THE POST-EMBRYONIC DEVELOPMENT AND FUNCTIONAL MORPHOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM IN GRAPHOCEPHALA FENNAHI (HOMOPTERA : CICADELLIDAE)

by

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ABSTRACT

A number of fields of study are brought together to provide a deeper understanding of the reproductive system in Cicadellids. The external genitalia of Graphocephala fennahi (Young) are discussed, together with their postembryonic development. Associated with this, the histology and development of the internal organs of reproduction and the abdominal musculature are described in detail.

The gonapophyses and basal genital structures are for the first time in Cicadellidae examined with the scanning electron microscope and this, combined with behavioural observations, permits a better functional understanding of the ovipositor. By comparing the genital structure in three species of British Cicadellids in relation to their oviposition habits it is possible to demonstrate the adaptive nature of ostensible taxonomic characters and to illustrate clearly that variation in ovipositor structure principally of the gonapophyses can be associated with characteristics of the oviposition site.

The development and histology of the reproductive system is followed from the earliest nymph and the origin of the efferent ducts described. A detailed account of the adult reproductive system and oogenesis is provided, including some electron-microscopic observations. Oogenesis in the telotrophic ovariole and the probable origin of certain nutritive substances is determined, using histological and cytochemical techniques. The ovarioles of Graphocephala were found to be contained within an ovariole sheath and an electron microscopic study of its structure was made. The sheath contains "lumen cells" and their possible phagocytic function is discussed.

To provide a clearer understanding of the terminal abdominal segments the genital musculature was examined and compared with the typical pregenital abdominal musculature. Myology was followed from the basic nymphal arrangement and the

associated myogenesis examined and found to occur along four major routes involving two different methods of free myoblast incorporation. A table is drawn up which allows the direct comparison of nymphal and adult muscles and the status of the nymphal muscles in the adult.

Some functional aspects of the reproductive and genital systems are combined in a discussion of the sexual behaviour of G. fennahi, concerned with pre- and post-mating behaviour and also with oviposition. Data relating to the epigamic communication between receptive pairs is presented and the importance of substrate-borne vibrations in G. fennahi indicated.

The results are discussed in relation to other work on the postembryonic development of the reproductive system and on sexual behaviour.

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INTRODUCTION

Graphocephala fennahi (Young) was first introduced into Britain from the United States in 1931 - 2, at which time it was confused with Graphocephala coccinea, and it was not named as a separate species until 1977. In America G. fennahi has a more restricted range than G. coccinea and is more selective in its host plants. In Britain it is only occasionally collected from plants other than Rhododendron, and the eggs are found only in the overwintering buds of this genus.

The role of leaf-hoppers as vectors of certain plant virus diseases has led to extensive research to define the vector-disease relationships. A major problem associated with such investigations concerns the biology of the vector and this forms the object of the present study. While G. fennahi is not associated, in any part of its range, with economically important plant diseases, it is linked with the spread of bud-blast in Rhododendron caused by the wound-parasite fungus Pycnostysanus azaleae.

Despite its relatively minor economic importance a suitable population of G. fennahi was available and it was considered that much valuable information on Cicadellid biology and morphology could be gained from an intensive study of a single species.

Extensive studies are reported in the literature which adequately describe Hemipteran morphology, particularly that of the head, and many of these dealt with the Auchenorryncha (e.g., Ali 1958; Butt 1943; Duporte 1946, 1957; Evans 1946, 1957; Ferris 1943; Kramer 1950; Muir & Kershaw 1911, 1912; Parsons 1964; Ribaut 1952; Ross 1957; Snodgrass 1927, 1938; Spooner 1938). Much of this work has led to controversies regarding the homologies and terminology of the various sclerites, but it is not intended that this aspect of Cicadellid morphology be pursued further in this study.

The present account is restricted to the genital region and associated reproductive system of the female, and attempts to describe these regions on a functional and developmental basis. While the morphology of this region has an extensive literature, it is mostly superficial and incomplete; the development of the systems and their function in sexual behaviour are fields in which the literature is extremely limited.

The first writer to describe the ovipositor of Auchenorrynchan Homoptera was Aristotle. Illustrations and descriptions are provided by Malpighi (1687) and illustrations by Reaumur (1740). All the early work was concerned with unidentified Cicadidae, the small size of most Cicadellids preventing their study until the nineteenth century. The first work of importance on the Auchenorryncha was that of Dufour (1825), in which he studied the digestive and reproductive systems of a cicada. Two papers by Doyere (1837) discussed the structure and function of the Cicadid ovipositor, and showed that the movement of the gonapophyses is brought about by the second gonocoxa and the associated musculature. The extensive work on insect female genitalia by Lacaze-Duthiers (1852) included a study of the Cicadellid Tettigella (= Cicadella). Verhoeff's (1894) comparative studies of female Heteroptera and Homoptera include a description of the Cicadellidae and Cercopidae and places these two families separately from the Membracidae.

Much recent work has been concerned with the homologies of the genital structures and the use of the genitalia as taxonomic characters. Several works that describe the morphology of the Cicadellid ovipositor have produced some rather confusing terminology. The study of Snodgrass (1933) on Amblydisca gigas is important, because it provides a useful terminology and several recent studies follow this system (Cunningham & Ross 1965; Helms 1968; Nielson 1965). Scudder (1957,

1958, 1959, 1961a, 1961b, 1964) has questioned some of Snodgrass's conclusions and developed his gonangulum theory for the ovipositor. This sclerite is present in one form or another in the Thysanura, Grylloblattodea, Orthoptera, Dictyoptera, Odonata, Psocoptera, Thysanoptera, Hemiptera and Hymenoptera. Scudder (1961a) considers it a very important sclerite of the ovipositor because of its functional relationship with both the first and second gonapophyses. Some aspects of the gonangular theory have been questioned by Stys (1959) and Dupuis (1963).

Balduf (1933) provides a detailed study of the ovipositor in Draeculacephala mollipes and provides some useful terminology, though the present study, with the aid of the electron microscope, has demonstrated that some of his interpretations are incorrect. In a detailed paper, Balduf (1934) describes the distal region of the gonapophyses within the genus Empoasca and indicates several characters of possible taxonomic importance. Cunningham & Ross (1965) also indicated that the area at the base of the ovipositor in Empoasca provided good diagnostic characters.

Similar comparative morphological studies have been made in other genera. Wagner (1950, 1953) described the female genitalia and the distal region of the second gonapophyses in the genus Macropsis. Ribaut (1952) also made a similar study in this genus. The detailed comparative study and descriptive terminology provided by Readio (1922) for the second gonapophyses of Cicadellids constitutes one of the most thorough works on these structures. These studies are purely taxonomic, but in the present work the extent to which these taxonomic differences are adaptive is discussed further in Chapter 5 and in the general discussion.

Considerable confusion has arisen concerning the terminology used for the description of Cicadellidae and at least four systems of nomenclature can be recognised in works concerned with the female genitalia. In the present study that of Scudder has been adopted.

Few descriptions are available in the literature which adequately describe the development of the external genitalia or reproductive systems of Cicadellids and a comprehensive study of the adult female reproductive system is not available. The early developmental works of Christophers & Cragg (1921), Kershaw & Muir (1922), Singh Pruthi (1925) and George (1928) are very superficial and differ greatly in their conclusions. The monograph of Myers (1928) on Cicadidae provides little information on the development of these systems. The work of Metcalfe (1932) agrees with the findings of George (1928), but her descriptions are over-simplified, and in all of these early works little attention is paid to the development of the basal components of the ovipositor.

The more recent work of Helms (1968) on Empoasca fabae, while useful, does not provide adequate detail and the present author suggests that Helms incorrectly aged some of his nymphal material, since he states that rudiments of the external genitalia were present from the first instar. This has not been reported by other workers and was not found to occur in Graphocephala. It is generally accepted that in the Cicadellidae the female external genitalia is first apparent in the third instar.

The work of Kathirithamby (1971) is concerned largely with the morphology of Cicadellid nymphs, though the descriptions of the female genitalia are not sufficiently detailed, and no attempt is made to describe the histology of the parts.

The detailed histology of the female reproductive system has not previously been described within the Auchenorryncha, though several studies are available for the Heteroptera (Bonhag & Wick 1953; Masner 1966; Oppong-Mensah & Kumar 1974; Pendergast 1957; Ramamurty 1969, 1971; Wick & Bonhag 1955). The present study on Graphocephala demonstrates differences between the Auchenorrynchous Homoptera and the Heteroptera.

A study of the reproductive and genital systems is important, because characters from this region are being used increasingly in taxonomy and the development of an appropriate terminology for these structures requires a knowledge of their developmental origins, especially if the terms are to be used comparatively and to indicate homologies. In addition, the form and function of the reproductive systems throw light on the behaviour since the systems may fail to function under certain conditions. Such considerations are of obvious ecological importance.

Though the development of the ovaries and germ cells are considered in some detail, no attempt is made to describe vitellogenesis fully, since little contribution could be made to this rapidly developing field without making it a major part of the study and putting the investigation on a physiological or even biochemical footing. The subject of vitellogenesis in insects has recently been reviewed by Huebner et al (1975); Moloo (1971); Telfer (1965) and Wightman (1973), though no information is available within the Auchenorryncha.

In order to make a comprehensive study of the Cicadellid genital region the associated musculature is described and compared with that of a generalized pregenital abdominal segment. Much of the work on myology and myogenesis of insects has been restricted to the flight and thoracic musculature, both at the ultrastructural (Auber 1967; Termier & Lauge 1976) and light microscope level (Hinton 1959; Pringle 1965; Tiegs 1955). The most comprehensive study of myogenesis in the Cicadellidae is that of Tiegs (1955) in the flight musculature of Erythroneura. The genital musculature has been described in the Heteropteran Oncopeltus fasciatus (Bonhag & Wick 1953), but this does not allow a profitable comparison with Cicadellids due to the different mechanisms of copulation and oviposition, and hence differing sets of muscles to carry out these functions.

Within the Auchenorryncha, descriptions of the abdominal musculature are provided by Maki (1938), Myers (1928) and Vasvary (1966) for Cicadoidea, and by Kramer (1950) for the Membracid Ceresa bubalus, but these are all brief and incomplete descriptions. An adequate description of abdominal musculature in the adult Cicadellid is lacking in the literature. No account of myogenesis of the abdominal muscles could be found for the Auchenorryncha. In the present study a detailed description of the myology of the adult female of G. fennahi is given, together with an account of myogenesis from the nymphal condition, as seen in the second instar nymph, up to the definitive adult condition, and provides the most complete extant account of Cicadellid musculature.

In addition to a detailed description of the reproductive and genital systems and their development, together with the associated musculature, the present work includes a study of the reproductive behaviour of G. fennahi concerned with pre-mating, mating and oviposition activities, and with the conditions necessary for the successful completion of sexual behaviour. This is a field of considerable interest, though one that has received little attention in recent years. Such morphological studies of the genital region as have been made have not previously been combined with a functional approach, though for the ecology and bionomics of these insects to be understood, a deeper knowledge of their reproductive behaviour is essential.

The literature on the reproductive biology of Cicadellidae is largely concerned with oviposition behaviour (Balduf 1933; Carlson 1967; Carlson & Hibbs 1962; Funkhouser 1917; McMillian 1960; Readio 1922), though the descriptions are usually superficial. During the last decade, much interest has arisen in communication and the roles of acoustic, visual and chemical stimuli in interactions between insects. In the Auchenorryncha, much of this work has been directed towards acoustic communication, but more recently substrate-borne vibrations and the associated sensory

structures have attracted attention. Ossiannilsson (1949) was the first to obtain sound recordings from Cicadellids and ascribed certain functions to the different call characteristics. Pringle (1954) provided a detailed study of sound production within the Cicadidae. Strübing (1958, 1959) recorded sounds from a number of Delphacids and suggested that they may function in bringing sexually active males and females together. More recently, Claridge & Howse (1968) and Howse & Claridge (1970) have examined sound production in three British species of Oncopsis and suggested a possible receptor in Johnston's organ near the base of the antennal flagellum. In the present study no sounds could be detected from Graphocephala during sexual encounters.

Recent work on communication by substrate-borne vibration has been carried out in several insect groups (Dambach 1976; Ichikawa 1976, 1977; Orchard 1975; Rupprecht 1974, 1975). Within the Auchenorryncha, much of this work has been conducted by the Japanese on Delphacids (Ichikawa 1977; Ishii & Ichikawa 1975; Takeda 1974), though Claridge & Howse (1968) have also suggested the possibility of substrate-borne vibrations as a means of communication within the Cicadellidae. In the present study special equipment was constructed to monitor substrate-borne vibrations produced by G. fennahi during courtship and interesting results were obtained.

In addition to this, oviposition behaviour was examined using video-filming techniques, which allowed accurate observations of the functioning genitalia, which could then be correlated with structure.

The present work therefore represents a contribution from many viewpoints to the structure, development and functional significance of the female reproductive system in Cicadellids, as illustrated by Graphocephala fennahi, allowing the genital structures to be linked with the associated musculature and reproductive behaviour.

As such, it bears on a number of less complete previous studies, and suggests several subjects for further investigation.

CHAPTER 1

EXTERNAL GENITAL REGION OF THE ADULT FEMALE

1. Introduction

The true segmentation of the Hemipteran abdomen has in the past been misunderstood, due to the modifications afforded to the first two segments which form the articulation with the thorax. The first segment has been considerably reduced and can be easily overlooked. The first abdominal notum has become partially fused with the second, and the venter of the first segment is largely membranous and indistinct, being overlapped by the coxae of the metathoracic legs. Evans (1945) mentioned that the first abdominal tergum of Putoniessa nigra was concealed by the scutum of the metathorax. This was not found to be the case in Graphocephala fennahi. Kramer (1950), during his description of the first two abdominal segments of Alauciza irrorata, claimed that the tergum of the first segment was composed of two parts, an anterior section connected to the meta postnotum and a posterior part connected laterally to the anterior part. No such connections could be determined in the present study in agreement with the unpublished work of Ali (1958).

The true segmental limitations can readily be determined by the internal inter segmental ridges, upon which the dorsal and ventral longitudinal muscles are attached. In the female Cicadellid, the abdomen can be described in terms of three zones : (i) Segments one and two are reduced. It is in this region that the tymbal apparatus is present. Though this structure was known to be present in Typhlocybids, evidence for its occurrence in Cicadellids was not put forward until the works of Ossiannilsson (1949). Ribaut (1936) had used the presence of a tymbal apparatus as one of five characters for distinguishing Typhlocybids from other Cicadellids. It is now known that the structure of the first and second

abdominal segments is affected by the development of this organ though in Graphocephala, as was determined by the present study, it may be reduced and perhaps non-functional.

(ii) Segments three to seven inclusive represent the typical segmental form, and are usually considered to carry no distinctive taxonomic characters of value in the definition of higher categories.

(iii) Segments eight to eleven. Though ten and eleven are effectively reduced, segment seven and the dorsal portion of segment eight are slightly modified and form a transition zone into the more highly modified terminal parts. The tergum of segment seven is similar to the terga immediately preceding it, but the sternum is enlarged and the posterior edge can be moved down and forward to expose the basal parts of the external genitalia normally concealed. This free posterior margin results from the greatly extended intersegmental membrane, which forms a hood like cap over the anterior parts of the basal genitalia. A membranous fold in this membrane represents, in Graphocephala, the eighth sternum. This third zone, including the appendages, constitutes the female genitalia and the abdominal segments that have been modified by their relationship with the genitalia.

The principal Pterygote groups of insects which possess well developed ovipositors are the Orthoptera, Odonata, Dictyoptera, Phasmida, Hemiptera and Hymenoptera. In the Thysanoptera it is always incomplete. An ovipositor may be found in members of other orders, either as a fully developed organ, or in a rudimentary form. It is, however, possible to generalize by saying that an ovipositor, formed from appendicular processes of the abdomen, is found only in those insects in which the female genital opening is on the eighth abdominal sternum or between the eighth and ninth sterna. If the genital opening is transposed to the ninth segment as in the Lepidoptera, an ovipositor is usually suppressed. The converse of this is not

necessarily true.

The appearance of the abdomen is dominated by the enlarged tergum nine, or pygofer. The two terminal abdominal segments appear, in cleared specimens, as an isolated unit suspended by membrane from the posterodorsal margin of tergum nine. Segment ten is cylindrical with lateroventral anteror projections; segment eleven is similar, but smaller, and usually lying partially within the preceding segment. Segment eleven bears a posterior ring which is drawn out anteriorly into a pair of apodemes; posterior to this ring several small sclerites encircle the base of a small, medially grooved, extension termed the anal style.

A generalized Pterygote ovipositor is composed of a shaft and basal mechanism usually together with a pair of accessory processes. The shaft is usually composed of two pairs of blade-like structures, the first and second gonapophyses which lie in a vertical plane. In some Orthoptera, e.g. Tettigonids, the accessory processes or gonoplacs are also included in the functional shaft. Here, the ovipositor blade is composed of three pairs of structures or either of the first and second gonapophyses may be reduced.

The basal structures are composed of essentially two pairs of plates, the paired first and second gonocoxae, which are associated with abdominal segments eight and nine respectively. In addition to these is the gonangulum, a sclerite that attaches ventrally to the base of the first gonapophysis and dorsally to the second gonocoxa and tergite nine.

In the Hemiptera the first gonocoxae are closely associated with the lower margins of tergum eight, and by the gonangulum to tergum nine; in the Orthoptera and Hymenoptera, they are displaced posteriorly and articulate with tergum nine or the second gonocoxa. In all cases, the second gonocoxa is always associated, anatomically, with tergum nine. Regardless of the position of the first gonocoxa, its

dorsal muscles always insert on tergum eight and those of the second gonocoxa on tergum nine.

The mid-ventral part of segment nine is membranous and deeply concage, forming a longitudinal groove, which encloses in the resting position the ovipositor sheath or gonoplacs and the shaft of the ovipositor formed by the first and second gonapophyses, which are united for most of their length by an interlocking device, permitting only a sawing action. Only the first gonapophyses are solidly connected to tergum nine via fusion with the gonangulum. The second gonapophyses, which move within the first, are fused to the second gonocoxa. The paired gonoplacs lie on either side of the ovipositor and ensheath the shaft in its normal resting position. Each gonoplac is membranously connected to tergum nine along its dorsoproximal edge, to the ventral end of the second gonocoxa and to its partner of the opposite side, above the ovipositor. The first gonocoxae are usually fused dorsally to produce an anterior cone. This complex is highly variable in leaf-hoppers and has been put forward by Cunningham & Ross (1965) as a possible taxonomic character. The second gonocoxae are each fused to the proximal end of the second gonapophyses by a single arcuate ramus, membranously joined to the anterior end of the gonoplacs, and moveable articulated with the gonangular anterior ridge. Through this articulation and the action of antagonistic muscles, the second gonocoxae initiate the sawing action of the second gonapophyses that is of major importance during oviposition.

Scudder (1959) concluded during his work on Heteroptera that the outer ramus of the first gonapophyses (Snodgrass 1935) was really a thickened sclerotization of the ventral edge in the same region as the ventral interlocking device. This structure unites the ventral flaps of the first gonapophyses and was described by Balduf (1933), it can often be very highly sclerotized. The anterior ridge (Snodgrass 1933) and

ramal plate (Kramer 1950) were considered to be parts of the gonangulum by Scudder (1960), and it is Scudder's terminology that will be employed in the present study. In his gonangulum theory he considers this to be the most important sclerite in the ovipositor, an idea that is supported by this study and which will be discussed in greater detail at a later point.

The mechanism of the Hemipteran ovipositor is comparatively simple compared to that of the Orthoptera, the musculature being relatively simple and composed only of those belonging to the first and second gonocoxae. Within the Hemiptera, there is no essential difference in the ovipositors of the Heteroptera and Homoptera. In each group some forms possess a well developed ovipositor, while in others it may be only poorly developed or absent.

In the Cicadellidae, the ovipositor is well developed but due to the very small size of most species, little work has been conducted on the genital region.

2. Morphology of the adult female external genitalia

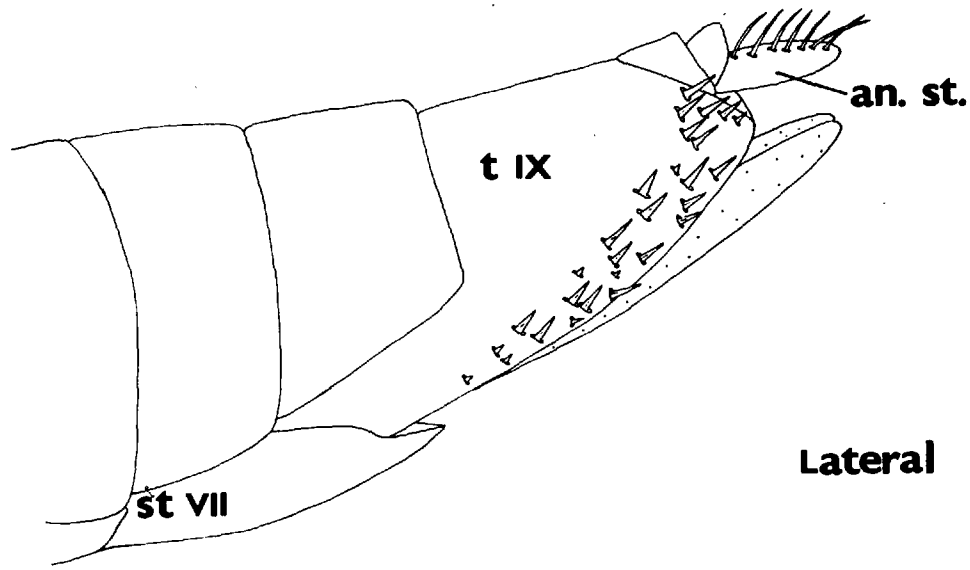
The ovipositor and basal components of the genitalia of Graphocephala fennahi will now be described; their development and origin will be discussed in Chapter 2. The genital region is shown in figs. 1.1 and 1.2.

(a) First Gonocoxa

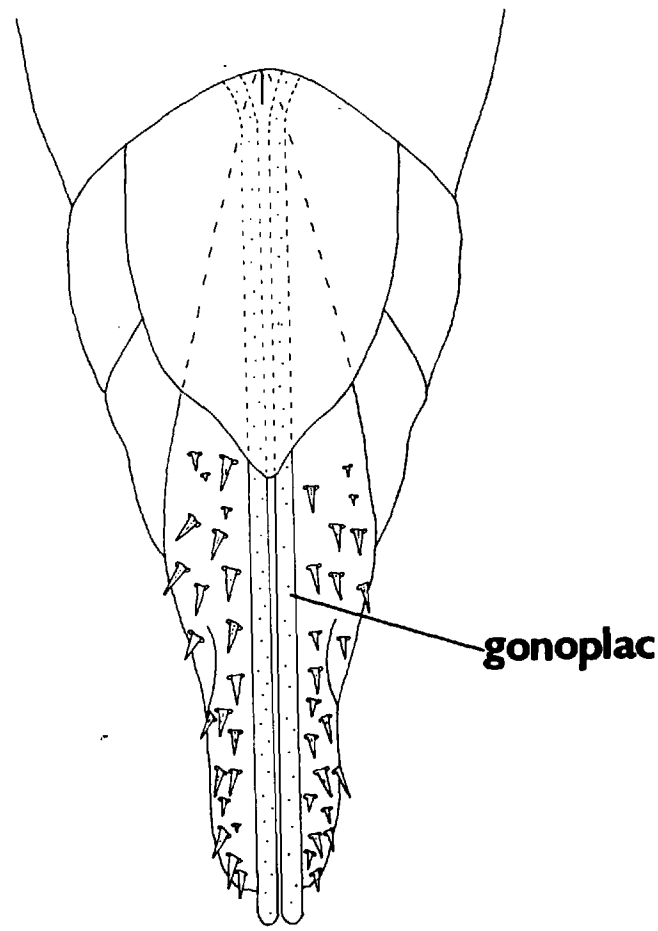
The position of the first gonocoxae, relative to the surrounding structures, differs considerably in different insects, according to the mechanism of the ovipositor. In some insects they clearly belong, anatomically, to the eighth segment, since they lie within the pleural region of that segment. This is the situation found in the Cicadellidae, where, though tergum nine has extended anteriorly, the first gonocoxae are situated directly beneath the tergite of segment eight. In other insects, they may be dissociated from the eighth segment and hinged directly to the second gonocoxae. A third possibility,

Fig 1.1

Genital Region



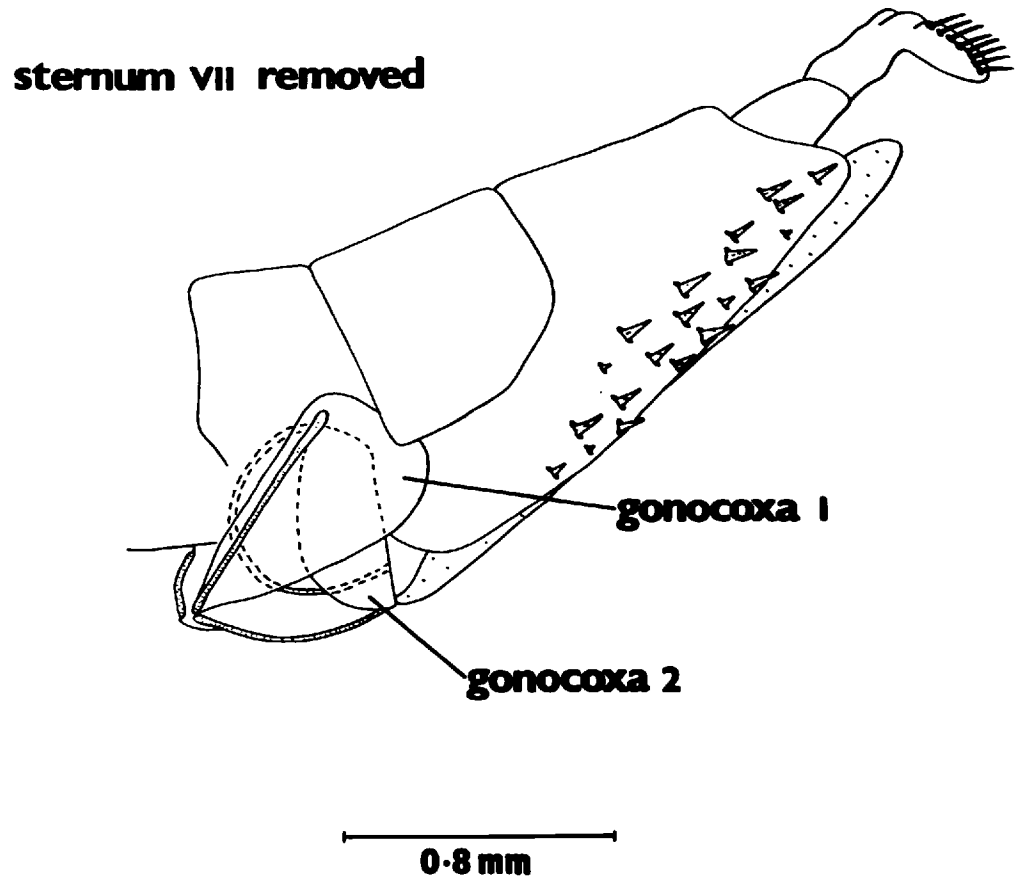
Lateral



Ventral

0·8 mm

Fig 1.2



though not known, is the articulation of the first gonocoxa with, or their attachment on either side to, the ninth tergum. If, therefore, the first gonocoxae appear to be derived in their development from the eighth sternum, the ontogenetic facts simply mean that at an early stage the bases of the first gonopods were not distinguishable from the true sternal area of this segment. Alternatively, if the first gonocoxae appear to be developed from tergum nine, it seems probable that their phylogenetic history is not fully recapitulated during their ontogenetic development. However, the invariable origin of the muscles serving the first gonocoxa on tergum eight is considered reasonable evidence for the derivation of the first gonocoxae from segment eight. As these sclerites always carry the first gonapophyses it is thought to be additional evidence for their representing the bases of the first gonopods.

