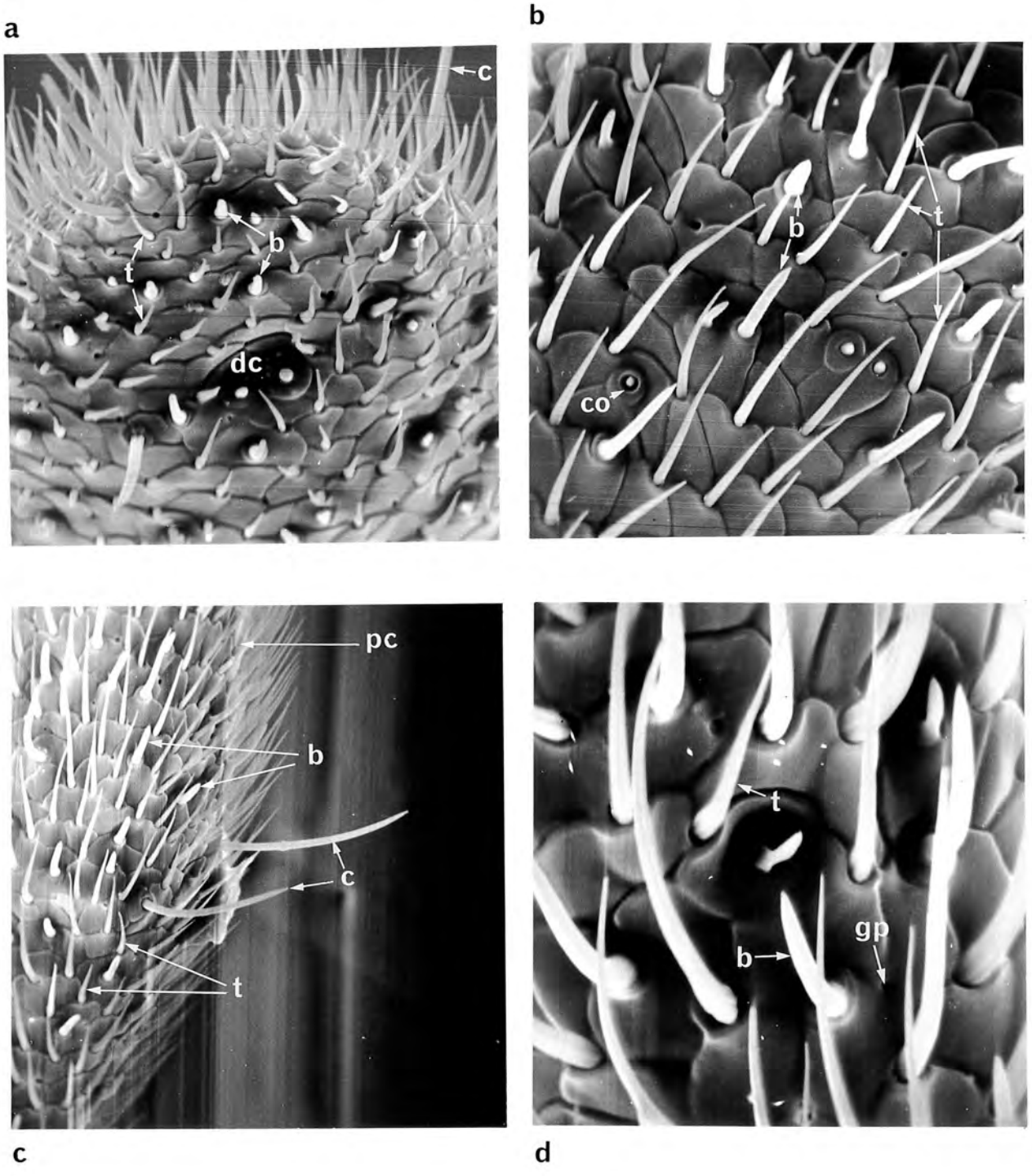
d

Figure 32 a. Male (x 178)
Left side of head showing compound eye and the left antenna. The differentiation of the antenna into scape, funicle and club is clearly shown and the position of the sensory fields indicated.

b. Male (x 1,720)
Junction between the proximal club segment and funicle showing differences in cuticular sculpturing and sensilla distribution.

c. Female (x 1,750)
Dorsal edge of the basal club segment showing a high density of trichoid sensilla (compare with fig. 34b).

d. Female (x 2,000)
Sensory field of middle club segment from one side. This is the view obtained when using light microscopy with mounted antennae.



c

d

Figure 33 a. Female (x 530)
Middle club segment shown from above with the distal club segment removed. The sensory field is clearly visible.

b. Female (x 2,150)
As above showing the sensory field at a higher magnification.

c. Female (x 910)
Sensory field on the proximal club segment and the junction with the middle segment.

d. Female (x 4,600)
As above at a higher magnification. The small sensilla basiconica typical of these areas are clearly visible.

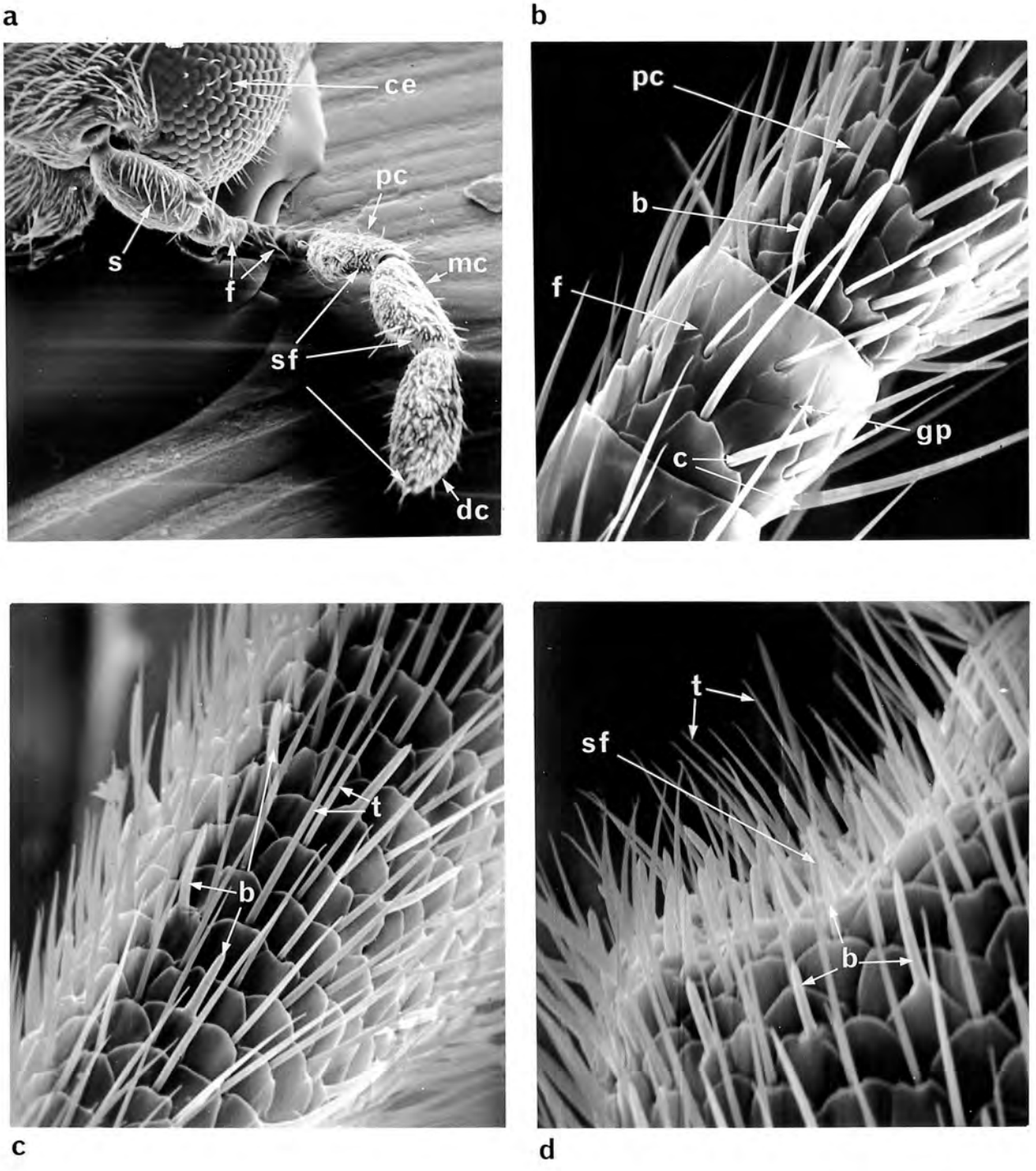

a
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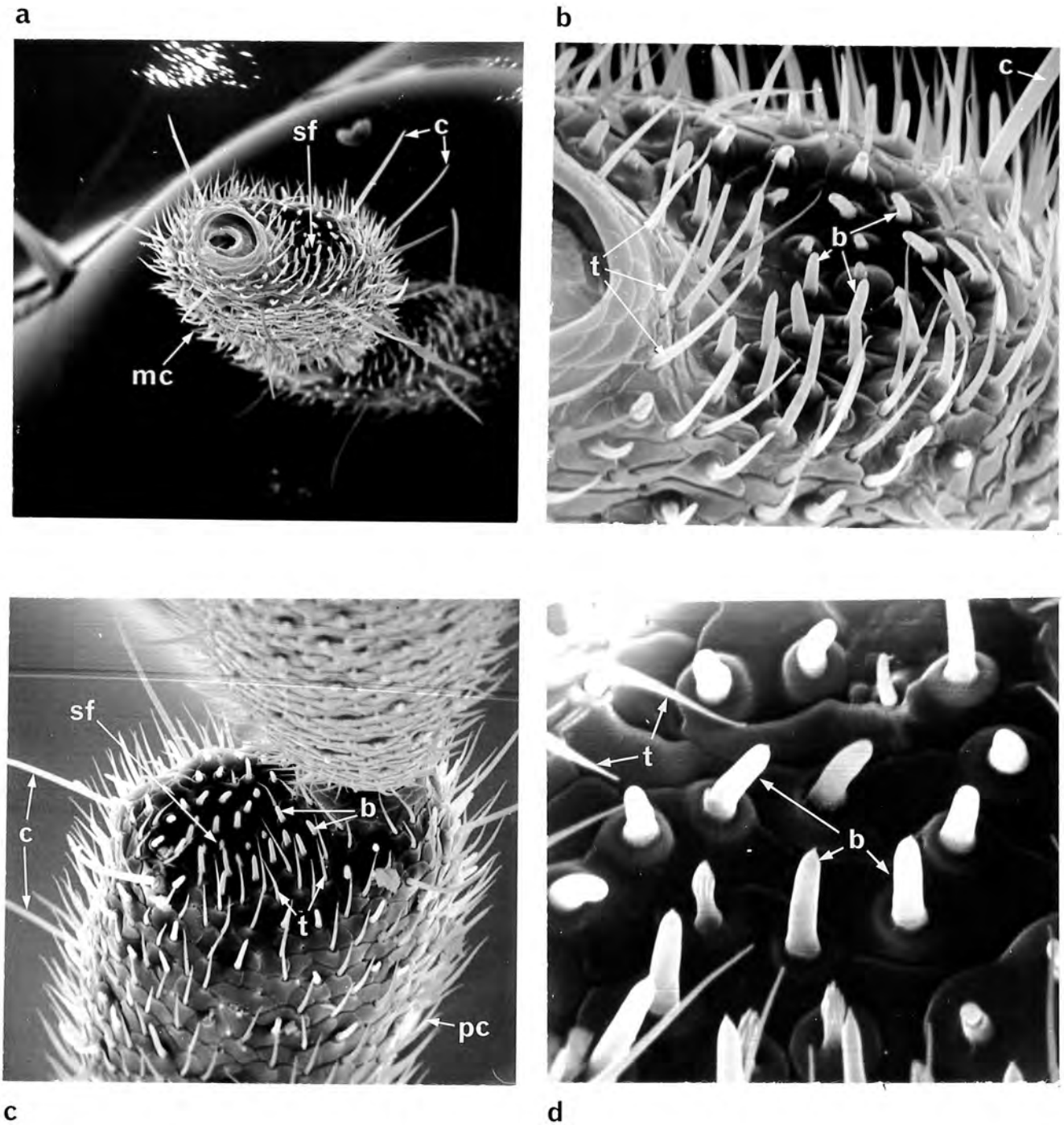
Figure 34 a. Female (x 1,820)
Extreme tip of the distal club segment showing the sensory field.

b. Female (x 2,220)
Area of the flattened surface of the distal club segment showing the comparative size and distribution of sensilla compared with the previous view.

c. Female (x 1,160)
Sensilla chaetica projecting almost at 90° from near the base of the proximal club segment.

d. Male (x 4,500)
Near the apex of the middle club segment showing a typical basiconic sensillum and two infrequently found club-shaped sensilla.


a
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sf
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pc
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d

Figure 35 a. Female (x 900)
Sensory field on the proximal club segment to be compared with that of a male (fig. 35 b.)

b. Male (x 960)
As above, showing the close similarity between the sensilla types and distribution of the sensory fields of both sexes.

c. Female (x 10,000)
Opening of a hypodermal gland pore

d. Male (x 9,000)
As above

vacuum-dried and surface-coated with gold (Echlin, 1971). A representative series of micrographs was produced in order to show the range of sensilla present and their distribution, but not to provide a quantitative assessment. A sample of micrographs is shown in figures 32-35.

