STUDIES ON THE SEX PHEROMONE OF THE VETCH APHID, MEGOURA VICIAE.

by

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ABSTRACT

Oviparae of Megoura viciae have been shown to release a sex pheromone from their hind tibiae. Morphological evidence strongly suggests that the pseudosensoria present on these leg segments are responsible. The involvement of the pheromone in the attraction of males to females, and in copulation, has been studied. A bioassay based on the short-range orientation responses of the males was developed and used to assess various factors which might affect chemical communication between the sexes.

Males become highly responsive to the female scent within the 24 h following the imaginal ecdysis and remain so for the rest of their lives. Their responsiveness does not vary during the daily 12-h light period. Copulation with females that are not releasing their pheromone does not affect their subsequent responsiveness to the pheromone. The presence of the secondary rhinaria on the male's antennae was found to be essential for a response.

Females show a daily pattern of scent release which changes as they age. Both the rate and duration of release increase daily to reach a maximum by the sixth day of adult life. Thereafter, they decline gradually. Pheromone liberation is under the control of an endogenous rhythm, but is also influenced by environmental factors.

The pheromone appears to be present in the tibiae only at those times at which it is released. In six-day-old insects the amount that can be extracted fluctuates markedly during a period when the rate of release into the atmosphere remains more or less constant. Simultaneous changes occur in the numbers of secretory vacuoles present in the cells of the pseudosensoria. An explanation of the differences between the rates of pheromone synthesis and release is tentatively advanced.

Mating does not directly affect scent release but

oviposition, which is stimulated by coitus, temporarily decreases the attractiveness of a female.

A summary of the ultrastructure of the gland cells is given.

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INTRODUCTION

The adult egg-laying females (oviparae) of most holocyclic aphid species bear on their hind tibiae circular plaques or tubercles which taxonomists variously refer to as sensoria, pseudosensoria, or pseudorhinaria. It has been suggested that they aid the ovipara in fixing its eggs to the host plant (Buckton,I876-83), that they are sensory receptors (Jones,I944; Roberti,I946; Pagliai,I968), or that they produce a sex pheromone which attracts the males (Flogel,I905a; Weber,I935; Smith,I936; Bodenheimer & Swirski,I957; Stroyan,I958). Hille Ris Lambers (I966) noted that they were glandular but did not speculate on the nature of the secretion. As no experimental work on these structures had been published their function in the Vetch aphid, Megoura viciae Buckton, was examined. Having confirmed that they do indeed secrete a sex pheromone, studies were made of the synthesis and release of the active material, and of the behaviour of responding males.

While this work was in progress Pettersson (I970a,I97I) advanced some preliminary evidence of a similar function in a species of Schizaphis.

LITERATURE REVIEW

Nomenclature

For successful mating and reproduction, insects must be able both to find members of the opposite sex and to elicit in their partners behaviour patterns that will allow insemination. The systems of communication by which these sequences of reproductive behaviour are mediated are often complex and undoubtedly involve more than one sense in any one species. It has become increasingly clear over the last few decades that in insect behaviour in general, as well as in the particular case of reproductive behaviour, chemical communication is pre-eminent. This is not a peculiar feature of insects, or even of arthropods in general, for Wilson (I970) has concluded, "... that chemical communication is the paramount mode of communication in most groups of animals".

Indeed, chemical messengers have been found to be involved in so many different intra- and inter-specific relationships that various systems of classification have been proposed. In I959, Karlson and Lüscher proposed the term "pheromone" for "... substances which are secreted to the outside by 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 a developmental process ".They added, "...strict species-specific activity is not required, certain overlaps between closely related species may occur ". Thus pheromones were differentiated from other stimulating substances, such as phagostimulants, flower scents, and insect repellents which had, in earlier classifications (see Karlson and Lüscher,I959), been included with pheromones in one class of substances. Micklem (I959), objecting to "pheromone" on etymological grounds, suggested as an alternative, the name "transcitant", while Kirschenblatt (I962) attempted to reintroduce an earlier, more complicated but not entirely

comprehensive system of nomenclature for chemical messengers. However for intraspecific chemical cues between individuals the term pheromone has now become more or less universally accepted.

In 1963, Wilson and Bossert subdivided pheromones according to the way their effects are expressed in the recipient organism. Those eliciting an immediate behavioural response in the receiving individual were termed " releasers ", while those acting in a more subtle manner by changing the physiology of the recipient, and so allowing it to display a different behaviour pattern, were called " primer " pheromones. To directly complement the term pheromone Brown et al (1970) proposed that those interspecific chemical messengers whose adaptive benefit favours the emitting organism should be designated " allomones " and those favouring the recipient, " kairomones ". More recently, Law and Regnier (1971) suggested that all substances transmitting information between individuals be called " semiochemicals " and that pheromones, allomones, and kairomones are the major types of such signals.

Sex pheromones

Sexual reproduction in insects involves a succession of different processes, many of which may be influenced by pheromones. The prolific literature in this field has been reviewed in varying detail and with different emphasis by many authors, e.g. Richards,1927; Karlson and Butenandt,1959; Karlson,1960; Butler,1964,1967a,1970; Jacobson,1965,1966,1972; Schneider,1966; Shorey,Gaston and Jefferson, 1968; Regnier and Law,1968; Pyatnova et al,1969; Engelmann,1970; Law and Regnier,1971; Shorey,1973. The present survey will therefore be confined to the particular topic of this thesis, namely, the way in which pheromones are involved in the sexual behaviour of adult insects up to and including copulation. Neither the chemical isolation , purification, and identification of the active compounds, nor the

physiology of the receptors which perceive these substances will be dealt with as they form no part of the present study.

For the sake of completeness certain other sexual processes in which pheromones have been implicated may be mentioned briefly;

(i) the control of sexual maturation in adult locusts, and of sexual development in the worker and reproductive castes of social insects (Butler,1964,1967a,1970; Barth,1969; Gary,1970; Stuart,1970),

(ii) the induction of higher chiasmata frequencies in maturing male locusts (Nolte et al,1970),

(iii) the prevention of multiple mating and/or insemination in various Diptera by substances produced by the male and passed to the female during copulation (Swailes,1971; Gwadz,1972; Hiss and Fuchs,1972; Terranova et al,1972; Downe,1973),

(iv) the stimulation of oviposition (a) by pheromones transferred from the male to the female during coitus in Diptera (Hiss and Fuchs, 1972; Terranova et al,1972), in Lepidoptera (Benz,1969; Holt and North,1970), and in Orthoptera (Pickford et al,1969), (b) by a pheromone released into the atmosphere by the male in Actias selene (Hubner) (Benz and Schmid,1968), or (c) by chemicals from other females (Shorey,1973),

(v) the inhibition of oviposition by pheromones from other females in parasitic Hymenoptera and in the flour beetle, Tribolium confusum Jacquelin du Val (Shorey,1973), as well as in Rhagoletis pomonella (Walsh) (Prokopy,1972).

Functions of pheromones

Sex pheromones may be perceived as olfactory or gustatory stimuli. They may be involved, in either a releaser or primer capacity, at any stage along the sequence of events which culminates in copulation. However, the manner of their involvement, and the degree to which sexual behaviour is dependent upon them, varies greatly between species. One of the most commonly described functions of a sex pheromone is the attraction of members of the opposite sex into the immediate vicinity of the pheromone-releasing individual. Most frequently this involves guidance of the male to the female, but the reverse situation is known from several groups of insects (Butler,I970; Jacobson,I972). Less commonly, pheromones produced by one or both sexes facilitate mating by effecting the aggregation of both males and females (Hardee,I970; Levinson and Bar Ilan,I970,I97I; Moser,I970; Bakke,I973; Downe and Caspary,I973). Mating within some aggregations may occur long after the insects first assemble.

Long-distance attraction

In comparision with visual or auditory systems pheromonal communication can be effective over relatively long distances (Engelmann,I970). Attraction of male insects to females over distances of several kilometres has been reported (see Shorey,Gaston and Jefferson,I968, and Butler,I970 for examples) but the validity of these claims is open to some doubt (Bosman,I970; Butler,I970; Shorey,I970). Kishaba et al (I970) reported that caged male Trichoplusia ni (Hubner) responded to synthetic female pheromone released more than I00m upwind during wind speeds of I-2m/s. However,Sower,Gaston and Shorey (I97I) have suggested that, because the pheromone release rate used by Kishaba et al is not known, their data do not necessarily indicate normal communication distances between females and males. In turn

Sower,Gaston and Shorey estimated, on the basis of the laboratory determination of female pheromone release rates and male threshold response levels, that at a wind speed of 0.5m/s communication distances may vary from less than Im to more than I00m depending upon the individual insects involved. In some species attraction seems to occur only over distances of a few metres (Strong et al,I970; Hidaka, I972).

Pheromones are distributed downwind from the individuals releasing them, and responding insects must consequently be attracted from this direction. Wilson and Bossert (I963) and Bossert and Wilson (I963) have discussed the shapes of the pheromone distributions or plumes and the factors which affect them. The most important properties of the plumes are their disrupted nature and the extreme dilution of the pheromone. Indeed, the sensory capacity of insects seems to be such that there is no effective concentration gradient throughout much of the plume, except in close proximity to the releasing individual (Butler,I970; Shorey,I970). Attraction of sexual partners over long distances cannot, therefore, be due simply to chemotaxis (a response to a chemical gradient). Following the classic experiments of Kennedy (I939) on upwind orientation in mosquitoes, and of Schwink (I956) on the responses of male Bombyx mori L. to the female scent, it has been thought that perception of the pheromone releases positive anemotaxis (upwind movement) and it is this which guides the responding insect towards the pheromone source. On leaving the plume insects show stereotyped behaviour patterns, usually consisting of crosswind and/or downwind casts, which tend to bring them within the plume again, and from where upwind orientation is resumed. However, as Shorey,Gaston and Jefferson (I968) have noted "... this is theory only, and precise information is not available as to how the stimulated insect integrates sensory information concerning relative pheromone concentrations, air velocity, and direction, and the movement of its body in flight in

in relation to topography ".

In I972, Farkas and Shorey questioned this theory and claimed that in Pectinophora gossypiella Saunders the method by which the male steers towards a pheromone-releasing female is not dependent on sensing the direction of the air movement. Moreover, they also claimed to have proposed an alternative mechanism for orientation to a distant odour source but freely admitted that they could not explain what must be the cardinal feature of any such mechanism - that of how the insect distinguishes the "upwind" from the "downwind" direction within the plume!.Indeed, they did not even substantiate their contention that the males' behaviour was independent of air movement. Their argument was based on the finding that some of the moths which were already flying upwind in a pheromone plume continued towards and reached a small target area surrounding the odour source after the air flow in the wind tunnel was suddenly stopped and the plume "frozen" into a (temporarily) static one. They concluded that the moths oriented equally well when the plume was in moving or in still air. Clearly, the insects could have been pointed in the upwind direction by an anemotactic response before the air flow was interrupted. Their demonstration that some of the males going in the upwind direction could, after the air flow had ceased, remain within the plume for a distance of up to I.5m is nevertheless interesting. Together with the similarity in the flight paths of male P.gossypiella in the moving and static plumes (Farkas and Shorey,I972), it suggests that some other type of behaviour, such as klinokinesis or klinotaxis, is also involved in keeping the moth within the plume. It seems likely that anemotaxis is responsible for the coarse, directional (upwind) movement of a responding male and that a supplementary mechanism increases the accuracy of the male's path towards the odour source. Shorey (I973)very recently stated that "In the absence of more definitive experimentation, we should conclude that orientation through an aerial

odor plume toward the source is a complex behaviour, probably involving a number of interacting mechanisms. Different combinations of mechanisms are probably used by different insects for this purpose.".

Certain behaviour patterns, such as the crosswind patrolling behaviour of Formica montana Emery and F.pergandei Emery males (Kannowski and Johnson,1969) and the alert postures adopted at the top of clods of earth or of grass stems by some male elaterids (Lilly,1959) and scarabids (Travis,1939), may increase the likelihood of meeting the odour stimulus which induces anemotaxis. In other species laboratory experiments have shown that the pheromone may first activate the recipient insect before eliciting flight and anemotaxis (Shorey,1964; Bartell,1968; Birch,1970a; Struble and Jacobson,1970).

Guidance of pheromone-stimulated insects in flight may be supplemented by other stimuli. Dahm et al (1971) have shown that auditory cues greatly increase the efficiency with which female Achroia grisella (Fabr.) locate pheromone-releasing males, and Kay (1969) has suggested that ultrasonic communication may likewise enhance pheromone attraction in Heliothis zea (Boddie).

Short-distance attraction and arrest

Close to a pheromone source scent-induced anemotactic flight behaviour may cease and the responding insects adopt an intensive searching behaviour in which visual stimuli may play an important role (Shorey,1970; Buschinger,1972). Male moths often exhibit circling movements with simultaneous wing vibrations which cause air to be drawn over the antennae in a direction parallel to the body axis. Schneider (1964) has suggested that this is a device for accurately locating a nearby pheromone-releasing female.

Attraction due to pheromones may, in some species, only occur over very short distances as, for example, in Rioxa pornia (Wlk.) (Pritchard,1967), in Deinocerites cancer Theobold (Provost and Haeger,

I967), and in three species of Culex (Gjullin et al,I967). Males of the gnat,Hippelates collusor (Towns.), are attracted to females over distances of I5 cm or less (Adams and Mulla,I968) while for males of the scale insect, Planococcus citri (Risso), the range is no more than 5 mm (Gravitz and Willson,I968). In many instances the behavioural mechanism by which this attraction is achieved is unknown, but it may be independent of air movement, and may involve both kineses and taxes (Shorey,I973).

Long-distance attraction in danaid butterflies is accomplished by visual means. However, a pheromone, applied to the female's antennae during flight by large everted brush-organs at the tip of the male's abdomen, acts as an arrestant by inhibiting flight in the female and causing her to alight on nearby herbage, where further steps in the courtship take place (Stride,I958; Brower et al,I965; Pliske and Eisner,I969). Similarly, the abdominal scent brushes of the males of many moth species may disperse a pheromone which inhibits escape responses in the females (Clearwater,I972). These organs are everted as the male approaches a sexual partner. Both sexes of Rhagoletis pomonella (Walsh) are visually attracted to apple fruits, the site of mating and oviposition. A scent released by both sexes at these sites prolongs the stay of the males on the apples and thereby increases the likelihood of the sexes meeting (Prokopy and Bush,I972). Courtship is released by visual stimuli (Prokopy et al,I97I).

General excitation

A general increase in the level of activity is a common feature of responses to sexual odours. In species in which chemical communication occurs over short distances it may be the primary characteristic of the response. Female Cochliomyia hominivorax (Coquerel) exposed to the male pheromone show increased preening and locomotor activities which result in a greater frequency of contacts

between the already aggregated sexes (Fletcher et al,1966).Copulation is initiated during these contacts. A contact pheromone present on the body surface of female mosquitoes of the genus Aedes stimulates their conspecific males to adopt a searching, figure-of-eight flight pattern once contact with the female is lost (Nijhout and Craig,1971). This occurs whether or not the encounter resulted in copulation, and presumably increases the likelihood of the male meeting another female.

Stimulation of copulation (aphrodisiacs)

Scents produced by female insects may stimulate males to make copulatory attempts. This may be accomplished without the need for additional non-chemical stimuli, as in Matsucoccus resinosae Bean and Godwin (Doane,1966), Aonidiella aurantii (Maskell) (Tashiro and Chambers, 1967), Rhyacionia buoliana (Schiffermuller) (Daterman,1968), Diatraea saccharalis (F.) (Hammand and Hensley,1970), and Choristoneura fumiferana (Clem.) (Sanders,1971). In Trichoplusia ni this releaser effect of the female pheromone may be enhanced by visual stimuli (Shorey and Gaston,1970). In other species, however, pheromones alone are insufficient to release copulatory behaviour but act as primers by lowering the thresholds of males to other stimuli associated with females, e.g. Musca domestica L.(Rogoff et al,1964), Lucilia cuprina (Wiedemann)(Bartell et al,1969), Apis mellifera L. (Butler,1967b,1971), Bombus pratorum L. (Free,1971), Tenebrio molitor L.(August,1971).

Stimulation of females by aphrodisiacs produced by males is probably of widespread occurrence. Nevertheless, evidence for this is largely circumstantial and based upon the exposure in many species of elaborate male scent brushes or hairpencils immediately prior to copulation (Aplin and Birch,1968; Pliske and Eisner,1969; Birch,1970a; Grant,1970). Apart from the arrestant responses already described, overt female responses to odours from these hair brushes have not been observed. Female Phlogophora meticulosa (L.), however, did not mate with

males from which these brushes had been removed (Birch,I970a). In the dipterans Cochliomyia hominivorax (Fletcher et al,I968) and Dacus tryoni (Frogg.) (Fletcher,I969), the females do show behavioural responses to the male pheromones which are typical of normal copulatory behaviour. In response to a male pheromone female Cerocoma schaefferi (L.) indicate their willingness to mate by a downward movement of the head (Matthes,I970).

Achievement of mating position and protection of spermatophore

In many Orthoptera and Dictyoptera in which the female mounts the male during at least the preliminary stages of copulation, the males possess glandular structures or areas on their terga (Roth and Dateo, I966), elytra (Richards,I952),or hind tibiae (Fulton,I93I; Vickery and Johnstone,I970) which the female palpates or on which it apparently feeds. The secretions seem to entice the female into a position above the male which allows genital contact to be achieved (Roth,I970). In some species they may prolong the stay of the female on the back of the male for long enough to prevent the spermatophore being eaten before sperm transfer is completed (Roth,I969). These secretions may also have an aphrodisiac effect (Butler,I967a).

Inhibition of sexual behaviour and of responses to sex pheromones

Although most sex pheromones have an excitatory affect upon the recipient insect some actually inhibit sexual behaviour. Male Tenebrio molitor produce, in response to the female pheromone, a secretion which reduces intraspecific competition by inhibiting the response of other males to the female scent (Happ,I969). On the arrival of a stridulating male at the entrance to her gallery a female Dendroctonus pseudotsugae Hopk. releases a substance which masks her own population-aggregating pheromone (Rudinsky,I968). Masking, which is reversible, is effective only within the immediate vicinity of the

gallery and appears to be a mechanism for regulating the sex ratio of the aggregating adults. Nijholt (1970) has suggested that in another scolytid, Trypodendron lineatum (Olivier) the male produces a mask to the female pheromone.

Soo Hoo and Roberts (1965) reported that male Rhopaea magnicornis Blackburn that were attracted to virgin females departed within one minute when the females were allowed to mate. This behaviour is highly suggestive of the presence of repellent or masking substances. Female Hippelates collusor that had passed the stage of maximum attractant production appeared to be repellent to males when tested in a laboratory olfactometer (Adams and Mulla,1968). A male Drosophila may direct the initial stages of his courtship behaviour towards flies of either sex. However, a chemical cue perceived while touching the other fly with a foreleg inhibits further sexual activity towards conspecific males or females of another species (Ewing and Manning,1963; Narda,1966). Pheromones may prevent interspecific matings by inhibiting responses of males from closely related species (Mitchell, 1972; Berisford and Brady,1973). This may be especially important in groups of species with chemically similar pheromones.

Sequences of behaviour

In a single species pheromones may be involved in many of the functions mentioned above. The types of activity they mediate do not occur in isolation but within sequences of behaviour that are often complex, and which lead ultimately to coitus. In some insects stimuli of both a chemical and a non-chemical nature are required before attempts at copulation are observed (Crane,1955; Stride,1958; Barth,1970). In others however, whole sequences of successive behavioural steps including copulation attempts can - at least under experimental conditions - be evoked merely by the presence of a pheromone, e.g. Bombyx mori (Schwink,1958), Trichoplusia ni (Shorey,1964), Rhopaea

magnicornis (Soo Hoo and Roberts,I965, Anagasta kuhniella Zell. (Traynier,I968), and Epiphyas postvittana (Wlk.) (Bartell,I968). Of course, under natural conditions other stimuli are probably involved, particularly during the later stages.

It has been shown that for Bombyx mori (Schwink,I958) and Epiphyas postvittana (Bartell and Shorey,I969a) these sequences represent hierarchies of responses, for successive stages are elicited only at successively higher pheromone concentrations. In nature, increasing pheromone concentrations would be experienced by an insect approaching a pheromone-releasing individual. These sequences are terminated prematurely if the threshold concentrations for the later stages are never encountered. In many instances the early steps in the sequence are always completed before the later ones are elicited, even though pheromone concentrations above the threshold for the later stages are continuously present (Shorey,Gaston and Jefferson,I968). In Choristoneura fumiferana however, a sufficiently strong pheromone stimulus may release wing buzzing and circling movements in males, without earlier stages being expressed (Sanders,I97I).

Although in some insects a single pheromone compound may be responsible for eliciting both attraction and copulatory behaviour (Shorey,I970), it has been suggested that in others different compounds or combinations of compounds may be involved in the elicitation of these different responses (Jacobson et al,I970; Brady et al,I97I; Butler,I97I; Redfern et al,I97I; Mansingh et al,I972).

Factors affecting pheromonal communication

Chemical communication does not take place with equal efficiency under all conditions. Environmental factors such as wind speed and temperature, and the physical properties of the scent molecules themselves, affect the size, shape and persistence of the pheromone

plume distributed downwind of the releasing insect (Wilson and Bossert,I963; Bossert and Wilson,I963). Other factors, relating to the physiology of both the pheromone-producing sex and the responding sex are also important, and will be surveyed in the following sections.

Calling behaviour

At certain times of day insects may adopt characteristic postures which, because they have been closely correlated with the pheromone-mediated attraction of the opposite sex (Feron,I959; Pritchard,I967; Traynier,I968; Sower,Shorey and Gaston,I97I), are referred to as " calling behaviour ". The attitudes adopted vary according to the species but are usually characterised by the inflation, eversion, or extrusion of certain parts of the body bearing the scent-producing glands or special evaporative surfaces (Lhoste and Roche,I960; Bornemissza,I964; Doane,I968). Calling is thus well developed in those species in which the scent glands form invaginations of the body surface. The everted glands may be held immobile (Sanders,I969; Percy et al,I97I; Fatzinger and Asher,I97I) or rhythmically expanded and contracted (Lhoste and Roche,I960; McFarlane and Earle,I970; Roelofs and Carde,I97I). In Phlogophora meticulosa (Birch,I970a) and Pectinophora gossypiella (Lappla,I972) the gland may be wiped on the substrate. Vibration of the wings may direct currents of air over the exposed glandular surfaces (Shorey,I964; Bodot,I967; Birch,I970a).

Age and sexual maturity

The liberation of a sex pheromone does not usually occur throughout the adult life of an insect but follows a pattern which varies according to the species (Hardee et al,I967; Happ and Wheeler, I969; Burkholder,I970; Minks and Noordink,I97I). Similarly, the responding sex is not, in general, equally responsive to the pheromone at all ages (Guerra,I968; Adeesan et al,I969; Bartell and Shorey,I969b;

Hammand and Hensley,I970). The pattern of scent release does not, however, necessarily coincide with the synthesis of the scent. Evidence that the odorous compounds, or their inactive precursors, can be stored prior to liberation has been presented by, for example, Sekul and Cox (I967), Jacobson et al (I968), Fletcher (I969), Birch (I970a,b), McFarlane and Earle (I970), Traynier (I970), and Bierl et al (I97I).

The production of viable eggs requires the integrated maturation of the reproductive system and behaviour patterns in both male and female insects. The age at which an insect releases, or responds to, a sex pheromone may therefore be expected to be correlated with other aspects of the insect's reproductive physiology. Indeed, the liberation of pheromones has been correlated in females of various species with vitellogenin secretion, yolk deposition, ovarian development, colleterial gland activity, susceptibility to insemination, and " sexual maturity " (Adams and Mulla,I968; Scales,I968; Shorey, McFarland and Gaston,I968; Barth and Bell,I970; Strong et al,I970), and in males with the ability to produce a spermatophore, and the onset of sexual activity (Roth and Dateo,I966; Fletcher,I969). Responsiveness to sexual scents has been correlated in females with ovarian development and " sexual maturity "(Feron,I959; Fletcher,I969), and in males with the ability to produce a spermatophore, accessory gland activity, completion of the synthesis of an aphrodisiac secretion, and " sexual maturity " (Shorey,Morin and Gaston,I968; Birch I970a; Happ,I970; Strong et al,I970; Carlson et al,I97I). Completion of the development of these different components of the reproductive physiology does not always occur simultaneously (Shorey,McFarland and Gaston,I968; Barth and Bell, I970; Strong et al,I970).

Factors that affect the rate of sexual maturation could logically be expected to determine the age at which an insect produces, or responds to, these sexual odours. Happ et al (I970) have shown that volatile compounds from adult male and female Tenebrio molitor accelerate

the rate of development and shorten the latency between the imaginal eclosion and attractant release in young adult females. In Aonidiella aurantii both development and the production of the sex pheromone were retarded when females were reared on potatoes rather than on lemons (Rice and Moreno,1969). Those individual Byrsotria fumigata (Guerin) females in which ovulation follows an unusual course also show parallel deviations in pheromone release (Barth and Bell,1970). However, female Musca domestica (L.) present a sharp contrast for individuals in which ovarian development has been experimentally suppressed still secrete odorous compounds which attract the males (Silhacek et al,1972).

Time of day and circadian rhythms

Attraction of insects to their sexual partners does not take place throughout the day but at times characteristic for each species (Jacobson,1972). This phenomenon is due to daily patterns of scent liberation by one sex and to differing levels of responsiveness to the scent in the other sex. The calling behaviour of female Anagasta kuhniella (Traynier,1970) and Trichoplusia ni (Sower et al,1970), and the level of responsiveness to the conspecific female pheromone in males of four species of noctuid moths (Shorey and Gaston,1965a) have been shown to be controlled by endogenous circadian rhythms. Presumably such endogenous rhythms occur in many species. Periods of sexual activity generally coincide with periods of high locomotor activity.

The amounts of pheromone that can be extracted from females of some species has been found to vary throughout the day (Wong et al, 1971; Nagata et al,1972), while in other species it remains more or less constant, despite well-defined periods of pheromone release (Shorey and Gaston,1965b; Traynier,1970). Both these situations indicate that there are daily variations in the rate of synthesis of the active compounds, for in those species without appreciable changes in pheromone content a constant rate of synthesis would lead to an accumulation of pheromone

between the periods of release.

In a few species chemical communication between the sexes may not be restricted to certain times of day, at least during laboratory experiments (Brady and Smithwick,I968; Jacobson,I972). Riddiford and Williams (I97I) have suggested that the willingness to mate at all times of day in Antheraea pernyi Guerin is due to selection which has taken place during the domestication of this silkmoth.

Copulation

The effect of mating upon the liberation of sexually active odours seems to vary according to the mating habits of each species. In those species that mate only once calling and pheromone release may be reduced or terminated after copulation (Shorey,Gaston and Jefferson, I968), although some variation between individual insects has been reported (George,I965; Keys and Mills,I968). Calling behaviour usually ceases immediately (Doane,I968), while the pheromone already present in the insect disappears more gradually, presumably by degradation and reabsorption (Lilly and McGinnis,I968; Nakajima,I970; Nagata et al, I972). The effect of cessation of attractant discharge may be enhanced by the production of masking or repellent substances (Soo Hoo and Roberts,I965), or by changes in the behaviour of the female (Doane,I968).

Scent release in multiple-mating species does not generally cease following the first mating (Bornemissza,I966; Roth and Dateo,I966; Shorey,McFarland and Gaston,I968). However, in female insects having multiple oogenic cycles pheromone release may be temporarily inhibited following copulation while eggs are laid (Adams and Mulla,I968; Bell and Barth,I970; Strong et al,I970; Minks and Noordink,I97I). Interestingly, the parthenogenetic strain of Pycnoselus surinamensis (L.), in which mating is not required, produces the sex pheromone continuously (Barth,I965). In Tenebrio molitor mating has been reported to accelerate attractant production in isolated females (Happ and Wheeler,I969).

Assessment of the effect of coitus upon the responsiveness to pheromones during the period immediately following copulation is complicated by the likelihood of habituation to the pheromone (see later) having occurred while mating. However, the long term effect of copulation probably parallels that on pheromone-release, the responsiveness of species mating only once being inhibited or reduced (Feron, I959; Fletcher et al,I966), while that of insects which mate more than once is not permanently affected (Shorey,I964). Similarly, in females with multiple oogenic cycles responsiveness may be temporarily inhibited following mating (Stay and Roth,I956; Roth and Dateo,I966). In mated female Nauphoeta cinerea (Olivier) this lack of responsiveness to the male pheromone is due to the carriage of the ootheca since mechanical removal of the latter or transection of the ventral nerve cord restores receptivity (Roth and Dateo,I966).

Seasonal physiology

Overwintering adult insects often show suppressed sexual activity as well as some atrophy of the reproductive system. Such insects may be expected to behave differently than at other times in the year. Both sexes of Anthonomus grandis Boheman respond to a male pheromone during flights to and from their overwintering sites in the autumn and spring respectively. However, for the mid-season generations reared in the cotton fields, attraction to these male substances is confined to the females (Hardee,Cross and Mitchell,I969). In diapausing Lygus hesperus Knight (Strong et al,I970) and Pyrrhocoris apterus L. (Zdarek,I968) the females do not produce, and the males do not respond to, the sex attractant present at other times of the year.

Shorey (I970) notes that seasonal fluctuations occur in the responsiveness of noctuid males in laboratory cultures in California but does not explain this phenomenon. The differences in the field responses of male Trichoplusia ni to the female attractant at

different times of the year, as described by Saario et al (1970), seem to be due to variations in the physical conditions of the environment rather than to physiological changes in the insects.

Variation between individuals

Variation between individuals has been reported with respect to pheromone production (Shorey and Gaston,1965b; Rahn and Labeyrie,1967; Minks,1971; Sower,Gaston and Shorey,1971), and to responsiveness to pheromones (Cowan and Rogoff,1968; Fletcher et al,1968; Sower,Gaston and Shorey,1971).In at least three of these instances (Cowan and Rogoff, 1968; Fletcher et al,1968; Minks,1971) the variation between the individuals was probably genetically controlled.

Crowding

Experimentally crowded female Tenebrio molitor emit more pheromone than do isolated females of the same age (Happ and Wheeler, 1969). This may be due to the acceleration of the maturation of young adult females by pheromones from other adults (see earlier). In contrast, Hardee,Cross,Mitchell et al (1969) have indicated that male Anthonomus grandis liberate more attractant when isolated than when kept in groups.

Food

The food of either the larval or imaginal stages may influence chemical communication between the adult insects in a number of ways.

In Trichoplusia ni (Saario et al,1970) and Pectinophora gossypiella (Sharma,Rice et al,1971) males fly just above the tops of the larval food plants and thus increase the probability of encountering the female pheromones. In other species larval food plants seem to increase the response to scent-releasing females (Rahn,1968; Scales,1968; Stockel,1971), but the mechanism underlying this phenomenon has not

been described.

Female Antheraea polyphemus (Cramer) (Riddiford and Williams, I967) and Laspeyresia caryana (Fitch) (Schroeder,I969) only release their sex attractants upon perception of odorous compounds from the respective foods of their larvae, Red Oak leaves and pecan nuts. In other insects host plant odours increase the response to the pheromones secreted by feeding insects. The response of both sexes of Anthonomus grandis to the male pheromone is increased by the presence of volatile substances from cotton buds (Hardee et al,I97I). It is in the Scolytidae however, that interactions between pheromones and host odours are most well known. In these beetles one sex attacks a host tree and on feeding produces a pheromone which, in conjunction with host compounds incidentally released, attracts other beetles (Wood,I970; Bakke,I973). With continued feeding by the attacking beetles the ratio of host tree odours to insect pheromones changes and this change is important in regulating the sex ratio of the aggregating population (Renwick and Vité,I969; Vité and Pitman,I969).

Male Anthonomus grandis will produce their sex pheromone on a variety of diets, but they liberate far more on cotton buds than on any other food (Hardee,I970; Tumlinson et al,I970). Removal of these weevils from the food source causes a rapid decline in scent release. Diet may also be important in determining whether some male moths synthesise their aphrodisiac secretions (Grant,I97Ia). In Musca autumnalis DeG. proteinaceous foods are necessary for the production of the female pheromone (Chaudhury and Ball,I973), while in Lucilia cuprina (Bartell et al,I969) and Protophormia terrae-novae R.-D. (Parker,I968) they are not essential. However, in the latter two species a protein intake may enhance the general level of sexual activity. Shorey et al (I969) have shown that odours from the foods of Drosophila and Lucilia directly stimulate sexual behaviour.

In Rhodnius prolixus Stal only fed males respond to the

pheromone produced by copulating pairs (Baldwin et al,I97I). Starvation of male Periplaneta americana (L.) did not significantly reduce the response to the female attractant (McCluskey et al,I969), whereas in Phlogophora meticulosa unfed males interrupted courtship if a food source was located (Birch,I970a).

Male Euglossine bees accumulate in the spongy tissue of their swollen hind tibiae odorous substances from the orchids they pollinate. It has been tentatively suggested that these compounds may be used in a modified form as pheromones to attract females to the copulation sites (Dodson et al,I969).

Moore (I967) has postulated that pheromone evolution has been closely correlated with the feeding habits of adult insects, and with the relative importance of vision in each species. In those species in which adult aggregation occurs at the food sources, or in which vision is involved in both food and female detection, long-distance attractants are relatively unimportant. In contrast, in those species with non-feeding imagos scent attraction has become extremely sophisticated. This hypothesis has been endorsed by Shorey et al (I969).

Habituation to the pheromone stimulus

Behavioural responses to sexually important chemical cues do not continue indefinitely in the absence of other stimuli. Prolonged or rapidly repeated exposures to a pheromone result in a marked waning of the response as the insect becomes " habituated " to the stimulus (Shorey,I964; Gaston et al,I967; Klun and Robinson,I970). This phenomenon seems to represent a mechanism which prevents the wasteful expenditure of energy by a male responding for long periods to a pheromone released by an individual that cannot be located.

In laboratory tests habituation may occur very rapidly (Bartell,I968). Following habituation the threshold to further stimulation by the pheromone is raised for a variable period, but full

recovery is usually completed by the normal time of mating activity on the following day (Shorey and Gaston,I964; Shorey et al,I967; Bartell and Shorey,I969b). Traynier (I970b) has suggested that the rate at which males of two lepidopteran species habituate is independent of the pheromone concentration to which they are exposed, provided the latter is above the threshold level for a behavioural response.