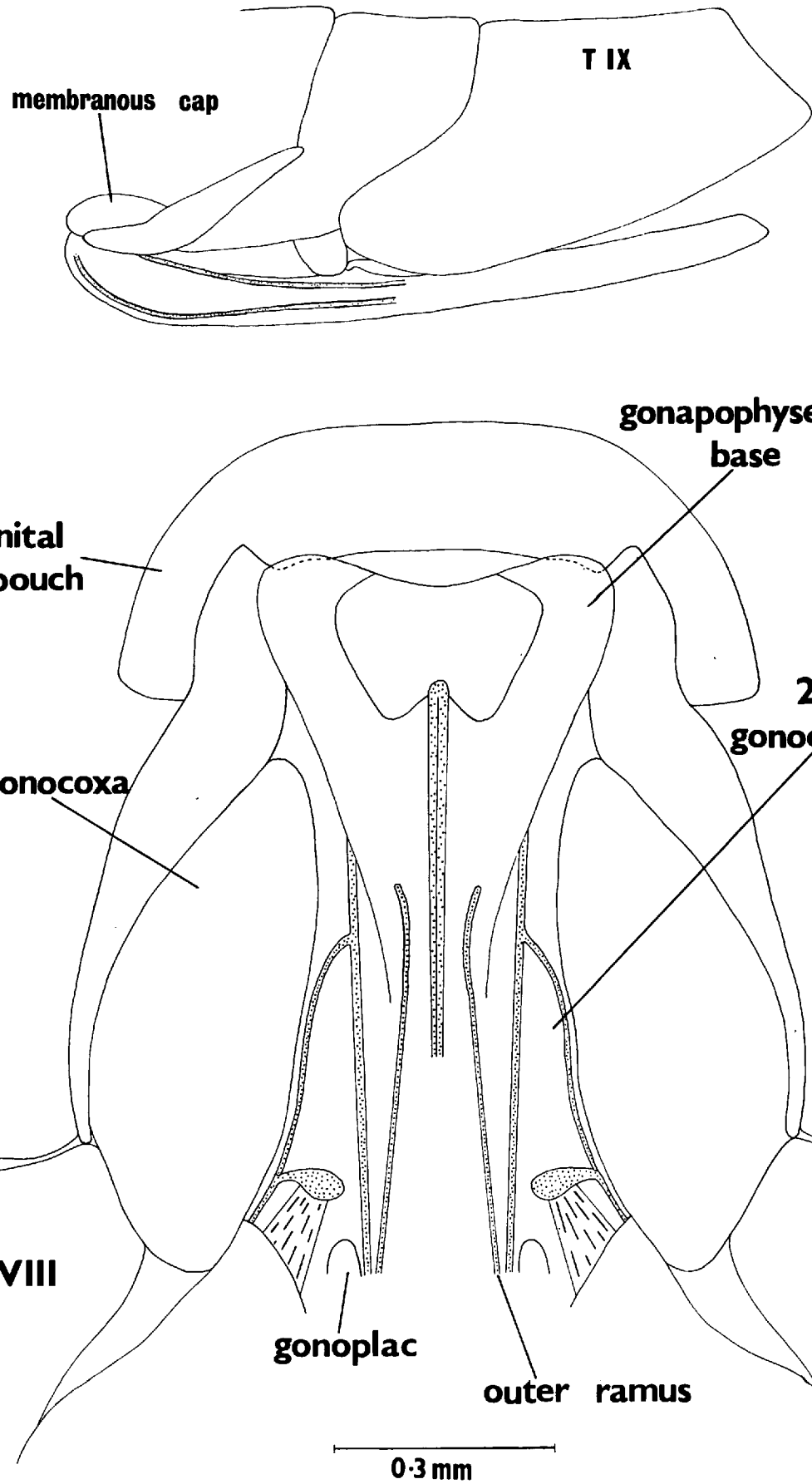
In Graphocephala, the first gonocoxae are rounded, triangular plates, which are attached posteriorly by a membrane to the posterior margin of tergum eight and connected, by the gonangulum, to the lower anterior part of tergum nine. In the Cimicomorph section of the Heteroptera, there is a marked tendency for the first gonocoxae to fuse dorsally with the eighth tergite.

In the resting Graphocephala, the first gonocoxa lies largely concealed by segment seven. The intersegmental membrane between segments seven and eight extends anteriorly from the lower margin of tergum eight along the dorsal margin of the first gonocoxa to the rudimentary and membranous sternum eight, situated beneath the anterior margin of the gonocoxa within the genital pouch (see fig. 1.3). The first gonocoxae are united on each side, above the genital pouch, by a dense white membrane that extends over the base of the gonapophyses and runs anteriorly almost to the apodeme on the base of sternum seven.

The dorsal margin of the first gonocoxa is strengthened by a sclerotized bar,

Fig 1·3

Lateral view of the genitalia with a ventral view of the basal parts



which articulates anteriorly with the proximal end of the outer ramus of the first gonapophysis. At the posterior dorsal end a head is formed by the expansion of the bar upon which inserts the two major first gonocoxal muscles which run posterolaterally to their origin on tergum eight. Mesally the first gonocoxa is joined to the gonangulum along the anterior gonangular ridge, thus restricting movement. Contraction of the first gonocoxal muscles produces a posterodorsal movement within the sclerite, which, because of the articulation between the sclerotized rod and the outer ramus of the first gonapophysis, depresses the distal part of the ovipositor.

(b) First gonapophyses

The ovipositor proper is composed of two pairs of broad, thin lanceolate blades, the first and second gonapophyses. The plane of these blades is vertical. They were originally hollow structures, which, in their definitive adult form, have become strongly sclerotized and laterally compressed. The outer wall is convex and the inner concave, thus allowing the two pairs of blades making up the shaft to fit compactly together. Several previous studies have been made of these blades in Cicadellids, of which the most thorough was the works of Balduf (1933; 1934). During the present study, when it was possible to use scanning electron microscopy in addition to light microscopy it was found that some of his original interpretations of surface structures were inaccurate, so detailed descriptions of the first and second gonapophyses will be given.

The first significant comparative morphological studies, where the gonapophyses of Cicadellidae have been described is that of China (1926) on a single species of his new genus Lasioscopus and four species from the genus Pogonoscopus. Cunningham & Ross (1965), studying Empoasca, described the ovipositor and basal parts and their descriptions have shown that these structures can provide excellent group characters for most species complexes recognised within this genus. Wagner (1950; 1953)

described the apical portions of the blades in ten European species of Macropsis. Ribaut (1952), Young & Beirne (1958) and Nielson (1965) have all given descriptions of gonapophyses and used them as taxonomic characters. However, all these authors followed Balduf's basic descriptions, and this is the first comprehensive study incorporating both light and scanning electron microscopy of the gross morphology. A series of transverse sections through the ovipositor was produced (figs. 1.4 and 1.5), from which it can be seen that distally the first gonapophyses contain numerous intra-gonapophyseal spaces within the spongy matrix, but throughout most of the length these have fused to produce two or occasionally three large cavities in the ventral region of the blade, and immediately ventral to the inter-locking device. These are probably strengthening devices.

Further along the first gonapophyses, a considerable change is seen in their structure. Distally they appear relatively undifferentiated; they are slightly shorter than the second gonapophyses with extremely heavily sclerotized walls, and contain cavities as seen in transverse section. Distally, the first gonapophyses taper to a point. Proximally, the broad, thin form becomes evident and the dorsal rasp is formed on the outer wall. A well developed, heavily sclerotized groove is present one quarter of the way forward from the distal extreme and this continues to the base of the blades. This structure will here be termed the rhachis. Immediately ventral to this groove are a series of pits which receive spines from the outer face of the second gonapophyses and also forms part of the interlocking mechanism. The proximal two thirds of the ventral edge has become modified into a flexible, membranous flap which carries at its distal end the inter-locking device uniting the first gonapophyses of either side. The development of this flap can be traced along the blades. While the outer wall retains its sclerotized structure, which is particularly well developed

Fig 1.4

Transverse sections along the ovipositor. Figures refer to distance in μm from the distal end of the 2nd gonapophysis

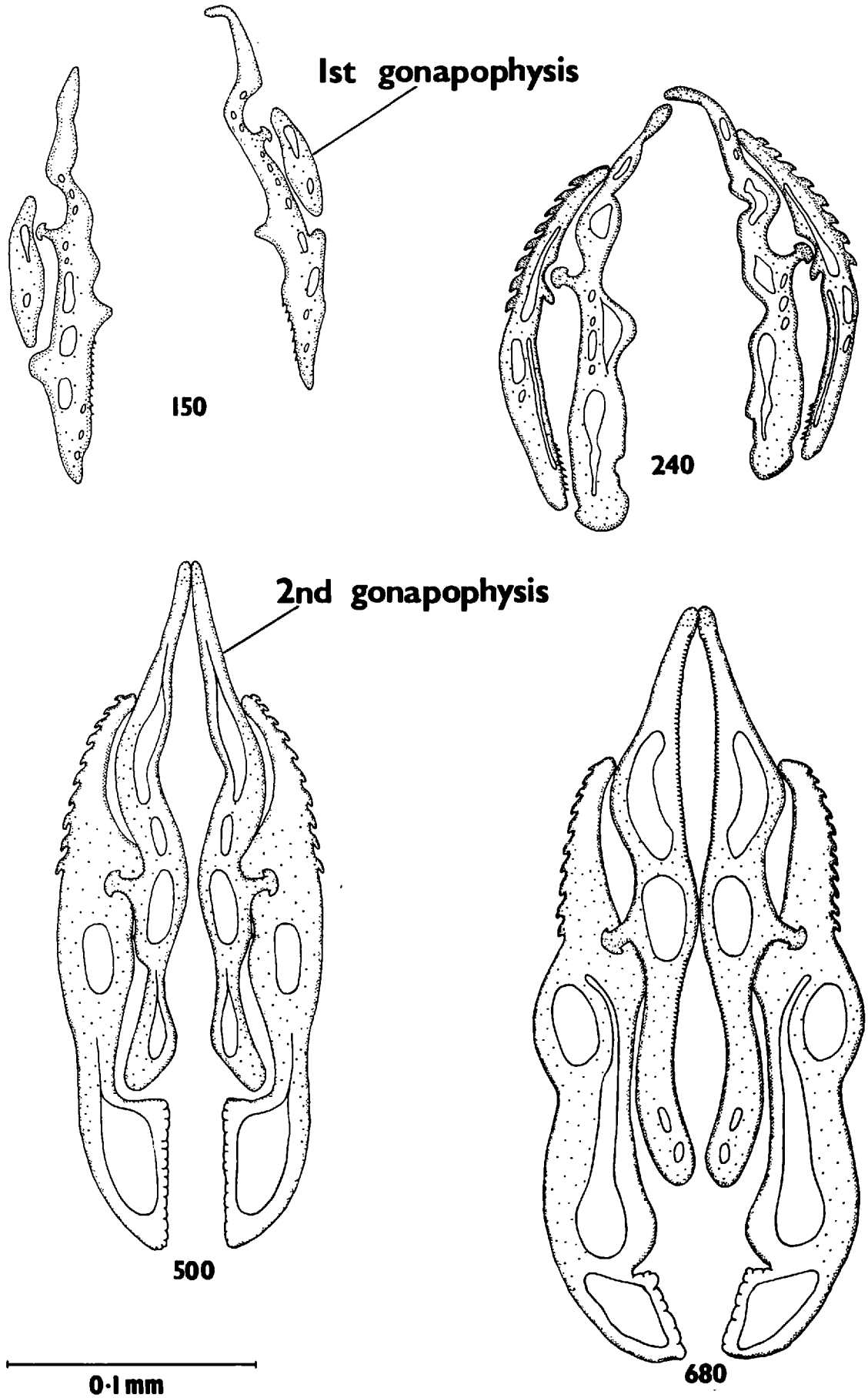
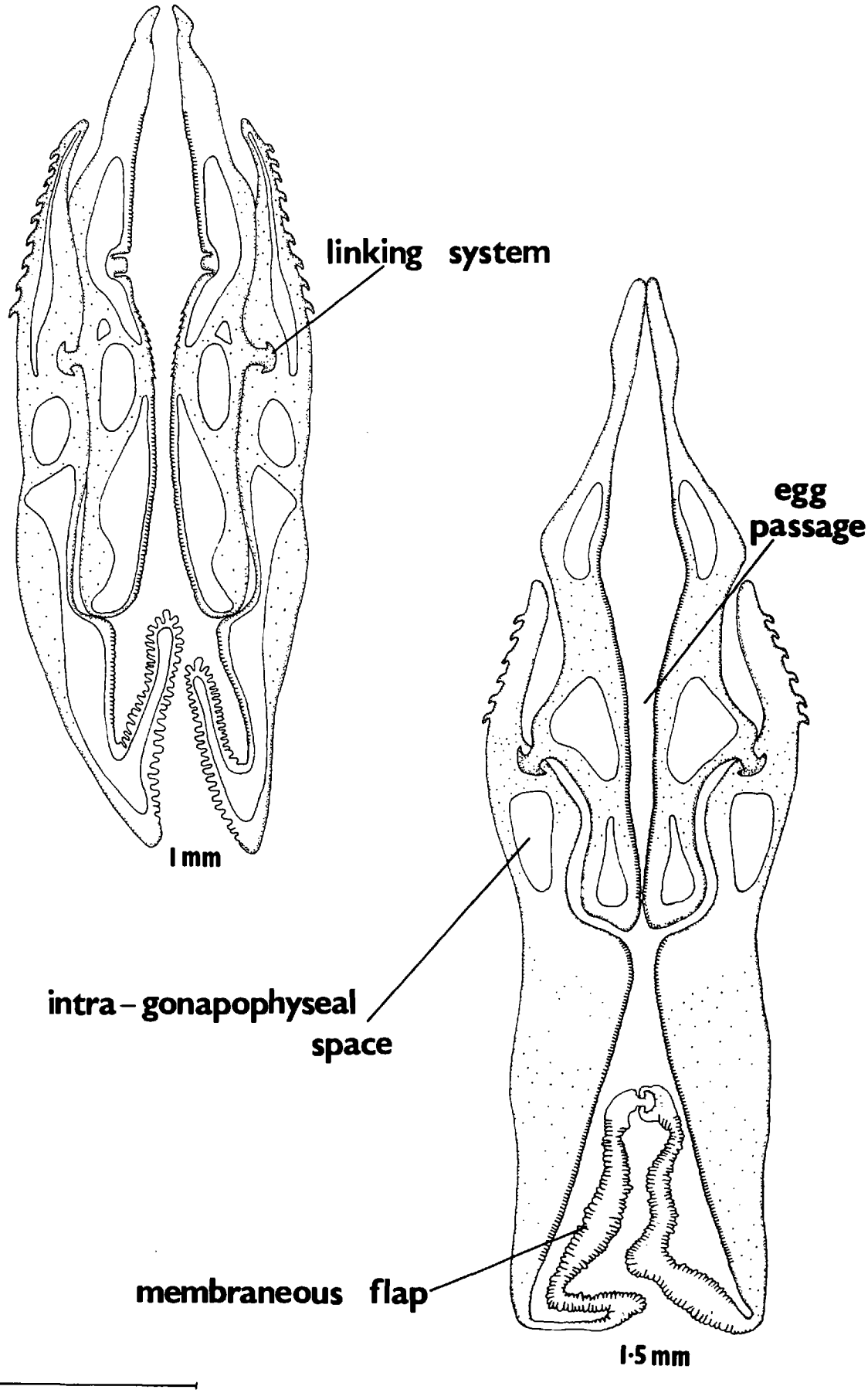


Fig 1.5

Transverse sections of the ovipositor, with distance from the distal end of the 2nd gonapophysis

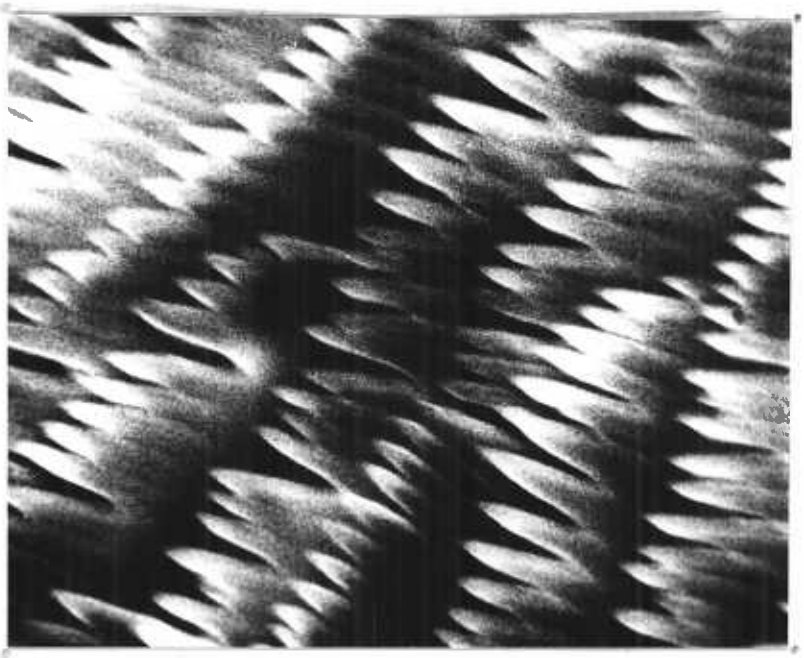


in the dorsal half, the inner wall retains its original sclerotized character only at the apex, the remainder having developed into scales, the anterior edge of which have been extended into a fringe of fine finger-like projections pointing towards the anterior end, or base, of the ovipositor (fig. 1.6). In transverse section this gives the appearance of a corrugated texture, and the structures appear to act as a "buffer" against the second gonapophyses and to reduce movement between them.

The apex and dorsal half of the outer wall in the distal third is made up of sclerotized blocks (fig. 1.7). As can be seen, the blocks in the dorsal and ventral position are somewhat different. Those dorsally are elongate, sloping towards the apex of the blade and form discrete blocks in surface view of approximately 16 μm x 3.5 μm (fig. 1.8). These stand out above the surface of the blade and are separated by deep channels. The blocks on the ventral edge appear as rows of overlapping scales of approximately 14 μm x 4.3 μm, again pointing towards the apex (fig. 1.9). There is a transitional zone of rounded scales between these two sets. Balduf (1933), Snodgrass (1933) and later workers have considered this to be the main rasping area and of major importance during oviposition. During work on oviposition in Graphocephala(Chapter 6) and video filming, it was shown that this region was not responsible for making the incision prior to oviposition, a function carried out largely by the second gonapophyses. Instead, this rasping area was responsible for clearing plant debris from around the incision and has little to do with actually cutting the hole.

In the middle region of the blades the discrete blocks of sclerotin break down to produce a more diffuse and smooth surface and then give way in the proximal region to lightly sclerotized scales whose anterior edge is also drawn out into a fringe pointing towards the base of the ovipositor. Video film revealed that the function of these scales is to anchor the ovipositor blade into the incision during oviposition with-

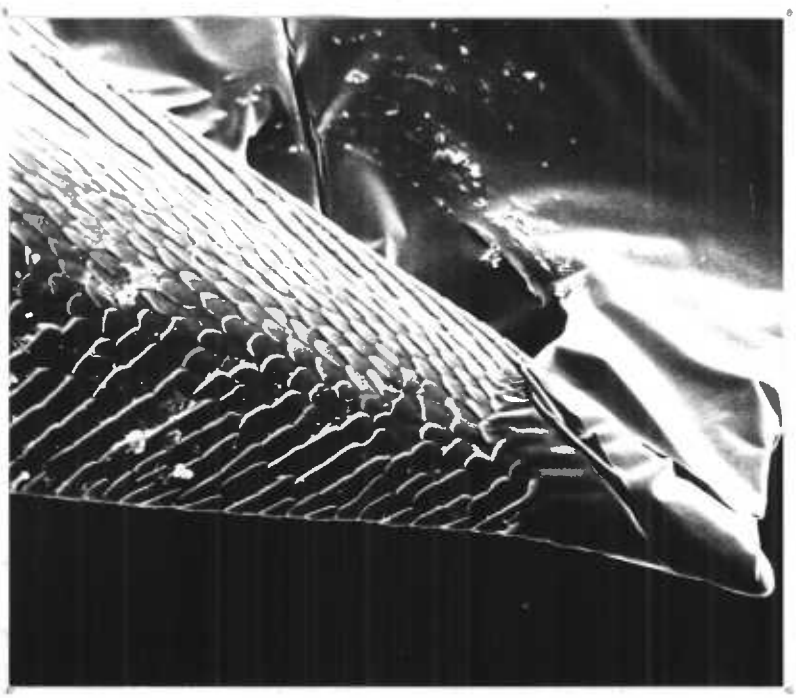
Fig. 1.6



Fringed scales on the inner wall of the 1st gonapophyses approximately 1mm from the proximal end.

x 7,500 20 KV Gamma 1

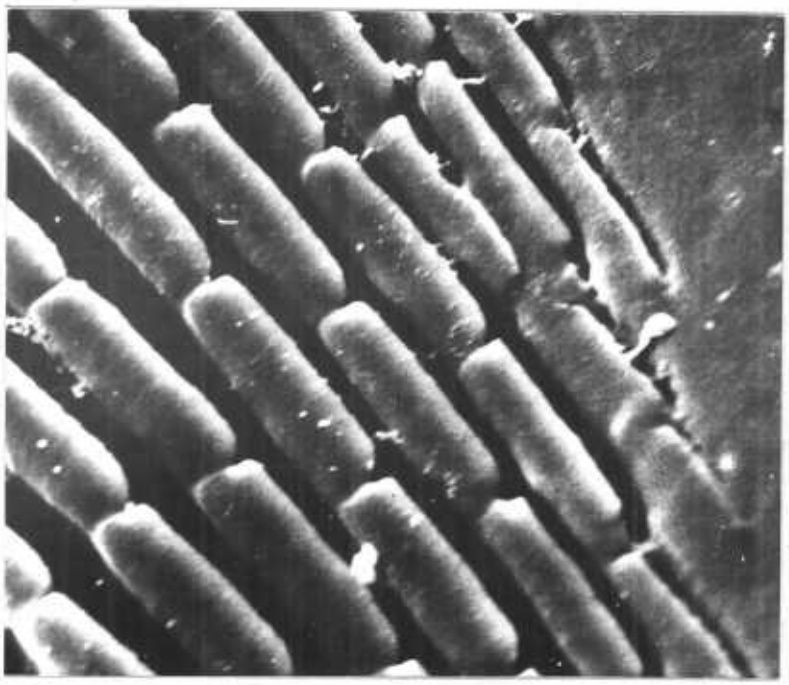
Fig. 1.7



Distal end of the 1st gonapophyses to show the sclerotized blocks.

x 500 10 KV Gamma 1

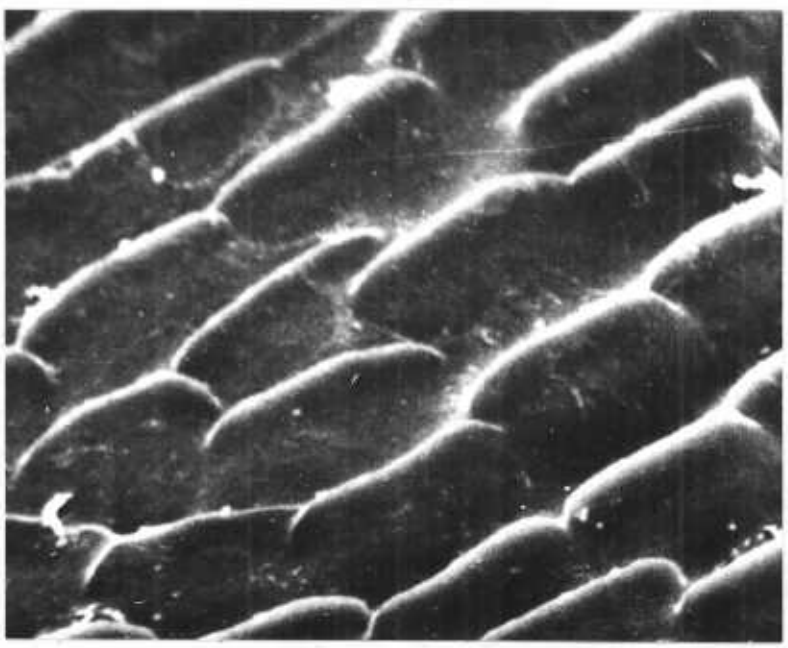
Fig. 1.8



Sclerotin blocks from the dorsal region of the distal 1st gonapophyses.

x 2,200 10 KV Gamma 1

Fig. 1.9



Sclerotin blocks from the ventral region of the distal 1st gonapophyses.

x 2,200 10 KV Gamma 1

out providing such a system that would prevent the retraction of the ovipositor afterwards. This agrees with the work of Micholeit (1974) working on Neuropteron (Raphidoptera).

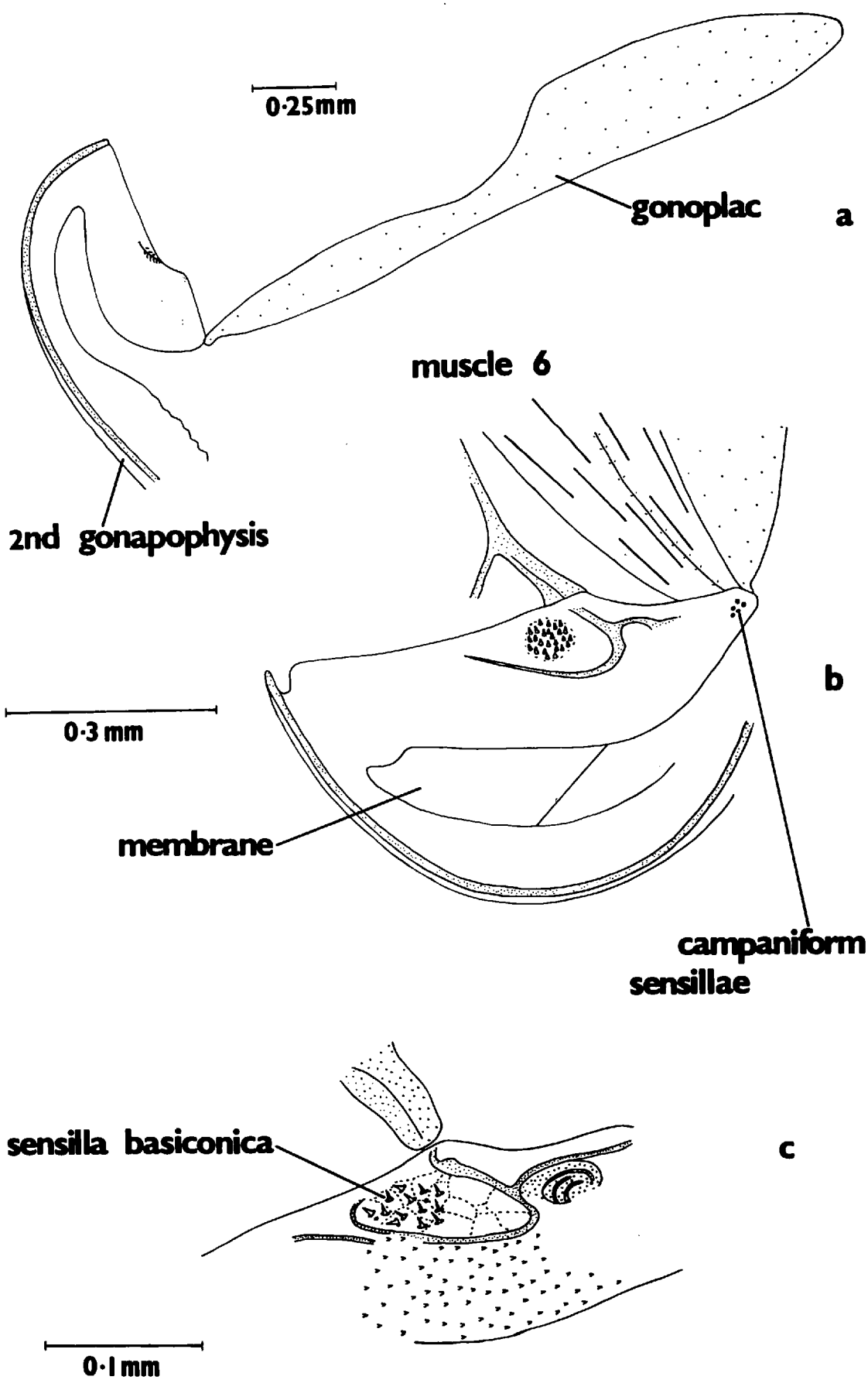
Snodgrass (1933) described the typical first gonapophysis as possessing two proximal rami, a ventral outer ramus, which in Graphocephala articulates proximally with the anterior angle of the first gonocoxa and continues distally as a heavily sclerotized portion in the ventral region of the blade. The second or dorsal inner ramus continues distally as a rhachis in Graphocephala, represented by the heavily sclerotized groove; this extends beyond the gonapophysis proper and expands into a head which is fused to the ventral edge of the gonangulum, which in turn is fused to the lower anterior angle of the ninth tergum.