The nomenclature adopted below for antennal sensilla types follows that formulated by Schneider (1964) and is generally accepted by most workers in this field.

(b) Antennal sensilla of Stegobium

Four distinct morphological types of sensilla were distinguished in Stegobium by means of the techniques described above.

(i) Sensilla chaetica

These are long (22.5-48.0 μm), thick-walled sensory bristles, tapering to a point with an articulated basal socket. They were found widespread over the surface of the body and were not confined to the antennae. Most sensilla chaetica were found on the distal surface of each club segment where they protruded almost at 90° from the surface of the antenna (figs. 33a, 34c), but greater concentrations were found on the other segments, especially the expanded basal segment or scape (fig. 32a).

Sensilla chaetica are thought to have a mechano-receptive function. This is suggested by their length (extending beyond the other sensilla), the articulated socket and the thick cuticularwall which excludes most other possible functions.

The structure of these tactile receptors has been described, much as above, for many other insect species. Payne, Moeck, Willson, Coulson and Humphreys (1973) found sensilla chaetica in 16 species of scolytid beetles studied. The ambrosia beetle, Trypodendron lineatum (Olivier) has sensilla chaetica of about 40 μm in length present in greater numbers than Stegobium, on the expanded club segment (Moeck, 1968).

(ii) Sensilla basiconica

Basiconic sensilla are found only on the three club segments of the

antennae of Stegobium. They are short, thin-walled pegs that may appear conical or cylindrical, tapering to a blunt point. These sensilla range from 3-12 μm in length and may be of two types. The smaller (3-6 μm), straight, round-tipped type (fig. 33d), often with an enlarged cuticular basal area (figs. 33b, 33d) is found mainly at the apices of the club segments. The longer type (6-12 μm) is curved at the base with a more pointed tip (fig. 35d) so that the sensillum lies almost flat against the surface of the antenna, pointing distally. Payne et al (1973) described a short (3-8 μm) and long (6-20 μm) basiconic sensilla type in scolytid beetles. The former type were round-tipped, flask-shaped pegs and the latter, long, cylindrical and pointed. Moeck (1968) also found two size classes of sensilla basiconica, 6-8 μm and 14-18 μm, both pointed, in Trypodendron lineatum. In Necrophorus sp. two sensilla basiconica types were distinguished (Ernst, 1969) of length : diameter proportions, 7:1 and 5:1 respectively.

The longer sensilla basiconica in Stegobium were found fairly evenly distributed over the club segments, but the shorter type were more or less confined to areas which will hereafter be called sensory fields. These were situated at the distal surface of the two basal club segments (figs. 32a; 33a, b, c; 35 a, b) in a flattened, slightly concave area near the point of articulation with the next distal segment. A larger, although less dense, sensory field was situated at the apex of the distal club segment (fig. 34a).

Very distinct sensory fields have been described for the 16 species of scolytids studied by Payne et al (1973). They have, typically, 2-3 distinct bands of basiconic sensilla completely encircling the club segment, sometimes with an additional band of trichoid sensilla. A similar arrangement was found in the pine weevil, Hylobius abietis L. (Mustaparta, 1973). The blowfly, Phormia regina Desvoidy has groups of sensilla basiconica of a single type, confined to antennal pits (Dethier, Larsen and Adams, 1963). Lewis (1971) found in the muscid, Stomoxys calcitrans Geoffr. a regional separation where the basiconic sensilla were situated proximally and centrally. A less

ordered distribution of sensilla was present in the grain beetle Oryzaephilus surinamensis where basiconic sensilla are mainly distal and lateral (Arbogast et al, 1972), and in Trypodendron lineatum, basiconic sensilla are distributed over the surface of the club except near the base (Moeck, 1968).

The number of sensilla basiconica in Stegobium was estimated at 400-500 per antenna (table 19). Borden and Wood (1966) calculated a mean of 343 per

Table 19 Numbers and distribution of basiconic and trichoid sensilla on the three club segments of two male and one female Stegobium antenna.

sex	sensilla	antennal club segment			total
		distal	middle	proximal	
male	basiconica	154	163	157	474
	trichoidea	549	405	414	1368
"	basiconica	152	157	143	452
	trichoidea	452	357	387	1196
female	basiconica	113	138	151	402
	trichoidea	529	439	385	1353

male antenna and 383 per female antenna in Ips confusus (LeConte): this difference was significant. In Hylobius abietis, 4,000-5,000 basiconic sensilla per antenna were estimated (Mustaparta, 1973).

Sensilla basiconica have been proved olfactory in function in some insects, which was previously suggested from studies on the fine structure of the cuticle wall (Slifer, 1961, 1967). In all cases so far examined, the cuticle wall has been shown to be perforated by pores of about 0.1 μm in diameter, or less (Slifer, Prestage and Beams, 1959). Dendritic nerve endings are thought to terminate close to these pores which bring them in direct contact with the air (Slifer, 1961; Dethier, 1963). These pores were not visible either in the light or SEM preparations of Stegobium antennae which would be expected, since EM sectioning is necessary to study the fine structure of these sensilla. The structure of the basiconic sensillum is

thus ideal for sampling air surrounding and streaming over the antennae.

By means of electroantennogram recording, Schneider and Boeckh (1962) presented direct evidence for the olfactory function of the basiconic sensilla of Necrophorus, Tenebrio and some saturniid moths. Morita and Yamashita (1959) recorded directly from a single large sensillum basiconicum on the antenna of a larval silk worm, Bombyx mori and its function was determined as olfactory.

A hygroreceptive function has been attributed to basiconic sensilla in Tenebrio and Dermestes sp. (Roth and Willis, 1951) and Oryzaephilus surinamensis (Arbogast et al, 1972). There may therefore be some degree of functional diversity of these receptors, or specialisation amongst the sensilla according to function.

(iii) Sensilla trichoidea

These hair-like sensilla are slender, tapering and sharply pointed, with no visible external socket. They form the majority of the sensilla types on Stegobium antennae, numbering between 1,100-1,400 per antenna for both sexes (table 19). Sensilla trichoidea were found in greater density towards the periphery of the club segments as opposed to the flattened surfaces (fig. 32c). Length varied from 13.5-21 μm and no real structural differences could be distinguished over this size range. The trichoid sensilla surrounding the sensory fields were generally shorter, with a more pronounced curvature (figs. 33b, c, d; 35a, b) but this is thought to reflect the tendency of the sensilla to follow the curvature of the antenna.

Moeck (1968) and Payne et al (1972) distinguished between three types of trichoid sensilla, differing in length, curvature and socket attachment in Trypodendron lineatum and scolytid beetles respectively. Those of Stegobium correspond most closely to the sensilla trichoidea type II which were described by the above authors as being 18-25 μm in length, smooth, sharply pointed and gently curved, without a socket. Lewis (1971) described a longer (30-45 μm) and shorter (20-25 μm) trichoid sensillum type in Stomoxys calcitrans.

Most authors mentioned above consider sensilla trichoidea to be olfactory in function. They have thin cuticle walls and surface pores like sensilla basiconica, although the pores tend to be confined to the apical region of the shaft. Electro-physiological investigation has verified an olfactory function for these sensilla and Schneider (1971) has shown them to be specialised sex pheromone receptors in Bombyx mori and some other moth species (Schneider, Lacher and Kaissling, 1964; O'Connell, 1972).

(iv) Sensilla coeloconica

These short pegs are located within cuticular pits and are found in very small numbers (less than 5) on Stegobium antennae (fig. 34b). They are thought to be olfactory in function in some insect species. In Apis mellifica (L) (Lacher, 1964) some were shown to respond to carbon dioxide, and others to humidity.

The small holes seen over the surface of the antennae (figs. 34a, b, c, d) are shown at high magnification in figures 35c and d. They were also found over the rest of the body and are thought to be the openings of hypodermal gland pores, also described by Moeck (1968). These openings can be inspected by means of a high-power light microscope. Using differential focusing, a slightly coiled canal can be seen to lead to a large, oval, blind-ending sac (30-40 μm long) which is probably the cuticular reservoir of such a gland (Hinton, H.E., pers. com.).

Although electrophysiological methods would need to be employed to determine which of the sensilla types was responsible for sex pheromone perception in Stegobium, this function would certainly be performed by either the basiconic or trichoid sensilla.

There was no discernible sexual dimorphism in sensilla types, numbers or distribution in Stegobium. Had more counts of sensilla been possible, significant differences in the number of basiconica might have been found. However, despite females being greater in body length than males, the antennae of the latter have longer club segments (Fowler, 1890) and the length:width

ratio of male antennal club segments has a mean of 2.4 as compared with females, 2.34 (Griffith, 1946). This might represent an evolutionary trend towards housing more olfactory receptors specifically concerned with sex pheromone perception.

Obvious sexual dimorphism has been shown in the cockroach, Periplaneta americana (L.) where 77% of male sensilla were olfactory compared with 64% in females (Schafer and Sanchez, 1973). Borden and Wood (1966) found significantly more basiconic sensilla in the female scolytid, Ips confusus (where males produce the pheromone), but following ablation studies they suggested that sensilla trichoidea were involved in sex pheromone perception. Both sexes of Bombyx mori have sensilla trichoidea, but only males respond to the sex pheromone (Schneider and Kaissling, 1957). In most Lepidoptera studied, only males have trichoid sensilla which are the pheromone receptors (Boeckh, Kaissling and Schneider, 1960).

C. Effect of antennectomy on the response of male Stegobium to the sex pheromone

Antennectomy experiments were performed in order to confirm that the antennal sensilla described above are in fact the sex pheromone receptors and to provide some further information on the distribution of the chemoreceptive regions of the antenna.

(a) Methods

To effect partial or complete antennectomy, individual males were secured beneath a strip of paper held down on either side of the body under a low power binocular microscope. The paper was initially slackened until the head of the beetle protruded, and then tightened sufficiently to prevent further movement. A fine scalpel was used to remove the relevant parts of the antennae. This technique was found to be simple, rapid and less damaging to the individual than other methods. Amos (1969) pressed individual dried fruit beetles (Carpophilus dimidiatus F. and C. hemipterus L.) into plasticine in order to immobilise them for antennectomy. Various anaesthetics such as diethyl ether for the scolytid, Ips confusus (Borden and Wood, 1966) and low

temperature for the grain beetle, Oryzaephilus surinamensis (Arbogast et al, 1972) have been used.

Newly emerged males did not survive well after operation, but when 5-day-old beetles were antennectomised, almost 100% survival was achieved. Very little difference in activity was observed between operated and control groups and longevity was even slightly increased amongst operated males (actual values were not recorded).

(b) Results

Three groups of 5-day-old males were treated by removing 1, 2 and 3 club segments respectively from both antennae. Three days were allowed for recovery from this operation. Moribund individuals were discarded and the remaining males (at least 50 in each group) were tested with a 6v extract. The results are shown in Appendix II, table 20, figures 36 and 37. The data for the control, unoperated group was taken from section IV part C.

Table 20 The percentage response of partially, or totally antennectomised males to a 6v extract over 8-19 days of age

no. club segments of antennae remaining	n	mean % response of males of 8-19 days to a 6v extract ± SE	comparison of means	
			$t_{n_1+n_2-2}$	P
3	62	87.03 ± 4.34	9.19	<0.001
2	55	36.18 ± 3.38	1.98	0.05
1	57	28.28 ± 2.09	6.48	<0.001
0	50	0		

Removal of all three club segments from the antennae resulted in complete failure of males to respond to the 6v extract. This confirms the morphological studies indicating that the pheromone receptor sensilla are located only on the club segments. Intermediate levels of response were elicited by the 6v extract from males with 1 or 2 club segments remaining. There was less

Figure 36. The effect of removing antennal club segments from male Stegobium on their subsequent response to a 6-day-old virgin female extract. The numbers in brackets represent the number of club segments remaining on both antennae.