Physical factors

Fluctuations in some of the physical parameters of the environment may greatly affect the attraction of one sex to another. Factors such as wind speed, relative humidity, temperature, and light intensity may act directly by being above or below threshold values for flight behaviour or other responses (Batiste,I970; Rice and Moreno, I970; Saario et al,I970). In addition, the effect of some variables, in particular temperature and light/dark cycles, is indirectly expressed via their influence on the entrainment of the endogenous circadian rhythms that underlie most sexual behaviour in insects (Shorey and Gaston,I965a; Sanders,I969; Sower,Shorey and Gaston,I970, I97I; Traynier,I970a).

Behavioural relevance

Chemical communication in some insects takes place only in certain physical situations or at behaviourally appropriate times. Queen honey bees, Apis mellifera, produce in their mandibular glands a pheromone which functions inside the hive to inhibit ovarian development in the workers and prevent the building of queen cells, while outside the hive it acts as a sex attractant for the drones (Gary,I970). Drones do not respond to the pheromone inside the hive, and even outside it do so only at certain heights above the ground (Gary,I962). Furthermore, the response of the drones to the queen's scent may be limited, in some instances, to certain topographical

areas (Strang,1970). Holldobler (1971) has suggested that in the ant, Xenomyrmex floridanus, the same substance may act as a trail pheromone when placed upon the ground and a sex attractant when released into the atmosphere. Males of Aglia tau (L.) may be attracted to the vicinity of females placed on a tree trunk five feet above the ground but only accurately locate the females if these are placed very near the ground (Stace,1961). Moreover, in Trichoplusia ni males will only enter a pheromone-baited trap provided certain visual stimuli are also present (Sharma,Shorey and Gaston,1971).

Male insects may show calling behaviour only until a female is attracted into the immediate vicinity, the everted scent glands then being retracted and the final stages of courtship guided by other stimuli (Bornemissza,1964; Pritchard,1967).

Species-specificity of response

The intimate involvement of pheromones in the mating behaviour of many insects suggests that they are ideally suited to play a major role in the sexual isolation of different species. In some instances chemical cues certainly fulfill such a function. In Drosophila (Narda,1966) and Rhinocoris (Parker,1969), for example, chemicals present on the surface of the insects allow the males to distinguish their conspecific females from other males and from females of other species. However, strict species-specificity of response to sex pheromones does not occur between all species for heterospecific responses have been reported by numerous workers (Jacobson,1972). Preisner (1969) has studied the interspecific responses of 600 species of moths from 27 families. In 1900 combinations of species tested he found 104 with interspecific effects, and 102 of these with reciprocal activity between the species of a pair. Such interspecific responses were only shown by fairly closely related species, and as the affinity of two species decreased so did the likelihood of interspecific responses.

This has been supported by the work of Lanier (I970) who found that female bark beetles of the genus, Ips, showed a level of response to the pheromone of a hybrid male which was intermediate to the levels shown to the pheromones of it's parent species. Clearly, closely related species may not necessarily be isolated by the chemical cues employed during their mating behaviour but other factors, such as temporal, spatial, ecological, behavioural, and anatomical differences, also serve to protect the integrity of a species.

Control of pheromone behaviour

Very little is known about the control of chemical communication in insects. Payne et al (I970) have shown that factors such as age, time of day, and light intensity, which greatly affect the behavioural response of male Trichoplusia ni to the female pheromone, did not affect the electroantennogram responses of the males to this scent. These authors concluded that the variations in the behavioural responsiveness of the males were therefore due to central inhibitory mechanisms, and not to peripheral filtering devices at the receptor level. The restoration of responsiveness to the male pheromone in pregnant female Nauphoeta cinerea by transection of the ventral nerve cord indicates that the central nervous system may be involved in the control of responsiveness (Roth and Dateo,I966).

Investigations into the control of the release of sexual scents have been concerned primarily with the involvement of endocrine factors. This work has recently been reviewed by Barth and Lester (I973). They concluded that endocrine factors can be expected to be involved in pheromone release in insects which are long-lived as adults and which mature their eggs after the adult moult, and perhaps also in species with short-lived adults " ... if there is some advantage in correlating mating with a particular environmental factor ". However, many species with short adult instars seem to lack this hormonal

control. The central nervous system is, in addition, likely to be intimately involved in the control of sexual behaviour in all species (Barth and Lester,1973).

Sex pheromones in different insect groups

Sexually important pheromones have been found in species from at least ten orders of insects - the Lepidoptera, Coleoptera, Diptera, Orthoptera (and Dictyoptera), Hemiptera, Mecoptera, Hymenoptera, Isoptera, Siphonaptera, and Neuroptera. As the occurrence of scents in these groups has been catalogued by Jacobson (1972), only the order most closely related to the Aphididae, the Hemiptera, will be surveyed here.

Hemiptera-Heteroptera

Odours involved in the mating behaviour of the Heteroptera have not been extensively studied. Most reports concerning these bugs present little more than very preliminary evidence for the existence of a chemical cue in the sexual behaviour.

Pheromones which may be operative over distances of several metres have been reported from Lygus lineolaris (Palisot de Beauvois) (Scales,1968), Lygus hesperus (Strong et al,1970), and Distantiella theobroma (Dist.) (King,1973). Contact pheromones or those operating over short distances, and which possibly have an aphrodisiac action, are found in Triatoma phyllosoma pallidipennis (Stal) (Valasquez,1965); Zelus exsanguis (Stal)(Edwards,1966), Dysdercus cingulatus F.(Osmani and Naidu,1967), Oncopeltus fasciatus (Dallas) (Lener,1967), Rhinocoris bicolor F. and R.tropicus Herrich-Schaeffer (Parker,1969), Pyrrhocoris apterus L.(Zdarek,1970), Vestula lineaticeps (Sign.) and Pisilis tipuliformis (F.)(Parker,1971), Rhodnius prolixus (Baldwin et al,1971), Dysdercus fasciatus Sign.(Brunt,1971), Podisus modestus(Dallas)

(Tostowaryk,I97I), and Nezara viridula (L.) (Mitchell and Mau,I97I). In many reduviids these compounds also serve the important function of inhibiting predatory responses of the males towards the females and allowing sexual activity to proceed (Parker,I969,I97I). Adults of both sexes of Cimex lectularis produce a scent which causes aggregation of the adult population and so presumably enhances the likelihood of mating (Levinson and Bar Ilan,I97I).

Hemiptera-Homoptera

Very few homopteran species have been shown to produce a sex pheromone. Males of Schizaphis arrhenatheri Pettersson (Pettersson, I970a,I97I) and Planococcus citri (Gravitz and Willson,I968) respond to ... retions from their conspecific females , but only from short distances. In Matsucoccus resinosae (Doane,I966), Aonidiella aurantii (Tashiro et al,I969), and Aonidiella citrina (Coquillett)(Moreno et al,I972) flying males respond to the female scents, and presumably at greater distances than in the two latter species. The role of chemical communication in the sexual behaviour of A.aurantii has been studied in more detail than in any other hemipteran, various aspects that have received attention being the response of walking males (Tashiro and Chambers,I967), the response of flying males (Tashiro et al,I969; Rice and Moreno,I970), the effects of mating (Tashiro and Moffitt,I968) and of adult food (Rice and Moreno,I969) upon pheromone production, the site of pheromone production (Moreno,I972), and the possible use of traps baited with attractive females in survey work (Shaw et al,I97I). Warthen et al (I970) have published the results of an incomplete study on the chemical nature of the active principle.

Sex pheromone glands

The occurrence and morphology of insect sex pheromone glands has been reviewed in detail by Richards (I927), Shorey,Gaston and Jefferson (I968), and Jacobson (I972). Only a summary will be given here. Scent glands may be found on any of the major divisions of the body and on their appendages. The majority are, however, associated with the abdomen, and more particularly with its posterior extremity. Generally they are formed from modified areas of the integumental epithelium or of the gut and its associated organs. They may be situated on inverted areas of the body wall, with their secretions being released into the atmosphere only when the glands are protruded during calling behaviour. In male Lepidoptera the glands may be associated with elaborate cuticular structures which are used to disseminate the pheromone. The internal reproductive system has been implicated in pheromone production in only two cases (Strong et al,I970; Hoyt et al,I97I). Comparatively little attention has been paid to the cytology of the glands, especially at the ultrastructural level. This aspect will be discussed later (p.I57).

In the Homoptera sexual scents have been shown to be produced in the pygidial glands of female coccids (Moreno,I972) and in the hind legs of oviparous aphids (Pettersson,I970a,I97I). Much less is known about sex pheromone production in the Heteroptera. Strong et al (I970) have tentatively suggested that the pheromone of female Lygus hesperus is produced in the spermatheca, while Gupta (I96I) has speculated that the thoracic glands found in many bugs may be the source of sexual odours. Experimental support is lacking in both cases.

MATERIALS AND METHODS

Aphids

Rearing and maintaining sexuales of Megoura viciae

The factors governing the production of the sexual morphs (sexuales) of M.viciae by parthenogenetic females have been studied in detail by Lees (I959).He found that the production of the apterous, egg-laying females (oviparae) was induced by short photoperiods and moderate to low temperatures,whereas the production of the smaller, alate males was independent of the photoperiod and occurred only at moderate temperatures (I5-20°C.) Of particular importance to the techniques used for rearing sexuales was the finding by Lees that far fewer males than females, on average one male per nine or ten females, are produced by any one mother. Moreover,the males are not produced at a constant rate throughout the progeny sequence of a mother but are confined to the middle batches of larvae (Lees,I959). It was for these reasons that particular attention was paid to collecting the sexuales from these batches.

A stock culture of parthenogenetic females (virginoparae) was maintained on broad bean plants (Vicia faba L.) under long-day conditions (LD I6:8) at 20.0°±I.0°C.

Oviparae and males were reared by subjecting their grand-mothers,and then their mothers, to short-day conditions at a reduced temperature. Groups of four to six young,adult virginoparae (grand-mothers of the sexuales) from the stock culture were transferred to groups of tick bean plants (V.faba) under a lamp glass and kept in a short photoperiod (LD I2:I2) at I5.0°C. All subsequent stages were held under similar conditions of light and temperature.The offspring of the virginoparae (mothers of the sexuales) were later isolated as young adults on tick bean plants by confining each female to one plant

under an inverted 3 x I" glass specimen tube.As all the larvae produced during the following week were oviparae these were discarded and the adults transferred to fresh plants for a further week. At the end of this period the tubes were removed and a lamp glass was placed over all the plants in each pot. One week later the aphids were sorted onto fresh plants according to sex. Last instar larval males, (recognisable by their wing pads) were sorted into one group,while larval oviparae,larvae too young to be sexed,and the mothers (still producing sexuales) were assigned to a second group. Thereafter the sexes were kept under similar conditions but in separate environmental cabinets.

Adult sexuales were collected daily,usually during the ninth and tenth hours of the light period,and kept in groups of a known age. Any larvae that had acquired wing pads during the previous 24h, and were thus now recognisable as males, were transferred from the female cabinet to the male cabinet. At no time were adults allowed access to adults of the opposite sex.

The tick bean plants were grown in sand and used as young seedlings at the hook stage of germination, whereas the broad bean plants on which the stock culture was maintained were grown in a peat compost. Temperatures within the environmental cabinets were maintained with an accuracy of ± I.0°C. The light intensity during the photoperiod was I05-I70 ft-c. or I50-200 ft-c. according to the cabinet.

Rearing and maintaining sexuales of Acyrthosiphon pisum Harris

Sexuales of Acyrthosiphon pisum Harris were not reared routinely but only for use in the species-specificity tests. As in M.viciae the males and oviparae of A.pisum are produced by the same virginoparae but, in contrast to M.viciae,the males of A.pisum are produced towards the end of the progeny sequence(Kenten,I955).

A group of middle instar larval virginoparae on tick bean plants were transferred from long-day conditions (LD I6:8) at I5.0°C. to short-day conditions (LD I2:I2) at I5.0°C. These females were allowed to mature and produce their offspring (mothers of the sexuales) which, when adult, were placed in small groups on fresh bean plants under a lamp glass. At weekly intervals the adults were transferred to fresh plants and their offspring (sexuales) kept on the same plants to mature. Under these conditions a few adults lived long enough to reach the end of their progeny sequence and produce a few males. Adult sexuales were collected in the same way as those of M.viciae . The same environmental cabinets were used for both species.

Age notation for adult sexuales

The day on which adult sexuales were seperated from the larvae was designated the first day of adult life, for individuals could have moulted into this instar as much as 24h before collection.

Insects used in experiments

Except where a definite statement to the contrary is made, all insects used in experiments were virgin, having had no opportunity to mate. Males were used only once on any given day to prevent possible habituation to pheromone stimuli complicating the results.

In many experiments aphids without certain leg or antennal segments were used. These appendages were removed with fine forceps or razor-blade knives under light diethyl ether anaesthetic on the day before the insects were needed.

Bioassay Techniques

Preliminary observations and development of a routine bioassay

No experimental work on aphid pheromones had been published before this project was started. There was,therefore,no firm indication that chemical signals were involved in the sexual behaviour of aphids. Consequently, the initial problem entailed a search for some behaviour pattern of the sexuales, particularly a male response to oviparae, which might be mediated by olfaction. A suitable bioassay,based on this behaviour pattern, would permit many other aspects of pheromone communication to be examined.

Most value would be derived from a bioassay in which the behavioural responses employed were unambiguous, which was quick and easy to conduct, and which could be used throughout the whole of the light period,if not the entire 24-h light:dark cycle. It was assumed from the start,however, that females were most likely to release their sex pheromone during the photoperiod as males are normally active during this time and are inactive in the dark period.These requirements were therefore borne in mind during the preliminary observations on the behaviour of the oviparae and males..

The methods of presenting chemical stimuli to test insects, as well as the responses they elicit, are very diverse and have been reviewed by Jacobson (I972) and Shorey,Gaston & Jefferson (I968).Most commonly,insects are exposed to odours by placing them in an air stream that has been passed over the individuals suspected of releasing a pheromone.Since this type of test is easy to arrange and can be used under many different conditions its feasibility with M.viciae was investigated. Male aphids did not react to an air stream that had been passed over oviparae either by wing-fanning,flight activity, or by extension of the genitalia - types of behaviour which occur in many insect species (Jacobson, I972;Shorey,Gaston & Jefferson,I968).

There was an increase in general locomotor activity and in upwind movement (anemotactic behaviour) when the air flow was switched on but this was either very short-lived or occurred whether or not females were present upwind. These initial trials with a wind-borne stimulus did not, therefore, yield any evidence for the presence of a sex pheromone. Males of M.viciae are reluctant to fly and flying males were not tested.

Attempts to activate resting males by placing oviparae nearby but hidden by gauze screens were far more successful, particularly as virginoparae and other males did not evoke this response. A bioassay dependent upon the activation of resting insects would have the advantage of great sensitivity, for this response would be the first step in any sequence of behavioural events, and would therefore be elicited by low pheromone concentrations. However, very stringent controls would be needed as it is also likely to be a very unspecific response. This type of test was considered to be unsuitable as a routine bioassay with M.viciae as the males, even in the absence of females, show daily cycles in which there are periods when they are all extremely active.

The frequency of copulation attempts made by males towards coloured plastic beads was found to be increased by the presence of oviparae beneath the gauze floor of an experimental arena. However, the high incidence of such behaviour even in the absence of females meant that any assay using this response would necessarily be time consuming and cumbersome.

Further investigations of the responses of males to oviparae held within the immediate vicinity were made with simple choice-chambers in which females and activated charcoal were placed in adjoining halves beneath a perforated floor. Males clearly aggregated above the females in preference to the charcoal. Nevertheless, this response in groups of males was not incorporated into a routine bioassay as observations on males attempting to copulate with plastics beads suggested that male

to male interactions might influence the results. It was considered desirable to devise a bioassay involving individual males.The behaviour shown by males at the border between the two halves of the choice-chamber indicated that this was possible.

When walking from the 'female side' into the 'charcoal side' males hesitated or stopped at the border,or at a short distance inside the charcoal half, increased the amplitude of their antennal waving, and then turned round and returned to the female half. This behaviour was not shown by males crossing the border in the opposite direction. Unfortunately,this did not provide a practicable assay. After being placed in one side a male might remain more or less inactive for long periods,particularly at those times in the light period when males are normally less active. It therefore became necessary to arrange for all males to cross a zone,permeated with odours from females beneath the floor,at any time in the light period.

Males removed from the food plant and placed at one end of a narrow pathway were attracted by a light at the other end,regardless of the time in the twelve-hour light period. Two olfactometers based upon this attraction of males to light were built and tested. In one, live insects were used as the pheromone source,and in the other,pheromone extracts. The principle behind both olfactometers was the same,namely, to provide a pathway along which males were able to walk and in so doing pass through two clearly delineated zones permeated with odours from test insects, or from pheromone extracts, held below the perforated floor.

Description of the 'single'olfactometer for use with live insects

(i) General construction

This perspex olfactometer (Fig.I) consisted of a pathway or removable floor suspended between two vertical walls and below which were placed containers holding insects being tested for pheromone

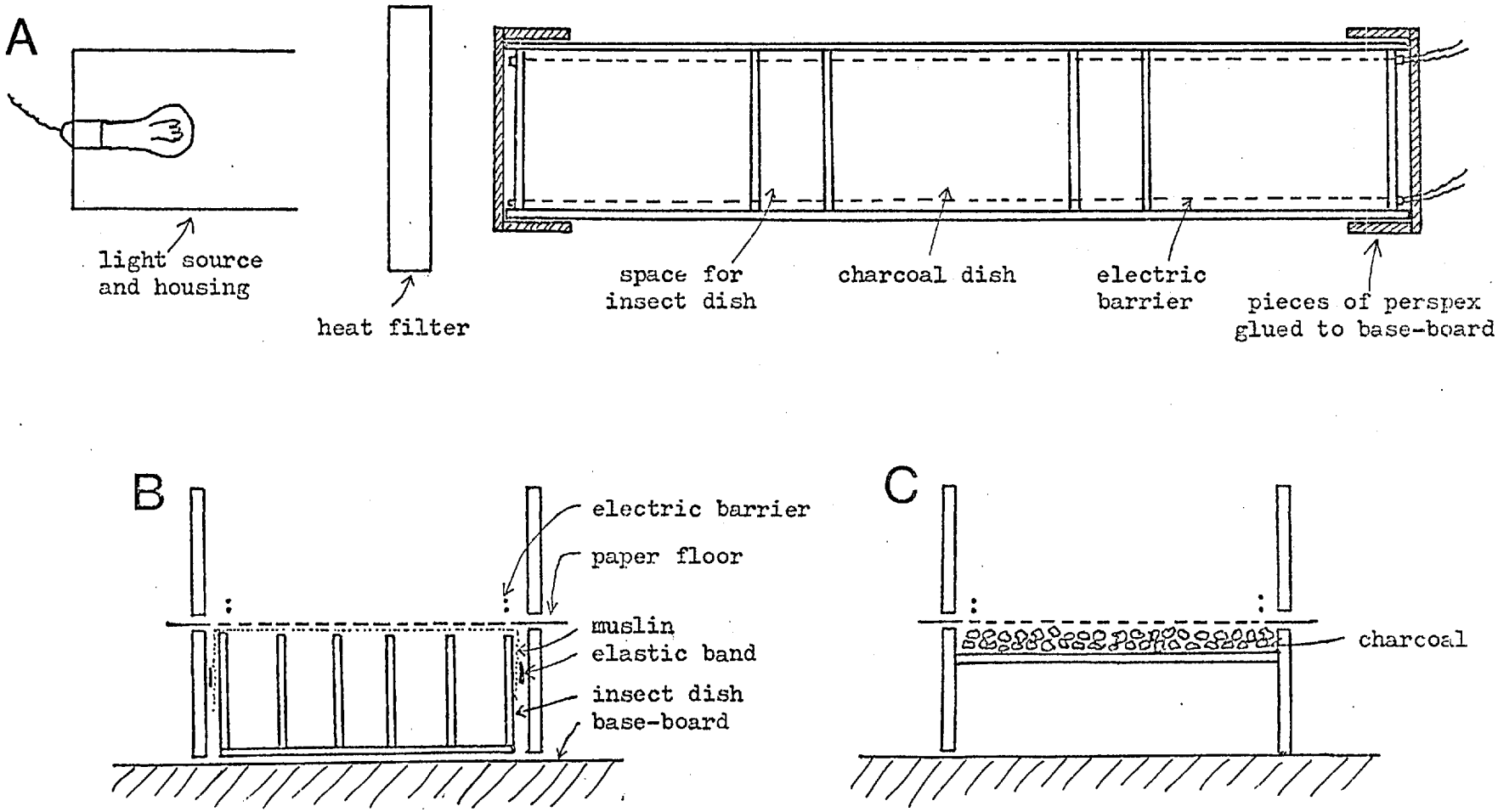


Fig. 1. Diagram of single olfactometer. A. Plan view of olfactometer, heat filter, and light source. Scale 1:2. B. Vertical section through odour zone showing insect dish and paper floor in position. Scale 1:1. C. Vertical section through charcoal dish. Scale 1:1.

production. Between the vertical walls (4.2 cm high and 28.0 cm long) three equally spaced dishes (7.6 x 5.0 x 0.6 cm) were fixed so that their tops reached the level of the floor, 2.0 cm above the base of the vertical walls. These dishes, receptacles for holding activated charcoal, were so arranged as to enclose two spaces,2.I cm long, running across the width of the olfactometer between the walls. The spaces were thus seperated by the central fixed dish and it was into these spaces that the moveable dishes containing test insects were placed. Five dishes,three fixed and two moveable,were thus arranged alternately along the length of the apparatus,and all were covered by the perforated paper floor. The latter was inserted via horizontal slits (0.2 cm deep and 27.0 cm long) in the vertical walls 2.0 cm above their bases, and overlapped the width of the olfactometer so that its edges extended through the walls on either side.

The disposable floors were made of perforated graph paper. For normal use the ruled side (millimetre ruling) was placed downwards but when the paths taken by males were recorded the ruled side was placed uppermost so that the male activity could be accurately transcribed onto a similar piece of graph paper.Floors were perforated in a reproducible pattern by means of a hand sewing machine without cotton in the needle. Using the ruled side of a piece of graph paper as a standard template several sheets were perforated simultaneously. The perforations thus produced had an outside diameter of 0.75-I.00 mm but inward fraying of the edges reduced the effective size of each hole. Centre to centre spacings of adjacent holes within and between rows was 2.5mm.

Initially floors were perforated throughout their entire length (26.5 cm) to avoid any physical change in the floor at the 'odour zones' compared to the rest of the pathway. Activated charcoal was included beneath the floor to sharpen the gradient between odour-free and odour-permeated zones. However,with the increased use of the

olfactometer the task of preparing the floors became so unduly time-consuming that the perforations were later restricted to the two test zones. This change did not noticeably affect male behaviour.

Preliminary trials indicated that many males climb upon the olfactometer walls instead of remaining on the paper floor. "Fluon" applied to the walls of a prototype olfactometer prevented this but did not prevent males spending many minutes attempting to do so. An 'electric barrier' was therefore placed along the borders of the pathway inside the vertical walls. Each barrier consisted of two independent strands of wire, 0.I9mm in diameter and I.5mm apart, connected to the same I2V power supply as the light source (see below). The lower wire was positioned approximately I.5mm above the floor. These barriers reduced the effective width of the pathway along which the males could walk from 5.0cm to 4.2cm. Males coming into contact with both strands of wire completed the circuit and received an electric shock. However, extensive use of the olfactometer showed that a vast majority of males never came into actual contact with the barrier, for the latter seemed to act more as a visual deterrent than a physical one. Most males that did touch the barrier contacted only one wire and reacted by discontinuing their attempts to climb it, and to these males it seemed to act as a physical obstacle. The current was only necessary to deter the minority of males who otherwise persisted in their attempts to reach the walls.

Dishes (I.90 x 4.50 x I.95 cm) for holding test insects below the floor were made of clear perspex and fitted closely into the two spaces in the olfactometer so that they just overlapped the width of the pathway between the electric barriers (Fig IB). Each dish was partitioned into five compartments (I.6 x 0.7 x I.8 cm) into which were put one or more aphids. Odours from a group of insects were thus spread evenly across the width of the pathway. Aphids were prevented from climbing out of the dishes onto the underside of the paper floor

by nylon gauze held over the top of the dishes with an elastic band.

Except for the perspex supports for the electric barriers, the dishes holding test insects, and the paper floors the olfactometer was painted a matt black.

(ii) Light source and arrangement with respect to olfactometer

The light used to attract males along the pathway was provided by a I2V,6W car bulb mounted in a shiny aluminium housing with the centre of the bulb I.6cm above the level of the olfactometer floor and IO.5cm from its nearest end. This source was not so attractive as to prevent males from responding to odours produced by insects below the perforated floor. Indeed, all experiments were conducted in a darkened room for other light sources sometimes competed with the experimental source and prevented males from proceeding along the apparatus.

In order that the olfactometer should be in the same position with respect to the light source in all experiments, the latter was fixed to a wooden base-board while the olfactometer was positioned between pieces of perspex glued to the board. Light intensity readings at various positions along the pathway were recorded with an Everett Edgecumbe Auto-Photometer and are given in Table I. A piece of perspex, I.2cm thick, fixed between the light source and olfactometer served as a heat filter and prevented the appearance of a temperature gradient along the pathway.

Experimental procedure with single olfactometer

Males placed in the end of the olfactometer furthest from the light would, in walking towards the light, pass successively through the two odour zones above the insect containing dishes. They respond equally well when oviparae are placed in the first or second compartments. The first compartment was therefore reserved for 'control' material, either an empty dish or appropiate aphids according to the experiment.

The second compartment, nearest the light, contained aphids being tested for pheromone production.

Table I. Light intensity at various positions along pathway.

Position along pathway	Light intensity (ft-c)
Male starting position *	4.0-4.5
Middle of first odour zone	6.5-7.6
Middle of second odour zone	I7.5-I8.6
Male removal point *	55.0-58.0

* For further explanation see below.

In the first few experiments - those reported on pages 60 to 63 - the insects serving as the odour source were placed in the olfactometer, a paper floor inserted, the light switched on, and five minutes allowed for the insects to settle down and the scent they produced to diffuse through the perforations in the paper floor. At the end of this period a male was placed in the olfactometer and allowed to walk towards the light, its behaviour being noted as it passed through each of the odour zones. When it reached the end of the pathway (see below) it was removed and another male tested. The process was repeated until a replicate of ten males had been run. The floor was discarded after each replicate.

In all subsequent experiments the procedure was modified by replacing the floor after each male had been tested. This technique avoided testing successive males on a floor that was progressively contaminated with adsorbed pheromone. Insects were placed in the two compartments below the floor and allowed four minutes to settle down before the floor initially inserted was replaced by a clean one.

One minute was allowed for pheromone to diffuse through the perforations before the first male was introduced. After testing this male, the floor was replaced, one minute allowed, and the second male tested. The process was repeated for a replicate of ten males. All floors were discarded after being used once.

The males were always started from a standard position in the olfactometer, namely, in the centre of the floor and I.5cm from the end. If the aphid walked away from the light it was replaced on the starting point and after three such failures was discarded. This only happened on very rare occasions. Males were allowed 30 s to start walking towards the light before being gently touched on the end of the abdomen. This stimulation did not affect their subsequent behaviour in the first(control)zone. Those that did not start after this treatment were discarded. Again, this happened only rarely. All males were removed from the apparatus after they had walked to within I,5 cm of the other end of the floor or had spent more than 60 s in the second zone.

Electric shocks received by males during contacts with the fence did not usually affect their response to the scent produced by oviparae. On very infrequent occasions,however,violent reactions to the fence appear to have caused a male to hurriedly pass through a test zone without responding. These individuals were tested again.

In most experiments only five insects were placed in each dish held below the floor, one aphid in each compartment in the dish. While a single male in a replicate of ten was being tested the other nine were held in corked tubes in an illuminated cabinet in the same constant temperature room.

After each replicate the moveable dishes and the ends of the fixed dishes bordering the two compartments were thoroughly cleaned with paper towelling soaked in methylene chloride.No contamination from successive replicates was ever recorded. Furthermore, the moveable dishes were alternated between test and control insects so

that a stringent check on possible contamination was automatically exercised. A pool of dishes was used in rotation so that even during continuous testing any one dish was used several hours, or even days, after it was last used.

Description of the 'double' olfactometer for use with pheromone extracts

(i) Reasons for using a different olfactometer

After many unsuccessful attempts it was eventually found possible to obtain solvent extracts of the pheromone from oviparae. These could be assayed by putting filter paper impregnated with them into the dishes below the olfactometer floor, in the place of live females. However, there were two reasons for adapting the design of the olfactometer described above to one more appropriate for use with extracts. First, the aphid pheromone extracts did not appear to be as potent as similar extracts made from insects in other orders. Secondly, Shorey & Gaston (1965b) have shown that changes in the rate of volatilisation of the pheromone of female Trichoplusia ni from pieces of filter paper at 25-27°C followed the pattern of a first order reaction for the first 30 min, i.e. the rate was proportional to the concentration of pheromone present. If the same is true for the M.viciae pheromone, it is clear that by using a single dose of extract at the beginning of a replicate successive males would not be exposed to the same concentration of pheromone. It was decided, therefore, to use separate doses for each male so that individual insects passed through the zone directly above the filter paper at a standard time after the latter had been impregnated. This olfactometer was consequently designed to reduce the amount of pheromone used, principally by narrowing the pathway and thus decreasing the area of filter paper to be impregnated with extract.

(ii) General construction

Preliminary tests indicated that a pathway 2 cm wide was sufficient to allow males to respond to the pheromone without coming into too frequent contact with the electric barrier. It was also found that two males in separate pathways could be observed simultaneously. A double olfactometer of two parallel pathways with a common central wall was accordingly made from aluminium and tin plate (Fig.2). Each pathway was 2 cm wide between the electric barriers. In addition, the construction of this olfactometer differed from that of the single olfactometer by the features described below. Both olfactometers were painted a matt black.

Since the extracts as well as the floors were changed for each male, gaps were left in the side walls below the level of the floor to facilitate the rapid positioning and removal of the moveable dishes (Fig.2B). The height of the walls above the floor was reduced to I.0 cm to improve the views of the male in the far pathway for a laterally situated observer.

Floors for adjacent pathways were kept as a single piece of paper, this double-floor being slipped into position through the slits in all three vertical walls. As for the single olfactometer, floors were initially perforated along their entire lengths but later only in the region of the two odour zones.

The moveable dishes (Fig.3) were made by folding tin plate in such a way as to form a shallow tray which held a piece of filter paper, I.8 x I.9 cm, 0.5 cm below the perforated floor.

(iii) Light source and arrangement with respect to olfactometer

A I2V,6W light bulb in an aluminium housing was positioned at the end of each pathway (Fig.2A), I.6 cm above the level of the floor. The light source and olfactometer were again held in fixed positions on a base-board. Light intensities recorded along the length of the pathways were similar to those given for the single olfactometer.

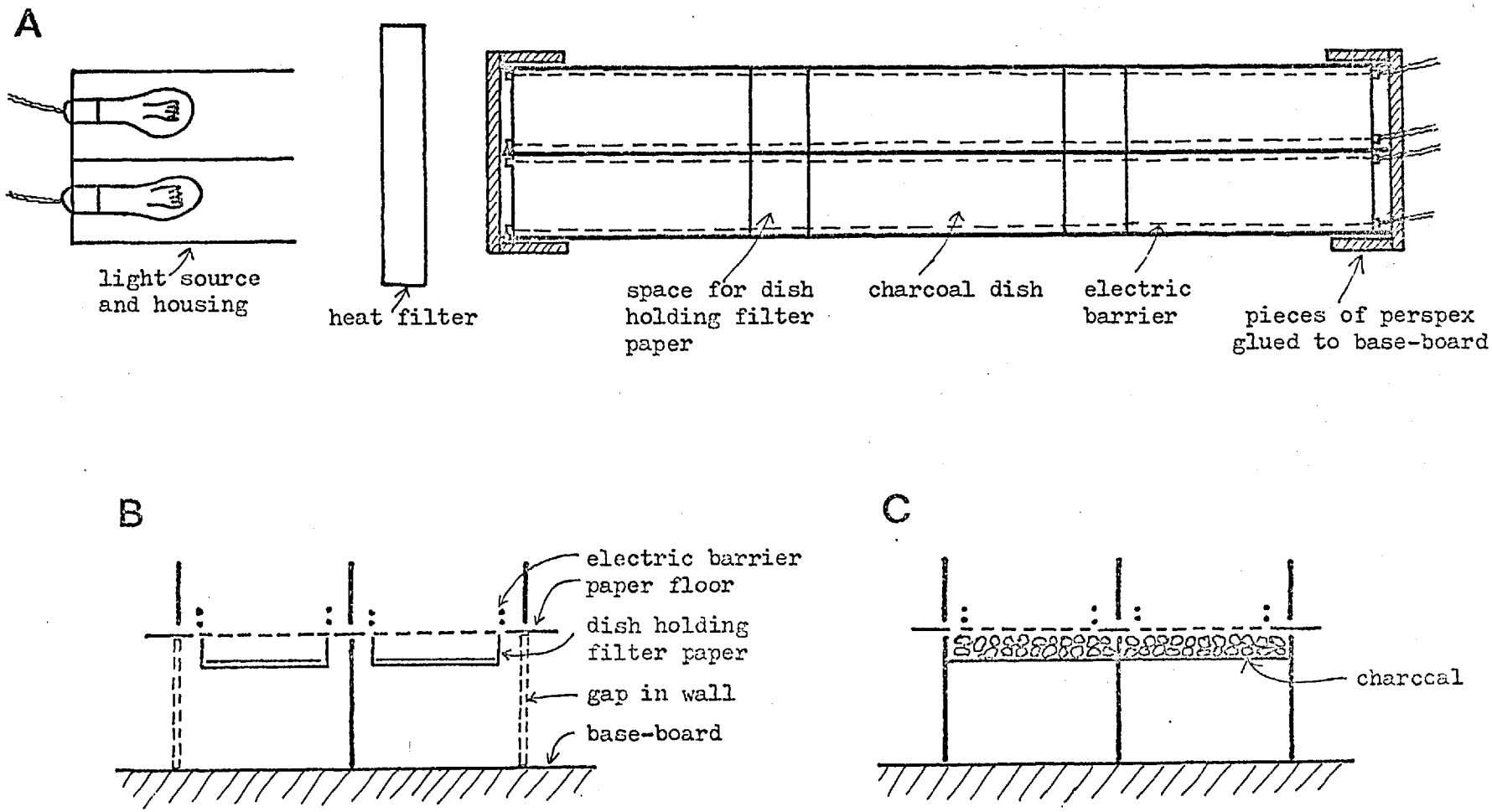


Fig. 2. Diagram of double olfactometer. A. Plan view of two pathways (floor not inserted), heat filter, and light source. Scale 1:2. B. Vertical section through odour zone showing perforated floor in position and the dishes holding the filter paper pieces beneath it. Note the supports for the dishes are not included as they would not be seen in such a section (see Fig.3). Scale 1:1. C. Vertical section through charcoal dishes. Scale 1:1.