The inter locking devices of the first and second gonapophyses will be described in detail under a separate section.

(c) Second Gonocoxa

The second gonocoxae are always associated with segment nine and are mesad to, and when the insect is at rest, mostly concealed in lateral view by the first gonocoxae. In Graphocephala they are very strongly sclerotized, elongate plates that lie in a vertical plane; the second gonapophysis being solidly attached to the antero-dorsal margin. The base of the gonoplac is membranously connected to the postero-ventral margin. The second gonocoxa also articulates with the ventral end of the posterior arm of the gonangulum, about the mid point of its posterior edge (fig. 1.11). This important articulation forms the fulcrum upon which the second gonocoxa is rocked by two groups of antagonistic muscles which insert on the anterior and posterior corners and originate on tergum nine. The point of articulation is not part of tergum nine. It is through this articulation that movement of the ovipositor blade is initiated.

Fig 1.11


2nd Gonocoxa
0·25mm
gonoplac
a
muscle 6
2nd gonapophysis
0·3 mm
b
membrane
campaniform
sensillae
sensilla basiconica
c
0·1 mm

At the base of this articulation the gonocoxal wall is thickened and drawn out into a strong ridge surrounding a depression (fig. 1.11c). The enclosed area is oval in shape and the sclerotization considerably reduced, a cellular surface pattern being readily discernable. Within this region a major group of basiconic sensilla or small trichoid sensilla are located, forming a distinct ring at the base of the articulation and numbering approximately fifteen. The structure and function of the sensilla will be discussed in a later section.

A second, more discrete group is present in the posteroventral corner of the second gonocoxa, immediately beneath the insertions of the ventral second gonocoxal muscles. This group consists of two or three basiconic sensilla and two campaniform sensilla. Though not present in Graphocephala, a third group of sensilla is found in Ulopa reticulata, consisting of large campaniform sensilla distributed on the thickened ventral edge of the second gonocoxa. These will be discussed in Chapter 5.

The outer surface of the gonocoxa is covered by numerous small spines which can be seen in fig.1.11c, the apices of which are directed ventrally.

The powerful musculature and rich supply of sensilla present on the second gonocoxa of Graphocephala fennahi suggest it to be of considerable importance in the initiation and control of movement in the ovipositor shaft.

The sensory structures in this region have not previously been considered. Kathirithamby (1971) briefly mentioned the main group in the region of the gonangular articulation but no description was given.

(d) Second gonapophyses

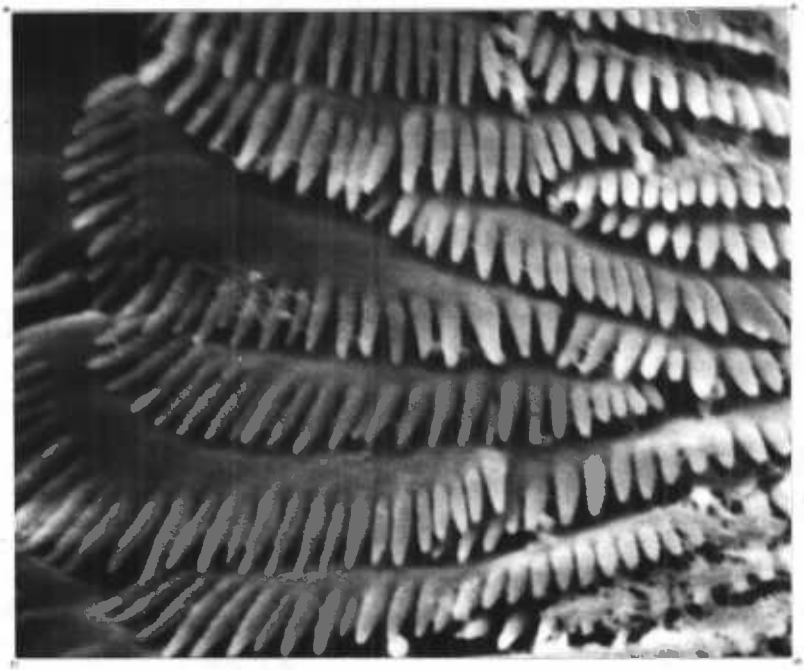
In general structure this pair of blades closely resembles the first gonapophyses to which they are applied. In the definitive adult condition the second gonapophyses

(inner valvulae of Snodgrass 1933) lie within, and partially above the first (fig. 1.5). The walls, particularly in the distal region, are heavily sclerotized, though more proximally, sclerotization is reduced. Considerably more intra-gonapophyseal spaces occur in this pair of blades than the first. Distally they comprise a series of small spaces throughout the depth of the blade (fig. 1.4), proximally, in the region of the egg passage and well formed linking mechanism, the spaces fuse to produce three or four large spaces concentrated around the rhachis and the ventral portion of the blade. There is a tendency for the spaces to be symmetrical in each blade.

In its proximal two-thirds the medial wall has been modified into a corrugated, cushion-like buffer which lines the surfaces over which the egg passes during oviposition. Video recording of oviposition indicates that the egg is expelled from the ovipositor ventrally, approximately one third from the distal edge. This agrees with the distribution of the buffer zone, the distal region of the blade being heavily sclerotized on both the inner and outer wall. An examination of the buffer zone with the scanning electron microscope revealed that it is composed of elongated scales, the proximal edge of which is drawn out into a fringe 2.2 μ m long (fig. 1.12). A backwardly directing fringe would provide a better hold and control of the egg, and alternating movements of the blades would act as a ratchet to move the egg along the ovipositor. The zone between the highly sclerotized dorsal margin and the buffer was flexible and of a rather soft texture. The curvature of the mesal wall is such that when the two second gonapophyses are opposed, in transverse section, the gonapophyseal space contained between them is divided into a dorsal and ventral portion. It is the ventral portion that acts as the egg passage.

Each second gonapophysis has a single, basal ramus which extends basal to the gonapophysis proper and is solidly attached to the second gonocoxa. In the curved portion (as shown in fig. 1.11a), the walls of the ramus are made up of over-

Fig. 1.12



Scales from the medial wall of the 2nd gonapophyses in the region of the egg passage.

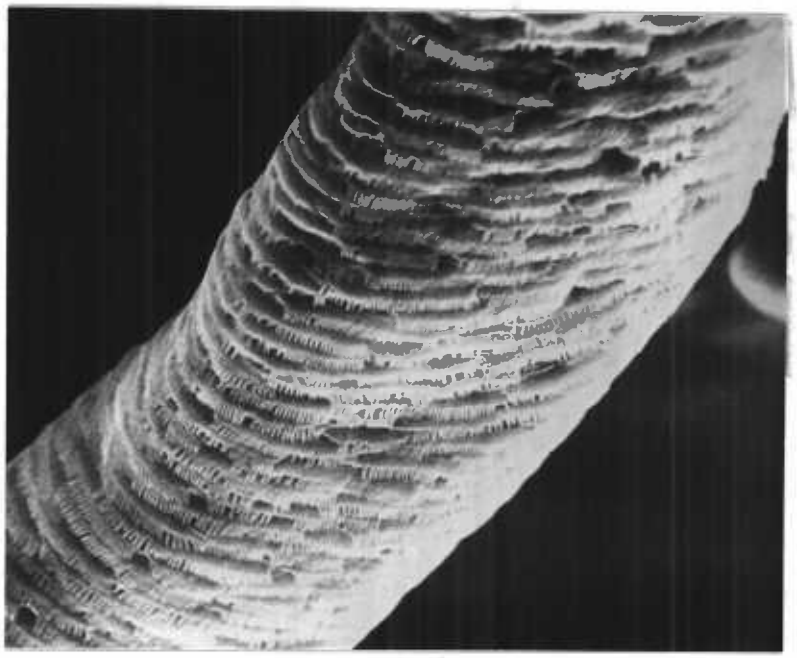
x 6,000 10 KV Gamma 1

lapping scales (fig. 1.13), the free edges of which are also pulled out into a fringe about 1 μm long (fig. 1.14). The function of the scales and fringe in this region is not known. The ventral rhachis forms the linkage mechanism with the inner rhachis of the first gonapophysis. This is the T-shaped process of Balduf (1933) and its structure was confirmed in this study. When the ridge is viewed with the scanning electron microscope, the impression of its structure is somewhat different. Though light microscopy clearly depicts slight lateral flanges, the electron microscope shows that it is made up of units 17 μm long (fig. 1.15), which extend the length of the second gonapophysis. It is thought that Balduf's interpretations of the linking mechanism are somewhat diagrammatic and it is simpler than he suggested. It is agreed that the first and second gonapophyses slide on this track-like connection, but movement is considerably restricted by the change in orientation of the tongue and groove. The transverse sections (1.4 and 1.5) clearly show that the tongue is directed dorsally in the distal part of the blade and ventrally in the proximal parts. This change in direction through approximately 45° must reduce movement considerably. This feature was not mentioned by Balduf (1933; 1934) in his studies of Draeculacephala or Empoasca and the linking mechanism has not been studied in detail since those early works.

The second gonapophyses are united in their proximal 500 μm by a thin membranous fold which can be seen clearly under a dissecting microscope. For the remainder of their length they are free, though closely applied. This basal connection remains flexible and permits a considerable amount of independent movement, by the two blades between the first gonapophyses.

The dorsal margin of each second gonapophysis bears a series of coarse, asymmetrical teeth, the edges of which are serrated. These angular teeth occur on

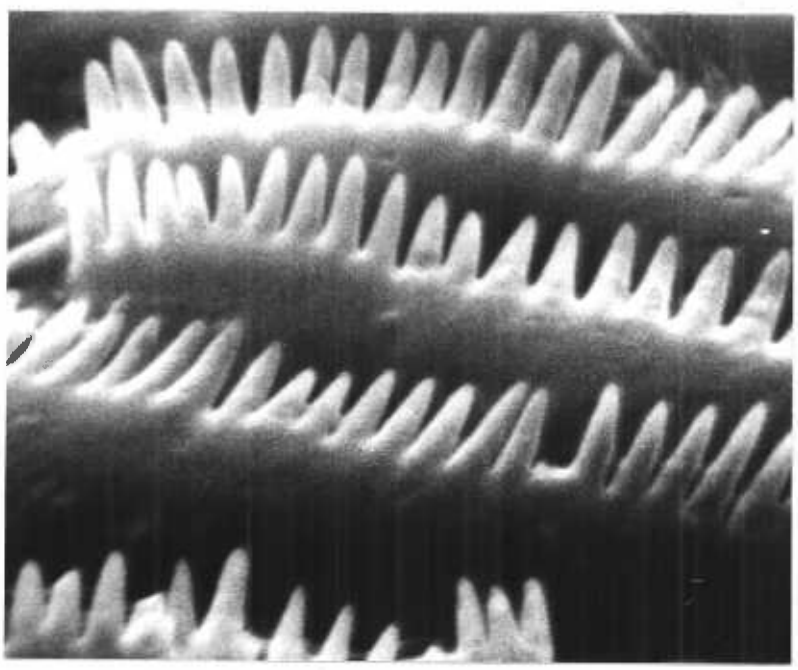
Fig. 1.13



Extreme base of the 2nd gonapophyses as it flexes up towards the 2nd gonocoxa.

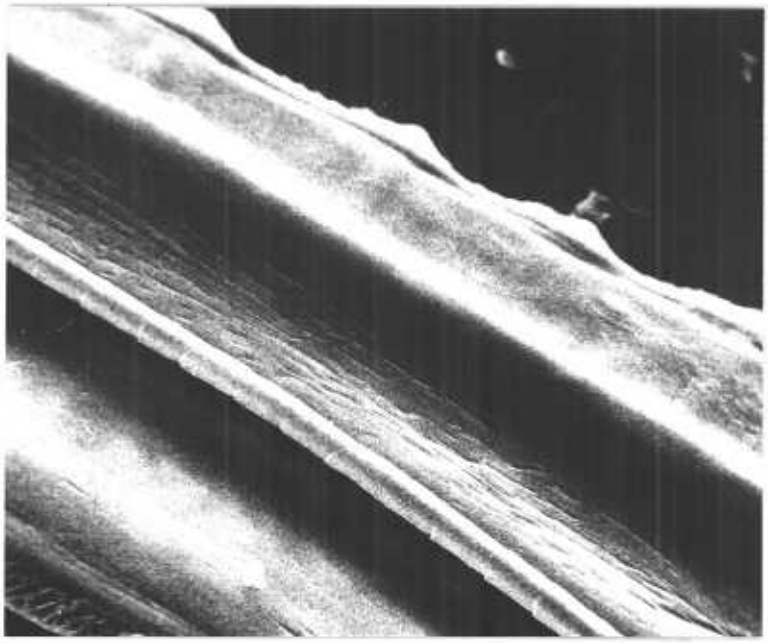
x 1,100 20 KV Gamma 2

Fig. 1.14



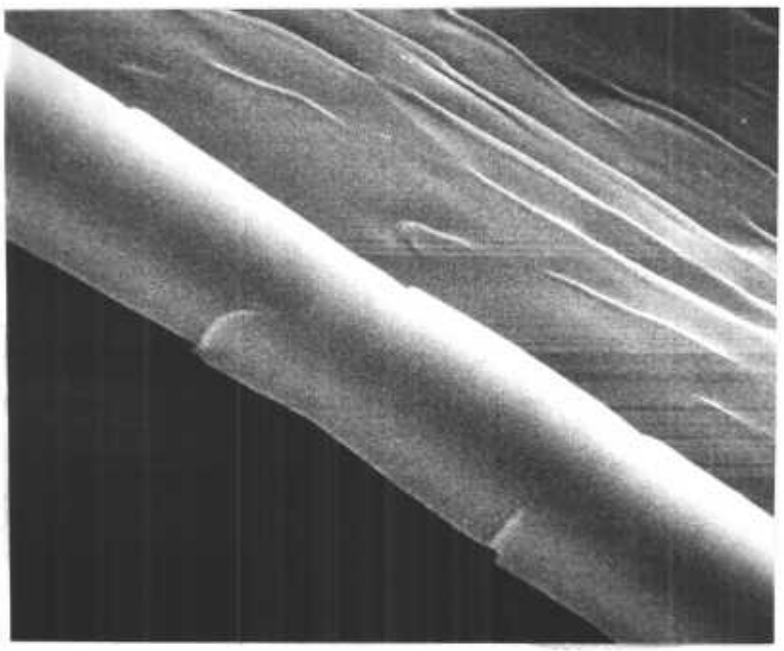
High power of the fringe as above.

x 11,000 20 KV Gamma 2



To illustrate the linking mechanism (basal rami) on the outer wall of the 2nd gonapophyses.

x 400 20 KV Gamma 1



Linking mechanism as above indicating its spiralling construction.

x 2,200 20 KV Gamma 2

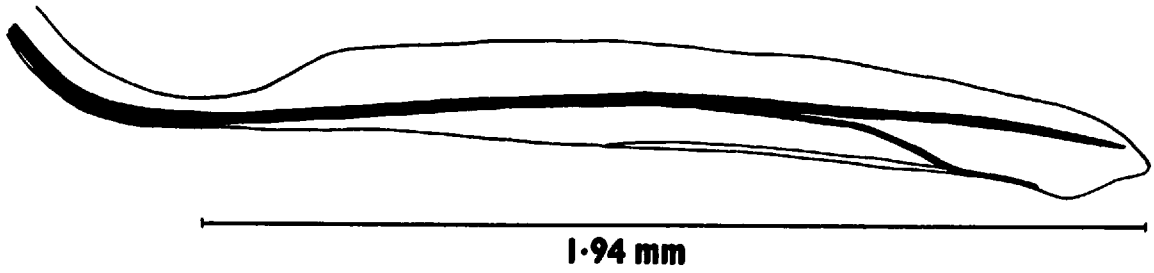
the distal three quarters of the blade and have short, acute anterior edges, the posterior edge being longer and more gradual (fig. 1.16). The form of these teeth suggests that the most powerful cuts are made during posterior thrusts. In the basal quarter, the teeth are of lower elevation and more rounded and probably do not function in cutting of the plant tissue. The distal tip is rounded and bears single-pointed, posteriorly directed teeth on the sub-apical face (fig. 1.17a). A ventral view of the teeth, (fig. 1.17b), indicates that they would provide a very efficient rasping area to supplement the dorsal teeth. Additional dentition (fig. 1.18) is also present along the short ventral cutting edge in the form of strongly sclerotized scales projecting beyond the edge of the blade.

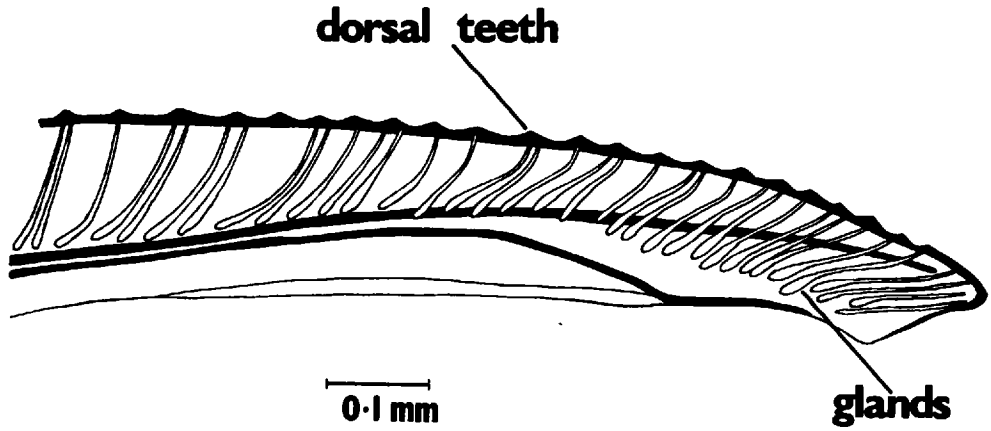
It can be seen from this description that the cutting surfaces of the second gonapophyses are highly developed and quite diverse in form. Previous authors have only considered the dorsal teeth (the caudal cutting surface of Balduf 1933) to be of prime importance during oviposition. In contrast to previous descriptions (Ali 1958; Kathirithamby 1971; Young 1977) which failed to recognize the three major cutting areas, the present author suggests that it is the second gonapophyses that makes the incision during oviposition, the first gonapophyses anchoring the ovipositor and providing a rigid shaft within which the second gonapophyses can function.

In addition to the cutting areas, the second gonapophyses also possess important sensory areas concentrated beneath the dorsal teeth and in the tip (fig. 1.19). These will be discussed later. Glandular, multi-cellular glands (fig. 1.16) also open on the inner surface of the blade, ventral to the dorsal teeth. In light microscopic preparations these are clearly seen as dermal glands, the glandular portion of which, except in the extreme apical position, lies immediately dorsal to the ventral rhachis and contains four to five large, strongly basiphilic nuclei. The gland opens to the surface via a

Fig 1.16

2nd Gonapophysis





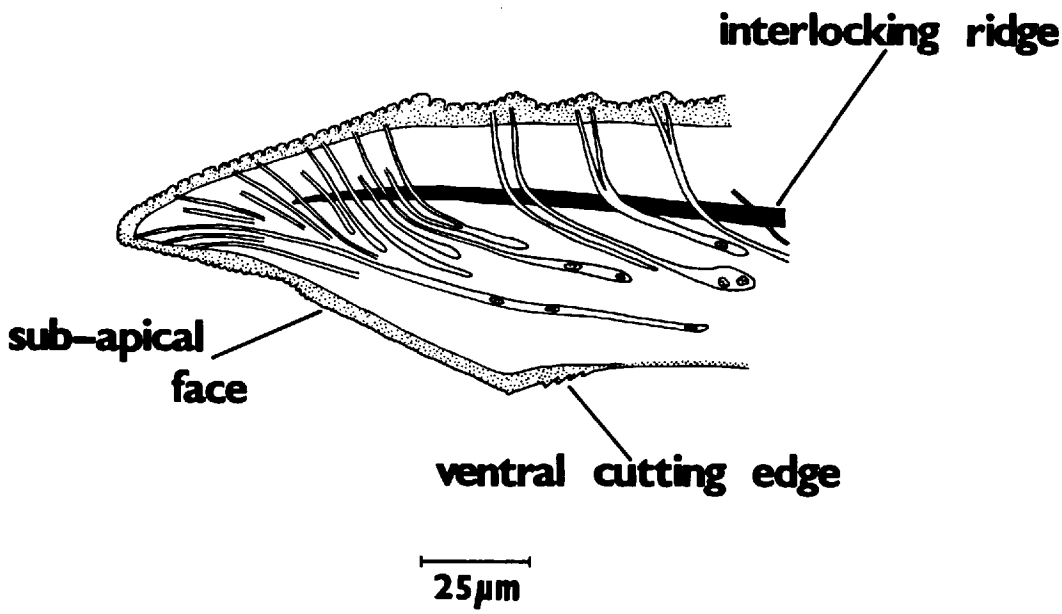
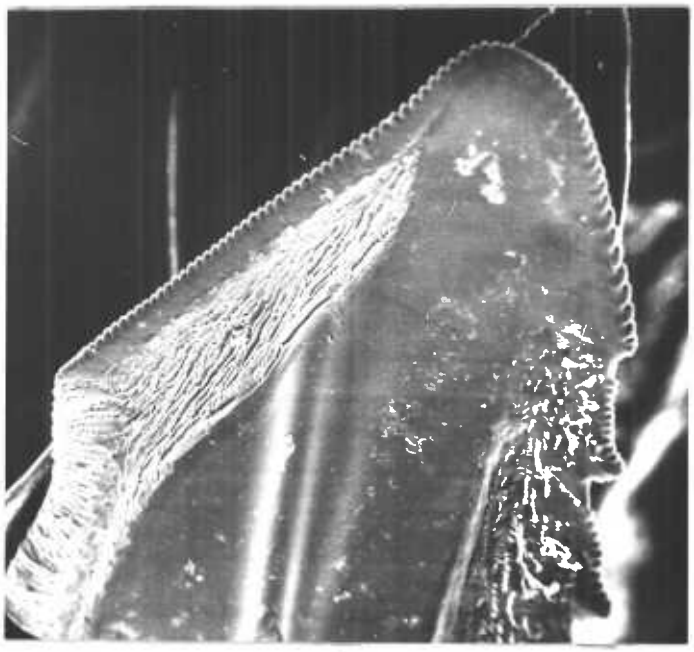


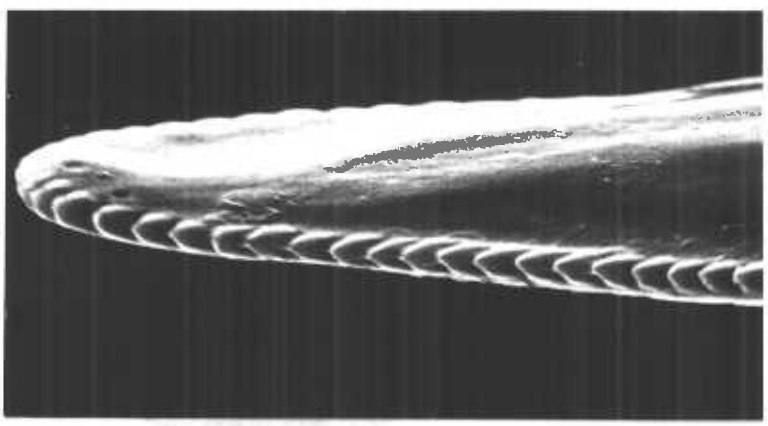
Fig. 1.17a



Distal end of 2nd gonapophyses to illustrate the dentition of the sub-apical face viewed from the inner surface.

x 600 10 KV Gamma 1

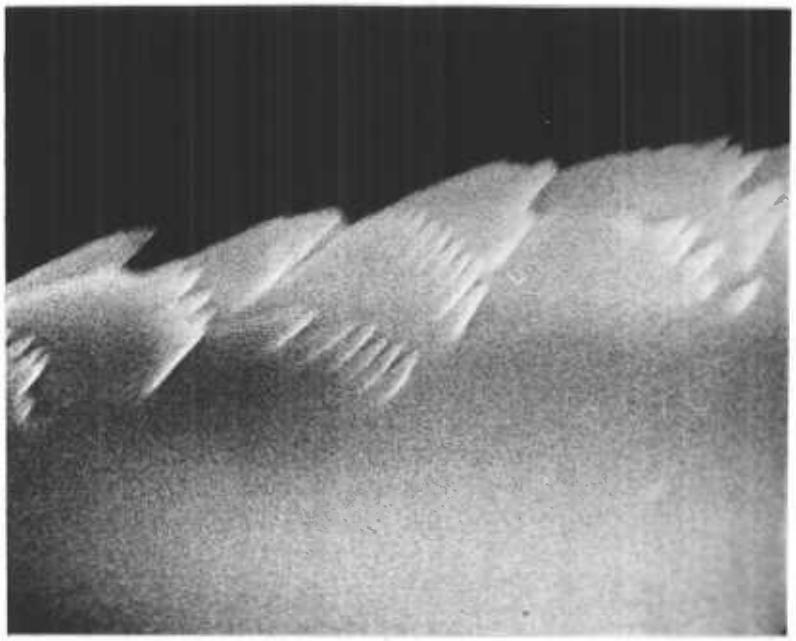
Fig. 1.17b



Ventral view of the sub-apical face illustrating the cutting area.

x 1,300 20 KV Gamma 1

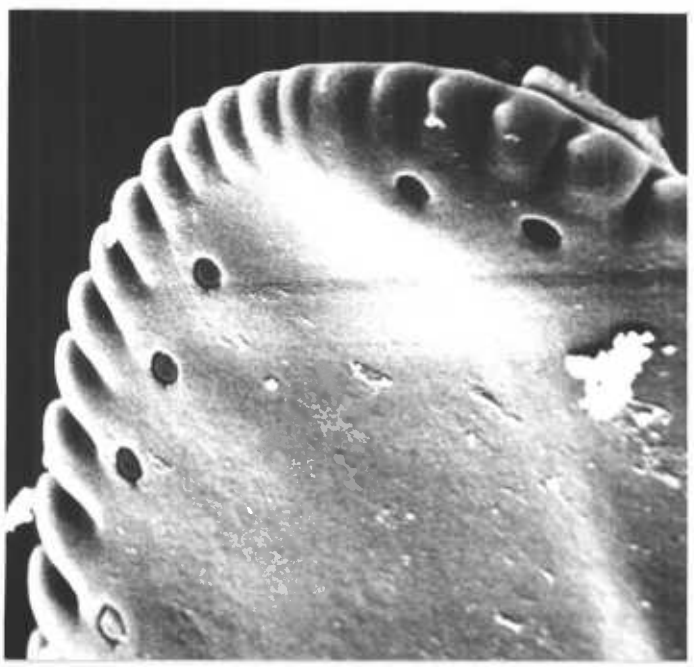
Fig. 1.18



Heavily sclerotized projecting scales on the ventral cutting edge of the 2nd gonapophyses.

x 4,000 20 KV Gamma 1

Fig. 1.19



Distal tip of the 2nd gonapophyses showing several campaniform mechano-receptors and a single sensillum coelonconica.

x 2,300 10 KV Gamma 1

long duct which occasionally branches near its distal end. The functions of the secretions has not been determined, but they may act as a fungicidal substance to protect the eggs or as a lubricant.

(e) Linkage of the gonapophyses

This is an important mechanism if the blades composing the ovipositor are to function in a co-ordinated manner. The only comprehensive description is by Balduf (1933) for Draeculacephala. Other authors, when describing the ovipositors of Cicadellids, only record that a tongue and groove system is present. Two linkage systems exist within the ovipositor of Graphocephala : (a) That which connects the first and second gonapophyses of each side. (b) A ventral connection between the first gonapophyses of each side.

The first and second gonapophysis of each side are linked by a relatively simple sliding tongue and groove mechanism. This system appears to be general in the Cicadellids and was briefly described by Snodgrass (1933) in Amblydisca gigas. It is common in all other orders which possess a well developed ovipositor. Though the mechanism is constant, the condition seen in some Orthopteroids, e.g. Acheta, differs in that it is the gonoplacs that are ventrally ridged and interlock in the groove on the dorsal surface of the first gonopophyses.