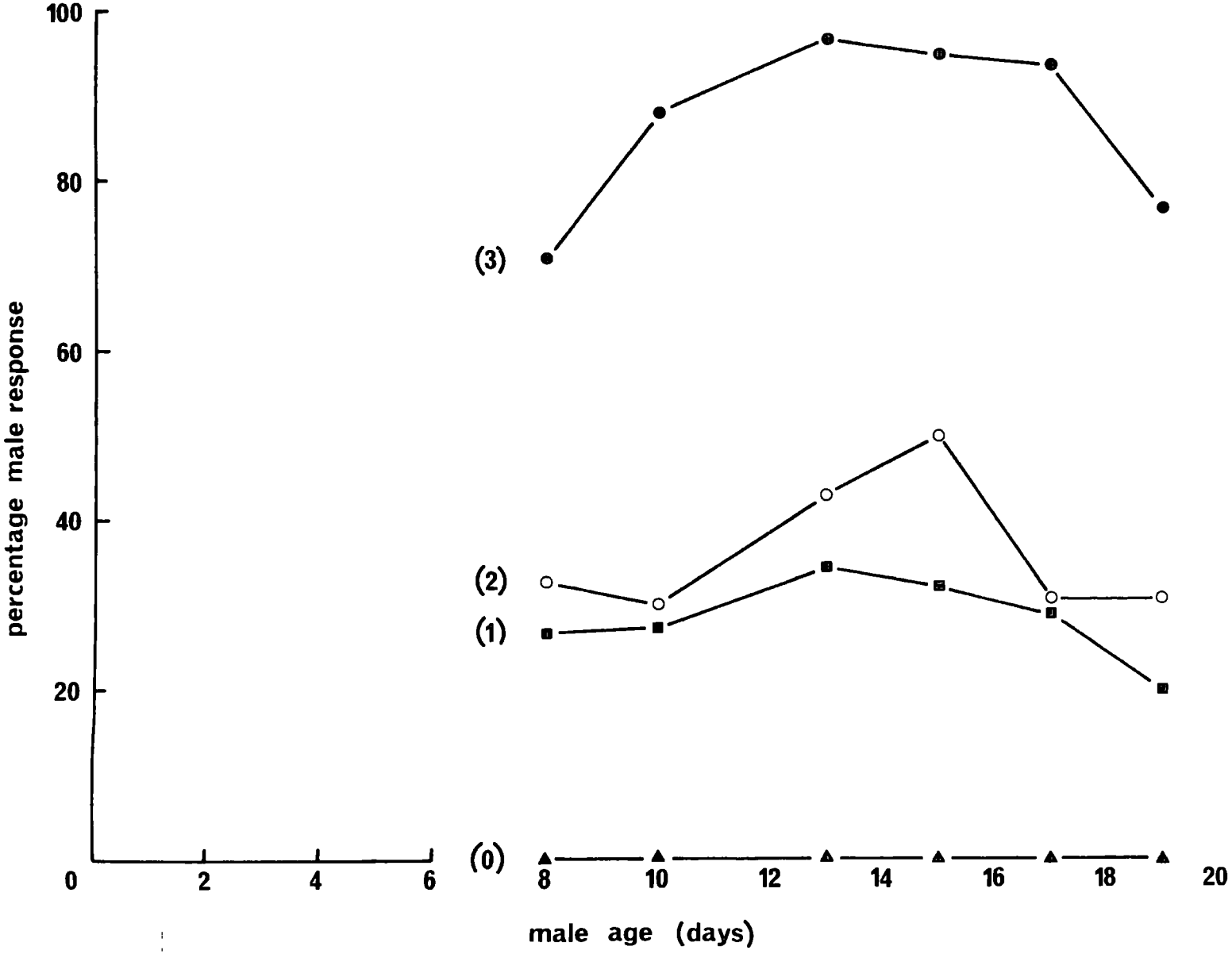
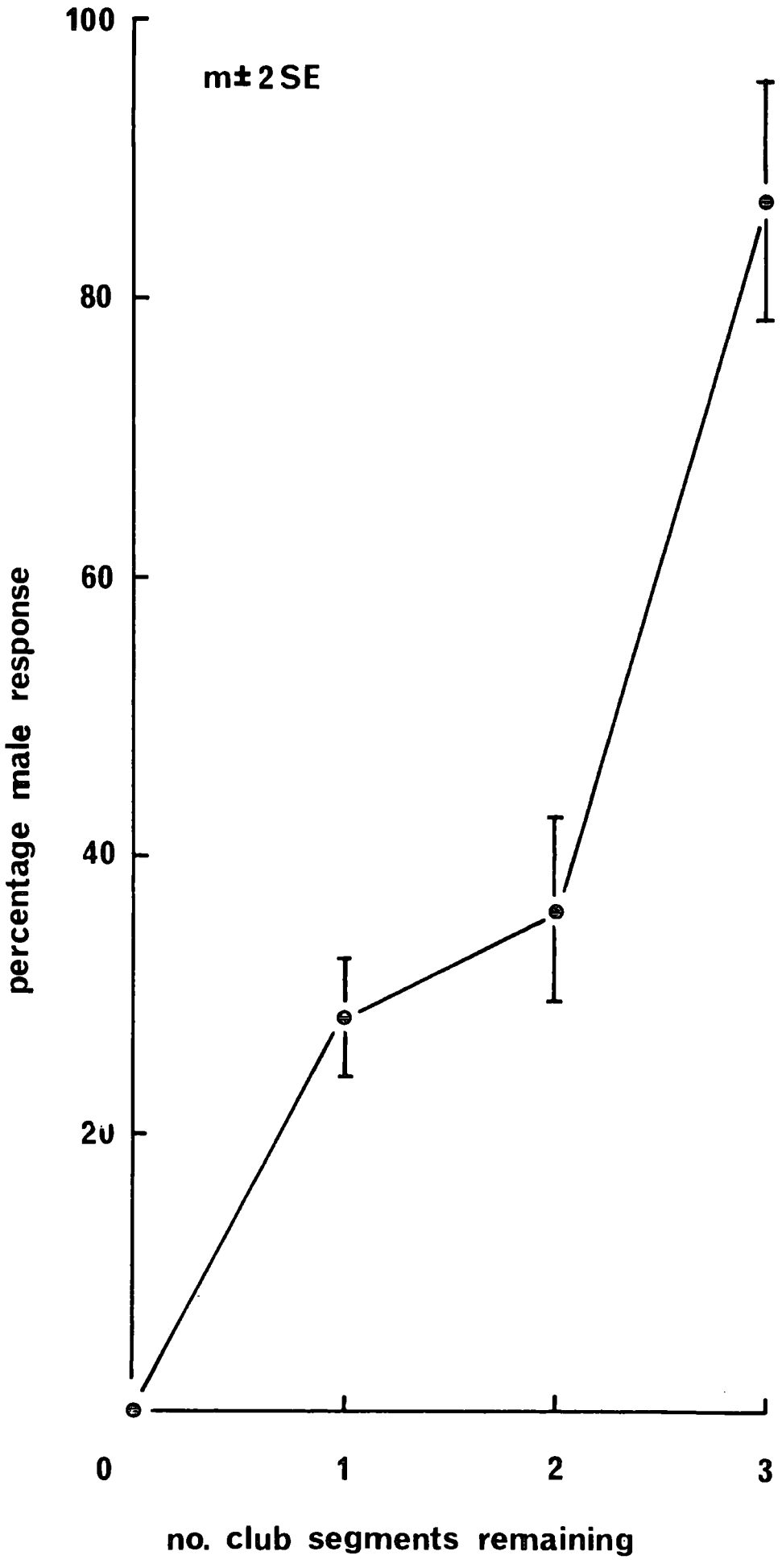

100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
percentage male response
male age (days)
(3)
(2)
(1)
(0)

Figure 37. Mean percentage response of male Stegobium over 8-19 days of adult life after removal of 0, 1, 2 and 3 club segments of both antennae.


100
m±2SE
80
60
40
20
percentage male response
0
1
2
3
no. club segments remaining

difference in response between males with 1 and 2 intact segments than those with 2 and 3 or 0 and 1. This may indicate that there are more sex pheromone receptors on the distal and proximal club segments than the middle segment. Alternatively, permanent post-operation shock might affect behaviour, and hence the decrease in male response following amputation of the distal segment might be therefore exaggerated.

A pronounced change in general male behaviour was observed following complete antennectomy. During bioassay experiments, males repeatedly displayed activity which may be termed "wing-opening". The elytra were raised and wings expanded as if to take flight, but in fact this position was held for several seconds while rotating slowly on the spot. Although seeming to be an attempt to orientate in some way, this behaviour on no occasion resulted in location of the pheromone source. Since hygroreceptors are usually found in high concentrations on the antennae (Roth and Willis, 1951), this activity might be an attempt by the insect to gain information regarding the humidity of the environment by means of receptors located elsewhere on the body. Antennectomy abolishes the humidity response of Oryzaephilus surinamensis (Arbogast et al, 1972) which is also true of many other species, but reports of "wing-opening" behaviour have not been found elsewhere in the literature.

D. Effect of antennectomy on male mating success

From the results of the previous experiments, it may be supposed that antennectomised males would be less successful in locating and copulating with females than would normal, unoperated males. Tobin and Smith (1971) found that control male Lasioderma serricorne located virgin females in less than 60 s, usually 27 s but antennectomised males required much longer, with only 20% copulating successfully.

The mating success of antennectomised male Stegobium was roughly ascertained by the following experiment. A group of 20 males was antennectomised at 5 days old as described in the previous experiment. A control group of 20 males was left intact, and at 8 days of age, each group was placed together

with twenty 6-day-old virgin females beneath an inverted 15 cm-diameter petri dish on filter paper. The numbers of pairs "in copula" was recorded at 5-min intervals for a total of 30 min (table 21). Each group, including

Table 21 Copulation success of a group of 20 antennectomised males placed with 20 females, compared with a similar group of control males

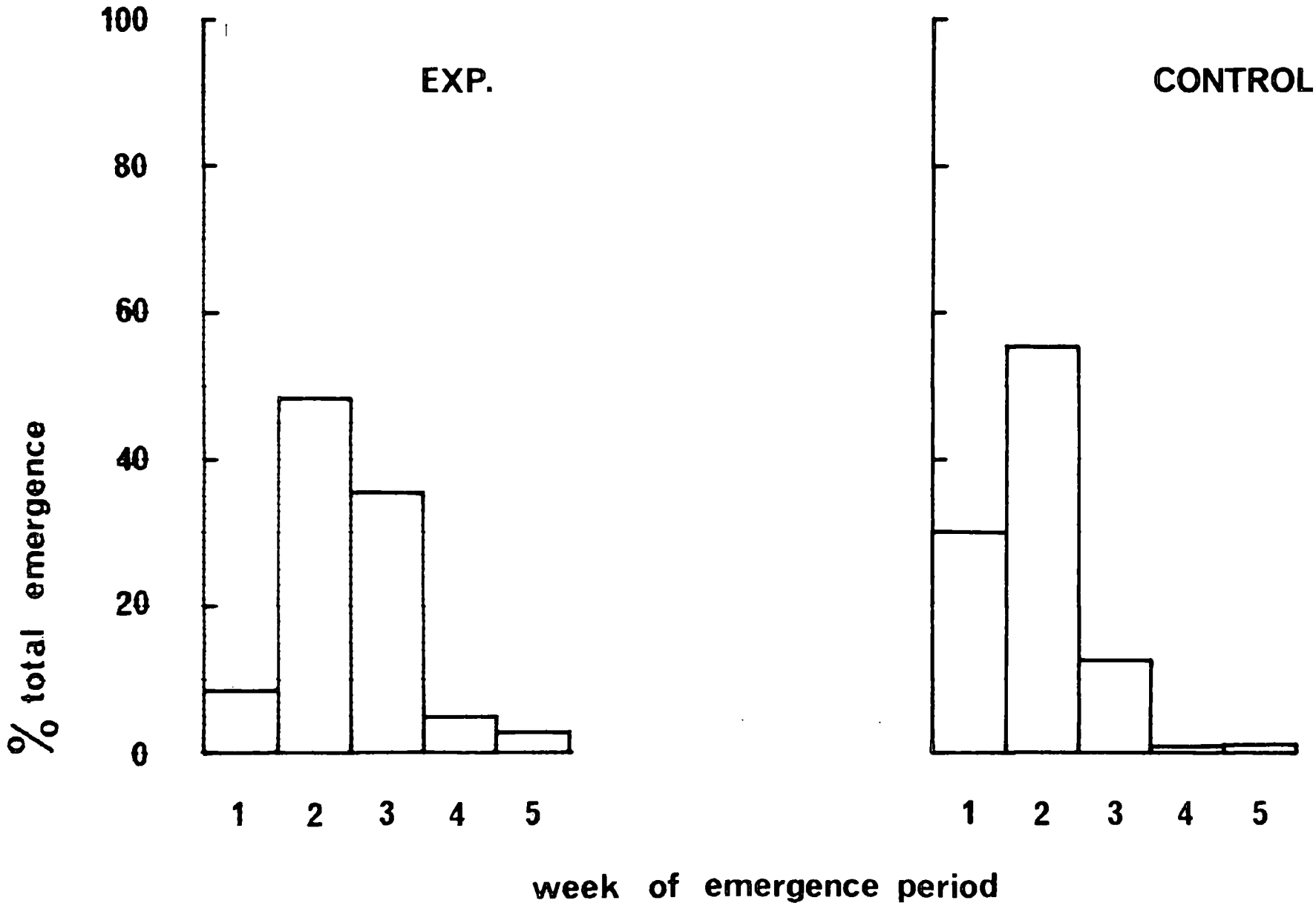
time (min)	no. pairs copulating	
	antennectomised males	control males
5	0	10
10	0	16
15	0	16
20	0	16
25	2	16
30	2	14

the females, was then transferred to a separate culture jar and the number of adults emerging from these cultures was counted at weekly intervals over the 5-week emergence period (fig. 38, table 22). It can be seen that over 85%