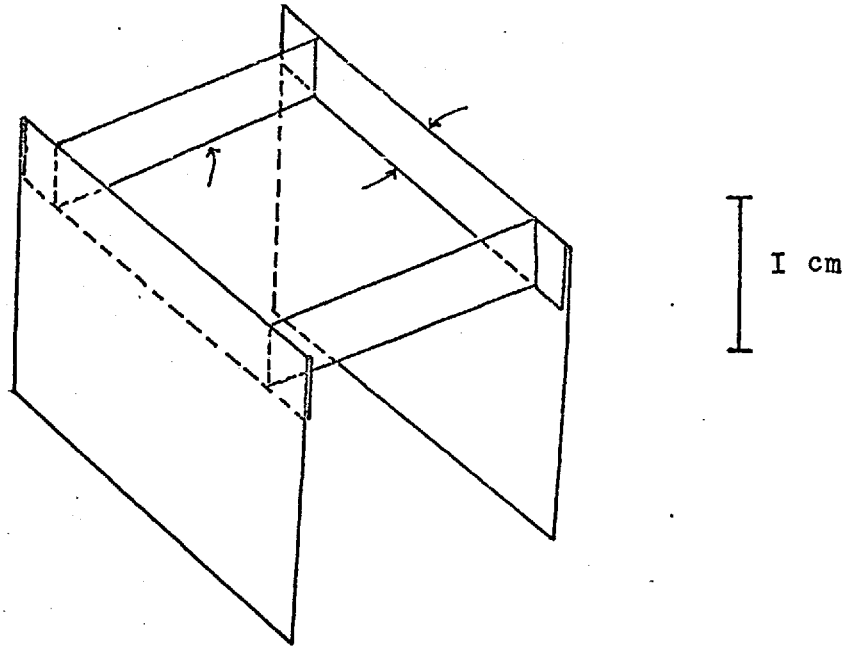


Fig.3. Dish used for holding pieces of impregnated filter paper beneath the olfactometer floor. The arrows point to bends in the tin-plate.

Experimental procedure with double olfactometer

The experimental technique adopted for testing extracts was essentially similar to that used for testing live females. A floor was inserted into the olfactometer and a piece of filter paper evenly impregnated with methylene chloride. The solvent was allowed to evaporate (aided by gentle blowing), the paper halved and the pieces, each I.8 x I.9 cm, placed in two moveable dishes which were then inserted in the spaces below the floor furthest from the light. This procedure was repeated with another piece of filter paper which was impregnated with an equal volume of methylene chloride pheromone extract and the dishes placed in the remaining spaces. The light was switched on and one minute after the extracts had been placed beneath the floor a male was introduced into each pathway of the olfactometer. After

testing the males the floor and all the filter papers were removed and discarded. A new floor was inserted and the process repeated for another pair of males, until a replicate of ten males had been completed. Males tested in this manner entered the zone above the extracts during the last half of the second and the beginning of the third minutes after the filter paper had been placed beneath the floor.

Volumes of solvent and extract used were based upon the finding that 10 µl would evenly saturate a piece of filter paper 1.0 cm square without excessively soaking it. Sixty-eight µl were therefore pipetted onto each rectangle of filter paper (in area, twice 1.8 x 1.9 cm^2) before it was halved. All pheromone concentrations were expressed as a number of female equivalents (FE) per square centimetre of filter paper (see below). During experiments unused extract was kept in a tightly stoppered 1-ml volumetric flask on ice (from a refrigerator at about -20°C.) in a vacuum flask.

Each dose of extract was placed in a clean,moveable dish. The dishes and double olfactometer were cleaned with methylene chloride at the end of each replicate.

Preparation and storage of pheromone extracts

The pheromone produced by females in their hind tibiae was extracted according to the following procedure. Groups of 6th-day,adult, virgin oviparae were anaesthetised with diethyl ether and their hind legs removed at the trochanter/femur joint using fine forceps. The legs were dropped into a 1-ml volumetric flask, covered with 1 ml of methylene chloride, and then stored under nitrogen in a refrigerator at about -20°C. After soaking for 24 h the legs were vacuum filtered from the solvent, and the filtrate stored again under nitrogen at -20°C. Solutions from successive extractions were stored until enough had accumulated for a given experiment. Extracts were normally used as soon as possible after their preparation, but some were found to be fully

active after six weeks. Storage for periods much longer than this resulted in some loss of potency. Pheromone solutions soon became inactive when stored in air at 4°C. No difference was noted in extracts made with general purpose or analytical grade methylene chloride.

Stock pheromone solutions were diluted and divided into aliquots, sufficient to test a replicate of ten males, which were then stored in individual I-ml volumetric flasks under nitrogen at -20°C. This procedure minimised the number of times a flask containing an extract was removed from the refrigerator and opened.

Pheromone concentrations were expressed as the number of female equivalents (FE) contained in one millilitre of solvent. A female equivalent represented the pheromone extracted from the two hind legs of a single ovipara. As IO µl of extract or solvent were applied to every square centimetre of filter paper used in the olfactometer, the concentration of pheromone on an area of filter paper (FE/cm^2) was obtained simply by dividing the concentration of pheromone in solution (FE/ml) by IOO. For example, a 20.0 FE/ml solution gave 0.20 FE/cm^2 when applied to the filter paper.

Description of arena used to demonstrate tropotaxis

The ability of males to respond tropotactically was investigated in an arena with a central area permeated with pheromone. To minimise concentration differences within this zone the pheromone-producing oviparae were held beneath the floor in a grid of small compartments. The square arena (Fig.4) consisted of a tin-plate dish containing 8I (9 x 9) compartments (0.75 x 0.75 x 0.75 cm) into some of which were placed individual females. A fine nylon gauze was placed over the dish and held in position by a close-fitting perspex wall, 2.5 cm high. A square of perforated graph paper, placed ruled side uppermost on top of the gauze, prevented males from seeing the oviparae and enabled the paths taken by males to be accurately transcribed. The

arena was placed on a bench about I.4 m below a light on a nearby wall.

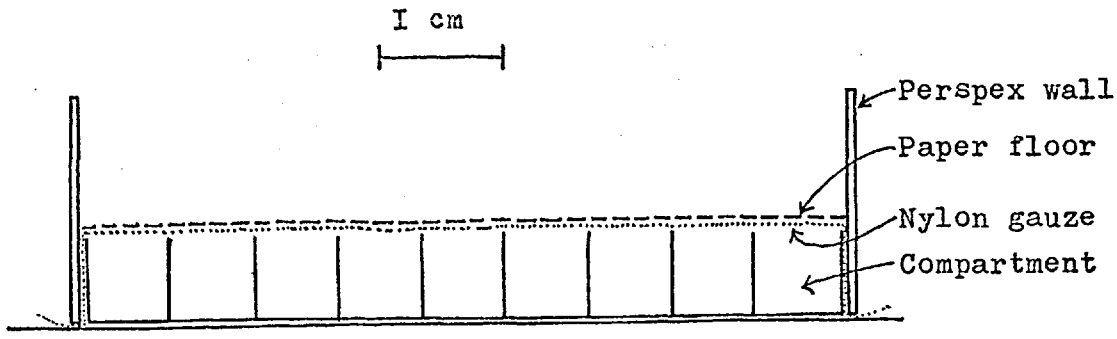


Fig. 4. Sectional diagram of arena used to demonstrate tropotaxis.

Description of arena for studying copulatory behaviour

The effect of the female pheromone upon the copulatory behaviour of individual males towards plastic beads was assayed in small, circular arenas. Each arena (Fig.5) consisted of an aluminium ring (3.5 cm internal diameter), 2.2 cm long, which was stood on end on a glass base-plate. Tin-plate strips fixed into the base of the ring divided this end into four quarter-circle compartments for holding individual pheromone-producing insects. Circular, perforated, graph paper floors (3.5 cm diameter) dropped, ruled side downwards, onto the dividing strips confined these insects to the compartments. Five plastic beads were placed in standard positions on the floor, one in the centre of the arena and one above the middle of each compartment at about two-thirds the distance between the centre and the wall (Fig.5B). Each cylindrical bead ("Blue-Box Toys", Hong Kong) was approximately

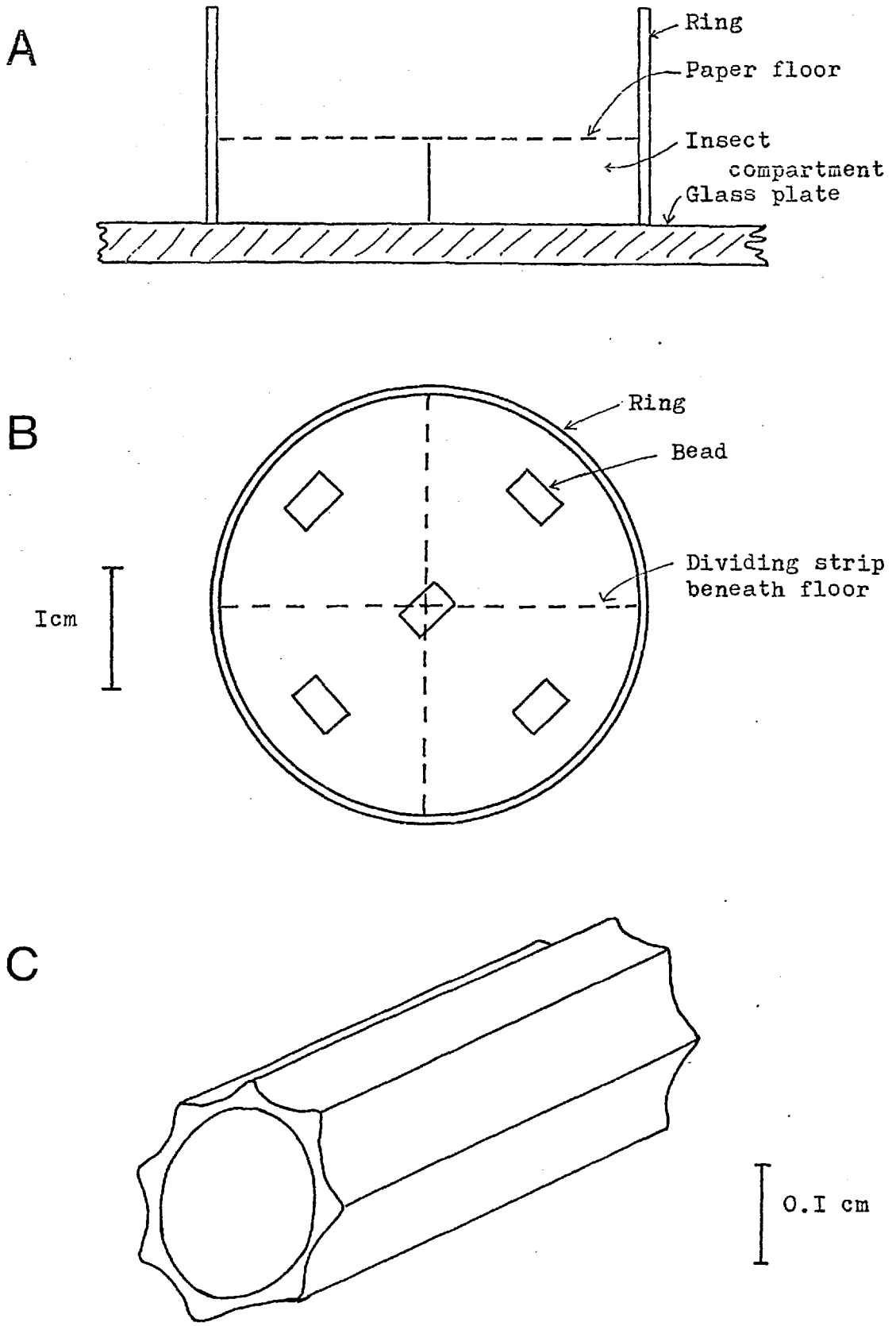


Fig. 5. Arena used to study copulatory behaviour of males.

A. Vertical section (beads not shown). B. Plan view (beads shown). C. Type of bead used.

2.5 mm in diameter and 4.0 mm long with about eight small longitudinal ridges around its circumference (Fig.5C). Test males were confined to the floor by a "Fluon" coating applied to the wall above the level of the compartments. The manner in which these arenas were used is described later.

Experimental conditions

All experiments were conducted in a constant temperature room at I4.9° to I5.9°C.(measured beside the olfactometer or arena being used). The humidity was not controlled but was recorded with wet and dry bulb whirling hygrometers and found to vary from 45 to 78% RH.

Pheromone that emanated from the experimental apparatus was not systematically removed from the room. However, the room was very small and after each replicate the door was left open for several minutes. Combined with the effects of a fan constantly in motion this probably led to a rapid exchange of air in the room. There were no indications that an accumulation of pheromone in the room ever occurred, even with rapidly repeated replicates.

RESULTS

Male behaviour in the olfactometers

Type of orientation

In the absence of pheromone male aphids walk steadily towards the light without any change in behaviour at either odour zone.However, immediately a male encounters pheromone from live oviparae,or from extracts, it begins to walk more slowly, shows a greatly enhanced rate of turning (Figs.6 and 7), and waves its antennae with greater vigour. It returns promptly to the pheromone region should it stray beyond the boundary. Normally, a male continues to respond in this manner for a few minutes, and some individuals for up to I2 minutes. There is, nevertheless, a progressive waning in the response with time for the rate of turning gradually declines, the walking speed increases, and the return to the odour zone becomes less prompt.

The response thus seems to involve both orthokinetic (slowing of walk) and klinokinetic (increased rate of change of direction) components which, by reducing a male's linear progression, keep it within the pheromone region. In this sense the odour is acting as an arrestant (Dethier et al,I960). However, the apparent directness with which males return to the pheromone zone after crossing the boundary suggests that klinotaxis or tropotaxis, or even both, also occurs. Since these types of behaviour are directed responses to a chemical gradient (Fraenkel and Gunn,I96I) the scent in this situation acts as an attractant sensu stricto (Dethier et al,I960).An experiment reported below confirmed that males are capable of responding tropotactically.

The response to a steep chemical gradient proved useful in identifying individual oviparae that were releasing much less pheromone than other members of the group of five held beneath the olfactometer

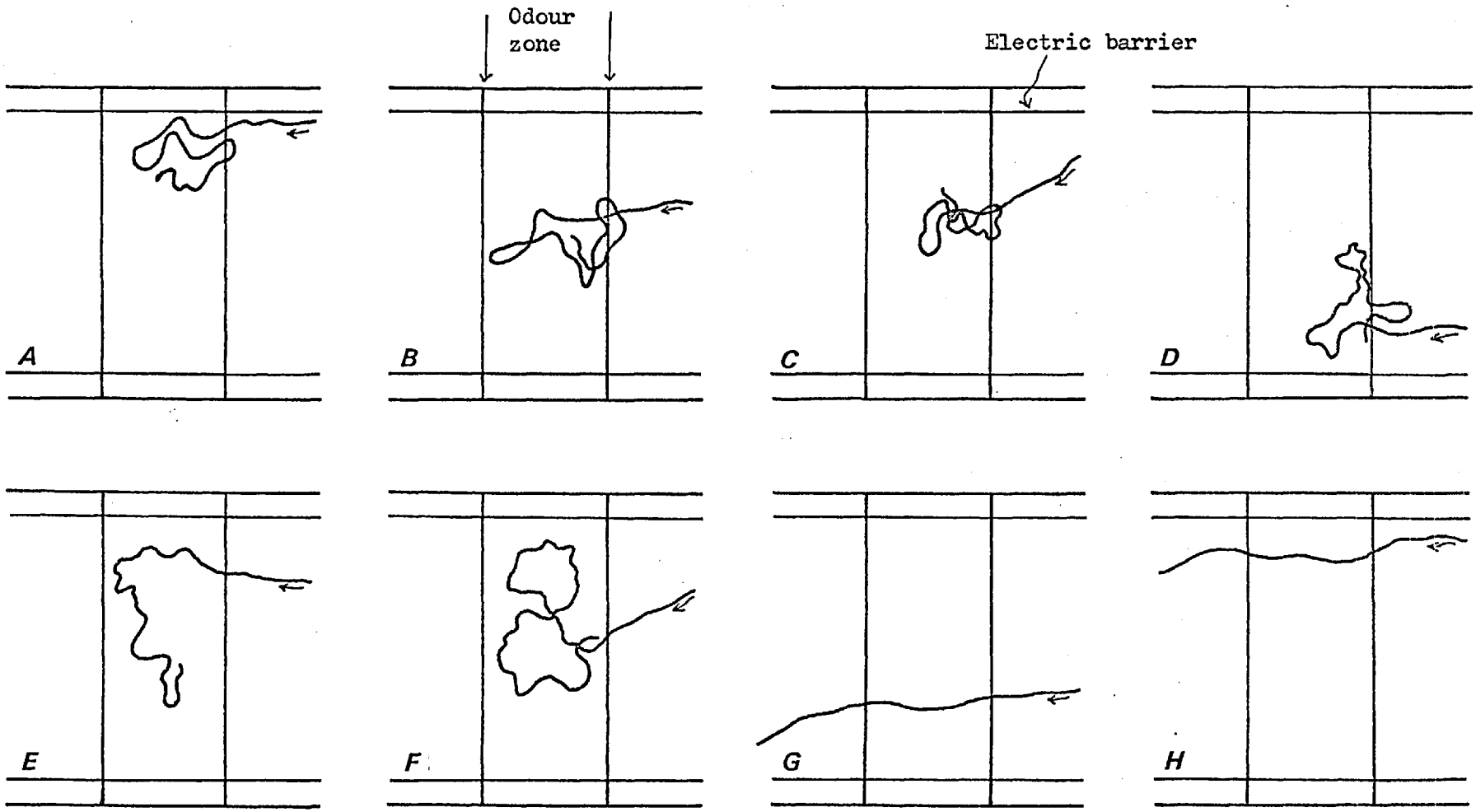


Fig.6. Behaviour of males in single olfactometer. A-F. With normal oviparae below odour zone. 60 s activity (after males first reached zone) given. G-H. With oviparae deprived of their hind tibiae below odour zone. Males initially walking from right to left. Scale 1:1.

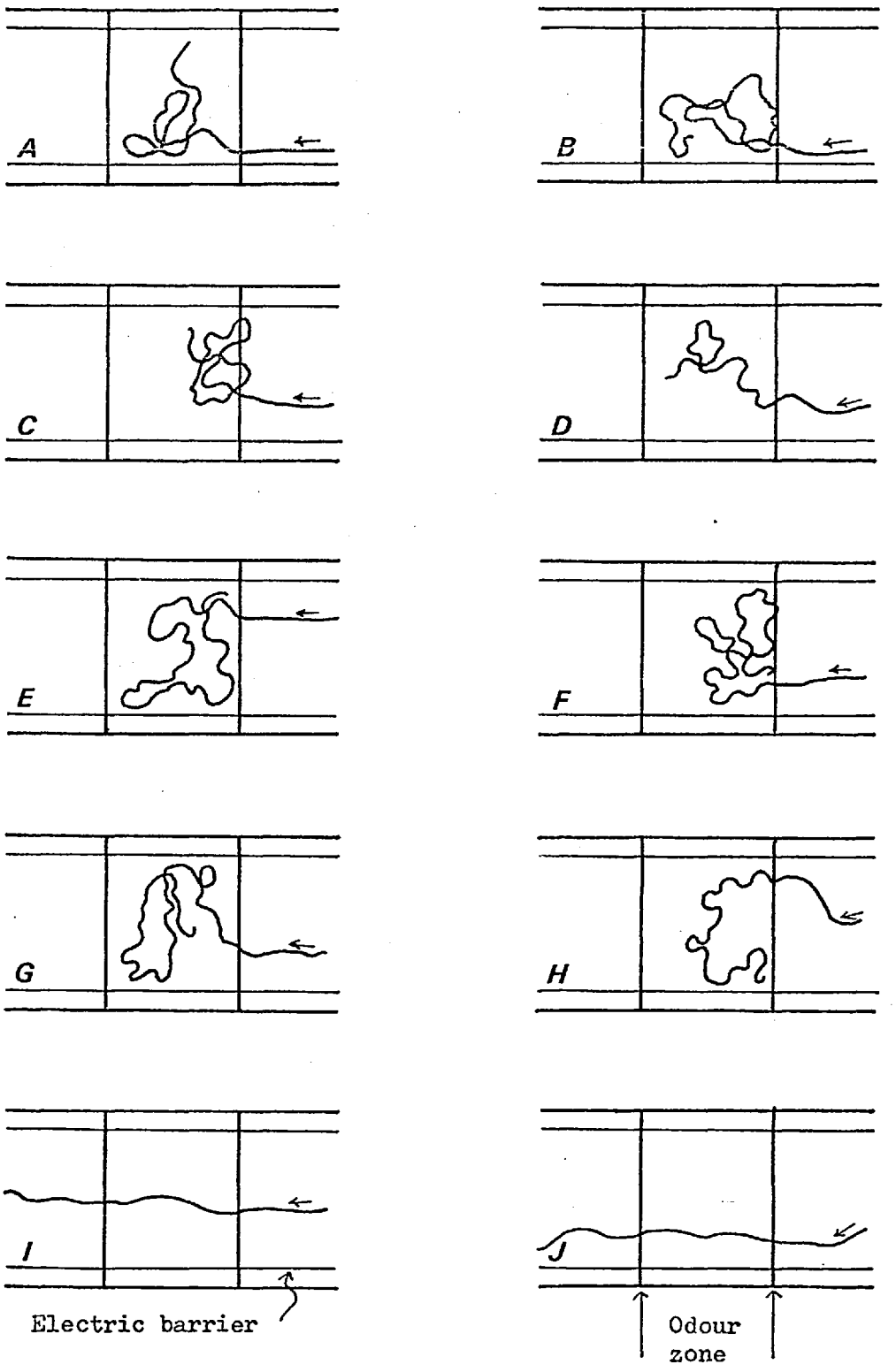


Fig.7. Behaviour of males in double olfactometer. A-H. With pheromone extract-impregnated filter paper beneath floor (0.40 FE/cm^2). 60 s activity (after males first reached zone) given. I-J. With solvent-impregnated filter paper beneath floor. Males initially walking from right to left. Scale 1:1.

floor. Successive males would consistently turn back into the regions directly above pheromone-releasing females if they wandered into areas above the relatively unattractive oviparae.

Criteria for response in routine bioassay

For the purposes of routine testing males were regarded as responding if they showed increased turning movements (klinokinesis) and remained in the odour zone for at least 30 s. Orthokinesis, however, was not included as a requirement for, despite its prominence in the behaviour of a large majority of males, some individuals showing klinokinesis failed to walk more slowly. Each male was scored as responding or as failing to do so.

Most males spent considerably less than 30 s crossing an odour zone when the pheromone was absent. In one experiment that encompassed the entire light period, the times taken for males to traverse the control zone were recorded. It was found that of the 600 insects tested 70% crossed in under 10 s and 96% in less than 17 s (Fig.8). Turning movements performed within the odour zones but which lasted for less than 30 s were very rarely seen. The criteria used to determine a response were therefore distinct and unequivocal.

All males that responded were allowed to remain in the odour zone for 60 s. However, some individuals stopped responding and continued towards the light before one minute had elapsed. This type of behaviour was of more common occurrence when extracts rather than live females were used as the pheromone source, possibly because of the lower stimulus levels involved.

The angles turned during 60 s were measured for males, varying in age from 2-17 days, that were tested during the 2nd-8th hours of the light period with 6th-day oviparae as the pheromone source. The amount of turning varied from 822° to 1,855°, with a mean of 1,382°, for the 25 tracks analysed. These values are, of course, the result of

movements made both in the odour zone and at its boundary , and are thus due not only to klinokinesis but also to tropotaxis and/or klinotaxis.

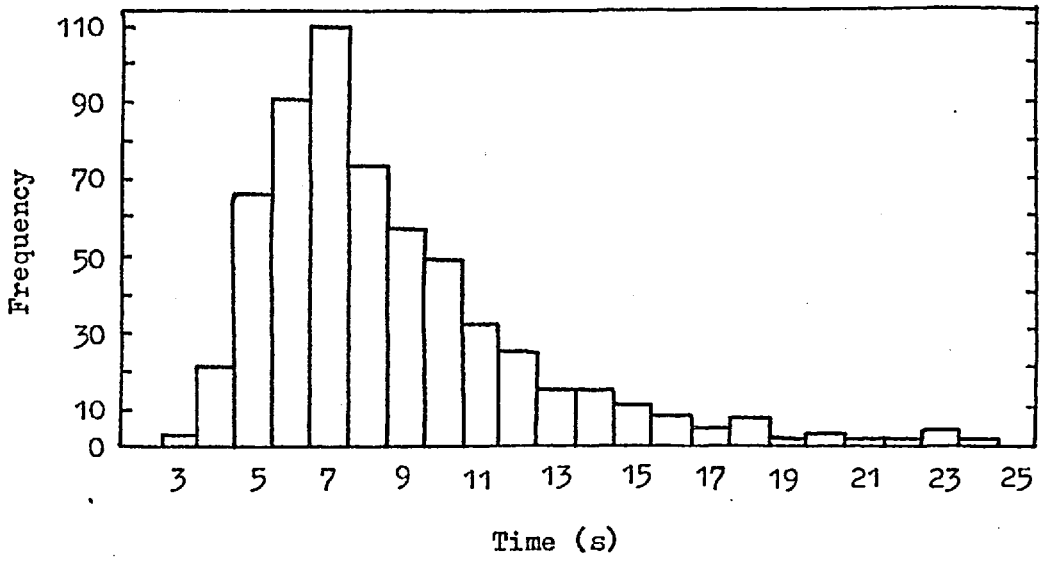


Fig.8. Frequency distribution of times taken by males to walk through the control zone in the single olfactometer.

Tropotaxis

Animals are said to be capable of tropotaxis if, after unilateral removal of appropriate receptors, they show circus movements in a uniform stimulus field (Fraenkel & Gunn,I96I). This capability in male aphids was investigated using the arena described on page 50. Twenty-five 5th-7th-day virgin oviparae, selected during the 5th-7th hours of the light period, were placed in the central 25 compartments (5 x 5) and the dish covered with the nylon gauze. Five minutes later a floor was dropped into position. After allowing one further minute a male was placed in the centre of the arena and its behaviour traced until either it had reached the wall or at least two

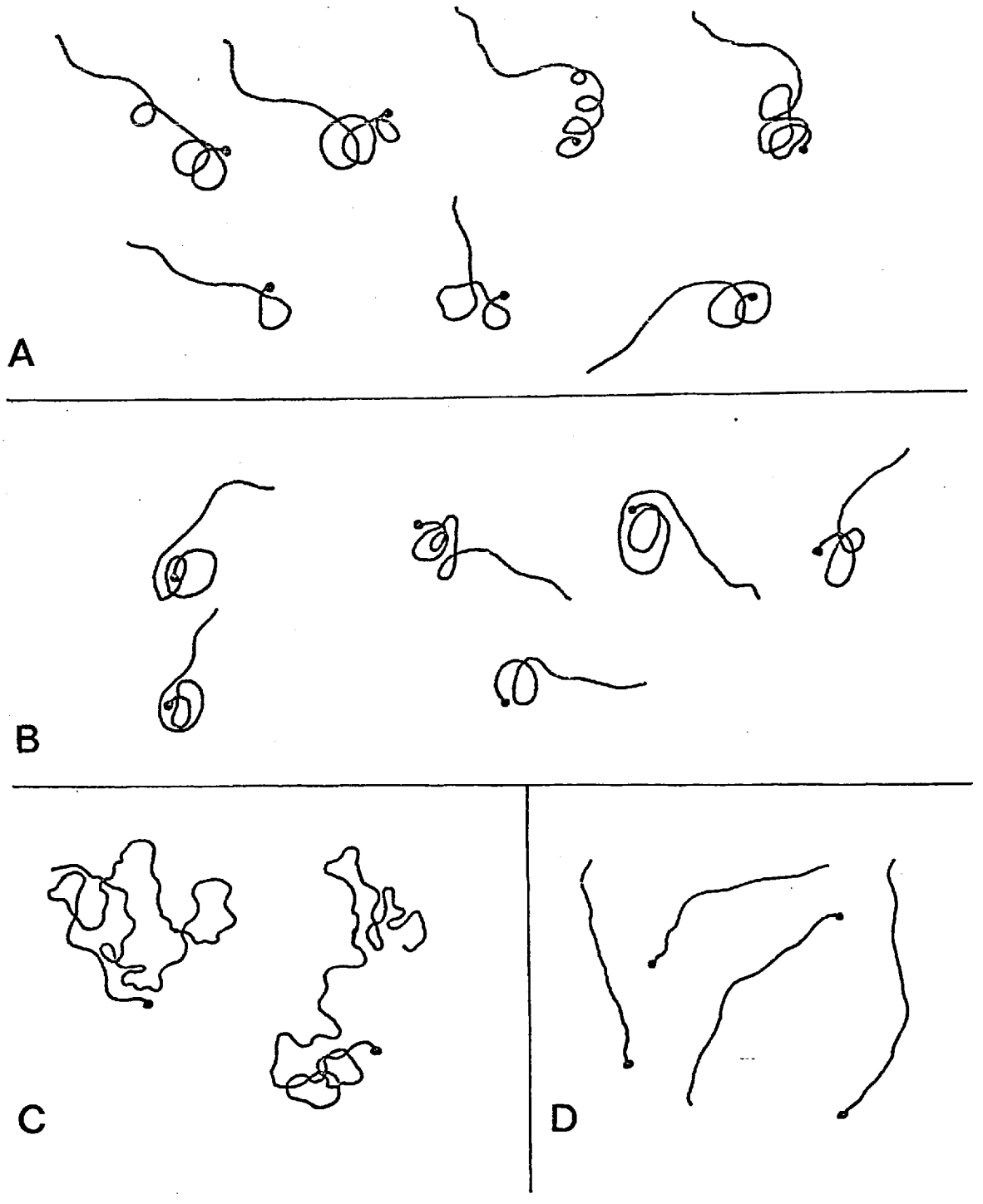


Fig.9. Demonstration of tropotaxis. A-B. Circus movements made in the presence of pheromone by one-antenna males which had left and right antenna remaining respectively. C. Paths of normal males (both antennae remaining) in the presence of pheromone. D. Paths of one-antenna males in the absence of pheromone. All tracks start at the black dots. Scale I:I.

minutes had elapsed. Floors were replaced after each male had been tested. Individuals (2-8 days old) with both antennae intact, or with one antenna removed at the base of the third segment, were tested both in the presence and absence of pheromone-producing females.

The 25 males with one antenna responded to the pheromone on 38 of the 4I occasions on which they were tested. All responses consisted of circus movements in which the direction of turning was towards the side of the remaining antenna (Fig.9). Some reactions were very short-lived, however, and consisted of little more than a single revolution. Circus movements were not shown by one-antenna males in the absence of the scent or by normal males in either situation. Responses in both types of males were typical with respect to the reduction in walking speed and increased antennal waving.

Response of adult sexuales to different aphid morphs

Response of males

To determine whether other morphs besides oviparae evoked a response in males, the latter were also tested against apterous virginoparae (apterae),alate virginoparae (alatae), and other males. Since, at this time, nothing was known of possible daily patterns of pheromone release in oviparae, or of responsiveness in males, each morph was tested at three different times in the light period,namely, during the 2nd-3rd, 6th-7th, and IOth-IIth hours.Initially, 6th-day adults, in groups of five, were placed below the second odour zone as in preliminary experiments most consistent results were obtained with oviparae of this age. No insects were placed beneath the first zone. Virginoparae were kept under similar conditions to the sexuales (LD I2:I2 at I5.0°C.) throughout their larval and adult lives. Males were used when 4-8 days old as previous trials had indicated that there were no differences in their ability to respond. The experiment

was run in triplicate and the results are presented in Table 2.

Table 2. Numbers of males responding to various aphid morphs at three times of day.

Morph	Time in light period		
	Hours 2-3	Hours 6-7	Hours IO-II
Oviparae	IO, 8, 9	IO, 8, 8	0, 2, 0
Alatae	0, 0, 0	0, 0, 0	0, 0, 0
Apterae	0, 0, 0	0, 0, 0	0, 0, 0
Males	0, 0, 0	0, 0, 0	0, 0, 0

Clearly, males reacted only to oviparae and the numbers doing so varied according to the time in the light period.To confirm that they do not respond to the other morphs of different ages males were further tested against alatae, apterae, and other males of each daily age group from 2-5 and 7-I2 days inclusive, at each of the three times in the light period. No responses were recorded.

Responses of oviparae

Having found that males responded exclusively to oviparae an experiment was conducted to see if oviparae themselves respond to males or to other oviparae. Sixth-day oviparous females were tested against males that were 2,4,6,8, and IO days old, while other sexual females, at similar ages to these males, were tested against 6th-day oviparae. All combinations were tested at each of the three times in the light period as described above. In all instances the dish in the control compartment was left empty.

The oviparae were initially more reluctant than males to approach the light, but, having started, they advanced steadily along the olfactometer. Nevertheless, no reaction whatsoever to males, or to other oviparae, was observed. The male response to oviparae therefore appears to represent a means of locating a sexual partner, rather than being a general aggregation response common to all morphs, or even to both sexes of the holocyclic generation.