In Graphocephala, the tongue is carried on the outer wall of the second gonapophyses and has been described in an earlier section. The groove is present on the mesal surface of the first gonapophysis and represents the heavily sclerotized first rhachis. The positions of the inter-locking devices are such that the dorsal edge of the second projects considerably above that of the first (figs. 1.4 and 1.5), so exposing the dorsal teeth of the second gonapophyses. Proximally this system is supplemented by spine-like projections from the outer wall of the second gonapophyses which insert into corresponding grooves in the first gonapophyses.

A transitional sequence in this mechanism can be followed along the length of the ovipositor. In the distal quarter of the shaft the groove of the first gonapophysis is incompletely formed so that the mechanism is not functional (fig. 1.4) and in this region the two blades are not joined. Proximally the mechanism is complete and the two blades are held inseparably together. It should be noted that in the newly emerged adult sclerotization of the ovipositor is incomplete, so that the ovipositor is not functional. Sclerotization continues until the definitive thickness is attained within twelve days of adult emergence. Sclerotization progresses most rapidly in the outer walls of both blades, the medial walls remaining cellular with prominent nuclei until a relatively late stage. During these early stages the region surrounding the inter-locking mechanism remains spongy with numerous intra-gonapophyseal spaces. Sclerotization continues until the majority of the spongy matrix has been displaced by the heavily sclerotized walls, and the spaces have come to lie above and below the mechanism, parallel to it and thus lightening and strengthening the blades.

The ventral part of the first gonapophyses, except in the extreme distal region, is very convoluted and expands into a flexible membranous flap which curves upwards and towards the mid-line in the plane of the ovipositor (fig. 1.5). For the proximal two thirds of their length the first gonapophyses are united inseparately by a linkage mechanism that is located on the distal end of the flap (fig. 1.5). In dissections the two blades can only be separated by sliding one lengthwise along the other. The structure of this mechanism is relatively simple and constant throughout its length, being a modified tongue and groove with no accessory inter-locking devices. The degree of sclerotization surrounding the link decreases distally though the tongue remains very heavily sclerotized. The joint would resist lateral stresses while still allowing longitudinal movement between the blades. The convoluted membranous

flap would permit considerable extension during the passage of an egg.

Balduf (1933) described this joint in Draeculacephala as a ball and socket joint and in the proximal region additional terminal and lateral processes that complement the main joint. Such structures were not present in Graphocephala.

The various linking mechanisms of the ovipositor are such that relatively little independent movement is attainable in the individual components. It seems likely that while a small amount of alternating movement is possible between the second gonapophyses, which would permit movement of the egg down the shaft, the ovipositor functions more as a complete unit during incision. The distal portions of the second gonapophyses project beyond those of the first, allowing its cutting areas to function, the dorsal teeth operating during posterior abdominal thrusts and contraction of the antagonistic muscles 4,5,6 and 7 of the second gonocoxa. The lateral rasps of the first gonapophysis clear away debris and the lateral blades themselves provide a shaft within which the second gonapophyses can function.

(f) Gonangulum

This sclerite has caused much debate and is known by several synonyms : Anterior plate (Snodgrass 1935), Triangular plate (Snodgrass 1925; 1956), Sclerites m + n (Snodgrass 1933) and in some orders it has been termed a valvifer. Scudder questioned some of Snodgrass's work in a series of papers (Scudder 1957, 1958, 1959, 1961a, 1961b and 1964), where he develops a new theory of the ovipositor, which he calls the gonangulum theory. The gonangulum, found in Dictyoptera, Grylloblattodea, Hemiptera, Hymenoptera, Odonata, Orthoptera, Psocoptera, Thysanoptera and Lepismatoid Thysanura (Scudder 1961b), is a sclerite attached ventrally to the base of the first gonapophysis and articulates with the second gonocoxa. Scudder (1961a) considers it the most important sclerite, because it

affects the movement of both the first and second gonapophyses. The anterior ridge (Snodgrass 1933) and the ramal plate (Kramer 1950) are both considered a portion of the gonangulum (Scudder 1960). Scudder (1957) suggested that its widespread occurrence indicated a considerable selective value; even in the most highly evolved forms, such as Hymenoptera, it has been retained in a form which is functionally and morphologically very similar to that seen in the primitive Lepismatidae.

Snodgrass (1933) described the mechanism of the ovipositor in several orders and the role of what was equivalent to the gonongulum is the control of these movements was shown to be of great importance. It is interesting to note that such groups as the Pentatomidae and Coreidae (Heteroptera) which do not oviposit in tissue and hence do not employ a sawing action of the ovipositor have a reduced or weakly sclerotized gonongulum. They oviposit on the surface of tissue. From the same sub-order the Miridae and Nabidae, which oviposit in plant material, possess a sclerotized gonongulum. Such indications of the oviposition site by the state of development of the basal genitalia will be developed in greater detail in Chapter 5.

Scudder's (1961) work on Thermobia confirms that of Snodgrass (1935a), who suggested that the triangular plate, which Scudder termed the gonongulum, develops from the second gonocoxa. In Graphocephala, it was seen to develop in the early third instar from the anterolateral portion of sternum nine. This is in general agreement with other authors who studied development in the Exopterygotes. Snodgrass (1935b) stated that during the early post-embryonic development of Acheta domesticus, a pair of small lateral sclerites (x) is present on the ninth segment, these persist through development, increase in size and adopt a more dorsal position.

Qadri (1940) also states that on either side of the lateral valves (equivalent to Scudder's gonoplacs), part of the ninth sternum failed to be absorbed into the ovipositor valves and persisted until the final moult, when it fused with the posterior margin of the first pair of valvifers (gonocoxa). The first gonocoxa is connected to the base of the first gonapophysis and posteriorly has a prolongation, which Snodgrass (1935b) termed y, which inserts between the base of the gonoplac and gonongulum. The latter gradually becomes closely associated with tergum nine. In the adult the gonongulum (x) is fused with sclerite y of the first gonocoxa and posteriorly articulates with tergum nine dorsally and the second gonocoxa ventrally. Work by Gupta (1950) and Stys (1959) also suggests the development of the gonangulum from sternum nine or ventrally between segments eight and nine. Scudder (1971), however, believes the gonangulum is derived from the second gonocoxa.

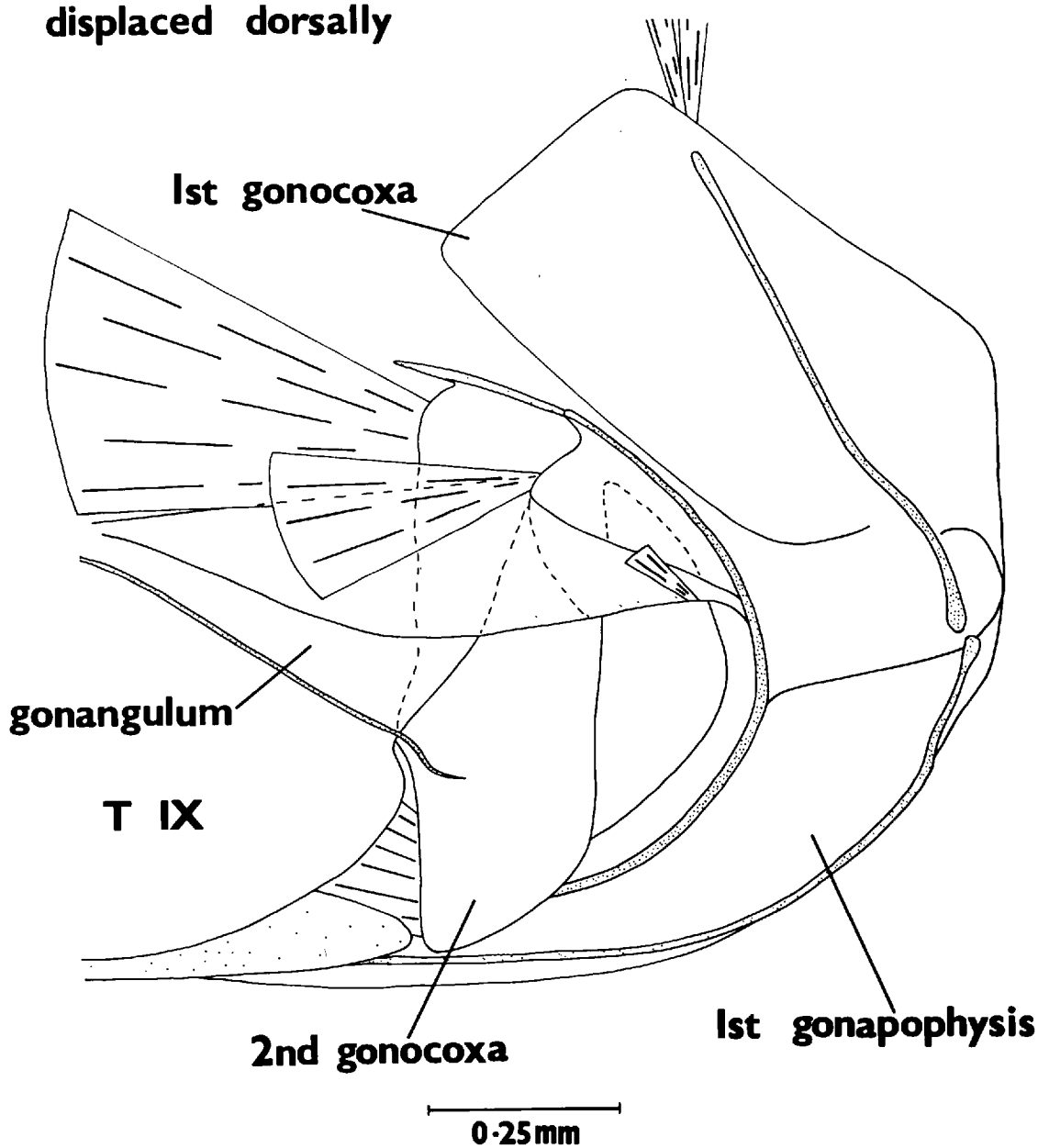
In the Hemiptera, the gonangulum is usually a triangular or inverted V-shaped sclerite. In Graphocephala (fig. 1.20) the anterior end of the outer ramus, belonging to the first gonapophyses, is expanded into a small head which is fused to the ventral edge of the anterior part of the gonangulum. The posterior dorsal edge of the gonangulum is fused to tergum nine. The posteroventral edge articulates with the second gonocoxa at the mid-point of the latter, allowing antagonistic gonocoxal muscles to rock the second gonocoxa about this fulcrum.

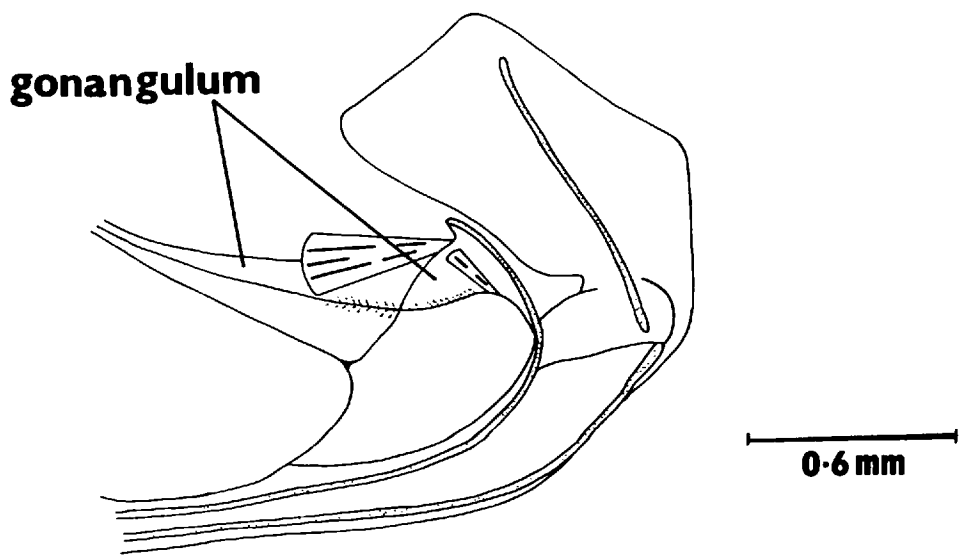
(g) The gonoplac

This arises from the posterior angle of the second gonocoxa and was termed the third valvula by Snodgrass (1936). The gonoplac is narrow at its proximal half, broad and spoon-like at its distal half and is membranously connected via the outer edges of its anterior half to the ventral edge of the ninth sternum in the region of the mid-ventral groove in segment nine, so that the two gonoplacs form a sheath, which,

Fig 1.20

Lateral view of the basal genitalia, 1st gonocoxa displaced dorsally





in the resting position, encloses the ovipositor. The inner edge of the anterior half of each gonoplac is joined by a broad area of membrane to the corresponding edge of its homologue from the other side. The free end of the gonoplac is retained within the groove by spines present in sternum nine which fit into corresponding pits on the opposed surfaces of the gonoplac. A transverse section through the distal part of a gonoplac (fig. 1.21) shows the heavily sclerotized walls, of which the inner possesses bifid setae which may function in the retention of the ovipositor. Also present on this inner wall are plugges pores (see fig. 1.21) the function of which is not known. Large nuclei with deeply staining chromatin material are present within the cytoplasm.

The above description outlines the definitive condition of the adult Cicadellid ovipositor based on Graphocephala fennahi. When compared to that of other orders the ovipositor may appear relatively simple. It is nonetheless a highly efficient organ and though the approach taken here has not been that of a taxonomist, and the purpose has not been to describe taxonomic characters, several have, in fact, been suggested.

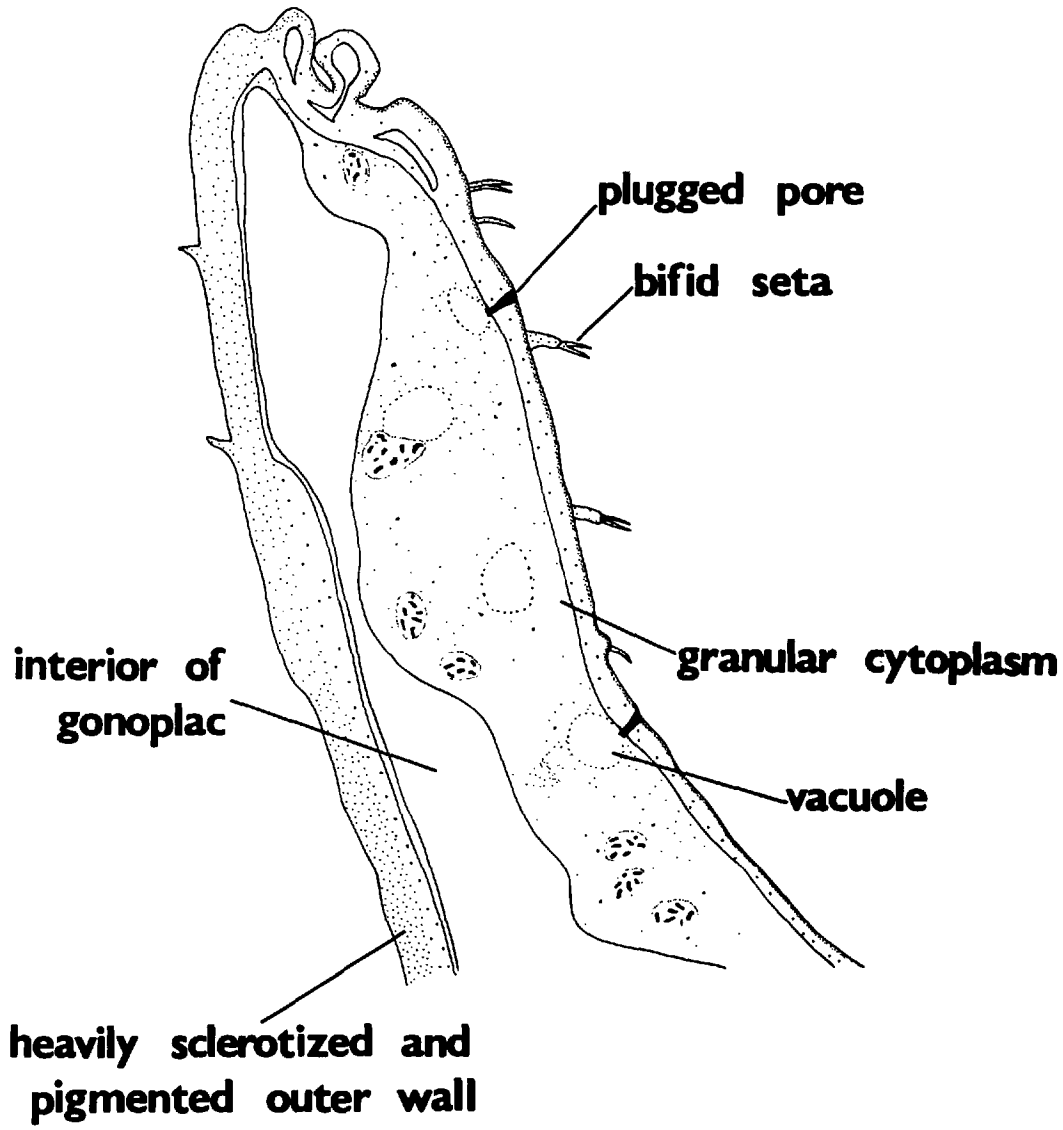
This completes the description of the ovipositor of Graphocephala fennahi; its postembryonic development is dealt with in Chapter 2. The sensilla present on the surface of the gonapophyses and second gonocoxa are described in Chapter 6 under the control of oviposition. The genital musculature, controlling movement within segments eight and nine, is described in Chapter 4.

3. Production of movement in the basal structures and its transmission to the ovipositor shaft

Though the several components of the ovipositor retain a limited amount of independent movement, the action of the blade as a single, coordinated unit is brought about largely by the presence of two structures : (a) The gonangulum which

Fig 1.21

Transverse section through the dorsal region of the distal gonoplac



links the second gonocoxa and the base of the first gonapophyses allowing movement in one to be transmitted to the other. (b) The quarter spiral twist by the tongue and groove of the first and second gonapophyses which limits movement between the two blades.

Movement of the Cicadellid ovipositor is simple when compared to some other orders, e.g. Orthoptera. In Tettigoniids, the ovipositor is rotated through 90° during mating and/or oviposition, which requires a complex musculature. In Graphocephala movement of the ovipositor only occurs in the vertical direction in the longitudinal plane of the body.

The whole of the genital region is depressed, by contraction of the lateral muscles in segments seven and eight. The ventro-medial groove on segment nine is opened by the contraction of muscle 10, a tergo-sternal muscle. This allows the depression of the ovipositor out of its sheath by the contraction of the gonocoxal muscle 4 (fig. 4.3). Muscles 1 and 2 of the first gonocoxa also function in the depression of the blade. The initial incision is made by the gross backward thrusting of the abdomen requiring no independent movement by the ovipositor during which time muscles 8 and 9 maintain the ovipositor in position. Further penetration of the plant tissue is brought about by the sawing action of the second gonapophyses. This movement is initiated principally by the alternating contraction of the two sets of antagonistic muscles of the second gonocoxa, muscle 4 on the one hand, and muscles 5, 6 and 7 on the other.

The massive musculature associated with the second gonocoxa suggests that this silvite is of prime importance in the movements of the ovipositor. The pivoting of the second gonocoxa by the gonangulum allows for a highly efficient system, and the muscular arrangement suggests that it is a posterior thrust of the ovipositor which is functional in the cutting of the plant tissue. The linkage mechanism ensures that

movement applied to one blade is also transmitted to the other.

4. Discussion

Considerable debate has arisen concerning the terminology and homologies of the basal structures of the ovipositor, some of which can be resolved by developmental studies and will be discussed further in the next chapter. Scudder, on a higher family level, claims to have solved many of these difficulties, and his system is generally agreed with in this study. The present work disagrees in part with that of Ali (1958) and Kathirithamby (1971), who also worked on Graphocephala in some of their interpretations of the basal parts, and hence their possible function. It is agreed with Scudder that the gonangulum is one of the most important sclerites in the control and co-ordination of movement in the ovipositor, though it is considered that the interlocking devices of the blades are also of considerable importance, a factor that appears to have been neglected by several previous authors, though Smith (1969) has described them for the Hymenoptera.

Some characters of the gonapophyses, determined in this study are somewhat unusual : (a) the inter-locking mechanisms between the first and second gonapophyses: Though present it is not functional in distal third of the blades and also the change in direction of the mechanism along the ovipositor. (b) The relatively long period necessary to attain full sclerotization of the blades after the final moult.

CHAPTER 2

DEVELOPMENT OF THE FEMALE EXTERNAL GENITALIA

1. Previous work on the developmental morphology of the female genitalia in the Hemiptera

Early works on the structure and development of the reproductive system and external genitalia in the Hemiptera include those by Christophers & Cragg (1921), Kershaw & Muir (1922), Singh Pruthi (1925) and George (1928), but they differed considerably in their conclusions. Metcalfe (1932) agreed with the findings of George but in all studies the descriptions are over simplified with little attention being given to the basal components of the ovipositor. The more recent studies of Scudder (1959, 1961a, 1961b, 1964) go some way to describe the development of the ovipositor, though he takes a phylogenetic standpoint and is concerned with the higher taxonomic groups. No detailed account of the development of the external anatomy of a Cicadellid could be found in the literature. The work of Helms (1968) made no attempt to provide a detailed description of development in Empoasca fabae, referring the reader to the older works of Kershaw & Muir (1922), who provided a general description within the Auchenorryncha, the work of Metcalfe (1933) on the Cercopid Philaenus spumarius, and that of Dupuis (1949) on the Cicadellid Ledra aurita. However, none of these descriptions provided any real detail. Kathirithamby (1971) provided a generalized description of the development of the external genitalia in Cicadellids, based upon six species, but her work does not constitute an authoritative study.

The more recent works concerned with the postembryonic development of insects (Lawrence 1970; Kudryasheva 1972; Lawrence 1975; Sedlak & Gilbert 1975) have avoided a descriptive approach and extended into the physiology and ultrastructure of single cell types, though the fundamental descriptions are still lacking.

Some orders have been better reviewed than others. The Orthoptera have been well described recently (Cejchan 1960; Dobosh 1969; Saenger & Helfert 1976). The structure and development of the external genitalia in the Psyllinae was extensively described by Zucht (1972).

From the above outline of the literature it can clearly be seen that a comprehensive study of the postembryonic development of the external female genitalia is required for the Cicadellids and it is intended that the present study will go some way towards that.

2. Method

The specimens used in the present study were hatched from eggs maintained in a constant temperature room. Within twelve hours of hatching the nymphs were individually sleeved on cut Rhododendron and kept at 21°C and a light regime of 16 hours light, 8 hours dark. A total of 100 nymphs were cultured in this way, of which, at the third instar, 61 proved to be female, and the remainder were disregarded. For each instar 10 nymphs were prepared for scanning electron microscopy as outlined below, the remainder were observed using a Wild M6 stereoscope dissecting microscope. The genital region of the fourth and fifth instar nymphs was dissected to determine the development of the basal components of the ovipositor.

Material for electron microscopy was either fixed in 50% acetone for 3 hours at room temperature of prefixed in 2% isotonic osmium tetroxide for 2 hours at 4°C followed by post-fixation in 4% isotonic glutaraldehyde for 4 hours at 4°C. All solutions were buffered at p.H. 7.3 - 7.4 with a cacodylate buffer. Material was dehydrated overnight with acetone, under vacuum, in a desiccator prior to substitution and critical point drying with liquid carbon dioxide. Various methods of dehydration were employed, including air drying, freeze drying and condensation under vacuum in a rotary condenser, but critical point drying provided the best, reproducible results.

Specimens were attached to a stub by double sided adhesive tape, gold coated in a Polaron E 5000 sputter coater and viewed in a Cambridge Stereoscan Type 96113 Mark 2A scanning electron microscope.

Some difficulties were experienced with natural wax on the specimen. Normal methods of wax removal failed, including a methanol/chloroform mixture, and the best results were obtained if specimens were washed in ultra-distilled water in a Kerry sonicator for approximately two minutes prior to fixation.

3. Development of the ovipositor in Graphocephala fennahi

No previous study of this type has been undertaken within the Cicadellids. The gross appearance of the abdomen undergoes considerable change from that of the first instar nymph through to the adult abdomen (fig. 2.1). During the early instars, abdominal segment nine is large and bulbous when compared to preceding segments and possesses a terminal group of setae. The ventral flexing, typical of the nymphal abdomen, is lost during the final moult, the adult abdomen being characterised by the greatly enlarged tergite nine or pygofer, which is flexed dorsalwards.

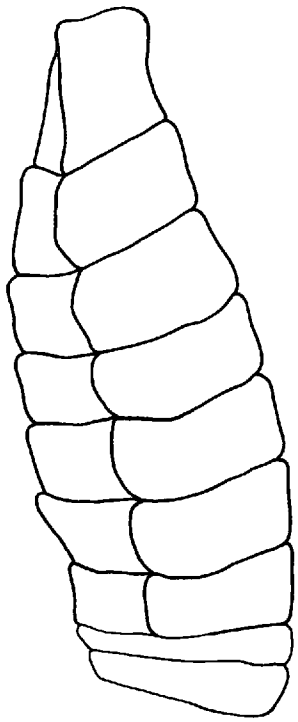
No externally visible genital buds are present on the ventral surfaces of segments eight and nine during the first and second instars (fig. 2.2). This disagrees with the findings of Helms (1968) who stated that in E. fabae external genital rudiments were visible from the first instar. This seems unlikely to the present author, who suggests that Helms may have incorrectly determined the age of his instars.

(a) Development of the gonapophyses.