Table 22 Emergence of the offspring from two groups of 20 pairs of Stegobium; an experimental group, in which the males had been antennectomised, and a control, unoperated group.

week of emergence	experimental		control	
	nos.	%	nos.	%
1	58	8.5	237	30.2
2	329	48.3	435	55.3
3	242	35.5	99	12.6
4	33	4.9	7	0.9
5	19	2.8	8	1.0
total	681		786	

Figure 38. Emergence of the offspring from two cultures of 20 male and 20 female parent beetles. The parental males of the experimental group were completely antennectomised, and those from the control group left intact (see text).


EXP.
CONTROL
100
80
60
40
20
0
% total emergence
1
2
3
4
5
1
2
3
4
5
week of emergence period

of the offspring from the control pairs emerged by the end of the second week in comparison with 57% of those from the experimental group. The latter produced about 85% of the total number of offspring of the control group.

These differences are not very great and the tests should be repeated in order to confirm the trends shown. However, copulation is quite clearly delayed in the absence of sex pheromone stimulation of males; only 10% of the antennectomised group were copulating after 25 min in conditions of close proximity with females at their peak of pheromone emission. After 10 min of contact with females, 80% of the control males were copulating.

The importance of the role played by the sex pheromone in the location of females by males is stressed. It may be presumed that given time, and a fairly confined area, antennectomised males encountered females by chance and it is possible that gustatory receptors on the maxillary and labial palps might have enabled males to recognise females and stimulate them to copulate.

The small reduction in numbers of offspring produced by the experimental group (table 22) might reflect a smaller number of females fertilised, delayed fertilisation of females resulting in reduced fecundity (suggested by the delayed peak of emergence of the offspring) or it might be natural variation.

E. Discussion

The chemosensory sensilla on the club segments of the antennae of male Stegobium are responsible for sex pheromone perception and their removal completely abolishes male response to a sex pheromone extract. Predictably, male location and courtship of mature females is delayed considerably following antennectomy. In terms of selection pressures and survival at low density, the importance of the sex pheromone to the species is thereby greatly emphasized.

Although the results obtained from progressive removal of the club segments would suggest a high concentration of pheromone receptors on the distal segment, counts of actual numbers of sensilla do not support this

finding. Either a specific group of trichoid or basiconic sensilla are sex pheromone specialists and their distribution is uneven, or post-operation shock is responsible for this loss of male sensitivity which would remain constant for any degree of antennal amputation.

Sexual dimorphism is not apparent in Stegobium antennal sensilla types or distribution. Female response to her own pheromone release, or more probably to that of other females might play an important, and as yet undetermined part in this system. This will be further discussed in the final discussion. Alternatively, sexual dimorphism may manifest itself at the molecular level. Electroantennogram studies would be necessary to investigate this possibility.

IX GENERAL DISCUSSION

This study has been primarily concerned with the demonstration and investigation of a sex pheromone in the bread beetle, Stegobium paniceum. The importance of this method of communication between the sexes in the biology of the species has been appraised, and factors affecting its operation studied. The sex attractant system has been shown to enhance and increase the speed of male location of females at a time conducive to maximum reproductive efficiency and success.

A. Nature of the sex pheromone in Stegobium

Previous work has shown, that in the majority of insect species employing a sex pheromone, the female produces the attractant, to which only males respond. Jacobson (1965, 1972) listed 283 insect species in which this has been demonstrated, compared with 107 species in which the male produces the sex attractant. Stegobium provides an example of the former, larger category.

It is not always simple to distinguish between sex and aggregating pheromones as was demonstrated by the pheromone system of Anthonomus grandis (page 4), which shows characteristics of both.

The behaviour of male Stegobium in the arena during ether and air extract assay experiments suggested that the pheromone initiated both orientation towards the odour source and copulatory behaviour, since males tested concurrently would readily mount each other on, or very near, the pheromone-treated disc. Males were never observed mounting each other in the absence of the pheromone, or on the control disc of the test arena. There was a marked negative relationship between potency of the pheromone extract and time taken to locate the treated disc in these experiments, which strongly suggests the presence of a distinct orientation phase of the response, although this was not directly measured. Males had only to cover about 5 cm from the point of release to the treated disc, but this was

commonly achieved 2-3 s in response to 0.1 female equivalent of a 6-day-old virgin female extract. Orientated movements rather than random walking must be responsible for male location and response to the sex pheromone extract.

Visual stimulation, perhaps by a Stegobium-sized object, might be important in invoking copulatory attempts. Solitary males merely circled within the pheromone-treated disc, displaying no further sexual behaviour. Doane (1968) and Shorey and Gaston (1970) found that visual stimulation was essential in the copulatory behaviour of some Lepidoptera, including Trichoplusia ni.

From the above observations on Stegobium, it is clear that females produce a volatile substance which attracts only males. This has been shown to operate over a distance, however small, and excite copulatory behaviour in the presence of other males. This evidence would suggest that a sex pheromone is operating in this species, rather than an aggregating pheromone or aphrodisiac according to the definitions outlined on pages 4-5 of this thesis.

B. Behavioural response of males

(a) Distance

Since Stegobium is a small, essentially pedestrian insect, its range of sex pheromone communication is most unlikely to equal that of larger flying insects. Moreover, under natural conditions, especially in temperate climates, active movement of this beetle is largely confined to the interior of infested premises. However, location of a female by a male may be possible over distances of several metres. The habitat of Stegobium is very much one of three dimensions compared with the field situation, where orientation of insects is largely controlled or directed by surface wind currents. Air movements will certainly exist in a warehouse situation, but they will probably be less consistent and more turbulent.

There are several factors which limit the distance over which orientation of insects to a chemical attractant can be achieved. These include the

emitter's output rate and duration of emission, the receiver's sensitivity threshold concentration, and wind turbulence and velocity. Theoretical distances may be calculated for pheromone communication by means of a formula which takes the above factors into account. For this formula to be used, knowledge of the chemical identity of the pheromone is necessary (Bossert and Wilson, 1963; Wilson, Bossert and Regnier, 1969).

Since Stegobium males display no recognisable behavioural indication of pheromone perception, distances involved cannot be measured directly. However, individual male success in choosing the correct direction of a standard pheromone sample placed at varying distances from the male could be assessed. This should be determined initially in "still air", and factors such as air movement and variable emission rate could be introduced into this system. Clearly, a pheromone-free environment would be essential for such tests.

(b) Orientation

In many flying insects where a pheromone communication system is present, positive anemotaxis has been demonstrated. This involves movement towards, or directly into the air flow. The pheromone acts as a "releaser" for this behaviour. Positive anemotaxis operates in Drosophila melanogaster (Kellog, Frizel and Wright, 1962) and Aedes aegypti (L.) (Kennedy, 1939; Wright, 1962) in both of which species, vision is used to follow the ground pattern as an aid in steering. In a more recent series of experiments with male Cadra cautella, Kennedy and Marsh (1974) have demonstrated guidance towards a pheromone producing female by means of anemotactic optomotor reactions. Loss of the odour stimulus changes the anemotactic angle from upwind to an across-wind orientation, which is frequently reversed.

It would be interesting to evaluate the importance of flight in Stegobium and determine the factor(s) stimulating this behaviour. No positive reference to flight in this species has been encountered in the literature, but during the course of the present research it has been observed on many occasions.

Only once did a male fly from the test arena, suggesting that there is either no connection with pheromone orientation, or that it is a long range anemotactic response. The relatively rare incidence of flight would favour the former interpretation, although further work in this direction would be justified.

The mechanism of behavioural orientation towards an odour source has not been investigated in this study except for a short series of tests designed to indicate whether a chemo-orthokinesis response was involved whereby the rate of linear progression changes in response to pheromone stimulation. The results of these experiments, together with details of test procedure are shown in Appendix IV. No tendency towards an increase in the rate of locomotion in the presence of a pheromone source was demonstrated. Indeed, it was significantly reduced when live females were used as the source of attractant. Traynier (1968) found that in Anagasta kühniella, a chemo-orthokinesis reaction occurred near the pheromone source and that a reduction in flight speed resulted from an increased concentration of the attractant. The result of a similar phenomenon may have been responsible for the reduced rate of progression in male Stegobium in close proximity to 10 pheromone-emitting virgin females.

A far more comprehensive series of tests would be necessary to investigate the possibilities outlined above and for the mechanism of orientation in Stegobium to be fully understood. The apparent reduction in male linear progression might have resulted from the initiation of a taxis response in the presence of a steep concentration gradient. An insect might make successive comparisons of odour concentration by swinging both antennae from one side of the body to the other (chemo-klinotaxis) or simultaneous comparisons of both sides (chemo-tropotaxis). In either case, the responding individual turns towards the side of maximum stimulation, thus moving up the concentration gradient towards the source.

(c) Response thresholds

In many insects, behavioural response to a sex pheromone is composed of a series of fixed steps, each of which requires a higher pheromone concentration than the previous one. In a natural situation, therefore, an insect approaching the pheromone source would pass through all the necessary stages of behavioural response, normally culminating in copulation. Shorey (1973) called this phenomenon a "response hierarchy". Epiphyas postvittana displays four well-defined behavioural steps in its response sequence; antennal elevation, activation (including flight), upwind orientation and copulatory movements. In laboratory assay, greater concentrations of pheromone were necessary to elicit the successive elements in this response sequence (Bartell and Shorey, 1969b).

Stegobium is not obviously suitable for this type of study since the behavioural response appears not to fall into well-defined units. Moreover, assay of serially diluted female ether extracts was not designed to demonstrate male response at different levels. In effect, the extrapolated response threshold concentration of 0.0004 female equivalents for males might be much higher than the concentration necessary to stimulate behaviour at a lower level in the "hierarchy", for example, male activation.

C. Female pheromone production and its timing in relation to reproductive events

(a) Synthesis

The glandular tissue responsible for pheromone synthesis has not been identified in Stegobium, but as shown by the results given in Appendix V, it is thought to be located in the abdomen of the female. The presence of a small degree of male response (maximum 30-40%) to diethyl ether extracts of the head and thoraces of females suggests that the pheromone is adsorbed into the epicuticular waxes as Tschinkel et al (1967) demonstrated in Tenebrio molitor.

(b) Pheromone content and emission rate relationship in virgin females

Although adult female Stegobium remain in the pupal cocoon for 4-5 days

after eclosion, about 20% of males found 0-day and 1-day-old female ether extracts attractive. Live females and air extract discs from females of these age groups were not attractive to males. Clearly, pheromone synthesis, probably beginning in the pupal stage, is under way during the early adult period of ovary maturation in the cocoon. Emission of the sex pheromone was not indicated by male response to air extract discs, or to live females in gelatine capsules until females were 3 days old. Pheromone release rapidly reached its maximum levels (60-70% male response to air extract discs) in 4 to 5-day-old females. The loss of pheromone by emission might deplete the synthesised store of attractant resulting in maximum pheromone content levels being delayed until females were 5 or 6 days old.

As discussed in section VI, part C(a), the premature peak of pheromone emission twice recorded from 12-hour-old females (indicated by 30% and 5% male response respectively) might be unavoidable, due to "leakage" through the cuticle before protein tanning is complete.