Proof of pheromone communication and position of glands

Males can only detect the presence of insects below the olfactometer floor by their olfactory and auditory senses. Tactile stimulation is prevented by the nylon gauze and paper floor, while a visual response is impossible because the low angle of the light source results in the perforations being filled with shadow. Stridulation has been recorded in one genus (Toxoptera) of aphids (Williams,1922; Eastop,1952; Broughton & Harris,1971). Sound production occurrs when the insects move their abdomens rhythmically up and down, and is due to the movement of abdominal, cuticular ridges over modified, peg-like hairs on the hind tibiae. M.viciae does not behave in this way and does not possess these anatomical modifications. Evidence that the male response to oviparae is evoked by a pheromone was obtained by placing females deprived of various leg segments, or the leg segments themselves, below the test zone. Males, 4-8 days old, were tested against groups of five 6th-day oviparae during the 2nd-7th hours of the photoperiod. The leg segments placed in the olfactometer were removed from five females immediately prior to their use. The dish below the control zone was left empty. Categories of insect material tested for pheromone production, and the numbers of males that responded in the five replicates, are given in Table 3.

Table 3. Numbers of males responding to oviparae without various leg segments, and to detached leg segments.

Material below test zone	Replicates	Mean
Normal oviparae	9, 7, 8, 9, 9 *	8.4
Oviparae without metatibiae and tarsi	0, 0, 0, 0, 0	0
Oviparae without mesotibiae and tarsi	9, 6, I0, I0, 9 *	8.8
Metatibiae and tarsi alone	I0, 9, 8, I0, 9 *	9.2
Mesotibiae and tarsi alone	0, 0, 0, 0, 0	0

* No siginficant difference ($p > 0.05$) with Mann-Whitney U test.

These results demonstrate that the male response to oviparae is dependent upon some factor closely associated with the hind legs, but not with the middle legs. Since isolated tibiae and tarsi appear to be incapable of sound production the response must be elicited by an olfactory stimulus. Other stimuli are obviously unnecessary, as the hind leg segments alone evoked as high a level of response as did intact oviparae. These findings were confirmed in further tests with methylene chloride extracts of the meso- and metatibiae of oviparae, of oviparae without their hind tibiae, and of the hind tibiae of virginoparae. Responses, resembling in every way those shown to live oviparae, were elicited by extracts of the hind tibiae of oviparae but not by other extracts or by the solvent alone.

The metatibiae of oviparae differ from those of virginoparae mainly in possessing, on their basal halves, numerous domed plaques or "pseudosensoria". Ultrastructural studies summarised later in this thesis have shown that the cells underlying these structures have a glandular appearance, and it is assumed that they are responsible for

the production and release of the pheromone. For this reason the tibial structures will be referred to as "scent plaques", a more appropriate term first used by Stroyan (1957).

In all subsequent experiments employing live females as a source of pheromone, other females without their hind tibiae were used as control insects below the first odour zone. No response to such oviparae was ever recorded.

Male responsiveness throughout the light period

In order that males could be used routinely to indicate the presence of pheromone it was necessary to determine whether their responsiveness to the female scent varied during the day. This was investigated using a standard pheromone stimulus.

A method of preparing active pheromone extracts had not been developed when this experiment was first conducted. Consequently, the standard pheromone stimulus had to be provided with live females. Initial tests showed that males responded equally well to oviparae during the 3rd-5th hours, inclusive, of the light period. Observation also indicated that oviparae show a daily change in the position in which the abdomen is held relative to the substrate (see later); and that this pattern could be readily phase-shifted by altering the time of light-on in the light:dark regime. It was assumed that any daily variation in pheromone release would be similarly entrained by daily light cues. Four groups of females were therefore placed in LD I2:I2 regimes which were out of phase in such a way that the 3rd-5th hours of their light periods formed a continuous series spanning the I2 h of the male light period. Oviparae were transferred to these staggered cycles on the day of the imaginal ecdysis and were used as a pheromone source during the 3rd, 4th, or 5th hours of the photoperiod on the 6th day of adult life. A replicate of I0 males (5-I0 days old) was

tested during the middle 30-40 minutes of each hour in the male light period. Separate groups of five females were used for each successive hour. The results obtained are presented in Table 4.

Males proved to be equally responsive to the female pheromone throughout the light period. However, the stimulus used was obviously very potent for in 45 of the 60 replicates 9 or I0 males responded. Indeed, the numbers were so close to the maximum that some differences in responsiveness may have been obscured. The experiment was repeated when pheromone extracts became available and it was possible to use a lower stimulus concentration.

Males were consequently tested during the Ist, 3rd, 5th, 7th, 9th, and I2th hours of the photoperiod against pheromone extracts diluted to give a concentration of 0.20 FE/cm^2 on the filter paper placed below the olfactometer floor . In previous tests 50-70% of the males reacted to this stimulus. Methylene chloride only was applied to the filter papers below the control zone. Five replicates were completed and the results are given in Table 4.

The results obtained with this lower stimulus concentration confirmed the findings presented above , and failed to show any differences in male responsiveness throughout the photoperiod. No responses to the solvent were observed in this or any subsequent experiment in which extracts were used.

Table 4. Numbers of males responding to two standard pheromone stimuli at different times of day.

Hour in light period *	Pheromone source			
	Live oviparae		Extract	
	Replicates	Mean	Replicates	Mean
I	9,IO,IO,IO, 7	9.2	8, 7, 4, 7, 8	6.8
2	IO,IO, 9, 9, 7	9.0		
3	7, 9,IO, 9, 8	8.6	5, 7, 7, 6, 5	6.0
4	IO, 9,IO, 8,IO	9.4		
5	9,IO, 9, 7,IO	9.0	5, 7, 5, 7, 7	6.2
6	9, 6, 9, 9,IO	8.6		
7	IO, 7,IO,IO, 6	8.6	7, 6, 7, 6, 5	6.2
8	IO, 8,IO,IO, 9	9.4		
9	IO,IO, 7, 8,IO	9.0	7, 6, 6, 7, 7	6.6
IO	8,IO,IO,IO, 9	9.4		
II	9,IO,IO, 9, 9	9.4		
I2	IO, 8, 9,IO, 8	9.0	7, 5, 5, 7, 6	6.0

* With either pheromone source there was no significant difference, ($p > 0.05$), between the results for any two hours using the Mann-Whitney U test.

Male responsiveness with age

Adult males

The responsiveness of adult males of different ages also required investigation. Initially, males were tested against pheromone from live oviparae but the high response level obtained made it desirable to repeat the experiment when extracts became available. Females in the 3rd-5th hours of their light period or extracts (0.20 FE/cm^2) were used as a stimulus source (see previous section). Tests were conducted during the first nine hours of the male photoperiod. Five replicates of each experiment were completed, and the results are presented in Table 5.

Males were equally responsive at all ages from 2 to 20 days when tested at either pheromone concentration. A tendency for older males to react less frequently towards the weaker stimulus was nevertheless apparent, but only 22-day-old insects were significantly less responsive than the 2-day old ones. ($p < 0.05$, Mann-Whitney U test). Males older than 22 days were not assayed for, although some individuals survive in the laboratory for as long as four or five weeks, most die during the third and fourth weeks. All subsequent experiments were conducted with males less than 15 days old.

In the present experiments it was assumed that because 5-10-day-old males had been shown to be equally responsive throughout the light period, that the responsiveness of males of all ages was similarly unchanging. No evidence to the contrary was obtained despite the replicates in each age group being tested at different times in the photoperiod.

A few males less than 24 h old were assayed separately against 6th- and 7th-day oviparae. Responses were shown by some individuals within 5-12 h after the adult ecdysis, and by all males within 24 h.

Table 5. Numbers of males of different ages responding to two standard pheromone stimuli.

Male age (days)	Pheromone source			
	Live oviparae		Extracts	
	Replicates	Mean	Replicates	Mean
2	9, 9, 8,I0,I0	9.2	8, 7, 6, 6, 5	6.4
3	9, 9, 9,I0, 9	9.2	8, 5, 5, 6, 7	6.2
4	8, 7, 8,I0,I0	8.6	5, 5, 7, 6, 7	6.0
5	I0,I0,I0, 9, 7	9.2	7, 6, 8, 5, 5	6.2
6	9, 8, 9,I0, 9	9.0	6, 6, 5, 5, 7	5.8
7			5, 5, 8, 4, 8	6.0
8	I0,I0, 7, 9,I0	9.2	5, 6, 6, 7, 7	6.2
I0	7,I0, 8, 9,I0	8.8	5, 6, 6, 6, 7	6.0
I2			5, 7, 5, 5, 7	5.8
I4	I0, 9, 8, 8,I0	9.0	6, 7, 6, 6, 5	6.0
I6			5, 4, 7, 7, 7	6.0
I8	9, 8, 9, 9, 9	8.8	6, 7, 5, 5, 5	5.6
20			5, 7, 5, 6, 5	5.6
22	9, 9, 9, 8,I0	9.0	5, 7, 5, 4, 4	5.0

Larval males

Last instar larval males were exposed to two concentrations of pheromone from oviparae (5 or 25 females in the dish below the test zone) at three different times of day, namely, the 2nd-3rd, 6th-7th, and I0th-IIth hours after light-on. Some oviparae were kept in a LD I2:I2 regime out of phase with the males so that the 2nd-3rd or 6th-7th hours of their light period coincided with the times of

testing. Sixth-day oviparae were known, by this time, to release equal amounts of pheromone during the 2nd-7th hours of the photoperiod. Two or three adult males were shown to respond to the oviparae before each replicate of IO larvae was tested. All combinations of stimulus intensity and times of testing were run in triplicate.

No response whatsoever was shown to either the test or control (without hind tibiae) females by any of the I80 larvae used.

Other aspects of the reproductive physiology of males

Responsiveness to a sex pheromone has been correlated, in many species, with other aspects of the insects' reproductive system, (p. 20). In male M.viciae the maturation of the reproductive system, the willingness to copulate, and the ability to inseminate females was briefly examined. The descriptions given below are based on virgin insects only.

(i) Sperm maturation and storage

The main features of the internal reproductive organs of male M.viciae are illustrated in Fig.IO. The two testes, each consisting of three globular lobes, are joined together and lie in a median position. Vasa deferentia pass posteriorly from the testes to the ejaculatory duct and,in males in which mature sperm is stored, become dilated immediately below the testes to form seminal vesicles. Many stages of spermatogenesis are evident during the last larval instar and the first week following the imaginal ecdysis. Mature sperm may be observed in some 2nd-day adults, but only in older males is it present in all individuals. In IOth-day insects only the later stages of spermatogenesis are seen, while in males I6-25 days old the testes contain only mature sperm. The development of the seminal vesicles follows the accumulation of mature sperm, being negligible in 2nd-day males,very variable in 3rd-day insects,and well developed in 4th-day individuals. Thereafter, they become increasingly distended.

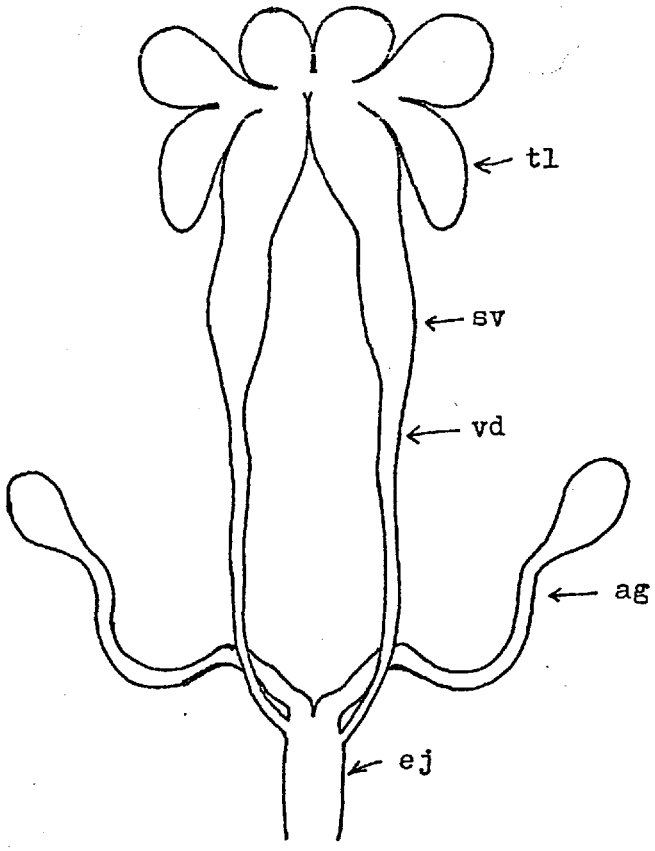


Fig. IO. Outline diagram of internal reproductive organs of male M.viciae.

ag = accessory gland and duct, ej = ejaculatory duct, sv = seminal vesicle, tl = testis lobe, vd = vas deferens. The lobes of the testes are somewhat variable in shape.

(ii) Development of accessory gland

An accessory gland from each side of the abdomen connects with the ejaculatory duct between the points of insertion of the vasa deferentia. In larval males each gland consists of a simple, thick-walled, blind-ending tube. However, during the days immediately following the adult moult a terminal, globular chamber becomes differentiated. A white translucent material containing minute droplets or granules in suspension accumulates within this chamber. This secretion

first appears in 2nd-day adults, and thereafter the amount stored and the size of the reservoirs gradually increases.

(iii) Copulation and insemination

When placed in I.0 x 0.5 " stoppered tubes with 6th-day oviparae during the 5th-6th hours of the photoperiod, males 3-28 days old readily copulated with, and inseminated, the females. However, first-day adults remained inactive and failed to copulate, despite the fact that many males of like age responded to the pheromone in the pathway olfactometer. The level of copulation increased in 2nd-day adults but remained lower than for older insects .Nevertheless, those that did mate also inseminated their partners. It seems that copulation does not readily occur until mature sperm has been produced.

The onset of responsiveness to the female pheromone appears to slightly precede the age at which storage of mature sperm begins. Reactions of very young adults to the pheromone involve orientation towards the source, and perhaps arrest within its immediate vicinity, but not copulation behaviour. It may be that in these males the visual responses involved in mating (see later) have not yet developed.

Male response and pheromone concentration

A knowledge of the relationship between the number of males which respond and the concentration of the pheromone stimulus is important both in the design of experiments and in the interpretation of results. Males were therefore tested against various dilutions of the extracted pheromone.

Extracts prepared from three groups of 6th-day oviparae were pooled. The hind tibiae were removed during the latter half of the 4th hour and the first half of the 5th hour following light-on. This particular time was regarded as being most suitable as it coincided with the middle of the period of maximum scent release (see following

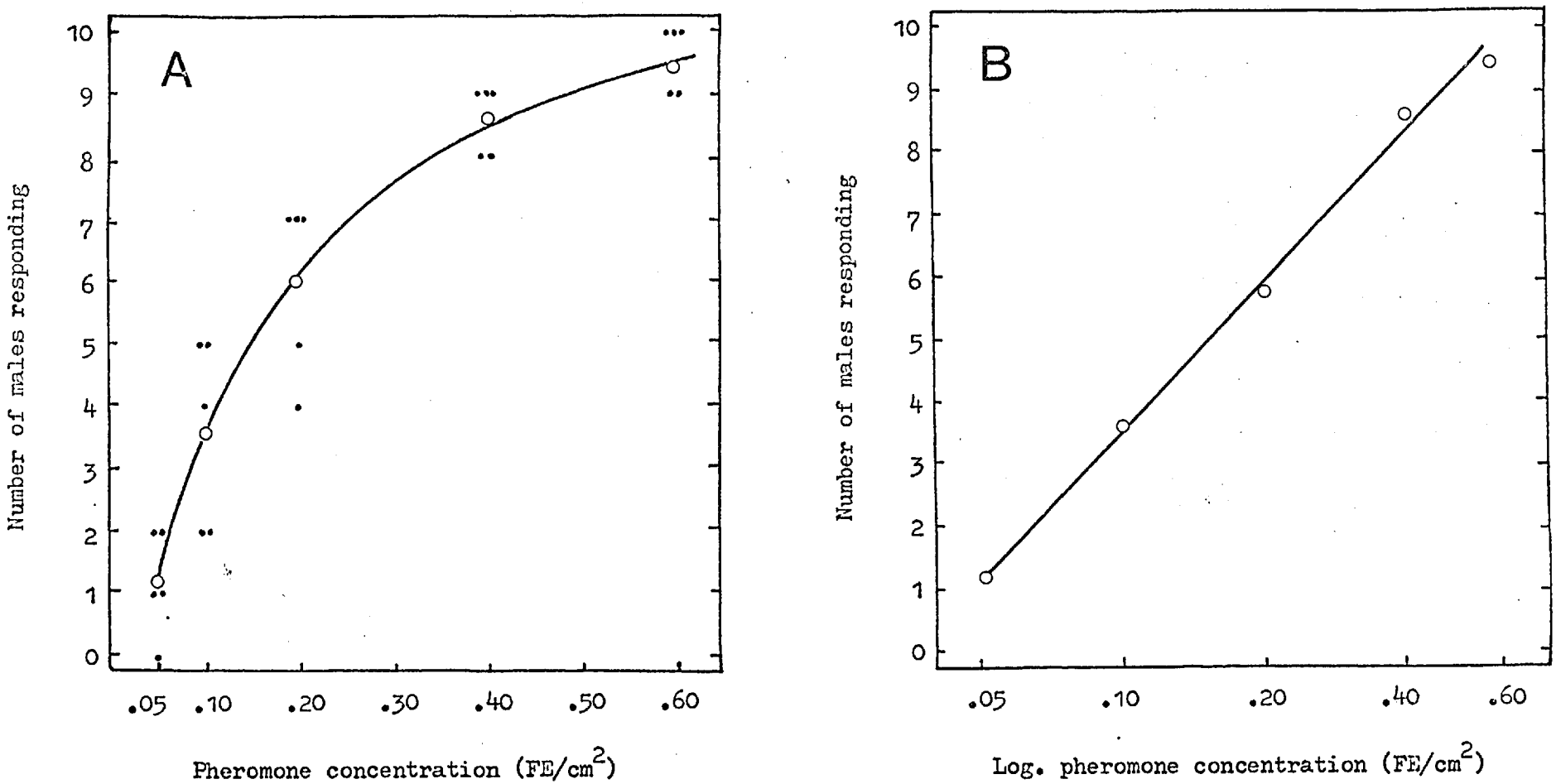


Fig.11. Numbers of males responding to different concentrations of pheromone. A= Means (open circles) and individual replicates (small,closed circles). B= Means only.

section), and was thus well removed from any changes in pheromone content which might be associated with an increasing,or a decreasing, rate of release. This choice was, indeed, fortuitous for it was later shown that a maximum amount of pheromone is present at this time. It has been demonstrated that males are equally responsive throughout the light period, and over a wide range of ages, when tested against stimuli which elicit a response in 50-100% of the males. In the present experiment it was assumed that the same would be true at lower stimulus levels. Five pheromone concentrations ranging from 0.05 to 0.60 FE/cm^2 were used and five replicates at each concentration completed. The results are presented in Fig.II.

Progressively fewer males responded with each increased dilution of the extract. The differences between successive concentrations proved to be significant ($p < 0.05$, Mann-Whitney U test) in all cases except for 0.60 and 0.40 FE/cm^2. The decrease was not, however, constant over the whole range, but accelerated progressively. For example, a two-fold decrease in concentration between 0.40 and 0.20 FE/cm^2 resulted in a 1.43-fold drop in the response level,whereas between 0.10 and 0.05 FE/cm^2 there was a three-fold difference in response. The relationship between response and the logarithm of the pheromone concentration was, nevertheless, found to be linear. It is striking that a mere twelve-fold difference in the stimulus concentration produced so great a fall in the numbers of males responding.

Daily patterns of pheromone release in females of different ages

Pheromone release

Previous results have indicated that the rate of scent release by oviparae varies during the day. The number of males responding to 6th-day females was not the same at three different times in the light period (Table 2), despite the lack of variation in male

responsiveness during this period. In addition, 6th-day oviparae appeared to be a more consistent source of a potent pheromone stimulus than were females of other ages. The pattern of scent release throughout the light period was therefore determined in females of different ages.

Groups of five oviparae were tested for pheromone production during the middle 30-40 min of a given hour;different groups being used for successive hours. From the number of males responding and the dose-response curve presented in the previous section, the relative amounts of pheromone released at different times of the day were estimated. The mean levels of male responses, and the general patterns of scent release for females during the 2nd,4th,6th,8th,I0th and I4th days after the adult ecdysis, are presented graphically in Fig.I2, while detailed results are given in Table 6.

Not only do the results confirm that oviparae show a daily pattern of pheromone release but they also demonstrate that the profile changes as the females grow older (Fig I2). There is an obvious waxing and waning in the total amount of scent liberated each day, with a maximum output occurring on day 6. However, at all ages from 2 to I4 days release begins shortly after light-on and rapidly reaches a peak by the second hour of the light period. This daily peak is then maintained for a variable time before the rate falls gradually to a level at which no male response is elicited. The rapid change in the daily pattern occurring between days 2 and 6 involves both an increase in the level of the daily peak and a great extension in the time for which it is maintained. Following day 6 there is a decline in the amount of pheromone produced. At first this occurrs rapidly but after day 8 it is more gradual. It involves a systematic reduction in the duration of the daily peak and a marginal decrease in the level that the latter attains.

As might be expected, differences between replicate groups

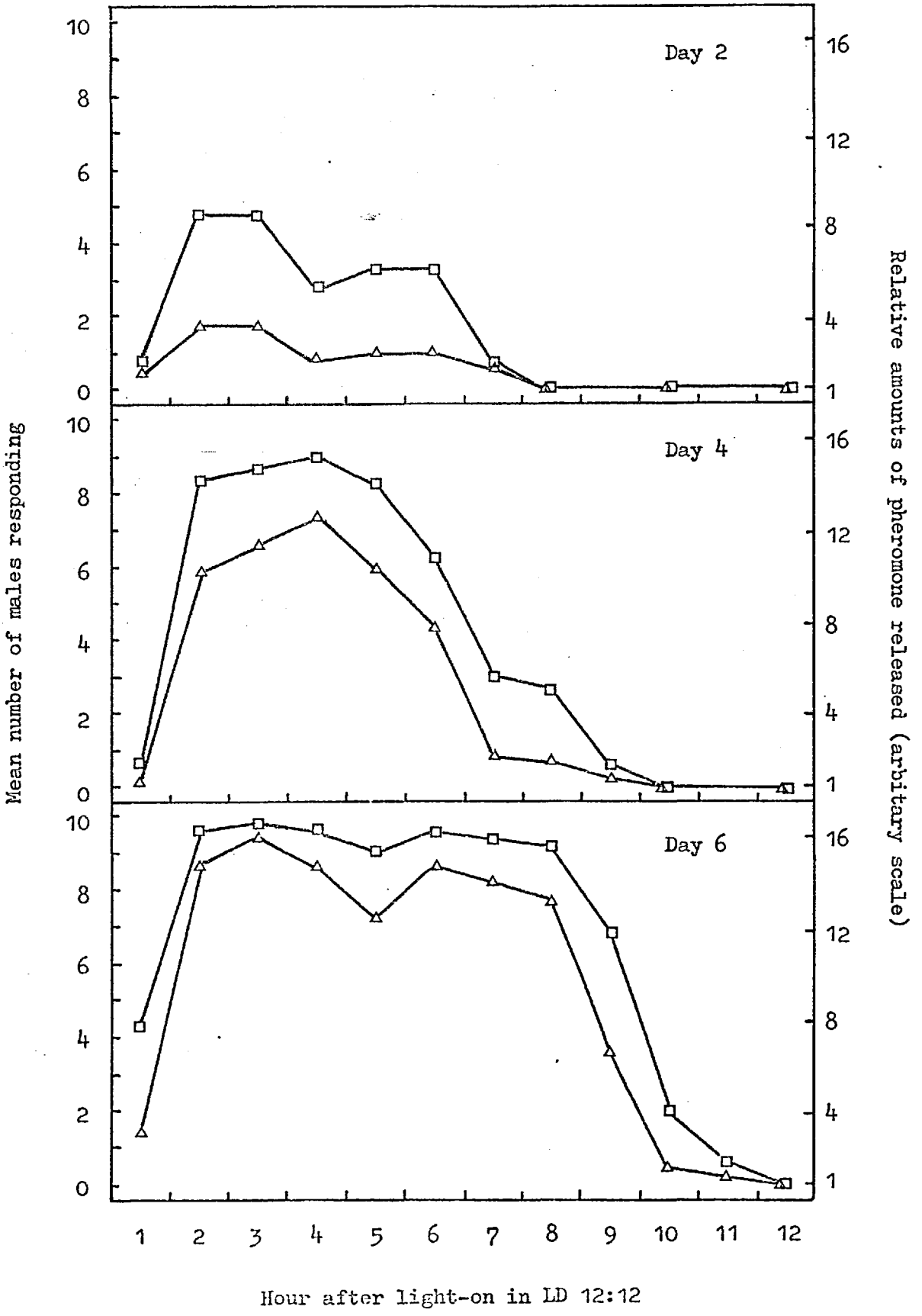


Fig.12. Pattern of pheromone release of oviparae at various ages during the course of the light period in LD 12:12. Squares = mean number of males responding during a given hour. Triangles = relative amounts

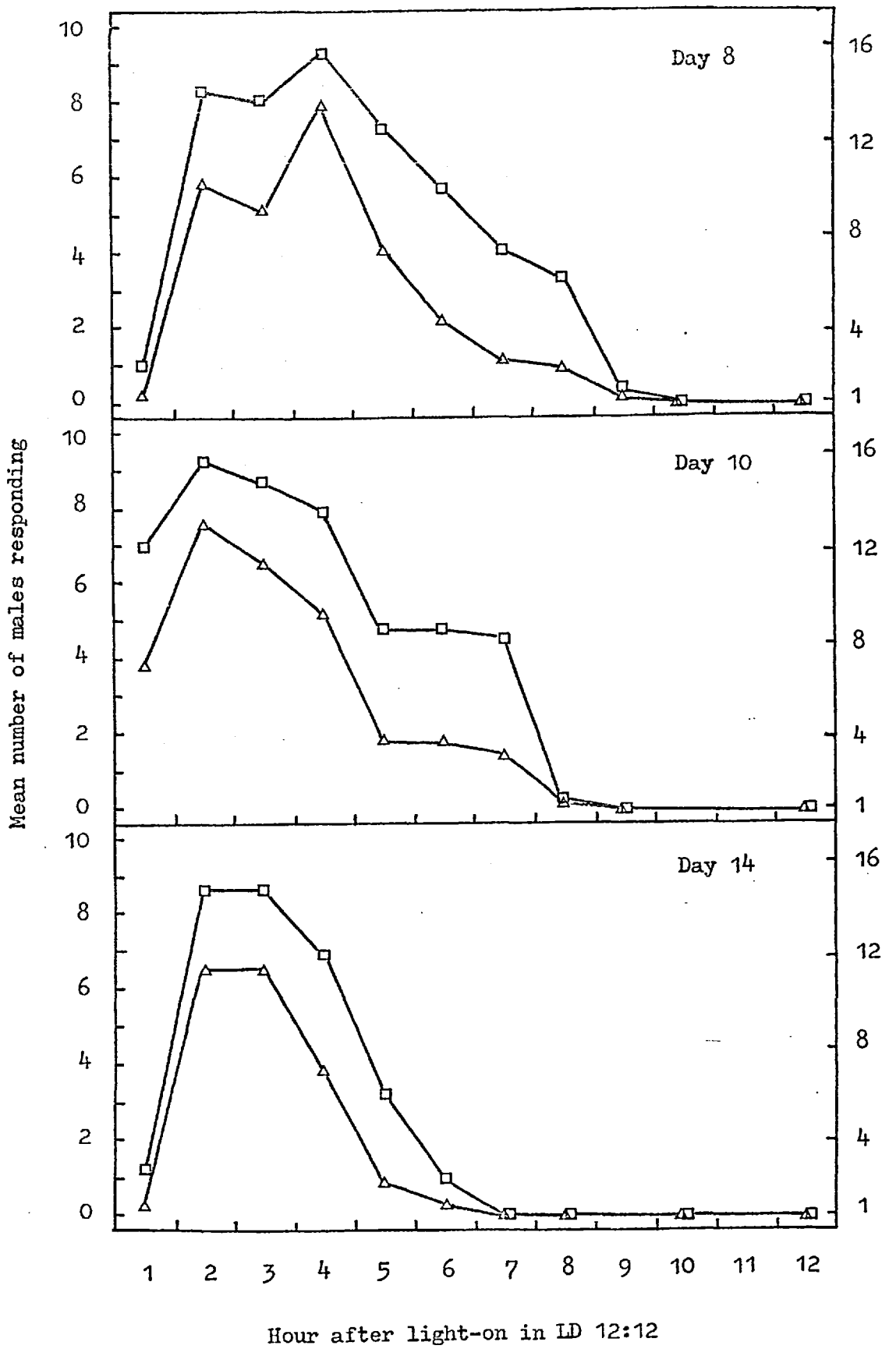


Fig.12. (continued) of pheromone released - 4 on the arbitary scale represents the amount of pheromone which elicits a response in 50% of the males.

Table 6. Numbers of males responding in individual replicates during investigation of daily pheromone release patterns in oviparae of different ages.

Hour in light period	Female age (days) 2	4	6	8	10	14
1	0, 2, 0, 1	1, 1, 0	5, 5, 7, 0, 4	0, 3, 0	8, 7, 6, 7	0, 4, 0
2	3, 4, 8, 8	8,10, 7	9, 9,10,10,10	7,10, 8	10, 9,10, 8	10, 9, 7
3	7, 5, 6, 5	8, 8,10	9,10,10,10,10	8, 8, 8	8, 8, 9,10	10, 9, 7
4	2, 0, 1, 8	9,10, 8	10,10,10, 8,10	10, 9, 9	8, 8, 7, 9	8, 6, 7
5	5, 0, 4, 4	8, 9, 8	7,10,10,10, 8	6, 7, 9	4,10, 4, 1	1, 7, 2
6	1, 5, 5, 2	7, 7, 5	10,10,10, 8,10	4, 8, 5	5, 0, 8, 6	3, 0, 0
7	0, 0, 0, 3	3, 4, 2	10, 9, 8,10,10	3, 0, 9	4, 4, 5, 5	0, 0, 0
8	0, 0, 0, 0	2, 5, 1	10,10,10, 8, 8	1, 5, 4	0, 0, 0, 1	0, 0, 0
9		0, 2, 0	4, 5, 9, 7, 9	0, 0, 1	0, 0, 0, 0	
10	0, 0, 0, 0	0, 0, 0	6, 0, 0, 4, 0	0, 0, 0		0, 0, 0
11			3, 0, 0, 0, 0			
12	0, 0, 0, 0	0, 0, 0	0, 0, 0, 0, 0	0, 0, 0	0, 0, 0, 0	0, 0, 0

of insects of the same age were most pronounced during the decline which follows the daily peak (Table 6). Moreover, pheromone release rates of individual oviparae within each group of five were also more variable at this time than at the time of maximum release. For most age categories the levels of response during the daily peaks were very consistent. Indeed, on day 6 they were so consistently high that of the 350 males tested during the 2nd to 8th hours inclusive, 331 responded. Response levels so near the maximum could theoretically conceal variations in the rate of scent release within this period. However, when the pheromone produced by oviparae is first adsorbed onto filter paper and the latter then tested, fewer males respond than when the oviparae are used directly as the pheromone source. Experiments in which the chemical signal released by 6th-day females was attenuated in this manner failed to show any striking differences in the release rate during the 7-h peak period. For the purposes of subsequent experiments it was assumed that no differences exist.

The onset of scent liberation was normally evident by the third quarter of the first hour and was well advanced by the last quarter. Responses for the first hour were therefore mainly recorded during the latter half of each replicate. The 10th-day oviparae used in these particular tests seem to have been exceptionally prompt in releasing their pheromone.Tests with other groups of 10th-day oviparae yielded lower results similar to those obtained with other age categories.

Females may survive in the laboratory for three or more weeks but many die during the third week. Oviparae older than 14 days were not tested because the results may have been biased by abnormal behaviour in females nearing death.It had been noticed,for instance, that just before dying virgin females may lay many more eggs than are ever laid on a single day by healthy females of the same age.

The present findings clearly illustrate that the number of

males responding to a pheromone stimulus can be a misleading indicator of the amount of pheromone present unless the dose-response curve is taken into consideration. For example, half as many males responded to 2nd-day females during the 2nd to 3rd hours of the photoperiod as did to 6th-day oviparae, but the older females were actually releasing four times as much pheromone as the younger insects.

A few groups of females were assayed for pheromone release at various times during the first 24 h after the imaginal ecdysis. Tests were conducted during the 2nd and 3rd hours of the light period. The results are presented in Table 7. Some oviparae released pheromone within I5-2I h of the adult moult but individual insects within these age groups were very variable in this respect . It seems reasonable to suppose that the age at which a female first releases its pheromone depends largely upon the time of ecdysis in relation to the normal time of scent release in the LD I2:I2 regime.

Table 7. Numbers of males responding to young,adult oviparae.

Female age (hours)	Replicates
2-4	0, 0
up to I5	0, 6, 0
I6-I7	2
20-2I	0, 6

Other aspects of the reproductive physiology of females

It would not be surprising if the striking changes in the daily pattern of pheromone liberation were correlated with other changes in the reproductive physiology. Accordingly, some preliminary observations were made on the maturation of the ovaries, on oviposition, and on copulatory behaviour.

(i) Maturation of ovaries

The number of eggs with fully formed chorions found within the ovaries of adult females was taken as a measure of their maturity. Since the posterior pole of the egg becomes chorionated last of all, care was taken to count only those in which the hook-like protuberance found in this position - presumably a micropyle - had been formed. The results for groups of 20 oviparae of each daily age category from I to I4 days are given in Fig.I3. In virgin insects there is a steady accumulation of mature eggs after ecdysis, but the rate decreases slightly after day 7. Some of the first-day oviparae that had been shown to be attractive to males in the olfactometer were dissected and their ovaries examined. No fully chorionated eggs were found.

(ii) Oviposition

Oviposition in unmated females begins about day 6 and then continues at a very low, but gradually increasing, level. The numbers of eggs laid during the first I4 days after ecdysis by virgin oviparae kept in groups of 20 are given in Table 8. Since a total of only 32 eggs were laid by I00 females, a maximum of 32% of the females could have oviposited during this time. Examination of the ovaries of these females showed that fewer individuals were actually involved.Mating stimulates oviposition for a variable period, the length of which may depend upon the age of the female. Mated oviparae may lay up to 3, or possibly 4, eggs per day, and may begin to oviposit as early as day 4.