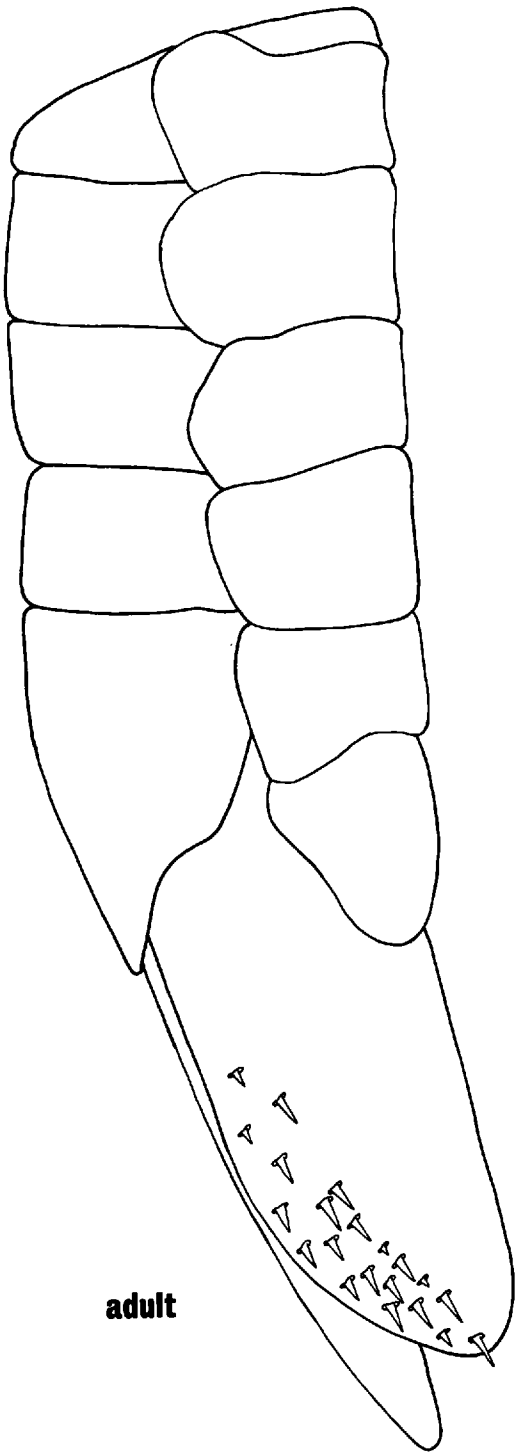
Transverse and parasaggital sections through abdominal segments eight and nine of G. fennahi revealed the presence of developing genital rudiments in the venter of these segments. During the early part of the first instar the epidermis

Fig 2.1

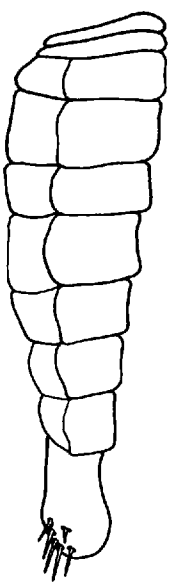
Comparison of gross abdominal form



IVth instar



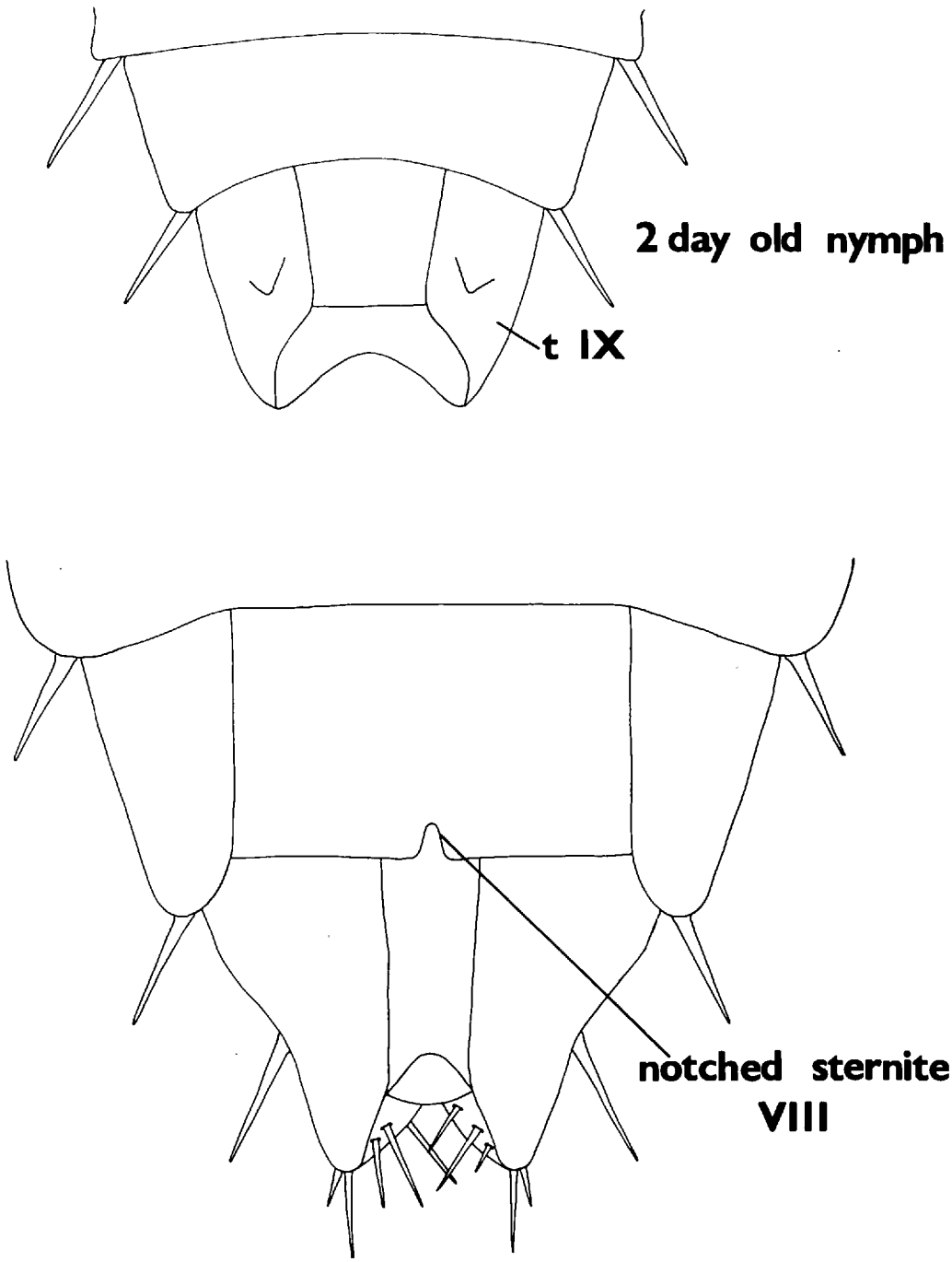
adult



early III

Fig 2.2

Ventral view of segments VIII and IX



of the sterna in the genital region appeared normal, composed of a single layer of cuboidal cells approximately 4.0 μm thick, with well defined, slightly basiphilic, nuclei. During the late first and early second instar (approximately 6 days old), the epidermis in the posterior two-thirds of segment eight became hypertrophied, differentiating into a median proliferation some 15 - 25 μm thick, with thinner lateral zones which lay beneath the longitudinal ventral muscles. In the posterior zone of the median proliferation, a pair of postero-laterally directed lips develop which represent the precursors of the first gonapophyses. The anterior part of the proliferation and the region between the developing lips indicates the site of origin of the spermatheca. A transverse section through the proliferation reveals a large number of strongly basiphilic nuclei stacked several layers thick and measuring 3.5 μm diameter; occasional mitotic figures were present.

At the same time the epidermis in the anteroventral region of segment nine was also undergoing proliferation to produce a layer approximately 15 μm in thickness. Almost midway along the sternum, medially, lips similar to those described in segment eight, developed by the infolding of the proliferation to produce the rudiments of the second genital buds.

Multiplication of the epidermal tissue in the ventral region of segments eight and nine continued through the second instar, the two pairs of genital buds continuing to thicken and extend posteriorly. Transverse sections through the buds show that the distal 10 to 15 μm are solid composed of strongly basiphilic, spherical nuclei 2.5 μm diameter; cell walls are not distinguishable and the granular cytoplasm stains deeply with haematoxylin. By the mid second instar the anterior lips are 35 - 40 μm long, and the posterior lips 23 - 27 μm long. Except for the solid distal cap the rudiments are hollow with a lining of epidermal tissue some

8.0 μm thick.

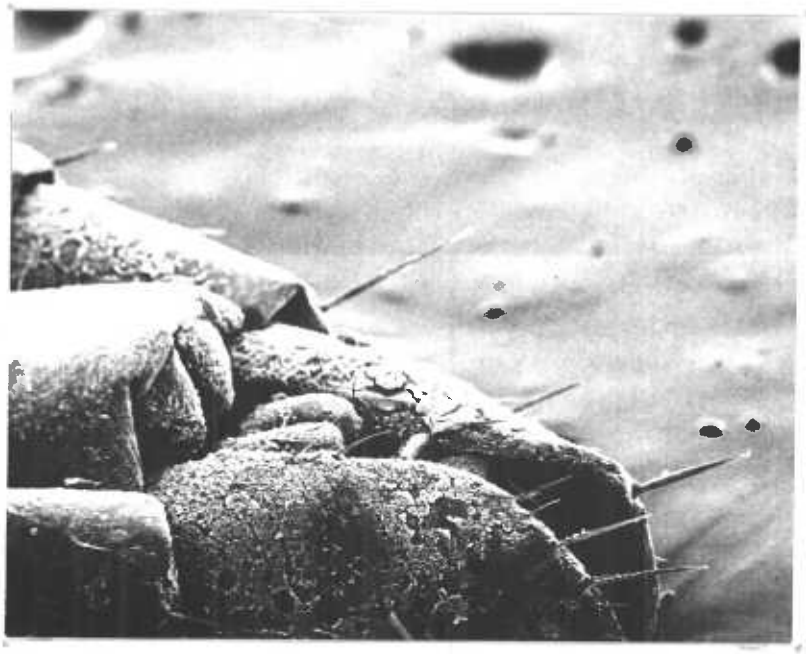
External genital buds were not visible until the early third instar (approximately 16 days old), when one pair of primary genital rudiments was present medially on the sterna of segments eight and nine. The anterior rudiments were borne on the posterior margin of sternum eight and the posterior rudiments arose one third of the distance from the anterior margin of sternum nine. These findings disagree with those of Kathirithamby (1971), who stated that the rudiments arose from the posterior margin of the eighth and ninth sternites. A lateral view during the third instar (fig. 2.3) shows that the anterior rudiments appear as vertical structures which emerge from the posterior margin of the depressed eighth sternum.

Throughout the third instar the rudiments continue development as two distinct pairs of structures. In transverse section they are seen to be closely applied to sternum nine immediately lateral of the mid-line. Continued division of the epidermal tissue within both pairs of rudiments results in a decrease in the diameter of each rudiment lumen to approximately 4.0 μm, each of them opening independently into the haemocoel. The rate of extension is greater in the posterior rudiments during the third instar and at the late third instar the anterior rudiments do not conceal the bases of the posterior ones.

The photomicrographs show that the anterior rudiments are not closely applied to each other in the mid-line (fig. 2.4 and 2.5), but are directed posterolaterally. The posterior rudiments remain closely applied throughout their length and distally are directed slightly ventrally. By the late third instar (fig. 2.5 and 2.6) the posterior rudiments have extended to the distal edge of sternum nine, which has already begun to sink between the lateral edges of tergite nine.

During the third instar considerable differentiation of the genital disk occurs, and the unstructured appearance of the cell mass is lost, all the ectodermal tissue

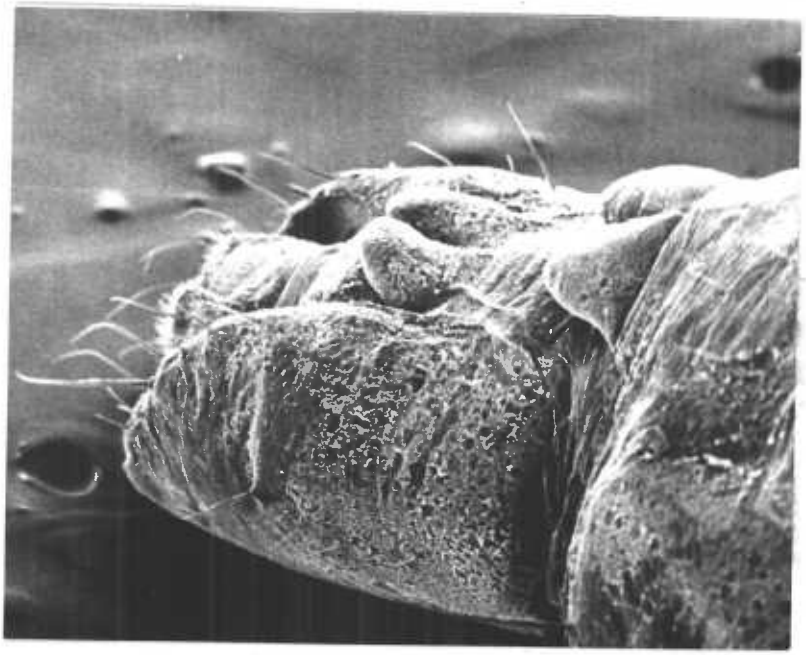
Early III instar



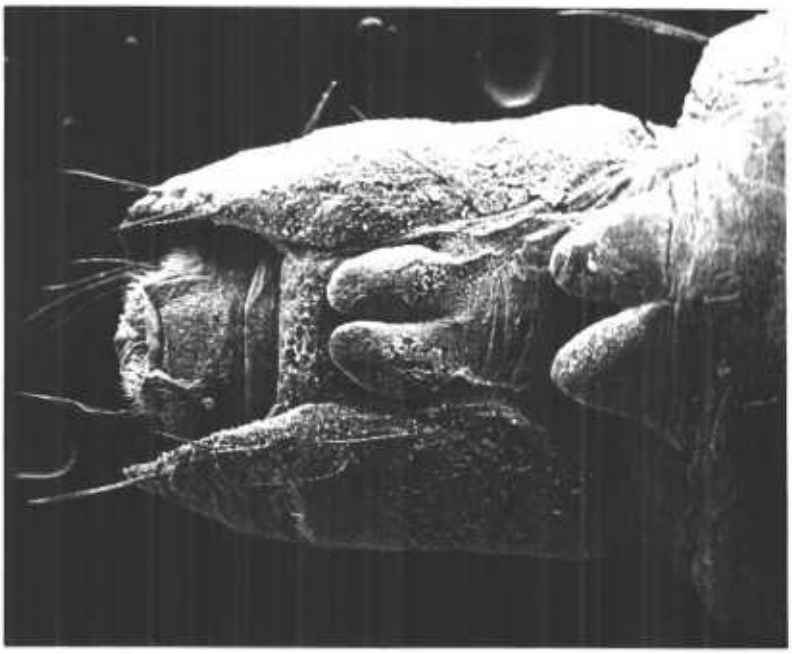
Lateral view x 120 10 KV Gamma 2

Fig. 2.4

Mid III Instar



Lateral view x 115 10 KV Gamma 2



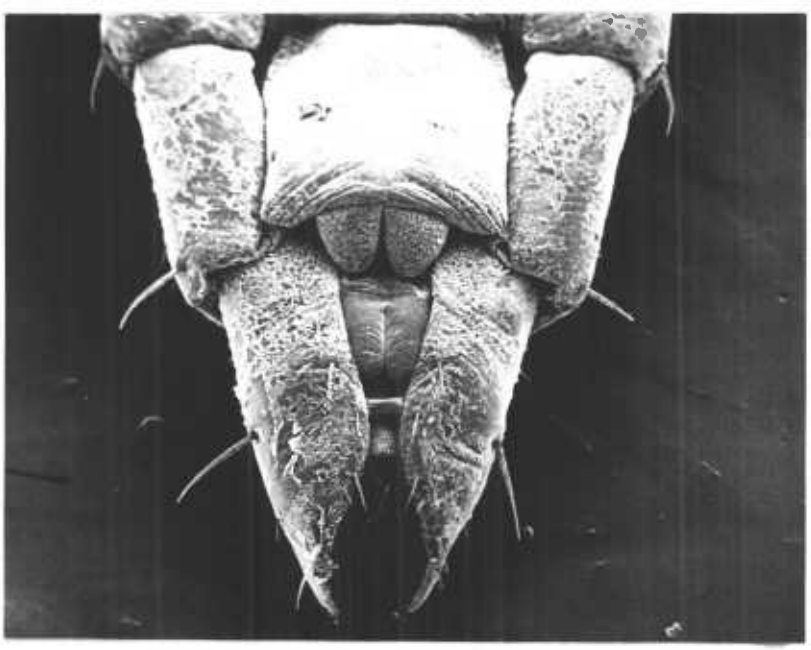
Ventral view x 110 10 KV Gamma 2

Fig. 2.5

Mid III Instar

Lateral view x 230 10 KV Gamma 2

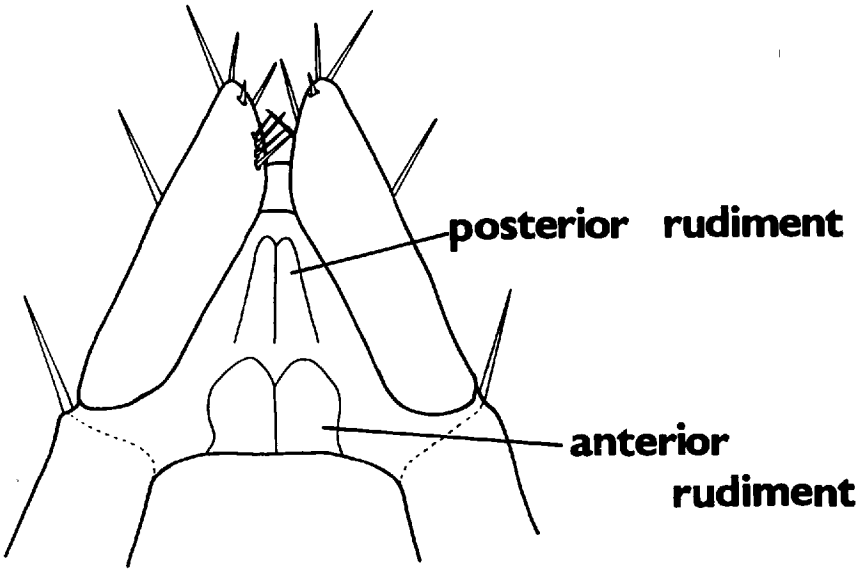
Late III Instar



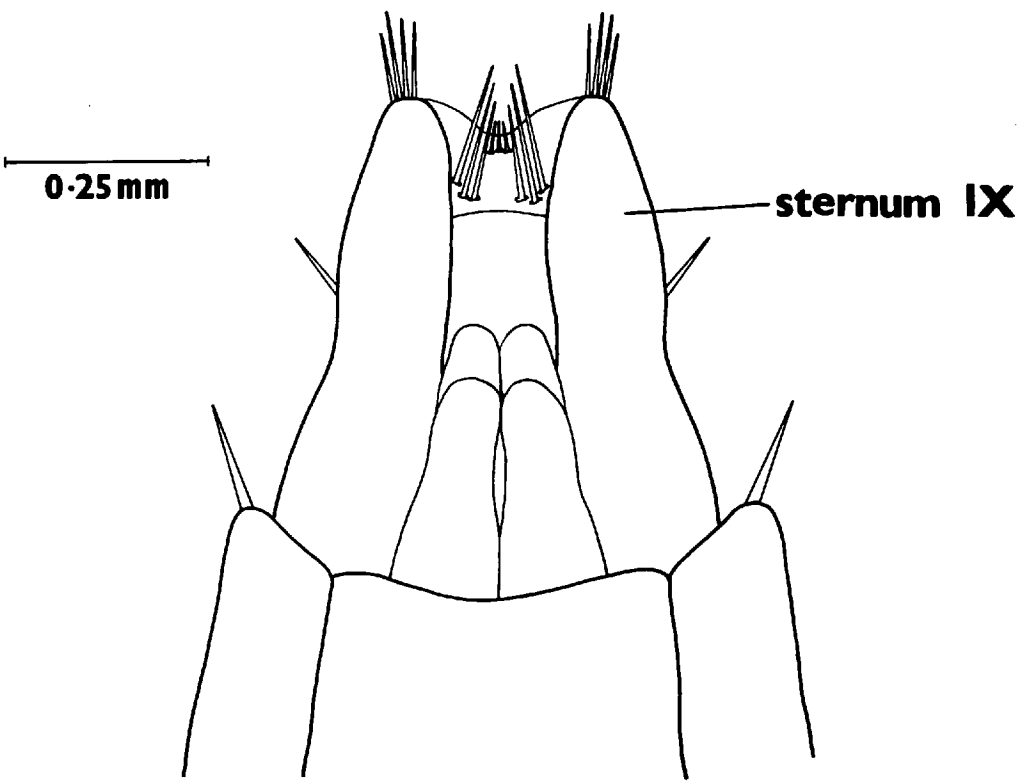
Posterior view x 112 10 KV Gamma2

Fig 2.6

Ventral view of segments VIII and IX



Mid III instar



Early IVth instar

being incorporated into the developing genitalia or efferent genital ducts. The ectodermal tissue making up the external genitalia retains its strongly basiphilic character, and the nuclear diameter has increased to 3.0 μm.

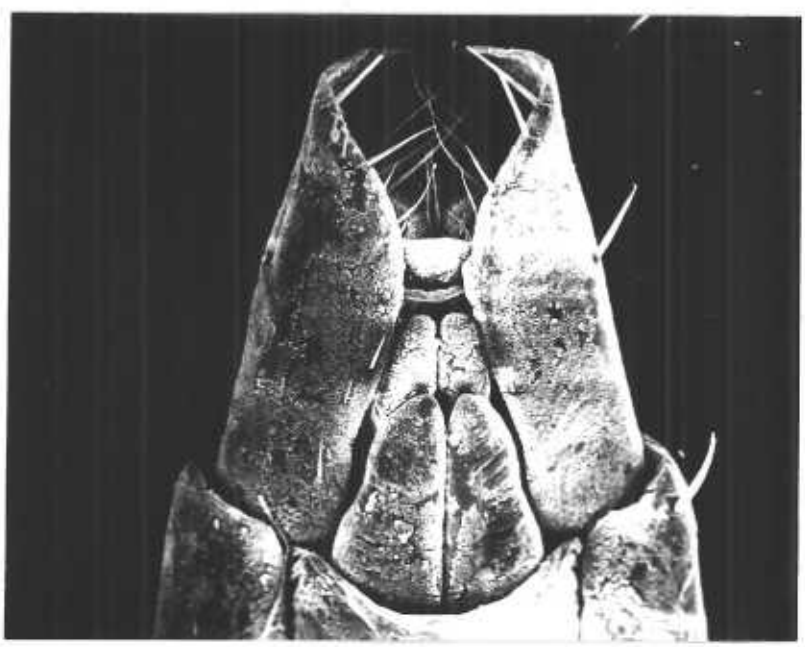
Considerable growth of the anterior rudiments occurs around the third nymphal moult, and by the early fourth instar (fig. 2.6 and 2.7) they conceal the bases of the posterior rudiments. Their bases have broadened to cover the ventral surface of segment nine, the entire sternum of which, except for the extreme anterior portion, has become infolded to sink beneath the lateral edges of tergite nine, forming a median ventral groove in which, during the adult stage, the ovipositor lies. The groove is brought about by the proliferation of epidermal cells associated with the lateral boundaries of tergite nine and the ventral growth of this region around the sternal region so effectively burying it. This process continues through the fourth instar to produce a well developed groove, the dorsal and much of the lateral surface of which is composed of sternum nine, and the extreme lateral edges of tergum nine. The groove runs ventrally for the whole length of segment nine, except in the extreme anterior part.

The appearance of the posterior rudiments during the early fourth instar is similar to that seen during the third instar, and they do not project beyond sternum nine. In transverse section they are round or oval, each rudiment containing a narrow lumen continuous anteriorly with the haemocoel in segment nine. The epidermis shows no cellular boundaries under the light microscope, and the nuclei, stacked three or four deep, together with the cytoplasm, retain their strongly basiphilic character.

During the mid fourth instar, when the nymph is approximately 36 days old, a groove appeared anteriorly on each posterior ovipositor rudiment and ran posteriorly, longitudinally bisecting each rudiment into an inner and outer portion. The division was asymmetrical, the inner portion being larger than the outer, and forming the

Mid IV Instar

Lateral View x 115 20 KV Gamma 2



Posterior view x 125 20 KV Gamma 2

rudiments of the second gonapophyses and gonoplacs respectively. For ease of description, these will be termed the posterior and lateral rudiments. Histologically the division starts as an infolding of the epidermal tissue, which drags the cuticle with it to produce, initially, a shallow groove. Within 36 hours of its first appearance, the groove has deepened and extended posteriorly to bisect the rudiment. Transverse sections through the late fourth instar nymph reveals the posterior and lateral rudiments as discrete structures and differential growth is initiated before the end of this instar (fig. 2.8).

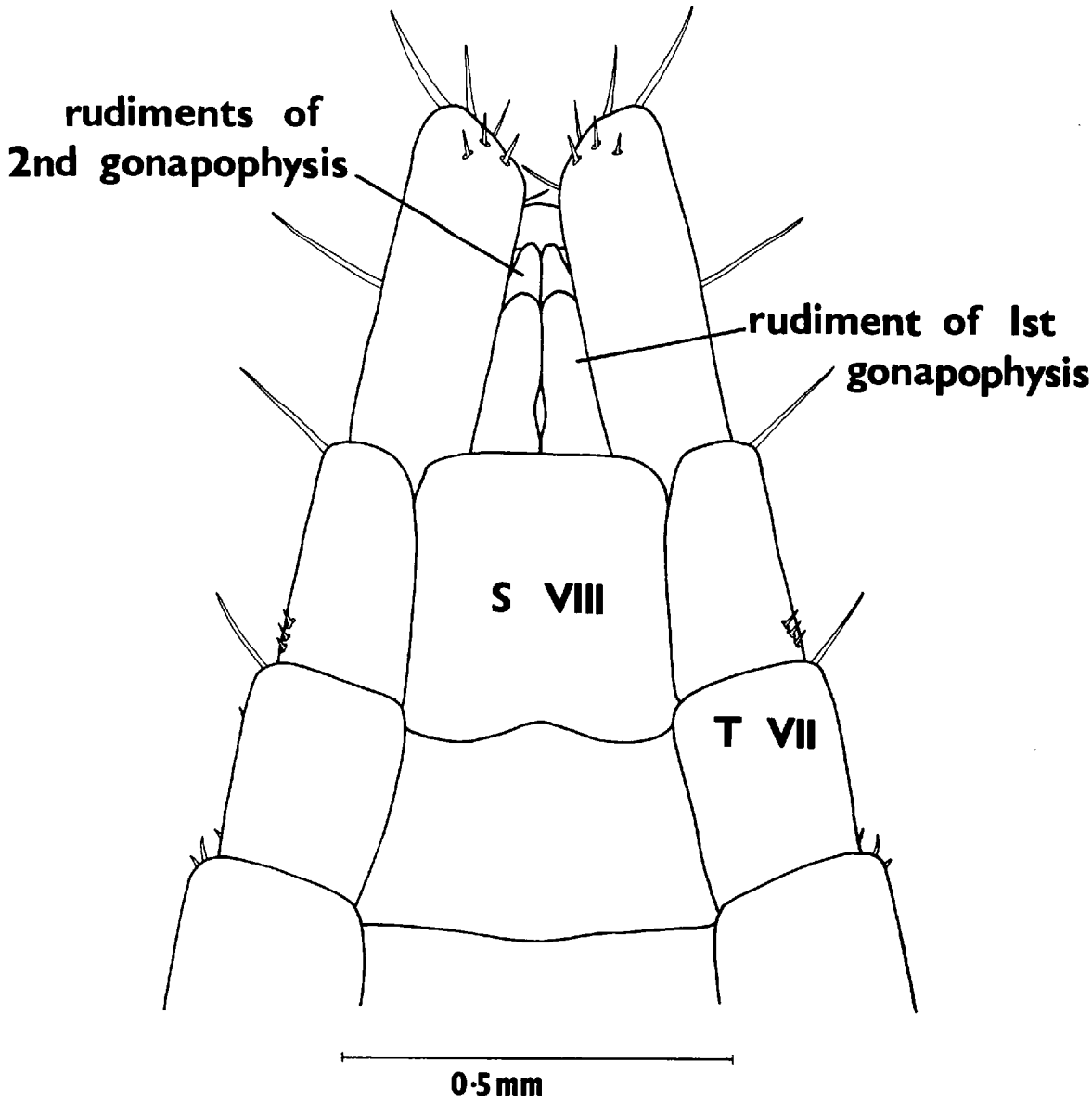
By the late fourth instar the anterior rudiments lie ventral to the posterior, so that only the distal end of the latter are visible. The lateral rudiments have undergone elongation to the distal end of segment nine and are flexed slightly ventralwards. For most of their length they are obscured by the lateroventral fold of tergite nine, but their distal ends curve in towards the mid-line around the posterior rudiments and touch in the mid-line (fig. 2.9). This is the condition at the end of the fourth instar.

Relatively little growth of the genital rudiments occurs during the fourth nymphal moult. By the mid fifth instar (fig. 2.10) the lengths of the rudiments are still similar to those seen in the previous instar. The posterior rudiment projects approximately 215 μm beyond that of the anterior pair, a condition also seen in the adult, where the second gonapophyses project approximately 320 μm beyond the first. The lateral rudiments continue elongation, their distal ends projecting posteroventrally and curving around the distal extremes of the other genital rudiments.