In 3 to 4-day-old females, terminal oocyte length had reached its maximum, and mature oocytes had moved down into the calyx in synchrony with attainment of substantial levels of pheromone emission. Copulation may presumably occur immediately after emergence from the cocoon. Oviposition was first recorded when females were 4-5 days old.

(c) Copulation

The effect of mating upon female pheromone emission in Stegobium is by no means fully understood. The pheromone content of mated females was only slightly reduced (to about 85% of that extracted from similar-aged virgin females calculated by using figure 12 as a calibration curve) in 6-day-old females, although it subsequently decreased more rapidly in mated, as compared with virgin females of the same age groups. This might be an indirect effect of earlier senescence, since egg-laying, after mating, constitutes a major loss of stored food reserves.

Pheromone emission oscillated markedly after copulation. In two series

of experiments, two peaks of pheromone emission on day 7 and 9 alternated with very little male response to air extracts of 6 and 8-day-old mated females. Since both groups of 25 females displayed this oscillation at exactly the same times, unavoidable, or uncontrolled loss of pheromone is not thought to be occurring. With the available evidence, it can only be presumed that the peaks of pheromone emission coincide with periods when females need to copulate for a second (or third) time in order to maintain maximum fertility of eggs produced. It was shown that the number of eggs laid per female was not influenced by multiple mating. Since fertility of eggs was not estimated in this study, the question cannot be elucidated further.

Multiple mating was observed frequently after the onset of oviposition and it may be supposed that pheromone emission can be controlled by females so that a male is attracted when necessary, while oviposition periods remain undisturbed.

During the oviposition studies (section VII, part E) in the course of which the number of eggs produced per female was counted every day, females were kept singly, or with one male. No pattern of egg laying was evident, although the air extract results might have suggested that greater numbers of eggs would be laid on alternate days. The hypothesis proposed here is that by placing females in groups of 25 for the purposes of air extraction (they were kept together permanently, not only for the actual extraction periods) oviposition and pheromone emission became synchronised and cyclical. This might be a direct response of females to each other, with the obvious potential advantage of attracting more males by combined pheromone output, or a result of a synchronising event common to these female groups. The uniformity of age of the females (eclosion occurred at time t $\pm$ 30 min) might have resulted in a high degree of synchrony of the group throughout the experimental period, or the point of separation from males at the beginning of day six might have "set" the timing of future events.

The effect of grouping the females in the air extract experiments might

be of consequence other than in the synchronisation of oviposition and pheromone emission in Stegobium. Pearl (1932) and Sameoto and Miller (1966) found that female Drosophila melanogaster and D.simulans kept in groups produced fewer eggs per female than isolated flies, probably as a result of interference between individuals. Husain and Mathure (1945) demonstrated that crowded Schistocerca gregaria matured their eggs more rapidly than isolated females. In this case, increased activity was found to enhance neurosecretary hormone release necessary for egg maturation (Highnam and Haskell, 1964). In Locusta migratoria, however, the reverse effect was observed as a result of crowding (Norris, 1950).

It is apparent from the examples given above, that grouping of female Stegobium might have had a pronounced effect upon the results obtained in the air extraction experiments with particular emphasis on the reproductive system. Apart from a possible synchronisation of mating and oviposition in grouped females, timing of egg maturation, repeated copulation and oviposition might have been influenced. In order to fully appreciate the effect of mating upon pheromone emission in female Stegobium, the importance and significance of repeated mating must be determined, and the mechanism of physiological control of pheromone synthesis and emission investigated. Modification of the air extraction technique in order to monitor pheromone emission in individual females might be a suitable approach to such a study.

D. "Wild" and laboratory strains

Shorey (1974) has recently criticised comparative work on the sex pheromone of different strains of a single species on the grounds that monthly, seasonal and other periodic variations might lead to differences in the pheromone "balance" of the strain. One might, for example, be testing the "high point" of one strain and the "low point" of the other, giving differences which are only real on a temporary basis. Sower et al (1972) found that unexplained population density changes in laboratory strain Trichoplusia ni resulted in marked variation in the amount of extractable

pheromone from females, and its release rate.

The laboratory strain of Stegobium was given no known environmental clues beyond the daily photoperiod and there were no noticeable variations in population density in the cultures. The environmental conditions and population density from which the "wild" strain was taken is not known apart from the temperature and humidity measurements in the bakery (page 9). The pheromone content of "wild" females was very similar to that found in laboratory females when extracts of both were bioassayed with laboratory males, but this might not necessarily be the case with a strain of beetles from a different population density situation. A study of a series of infested sites, with subsequent laboratory testing of extractable pheromone from females would be necessary to examine this problem.

Although the intensity of male response might vary in "wild" Stegobium populations, the general pattern of male response over their life span probably remains fairly constant. The very rapid rate of increase in the pheromone response of young males of the "wild" form at sexual maturity (3-5 days) is thought to indicate a significant selective factor which has declined in importance with continuous laboratory rearing over a long period.

The sex pheromone system of some insect species is known to be non-uniform throughout their geographical range. Lanier (1972) has demonstrated this effect in Ips pini (Say) in North America, where there are great differences between the pheromone systems of eastern and western populations.

Azab (1954) described two races of Stegobium, an American "tobacco race" and a European "flour race", both of which displayed marked preferences for their respective larval food medium for oviposition sites. It would be interesting to investigate the pheromone systems of these strains comparatively.

In a stored-product insect, where populations might tend to become insular, gene-flow between breeding populations might be rather restricted. Races and sub-species could easily arise from this situation with the sex pheromone, perhaps eventually operating as an isolating factor (if small

changes occurred in its chemical structure) as well as food preference.

E. Further research and application of results

Apart from the topics for further investigation already outlined in the present section and throughout this thesis, studies which would either contribute to the understanding of the sex pheromone system or provide possible application for the system, are given below.

(a) Physiological control of pheromone synthesis and emission

Barth (1965) proposed the hypothesis that in insects which matured more than one batch of eggs, pheromone production and release is probably under hormonal control. He proved this to be the case previously (Barth, 1961, 1962) in the cockroach species, Byrosotria fumigata (Guerin) and Pycnoscelus surinamensis L. The corpus allatum was found to be responsible for pheromone secretion in these species, and in Tenebrio molitor (Menon, 1970). Barth (1965) similarly expected short-lived adult insects, which mature only one batch of eggs to be independent of hormonal control with respect to pheromone production which he demonstrated in the Chinese silkmoth, Antheraea pernyi (Guérin-Méneville). Similar studies involving corpus allatum ablation have shown that pheromone production in Bombyx mori, a short-lived, non-feeding adult is not under hormonal control (Steinbrecht, 1964) nor is it in the wax moth, Galleria mellonella L. (Röller, Piepho and Holz, 1963). Barth (1965) suggested that in short-lived species there might be an automatic "switch-on" of pheromone production at sexual maturity and that there is thus no necessity for direct and continued hormonal control. He commented upon the shortage of information on species with an intermediate life span and the concentration of work on only extremely short or long-lived species. In this context, Stegobium would probably qualify as a species of intermediate life span. As multiple mating has been observed and control of pheromone emission suggested, neuroendocrine control of the pheromone system might be possible. Allatectomy of Stegobium might, however, prove difficult because of the very small size of the insect.

(b) Chemical identification of the pheromone

Purification and chemical identification of the pheromone produced by female Stegobium has not been attempted in the present study. Complex and specialised biochemical and biophysical techniques are required for analysis of a pure pheromone sample, including gas chromatography, ultraviolet and infrared spectroscopy to determine functional groups, and mass spectrometry to determine molecular formulae and fragmentation.

Such work has often revealed complexes of two or more compounds making up a sex attractant. The aggregation pheromone of the boll weevil, Anthonomus grandis is a mixture of four compounds (Tumlinson, Hardee, Gueldner, Thompson, Hedin and Minyard, 1969). Ikan et al (1969) and Yinon, Shulov and Ikan (1971) identified the assembling scent of Trogoderma granarium to be a mixture of five fatty acid esters, each of which is as attractive alone as the whole mixture. Tamaki, Noguchi, Yushima and Hirano (1971) identified two components of the sex pheromone of Adoxophyes orana. Neither was attractive alone, but a mixture of both was highly attractive.

It would be a worthwhile study to identify the sex pheromone of Stegobium, not only for the sake of determining its chemical formula, but to enable a synthetic version of the pheromone to be used in bioassay experiments in precisely known quantities.

(c) Ecological implications and importance of the sex pheromone in the natural habitat.

In order to fully appreciate the role of the sex pheromone in Stegobium, it would be important to demonstrate and analyse the part played by the sex attractant in natural conditions and its influence upon the population dynamics of the species. Undoubtedly, survival of populations at low density and population increase is enhanced by this system of communication between the sexes. At high density, however, population increase might be restricted as a result of saturation of the atmosphere with the pheromone, and ensuing male "confusion" or failure to orientate towards a single pheromone source (ie. an emitting female) might result. In order to study these effects, it

would be necessary to determine the minimum density required for survival and the effect of density upon population growth and female effective fecundity. An investigation of sub-populations of beetles in parts of infested buildings would be an essential part of such a study.

Azab (1954) found that female Stegobium require a suitable oviposition site before eggs are laid, which is often the female's own larval food medium. He also suggested that location of such materials was by olfactory means. This was demonstrated by means of an experiment in which cotton wool was contaminated with the odour of food material, in which females readily laid eggs: uncontaminated cotton wool failed to stimulate females to oviposit. These results suggest the possibility that mature females might tend to collect, perhaps before they are fertilised, at a suitable oviposition site. Furthermore, males might also respond and orientate toward such food materials, and use the sex pheromone at closer range for final location of the female.

(d) Biological control

Since Stegobium poses a problem of hygiene in food storage and processing buildings, the possibility of using the sex pheromone as a non-insecticidal control mechanism is worthy of consideration. There are several approaches to this problem. Male inhibition or "confusion" as mentioned above might be achieved by artificially permeating the air with the pheromone resulting in habituation and failure to orientate within the background level. Alternatively, the pheromone could be used to bait traps, where attracted males are then exposed to a toxicant or chemosterilant.

The range of male orientation to various concentrations of a pheromone source under natural conditions would have to be established before the feasibility of the above measures could be assessed. For example, pheromone-baited traps would not be practicable if males were receptive only over 1-2 m to a concentrated bait. However, should this not be a problem, a suitable toxicant or chemosterilant would have to be found experimentally. Clearly, a sterilising agent which did not reduce male competitiveness for females,

compared with untreated males, would be essential. Male sterilisation has often proved more effective in insect control than direct elimination of males.

Before consideration of any such exploitation of the sex pheromone of Stegobium, a greater understanding of the system and its role in the biology of the species is clearly necessary.

SUMMARY

1. Stegobium was reared in the laboratory at 30°C and 75% r.h. Under these conditions, the developmental period from egg to adult was approximately 43 days. Methods of insect culture and rearing of experimental insects are discussed.

2. In arena assay experiments, chemical attraction of male Stegobium to live females confined in punctured gelatine capsules was demonstrated. Males were not attracted to other males, and females failed to respond to either sex.

3. Maximum response of males to live, encapsulated females occurred over the first 5 min of the 30-min assay period, reaching almost 50% positive response.

4. Males first responded to live females of 3-4 days old but reached maximum response levels to 7 to 8-day-old females. Older females became less attractive to males. Males first became responsive to live females at the age of 5 days, and from 6-7 days onwards they maintained a fairly high and constant level of response which decreased shortly before death (17-20 days).

5. Male response to diethyl ether extracts of females followed a typical, almost linear, regression phase over approximately the first 10 days of adult life, followed by a plateau phase, during which maximum male response levels were maintained for about 6 days. Response levels declined with male senescence at 17-20 days of age.

6. The age at which female Stegobium were extracted in ether produced significant differences in male response. Newly eclosed (0-day-old) and 1-day-old females elicited a small degree of response from males, barely exceeding 20%. Maximum male response was elicited by 6 and 9-day-old virgin female extracts (90-95%), although 16 and 23-day-old virgin female extracts provoked considerable response from males, reaching about 85% and 74% respectively.

7. Solvent extracts of females which had previously mated were only a little less attractive than virgin females of the same age. The difference in male response was significant at about 8% over 1-16 days of the male life span.

8. Males which had been in continuous contact with females, or which had been exposed to a pheromone extract for the first 6 days of adult life, exhibited a much diminished response to a 6v extract in comparison with control males. This was thought to result from adaptation of the chemoreceptors and the consequential increase in the threshold level of male response. Normal male response levels were partially but never fully recovered when the stimulus was removed, but the response of males kept in continuous contact with a pheromone source was considerably subdued, never exceeding about 20%.