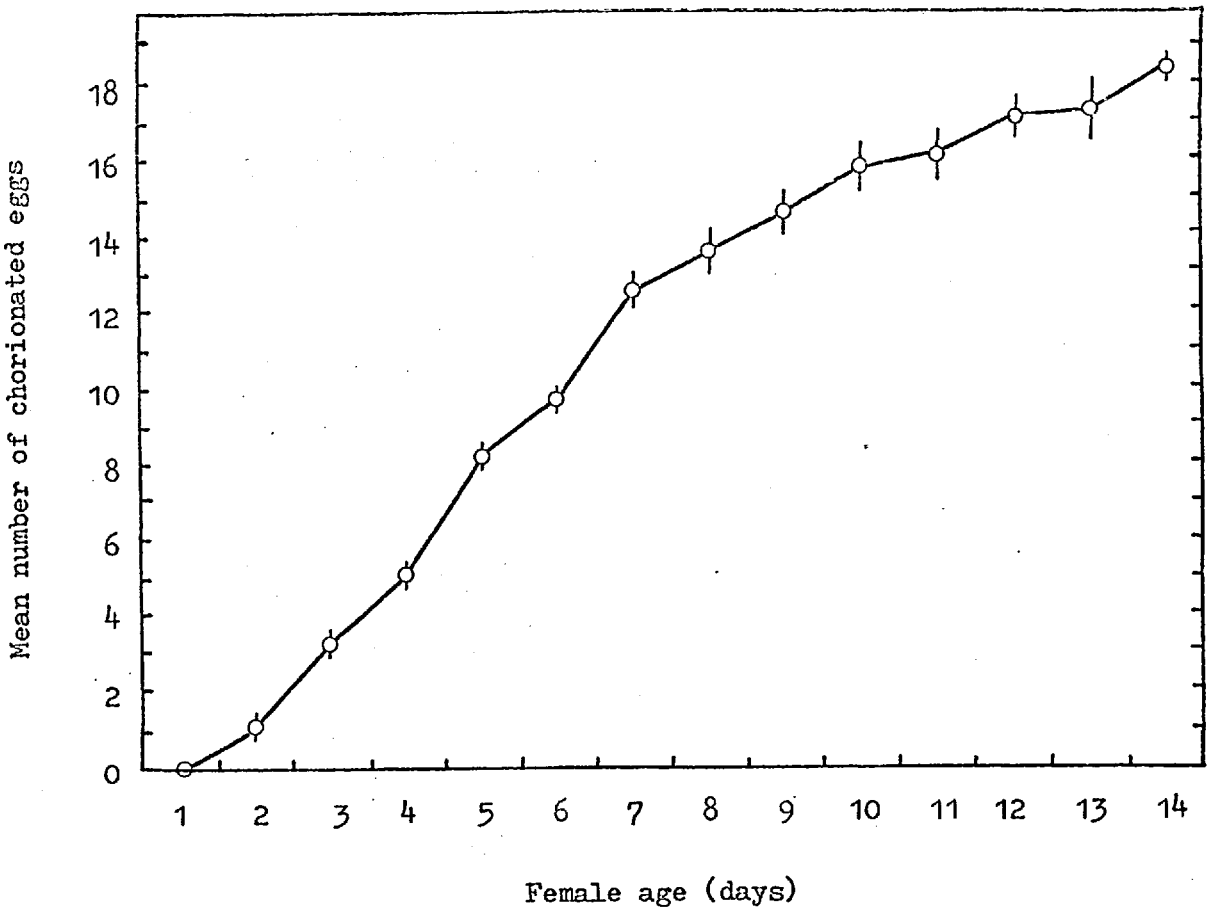


Fig.I3. Mean number of fully chorionated eggs in oviparae of different ages. Vertical lines represent S.E. of the mean. Day I females were dissected immediately after ecdysis.

Table 8. Mean numbers of eggs laid by virgin oviparae during the first I4 days following the adult ecdysis.

	Female age (days)									
	I-5	6	7	8	9	I0	II	I2	I3	I4
Total number of females	I00	I00	I00	I00	I00	I00	I00	I00	99	99
Eggs/female	0	0	.0I	.0I	.02	.02	.05	.03	.05	.I3

(iii) Copulation and insemination

Females of all ages from I h to 2I days after the adult moult were found to be willing to copulate, and to be susceptible to insemination, when placed in I.0 x 0.5 " stoppered tubes with males during the 5th-6th hours of the photoperiod.

Clearly, the presence of mature eggs in the ovaries is not a prerequisite for the production of the sexual scent. Mating and insemination can occur immediately after ecdysis, and, presumably, before pheromone release begins. The age at which maximum scent liberation occurs (day 6) does not coincide with the earliest possible age for oviposition. However, a slight decrease in the rate at which eggs become mature, and the initiation of a low level of oviposition in virgin females, do occur simultaneously with the decline in the quantity of pheromone released daily. Of course, it is not known whether these changes are functionally related.

Behaviour of oviparae during pheromone release

At certain times of day, apparently coincident with pheromone liberation, oviparae of M.viciae adopt an unusual posture by raising the end of the abdomen to a position well above the substrate. So pronounced is this behaviour that, in extreme cases, the abdomen may be held at an angle of almost 90° to the surface of the support. Usually, one or both hind legs are also raised from the substrate and held wide apart and immobile. More rarely, the middle legs are similarly involved and the aphid maintains contact with the plant stem only through its forelegs and rostrum. This attitude is shown whether the insect is in a horizontal or vertical position, and may be maintained for long periods.

In sexual females the adoption of this stance is not associated with feeding, crowding, or oviposition for it occurs in insects

that are unable to feed (in an empty tube), that are isolated , or that are not old enough to oviposit. Similar, but less exaggerated postures have also been observed in adult and larval virginoparae of M.viciae, and of other species, in both laboratory and field colonies. However, the conditions under which this behaviour occurs in these morphs differ from those for oviparae. Parthenogenetic females only show it when they are apparently feeding, and even then, more frequently in crowded colonies than when isolated. In such colonies it appears to enable greater numbers of aphids to occupy a given length of host stem. Isolated virginoparae do not maintain this attitude for very long. The more consistent and impressive behaviour by oviparae may indicate that it is concerned with sexual behaviour and particularly with dispersal of the pheromone. Changes in this stance were followed throughout the day in females of various ages.

Insects were individually confined to young bean plants under inverted 3 x I " tubes, 24 h before being observed. At hourly intervals during the light period each insect was observed and recorded as showing the ' abdomen up ' behaviour or not. Only cases in which the abdomen was unequivocally raised at an angle to the substrate (plant stem, glass tube, or sand in pot) were given positive scores, borderline cases being counted as not showing this posture. Oviparae in the last larval instar, and adults 2,4,6,8,I0, and I4 days old, as well as adult, apterous virginoparae of mixed ages, from 3-I4 days, were tested. The latter, like the oviparae, had been reared and maintained in LD I2:I2. The results are summarised in Fig.I4.

Obviously, the numbers of adult oviparae exhibiting this posture, and the time for which they maintain it, depends upon their age. Few females do so immediately after light-on,but the numbers increase rapidly to reach a peak by the 2nd or 3rd hour.This peak is then maintained for a variable period before the numbers gradually return to a low level before light-off. Although some females quickly

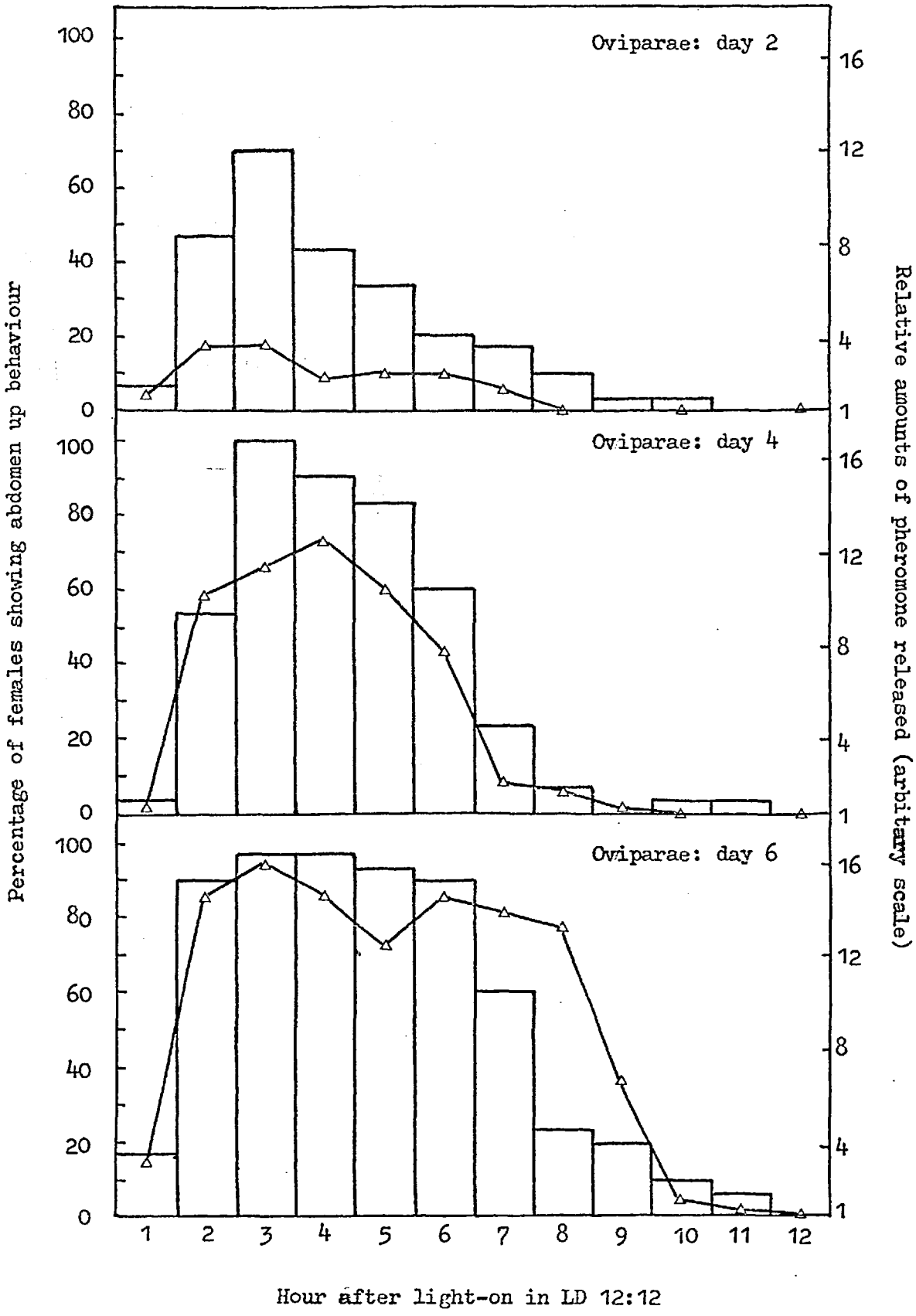
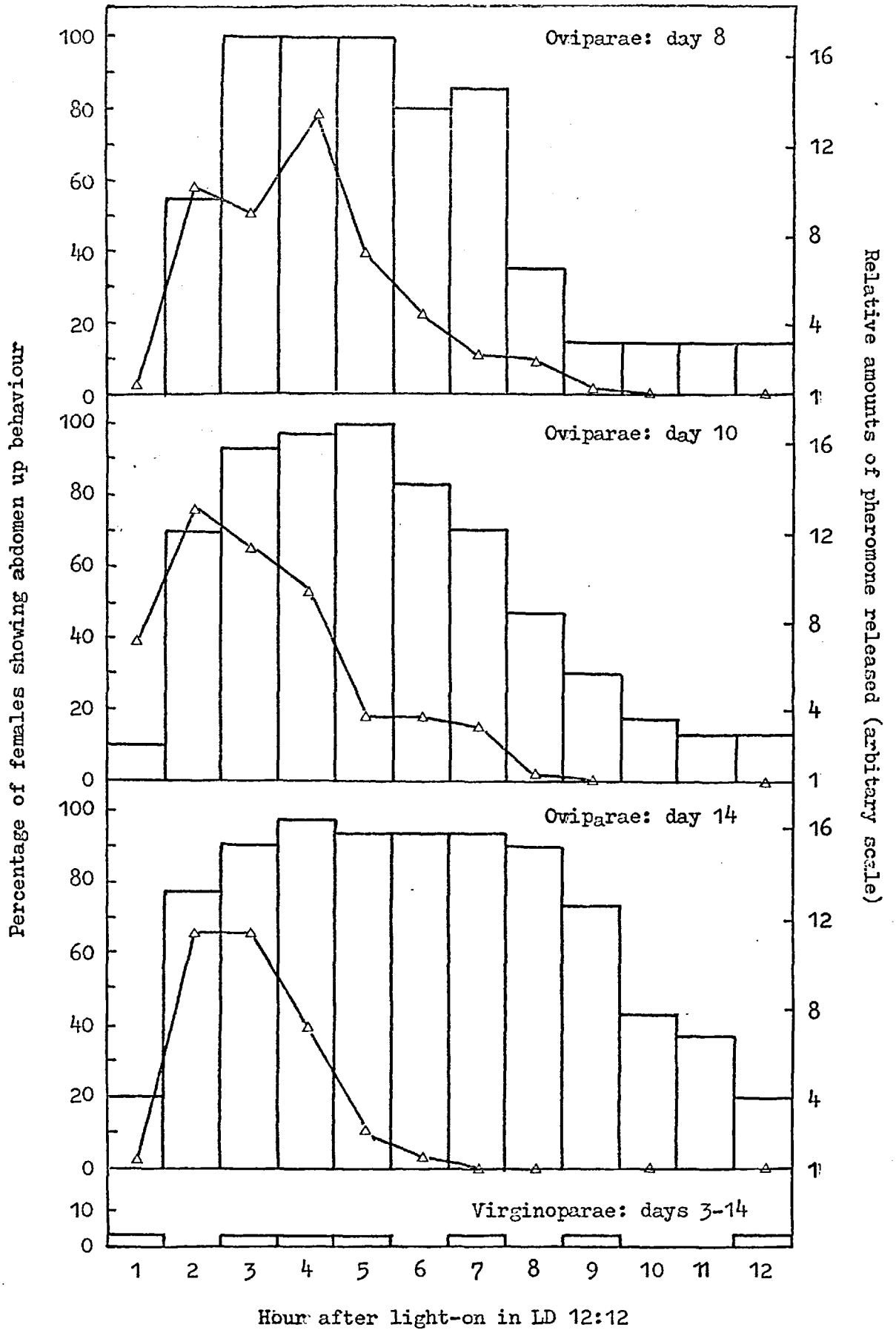


Fig. I4. Percentage of oviparae and virginoparae of various ages showing abdomen up behaviour (histogram) at different times of day. Results for all age groups are from two replicates of 15 females, except for 8th-day oviparae for which only one replicate was completed. The relative amounts of pheromone released by females of like age (graph) are included for comparision (taken from Fig. 12).


Oviparae: day 8
Oviparae: day 10
Oviparae: day 14
Virginoparae: days 3-14
Percentage of females showing abdomen up behaviour
Relative amounts of pheromone released (arbitary scale)
Hour after light-on in LD 12:12

elevate their abdomens to their highest positions, the majority proceed more gradually. Of the females that showed this stance during the first two hours of the light period only 40.4% also raised one or both hind legs, whereas during the 3rd and 4th hours 80.4% of them did so. Individuals may continue to behave in this manner for long periods with little or no interruption. Some I4-day old females, for example, were given positive scores in II consecutive hourly observations. Cessation of this behaviour is generally gradual and somewhat hesitant for the abdomen may be raised and lowered a few times before the aphid finally settles into the typical resting position. Clearly, postures which were not given positive scores in this experiment, but which might represent much less pronounced abdomen up stances were common during these increasing and declining phases. At these times, for example, many oviparae hold their bodies parallel with, but away from, the plant stem by straightening their legs. This contrasts with the resting position in which the plant is gripped closely, the abdomen is held fairly tight against it , and the legs are drawn up close to the body. Disturbance of oviparae in the abdomen up position causes them to rapidly lower their bodies and grip the plant. If the disturbance does not continue the posture is resumed within a few minutes.

As several larval oviparae moulted into adults during the experiment only six larvae were observed throughout the I2-h period. Only a single insect showed any abdomen up behaviour, and this on two occasions 7 h apart. Similarly, very few virginoparae elevated their abdomens. Only six of the 30 females observed contributed to the total of seven positive scores. None raised their hind legs although other virginoparae have been observed to do so on other occasions.

These findings confirm that there are very real differences in the occurrence of this behaviour between adult oviparae and virginoparae, and between adult and larval oviparae. They also

emphasise the coincidence between the daily patterns of pheromone release and of raising the abdomen. Indeed, it is only when pheromone production in older insects begins to be curtailed that the two processes diverge appreciably. The elevated abdomen stance is therefore regarded here as a type of calling behaviour. However, it is recognised that pheromone liberation can probably take place without this behaviour occurring and, conversely, that adoption of the calling posture does not necessarily mean that scent molecules are being released - at least, at a high enough rate to evoke responses from males in the olfactometer.

Endogenous nature of the daily pattern of pheromone release

To determine whether scent liberation is under the control of an endogenous rhythm it was followed in females which were held in constant dark (DD). As insects placed below the olfactometer floor are normally exposed to light, and as attempts to construct a light-proof dish which allowed the pheromone to diffuse freely outwards were unsuccessful, the pattern of scent release had to be followed indirectly. This was accomplished by allowing the pheromone to be adsorbed onto filter paper which was then tested in the olfactometer.

Pieces of filter paper, equal in size to those normally impregnated with pheromone extracts, were placed in five 2 x I" tubes. Five oviparae were added to each tube, and the latter corked and left in a horizontal position for one hour. Each filter paper was arranged next to the cork and the females shaken onto the middle of the paper. The insects usually remained on the paper, although they dispersed over its area during the course of the hour. At the end of this period the females were removed and the tubes resealed. To reduce evaporation of the adsorbed pheromone during the interim period before the papers were tested, the tubes were placed in a vacuum flask with ice taken

from a refrigerator at -20°C. Each piece of paper was cut in half and the two fragments placed beneath the pathways of the double olfactometer. Testing was completed less than 25 min after removing the oviparae. The concentration of the adsorbed pheromone was not appreciably reduced during the temporary storage for the numbers of males responding to the first two papers, and to the last two papers, from each replicate group of five were not significantly different ($p > 0.05$, Chi-squared test).

Oviparae were placed in DD following normal light-off in the LD I2:I2 regime on day 5, and were tested during the first and second subjective photoperiods in DD, namely, on days 6 and 7 respectively. Testing was started one hour earlier on the second day in DD so that any phase-advance in the release pattern could be detected. One group of females subsequently subjected to DD was transferred, on day I, to a LD I2:I2 regime three hours out of phase with that of other oviparae. All manipulations of females in the dark were kept as brief as possible and were performed under dim red light, using a filter with a sharp cut-off in its transmission spectrum at wavelengths shorter than 600 nm. Other females were not placed in DD but were tested during the photoperiod in LD I2:I2 on day 6. The numbers of males that responded are given in Table 9. The relative amounts of scent released by the filter papers were obtained by reference to the dose-response curve presented earlier and are shown in Fig.I5.

The filter papers exposed to the females maintained in LD I2:I2 elicited far fewer responses than are usually obtained with live oviparae.One third to one half as much pheromone appears to have been released from the papers as from live oviparae (compare Figs. I2 and I5). Presumably, a major factor in the attenuation of the chemical signal is that some of the released pheromone is not adsorbed by the filter paper. In addition, the rates of evaporation of the

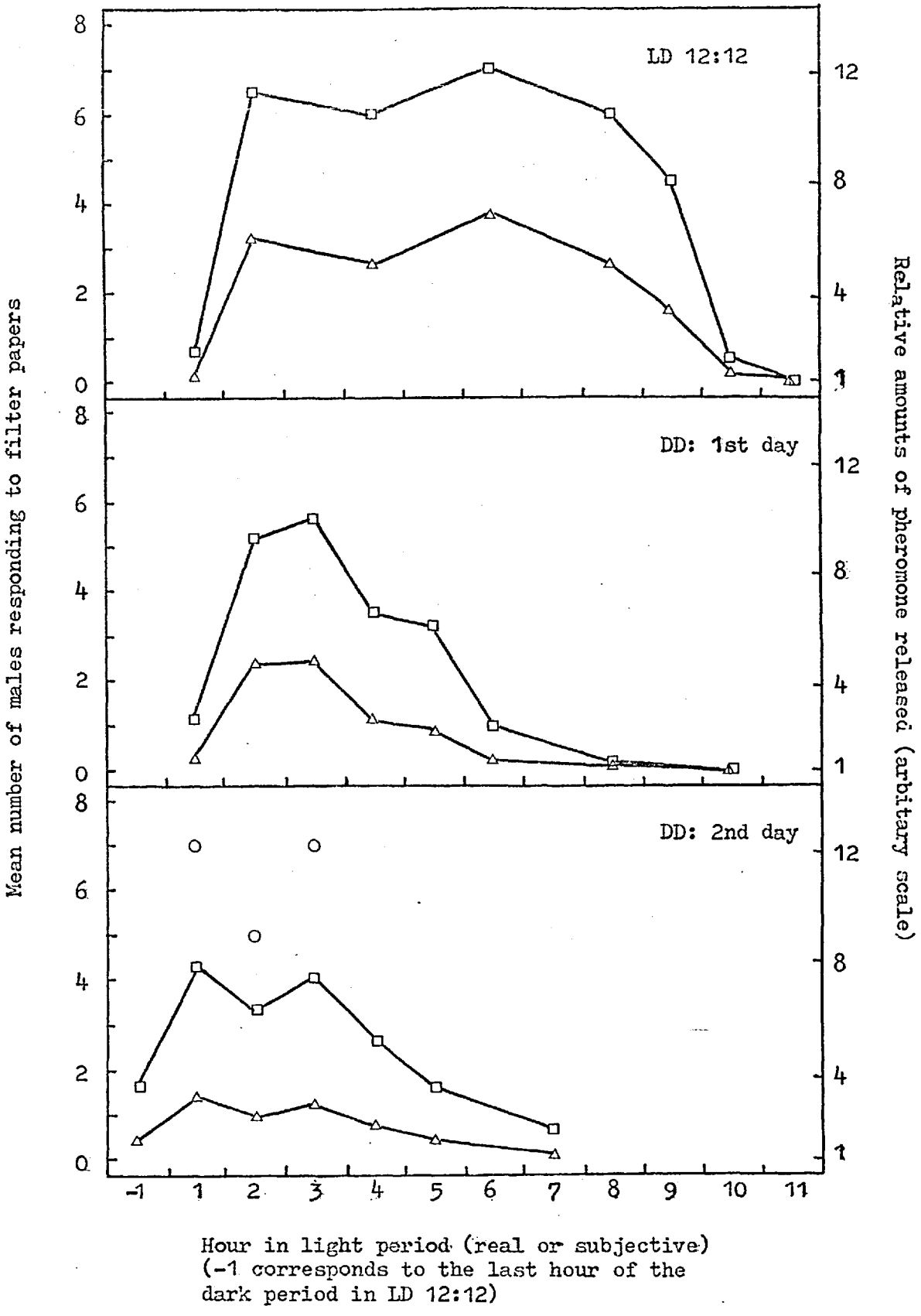


Fig. 15. Pheromone release in DD. Squares = mean number of males responding to filter papers exposed to oviparae held in LD 12:12 or in DD. Circles = highest number of males responding in individual replicates during the 2nd day in DD. Triangles = relative amounts of pheromone released by the filter papers.

Table 9. Numbers of males responding to filter papers exposed to oviparae held in LD 12:12 and in DD.

Hour in photoperiod (real or subjective)	Female light:dark regime		
	LD 12:12 (day 6)	DD - 1st day (day 6)	DD - 2nd day (day 7)
-1*			2, 2, 1**
1	1, 0	2, 3, 0, 0**	4, 7, 2
2	8, 5	6, 6, 7, 2	5, 1, 4
3		4, 5, 8	4, 1, 7
4	7, 5	2, 4, 2, 6	3, 0, 5
5		2, 2, 4, 5	1, 0, 4
6	8, 6	2, 0, 2, 0	
7			1, 0, 1
8	8, 4	0, 1, 0, 0	
9	6, 3		0, 0
10	1, 0	0, 0, 0	
11	0, 0		

* Hour immediately before beginning subjective photoperiod in DD.

** Group of females held in LD 12:12 regime three hours out of phase with that of other females before being subjected to DD.

scent from filter paper and from insect cuticle may possibly be quite different. The reduced efficiency of the experimental technique should be borne in mind when the results obtained with females in DD are examined.

Pheromone liberation occurred in DD and was coincident with the times of release in LD I2:I2. However, the profile in DD differed from that seen in LD I2:I2. A pronounced peak occurred during the first three hours of the subjective photoperiod and was followed by a decline in the rate of release. The latter phase was gradual on the first day in DD but was more abrupt on the second day (Table 9). Nevertheless, scent liberation continued, although at a very low level, for almost as long as in LD I2:I2. The results for the second day in DD were more variable than for the first for the peaks in the individual replicates occurred in three separate hours (Table 9). The mean of these replicates (given in Fig.I5) is therefore misleading for it has a profile much less peaked than the actual course of scent release.

The occurrence of at least two daily peaks of pheromone release in DD indicates that this behaviour is controlled by an endogenous rhythm, presumably with a circadian periodicity. Nevertheless, exogenous factors are important in influencing the shape of the daily release curve and in entraining it to the LD I2:I2 regime. After exposing one group of females to a phase delay of three hours the pattern of release was shifted by a similar amount; and this delay was maintained in DD. Environmental factors could not, therefore, have determined the position of the daily peaks in DD. Entrainment to the light regime was, of course, used in previous experiments to provide pheromone-releasing oviparae throughout the course of the male photoperiod. A prominent peak at the beginning of the period of release appears to be a consistent feature of the daily pattern for it is shown both in DD by 6th and 7th-day oviparae, and in LD I2:I2 by females of all ages from 2-I4 days (Fig.I2).

Amount of extractable pheromone and time of day

Following the demonstration that the sexual scent is liberated only at certain times of day, it was of interest to establish whether the amount of pheromone present in the tibiae shows similar variations. Males were therefore tested against extracts prepared at eight different times in the day.

Hind tibiae from 6th-day oviparae were removed during the middle 10-30 min of a given hour and extracted. Samples were taken at up to eight times a day depending on the availability of females. Oviparae were, however, in relatively short supply at this time, and the experiment was conducted over a period of several weeks. Consequently , although replicate extracts prepared at the same time of day were obtained from different daily batches of oviparae , they were not mixed prior to testing but were assayed as they became available. In this way storage of the extracts for long periods was avoided. All tests were performed during the 3rd-8th hours of the photoperiod.

Initially, pheromone from groups of 20 oviparae was extracted in I ml of solvent and the undiluted solution tested directly at the rate of 0.20 FE/cm^2. However, owing to the inactivity of many of the extracts prepared at five of the sampling times, groups of 60 or 80 insects were finally extracted and the solutions tested at 0.60 and 0.80 FE/cm^2 respectively. The numbers of males responding in individual replicates are given in Table 10. Using the dose-response curve (p.72) as a reference, the relative amounts of pheromone present at the eight different times were estimated. These are shown in Fig.16.

Clearly, the amount of extractable pheromone present in the hind tibiae changes considerably during the course of the light period. Active material does not appear to be present before the onset of its release (Fig.12) but accumulates very rapidly during the hours

immediately after light-on to reach a maximum by the 4th hour. Indeed, between the Ist and 4th hours there is more than a 20-fold increase in the amount of pheromone present. About 80% of this increase appears to occur between hours 2 and 4. The peak is not maintained for very long , however, for the content declines gradually after hour 4 so that by the IOth hour only 5% of the maximum amount remains. A further reduction occurs before the end of the light period.

Table IO. Numbers of males responding to extracts of the hind tibiae of 6th-day oviparae prepared at different times in the day.

Time of preparing extract (LD I2:I2)	Concentration (FE/cm^2) 0.20	0.60	0.80
Dark period:			
Last hour	0, 0, 0, 0		0, 0, 0, 0
Light period:			
Hour I	0, 0, 0, 0		0, 0, 0, 0
2	I, I, I, 0	5, 6, 5, 4	
4	6, 9, 7, 5		
6	5, 5, 5, 4		
8	3, 2, 3, 3		
IO	0, 0, 2		2, I, I, 0
I2	0, 0, 0, 0		0, 0, 0, 0

Presence of the pheromone within the tibiae coincides with the period of its release. None could be detected within the legs at times when no release occurs. Nevertheless, the release rate is obviously not proportional to the amount of pheromone present. Although 6 day-old oviparae maintain a more or less constant rate of release

from the 2nd to the 8th hour of the photoperiod (Fig.I2), their pheromone content during this period undergoes a rapid five-fold increase and then a more gradual three-fold decrease.

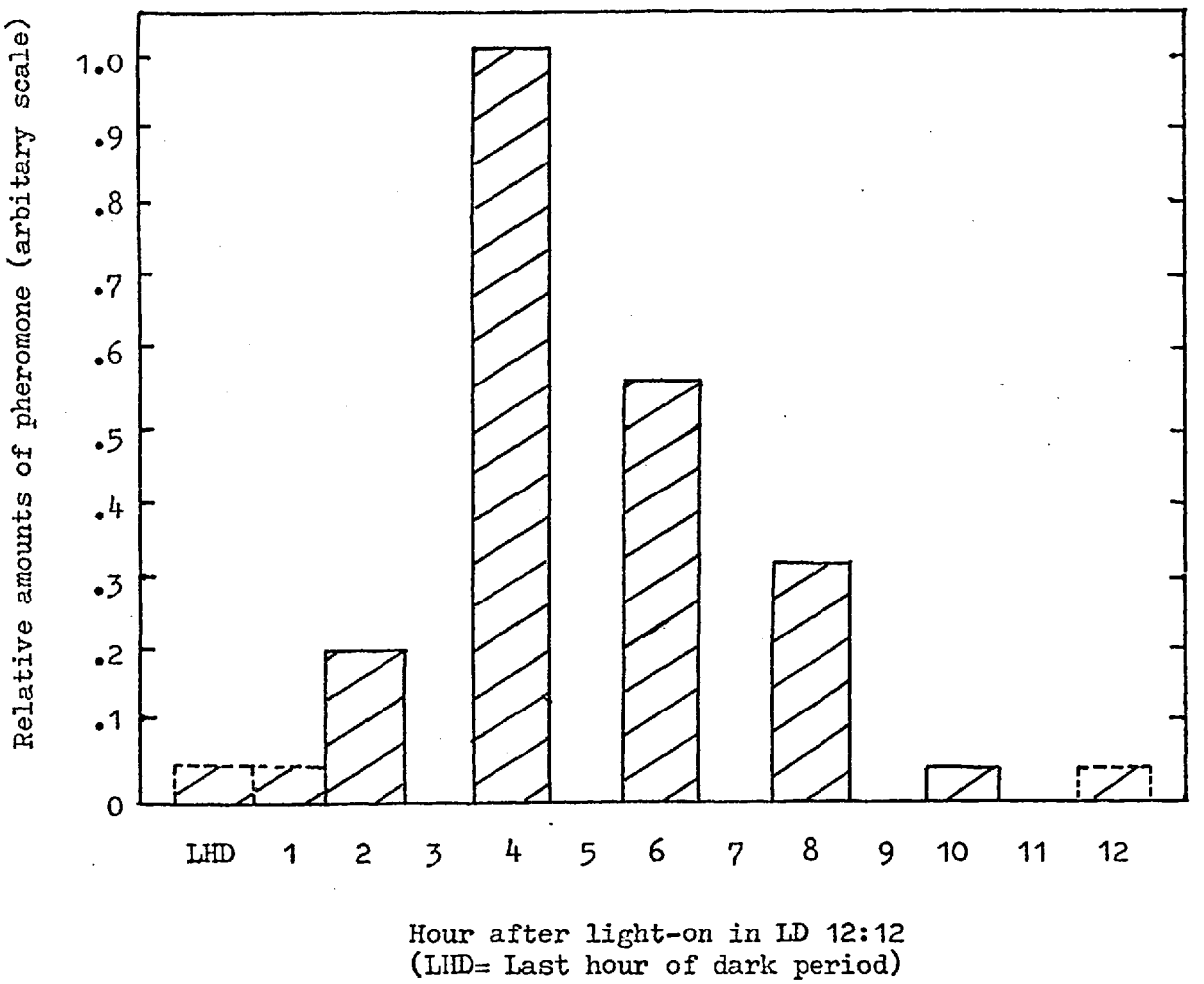


Fig.I6. Relative amounts of pheromone present at eight different times of day. Amounts expressed as arbitary scale from 0 to I.0, with maximum content (hour 4) as I.0. The amount present during LHD, hour I, and hour I2 is less than that indicated by the dotted column.

Effect of copulation on male response

The oviparae of M.viciae can be deprived of their pheromone glands without impairing their ability to mate and without preventing males from readily copulating with them. This enabled the effect of copulation on subsequent male responses to a pheromone stimulus to be assessed immediately after mating. By using females without their hind tibiae males were not exposed to pheromone during coitus. Any complications that such exposure might have caused were thereby avoided.

Virgin males were placed in I x 0.5 " stoppered tubes with two 6th-day oviparae that had been deprived of their hind tibiae. Each male was watched and as soon as it had finished copulating with one of the females it was tested against a pheromone extract at a concentration of 0.20 FE/cm^2. All males passed into the pheromone zone within 3-I0 min of the copulatory act terminating. Other males were placed in empty tubes for I0-20 min before being assayed. All tests were conducted during the 3rd-7th hours of the photoperiod. Five replicates of I0 individuals were completed for both mated and unmated males. The results are presented in Table II.

Table II. Numbers of mated and unmated males responding to a pheromone extract (0.20 FE/cm^2).

Males	Replicates	Mean
Mated	4, 6, 5, 6, 5	26
Unmated	5, 5, 4, 4, 7	25

There was no significant difference ($p > 0.05$, Mann-Whitney U test) between the numbers of mated and unmated males responding to the pheromone extract.