Sections through the genital region during the early fifth instar indicate that sclerotization of the genital rudiments had begun, though, throughout this instar the cuticle maintained a fushsinophilic reaction, indicating that sclerotization was not

Fig 2.8

Ventral view of a late IV instar nymph



rudimentary gonoplacs largely obscured by tergum IX

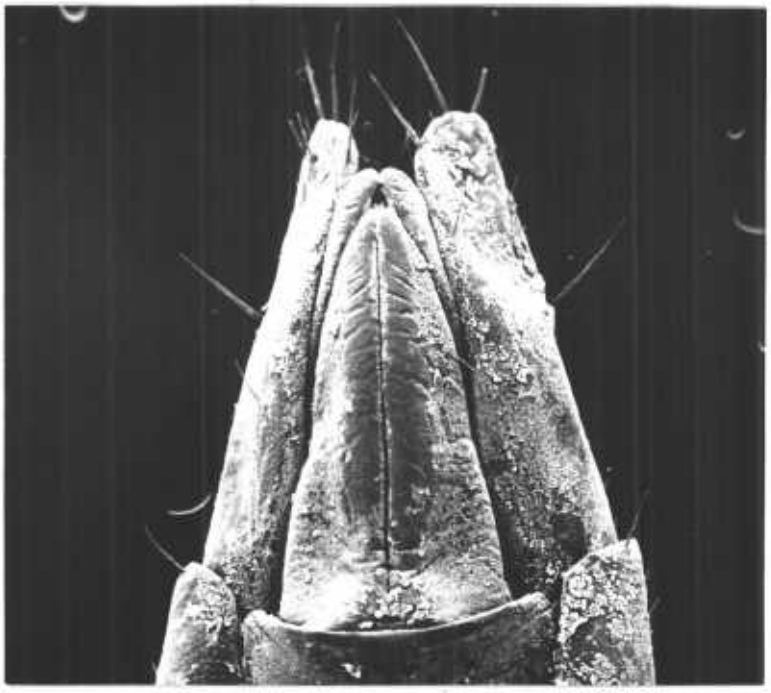
Late IV Instar

Posterior view x 120 20 KV Gamma 3

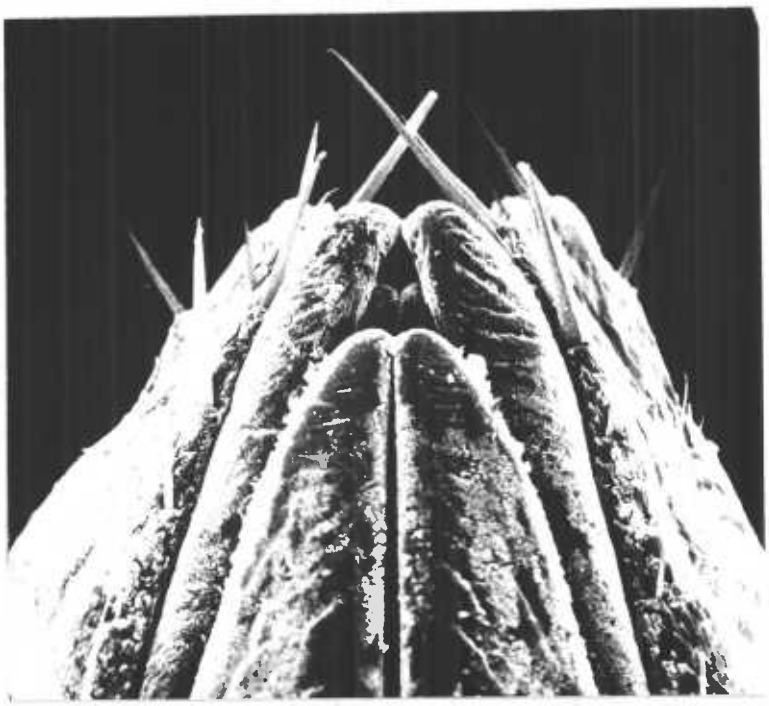
Distal part of 1st and 2nd Gonapophyses
x 250 20 KV Gamma 2

Fig. 2.10

Mid V instar



Ventral view x 56 10 KV Gamma 2



Distal part of 1st and 2nd Gonapophyses
x 220 20 KV Gamma 1

complete. By the end of the fifth instar (2.11) the rudiments had darkened, showing increased sclerotization of the parts, particularly the distal regions of the rudiments. In transverse section the rudiment lumina were clearly seen, and in both anterior and posterior pairs remained continuous with the haemocoel. The lateral rudiments did not contain a lumen. A dorsal view of the mid fifth instar nymph shows that the genital rudiments are just visible at the posterior end of the abdomen (fig. 2.12), and it is in this condition that the nymph enters the final moult.

Transverse sections through the genital region during the latter half of the fifth instar allowed the pharate condition to be observed and sclerotization of the definitive ovipositor blades. The pharate stage was first apparent in the genital region at approximately the 59th day, and by the end of the fifth instar (64 - 65 days) the pharate gonapophyses could readily be separated and withdrawn from the genital rudiments. At this stage the blades were well formed, strongly laterally compressed, though shorter than those of the adult (total length of the pharate second gonapophysis just prior to the final moult was 2.43 ± 0.15 mm compared to the adult length of 2.66 ± 0.09 mm) and the dorsal teeth were already formed. Each pharate blade contained a single intragonapophyseal space, which was blocked anteriorly and not continuous with the haemocoel. Sclerotization was well developed, and the blades showed dark brown pigmentation. The linkage mechanism was very poorly developed, and similar to that seen in the distal part of the adult blade (fig. 1.4). Proximally the inner ramus of the first gonapophyses continued anterodorsally to expand into a small head termed plate m by Snodgrass (1935). By the end of the fifth instar this had already fused with the developing gonangulum.

(b) Development of the basal components of the ovipositor

(i) Development of the gonocoxae

These arise anterodorsally to the bases of the anterior and posterior rudiments

Fig 2.11

Ventral view of segments VIII and IX of a mid Vth instar nymph

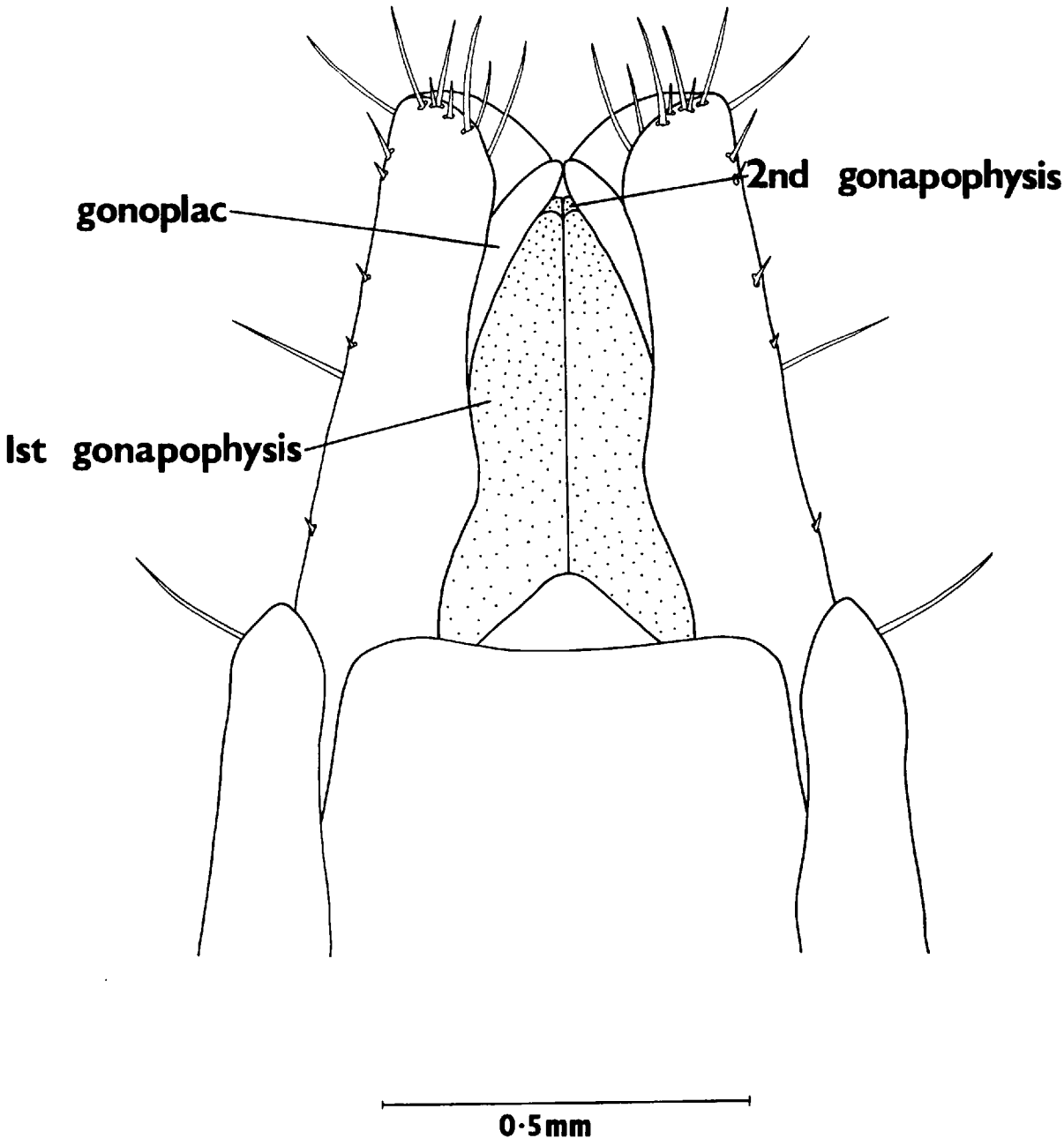
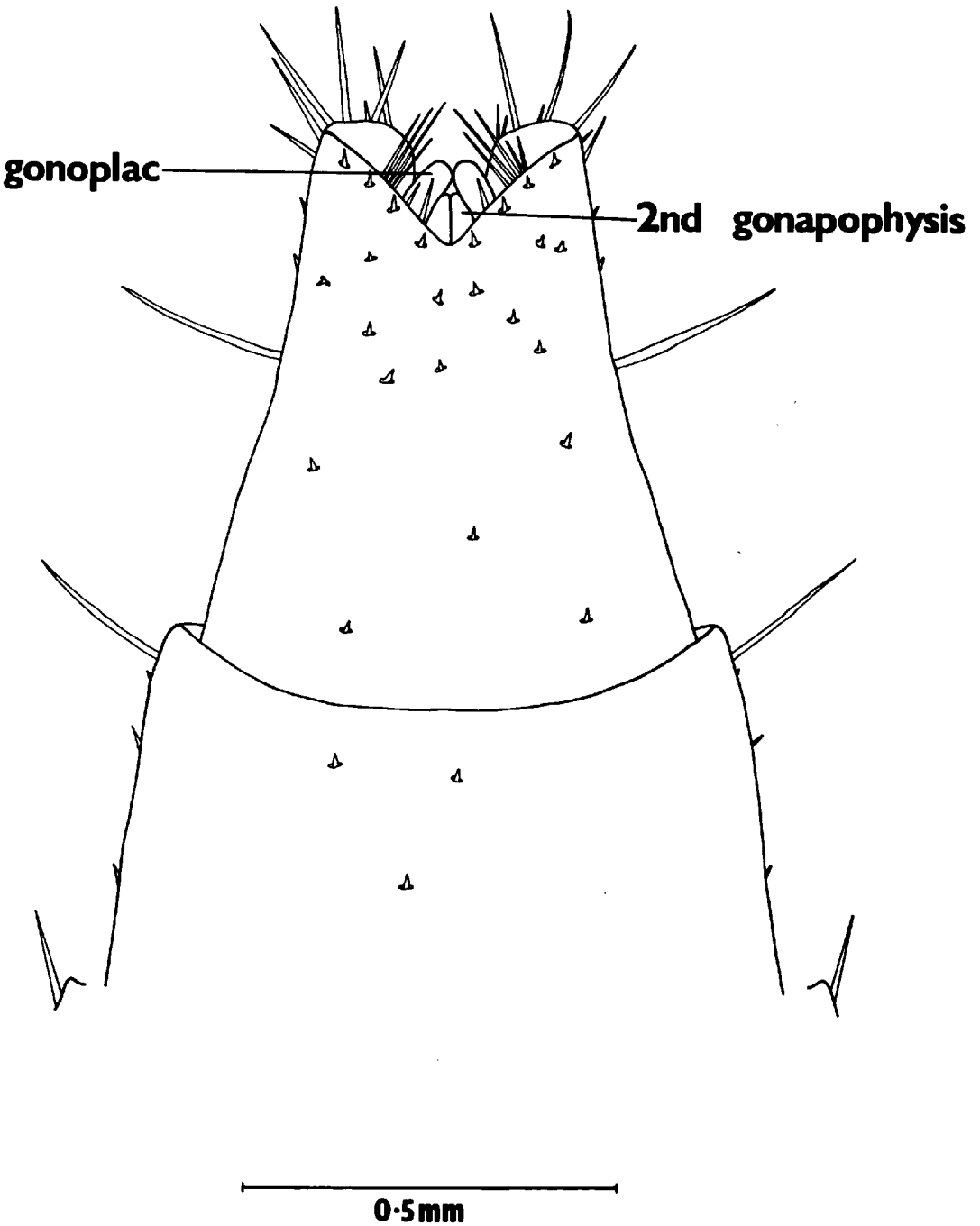


Fig 2.12



Dorsal view of segments VIII and IX in a mid Vth instar nymph

and are evident externally from the mid third instar. In transverse and parasagittal section they were seen to differentiate from the lateral zone of the genital disks in segments eight and nine during the late second instar after the differentiation of the anterior and posterior lips. The rudiment of the first gonocoxa arises mid-way along segment eight in the dorsal region of the sternum as a local thickening of the genital disk. During the early second instar the epidermal tissue in this region forms a single layer of cells approximately 4.0 μm thick, the nuclei being slightly basiphilic, and the cell boundaries indistinct. During the mid second instar this lateral zone enters a phase of proliferation, mitotic figures being present within the cytoplasm; it thus produces a lateral thickening, approximately 20 x 16 μm and 12 μm thick on each side of segment eight. The nuclei in this region were spherical, approximately 3.5 μm in diameter, strongly basiphilic and densely packed.

The rudiments of the second gonocoxa arise in essentially a similar manner on the anterodorsal edge of sternum nine in front of the developing posterior rudiments by a proliferation of the anterolateral genital disk in segment nine.

By the late third instar, the developing first and second gonocoxae can be seen as discrete sclerites lying dorsolaterally to the bases of the anterior and posterior rudiments, the second gonocoxa appearing to lie in the intersegmental fold between segments eight and nine. Both sclerites, at this stage, are triangular in form and subsequent development can only be followed by sectioning and dissection, since the first gonocoxa become concealed by the posterior development of sternum seven and the second gonocoxa, due to growth of the lateral margins of tergum nine, comes to lie beneath the lateral fold of tergum nine. Little development occurs during the fourth and early fifth instars. The rudiment of the first gonocoxa undergoes little growth and its nuclei retain their strongly basiphilic character. The rudiment of the second gonocoxa undergoes some elongation in the dorsoventral plane towards the

condition seen in the adult and both pairs of gonocoxa show progressive sclerotization during nymphal development.

During the mid fifth instar the dorsal margin of the first gonocoxa undergoes considerable sclerotization and its anterodorsal margin becomes closely applied to the anterior edge of the anterior rudiment, fusion does not occur until the final moult. Similarly, during the mid fifth instar the proximal end of the posterior rudiment extends anterodorsally to fuse with the anterodorsal edge of the developing second gonocoxa to form the definitive adult condition. At the same time, the proximal end of the lateral rudiment is connected by membrane to the posteroventral edge of the second gonocoxa. Transverse sections through the developing second gonocoxa during the mid fifth instar reveal that the sclerite, being laterally compressed, is approximately 20 - 25 μm in thickness with a thin cuticle and no intra-gonocoxal spaces, the interior being filled with strongly basiphilic nuclei of approximately 4.0 μm diameter. In the adult condition the sclerite undergoes considerable sclerotization, and by the twelfth day of adult life there is no cellular component remaining.

(ii) Development of the gonangulum

Much of the work into the development of this sclerite has been conducted in the Orthoptera (Snodgrass 1935; Qadri 1940; Gupta 1950; Scudder 1957). Where, though the terminology differs, there is general agreement that the gonangulum develops from sternum nine. It has been shown that primitively the gonangulum, in the development of Lepismodes, arises from the second gonocoxa. (Snodgrass 1935; Scudder 1961). Stys (1959) in a general paper on the origin of the pterygote ovipositor, stated that in Hemiptera the gonangulum is derived from the intersegmental membrane between segments eight and nine, which underwent secondary sclerotization.

In the present study of Graphocephala fennahi a small sclerite, later determined to be the gonangulum, developed from the anterodorsal edge of sternum nine immediately

posterior to the rudiment of the second gonocoxa. This developed in the late second instar from the posterior portion of the lateral zone of the genital disk that gave rise to the second gonocoxa. During the late third instar, it appeared as an irregular four sided sclerite approximately 8 μm x 10 μm which underwent progressive growth through to the fifth instar, when it came to lie dorsal to and slightly posterior of the second gonocoxa. During the fifth instar it underwent rapid growth to produce a five sided sclerite, fused anteriorly with the ramal extension of the first gonocoxa, tergite nine posterodorsally and articulating with the second gonocoxa ventrally.

4. Discussion

The present section provides a detailed description of the development of the external genitalia in the Homopteran G. fennahi; it represents the first comprehensive study within the Auchenorryncha and in respect of some features it provides information lacking in the earlier descriptions of development of insects generally. While the general description agrees with those of Snodgrass (1935) and Scudder (1959; 1961; 1971) the development of the posterior rudiment is clarified, and it is shown to involve actual division of a single genital bud and not the outgrowth of separate proliferations on each side (Scudder 1964). The development of the basal components is described and they are demonstrated to be of sternal origin and are separate, in their origin, from the gonapophyses. The present study disagrees with the findings of Stys (1959), and it is shown that in G. fennahi the gonangulum is also of sternal origin and its connections with the first and second gonocoxa and tergum nine are formed only in the last nymphal instar.

The question of sternal or appendicular origin of the female genitalia remains to some extent unclear, and it is unlikely that any major contribution can be made in this direction from studies of a single group, particularly a specialized group such

as the Hemiptera. The question is complicated, due to the pterygote abdominal sternum very probably being composed of cells from three embryonic fields. This fact has been acknowledged since Haase (1889), the fields being a median true sternal part and two lateral zones developed from segmental appendage rudiments. This may suggest that since, in Graphocephala, the gonapophyses are medial outgrowths, they may be of true sternal origin. The argument is complicated in the Orthoptera, where in Gryllus the second gonapophyses and gonoplacs arise laterally, supposedly in the appendage zone of the sternum.

It would not be justified to make far-reaching morphological statements without referring to the primitive condition as seen in the Thysanura, where the boundary between the sternal and coxal components, though clear in the pregenital region, remains uncertain posteriorly and the relationship between the gonapophyses, the coxae and sternum is not clear, so that the medial origin of the gonapophyses in Cicadellids is not, in itself, proof of a sternal or appendicular origin.

Matsuda (1958) considers it unlikely that embryonic limb rudiments should be suppressed only to reappear at a later stage as genital rudiments and advocates a sternal origin. It has, however, been argued by a number of authors (Cain 1955; Scudder 1964) that completely novel organs rarely arise in evolution, most structures forming by the modification of pre-existing structures (Michener 1942; Tuxen 1970). New information may be gained by vital staining, micro-dissection or radio-active labelling within the embryonic stage, but this represents a major problem in its own right and not the object of the present study.

CHAPTER 3

DEVELOPMENTAL ANATOMY OF THE FEMALE REPRODUCTIVE SYSTEM

1. Introduction

This investigation was conducted in order to provide a more complete understanding of the structure and functioning of the female reproductive system in Hemiptera.

Much of the early anatomical work was performed on the Cicadidae, largely due to practical reasons, the small size of most Cicadellid species preventing their study at this time. The first work of consequence to deal with the reproductive system, within the Auchenorryncha, was that of Meckel (1808) on Tibicen plebeia. He was the first to describe the single long, thick median accessory gland which opens near the aperture of the vagina at the base of the gonapophyses. This is now a well known character of Auchenorryncha anatomy. The work of Dufour (1825) provided a detailed study of the anatomy of the Cicadid alimentary and reproductive systems. The two papers by Doyère (1837) were principally concerned with the external genitalia of cicadas, but he also described the glandular components which open into the genital chamber, Holmgren (1899), comparing the reproductive systems of Cicadellids, Cercopids and Fulgoroids, agreed largely with the findings of Doyère and Dufour on cicadas, though the latter, in addition to the unpaired median accessory gland, also possess paired glands at the anterior junction of the median oviduct. Holmgren was unable to detect such glands. Berlese (1909) failed to describe the median accessory gland in Cicadidae, but otherwise followed the descriptions of Dufour closely. Gadd (1910), studying seven species of cicadas, found that characters of the median accessory gland, principally length, were species-specific. Meyer's(1928) detailed descriptions of cicada anatomy only briefly mention that of the reproductive system.

There was a movement, during the early decades of the present century, to describe the developmental anatomy of the reproductive system, and several such studies were made within the Auchenorryncha, (Christophers & Cragg 1921; Kershaw & Muir 1922; George 1928; Metcalfe 1932). These studies provide an introduction to the subject, despite their obviously incomplete character. More recent work into the developmental anatomy and histology within the Auchenorryncha has been restricted to that of Helms (1968). In his study, Helms briefly describes the development of the system. No attempt was made to describe oogenesis and vitellogenesis, and the mature adult system is not mentioned. The work of Kathirithamby (1971) was restricted entirely to nymphal development of the external genitalia, and a description of the reproductive system is lacking in her account.

Considerable work has recently been carried out in the Heteroptera, particularly the Miridae (Davis 1955; Masner 1966; Ramamurty 1970; Davenport 1975). The work of Bonhag & Wick (1953) and Bonhag (1955a; 1955b; 1958) on Oncopeltus fasciatus is well known, though the later papers are restricted to the mature ovary and only concerned with oogenesis.

Within the Miridae, Reduviidae, Nabidae and Anthocoridae, considerable differences are known to occur in the arrangement of the genital organs and ducts, compared to other Hemiptera making comparisons between the Heteroptera and Homoptera difficult. For instance, so far as is known, the definitive spermatheca of insects arises posterior to the opening of the median oviduct. In the Miridae, however, the sperm-receiving organ is anterior to the opening of the median oviduct, and it is not known if this organ is homologous with the spermatheca of insects generally.

There has been a tendency in the last 10 - 15 years for the structure and developmental anatomy of the gross reproductive system to be ignored in preference to physiological studies of the ovaries and the role of endocrine factors associated

with oogenesis and vitellogenesis (Telfer 1965; Moloo 1971; Zinsmeister & Davenport 1971; Huebner & Anderson 1972; Laverdure 1972). The fact that these studies have been performed in Hemiptera is not necessarily due to interest in the teletrophic ovary, but rather because some Heteroptera can easily be maintained in laboratory cultures.

The present study is not concerned with the hormonal control of the reproductive system; instead the developmental anatomy of the reproductive system from the earliest first instar nymph was studied in some detail using conventional light microscopic techniques. The histology of the adult system was then described, together with an electron microscopic study of the ovary.

2. Methods

Graphocephala fennahi was reared from eggs in a constant temperature room, as described in Chapter 2. In order that the chronological development of the reproductive system could be followed, slides were prepared at closely spaced intervals, during the nymphal and adult stages. Decapitated specimens were fixed in Bouin's fixative and formol saline, the former providing the best results. Penetration and fixation in both cases were aided by the use of a vacuum chamber at 55°C for 40 minutes. Material was dehydrated in alcohol or dioxan, cleared in xylene, in the case of the former, and embedded under vacuum in pure paraffin wax (M. pt. 56°C). Blocks were prepared for transverse, horizontal and parasagittal sections which were cut at 6 and 8 μm. In the fifth instar and adult stages, the complete reproductive system was removed under insect saline, by vivisection. This provided better results compared with whole-abdomen preparations due to the reduction in sclerotized material.

For general histological observations the following stains were used; Mallory's Phosphotungstic Haematoxylin, Heidenhain's Iron Haemotoxylin and Mallory's Triple Connective Tissue stain. All were useful general stains, the first two allowing detailed

histological descriptions while the Triple Connective proved particularly valuable during oogenesis.

A small amount of cytochemical work was undertaken, principally for the identification of nuclei acids during oogenesis. The following techniques were employed: (a) The Feulgen reaction for DNA by the release of aldehyde groups from the deoxypentose sugar groups of the nucleic acid by mild acid hydrolysis. The aldehydes were then caused to react with leucofuchsin which turns a reddish-purple colour and the RNA gives a pale green colour reaction. The best results were obtained after formol-saline fixation. (b) RNA was detected, using Trevan & Sharrock's stain as modified by Cullings (1963), using Pyronin Y and purified methyl green. The stain was diluted in an acetate buffer at pH 4.8. The results were RNA - red, DNA - green.

Specimen preparation for transmission electron microscopy was as follows :

Fixative A - 2g Paraformaldehyde + 25 mg Calcium chloride + 20 ml distilled water.
Heat to 65°C and add 0.2 ml 1M sodium hydroxide.
Cool to room temperature and add 10 ml 25% glutaraldehyde.
Make up to 50 ml with 0.2 M cacodylate buffer at pH 7.4.

Fixative B - Equal volumes of 5% osmium tetroxide and 0.2 M cacodylate buffer at pH 7.4.

Material was fixed for one hour at 4°C in fixative A, then washed in 0.12 M cacodylate buffer for half an hour (2 changes), and post-fixed in fixative B for one hour at 4°C. Material was washed in 0.1 M sodium acetate prior to block staining in 0.25% aqueous uranyl acetate for one hour. After washing in sodium acetate, the material was dehydrated in acetone and blocked up in Araldite. At the 70% acetone stage the material was block stained in 1% phosphotungstic acid and 1% uranyl acetate. The

material was section stained in Reynolds stain (lead citrate) and viewed in a Philips 301 transmission electron microscope.

3. Gross anatomy of the adult female reproductive system

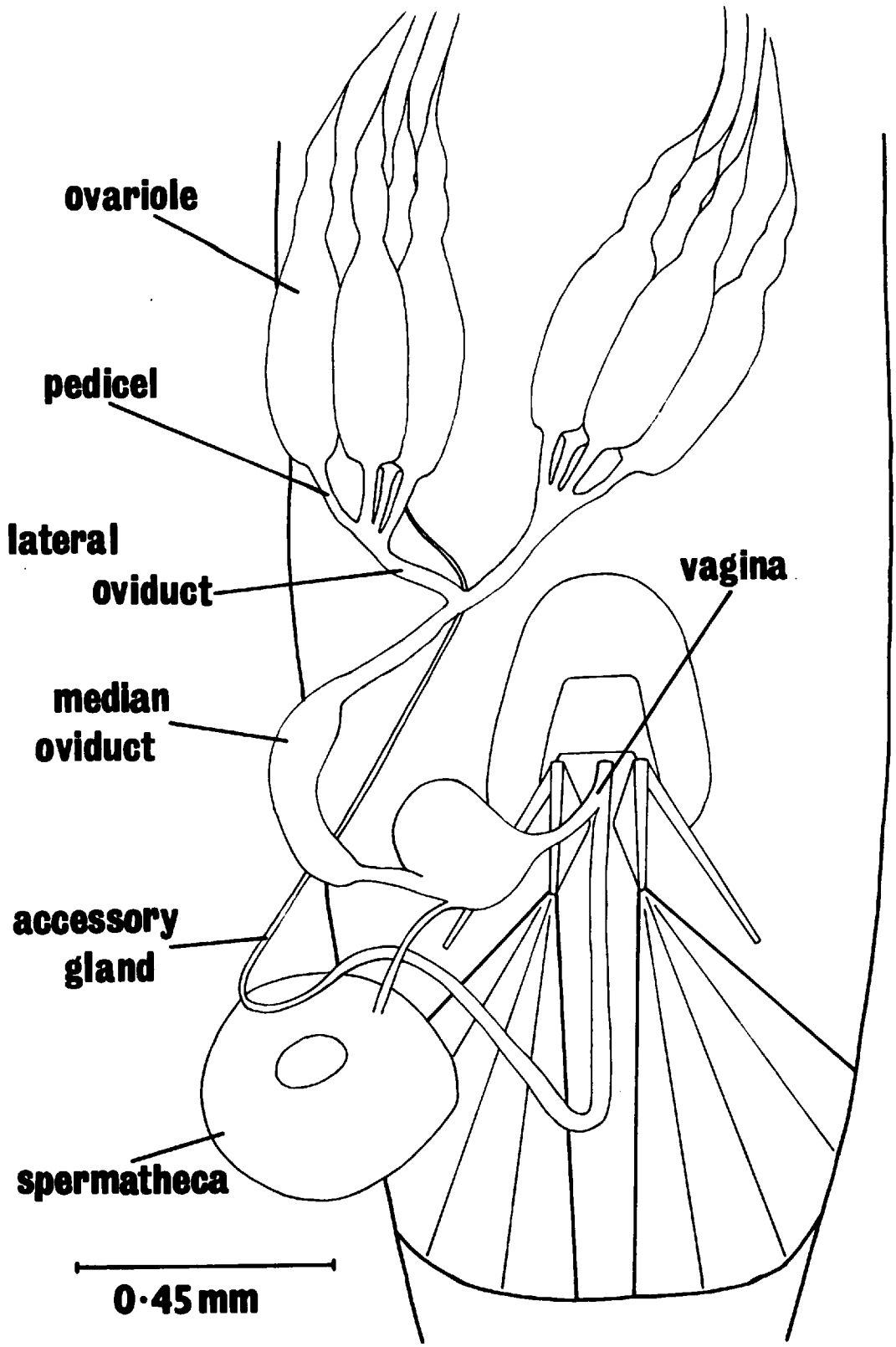
It is intended that a brief description of the definitive adult system is given before its development through the nymphal stages is described.