9. By means of serial dilution of a 6v extract, and bioassay with males, an approximate minimum response threshold was extrapolated to 0% male response at a concentration of 0.04 females per ml solvent (0.0004 female equivalents).

10. Individual males tested daily for their response to a 6v extract revealed that males with a shorter than average life expectancy tended to be less responsive to the sex pheromone than longer-lived males. If a male responded positively on day n it was calculated that this positive response would be repeated on day n+1 on 87% of occasions. After a negative response on day n, the probability of a positive or negative response on day n+1 was about 50%. Almost 40% of males failed to respond on the day prior to death regardless of their previous response record.

11. Pheromone was collected by adsorption from an air stream which had previously passed through a chamber containing live females. This method of air extraction and subsequent bioassay of the pheromone collection discs with males revealed that pheromone emission in virgin females began on about day 3 and maximum emission rates were attained by 4-5 days.

12. After separation from males on day 6, pheromone emission rates of mated females rose and fell sharply on alternate days until day 10-11, when the response of 10-day-old males to air extracts became less erratic. Possible explanations of this phenomenon are discussed.

13. The air extraction technique was shown to collect only a certain (and probably small) proportion of the total pheromone output from females, since three collection discs placed in series elicited almost equal responses from males. Collection for 2 h instead of 1 h increased the amount of pheromone adsorbed on to the disc, but extraction of 50 females instead of 25 did not result in a greater male response to the collection discs.

14. The reproductive system of females between 0 and 5 days was examined by dissection. Three-day-old females were shown to contain some mature oocytes in the calyx. The terminal oocytes in the ovarioles reached maximum length by day 3, but the whole ovary continued to increase in length up to 5 days of age, mainly as a result of swelling of the calyx with mature oocytes.

15. Histological sectioning of the ovaries showed that 1-day-old females contained no true follicles in the ovarioles, although by day 2 the follicle cells had passed through the columnar to the cuboidal stage of development. Three-day-old females were again revealed to contain mature chorionated oocytes, which greatly increased in numbers by day 4.

16. Copulatory behaviour is described. Transference of the spermatophore during copulation was found to occur during the back-to-back phase as demonstrated by the egg production of females separated from the male at various stages during copulation.

17. Oviposition records were kept for individual females. Virgin females produced 1.5 eggs/female, all of which were infertile. Mated females produced 41.7 eggs/female, the percentage fertility of which was not determined. The life span of mated females was only about 71% that of virgins.

18. If males were kept with females throughout their adult life span, fewer eggs/laying female were produced than if the male was removed after 4-6 days. However, a greater percentage of females were fertilised when pairs were left together, than when separated.

19. Females which started ovipositing on day 4 produced almost 60 eggs/female. Those starting to produce eggs between days 6-8 laid less than 40 eggs/female. Female fecundity is therefore shown to decrease rapidly with age.

20. Four types of antennal sensilla were identified in Stegobium all of which appeared to be present in both sexes: sensilla chaetica, sensilla basiconica, sensilla trichoidea and sensilla coeloconica. These were studied by means of light and scanning electron microscopy. Possible functions of the sensilla are discussed. Approximate numbers of each sensilla type were estimated and their distribution on the club segments of the antennae investigated.

21. Complete antennectomy, or removal of the three club segments resulted in total inhibition of male response to a sex pheromone extract. The removal of successive club segments progressively reduced male responsiveness.

22. The mating success of antennectomised males was greatly reduced. When placed with mature females, only 10% of the operated males had copulated after 25 minutes: 80% of a control group of males had copulated after 10 min contact with females.

APPENDICES

I	Comparison of solvents for extraction of the sex pheromone from Stegobium females	p. 109
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APPENDIX I COMPARISON OF SOLVENTS FOR EXTRACTION OF THE SEX PHEROMONE FROM *STEGOBIUM* FEMALES

Female extracts were prepared exactly as outlined in section IV for the diethyl ether extracts, using four different solvents; diethyl ether, water, benzene and absolute ethanol. The comparative efficiency of the resultant extracts was tested by means of arena bioassay experiments using at least a hundred 10-day-old males for each single extract. Two separate extracts, each with fifty 6-day-old virgin females were prepared for each solvent at a concentration of 10 females per ml, and the percentage response of males to each extract was calculated (table 23).

Table 23 Male response to extracts of 6-day-old virgin females using four different solvents.

solvent		storage temp. (°C)	% response of 10-day-old males
diethyl ether	(i)	-10	87.9
	(ii)		83.7
water	(i)	+10	0
	(ii)		0
benzene	(i)	+10	70.5
	(ii)		75.3
absolute ethanol	(i)	-10	76.3
	(ii)		75.6

Maximum response was elicited from males by the diethyl ether extracts, with those prepared using benzene and ethanol only proving a little less efficient. Water was not shown to be a suitable solvent for the sex pheromone.

On the basis of these tests, diethyl ether was chosen as the most suitable solvent for pheromone extraction from female *Stegobium*. Other factors in its favour include its very high volatility and relative harmlessness (compared with benzene) to the handler.

APPENDIX II RESPONSE OF MALE STEGOBIUM TO DIETHYL ETHER PHEROMONE EXTRACTS

The percentage response of male Stegobium to the complete series of diethyl ether extracts prepared and discussed in sections IV, V, VIII and Appendix V of this thesis are shown in table 24.

Three sets of control experiments which tested the response of about 500 females to a 6-day-old virgin female extract, about 300 males to a 6-day-old virgin male extract and about 400 females to a 6-day-old virgin male extract produced no response over their adult life span. All control experiments were conducted with extracts at a concentration of 0.1 female/male equivalent.

Abbreviations for table 24:

v = virgin

m = mated

l = laboratory

w = "wild"

ph^{1-6} = exposed to pheromone for days 1-6 of adult life

ph^{1-18} = " " " " " 1-18 " " "

ind. = individual male testing

abd. = abdomen only extracted

h+th = head and thorax only extracted

$ant.^{0}$ = no antennal club segments remaining intact

$ant.^{1}$ = 1 " " " " "

$ant.^{2}$ = 2 " " " " "

Table 24. Complete results of diethyl ether pheromone extract bioassay tests.

Female extract:										
age(days) :	0	1	3	6	9	16	23	6	12	16
mating status :	v	v	v	v	v	v	v	m	m	m
strain :	1	1	1	1	1	1	1	1	1	1
conc'n(FE) :	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
other details :										
Test males:										
mating status :	v	v	v	v	v	v	v	v	v	v
strain :	1	1	1	1	1	1	1	1	1	1
Other details :										
age : 1	1.7	0.0	0.0	1.5	3.3	0.0	0.0	0.0	0.0	0.0
2	1.6	2.8	1.4	14.9	20.7	1.7	9.7	7.9	3.2	1.4
3	1.7	3.9	7.0	19.8	23.8	14.5	14.3	16.2	12.5	1.5
4	3.1	7.5	10.9	32.9	33.8	13.5	16.7	18.6	18.9	1.4
5	3.0	7.2	15.6	43.6	42.1	26.5	16.7	30.0	15.8	5.6
6	6.3	7.5	16.4	50.6	50.0	31.6	21.3	52.4	23.3	8.3
7	6.9	7.1	33.5	64.9	70.7	31.5	27.1	50.6	36.0	11.9
8	15.1	11.7	43.5	71.4	73.3	42.4	31.7	71.4	42.2	21.9
9	16.4	14.3	56.9	81.8	83.1	51.6	26.9	72.3	48.7	24.3
10	17.3	14.3	61.5	87.9	90.0	67.8	48.2	79.7	54.7	27.3
11	28.0	21.0	69.5	93.9	89.4	69.8	52.4	87.5	59.3	27.5
12	33.8	22.1	68.7	92.8	84.4	86.7	64.6	84.0	63.9	30.1
13	17.9	20.6	54.6	96.9	96.8	84.0	71.7	91.8	82.8	49.1
14	10.6	15.1	66.2	93.5	90.0	85.0	75.0	95.3	83.3	41.9
15	11.6	31.6	73.3	95.3	90.7	83.5	71.0	91.4	71.4	47.5
16	16.1	27.5	64.1	96.7	95.0	83.6	76.2	91.7	67.7	56.6
17	9.8	23.1	66.3	94.0	96.7	76.1	70.0	86.9	52.6	65.1
18	3.2	22.3	60.6	91.7	91.8	63.8	58.1	17.7	56.7	61.3
19	0.0	14.3	53.3	76.7	83.9	50.0	44.8	56.3		52.0
20			42.1	71.4	62.5		28.6			47.3
21										
22										
23										

Table 24 cont'd.

	6 v 1 0.1	6 v 1 0.1	6 v 1 0.1	6 v 1 1.0	6 v 1 0.01	6 v 1 0.001	6 v 1 0.0005	6 v 1 0.1	6 v w 0.1	6 v w 0.1
	m 1	v 1 ph^{1-6}	v 1 ph^{1-18}	v 1	v 1	v 1	v 1	v w	v 1	v w
1			0.0	4.6	0.0	1.3	0.0	1.6	0.0	0.0
2			0.0	28.9	7.5	6.7	0.0	15.6	4.4	8.5
3			0.0	40.0	21.9	4.6	6.7	36.8	13.2	37.1
4			4.1	44.0	23.8	4.5	7.2	42.4	20.2	37.1
5			6.1	51.6	33.3	9.2	13.1	40.3	31.4	49.7
6			12.3	62.3	43.1	30.6	20.6	46.5	41.9	46.8
7	32.5	21.4	19.1	67.7	54.9	21.1	18.1	51.9	54.3	46.4
8	55.1	39.0	22.2	91.4	68.2	41.6	19.7	60.9	66.4	56.9
9	48.5	43.0	22.2	93.8	73.9	41.7	22.6	68.3	76.7	54.2
10	56.9	55.1	21.1	97.2	83.3	57.5	16.7	75.8	86.1	53.7
11	70.3	55.7	20.6	98.3	75.4	59.7	20.0	72.0	85.7	58.8
12	75.5	63.9	17.4	96.7	78.7	62.9	12.6	78.4	84.8	57.8
13	72.9	72.3	22.1	98.3	87.0	62.5	19.4	73.6	89.7	61.8
14	71.5	78.9	19.3	96.7	86.7	48.3	17.2	79.2	83.9	59.0
15	74.5	81.2	19.5	96.8	87.7	53.3	20.0	76.3	82.4	57.3
16	71.4	78.8	15.3	93.6	78.3	59.2	20.8	67.6	84.8	53.6
17	54.5	80.8	20.0	87.6	77.4	48.3	0.0	67.6	76.1	44.9
18	43.0	72.7	9.5	81.8	67.2	36.7	3.1	48.5	71.8	34.9
19	35.0	62.5		64.0	44.7	29.5	5.7	45.4	61.1	42.8
20					28.6		0.0			
21										
22										
23										

Table 24 cont'd.