Effect of copulation and oviposition on pheromone release

All experiments on pheromone release described above have been concerned with unmated insects. The effect of mating upon scent liberation by oviparae was therefore a matter of some interest.

Virgin oviparae, 6 days old, were placed individually in I x 0.5 " stoppered tubes containing two males. All ensuing copulations were timed. When IO or more females had each copulated at least once for a period of five minutes or longer, those that remained unmated were discarded. This time criterion was chosen since copulations of a much shorter duration do not normally result in insemination. In this way a group of females that had all mated within a I6-20 min period was obtained. The oviparae were then separated from the males and placed on bean plants under a lamp glass. During the 2nd and 4th hours after coitus separate groups of five insects were tested for pheromone production and then returned to the plants. On the following day two groups of five females were removed from the pool of mated insects and were again tested for pheromone production, that is, 23-27 h after copulation. Other oviparae were treated in a similar manner except that they were placed in empty tubes and not exposed to males. One group of five of these unmated females was tested for pheromone production during the 3rd hour after removal from the tubes, and two groups were assayed during the 23rd-27th hours. After the second day all mated females were dissected and their spermathecae examined for the presence of spermatozoa. The numbers of eggs laid by both mated and unmated insects were recorded. Mating was always accomplished during the 4th hour of the photoperiod. The results are presented in Table I2.

Table I2. Numbers of males responding to mated and unmated oviparae.

Hours after removed from tubes	Mated females	Unmated females
2	7, 3, 9, 9	
3		7, 9, 8,I0
4	6, 5, 0, 8	
23-27 *	8, 9,I0, 8,I0,I0	9,I0, 9, 9, 9,I0

* Not tested in fourth replicate of experiment.

There was no significant difference ($p > 0.05$, Chi-squared test) between male responses to mated and unmated females during hours 23-27, between mated females at hour 2 and unmated females at hour 3, and between mated females at hours 2 and 4. However, there was a highly significant difference ($p < 0.00I$) between the numbers of males responding to mated females at hour 4 and unmated females at hour 3. Taken by themselves these findings suggest that mating does indeed affect pheromone production in some way which varies with time. It would appear that scent release is somewhat inhibited at 4 h, but not at 2 h or at 23-27 h, after coitus. However, other observations made during this experiment indicate that mating has no direct influence on pheromone production, but that oviposition probably has a marked, although short-lived, effect which could account for the above results.

The behaviour of successive males showed that the liberation of scent by individual females was extremely variable after mating. In all cases where some females appeared to be releasing less scent than others a correlation with some aspect of oviposition could be found. These females were found (a) to have laid an egg immediately

before being tested,(b) to have laid an egg while in the olfactometer, (c) to have laid an egg within 2h after being tested, or (d) to have an egg in the posterior part of the common oviduct (visible through the body wall). In some individuals belonging to category (a) pheromone output increased towards the end of the test. In contrast, in those oviparae that laid eggs immediately after the assay, scent liberation tended to decline during the last half of the test. For example, in the second replicate at 2 h after coitus 3 out of I0 males responded, all of these being within the first five tested. Only some of the females appeared to be secreting the pheromone at the start of the replicate and none appeared to be doing so at the end. All five oviparae laid an egg within I.5 h after being tested.

Unmated females were not noticeably variable with respect to their rate of pheromone release. Neither did they lay any eggs near or during the times they were assayed for pheromone production.Indeed, of the 52 unmated females used, only one laid an egg within the 27 h of the experimental period, whereas each mated oviparae laid,on average, more than three eggs. It appears, therefore, that pheromone liberation is reduced, or the pheromone masked, for a short period before and after oviposition. Release of the sexual scent is resumed soon after the egg has been deposited. Presumably, inhibition of pheromone production occurs with each egg laid. Females of category (d) probably cannot be inseminated.

The variability of scent production in individual insects was more pronounced during the 2nd and 4th hours after coitus than during 23rd-27th hours. This difference, and the lower level of response during the 4th hour after mating, can be explained by the effect of copulation on oviposition. Mating in 6th-day oviparae stimulates oviposition for a number of days. Eggs are laid within a few hours of coitus but progressively fewer are laid on successive days. Consequently, one would expect that during the 23rd-27th hours after mating fewer

insects would oviposit than during the 2nd-4th hours.Less frequent oviposition is thus correlated with a less complete inhibition of pheromone production.The latency of the stimulatory effect appears to be such that more females are affected by oviposition during the 4th hour after copulation than during the 2nd hour.

Most of the mated females were inseminated, the actual numbers being I2/I3, 9/I0, I2/I3, and 8/I0 in individual replicates.

To summarise: it appears that mating and insemination do not in themselves directly affect pheromone production but that oviposition, which is stimulated by mating, does temporarily inhibit the numbers of males responding to females. Visual responses of males towards these olfactorily unattractive females were not tested.

Antennal receptors and responses to the pheromone

Description of antennal receptors

The antennae of adults of both sexes of M.viciae are long, highly mobile structures composed of six segments. As males deprived of their antennae fail to respond to the female scent these structures were examined to determine the types of receptors present.

The material was prepared for examination in one of two ways. Initially, antennae were removed from the aphid, warmed in dilute potassium hydroxide for a few minutes,and mounted in DPX. Although this treatment facilitated the counting of the receptors on the heavily-tanned, basal segments, it had the opposite affect on those of the fragile terminal segment. The latter were decolourised so effectively as to become difficult to see - an effect which was compounded by a tendency for the weakened segment to wrinkle and fold during preparation. Other antennae were therefore mounted directly in polyvinyl lactophenol - a combined clearing agent and mountant - and examined a few days later. The sensillae on the last segment of these antennae were easily

counted, while those on the basal segments were less readily discernible. The antennae of adult males are described below. Measurements of the individual segments are given in Table 13, while the types and numbers of receptors present are summarised in Table 14.

The cylindrical scape or basal segment is by far the broadest of the antennal units. It is surmounted by the second segment, or pedicel, which forms a truncated cone between it and the flagellum. The latter is composed of four successively narrower, cylindrical segments, the last of which is the longest and most distinctive. This terminal unit is clearly divided into a short, thick basal portion that narrows abruptly to the second,long,thin,whip-like portion or processus distalis. The cuticle of the distal half of the antenna is thrown into shallow folds.

Table 13. Dimensions of antennal segments of male M.viciae.

Segment	Length(μm)	Width(μm) *
I	121-147	M: 125-150
2	79-121	B: 88-110 A: 55-68
3	693-838	M: 31-37
4	649-792	M: 22-26
5	561-693	M: 20-24
6 (Base)	187-242	M: 18-22
6 (Processus distalis)	1045-1309	M: 11-13
6 (Both parts)	1243-1540	
1-6	3448-4070	

* Width measured at the base of the segment (B), midway along its length (M), or at its apex (A).

A great diversity of receptors is found on the antenna. Long, slightly curved hairs with spatulate or oar-shaped tips occur on all segments, except, in some instances, the last. They tend to become progressively shorter on the more distal segments, being 33-78 μm long on the scape and 25-35 μm long on segment six. They are mounted in sockets and are almost certainly mechanoreceptors. The ultrastructure of hairs present on the hind tibiae of oviparae, which are identical in external appearance to these antennal receptors, supports this view. A single, oval to circular, domed structure, 4-7 μm in diameter, which closely resembles a campaniform sensilla is also present on the dorsal surface of the apical third of the pedicel. Krzywiec (I968) refers to this structure in Macrosiphum rosae (L.) as Johnston's organ. However, this classification appears to be erroneous as Johnston's organ in other insects (Snodgrass,I935; Dethier,I963), including Aphis fabae Scop.(Johnson,I956), is inserted on the intersegmental membrane between the second and third segments. Krzywiec (I968) also states that, in other aphid species, two organs resembling campaniform sensillae are present on the dorsal surface of the proximal part of segment three. Two structures bearing some resemblance to Krzywiec's illustrations are, indeed, present in M.viciae but they are considerably smaller than those described by Krzywiec and are very indistinct, even when viewed under an oil immersion lens. They are assumed to be cuticular papillae without a sensory function.

All other antennal receptors have an external appearance which suggests a sensitivity to chemical stimulation. On the ventral surface at the apex of the pedicel there are 0, I, or 2 tapered pegs in small oval to circular pits, 2-3 μm in diameter. The pits are usually closely spaced and may occasionally be fused to form a figure-of-eight-shaped cup containing two pegs. Some, although possibly not all, of the pegs protrude from the pits for a distance of 2-3 μm.

Table 14. Receptors present on the antennae of adult male M.viciae.

Segment	Receptor	Numbers of receptors per antenna		Antennae counted
		Mean	Range	
I	Spatulate hair	9.0	6-11	63
2	Spatulate hair	4.9	3-7	62
2	Campaniform	I.0	0*or I	62
2	Coeloconic peg	I.3	0, I,or 2	62
3	Spatulate hair	I8.7	I3-25	56
3	Secondary rhinarium	27.5	2I-36	56
4	Spatulate hair	9.2	6-I4	64
4	Secondary rhinarium	9.9	4-I4	64
5	Spatulate hair	8.0	4-I2	63
5	Secondary rhinarium	8.2	4-I3	63
5	Primary rhinarium	I.0	I or 2*	63
6	Basal portion:			
	Spatulate hair	I.9	0-4	67
	Blunt-tipped hair	I.2	0-3	67
	Both types hair	3.I	2-5	67
	Primary rhinarium	I.0	I	67
	Accessory rhinarium	6.0	4*; 5*;or 6	67
6	Processus distalis:			
	Blunt-tipped hair			
	(along processus)	4.5	2-7	59
	(at tip)	4.0	2*; 3*;or 4	59
6	Total blunt-tipped hair	9.6	7-I3	59

* Only in exceptional cases.

Krzywiec (I968), who first described these receptors in aphids, called them "rhinariellen" and regarded them as olfactory receptors. This author also claimed that the numbers of rhinariellen present reflects the phylogenetic position of the aphid species, and mentioned that, in general, members of the Aphidinae possess one per antenna. In M.viciae, which belongs to the latter subfamily, some males clearly possess two of these coeloconic pegs on one or both of their antennae.

Along the outer, ventro-lateral aspects of the third to fifth segments are 37-59 circular plate organs,4-20 μm in diameter. These are the secondary rhinaria or secondary sensoria of aphidologists. They have long been thought to be sensitive to air-borne odours, e.g. Flogel (I905b),and the ultrastructural studies of Slifer et al (I964) support this contention. A similar plate organ, but which is surrounded by a coronet of inwardly-pointing,cuticular projections is present on the ventral surface at the apex of segment five, and at the distal end of the basal portion of segment six. These are called primary rhinaria by aphid taxonomists and are slightly larger (I7-25 μm in diameter) than the preceding sensillae.

Clustered, in an irregularly arranged group around the ventral and distal edges of the primary rhinarium on segment six, are 4-6 (usually 6) receptors, each in a pit 3-5 μm across. There are, at least, three different types of these sensillae, as can be clearly seen from Fig.I7. The edges of their pits may also be ornamented with cuticular projections. The receptors, however, do not project far, if at all, beyond the mouths of the pits. Krzywiec (I968) refers to these organs as accessory rhinaria.

Finally, 7-I3 blunt-tipped hairs, 7-I9 μm long, occur exclusively on the terminal segment. These are probably contact chemoreceptors for in both males (present work) and virginoparae (Slifer et al,I964) their tips stain with crystal violet (Slifer,I960). The processus distalis bears only receptors of this type arranged in



Fig.17. Stereoscan of the complex of receptors present on the basal part of segment six. The cuticle of the primary rhinarium (arrow) is domed in life. Three types of accessory rhinaria (a,b,c) can be seen within the pits bordering the primary rhinarium. t = tip of blunt-tipped peg or hair, pd = base of processus distalis. X 5,360

a group of 2,3 or 4 (usually 4) at its tip and 2-7 irregularly spaced along its length. The development of such a long support for so few sensillae, indeed, suggests that the latter need to be placed near, or in contact with, the source of stimulation.

The antennae of last instar larval males and of adult oviparae differ from those of adult males principally in the numbers of secondary rhinaria they possess. Larval males have none, while adult oviparae bear II-23 on the third segment and none on the fourth and fifth. The other types of chemoreceptors described above are present in both these forms. This finding contradicts the statement by Krzywiec (1968) that the coeloconic pegs or rhinariellen present on segment two are, like the secondary rhinaria, acquired only at the adult moult.

Responses to pheromone after removal of antennal segments

To determine which of the antennal receptors are necessary for the response to the pheromone males were tested in the pathway olfactometer after various antennal segments had been removed. Tests were conducted during the 2nd-7th hours of the photoperiod using 6th-day oviparae as the stimulus source.The categories of insects assayed and the numbers that responded are summarised in Table I5.

The results indicate that the secondary rhinaria are the principal receptors involved in the detection of the female scent. Removal of the primary rhinaria (category C) and of the other apparent chemoreceptors on segment six (categories A and B) did not significantly reduce the numbers of males reacting to the pheromone, whereas ablation of segments bearing the secondary rhinaria (categories D and E) produced a dramatic drop. When the only chemosensory structures retained were the coeloconic pegs of segment two (category E) no males responded. It appears, however, that the numbers of rhinaria left intact is also important if a behavioural response is to be elicited (compare categories C and D). The percentages of secondary sensoria

Table 15. Numbers of males responding to the female pheromone after removal of certain antennal segments.

Antennal segments removed	Replicates	Total
A. Processus distalis of segment six	7, 8, 9	24
B. All segment six	9, 9, 9	27
C. Segments five + six	9, 8, 7	24
D. Segments four to six inclusive	3, 0, 2	5
E. Segments three to six inclusive	0, 0, 0	0
F. None. Control males	10, 7, 10	27

Categories A,B,C, and F are not significantly different from one another (Chi-squared test, $p > 0.05$), whereas categories D and E are significantly different from categories A,B,C, and F ($p < 0.001$).

left after various segments have been removed have been computed from the figures presented in the preceding section and are given below.

Segments removed	Percentage of rhinaria remaining
6	100
6+5	73-88
6+5+4	52-71
6+5+4+3	0

This preliminary experimental evidence of an olfactory function for the secondary rhinaria thus supplements the histological evidence provided by Slifer et al (1964). Nevertheless, it should be remembered that the present findings merely show that the rhinaria are necessary if a behavioural response is to be evoked by the pheromone. They do not exclude the possibility that other types of sensillae may

also be sensitive to the female scent. Further studies using varnish or paint to cover specific antennal segments were not attempted because of uncertainty of the accurracy of such a technique.

Species specificity of pheromone communication

Response in pathway olfactometer

Since oviparae of other aphid species possess structures similar in appearance to the scent plaques of M.viciae it was assumed that many may also produce a sex pheromone. It was of interest, therefore, to see whether interspecific responses involving M.viciae sexuales could be obtained, using the pathway olfactometer. As a green strain of A.pisum was readily available the sexuales were cultured and used in this study.

M.viciae males were tested against groups of five normal oviparae, oviparae without their hind tibiae, and virginoparae of A.pisum during the 3rd-8th hours of the light period. Normal oviparae or virginoparae were placed in the test compartment while, in each case, oviparae deprived of their hind tibiae were confined beneath the control zone. Separate batches of females were used with successive replicates of 10 males. Thirty males were tested against each of the two female morphs of A.pisum. Unfortunately, tests with the apterous males of A.pisum were less extensive. The three individuals that were available were assayed with pheromone from groups of 6th-day M.viciae oviparae during the 5th hour of the photoperiod. The males were 7,11, and 18 days old respectively.

Twenty-three out of 30 M.viciae males responded to intact oviparae of A.pisum but none did so to tibia-less oviparae or to virginoparae. The responses were indistinguishable from those shown towards homospecific females. Twenty-one of the 23 responses lasted for more than 60 s and the remaining two responses for 45 and 53 s

respectively.

Similarly, all three A.pisum males responded to normal oviparae of M.viciae but not to oviparae without their hind tibiae. The responses of individual males continued for 200,61, and 128 s respectively, and were identical to those shown by M.viciae males to females of either species.

Responses by males of M.viciae and A.pisum are obviously not restricted to the conspecific females. Moreover, they appear to involve similar behavioural mechanisms. All responses,whether within or between species, were dependent upon the presence of the hind tibiae of the oviparae.

Copulation tests

Males of both M.viciae and A.pisum readily copulated with the heterospecific oviparae whether or not the hind tibiae of the females had been removed. The behaviour of males towards females, and of females towards males, appeared to be identical in both homospecific and heterospecific pairs. Any chemical stimulation perceived when the sexes are in contact does not inhibit mating.

Arousal of inactive males by the pheromone

Earlier observations indicated that the presence of nearby oviparae evoked other types of male behaviour besides the arrestant response used in the routine bioassay. For example, resting insects were aroused and stimulated to move towards the females. An experiment was conducted to determine whether this arousal response could be elicited by the sex pheromone.

Tests were made during the 1st-2nd and 9th-12th hours of the light period for two reasons. First, males are, in general,spontaneously active during the 3rd-8th hours of the photoperiod and almost totally

inactive at other times. Secondly, although in tests with the pathway olfactometer males were found to be responsive to the pheromone throughout the day those assays necessarily involved the artificial arousal of any inactive insects before the latter were exposed to the female scent. Evidence from undisturbed insects was therefore needed to confirm that males are normally responsive during their quiescent phases. Each insect was placed on a bean plant, 2 cm high, covered with a loosely-stoppered, 3 x I" glass cylinder, and allowed to settle down and become inactive during the course of the daily locomotor rhythm. A piece of filter paper, I cm square, impregnated with a pheromone extract (0.40 FE/cm^2), or with methylene chloride only, and from which the solvent had been allowed to evaporate, was placed very carefully into each tube. It was lodged against the plant or the tube so that its nearest edge was no more than I cm, and usually less than 5 mm, from the male. Any activity shown during the following IO min was recorded. Insects that showed any sign of having been disturbed during the introduction of the impregnated squares were discounted.

There were marked differences in the reactions shown to the filter papers. Only II aphids were aroused by the control papers and many of these merely waved their antennae for a short period but did not walk, while those that did move left the plant and walked away from the filter paper. In contrast, 72 of the 75 males activated by the pheromone approached the filter paper and spent some time walking back and forth over its surface. Some even attempted to copulate with the edge of the paper or with the bean plant immediately above the square. The other 3 activated males showed only prolonged antennal waving and no other movements. All I24 insects that were not aroused maintained, throughout, the resting posture characteristically adopted by males, and, indeed, by oviparae, in their quiescent periods. In this the body is kept tight against the plant and the antennae held close

together and low over the back of the abdomen. In males the antennae are held beside the wings, but are not covered by them. The results are summarised in Table I6.

Table I6. Numbers of inactive males aroused by filter papers impregnated with pheromone extract (0.40 FE/cm^2**),or with methylene chloride, at different times of day.

Hour in light period	Pheromone		Methylene Chloride	
	Males tested	Males aroused	Males tested	Males aroused
I	I3	I0	I3	0
I-2	I2	I0	I0	0
9	I4	I2	I3	I
9-I0	I2	9	I3	2
I0	I2	8	I3	5
II	I2	I0	I2	3
II-I2	I2	7	I4	0
I2	I3	9	I2	0
Total	I00	75	I00	II

** Another sample from the same stock solution elicited a response in 40-70% of the males when tested in the pathway olfactometer at a concentration of 0.20 FE/cm^2.

These findings complement previous results and confirm that males are responsive to the female scent at those times in the light period when they are inactive. The behaviour of males placed on the same plants as oviparae supports this conclusion. Such males are often active and copulate with females during the 2nd hour of the light period - a time when the oviparae are releasing their sex pheromone but when males in unisexual groups are inactive.

Copulation and insemination in relation to the female pheromone

Copulation behaviour

The pre-copulatory activities of M.viciae appear to be simple, although somewhat clumsy, and lack any recognisable courtship. The male approaches a female and clambers directly onto its back. Almost immediately the male curves the end of its abdomen ventrally and anteriorly, and with it, begins to probe the dorsal surface of the female. These exploratory thrusts may be directed towards any part of the ovipara, including the head. Once the male has succeeded in hooking the tip of its abdomen under the female's long cauda and has located the vagina, coitus ensues. Until this stage is reached, however, no protrusion of the male's genitalia occurs. Throughout the initial phase the male waves its antennae vigorously but does not use them to systematically stroke or touch any part of the female. Some individuals may probe the ovipara's abdomen with the proboscis but this does not continue for long and is not shown by all males. As soon as copulation begins, the male becomes completely immobile , and, with its abdomen in an almost upright position, remains in this cataleptic state until insemination is completed.

During the whole procedure the female usually remains passive. However, on occasions an ovipara may raise the end of its abdomen into an exaggeratedly elevated position by a smooth,rapid movement. This appears to facilitate the attainment of coitus either by increasing the accessibility of the female's genitalia, or by providing a better tactile stimulus for the probing tip of the male abdomen (see below).

Mating normally continues for 5-10 min (Table I7) and its termination is immediately preceded by slight movements of the male's legs or antennae. Muscular contractions of the posterior part of the abdomen of both sexes may also occur at this time.In those instances in which coitus lasts for 1-2 min only (Table I7) insemination is not

accomplished. These bouts are often characterised by continued fidgeting by the male, and are usually followed by a more sustained period of copulation in which the male adopts the cataleptic attitude, and in which the transfer of spermatozoa does occur.

Both sexes may mate repeatedly within a short space of time, either with the same or different partners.

Table I7. Frequency distribution of the duration of copulation.

Copulation time (min.)	I	2	3	4	5	6	7	8	9	I0	II
Frequency (Total=I25)	7	7	I	2	I8	44	23	I6	6	I	0

Copulation in other aphids seems to closely resemble that of M.viciae (Buckton,I876-83; Gadeau de Kerville,I902; Pettersson,I97I; Savary,I953; Smith,I936; Weber,I935). In Sappaphis pyri Fonsc., however, the antennae of the male are continuously quivered during coitus, although the rest of the body remains immobile (Savary,I953). Mating has been found to last for 2-6 min in S.pyri (Savary,I953) and for 5-30 min in Hyalopterus pruni (Geoffroy)(Smith,I936). In most species both sexes will mate repeatedly at short intervals.

Copulation and the pheromone

In the absence of pheromone males will readily attempt to copulate with a great variety of objects that provide appropriate visual and / or tactile stimulation. During the peak of their daily activity cycle males in unisexual groups may try to mate with the edges of the unfurled leaves of the host plant, with the sand grains at the base of the plant, and, more rarely, with the edges of the wings of other males. They will also try to mate with coloured plastic beads, colourless glass beads, balls of paper, and lumps of plasticine.

However, some objects are more effective at evoking this behaviour than are others. Males 'copulate' more frequently, and for longer periods, with those objects that possess ridges or protuberances under which they can attempt to hook their external genitalia, than with similar but smooth ones. Similarly, green or yellow objects are more effective at eliciting this activity than are white,black,red, or colourless ones.

The female scent alone does not, however, elicit copulatory movements, such as downward and forward flexing of the abdomen or protrusion of the genitalia. Males placed in a stream of air passed over nearby,pheromone-releasing females, or males responding to the pheromone in the pathway olfactometer, never attempt to copulate with the substrate, even though, in the latter case, the attractive females may be only a few millimetres away. Nevertheless, preliminary observations suggested that the female scent does affect the way in which males respond to coloured beads. This was therefore investigated further.

Tests were performed with individual males since initial trials with groups of insects had indicated that male-to-male interactions markedly influence their copulatory behaviour. For instance, when ten males were placed in a large arena (9 cm in diameter) with four coloured beads most males attempted to mate with one bead, in spite of insufficient space on that bead. This behaviour was not related to the characteristics of individual beads but to the presence of a male on a bead when another aphid reached it. Even with beads that evoke few copulation attempts,e.g. red ones, males remain longer if another male is already present, and especially if it is trying to mate with the bead, than when the bead is untenanted.

Male responses to beads of different colours were tested in both the presence and the absence of pheromone. The arenas, the beads, and the arrangement of the latter within the arenas have already been

described (p. 5I). The ridged, cylindrical beads were chosen in preference to larger,spherical ones because they evoked higher levels of copulatory behaviour. Four normal,6th-day oviparae (pheromone source) or oviparae without their hind tibiae, were placed under the floor of the arena 6 min before the male was introduced. Five beads of one colour - green,yellow,orange,pink,or red - were placed in position immediately before the male was added. The latter was always placed parallel to the central bead and 0.5 cm from it. All contacts made with any of the beads during the following 5 min were counted and timed. Those encounters in which the male attempted to mate with the bead were noted, as was the duration of this behaviour. Males were considered to be trying to mate with the bead when they showed a downward and forward flexure of the abdomen and probed the bead with the abdominal tip. Separate males, 3-I0 days old, were used for each test. A male assayed with beads of one colour in the absence of the pheromone was followed immediately by another male with beads of the same colour in the presence of the sexual scent. In each replicate the five colours were tested in varying succession. The experiment was repeated until I50 males had been assayed - I5 for each combination of colour and presence or absence of scent.All assays were conducted during the 3rd-8th hours of the photoperiod. The results are summarised in Tables I8 and I9.

As expected, the efficacy of the differently coloured beads in eliciting sexual activity varied considerably. Nevertheless, they fell into two groups - the green,yellow,and orange (GYO) were very effective, while the pink and red (PR) were much less so. Differences within these groups were statistically insignificant, except for those given in Table 20.

In the absence of the pheromone males attempted to mate only with the GYO beads while in its presence some sexual activity was shown towards beads of each colour. The pheromone did not,however,

Table I8. Total numbers of encounters made by males with coloured beads in the presence and absence of pheromone, and the numbers of these that resulted in copulation attempts.

Colour of beads	Pheromone absent			Pheromone present		
	Total contacts	Contacts involving copulation	Number of males that copulated	Total contacts	Contacts involving copulation	Number of males that copulated
Green	59	24	14	35	28	15
Yellow	60	19	11	36	26	14
Orange	65	15	8	42	27	14
Pink	74	0	0	57	7	5
Red	41	0	0	41	3	2
Total (out of 75)			33 *			50 *

* Significantly different ($p < 0.01$, Chi-squared test) - see Table 21 for tests with individual colours.

Table I9. Total time (s) spent by males in contact with, or copulating with, coloured beads in the presence and absence of pheromone.

Colour of beads	Pheromone absent			Pheromone present		
	Total contact time	Total non-copulation time	Total copulation time	Total contact time	Total non-copulation time	Total copulation time
Green	2928	1428	1500	2859	662	2197
Yellow	2383	1318	1065	3374	573	2801
Orange	2008	1527	481	2433	699	1734
Pink	805	805	0	928	675	253
Red	648	648	0	747	636	111
Total	8772	5726	3046	10341	3245	7096

Table 20. Differences within the GYO group of beads which were found to be statistically significant.

I. In the absence of the pheromone more males attempted to mate with the green beads than did with the orange ($p < 0.05$, Fisher Exact Probability test), and those that did so continued for longer ($p < 0.01$, Mann-Whitney U test).
2. In the presence of the pheromone individual males spent longer attempting to mate with the yellow beads than with the green or orange beads ($p < 0.01$, Mann-Whitney U test).

reduce the differences between the two groups for (a) the numbers of males that attempted copulation ($p < 0.05$, Fisher Exact Probability test), (b) the proportions of encounters that resulted in mating attempts ($p < 0.001$, Chi-squared test), and (c) the total times for which individuals maintained their copulation attempts ($p < 0.001$, Mann-Whitney U test) remained significantly higher for the GYO beads than for the PR beads.Indeed, it seems that the sexual scent had the biggest effect on male responsiveness to the GYO group for it produced more significant differences with these beads than with the PR beads (Table 2I).

Many interacting factors probably contributed to these results. For instance, it seems that both an increase in the number of sexual bouts and a decrease in the total number of contacts were responsible for the rise in the proportion of encounters that resulted in sexual activity. Moreover, the decline in the total numbers of encounters is likely to have been the result of a reduced speed of walking (orthokinesis) and of males spending more time trying to mate with the beads. Further, a non-sexual component in the

Table 21. Conclusions from statistical tests on the differences in the behaviour of males towards plastic beads of different colours in the presence and absence of the sex pheromone. NS = not significant ($p > 0.05$). S = significant ($p < 0.05$ or < 0.01).

Behaviour *	Colour of beads				
	Green	Yellow	Orange	Pink	Red
1. Numbers of males attempting to mate	NS	NS	S	S	NS
2. Proportion of encounters that resulted in mating attempts	S	S	S	S	NS
3. Total time spent by individual males in mating attempts	S	S	S		
4. Total time spent by individual males on beads without attempting to mate	S	S	S	NS	NS

* Tests used: Class 1 = Fisher Exact Probability test, Class 2 = Chi-squared test, Classes 3 and 4 = Mann-Whitney U test.

behaviour of the males may have facilitated a sexual response by causing them to stay on the beads. In the absence of the scent they remained longer on the GYO beads without showing any sexual activity than they did on the PR beads ($p < 0.01$, Mann-Whitney U test). This was not the case in the presence of the scent ($p > 0.05$), however, for there was a significant drop in the amount of time spent in this activity for the beads in the GYO group but not for those in the PR group (Tables I9 and 2I). Presumably, the reduction obtained with the GYO group was the result of increased copulation.

Thus, although the female scent increases the mating activity of males the effect is dependent upon other stimuli. The increase is most pronounced with coloured beads that elicit a fairly high level of sexual activity without the pheromone and may be insignificant with beads that are less visually stimulating. The tactile stimulation provided by the different beads must, of course, have been the same. It should be mentioned here that the beads also varied in the intensity or brightness of their colours; the pink, green, and orange being the brightest to the human eye, the yellow less so, and the red dullest of all. However, the present results and numerous preliminary observations with objects of widely differing colour intensities indicate that discrimination is due to the wavelength of the reflected light. Oviparae are a yellowish-green while the smaller, alate males are predominantly black.

Insemination and the pheromone

Males will copulate with oviparae that are not releasing their sex pheromone. In some mosquitoes, however, it has been established that young, virgin females and older, mated females may apparently mate without actually being inseminated (Lea, I968; Lea & Evans, I972). This refractoriness to insemination appears to be due to the females "..failing to give the cues that lead to male ejaculation "

(Gwadz et al,I97I). Tests were therefore conducted to see if the absence of the sexual scent prevented insemination in aphids.

Pairs of insects were placed in I x 0.5 " stoppered tubes during the 5th-6th hours of the photoperiod and watched. All ensuing copulations were noted. Three - 24 h later the females were dissected, their spermathecae mounted in Ringer, and examined for the presence of spermatozoa. Normal or antenna-less males were tested with normal oviparae or with those lacking hind tibiae. Males that were assumed to be habituated to the scent were also assayed. Earlier observations indicated that insects that had been held for about 20 min in a 3 x I " stoppered tube that had previously contained pheromone-releasing females failed to respond to the scent in the pathway olfactometer. Males were therefore placed individually in the copulation arenas without beads, but with four scent-releasing females below the floor. After I30 min exposure to the pheromone their ability to inseminate oviparae was tested. Control insects were kept in empty arenas. Finally, antenna-less males were paired with antenna-less females to check that the males themselves do not produce an olfactorily active substance that can be perceived by the female antennae and which might affect insemination. The results are summarised in Table 22. As those for the control insects (normal males and normal females) were so similar in all the experiments they have been grouped together in the table.

Although insects of both sexes were examined both before and after coitus no evidence for the production of a spermatophore was obtained. Instead, spermatozoa appear to be transferred to the female in numerous bundles. The heads of the spermatozoa in each bundle are embedded in a spherical,hyaline cap. Examination of the spermatheca of a recently mated ovipara under a low power microscope may give the impression that it is filled entirely with a mass of large,globular units (caps), the spermatozoa being seen only on

closer scrutiny. The caps are gradually broken down within the spermatheca and the spermatozoa set free. Occasionally this may occur within the seminal vesicles of the male ,especially in older insects. In other insects spermatozoa may be similarly transferred to the female in bundles (Wigglesworth,1965).

Table 22. The effect of various treatments on the insemination of females.

Female	Male	Pairs tested	Pairs mating	Females inseminated
Normal	Normal	69	66	63
Minus hind tibiae	Normal	36	36	35
Normal	Minus antennae	10	7	7
Minus hind tibiae	Minus antennae	10	7	7
Minus antennae	Minus antennae	12	9	9
Normal	'Habituated' to scent	15	11	11

It is evident that insemination can be successfully accomplished in the absence of the pheromone from the female's hind tibiae. Moreover, males are not prevented from consummating the sexual act by exposure to the scent for more than two hours immediately prior to the assay. These results therefore emphasise the relative unimportance of the tibial secretion in this phase of the reproductive behaviour. Without further information, however, it would not be safe to conclude that other chemical stimuli - perhaps present on the ovipara's body surface and perceived by receptors on the male's tarsi or external genitalia - are not involved.

Females remained susceptible to insemination after the

removal of their antennae.

Scent plaques

External appearance and distribution

Legs were removed at the trochanter/femur joint and mounted in polyvinyl lactophenol. Those from the left and right sides of the insects were mounted separately. Retention of the femur and tarsus prevented the tibia from twisting during preparation and enabled its posterior and anterior surfaces to be identified. Through-focusing allowed the numbers of plaques in exactly similar areas on these two faces to be counted. The distribution of the plaques along the tibia was determined by counting those present in successive spans of a microscope eyepiece graticule. Each span (0.317 mm) covered from I2.3 to I5.6% (mean = I3.8%) of the total length of a hind tibia of an ovipara (2.029-2.572 mm long, mean = 2.304 mm).

There are from I60 to 303 (Fig.I8) more or less circular, domed scent plaques on the swollen basal half of each hind tibia of an ovipara (Fig.I9). Each plaque bears numerous blind-ending pits on its surface. In two individual plaques there was found to be an average of 5.I and 5.7 pits/ μm^2 respectively. Most plaques occur singly (Fig.20) but 2.4 to 24.0% (mean = II.7%) per tibia are involved in figure-of-eight shaped 'double plaques' (Fig.2I) in which the boundaries of the two contributing plaques are still visible.'Triple plaques' (Fig.22) are also found but very rarely. Single plaques vary in diameter from 4-22 μm (Fig.23), while each of those involved in multiple plaques may be of any size within this range.