In the mature female a pair of ovaries occupy the greater part of the abdominal cavity, extending anteriorly from segment V to the metathorax. Each ovary is composed of four telotrophic ovarioles which lie beneath the alimentary canal. Each fully mature ovary measures approximately 2.4 mm in length, but they have undergone considerable growth during the first twelve days of the adult stage (fig. 3.1) and in an 8-day old virgin female each ovariole measures only approximately 380 μm in length. Each ovariole can be divided into four parts. (a) The anterior terminal filament, a mesodermal syncytium, the four filaments of each side uniting to form a suspensory ligament which passes anteriorly into the thoracic region where it is attached to the pleural epithelium in the metathorax, so effectively anchoring each ovary. (b) Apically the ovariole forms a pyramid-like germarium composed of trophocytes and oocytes. In all but the senescent female this is a zone of considerable mitotic activity, meiosis having been completed in the earlier stages. (c) The vitellarium lies immediately posterior to the preceding zone and in the mature female represents the largest part of the ovariole, usually containing one fully developed oocyte and 2 to 3 oocytes in various stages of vitellogenesis and previtellogenesis. The walls are composed of prefollicular and follicular tissue. This is the zone of oocyte development. (d) The final zone is the pedicel, which links the ovariole with the lateral oviduct. In Graphocephala, eggs are not stored in this region. The whole of the ovariole is covered by a muscular sheath. (p. 125).

The lateral oviducts are approximately 150 μm in length and pass to the anterior edge of segment seven, where they join, near the mid line, with short branches of the

Fig 3.1

Reproductive system of a 10 day old adult female



median oviduct. They are of mesodermal origin (p. 97) and possess a muscular sheath. The median oviduct can be divided into a number of regions according to the degree of muscular development and the secretary nature of its epithelial cells. Though it is of ectodermal origin, it is not lined by an intima. It passes posteriorly into the enlarged, thin walled vagina, which opens posteriorly between the gonapophyseal bases on the anterior part of segment eight. A single large spermatheca enters the vagina dorsally by a short, narrow tube. The glandular portion forms a large, spherical, thin walled sac which lies, in the natural position, on the right hand side of the abdomen (fig. 3.2) partially obscured by the median oviduct in segment six. In an inseminated female a pink mass of ejaculatory material is obvious within the spermathecal bulb.

The unpaired median accessory gland opens dorsally at the extreme end of the vagina. It runs posteriorly into segment nine, between the muscles of the second gonocoxa, before turning anteriorly to pass dorsal to the median oviduct. Behind segment six it tapers to a very fine tube, which passes between the ovaries and becomes closely associated with the terminal filaments of the left hand ovary. It ends freely in the haemocoel of the metathorax.

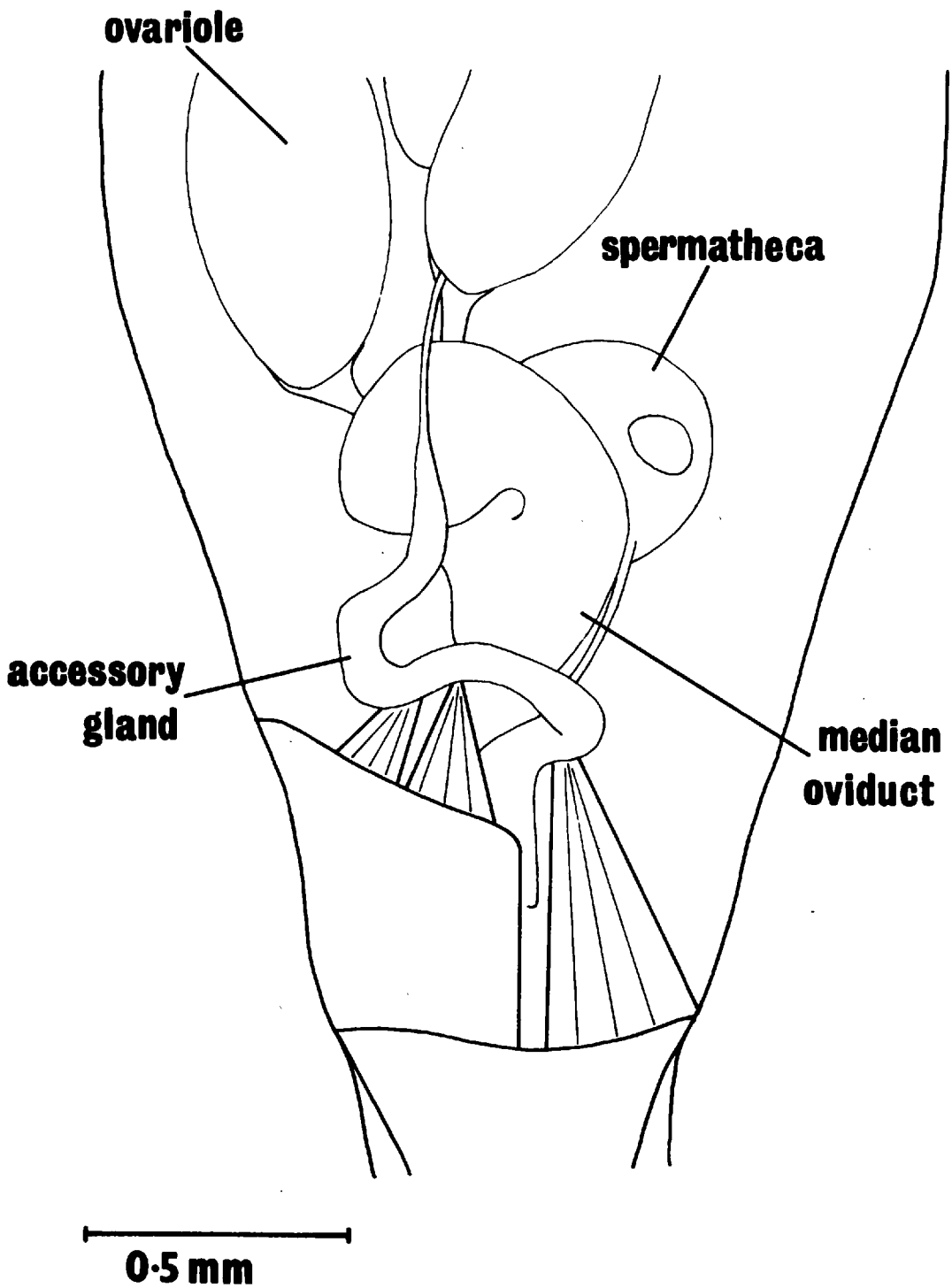
4. Postembryonic development of the reproductive system

(a) The ovary

Each ovary rudiment in the early first instar (2 days) is composed of four spherical ovarioles. Each ovariole in nymphs one hour after eclosion from the egg possesses a fine anterior and posterior stand which are non-nucleated cytoplasmic processes, and lies ventrolaterally in segment two beneath the alimentary canal. By six hours the ovariole is surrounded by a primary epithelial sheath of mesodermal origin that will surround the germinal tissue during later postembryonic development.

Fig 3.2

Mature female reproductive system, in situ.

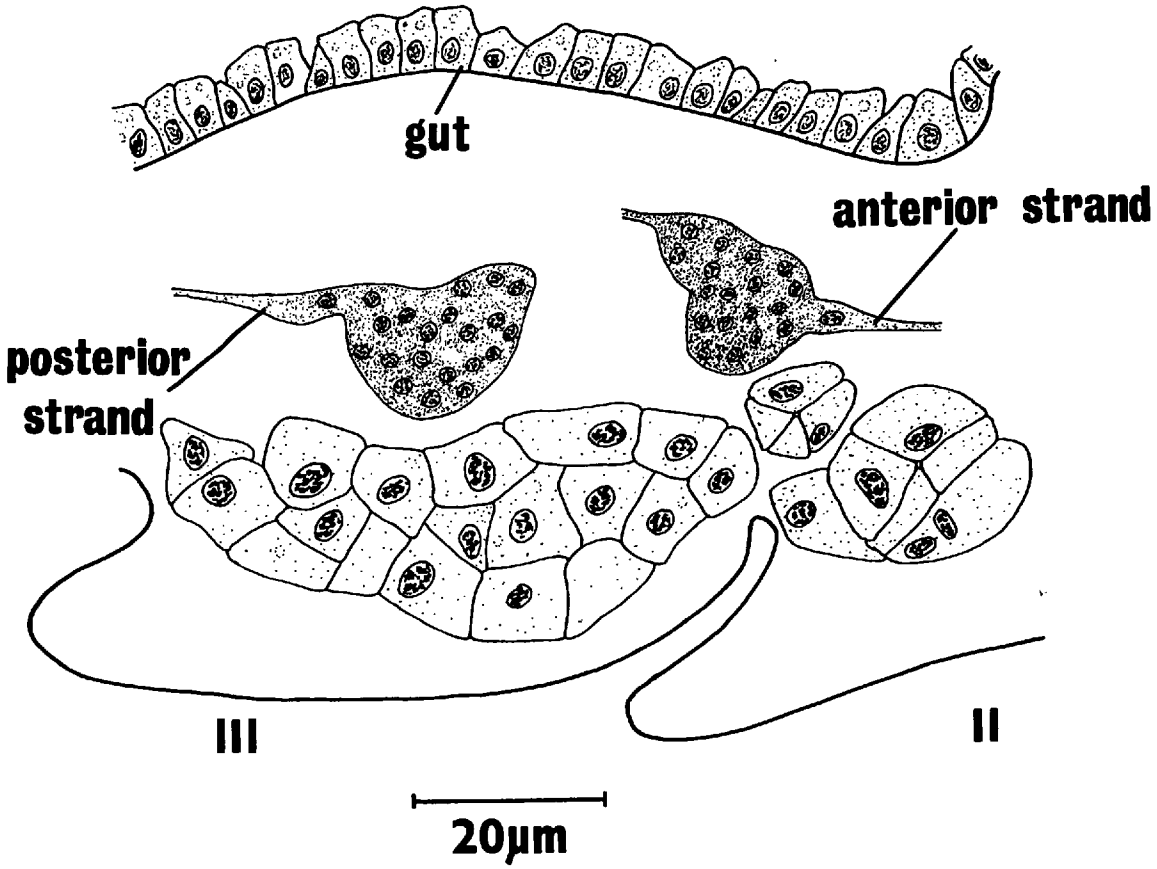


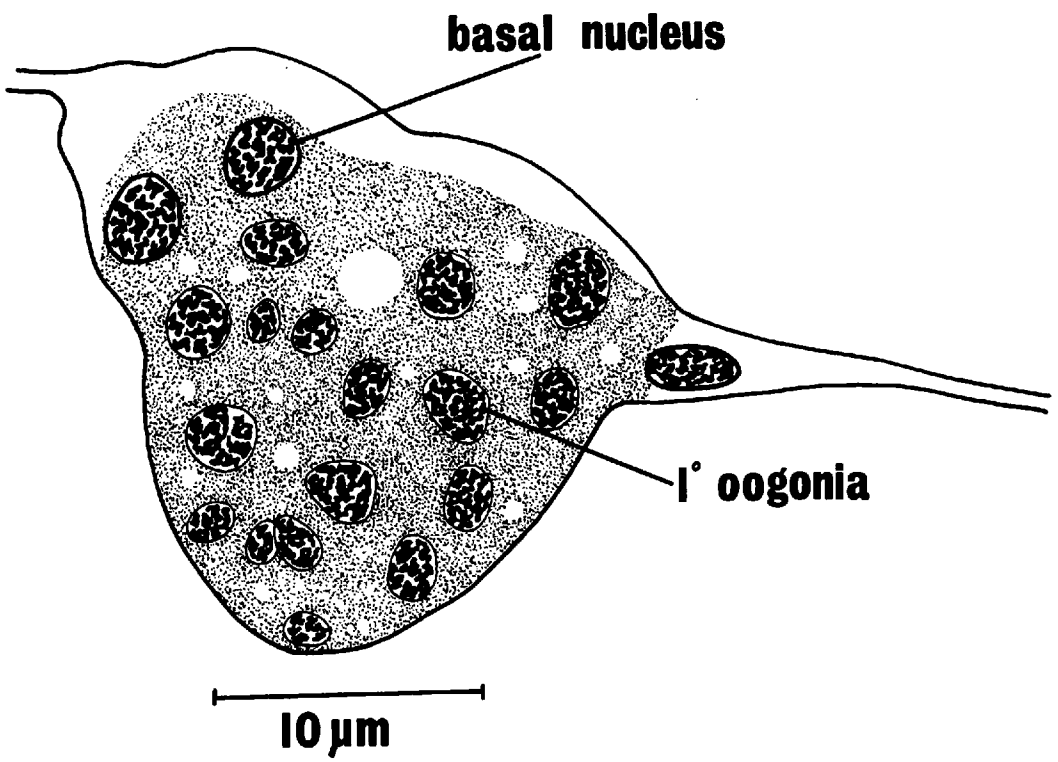
At thirty six hours, each ovariole rudiment, excluding the anterior and posterior strands, measures 14 - 16 μm in length and 10 - 12 μm in maximum width. During the early first instar, primary oogonia are distinguishable by their large spherical nuclei containing scattered basiphilic chromatin and intensely granular basiphilic cytoplasm (Fig. 3.3), cell walls were not apparent in light microscopic sections. The nuclei undergo division during the late first instar (5 - 6 days old) to produce secondary oogonia, which are retained through to the fifth instar. The absence of cell boundaries at the light microscopic level has caused confusion in the literature as to the syncytial condition and examination with the electron microscope is necessary to confirm this state.

During the first instar the anterior strands end freely in the posterior part of the first abdominal segment, not uniting to form a suspensory ligament, a characteristic feature of later instars. The histology of the anterior and posterior strands are similar and they form a mesodermal syncytium with small spherical nuclei irregularly distributed along their length. This contrasts with the findings of Helms (1968) in Empoasca fabae who described a cellular structure. Where the anterior strand is continuous with the body of the ovariole, the base is expanded and contains two large ovoidal nuclei separating the strand from the trophic and germinal tissue. In the late first instar, these basal nuclei undergo repeated mitotic division, the products of which migrate along the strands to form a regular single row of nuclei. This process is of interest because the basal nuclei are acting as a functional histoblast, from which the anterior and posterior strand nuclei are developed. Within this region the development can be followed from the non-nucleated state of the very young anterior and posterior strands, the mitotic division of the basal nuclei and the migration of the products into the strands to produce in the anterior strand, a condition similar to that found in the adult. A complete developmental sequence within this small area.

Fig 3.3

Ovariole rudiments in a 5-day old nymph





The primary epithelial sheath is represented by a single layer of cells which extend on to the anterior and posterior strands. Cell walls are clearly visible in this layer, each cell contains a single large spherical nucleus with a prominent nucleolus and dispersed basiphilic chromatin. This single-layered ovariole sheath persists throughout post-embryonic development and does not undergo further division. In the Heteropterans Oncopeltus fasciatus and Cydanus indicus (Wick & Bonhag 1955; Ramamurty & Medhi 1970) the primary sheath differentiates into an outer and inner epithelial sheath. The cells composing the sheath of G. fennahi are cuboidal or slightly flattened and during the first instar mitosis was not observed.

During the second instar, the ovariole shows an increase in size, becoming 22 - 25 μm in length and 12 - 14 μm in maximum width by the middle of the instar. The anterior strands possess a similar organisation to that seen during the late first instar. The cytoplasm remains translucent while the prominent nuclei are strongly basiphilic. The nuclear content of the individual strands has increased, and mitotic figures could be seen along their length. Growth of the ovariole and elongation of the anterior strand has brought the latter into segment one by this time, though there is no tendency for the individual strands to fuse. Their positions are maintained by large deposits of adipose tissue, which are connected by cytoplasmic strands to the body wall epidermis.

By the mid second instar the posterior strand is similarly organised, though ill-defined cell boundaries are present between the single row of ovoid nuclei, (approximately 2.0 μm diameter). Early in the second instar the posterior extremes of these strands fuse on each side to form the primordial calyx and lateral oviduct, which is also therefore of mesodermal origin. The germinal region of the ovariole increases in volume by mitotic division; in appearance it is essentially similar to

that of the first instar nymph, except for an increase in the number of secondary oocytes (fig. 3.4). No division into germarium and vitellarium was apparent.

Little change of form occurs in the third instar nymph, but growth continues, so that by the middle of the stage the rudiment measures 32-38 μm in length, and 17-18 μm wide. Mitotic division within the ovariole sheath has kept pace with this increase in volume, the epithelial layer still being composed of cuboidal cells with clearly defined cell walls. The anterior strands, now termed the terminal filaments, fuse anteriorly on each side to form two suspensory ligaments which end freely in the lateral part of segment one. The posterior part of the terminal filament is separated from the germinal zone by a thin transverse septum which arose as a constriction of the lateral walls and the basal part of the terminal filament becomes bulbous.

The posterior strand differentiates in the early third instar to form the pedicel and prefollicular tissue (fig. 3.5). The latter forms a compact mass of cells derived from the anterior part of the strand and which, during the third instar, forms a solid mass of clearly delineated spherical cells containing large basiphilic nuclei and obvious nucleoli. The pedicel is solid and composed of regularly arranged cells approximately three wide. The nuclei are smaller and less dense than those of the prefollicular primordia. During the third instar, considerable mitotic activity is apparent in both of these regions.

Ovariole length reaches 55-60 μm and 23-25 μm in width by the mid-fourth instar. The terminal filament takes on a more adult appearance and can be divided into a solid basal bulb, composed of a large number of small, spherical nuclei (2.5 μm diameter) which are densely basiphilic. No mitotic figures were seen in this region, though a considerable number of sections were examined. The cytoplasm appears highly granular and stains lightly with haematoxylin. This region is separated posteriorly from the germinal zone by a non cellular transverse septum, continuous with the basement

Fig 3.4

Ovariole rudiment in the late second instar

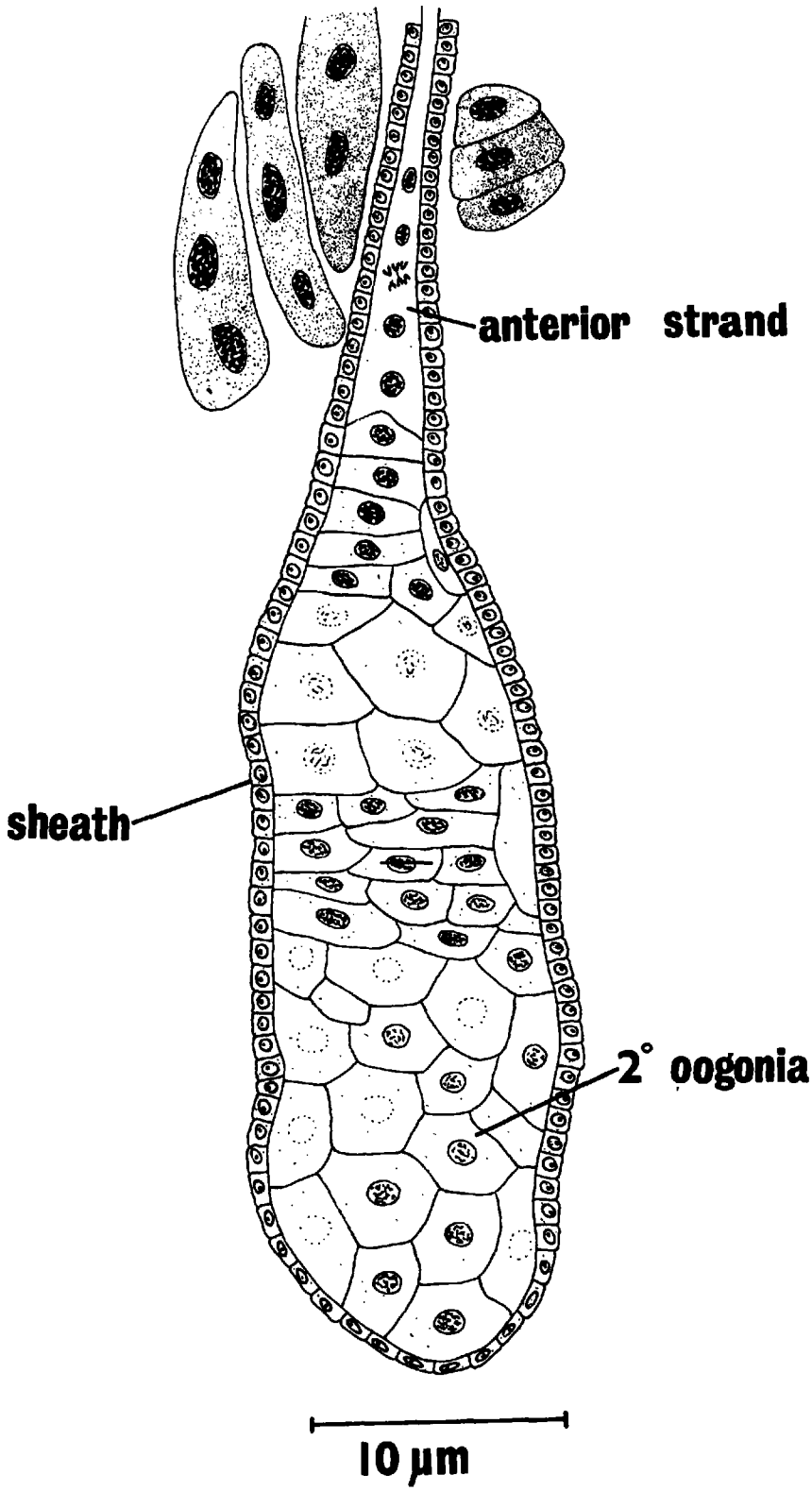
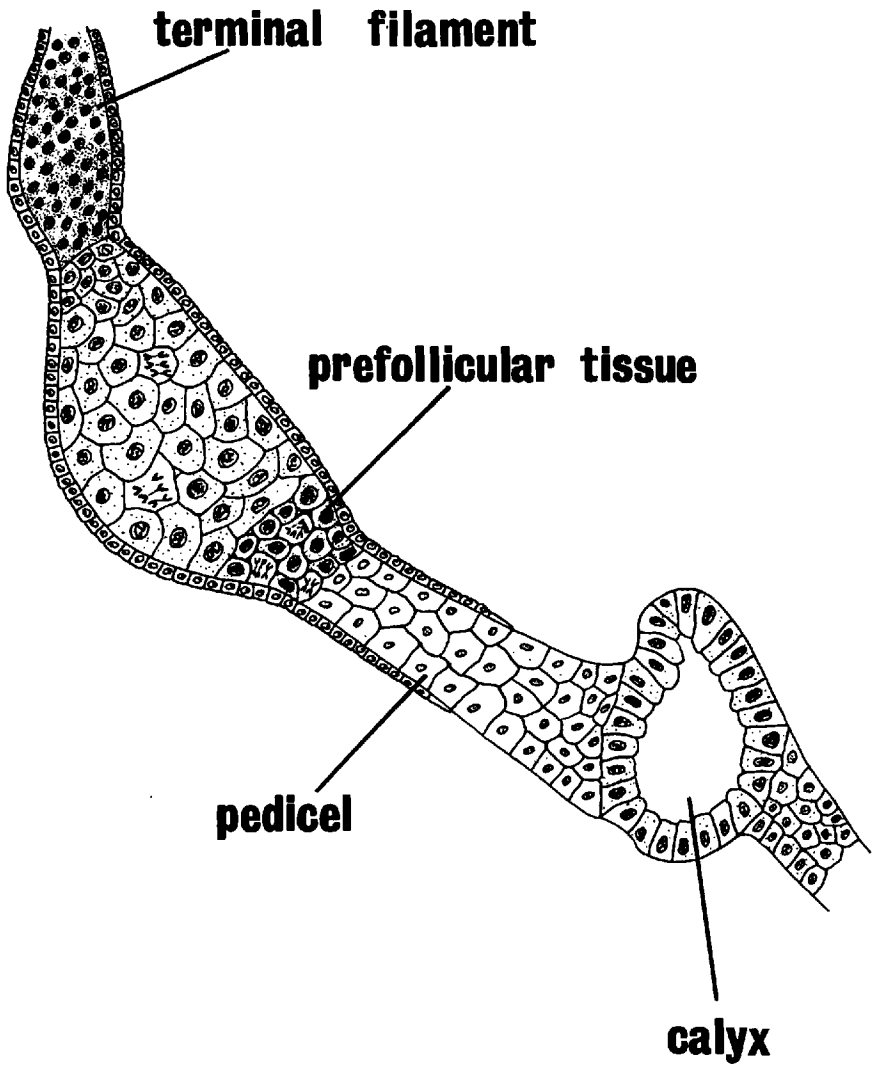


Fig 3.5

Oblique section through the ovariole of an early fourth instar nymph



membrane of the ovariole sheath. The anterior part of the filament is composed of a single row of spherical nuclei some 2.0 μm in diameter. Like the terminal filament, the suspensory ligament as far as can be determined by the light microscope is a true mesodermal syncytium.

The pedicel, derived from the posterior portion of the posterior strand, is well developed by the fourth instar, forming a hollow tube lined by a single layer of cells intermediate in appearance between the columnar and cuboidal condition (fig. 3.6). These cells contain small spherical nuclei (2-3 μm diameter) with strongly basiphilic dispersed chromatin and no obvious nucleoli. The cytoplasm is slightly basiphil, and contains numerous spherical granules. The apical cell wall (that towards the lumen) is strongly convex. Posteriorly the lumen is continuous with that of the developing lateral oviducts while anteriorly it is separated from the prefollicular mass by a transverse septum continuous with the basement membrane of the ovariole sheath. During the late fourth instar, the cells composing the pedicel walls are in an active growth phase, numerous mitotic figures being present.

The nuclei of the outer sheath are arranged in a single layer, though these may occasionally be two deep. Frequent mitotic divisions occur to allow for an increase in area in keeping with that of the ovariole. The cell boundaries become indistinct.

During the latter half of the fourth instar the nuclei of the prefollicular tissue become flattened (fig. 3.6a), approximately six times as long as wide and in transverse section it can be seen that these nuclei are distributed in the peripheral part of this zone. In the centre the arrangement is similar to that seen in the previous instar, the nuclei being spherical, some 3.0 μm in diameter, and evenly distributed in a mass. Cell boundaries are indistinct.

Little change occurs in the germarium during the fourth instar; there is an increase in volume due to mitotic division and the number of oogonia continues to increase throughout the instar.