	6 v 1 0.1	6 v 1 0.1 abd.	6 v 1 0.1 h&th.	6 v 1 0.1	6 v 1 0.1	6 v 1 0.1
	v 1 ind.	v 1	v 1	v 1 ant.^{0}	v 1 ant.^{1}	v 1 ant.^{2}
1	0.0	0.0	0.0			
2	12.0	4.3	0.0			
3	23.0	8.5	1.4			
4	49.0	17.5	0.0			
5	54.0	31.8	1.4			
6	60.4	44.6	1.3			
7	75.5	47.6	3.1			
8	81.8	60.6	7.4	0.0	26.3	32.7
9	75.3	74.3	4.5			
10	83.3	88.7	15.1	0.0	27.2	30.1
11	84.1	92.2	31.3			
12	86.7	98.3	38.3			
13	86.5	90.2	29.2	0.0	34.6	42.8
14	90.1	94.4	4.8			
15	95.0	85.5	5.0	0.0	32.4	50.0
16	89.6	90.3	1.7			
17	85.7	80.3	0.0	0.0	29.2	30.7
18	85.9		0.0			
19	63.0			0.0	20.0	30.8
20	71.5					
21	69.2					
22	50.0					
23	33.3					

APPENDIX III INDIVIDUAL MALE RESPONSE TO DIETHYL ETHER PHEROMONE EXTRACTS

The response of each individual male tested daily with a 6-day-old virgin female extract (section IV, part 7) is shown in table 25. For simplicity, only positive responses are marked (+) but all blank spaces indicate no response from males. The life span given for each male is exclusive of days when the insect was found moribund (completely inactive) or dead. In table 25;

m = moribund

d = dead

( ) = day of first positive response

Male no.	Age in days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	life span (days)	+ve	-ve	% -ve
1				+	+	+	+	+	+	+	+	+	+		+	m	m	d										15	11(4)	4	26.7
2						+	+	+	+	+	+		m	d														12	6(6)	6	50.0
3		+		+		+	+	+	+	+	+	+	+	+	+	+	+	d										17	14(2)	3	17.7
4					+			+	+	+	+	+	+	+	+	+	+	+	d									18	12(5)	6	33.3
5							+	+		+	+	+	+	+	+	+	+	+	d									18	11(7)	7	38.9
6				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d									18	15(4)	3	16.7
7			+		+	+		+	+	+	+	+	+	+		+	+	+	+	d								19	14(3)	5	26.3
8			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		d								19	16(3)	3	15.8
9		+		+	+		+	+	+	+	+		+	+	+	+	+	+	+	d								19	15(2)	4	21.1
10				+	+	+		+	+	+	+	+	+	+		+		+		d								19	12(4)	7	36.8
11		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d							21	19(2)	2	9.5
12														+	+	+	+	+	m	d								18	5(14)	13	72.2
13					+	+	+	+		+	+	+	+	+	+	+	+		+			d						21	13(5)	8	38.1
14		+		+	+	+	+	+		+	+	+	+	+	+	+	d											16	13(2)	3	18.8
15		+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	d									18	15(2)	3	16.7
16			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			d								19	15(3)	4	21.1
17				+	d																							4	1(4)	3	75.0
18					+			+	+	+	+	+	+	+	+	+	+	+	d									18	12(5)	6	33.3
19											+				+	+	+	+	+	+	d							20	7(11)	3	65.0
20					+	+	+		+	+	+	+	+	+	+	+	+	d										17	12(5)	5	29.4
21					÷	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	15(5)	4	21.1
22								+						+		+		+		+		d						21	5(8)	16	76.1
23				+		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d					22	17(4)	5	22.7
24			+	+	d																							4	2(3)	2	50.0
25		+					+			+		+		+	+	+	+		d									18	8(2)	10	55.6

Table 25. Daily response of 100 individual male Stegobium to a 6v extract.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	life span (days)	+ve	-ve	% -ve
26							+		+	+	+	+	+	+	+	+	+	+		d								19	11(7)	8	42.1
27									+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d				23	15(9)	8	34.8
28						+	+	+	+	+	+	+	+	+	+	+	d											16	11(6)	5	31.3
29				+	+	+		+	+		+	+	+	+	+	+	+	d										17	12(4)	5	29.4
30			+				+	+			d																	10	3(3)	7	70.0
31			+	+	+		+	+	+	+	+	+	+	+	+	+	+	d										17	14(3)	3	17.7
32				+			+	+	+						+	+	+	+		d								19	8(4)	11	57.9
33				+	+	+	+	+	+	+	+	+	+	+	+	+	+	d										17	14(4)	3	17.7
34						+	+	+	+	+	+	+	+	+	+	+	d											16	11(6)	5	31.3
35										+	+	+	+	+	+		+	+		d								19	8(10)	11	57.9
36						+	+	+	+	+	+		+	+	+	+	+	+	+	d								19	13(6)	6	31.6
37			+		+					d																		9	2(3)	7	77.8
38						+	+	+	+	+	+	+	+	+	+		d											16	10(6)	6	37.5
39			+					+	+	+	d																	10	4(3)	6	60.0
40		+			+			d																				7	2(2)	5	71.4
41			+	+		+	+	+	+		+	+	+		+	+	+	+		d								19	13(3)	6	31.6
42			+	+		+	+		+	+	+	+	+	+	+	d												15	11(3)	4	26.7
43		+					d																					6	1(2)	5	83.3
44		+	+	+	+	+	+	+	+	+	+	+	+	+	+	d												15	14(2)	1	6.7
45			+				+	+	+	+	+	+	+	+	+			d										17	10(3)	7	41.2
46			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	17(3)	2	10.5
47					+	+	+	+	+	+	+	+	+	+	+	+	d											16	12(5)	4	25.0
48				+	+		d																					6	2(4)	4	66.7
49			+	+		+	+		d																			8	4(3)	4	50.0
50		+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d					22	19(2)	3	13.6

Table 25 cont'd.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	life span (days)	+ve	-ve	% -ve
51			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d						21	19(3)	2	9.5
52					+	+	+	+	+	+	+	+		+	+	+		d										17	11(5)	6	35.3
53				+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	d								19	15(4)	4	21.1
54				+	+	+		+	+			+	+	+	+	+			+		+				d			24	12(4)	12	50.0
55				+			d																					6	1(4)	5	83.3
56							+	d																				7	1(7)	6	85.7
57							d																					6	0(-)	6	100.0
58						+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	13(6)	6	31.6
59				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	16(4)	3	15.8
60							+		+	+	+	+	+	+	+	+	+	d										17	10(7)	7	41.2
61								+				+	+	+	+	+	+	+		d								19	8(8)	11	57.9
62		+		+	+	+	+	+	+	+	+	+	+	+	+	d												15	13(2)	2	13.3
63				+	+	+	+		+	+				+	+	+		+	d									18	10(4)	8	44.4
64					+	+	+	+	+	+	+	+	+	+	+	+	+	d										17	13(5)	4	23.5
65				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	16(4)	3	15.8
66				+		+	+	+		+		+	+	+	+	+	+	+	+	+	d							20	14(4)	6	30.0
67						d																						5	0(-)	6	100.0
68				+		+	+	+		+	+	+	+	+	+		+	+	d									18	12(4)	6	33.3
69					+			+		+	+	+	+	+	+	+	+	+	+	+	d							20	13(5)	7	35.0
70				+		+		+		+		+	+	+	+	+	+	+	d									18	11(4)	7	38.9
71				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d						21	18(4)	3	14.3
72				+	+	+	+	+	+	+	+	+	+	+	+	+	d											16	13(4)	3	18.8
73			+	+	+	+	d																					6	4(3)	2	33.3
74				+	+		+	+	+	+	+	+	+	+	+	+	d											16	12(4)	4	25.0
75						+	+	+	+	+	+	+	+		+		+	+		d								19	11(6)	8	45.1

Table 25 cont'd.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	life span (days)	+ve	-ve	% -ve
76				+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	15(4)	4	21.1
77			+	+	+	+	d																					6	4(3)	2	33.3
78				+	+	+	+	+	+	+	+	+		m	d													13	9(4)	4	35.7
79			+				+	+	d																			8	3(3)	5	62.5
80				+	+	+		+				+	+		+	+	+	+	+	m	d							19	11(4)	8	45.0
81				+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	d								19	15(4)	4	21.1
82					+		+	+	+	+	+	+	+	+	+	+	+		d									18	12(5)	6	33.3
83				+	+	+	+	+	+	+	+	+	+	+	+	+	+	d										17	14(4)	3	17.7
84				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		d						21	17(4)	4	19.1
85					+	+		+	+	+	+	+	+	+	+	+	+	+		d								19	13(5)	6	31.6
86							+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	13(7)	6	31.6
87				+		+	+	+		+	+	+	+	+	+	+	+	+		d								19	13(4)	6	31.6
88			+	+		+	+	+	+	+	+	+	+	+	+	+	+	d										17	14(3)	3	17.7
89			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d								19	17(3)	2	10.5
90		+	+	+	+		+	+						+	+	+	+			d								19	10(2)	9	47.4
91						d																						5	0(-)	5	100.0
92			+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		d					22	18(3)	4	17.4
93						+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	d						21	15(6)	6	28.6
94				+	+		+	+	+			+	+	+	+	+	+	+	+		d							20	13(4)	7	35.0
95																											d	26	0(-)	26	100.0
96					+					+	+	+	+	+	+		+	+		d								19	9(5)	10	52.6
97					+		+	+	+	+	+	+	+	+	+	+	+	d										17	12(5)	5	29.4
98									d																			8	0(-)	8	100.0
99			+		+	+	+	+			+	+	+	+	+	+	+	+	d									18	13(3)	5	27.8
100						+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d							20	15(6)	5	25.0

mean life span = 16.39 $\pm$ 0.50
mean day first positive response = 4.43 $\pm$ 0.22

Table 25 cont'd.

APPENDIX IV THE EFFECT OF *STEGOBIUM* SEX PHEROMONE ON MALE ACTIVITY

A. Introduction and Methods

The effect of the sex pheromone on male *Stegobium* locomotion was briefly investigated in order to determine whether male response involved an increase in the general rate of male progression, or chemo-orthokinesis behaviour.

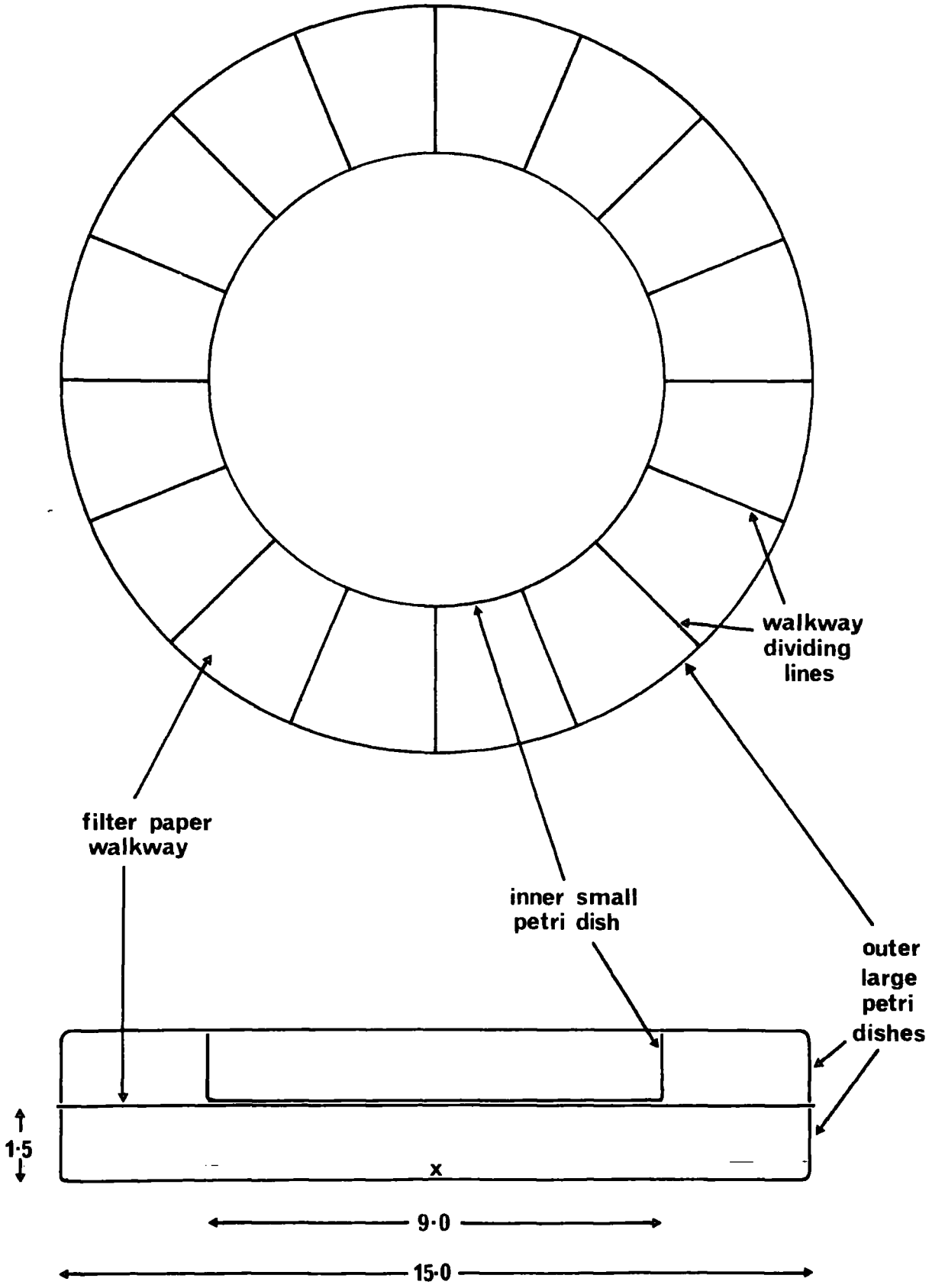
Figure 39 shows the apparatus designed for this series of tests. A filter paper circle (15.0 cm diameter) was enclosed and supported between two 15.0-cm-diameter petri dishes placed rim to rim. A plastic petri dish (9 cm diameter) was placed in the centre of the filter paper, its rim meeting the top of the upper large petri dish. An enclosed ring about 3 cm wide was thereby formed around the smaller petri dish. This peripheral area was divided into 16 segments marked in pencil on the filter paper (fig. 39), forming the "walkway" for males.