Although the numbers of glands vary enormously between individual aphids their general distribution along and around the tibia is remarkably consistent. This is clearly illustrated by comparision of the numbers and percentages of plaques present in each

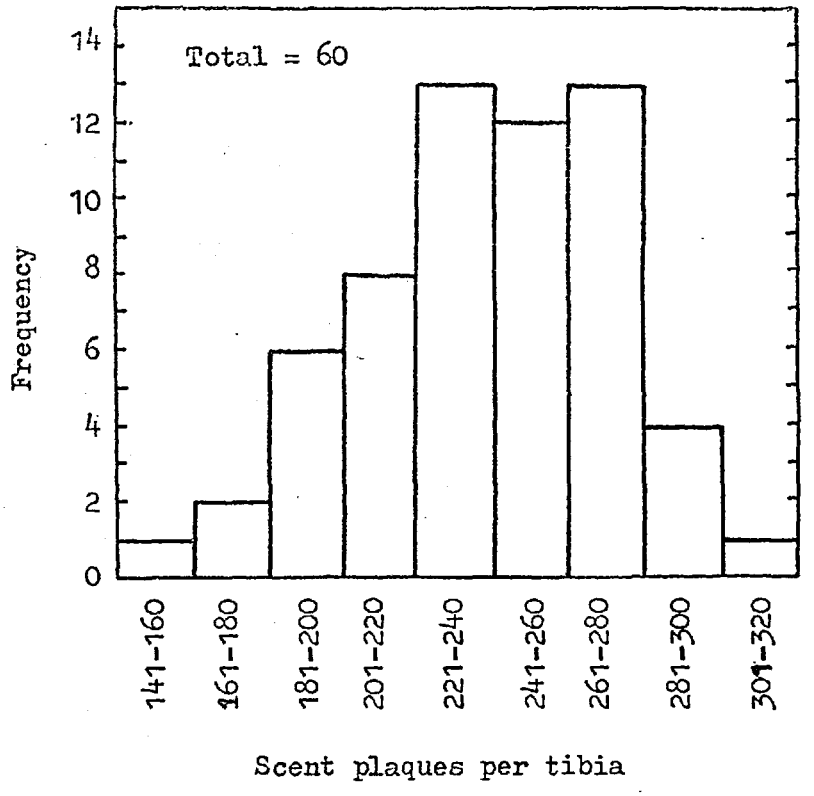


Fig.I8. Frequency distribution of numbers of scent plaques per tibia.

section (Table 23). On the more generously endowed legs the glands do not extend further down the tibia but are, instead, more closely spaced. On all legs relatively few glands are present on the inner or outer quarters of the tibial circumference, but are concentrated on the anterior and posterior faces. Slightly more are found on the anterior surface (mean = 55.0%) than on the posterior surface. This situation was reversed in only two of the 60 legs examined.

In M.viciae scent plaques occur only in adult oviparae and in no other morph. The shape of the hind tibiae reflects this difference. Those of oviparae expand from the base to a maximum diameter of 92-I24 µm (mean = I00 µm) before tapering towards the distal end. They are most swollen where the glands are most frequent,

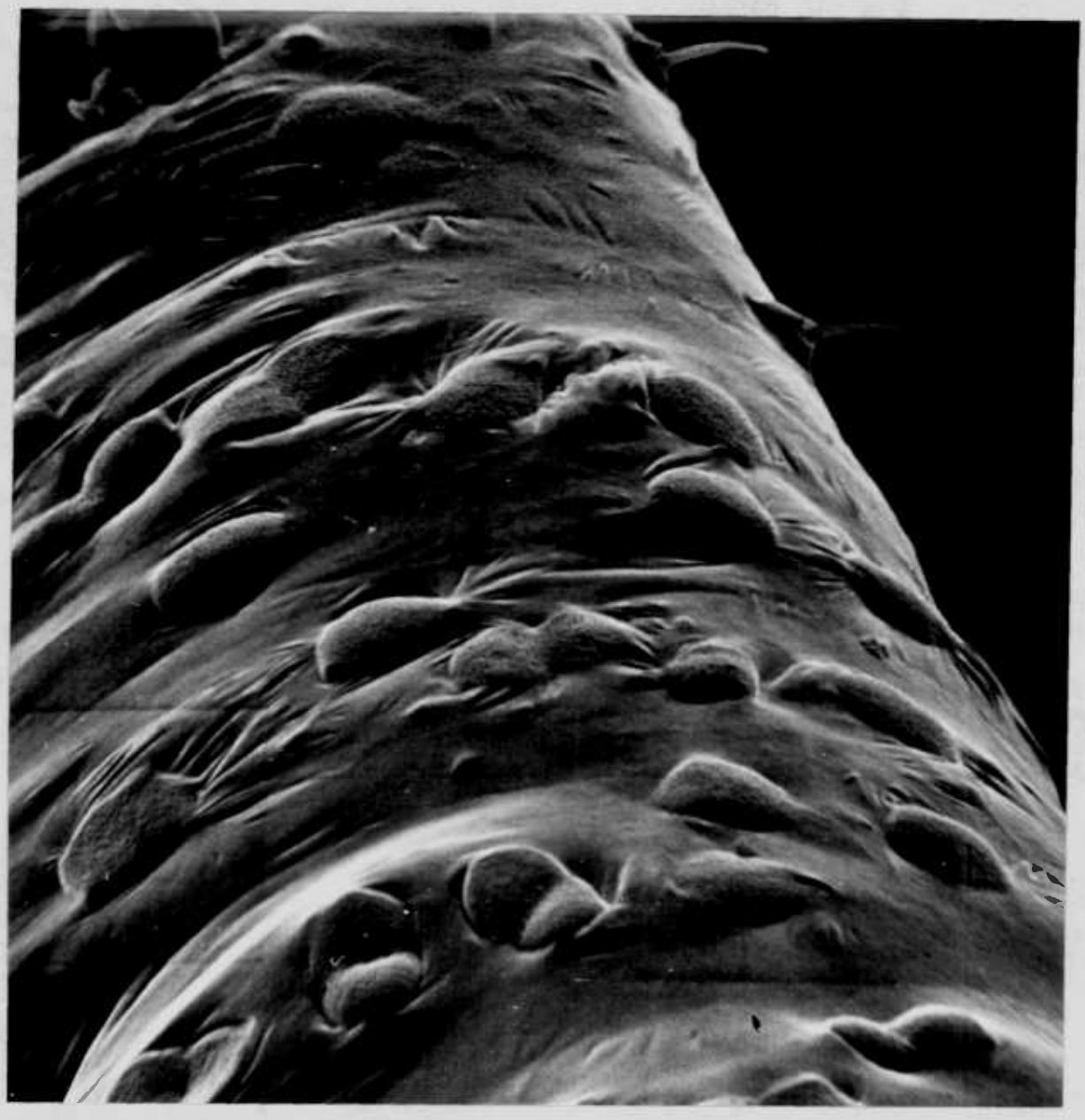


Fig.I9. General view of a hind tibia of an ovipara of M.viciae showing several scent plaques. Both single and double plaques can be seen. The straight lines surrounding the plaque in the left foreground are artefacts. X I,630



Fig.20. Single scent plaque. The mouths of the cuticular pits are clearly visible. X 8,830

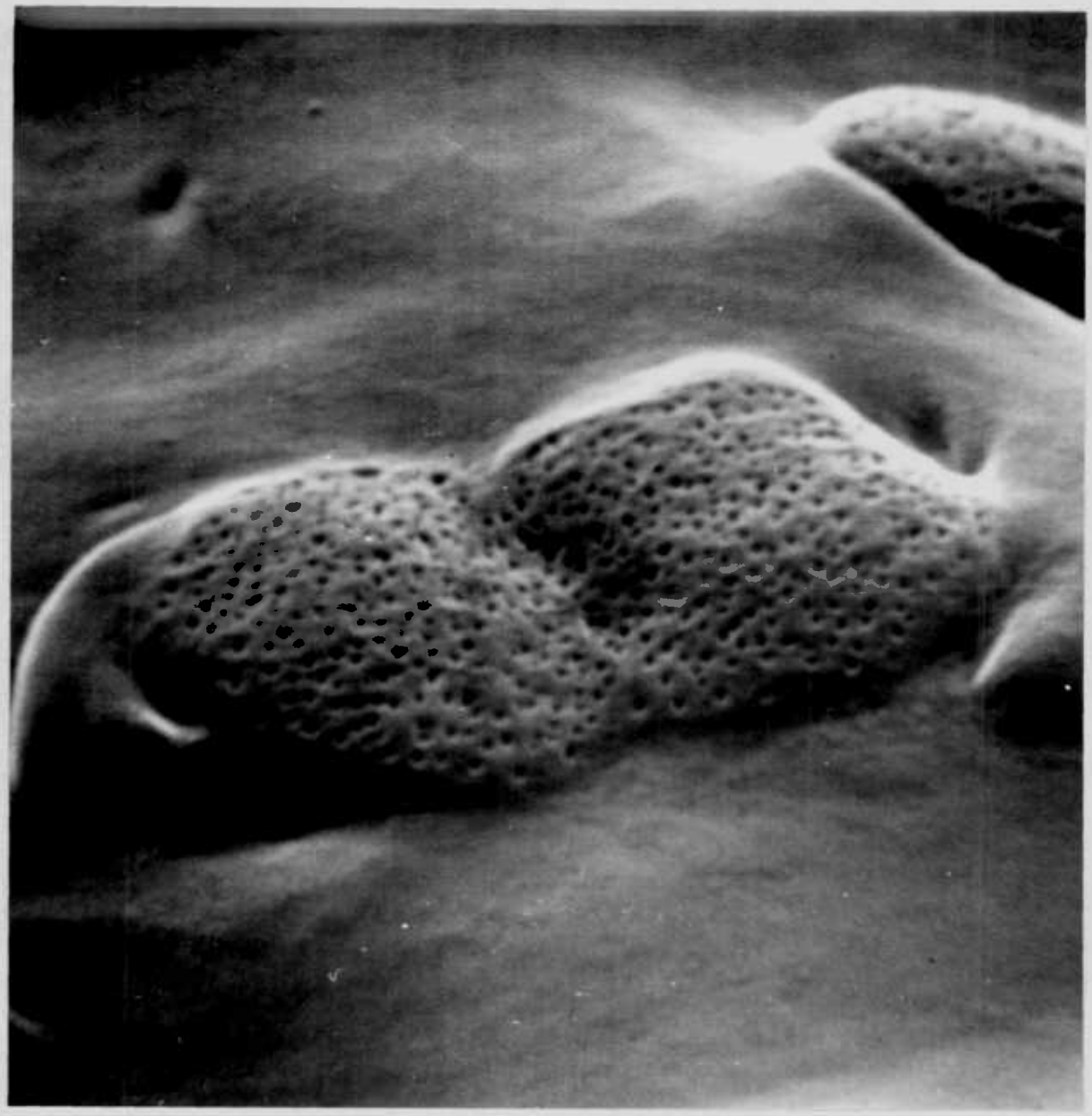


Fig.21. Double scent plaque. Plaques vary in the degree to which they are domed. The plaque in the background of this figure and that in Fig.22 are good examples of cupola-like plaques. X 8,540



Fig.22. Three-lobed or triple scent plaque. X 9,520

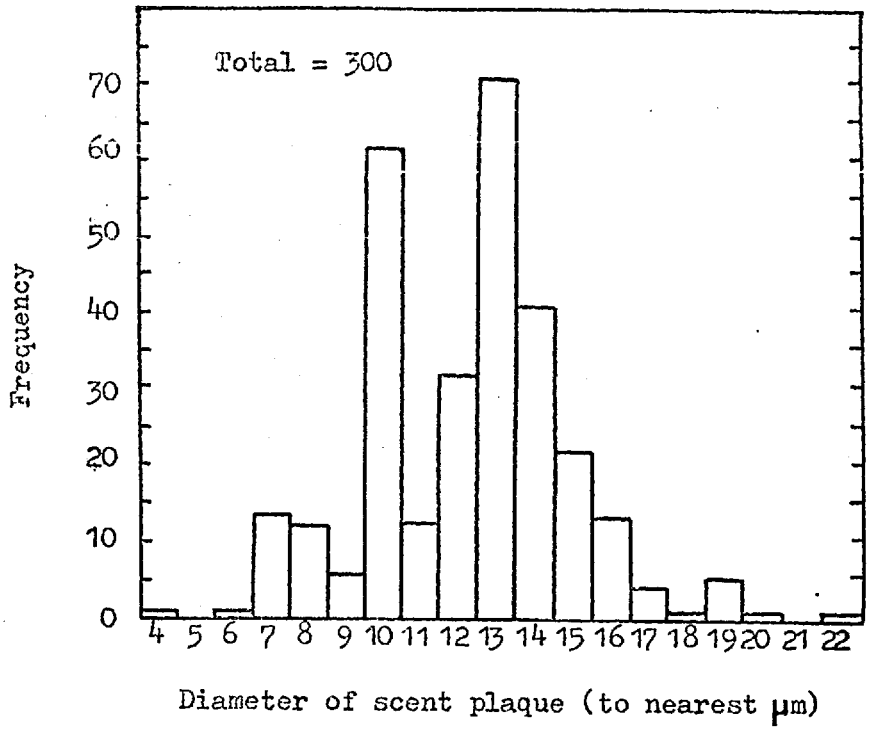


Fig.23. Frequency distribution of longest dimension of single scent plaques.

Table 23. Distribution of scent plaques along the hind tibiae of oviparae.

Successive spans of microscope eyepiece graticule	Numbers of plaques		Percentage of total	
	Range	Mean	Range	Mean
I (Basal end)	39-78	58.0	I9.I-30.5	24.4
2	54-I07	88.I	30.8-42.7	37.0
3	39-86	59.4	I9.8-3I.0	24.8
4	I3-47	26.5	6.7-I6.I	I0.9
5	0-I6	6.4	0-6.I	2.6
6	0-4	0.6	0-I.5	0.2
7	0-I	< 0.I	0-0.4	< 0.I
8 (Distal end)	0	0	0	0
Total	I60-303	239.0		99.9



Fig. 24. Mechanoreceptor hair on hind tibia of an ovipara of M.viciae. It's cuticle is ridged longitudinally. X 2,240

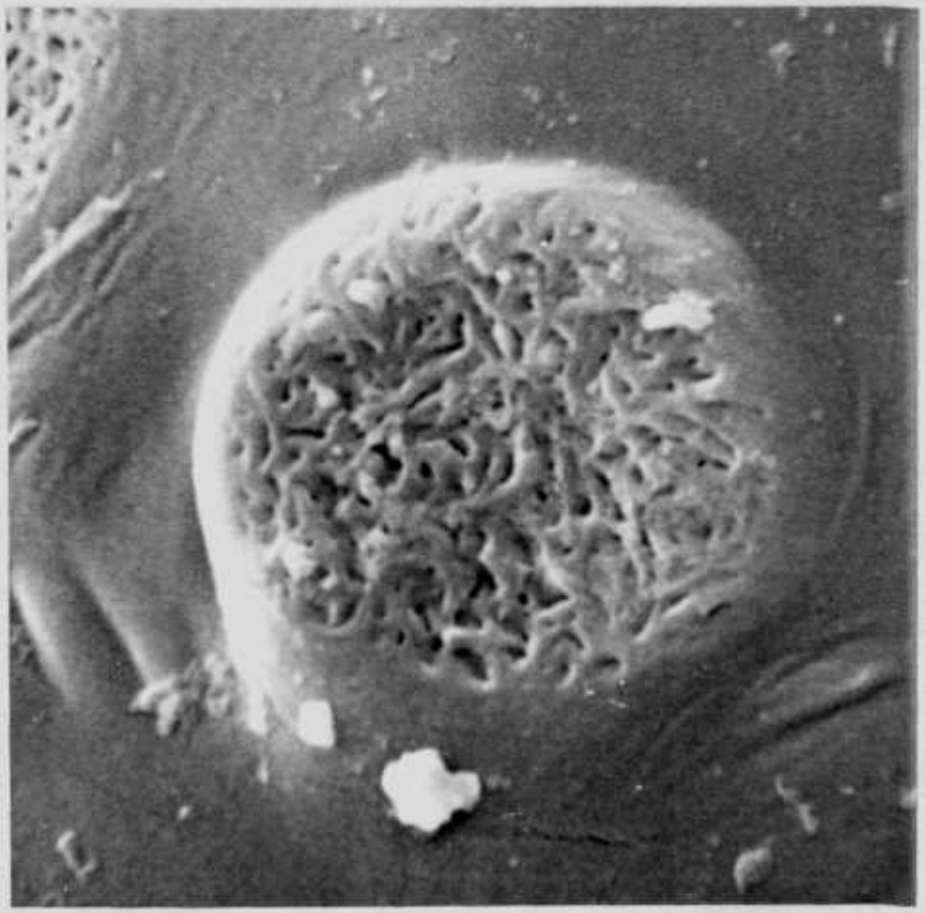


Fig. 25. Scent plaque on hind tibia of an ovipara of Aphis fabae. As in M.viciae there are pits in it's surface. A certain amount of collapse may have occurred during preparation of the specimen. X 8,740

namely, about one quarter of the way along their length. In contrast the tibiae of virginoparae are broadest at the base (77-9I μm, mean = 84 μm) and taper gradually towards the tarsus. Spatulate mechano-receptor hairs (Fig.24) are present on the tibiae of all morphs.

The plaques of Aphis fabae oviparae also possess surface pits (Fig.25).

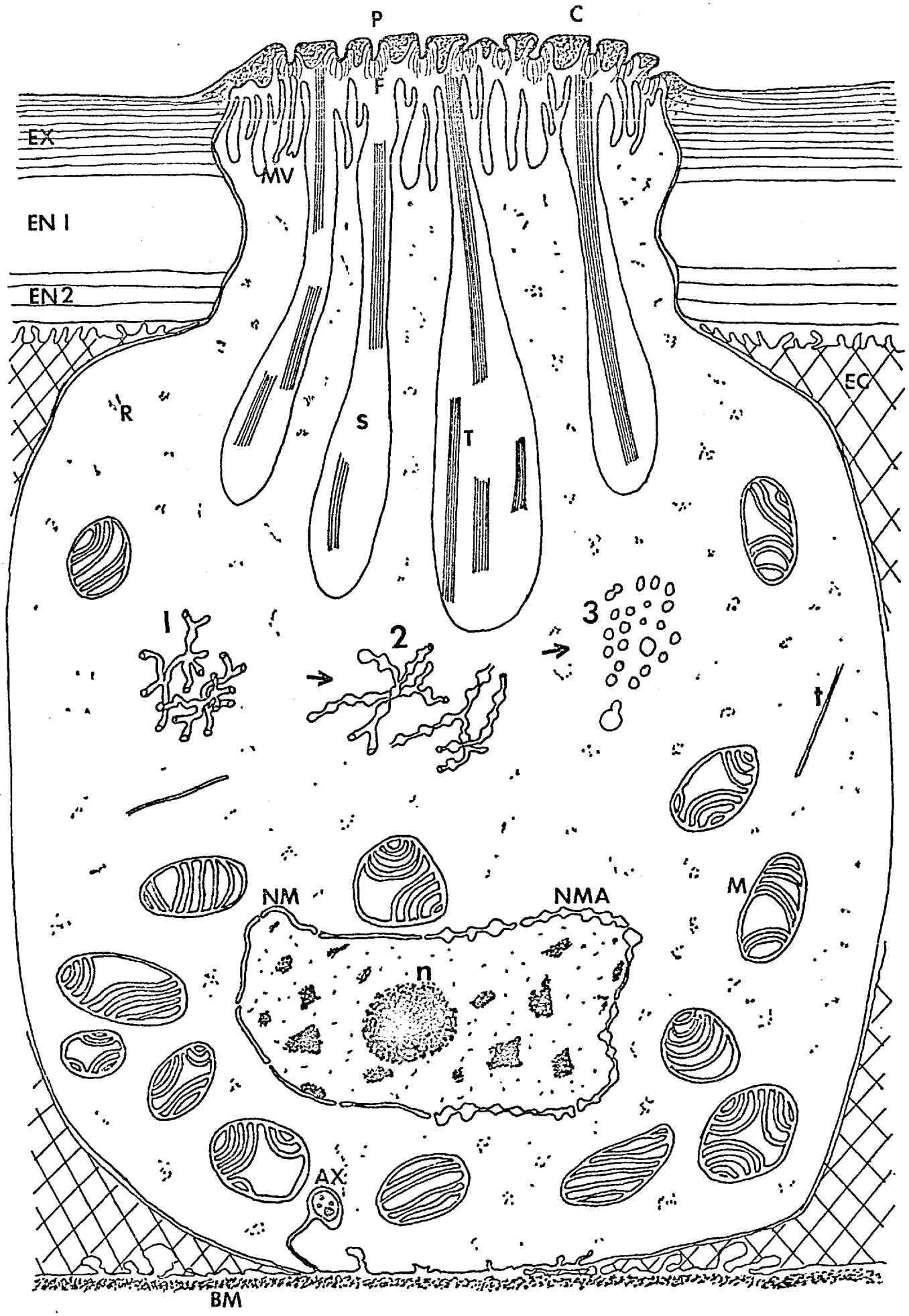
Summary of ultrastructure

The ultrastructure of the gland cells beneath the cuticular scent plaques of 6th-day adults was investigated using conventional electron microscope techniques. It is not proposed to present the detailed results here but merely to summarise the most important findings. Changes in the appearance of the cells during the period of scent release are of particular interest as they complement some of the results described earlier in this thesis.

The main features of the glands are illustrated in Fig.26. Each scent plaque overlies a single, large secretory cell, I5-48 μm deep and I0-36 μm wide. There are, therefore, two cells beneath the double plaques. The plaque consists of a relatively thin (0.37-I.I5 μm), exocuticular dome which caps a cylindrical hollow in the cuticle. It contains numerous thin-walled (20-68 nm), thimble-shaped pits, I00-590 nm deep and 22-I64 nm wide, the openings of which are visible externally (Figs.I9-22). Many filaments, I5-20 nm in diameter and somewhat woolly in appearance, are attached to each pit and extend down to, or just beyond, the inner surface of the cupola. Elsewhere the cuticle is very much thicker (5-9 μm in 6th-day insects) and consists of three distinct regions - laminated exocuticle, non-laminated endocuticle, and lamellate endocuticle showing typical parabolic striations. Pore canals are present but sparsely distributed. Normal squamous epidermal cells underlie this cuticle and extend right up to the edges of, but do not enter, the gap below each plaque . The latter is virtually filled by

Fig. 26. Diagrammatic representation of the main components of the scent plaque cell of M.viciae. The normal epidermal cells (EC) surrounding the gland cell are shown as cross-hatched areas. For the sake of clarity the different structures have not been drawn strictly to scale.

AX = sensory axon (from mechanoreceptor hair) which has become engulfed by the base of the gland cell
BM = basement membrane
C = exocuticular plaque which in section appears as wedges between the pits (P) - this cuticle does not show the laminations characteristic of the rest of the exocuticle (EX) and in this respect differs from the scent plaque of Schizaphis (Pettersson, 1971, Fig. 4 - or Fig. 27 C of this thesis)
EC = normal epidermal cells
EN I = non-laminated endocuticle
EN 2 = laminated endocuticle - this continues to increase in thickness until about the tenth day of adult life
EX = laminated exocuticle - only the exocuticle is present in newly emerged oviparae
F = filaments attached to cuticular pits of plaque
M = mitochondrion
MV = leaf-like folds in apical surface of cell which in section resemble irregular microvilli
n = nucleolus
NM = nuclear membrane as it appears in cells not engaged in vacuole formation
NMA = nuclear membrane as it appears in cells actively producing secretory vacuoles
P = pit in surface of plaque - the cuticle forming the pit stains rather differently from the cuticle of the rest of the plaque
R = ribosomes
S = extracellular sac
t = intracellular (cytoplasmic) microtubule
T = bundles of extracellular microtubules - there may be as many as 100 of these bundles within a single cell
1,2,3 = stages in the production of the secretory vacuoles
1 = branching, tubular, smooth endoplasmic reticulum (SER) as it appears before vacuole formation begins
2 = first stage in vacuole formation - the lumen of the SER becomes dilated at frequent intervals along its length
3 = completion vacuole formation - the dilated parts of the SER are blebbed off as membrane-bound vacuoles which then migrate to the apical surface of the cell and discharge their contents into the extracellular space beneath the plaque


P
C
F
EX
MV
EN I
EN2
EC
R
S
T
1
2
3
NM
NMA
n
M
AX
BM

the apical portion of the gland cell.

A globular to sausage-shaped nucleus is situated at the base of the secretory cell. It contains a prominent, central nucleolus and scattered chromatin, and may be up to I6 μm long. Pores are visible in the nuclear membrane. Clustered around the nucleus and packing much of the basal half of the cell are a great number of globular mitochondria, up to 2.5 μm in diameter. Their cristae are arranged in concentric whorls and two or three groups of whorls may be present in any one mitochondrion. An extensive system of tubular, smooth endoplasmic reticulum (SER) ramifies through the cell. Rough endoplasmic reticulum (RER) is either absent or present in very small amounts. Free ribosomes are plentiful.Isolated lengths of cytoplasmic microtubules, 20-25 nm in diameter and surrounded by a clear zone of cytoplasm (Porter,I966), are occasionally observed. The basal edge of the cell shows small inpushings and folds but these are not extensive. Sometimes one to several axons (in a group) are engulfed by this edge of the cell. They contain microtubules and mitochondria but no vesicles, and are almost certainly sensory axons from the tactile hairs present on the tibia. Presumably, they become surrounded by the gland cell as it expands during development. A basal lamella separates the cell from the lumen of the tibia.

In contrast, the surface of the cell immediately below the plaque is thrown up into many branching leaves or folds which, in vertical section, resemble irregular microvilli. The tubular SER enters these structures. At intervals the surface infoldings are enormously exaggerated to form voluminous extracellular sacs extending 'into' the cell for one to two thirds of its depth. Contained within these sacs, but at no point in contact with the plasma membrane, are bundles of extracellular microtubules many microns in length. Individual microtubules have a diameter of I5-I7 nm and a conspicuous helical substructure. Within the bundles they are arranged in a closely-spaced

hexagonal lattice, with each microtubule linked to its six immediate neighbours by arms protuding from their surfaces. The fascicles may appear straight and rigid or they may follow an undulating course. They terminate at, or just below, the cuticular dome, but their exact relationship to other structures found here is unclear. Some appear to abut onto the lower surface of the cuticle between the pits, some appear to end a short distance below this, while others seem to have a tenuous link with the lower ends of the pit filaments. The arrangement of the latter is likewise confusing for apart from those contacting bundles of microtubules, others seem to be attached to the apices of the pleats in the cell surface, while some apparently dangle down into the space between the cuticle and the cell. Small regions of septate desmosomes are very occasionally seen at the interface of adjacent gland cells or between gland cells and other epidermal cells.

Marked changes occur in the gland cells during pheromone synthesis and release. The most obvious of these is the production of electron-lucent vesicles (secretory vesicles) from the SER. At frequent intervals along its length the SER becomes distended into bulbous swellings which increase in size and eventually round up into numerous, membrane-bound,spherical vesicles 40-650 nm (usually 60-180 nm) in diameter. This process takes place simultaneously in all parts of a cell. The vesicles migrate to the apical portion of the cell and enter the surface pleats from where their contents are discharged by exocytosis. The perinuclear space undergoes similar distension in synchrony with that described for the SER. However, it is uncertain whether secretory vesicles are actually blebbed off into the body of the cell. Structures resembling Golgi complexes are seen within the cells and may possibly contribute to the production of vesicles but their role in this respect, if any, is clearly a minor one.

Simultaneous fluctuations occur in the staining properties of the material found in the intercristal spaces of the large

mitochondria. As the SER vesicles enlarge and accumulate this mitochondrial material becomes progressively more electron-dense so that in cells containing a maximum volume of vesicles the cristae are difficult to see. At other times the cristae are very obvious.

All these changes are first seen at the beginning of the light period and are maximally developed by the fourth hour. Thereafter they decline. The total volume of secretory vesicles appears to parallel the changes in the quantity of extractable pheromone present in the legs, at least, during the first half of the daily period of scent release. Far fewer vesicles are, however, present in the latter part of the period of release (e.g. hour 8) than during the first part (e.g. hour 2), although similar amounts of active secretion may be present (see Fig.16). It seems likely that the secretion accumulates in the extracellular space immediately beneath the cuticular plaque. During the fourth to eighth hours of the light period scent liberation is, of course, maintained at a steady level, while the numbers of vesicles declines progressively.

Surprisingly the cells of 6th-day females are not all active. Those that are active do not have synchronous cycles for adjacent cells may be in differing stages of vesicle production and discharge. It is true to say, nevertheless, that most cells have reached their peak by hour four. Very few cells commence vesicle formation after this.

During the period of scent liberation no variations whatsoever were observed in the appearance of the extracellular microtubules, the pit filaments, or the nerve axons at the base of the cells.

The ordinary epidermal cells differ markedly from the foregoing gland cells. They have attenuated and distorted shapes due to their position between the plaque cells. They reach from the leg lumen to the cuticle and possess lobed nuclei, well developed RER and Golgi

complexes, sausage-shaped and dumb-bell-shaped mitochondria, and regular arrays of microvilli on their outer surfaces. Cytoplasmic microtubules are a common constituent. A considerable amount of material seems to be transported from the leg lumen to their apices in large (510-800 nm), ovoid vesicles. The cells appear to be active in the continuing deposition of cuticle during adult life. No variation in their appearance during the light period could be detected.

Preliminary observations on the scent plaques of A.pisum showed them to have a similar structure to those of M.viciae.

DISCUSSION

Responses to the pheromone

Sex pheromones of female insects usually elicit more than one type of response from conspecific males (p.17). Since each response occurs at a particular range of pheromone concentrations , different reactions are evoked at varying distances from the stimulus source. These appear to be linked in an orderly behavioural sequence which enables a male, initially stimulated by a low scent concentration, to be guided into the immediate vicinity of the pheromone-releasing female, and then to successively initiate searching behaviour , courtship (where appropriate), and mating attempts. The secretion from the hind tibiae of M.viciae oviparae is likewise involved in diverse activities for it has been shown to arouse resting males, to attract walking insects to its source, and to stimulate copulation attempts with aphid-like objects. It is of interest, therefore, that all these aphid responses apparently require high concentrations of pheromone. None has been elicited at a distance of more than a few centimetres from an ovipara. The arousal of resting males must necessarily be the first step in any behavioural sequence, and is consequently the least likely to be dependent upon a concentrated chemical signal. Numerous exploratory tests, however, yielded no indication that this reaction could be evoked by a much weaker stimulus than was required for klinokinesis. It may be that M.viciae has no need for long range communication between the sexes as both males and females are produced on the same host plant by the same mothers.

Conclusions concerning the distance over which the pheromone is effective must, nevertheless, be tentative at this stage, for, some types of behaviour usually associated with long-range attraction have not been fully examined. It has not been possible, for instance, to determine whether anemotaxis can be released by the presence of the

pheromone. In a variety of tests using a wide range of wind speeds most males (70-100%) moved upwind even in the absence of the scent. This result would appear to be somewhat anomalous for it seems unlikely that in the field male aphids would always behave in this way.

Similarly, the degree to which flight is involved in the attraction of males to females remains unclear. Males of M.viciae were very reluctant to fly in the laboratory. However, as short flights and take-off attempts were occasionally seen, one would expect to see some change in male behaviour, such as wing spreading or fluttering, if the female scent stimulated flight. No such indications were ever obtained despite the use of a wide range of odour concentrations. This circumstantial evidence must, nevertheless, be balanced by the knowledge that other workers have found it difficult or impossible to demonstrate behavioural responses to a sex pheromone in the laboratory even though flying insects are attracted by a pheromone in the field, e.g. Casida et al (cited by Jacobson,1965) for Diprion similis (Hartig); Strong et al (1970) for Lygus hesperus; Roelofs et al (1971) for Zeiraphera diniana (Guenee); Berisford and Brady (1972) for Rhyacionia frustrana (Comstock). Moreover, in scolytid beetles the sex or population-aggregating pheromone may not elicit flight itself but does evoke responses in flying insects. Indeed, in Trypodendron lineatum and Dendroctonus pseudotsugae a reaction to the chemical stimulus is not shown until a certain amount of flight exercise has occurred (Bennett and Borden,1971).

With the exception of a few records of its occurrence on various host plants (Buckton,1876-83; Theobald,1926-29; Stroyan,1952, 1969; Bodenheimer and Swirski,1957) virtually nothing is known about the behaviour of M.viciae in the field. Attempts to redress this imbalance with observations on colonies at Windsor,Berkshire, were unfortunately thwarted for each year the aphids disappeared from apparently healthy host plants (Lathyrus pratensis L.) before sexuales were produced. It is not even known how often or how far males fly in

the field. Any endeavour to relate laboratory observations on this aphid to what might occur in the field must necessarily be of a speculative nature.

The occurrence of scent plaques on the hind tibiae of oviparae seems to be correlated with flight and/or migration of the males, for they are most numerous in species having winged males (Weber,I935) and in those showing host alternation (Hille Ris Lambers, I966). Accordingly, Weber (I935), Smith (I936) and van Embden et al (I969) have suggested that a female scent is involved in long-range attraction of migrating male aphids. Pertinent experimental evidence is scanty but does not lend support to this hypothesis. Tamaki et al (I970) were unsuccessful in obtaining any attraction of male Myzus persicae (Sulzer) to females in traps, in a wheel olfactometer, or in a Y-tube apparatus. The results of Pettersson's (I97I) attempts to determine the effective range of the pheromone from female Schizaphis are difficult to interpret, but seem to indicate that it is only a few centimetres. At present, therefore, it seems possible that males of some species may react to oviparae from a relatively close range, perhaps having located the host plant in much the same way as do gynoparae (Broadbent,I949; Kennedy et al, I959). Other factors, such as host plant chemicals and non-chemical stimuli from other aphids (Pettersson,I970b; Tamaki et al,I970), may be important in effecting the aggregation of males in situations where they are likely to meet oviparae.