The tests were carried out at 20 $\pm$ 2°C and males from the rearing room were left overnight at this temperature before testing the following morning. General activity of the insects was reduced at this temperature and it was anticipated that any change in male activity as a result of pheromone stimulation might be more exaggerated than it would be at 30°C.

At the beginning of a test, a single 10-day-old male was placed on the "walkway" and after 2 min, recordings of its progression rate began. The number of lines crossed by the male each minute for 5 consecutive minutes was noted. Ten virgin 6-day-old females in a punctured gelatine capsule were then placed in the centre of the lower petri dish (X in fig. 39), below the filter paper. After 2 min, recordings of line-crossings were made as before for a further 5 min.

The experiment was repeated with the introduction of an anti-bacterial assay disc treated with 50 µl of a 6-day-old virgin female ether extract in place of the live females. A control test was also performed in which a pure ether-treated disc and empty gelatine capsule were introduced after the first

Figure 39. Plan and elevation of the apparatus used to assess the rate of linear progression of individual Stegobium on the walkway before and after a pheromone source was placed at X.



Actual dimentions in cm.

5 min. For each of the three treatments, 10 individual males were tested, with clean glassware and a new filter paper "walkway" for each male.

B. Results and Discussion

Table 26 shows the number of lines crossed by each male in the three experimental situations. Since the activity of individual males varied considerably, the values for all 10 males were summed for each minute of the test and the mean number of lines crossed per minute over each 5-min period calculated (table 27).

Table 27 The number of lines crossed on the "walkway" by male Stegobium before and after exposure to a pheromone source

treatment	mean no lines crossed per min ± SE		$t_{(n_1+n_2-2)}$	P
	before treatment (A)	after treatment (B)		
50µl 6v extract	9.68 ± 0.66	9.04 ± 0.24	0.91	NS
10 females (6-day-old virgins)	10.08 ± 0.35	8.58 ± 0.26	3.44	0.01-0.001
control	11.04 ± 0.40	11.18 ± 0.35	0.26	NS

From the results of the previous diethyl ether extract bioassay tests, attraction of 10-day-old males to 50 µl of 6v extract would be expected if access to the source had been available in these tests. However, the pheromone extract did not appear to change the rate of male locomotion. The introduction of live females decreased the locomotion rate by about 1.5 lines per min, a significant, though not substantial, reduction in activity.

The live females probably constituted a more potent source of pheromone than the ether extract to experimental males and the subsequent reduction in the speed of male locomotion might indicate arrestment behaviour due to the close proximity of the pheromone source. Increase in the rate of turning, or change of direction of males might be expected as part of a chemo-orthokinesis response but was not measured in these experiments.

Table 26 Number of walkway lines (see fig. 39) crossed by male Stegobium each minute for 5 min before (A) and after (B) the pheromone source was introduced (see text).

male no.	(A) 1	2	3	4	5 (minutes)	I total	(B) 1	2	3	4	5 (minutes)	total
1	25	21	18	24	23	111	15	22	18	22	17	94
2	7	9	5	8	9	38	8	13	7	8	13	49
3	12	8	10	11	12	53	14	12	14	15	11	66
4	8	4	2	5	2	21	2	2	2	2	4	12
5	5	5	0	5	5	20	6	10	6	8	8	38
6	8	18	4	0	18	48	12	9	7	10	11	49
7	19	19	19	8	8	73	8	9	10	12	7	46
8	9	15	8	14	7	63	11	10	8	10	6	45
9	2	2	4	3	5	16	7	3	6	2	5	23
10	7	13	9	6	6	41	7	8	5	3	7	30
total	102	114	79	84	105	484	90	98	83	92	89	452
						II						
1	9	5	11	12	5	42	7	13	9	3	3	35
2	10	12	17	10	11	60	10	6	4	13	11	44
3	17	12	8	13	10	60	15	15	11	15	8	64
4	11	17	17	11	12	68	9	16	15	8	12	60
5	6	7	8	11	11	43	9	9	10	13	13	54
6	13	12	12	12	6	55	8	5	8	7	0	28
7	11	5	4	4	5	29	8	8	4	1	8	29
8	11	11	11	10	14	57	11	9	8	8	8	44
9	11	11	11	13	10	56	7	12	13	11	10	53
10	10	9	5	6	4	34	4	2	2	5	5	18
total	109	101	104	102	88	504	88	95	84	84	78	429
						III						
1	10	9	11	8	11	49	13	8	12	14	15	62
2	5	11	9	13	6	44	15	13	6	5	2	41
3	10	9	12	11	7	49	5	7	10	10	6	38
4	15	8	11	15	13	62	14	16	10	14	9	63
5	12	11	14	14	12	63	7	11	16	12	13	59
6	9	15	9	16	13	62	18	17	19	20	15	89
7	16	7	8	7	11	49	4	10	3	8	4	29
8	8	9	6	12	16	51	15	7	17	13	11	63
9	17	18	19	23	10	87	19	18	6	15	19	77
10	6	7	9	7	7	36	9	7	9	7	6	38
total	108	104	108	126	106	552	119	114	108	118	100	559

I 50 µl 6v extract introduced after first 5 min
II 10 females in a gelatine capsule introduced after first 5 min
III Control-empty capsule and pure ether sample introduced after first 5 min

Rather little positive information on the behavioural response of males to pheromone stimulation was provided by these experiments. It would appear, however, that the general activity of males in terms of locomotion was not increased during orientation behaviour.

Should the pheromone of Stegobium prove to be an "arrestant" rather than an "attractant", the observed reduction in the rate of locomotion (table 26 and 27) would be expected if chemo-orthokinesis is operating, when a male is exposed to a potent pheromone source.

APPENDIX V DIETHYL ETHER EXTRACTION OF TWO PARTS OF THE BODY OF STEGOBIUM FEMALES IN ORDER TO LOCATE THE GENERAL AREA OF PHEROMONE SYNTHESIS

A. Introduction and Methods

Pheromone-producing glands of insects are usually modified epidermal cells of the integument which may be located almost anywhere on the insect's body. Thomas (1970) identified pheromone-secreting cells which were distributed over the head, thorax, legs and abdominal segments of Schistocerca gregaria. Lepidoptera, however, often have specialised tergal glands which are everted during pheromone emission periods, or calling.

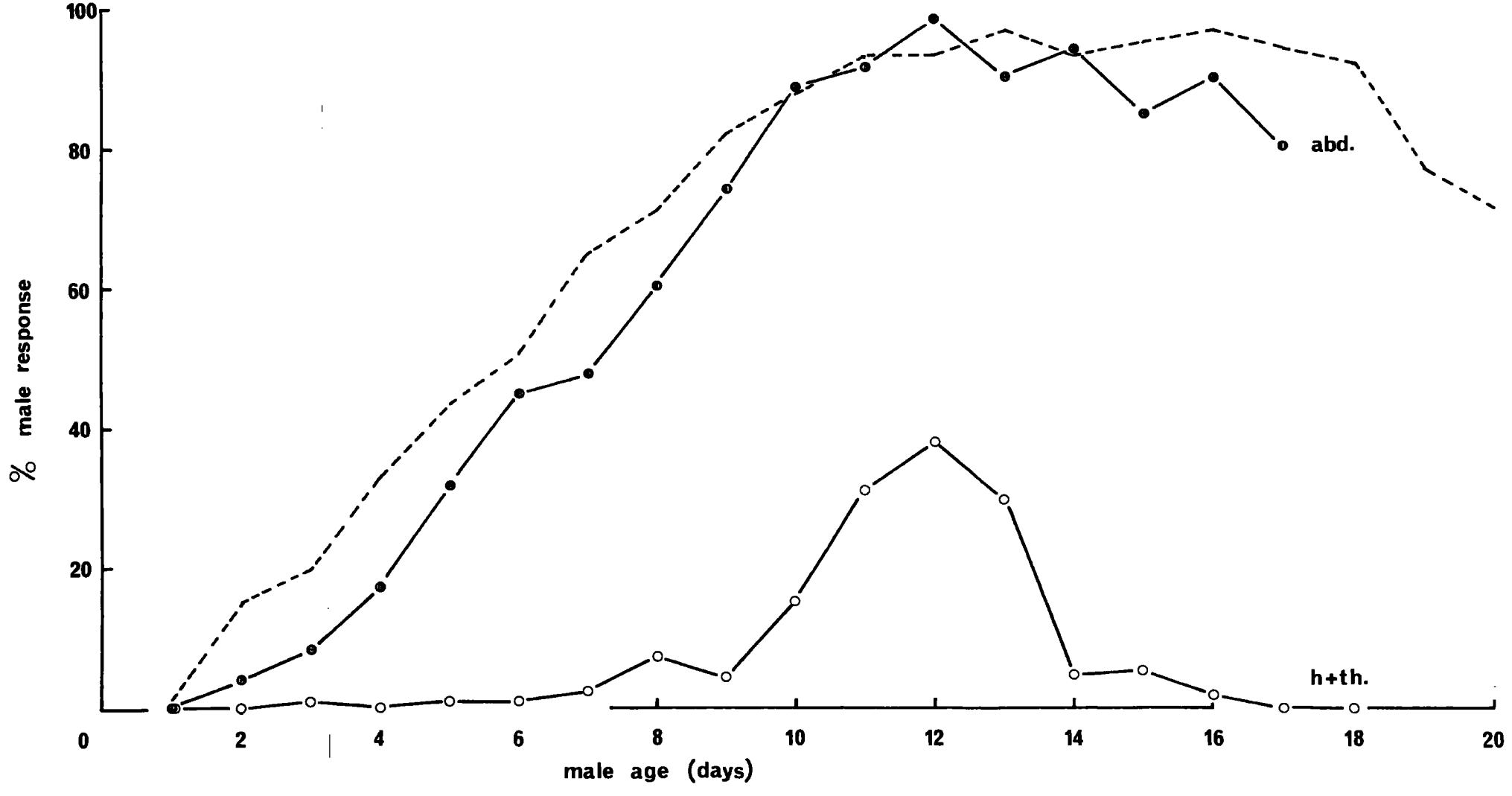
In order to try to identify the general region of pheromone synthesis and storage in Stegobium, two diethyl ether extracts of parts of females were prepared. The abdomens of 6-day-old live virgin females were severed from the thorax and head region and both parts were extracted immediately in ether at 10 parts per ml solvent. The extraction technique was otherwise exactly as described for whole females (Section IV, part A). Both extracts were bioassayed with at least 60 virgin males over their adult life span.

B. Results and Discussion

The percentage response of male Stegobium to the extracts is shown in table 24 of Appendix II and figure 40. The ether extract of female abdomens clearly elicited a much greater response from males than the extract of head and thoraces. The regression and plateau phases of male response to the former extract were distinct and the regression coefficient and plateau mean was calculated (table 5), whereas this was not possible for the latter extract. Statistical comparisons of the female abdomen extract with the corresponding extract of whole females (6-day-old virgin females at 10 females/ml ether) are shown in table 6. Only the paired mean differences of male response over 1-16 days were significantly different between the two extracts.

The presence of pheromone in the thorax and head region of the female body suggests either that there is a small site of synthesis in this area, or

Figure 40. The response of male Stegobium over their adult life span to ether extracts of the head and thorax (o ——— o) and abdomen (● ——— ●) of 6-day-old virgin females. Male response to a corresponding extract of complete females is also shown (broken line).


100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
% male response
male age (days)
abd.
h+th.

that the pheromone produced in the abdomen spreads over the female's body, perhaps in the wax layer of the cuticle. Tschinkel et al (1967) suggested that this occurred in Tenebrio molitor. A greater surface area for pheromone evaporation is available by means of this phenomenon at the expense of accurate control of pheromone emission.

Apart from scolytid beetles, which mostly synthesise pheromone in the digestive tract, Coleoptera sex pheromone-secreting cells are commonly located in the abdomen. Stanic, Zlotkin and Shulov (1970) found that the epithelium of the ventral intersegmental fold between the fifth and sixth abdominal sternites served as a gland for secreting sex pheromones in Trogoderma granarium. Henzell, Taylor and Lowe (1970) identified the first three abdominal segments on the dorsal surface of the female body of Costelytra zealandica as the site of pheromone production. However, Tschinkel et al (1967) have implicated the region of the metathoracic sternum and the first two abdominal sternites in pheromone manufacture in Tenebrio molitor, although the secreting glands were not identified.

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