Fraenkel and Gunn (I96I) have proposed that organisms might orient to chemical stimuli in a variety of ways - namely with orthokinesis, klinokinesis, klinotaxis, and tropotaxis. They envisaged that as an animal neared the stimulus source and the chemical gradient became steeper, it would progressively change from one type of behaviour to a more efficient one, for example, from klinokinesis to klinotaxis or from klinotaxis to tropotaxis. In this way its path would become

more direct as it neared the origin of the scent. To illustrate their point they reanalysed the behaviour of various organisms previously described by other workers, including that of male Bombyx mori responding to the female sex pheromone (p.329 of Fraenkel and Gunn,1961). They also suggested that the great mobility of insect antennae made it likely that klinotaxis was of great importance in this group. The responses shown by male M.viciae in the pathway olfactometers would seem to allow the type of orientation outlined by Fraenkel and Gunn. There are clearly several components in the behaviour of the aphids, and which operates at any one time appears to depend on the steepness of the pheromone gradient. Males show klinokinesis in the uniform pheromone field above an impregnated filter paper, and yet turn in a more directed manner at the edge of the odour zone where the gradient is pronounced. Although it is not known whether they can respond klinotactically as well as tropotactically it seems likely that they can. The turning movements made during klinokinesis would facilitate a transition to klinotaxis should the concentration gradient become sufficiently steep.

Insects may show klinokinesis in response to an increase or to a decrease in stimulus intensity (Kennedy,1965). Each type of behaviour may alone lead to the aggregation of the responding animals in a given area. David(1973) has discussed the mechanisms by which this might be achieved and has given examples from the literature of both types of klinokinesis. It is clear that with males of M.viciae an increase in pheromone concentration elicits klinokinesis. This behaviour, in conjunction with a simultaneous orthokinetic response, retains the aphids in the vicinity in which they first react to the pheromone. Whilst turning in this area they may encounter concentration differences which enable them to move nearer the source. In the absence of further stimulation habituation to the pheromone would allow them to leave the area. The simultaneous occurrence of an increase in turning behaviour

(klinokinesis) and a reduction in walking speed (orthokinesis) suggests that these responses are shown only within a short distance of the pheromone source. If, as has been suggested, they form the initial stages of a series of reactions which guide the males towards a female, and if they occur at a great distance from the female, it would be more efficient to increase, rather than decrease, walking speed so that the distance to the pheromone source could be covered more quickly. On a narrow plant stem orthokinetic and klinokinetic responses may together provide an efficient mechanism for bringing males within visual range of a nearby pheromone-releasing female.It should be noted that many authors recognise only one type of klinokinesis (Kennedy,1965) and that Shorey (1973), in his recent review of behavioural responses to insect pheromones, does not recognise the type shown by M.viciae.

Orientation to pheromone sources in other insects may involve, besides orthokinesis and klinokinesis, klinotaxis, e.g. Trogoderma granarium Everts (Levinson and Bar Ilan,1970); Anthonomus grandis (Hardee et al,1972); Lasioderme serricorne (F.)(Coffelt and Burkholder, 1972) and tropotaxis, e.g. Tenebrio (cited by Fraenkel and Gunn,1961). However, in Ips confusus (Le Conte) (Borden and Wood,1966) and Argyrotaenia velutinana (Walker) (Roelofs and Feng,1967) a tropotactic response has been discounted for insects deprived of one antenna either failed to make circus movements, or circled as much towards the side of the missing antenna as towards the intact one. In the spider mite, Tetranychus urticae Koch, the convoluted path of a searching male becomes straighter in the presence of the female scent (Penman and Cone, 1972), but the way in which this is brought about is not known.

The view that the aphid responses obtained in the pathway olfactometer represent short-range orientation behaviour is supported by the shape of the dose-response curve. The frequency of responses dropped dramatically with relatively little dilution of the stimulus. Most male aphids would therefore respond to the pheromone only within

the immediate neighbourhood of an ovipara for the concentration of a volatile chemical declines rapidly with increasing distance from the point of release (Bossert and Wilson,1963). The slope of the curve might have been accentuated to some degree by a lack of variation in the responsiveness of the males, for the insects used were taken from a laboratory clone. However, responses in other insects have been reported to occur over a range of pheromone concentrations differing by as much as a factor of 10^3 or even 10^7 (Roelofs and Feng,1967; Sekul and Cox,1967; Guerra,1968; Traynier,1970a; Hardee et al,1971; Coffelt and Burkholder,1972). Such results may be due to the use of more than one behavioural step as a criterion for a response in a bioassay, or to the use of a reaction that can occur at a great distance from the female. Indeed, Bartell and Shorey (1969a) have shown that for Epiphyas postvittana the slope of the dose-response curve increases for each successive step in the male's hierarchy of responses.

Copulation may be stimulated by the female tibial secretion but is not dependent upon its presence. This would appear to be advantageous in a species, such as M.viciae, in which the males are spontaneously active for much of the day, and in which the females are apparently willing to mate at all times and yet show striking changes in the daily pattern of pheromone release.

The nature of responses to sexually important chemical signals in other aphid species remains obscure, despite the recent publication of papers concerned with the pheromone of Schizaphis arrhenatheri (Pettersson,1970a,1971) and with male aggregation in Myzus persicae (Tamaki et al,1970). The latter authors suggested, but did not prove, that M.persicae oviparae produce a sex pheromone. They observed various types of male behaviour which may, in reality, have been evoked by a female scent, but alternative explanations were not excluded with critical experiments. They did show, however, that M.persicae males will attempt to copulate with other males in the absence of a female pheromone,

and that males may aggregate in response to both virginoparous and sexual morphs of M.persicae and to virginoparae of A.pisum.

Pettersson's (1970a,1971) work on S.arrhenatheri is more extensive and requires closer examination. In his routine testing for the presence of a pheromone he used a cruciform choice-chamber (Pettersson,1970a). Up to four materials to be tested for attractancy were placed in the olfactometer - one in each arm. Males, in groups of ten, were initially placed in the centre of the chamber and their positions noted at five minute intervals for fifty minutes. The ten readings thus obtained for each group of males were summed, and the numbers of insects found within any one arm were "...taken as a direct measurement of the attractivity of the actual stimulus " (Pettersson,1970a) provided by the material contained therein. Several aspects of this technique make interpretation of the results difficult. These are listed below. (i) It is by no means clear that during the course of an experiment all the males would necessarily encounter stimuli from each of the materials being tested. (ii) Successive readings were treated as though they were independent of one another. This condition does not seem to have been fulfilled for Pettersson (1970a - page 65) indicated that during the period of testing some males became inactive and settled in one position. (iii) The exact number of males from each group of ten which entered any one arm of the olfactometer was not determined. (iv) No consideration seems to have been given to factors such as male habituation to the pheromone stimulus, or aggregation responses towards other males, which may well have influenced the results. (v) There can be no distinction between individual males entering an arm of the olfactometer because of the material it contained and those entering as a result of random movements. (vi) Pettersson (1970a,1971) does not fully explain his own method of analysing the results. At best, therefore, this technique can provide only a rough guide as to

the presence of some kind of response. A conclusion that no response occurred cannot be made as any insect that entered an arm cannot be said to have, or have not, responded to a chemical stimulus. Furthermore, if the duration of a response to a pheromone stimulus was short (relative to the interval between readings), it is possible that the responses would not be reflected in the distribution of the males. Male behaviour in the olfactometer was not described. Consequently, nothing can be concluded about the mechanism of the response which male S.arrhenatheri show to the female pheromone.

Pettersson (I97I) also stated that the female scent was necessary if copulation was to occur. He bases his conclusion on the finding that males would not mate with oviparae when tested immediately after their antennae had been removed, although they had behaved normally before the operation. Obviously, his conclusion is meaningless. Under similar circumstances male M.viciae behave in exactly the same way as did S.arrhenatheri males, but if allowed to recover from the operation will readily copulate.

Stroyan (I958) noted that male Sappaphis showed " an excited but generalised reaction....when within a centimetre or two of the ovipara ". Of course, it is not known what type of stimulus elicited this response.

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Factors effecting male behaviour

Males of M.viciae are greatly outnumbered by their sexual partners (Lees,1959). However, all information so far obtained from laboratory experiments suggests they have a high potential for sexual activity. Copulation is curtailed until the second or third day of adult life, by which time mature spermatozoa have been produced and sensitivity to the female scent is well developed. Indeed, males become highly responsive to the pheromone during the 24 h following the imaginal ecdysis, and remain so for the rest of their adult lives.

Furthermore, both active and inactive males are responsive to this chemical signal throughout the entire light period in a LD I2:I2 regime. Nothing is known of their susceptibility to sexual stimulation during the dark period. As visual stimuli are so important in copulation, and as walking males rapidly become inactive in the dark, it seems unlikely that mating would take place in the absence of light.

Sensitivity to pheromones in other insects may be influenced by such factors as temperature (Shorey,I966; Bartell,I968; Klun,I968) and daylength (Shorey and Gaston,I965a). In the field, therefore, fluctuations in the environment might be expected to produce variations in the responsiveness of male aphids. The uniform sensitivity to the female scent recorded in the laboratory is nonetheless remarkable, and demonstrates that under appropriate conditions males maybe sexually responsive for much of the time.

M.viciae males mate repeatedly and seem well suited to life in colonies containing a majority of females. For instance, copulation with oviparae that are not releasing their sexual scent (hind tibiae removed) does not effect the responses of males exposed to the pheromone immediately afterwards. This is probably the first time that the effects of coitus , and of exposure to a sex pheromone, have been successfully separated. In other insects removal of the scent glands is not so easy. Males of Trichoplusia ni, for example, failed to react to the female scent four hours after mating (Shorey and Gaston,I964), but in this case copulation necessarily involved simultaneous exposure to the pheromone.

Secondly, males can continue to copulate after long exposures to the odour produced by oviparae. During the first mating(s) on any day a certain amount of habituation to the pheromone (p.I20) may reduce the effectiveness of the latter as a guide in the male's search for further sexual partners. This may be relatively unimportant, however, for having found and mated with one ovipara, a male would have

little difficulty in locating other females a short distance away on the same plant stem.

Finally, there is evidence that a male attempting to copulate with a plastic bead excites similar behaviour in nearby males. Presumably, this response would normally result in other males mating with other oviparae in a colony, and would undoubtedly be an asset in a species with a deficit of males. Some of the increase in copulatory behaviour recorded by Tamaki et al (I970) in tests with Myzus persicae may have been due to similar male to male interactions.

Larval males of M.viciae neither respond to the sex pheromone nor possess the antennal receptors (secondary rhinaria) which were found to be essential for the adult response. Pettersson (I97I) likewise concluded that the same type of receptors were involved in the response of adult S.arrhenatheri males, but had previously stated (Pettersson,I970a) that larval males did respond to the pheromone. Secondary rhinaria, however, are acquired only at the adult moult. The apparent anomaly requires further investigation.

Female behaviour

Variations in the rate of sex pheromone release with the time of day and the age of the producing insects have been reported for many species (Shorey,Gaston and Jefferson,I968; Butler,I970; Jacobson, I972). However, in all previous studies in which the effect of age on the rate of release has been investigated it has been assumed that the daily pattern is the same at all ages. Obviously, such assumptions may not necessarily be correct for in M.viciae the daily pattern changes as the females age. Indeed, observations on calling behaviour suggest that in some other species, at least, an increase in age may be accompanied by changes similar to those seen in M.viciae. Although in Choristoneura fumiferana (Sanders,I969) and Pectinophora gossypiella (Lappla,I972) calling occurs at the same time of day in females of all

ages, in Sanninoidea exitiosa (Say) (Jacklin et al,1967), Phlogophora meticulosa (Birch,1970a), and Anagasta kuehniella (Calvert and Corbet, 1973) it may be extended in older, unmated insects. Lawrence and Bartell (1972) have recently described marked differences in the numbers of female Epiphyas postvittana that extrude their scent glands, and the time for which they do so, on each of the first six nights of adult life. The adoption of a calling posture may not, of course, be an entirely reliable indicator of pheromone liberation as the results obtained with aphids indicate. It cannot provide any information on the rate of scent release.

Other aspects of the female aphids' reproductive behaviour could not be readily correlated with the striking developments in pheromone liberation. As adult oviparae of all ages are willing to mate it is surprising that maximum scent release does not coincide with, or even precede, the earliest possible time for oviposition (day 4), especially as at this age females already contain more mature eggs than are ever laid on any one day. However, since coitus stimulates oviposition for a number of days, sixth-day oviparae may be able to lay more fertilised eggs after one mating than can younger insects. Pettersson (1968), in contrast, recognised three phases in the lives of adult Schizaphis oviparae - a passive pre-copulatory period (PPP), an active copulatory period (ACP), and a passive post-copulatory period (PCP) - and later claimed (Pettersson,1970a) that the sex pheromone was produced only in the second of these (ACP). While it seems likely that an insect would not secrete a sexually-stimulating scent at a time when copulation is supposed not to occur, Pettersson's methods are not sensitive enough to allow firm conclusions to be drawn from low levels of "response". In addition, females in the PPP and the PCP were tested simultaneously, and therefore competed with each other, whereas females in the ACP were not assayed at the same time as other insect material. Pettersson (1970a,1971), moreover, advanced no information as to the

time of day at which any of his tests were conducted. If pheromone release, in any insect, follows a course similar to that in M.viciae testing individuals of different ages at a standard time of day could easily produce an incomplete and, perhaps, misleading picture.

The distinctive posture adopted by pheromone-releasing oviparae undoubtedly facilitates dispersal of the scent. Such behaviour may be widespread among oviparaous aphids for Pettersson (1971) noted its occurrence in Schizaphis arrhenatheri, S.dubia Huc., S.longicaudata H.R.L., and S.rufula (Walk.). He recorded the numbers of females of mixed ages showing this behaviour during the third to eighth hours inclusive of a light period of unspecified length. In all species except S.rufula the numbers decreased from a maximum at the beginning of the observation period (hour 3) to a low by the eighth hour, while in S.rufula the trend was reversed and there was a progressive increase during the same period. Stroyan (1958) did not mention this behaviour in Sappaphis but did state that the oviparae waved their hind tibiae. In M.viciae and A.pisum, however, the raised hind legs are conspicuous in being held absolutely immobile for very long periods. They are occasionally kicked out when the aphids are disturbed and, because of their elevated position, may appear to be waved, but this action seems to represent a defensive behaviour shown also by all virginoparae and by non-calling oviparae. It might be advantageous for oviparae to respond to the initial approach of a male in this way, for movement of the legs would surely enhance dissemination of the pheromone. Nevertheless, this does not form part of the normal pre-copulatory behaviour of M.viciae or A.pisum.

The calling posture of M.viciae may have another important function, namely, that of providing a visual stimulus for the opposite sex. Elevation of the abdomen would result in a maximum amount of female body surface being visible to a male walking along the same plant stem. It might also make the female more conspicuous in the sense

that its yellowy-green abdomen would possibly be viewed against a differently-coloured background, for instance, the sky, rather than against a green plant stem. Pettersson (I97I) regarded this behaviour as providing an important visual stimulus in Schizaphis. In Hyphantria cunea Drury (Hidaka,I972) and Trichoplusia ni (Shorey and Gaston,I970) visual cues associated with calling females seem to increase the efficiency of close-range orientation in pheromone-stimulated males.

Calling behaviour in different species varies not only in details of posture and movement, and in the time of day it is performed, but also in the lengths of the calling bouts. Individual females of M.viciae maintain the elevated-abdomen stance for long periods without interruption. Indeed, they show it throughout the entire daily period of pheromone release, and for even longer in older insects. Results obtained in the pathway olfactometer indicate that scent release similarly follows its daily course without interruption. Female Choristoneura fumiferana (Sanders and Lucuik,I972) likewise call for long periods, but those of Trichoplusia ni (Sower,Gaston and Shorey, I97I) and Epiphyas postvittana (Lawrence and Bartell,I972) do so in short, discrete bouts occurring within the general period of scent release characteristic of the species. In E.postvittana the number and lengths of bouts shown on any night depends upon the age of the insect (Lawrence and Bartell,I972), while in T.ni the bout length may even be influenced by the velocity of the prevailing wind (Kaae and Shorey, I972). Pheromone release by oviparae in the field would probably be influenced by environmental factors for in some Lepidoptera calling behaviour has been shown to be dependent upon the length of the photoperiod, temperature, and light intensity (Sower et al,I970; Sower,Shorey and Gaston,I97I; Sanders and Lucuik,I972).

The demonstration that scent liberation in M.viciae is based upon an endogenous circadian rhythm is of importance for two

reasons. First, although the involvement of such rhythms in chemical communication has been widely assumed, their presence has only been demonstrated in a few species (p.21). Secondly, this is the first endogenous circadian rhythm to be discovered in aphids. Under appropriate conditions moulting (Haine,1957; Haine et al,1964) and take-off behaviour (Davis,1966) of aphids may vary throughout the day, but these have not been shown to be under the control of an endogenous rhythm.

A most interesting aspect of the ovipara's physiology is the complexity of the relationship between synthesis and release of the pheromone. During the seven-hour period when the scent from six-day-old females is liberated at a more or less constant rate, the amount of extractable pheromone present in their hind tibiae undergoes a rapid five-fold increase and then a gradual three-fold decrease. This fluctuation is paralleled by changes in the numbers of vacuoles present in the gland cells, except during the latter part of the peak period of pheromone release (hours 4-8 of the photoperiod). At this time the vacuoles disappear from the cells even faster than the pheromone content of the legs declines. Clearly, some stage of the secretory process acts as a buffer and produces a stable rate of pheromone liberation (into the atmosphere), even though the rate of pheromone synthesis and accumulation changes markedly. Obviously, the penultimate stage in the secretory process - the emptying of the contents of the vacuoles into the extracellular space beneath the plaque by exocytosis - cannot be responsible for its rate varies considerably,both in individual cells and in the leg as a whole. The movement of the pheromone across the cuticle therefore seems the most likely candidate for this limiting role. If pheromone is discharged into the space beneath the plaque faster than it can be removed by transport across the cuticle, then a buffer pool would be created. So long as this pool remains in excess of a critical level, pheromone would be liberated into the

atmosphere at a constant rate, and one which would be independent of the concurrent rate of synthesis. This would account for the high rate of scent release continuing after most vacuoles have been discharged from the cell surface (e.g. during hour 8). In the absence of definite pores in the cuticle it is difficult to tell exactly how the odorous material reaches the outside. The structure of the plaques, nevertheless, suggests that the pits and, perhaps, their attendant filaments facilitate this passage in some way.

Direct control of pheromone synthesis does not appear to be accomplished by the nervous system, although it may be involved in any chain of command there might be. Some degree of independence of the secretory cells is apparent, however, for their activity is not always synchronised. The amount of independence may, indeed, be considerable for the epidermal cells of the locust seem to be more or less autonomous with respect to their cuticle-secreting activities (Neville, 1967). Furthermore, it is tempting to speculate that, as all the glands are not active at the same time, the disparity in pheromone release at different ages may be due to changing populations of cells being active for different periods of time.

The quantities of pheromone contained within the glands of other species are also known to vary throughout the day, but with peaks coinciding with the time of release (Wong et al,1971; Nagata et al, 1972). Nevertheless, in many others storage of the active material, or of its inactive precursors, takes place at ages, and at times of day, when no release occurs (Tashiro and Chambers,1967; McDonough et al,1969; Moreno et al,1972; and other references already given on p.20). In some the amount stored remains remarkebly constant (Shorey and Gaston, 1965b; Sower et al,1972). It seems possible that the substances may be retained in vacuoles within the secretory cells for in Trichoplusia ni (Jefferson et al,1966) and Choristoneura fumiferana (Percy and Weatherston,1971) the numbers of these do not change throughout the day.

In addition, the pheromone may be released onto the surface of an inverted gland (Sower et al,1972), into the lumen of a tubular gland (Moreno,1972), into the lumen of the gut (Bakke,1973), or even into a special reservoir (Fletcher,1969; Birch,1970b)and later exposed to the atmosphere by the eversion, protrusion, or compression of the gland or reservoir, by expulsion in the faeces, or by being displayed on the surface of a special evaporative organ. Storage outside the cells may result in an initial flush of scent molecules being released into the atmosphere when the insect first begins to call (Sower et al,1972). This would probably be followed by a lower rate of release more dependent upon the current activity of the gland cells (Sower et al,1972)

Since, in M.viciae, pheromone release changes dramatically with age it seems reasonable to assume that synthesis of the active material shows equally striking changes. However, because of the complicated relationship between these two processes in six-day-old oviparae, it is impossible to predict with certainty the time of day at which females of any other age might contain a maximum amount of extractable pheromone. The findings obtained with this aphid therefore have implications for work on other species. They illustrate that it cannot be assumed that calling behaviour, scent release, and pheromone content are linked in a simple fashion. Consequently, it cannot be inferred that if one of these follows a certain course the others must necessarily do the same. The results of Lawrence and Bartell (1972) support this view. They found that the numbers of Epiphyas postvittana females that called, the mean number of calling bouts per female, and the average length of each bout, rose to a peak in two- and three-day-old insects, dropped to a low point on days four and five, but rose again to an even higher peak on day six. The amount of pheromone contained by the females (extracted at a standard time of day) nevertheless increased progressively up to the fifth day and then dropped abruptly on day six. In Anagasta kuehniella the relationship

between pheromone content (again extracted at a standard time of day) and calling behaviour is similarly complex (Calvert and Corbet,1973).

As expected in a multiple-mating insect, copulation does not directly effect scent liberation in M.viciae. In contrast, the influence of oviposition is marked and raises several questions. How, for instance, is this influence achieved? A mated female may simply cease to produce the pheromone during the hour or so in which it lays an egg and during which it is unattractive to males. If this happens are the gland cells (a) prevented from synthesising the pheromone, and/or (b) prevented from discharging it at their apices? It seems unlikely that substances which have already been discharged from the cells could be inhibited from crossing the cuticle and evaporating into the atmosphere, unless chemical degradation processes are initiated. An ovipara might , on the other hand, become unattractive by producing a mask to her own, attractive scent. If this does occur, where is this substance produced? Presumably, such a signal would be effective over very short distances, so as not to negate the attractiveness of nearby pheromone-releasing females. The mask produced by a female Dendroctonus pseudotsugae on the arrival of a stridulating male at the entrance to her gallery operates in this way (Rudinsky,1968).

The mated females of other insects may call more intermittently than virgin females, and this may be due to oviposition (Sanders and Lucuik,1972). Of course, in many species the initiation and cessation of pheromone liberation may be accomplished simply by the protrusion and retraction of an eversible gland. In species having multiple oogenic cycles sex pheromone production by the female may cease for a number of days while oviposition takes place (Adams and Mulla,1968; Barth and Bell,1970; Strong et al,1970; Zdarek,1970; Minks and Noordink,1971).

Copulation in aphids is soon followed by a change in female behaviour associated with oviposition - the female goes off in search of a place to deposit its egg(s) and becomes temporarily unattractive

to males (at least, in the olfactometer). Male aphids have well-developed accessory glands but produce no spermatophore. It seems feasible that the accessory secretion is transferred to the female during coitus and, as in some other insects (p. 9), elicits oviposition. This is, however, entirely speculative.

Species-specifity of response

There seems to be little to prevent interspecific copulations if the sexuales of M.viciae and A.pisum meet, for the males respond strongly to the pheromone from heterospecific oviparae and show no reluctance to mate with them in the laboratory. Both species may hibernate on Lathyrus and Vicia plants (Bodenheimer and Swirski,I957). Closely-related species of Sappaphis have been observed to mate with each other (Stroyan,I958).

Interspecific responses to the pheromones from four species of Schizaphis were examined by Pettersson (I97I). Unfortunately, males of a given species were tested against females of four species simultaneously rather than separately. Detailed information about most of the twelve possible combinations of males with females was therefore not obtained. The results did, however, indicate a lack of strict specificity.

Sexual isolation of many aphid species probably occurs through spatial separation of the sexuales on different host plants.

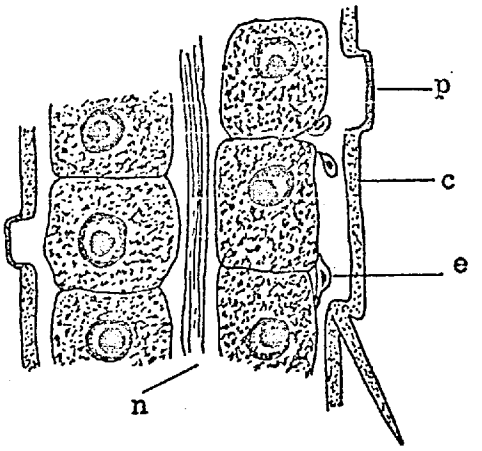
Structure of the gland cells

As only a summary of the electron microscope studies has been presented the ultrastructure of the gland cells will not be discussed at length. However, a brief comparision with that of other sex pheromone glands is given below.

The results of the studies of Flogel (I905a),Roberti (I946), and Pettersson (I97I) on the structure of aphid pseudorhinaria are

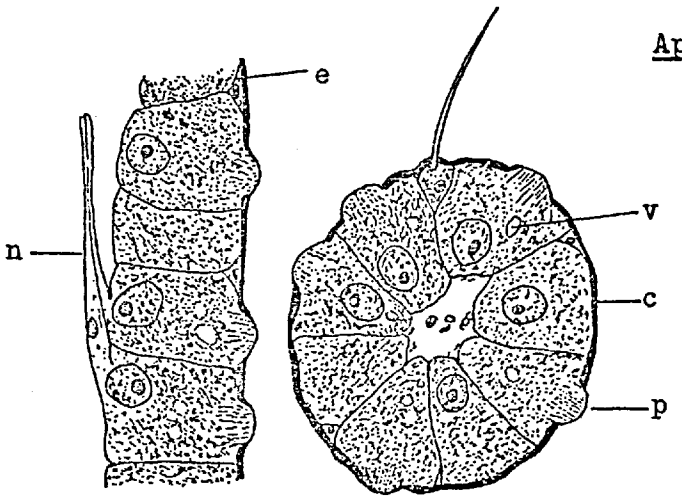
A

Aphis alni



B

Aphis frangulae Koch



C

Schizaphis sp.

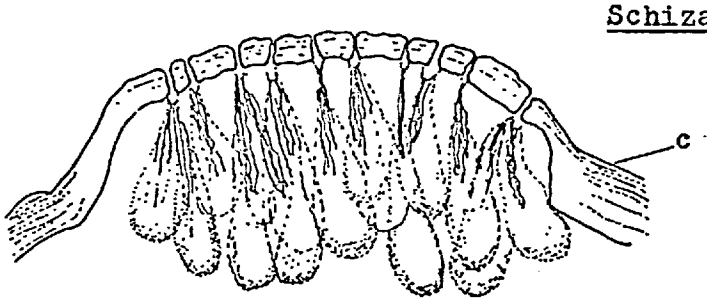


Fig. 27. Tracings of previously published figures depicting the scent plaques (pseudorhinaria) of oviparous aphids. A. From Flogel (I905a - Fig.24). B. From Roberti (I946 - Fig.83). C. From Pettersson (I97I - Fig.4). Pettersson's legend to this figure reads,"Schematical drawing of crossection through pseudorhinarium showing the glandular sacs and excretion channels leading to pores in the cuticle". Standard labels have been substituted for those of the original authors. c = cuticle, e = epidermal cell, n = nerves, p = plaque, v = vacuole.

summarised in Fig.27. Although they are very superficial they indicate a great similarity between different species. Both Flogel (1905a) and Roberti (1946) show hypertrophied secretory cells containing large nuclei with prominent nucleoli. Situated between these cells and the cuticle are relatively minute epidermal cells. In many of the sections of M.viciae hind tibiae it was not always easy, using a light microscope, to see that the epidermal cells extend between the gland cells to the lumen of the leg. The cells of Aphis frangulae (Roberti,1946) possess two other features which seem to closely parallel those of cells of M.viciae and A.pisum. First, the striations in the apices of the cells (Fig.27B) almost certainly represent the folded surfaces which lie beneath the cuticular domes, and to some extent, perhaps, the microtubule bundles present within the extracellular sacs. Secondly, the few vacuoles also shown are, according to the standards of M.viciae and A.pisum, far too large to be secretory vacuoles but are correctly situated to be portions of the extracellular sacs which push down into the cells from their upper surfaces. Roberti (1946) regarded these tibial structures as sensory receptors and claimed that the cells were innervated by branches of the pedal nerve. Flogel (1905a), on the other hand, was the first to speculate that these cells produced an odorous substance to attract the males and explicitly stated that the cells were not innervated. Sections of M.viciae hind tibiae showing cells with apparent connections remarkably similar to those illustrated by Roberti were often obtained. However , close examination, at both the light and electron microscope levels, never revealed this apparent innervation of the secretory cells to be real. In many cases the 'nerves' turned out to be either the tracheal trunk and its supporting cells, or one of the two nerve bundles which receives axons from the mechanoreceptive hairs (but does not supply the gland cells), or the tibial membrane (part of a pulsatile organ system which circulates blood around the leg) - all of which run the length of the tibia. The

similarity between the pseudorhinarial cells of A.frangulae and M.viciae suggests that the former are not sensory receptors and that Roberti (1946) was probably mistaken about their nerve supply.

Pettersson (1971) briefly examined the pseudorhinaria of Schizaphis using an electron microscope. His diagram (Pettersson,1971, Fig.4 - or Fig.27C of this thesis) includes only the extracellular parts of the gland (although he does not state this), i.e. the extracellular sacs (his " glandular sacs "), the microtubule bundles (his " excretion channels ") contained therein, and the cuticular plaque. It is clear (see the legend of Fig.27) that he believes that both the sacs and the tubules lead right up to pores (not pits) in the cuticular dome.

Little is known about the histology of sex pheromone-producing tissues in insects. Most studies have been conducted at the light microscope level, and relatively few species, e.g.Bombyx mori (Steinbrecht,1964; Waku and Sumimoto,1969), Trichoplusia ni (Miller et al,1967), Harpobittacus australis (Klug) (Crossley and Waterhouse,1969), Phlogophora meticulosa (Birch,1970b), Danaus gilippus berenice Cramer (Pliske and Salpeter,1971), and Choristoneura fumiferana (Percy and Weatherston,1971), have been investigated with the electron microscope. Although the cells of different species vary greatly, they seem to have certain features in common. These are, (a) a large size relative to the adjoining epithelial cells which do not secrete the pheromone, (b) a cuboidal, columnar, or goblet shape, (c) a large, centrally or basally placed nucleus, (d) numerous granules or vacuoles within the cytoplasm, and (e) an apical surface (through which the pheromone or precursor is released) that is greatly folded or bears numerous well-developed microvilli. It has been suggested that the vacuoles contain the pheromone or some precursor, and there seems to be some experimental (Schneider,1966) as well as circumstantial (Jefferson et al,1966) evidence for this.

In M.viciae vacuoles are formed from the SER. This is not surprising as the latter has been implicated in lipid synthesis in both vertebrates and invertebrates (Smith,1968). In at least three instances, however, it has been postulated that degradation of mitochondria within the secretory cells is directly involved in the synthesis of insect sex pheromones, and thus in vacuole or droplet formation (Crossley and Waterhouse,1969; Waku and Sumimoto,1969; Birch,1970b).

For many species the manner in which the pheromone is finally liberated into the atmosphere after it has been discharged from the cell surface is not clear. In Harpobittacus australis the odorous substances are carried to the gland surface by cuticular ducts (Crossley and Waterhouse,1969), while in many male Lepidoptera elaborate, hollow, cuticular hairs or scales are in some way involved in the dissemination of the pheromone (Weatherston and Percy,1969; Birch,1970b; Grant,1971b; Pliske and Salpeter,1971; Sellier,1972). In female Lepidoptera the pheromone is presumed to cross the cuticle overlying the secretory cells. Ducts passing through the endocuticle which might facilitate this have not been found in Trichoplusia ni (Miller et al,1967) but Waku and Sumimoto (1969) have postulated that in Bombyx mori the pore canals fulfill this role.

The bundles of microtubules found in aphids are most unusual in being extracellular. They are found in a situation which suggests an involvement in the movement of the pheromone to the outside. Their similarity with the bundles of intracellular microtubules of various protozoa further suggests that, as in the latter, they may be capable of movement (Mooseker and Tilney,1973). This is, however, purely speculative and details of such an involvement are difficult to envisage.

Glands thought to be associated with the production or storage of sexually important odours have been found on the legs of

various male Lepidoptera (Illig,1902), Hymenoptera (Cruz-Landim et al, 1965; Dodson et al,1969), and Coleoptera (Matthes,1970). Apart from the common features of secretory cells outlined above they bear little similarity to the aphid scent plaques.

Occurrence of pseudosensoria in aphids

Pseudosensoria do not occur in equal numbers in all oviparae. It has already been noted that they are most numerous in species having winged males (p.139). Indeed, there may be great variations between the species of a genus with some species that have apterous males bearing no pseudosensoria at all (Eastop,1972). Although generally situated on the hind tibiae, in some oviparae pseudosensoria may be found on the fore and mid tibiae,e.g. Neophyllaphis araucariae Takahashi (Carver,1971), or even on the hind femora, e.g. Schoutedenia sp. (Hille Ris Lambers, in litt.). They are commonly grouped to form double or multiple plaques such as occur in M.viciae (Smith,1936; Pettersson,1971).

Structures identical in appearance to the pseudosensoria of oviparae also occur in other morphs in some aphid species. A few examples are given in Table 24. In other species they are occasionally found on females having characters intermediate to those of virginoparae and of oviparae (Jacob,1964; Stroyan and Nagaich,1964; Pagliai,1968). Following a series of experiments on the effects of photoperiod and temperature on morph determination in A.pisum,Kenten (1955) concluded that the formation of pseudosensoria on the hind tibiae of females is determined late in the development of the aphid.

The role of pseudosensoria in those species in which they are normally found on the males and/or virginoparae (Table 24) remains to be elucidated.

Table 24. Some aphid species in which the virginoperous females and/or the males bear pseudosensoria on their legs.

Species	Morph*	Position**	Reference
Aphis ribiradicis Robinson	A,M	H	Robinson,1969
Acyrthosiphon malvae (Mosley)	M	H	Prior and Stroyan,1964
Cavariella konoi Takahashi	F	H	Stroyan,1964
Glyphina longiseta Richards	A,W	F,M,H	Richards,1968
Chaitophorus populeti Panzer	A	H	Eastop,V.F. (in litt.)
Nearctaphis sensoriata Gillette and Bragg	A	H	"
Neoceruaphis viburnicola (Gillette)	A	H	"
Lizerius ocoteae Blanchard	A	H	"

* A = apterous virginoparae, W = alate virginoparae, F = fundatrix, M = male

** F = fore tibiae, M = mid tibiae, H = hind tibiae

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