Fig 3.6

Transverse section through the pedicel of a 4th instar nymph

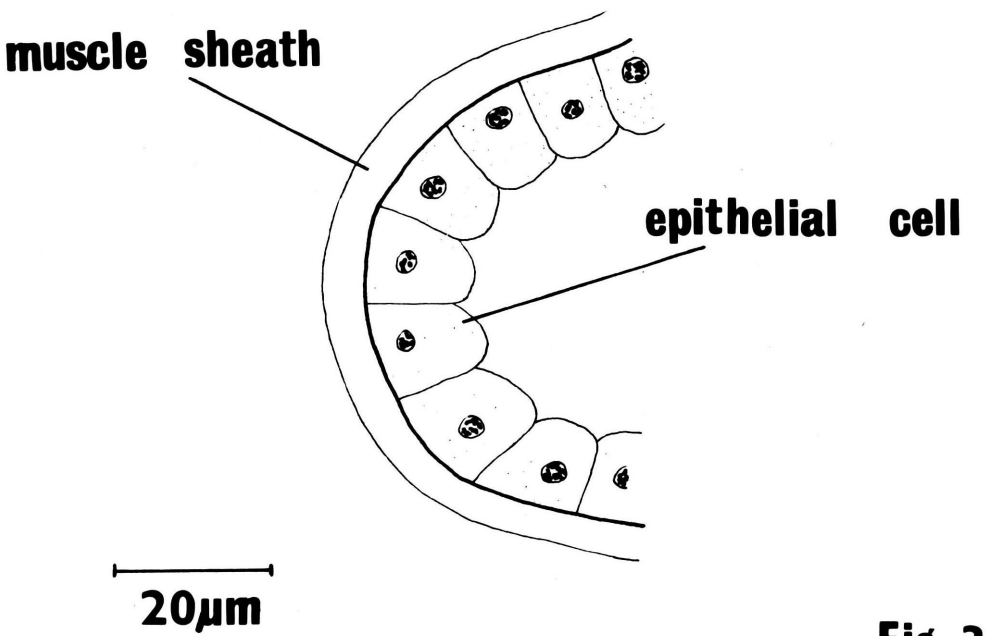
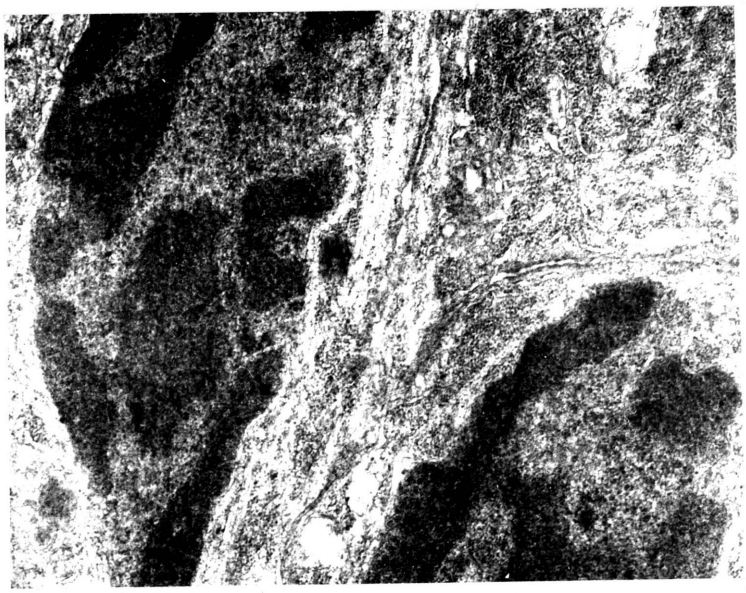


Fig 3.6a

Electron micrograph of follicle cell nuclei



x 13000

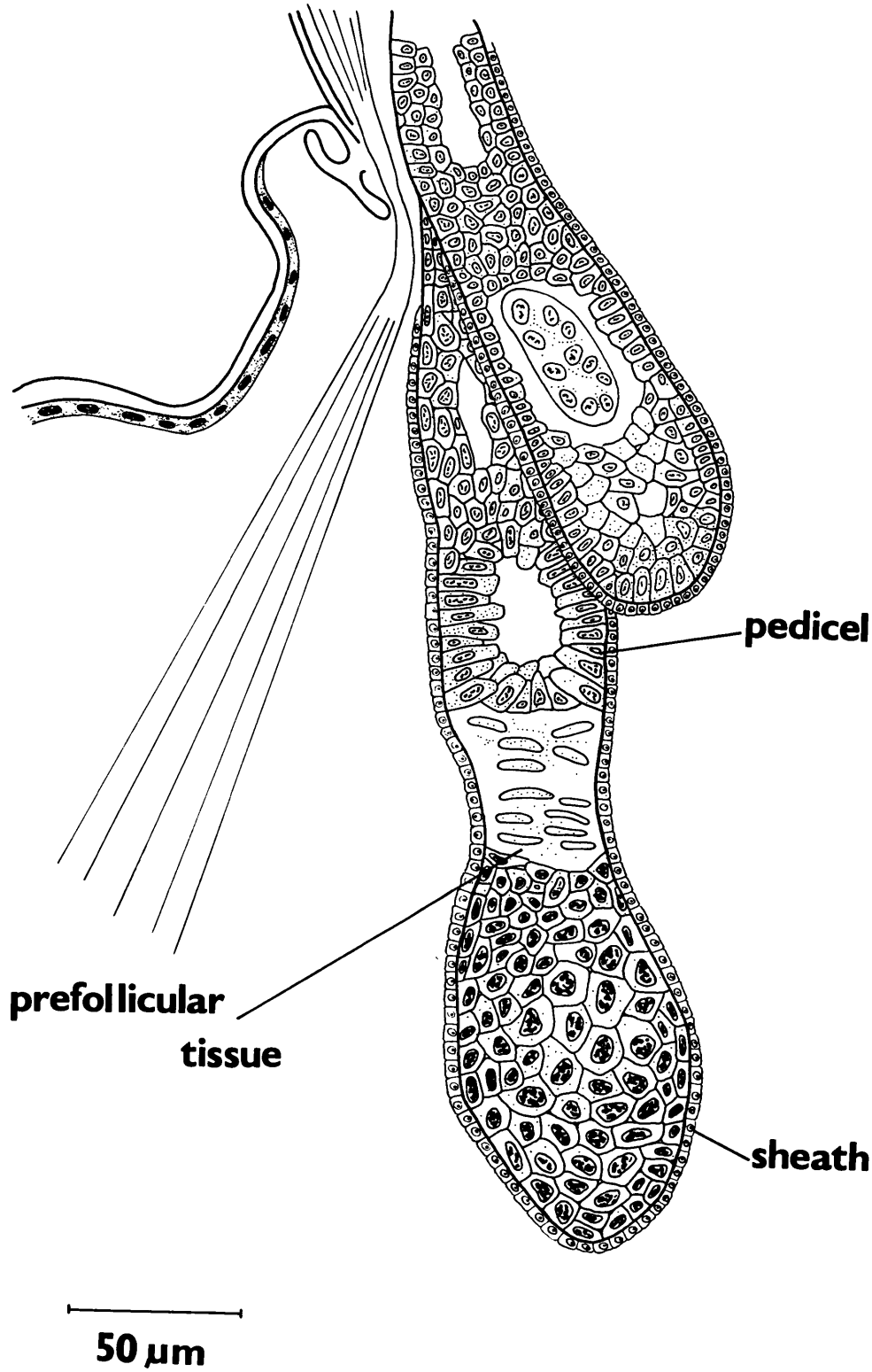
The ovarioles undergo rapid development during the fifth instar to a condition similar to that seen in the young immature adult. By the middle of the instar, each ovariole measures approximately 200 μm in length, and 30 μm wide. The ovarioles lie between segments two and three, lateroventrally beneath the large sac-like gut (fig. 3.7). The pedicel, approximately 150 μm long, extends posteriorly to segment four.

Histologically little change occurs in the terminal filament except for an increase in size, brought about by mitosis, both within the bulbous basal region and the fine syncytial thread. The attachment of each suspensory ligament on either side of the metathorax is achieved by the mid fifth instar. The pedicel similarly remains unchanged, compared to that of the fourth instar, except for elongation brought about my mitotic activity and growth. The transverse septum, separating the pedicel from the prefollicular tissue remains intact during the fifth instar, unlike the condition seen in Oncopeltus fasciatus (Wick & Bonhag 1955).

During the fifth instar the volume of prefollicular tissue is increased by mitotic division and the region containing the oogonia, the germarium, has become differentiated subequally into three distinct zones. Slightly more than the apical half is composed of large cells with very large nuclei of approximately 8.0 μm x 6.0 μm, each containing a nucleolus and very basiphilic chromatin material. Mitotic figures are common within this region. It is composed of undifferentiated cell types from which the several trophic tissues present in the definitive adult germarium arise. It is equivalent to zone I of Oncopeltus (Wick & Bonhag 1955). Immediately posterior to this is a small zone approximately 18.0 μm by 12 μm, characterised by a densely staining cytoplasm and regularly placed nuclei. No cell boundaries are apparent, though the existence of cell boundaries may be inferred from the regular spacing of the nuclei. In the adult this zone represents the trophic core. The posterior part of the germarium, anterior of the prefollicular tissue, is occupied by numerous oocytes, spherical in form with obvious cell boundaries. Their nuclei are centrally placed and contain a dense chromatin material.

Fig 3.7

Ovariole of a 5th instar nymph oblique section



This is the appearance of the ovarioles immediately prior to the final moult, little further differentiation occurring until the second or third day of adult life when the zonation becomes more obvious.

(b) Lateral oviduct

Rudiments of the lateral oviducts do not arise until early in the second instar (8-9 days), when fusion of the individual posterior strands of the ovarioles produces the calyx (fig. 3.5), the most anterior part of the lateral oviduct. During the second instar this anterior region forms a solid mass of cells, each with a clear cell boundary and a small irregular nucleus, which, together with the cytoplasm, is slightly basiphilic. During the third instar this pair of solid structures is the site of considerable mitotic activity and posterior elongation of the rudiment also occurs. By the late third instar each lateral oviduct extends posteriorly in a lateral position to a point mid-way through segment six. Here they bend medially and extend to the anterior end of segment seven, where they end loosely attached to the sternal epidermis by cytoplasmic threads. Throughout their course fat body cells are closely applied and also hold them in position. Transverse sections through the anterior lateral oviduct during the late third instar indicates that the developing calyx has, as a result of mitotic activity, become a hollow mass with a small eccentrically placed lumen approximately 3.0 μm in diameter. The nuclei seem not to be contained within distinct cell walls and are scattered throughout the tissue, and contain densely staining chromatin material distributed around the nuclear membrane. The cytoplasm is only slightly stainable, but contains numerous basiphilic granules. During this time the posterior strand of the ovarioles remains solid, some 6.0 μm in diameter.

During the fourth instar, contact is established between the anterior part of the median oviduct and the posterior extremes of the lateral oviducts. During the early to mid fourth instar, the lumen of the lateral oviducts is continuous from the calyx and ends blindly in a solid bulb, approximately 8 - 10 μm in diameter on either side of the mid-line in segment seven, immediately lateral of the longitudinal ventral muscle. By the late fourth instar (38 - 40 days), anterior extension of the median oviduct has allowed its

short, lateral branches to unite with the posterior bulbs of the lateral oviducts. This is the condition at the end of the fourth instar.

Early in the fifth instar the wall dividing the two ducts breaks down, allowing the lumen of the lateral ducts to become confluent with that of the median oviduct. This contrasts somewhat with the findings of Helms (1968) on Empoasca fabae in which the lateral and median oviducts become confluent in the early fourth instar. From this description, it is clear that what appears to be the posterior extreme of the lateral oviduct is derived from the median oviduct and so of ectodermal origin, while the remainder of the lateral oviducts, derived from the posterior strand of the ovarioles, is mesodermal in origin.

Immediately before the final moult the lateral oviducts are composed of tubes 10.0 - 12.0 μm in diameter, expanding at the calyx to 15 - 18 μm diameter. The oviduct wall is composed of columnar epithelium surrounding a small lumen of 3 - 4 μm diameter. This increases considerably during the adult stage. The lumen is continuous with that of the pedicel anteriorly and the median oviduct posteriorly.

(c) Median oviduct

As described in the previous chapter, the sternal epidermis of segments eight and nine becomes hypertrophic during the first and second nymphal instars to form the developing genital disks. Posteriorly, in each genital segment the disk forms a pair of lips which grow back and will ultimately form the external genital rudiments. This proliferation of the sternal epidermis begins during the middle of the first instar and at the same time, by intense mitotic activity of the ectodermal tissue in the anterior part of the developing genital disk in segment eight, a small solid ampulla-like structure is formed. This lies in the mid-line and is the precursor of the median oviduct. Mitotic division continues within the ampulla throughout the first instar to produce a hollow mass of undifferentiated ectodermal tissue approximately 15 μm long, 12 μm wide and 10 μm deep, partially buried within the genital disk (fig. 3.8a). In

Post-embryonic development of the median oviduct

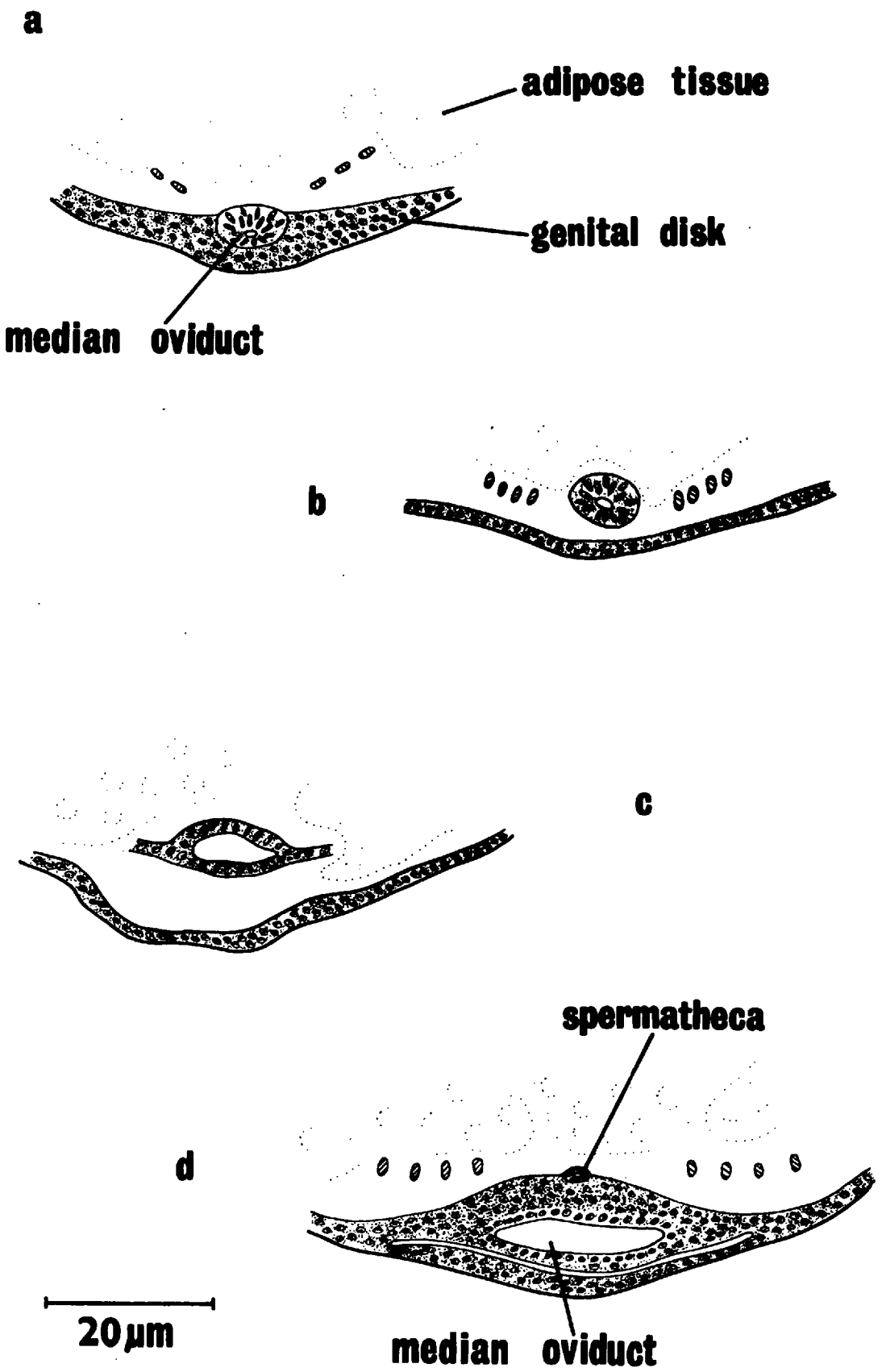
(a) Transverse section through the posterior part of segment seven of a mid third instar nymph. The rudiment of the median oviduct is partially buried within the genital disk.

(b) Transverse section through the posterior part of segment seven, slightly anterior to (a), of a late third instar nymph.

(c) Transverse section through segment six of a late fourth instar nymph to show the distal branching of the duct.

(d) Transverse section through the anterior part of segment eight of a fifth instar nymph to show the cellular appearance of the oviduct epithelium.

Fig 3.8


a
adipose tissue
genital disk
median oviduct
b
c
spermatheca
d
20µm
median oviduct

transverse and parasaggital section it appears as a syncytial mass surrounding a small lumen. The nuclei are small, approximately 2.5 - 3.0 μm in diameter, strongly basiphilic with no visible nucleoli; they are closely packed within a cytoplasm that stains faintly with haematoxylin. Several mitotic divisions were visible.

During the second, third and fourth instars, growth of the oviduct rudiment continues in both anterior and posterior directions. With the developing external genitalia the ampullae extends posteriorly and becomes dissociated from the anterior part of sternum eight (fig. 3.8b) and by the mid fourth instar it opens between the bases of the already well developed first gonapophyses as a slightly dorsoventrally compressed tube. Anterior growth continues, so that by the end of the third instar it ends blindly in the mid-line above the anterior part of segment seven. A transverse section through the median oviduct reveals a tube 12 - 15 μm in diameter with a single lumen 3 - 4 μm diameter. (Helms, 1968, described a double lumen in E. fabae). No cell boundaries were discernible and the spherical nuclei are stacked 3 - 4 deep. Associated with the outer wall of the duct are small discontinuous strands of striated muscle. In the early fourth instar the ampulla has separated from the sternal epithelium and the duct lumen is continuous to its blind exit between the gonapophyseal bases. During the mid to late fourth instar considerable development occurred at the anterior end of the duct, marked by mitotic activity and growth. The anterior extremity divides to produce solid lateral buds which extend anterolaterally over segment seven (fig. 3.8c) and establish contact with the posterior extremities of the lateral oviducts at the junction between segments six and seven. Fusion of the two ducts occurred during the late fourth instar and cleavage of the cytoplasm within the lateral branches of the median oviduct during the early fifth instar provided continuity between the lumina of the lateral and median oviducts.

Sections taken during the late fifth instar indicate that little change has occurred

though the definitive adult cellular appearance has developed. The epidermis of the duct is now a single cell thick, columnar and with the nucleus, situated near the basement membrane, possessing a diffuse chromatin. A well developed muscular sheath surrounds the duct in most of its length, being particularly well developed in the middle region, where it is differentiated into an inner layer of longitudinal muscle and an outer layer of circular muscle. The posterior part, near the vagina, remains undifferentiated (fig. 3.8d).

This description differs from that given for E. fabae by Helms (1968), who described a median longitudinal septum which divided the median oviduct into two lumina during the first four instars. This broke down during the fifth to produce a single duct similar to that of the adult. In Graphocephala, the duct is never subdivided. The present author agrees with Helms in the absence of a cuticular intima which is typically present on most ectodermal ducts (Snodgrass 1935). It is suggested that this may be due to the median oviduct arising from ampullar tissue and not as an invagination of the nymphal body wall.

(d) Spermatheca

The spermathecal rudiment arises during the early second instar from the hypertrophied tissue between the developing lips of the genital buds in segment eight. It is posterior to the median oviduct and appears as an invagination of the epidermis of sternum eight, the lumen being continuous with the space between the anterior genital rudiments. Its development during the second instar progresses by mitotic division which produces a bulbous projection between the rapidly elongating rudiments. In parasagittal section it appears as a cylindrical outgrowth, slightly displaced to the left of the abdominal cavity, with a narrow lumen 1.5 - 2.5 μm wide and a thick solid cap. As with the median oviduct, the walls contain closely packed nuclei which are spherical, intensely basiphilic

and approximately 2.5 μm in diameter. They are surrounded by very little cytoplasm (fig. 3.9a). Extension continues anteriorly during the third instar (fig. 3.9b and c), and by the late third instar the rudiment is differentiated into a stem and a globular apical region which is directed anteriorly. Growth of the two components continues by mitotic division during the fourth instar, when the stem measures approximately 50 μm in length and 8-9 μm in diameter, and the end bulb is some 80 μm in diameter. The spermatheca lies between the median oviduct and the gut. Development in the final instar is largely restricted to the end bulb. Its diameter increases to 110 μm, but few mitotic figures were apparent during this stage, so causing a considerable thinning of the wall and increase in lumen volume to produce a thin walled, much convoluted sac. Cell boundaries are not apparent. The lumen remains continuous, through the stalk, with the posterior dorsal wall of the vagina, which has extended posteriorly, and hence with the exterior. By the end of the fifth instar the stem wall contains a single row of nuclei containing prominent nucleoli. Cell boundaries, though present, are rather indistinct.

The spermatheca of G. fennahi is used for storage of sperm unlike that of E. fabae (Helms 1968) and spermatozoal pounches as described by Gadd (1910), Cogan (1916) and Evans (1931) in various cicadas and Cicadellids are not present in Graphocephala. The work carried out in the Heteroptera has revealed that the structural peculiarities of the spermatheca of the various groups provide a useful taxonomic character (Pendergrast 1957; Carayon 1964; Ramamurty & Medhi 1970). Similar detailed work has not yet been carried out in the Homoptera though similar peculiarities may be present within this group.

The mature adult spermatheca shows considerable complexity over that of the fifth instar nymph, principally in the convolutions of the inner wall of the end bulb.

Post-embryonic development of the spermatheca

(a) Transverse sections through the anterior and posterior part of segment eight of a second instar nymph to demonstrate the thickening and invagination of the genital disk.

(b) Transverse section through segment seven of a late third instar nymph to show the spermatheca and opening of the median oviduct which extends posteriorly into segment eight.

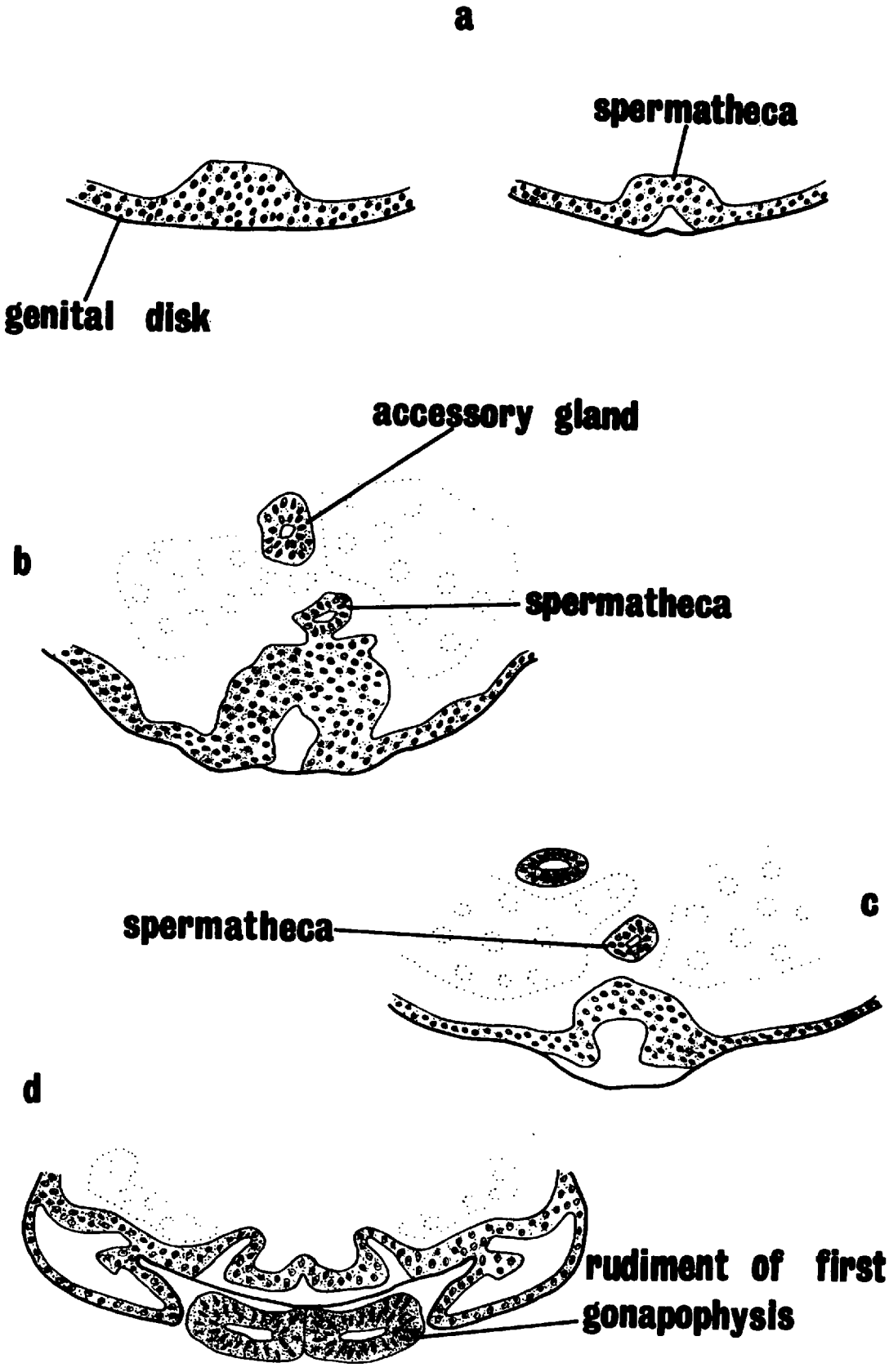
(c) Transverse section through the anterior part of segment eight of a mid fourth instar nymph to show the spermatheca and the posterior extension of the median oviduct.

Scale : ⟵——⟶ represents 20 µm.

(d) Transverse section through the anterior part of segment nine of a fourth instar nymph to show the gonapophyseal rudiments.

Scale : ⟵————⟶ represents 50 µm.

Fig 3.9


a
spermatheca
genital disk
accessory gland
b
spermatheca
spermatheca
c
d
rudiment of first
gonapophysis

These developments arise during the final moult and first two or three days of adult life (Fig. 3.21a).

(e) Accessory gland

The accessory gland takes its origin on the anterior margin of the genital disk in segment nine, occupying a medial position between the posterior rudiments of the external genitalia. Initially it appears as a small invagination of the genital disk, but at a later stage its lumen becomes continuous with that of the vagina into which it opens posterodorsally. During the third and fourth instars it extends anteriorly, lying above the median oviduct and to the left of the spermathecal rudiment, for approximately 60 μm to a point near the posterior edge of sternum seven. Growth occurs by mitotic division, serial transverse sections indicate that the lumen is continuous throughout the length of the duct, the duct itself being oval in section, and 13 - 15 μm diameter.

During the mid fourth instar the cells enter a phase of proliferation which results in the rapid anterior extension of the duct. By the late fourth instar it has extended anteriorly between the lateral oviducts, and the anterior fifth has become considerably more slender than the rest of the duct; the lumen does not extend into this distal region. During the fifth instar growth continues and the accessory gland can be divided clearly into two regions, a proximal half, approximately 180 μm in length and 20 μm in diameter, and a distal region of very small diameter, 8.0 μm. A lumen is present only in the proximal half, the fine distal strand being solid. The transition between the two regions is abrupt. During the fifth instar the accessory gland is displaced more strongly towards the left side of the abdominal cavity and the fine distal region becomes closely associated with the terminal filaments and suspensory ligament of the left ovary, ending freely within the first abdominal segment. Immediately before the final moult the definitive opening is achieved into the genital chamber

and dorsal to the external genital opening.

A large median accessory gland has been reported many times in the Auchenorryncha, Dufour (1834), Doyère (1837) and Myers (1928) described a similar structure in the Cicadoidea, Evans (1931), Gil-Fernandez & Black (1965) and Helms (1968) have also described such a gland in the Cicadellids. Kershaw (1910) described an unpaired median gland in the Fulgoroids. In addition to this, Cogan (1916) in his morphological study of the Cicadellids described the presence of large paired glands. This has not been confirmed, though the presence of small paired accessory glands which open into the genital tract near the median gland is well known in many Auchenorryncha. Such glands do not occur in Graphocephala.

5. Histology of the adult reproductive system

(a) Ovaries

The ovaries of the young, newly emerged female are similar to those seen in the late fifth instar nymph. They are small, 240 μm in length, opaquely white in appearance, and situated laterally in segments three and four, each surrounded by a large mass of adipose tissue. They undergo rapid growth during the first twelve days of the adult stage, after which the mature condition is achieved. In the eight day old female the ovaries are situated in segment three and are approximately 585 μm in length, the individual ovarioles entering the lateral oviduct one behind the other and having a mean length of 380 μm and maximum width of 96.5 μm. The pedicels, approximately 290 μm in length, extend posteriorly through to segment four.

By the twelfth day of adult life, the ovarioles occupy most of the abdomen posteriorly to segment six, each containing a mature oocyte, deep yellow in colour, and usually three other oocytes at various stages of previtellogenesis. The size of the oldest oocyte is 0.81 - 1.17 mm in length and mean maximum width of 0.41 mm. Dense

adipose tissue containing yellow lipid droplets surrounds the ovaries and is attached to the body wall by fine cytoplasmic threads. They are not attached to the internal organs.

During the first five to six days the epithelial sheath can be readily seen as a silvery covering over the entire ovariole. Its associated musculature is developing, and it is richly supplied with trachea. In the immature ovary it is wrinkled and forms a loose covering, but as oogenesis proceeds it becomes stretched over the enlarging ovariole.

i. The terminal filament

The terminal filament is a long, tapered syncytium containing distally a single row of numerous, irregularly arranged, slightly compressed nuclei, which are strongly basiphilic (Fig. 3.10). The syncytium is enclosed laterally by a continuation of the epithelial sheath and posteriorly is separated from the germarium by a transverse septum, continuous with the tunica propria and arising in nymphal development as a constriction of the apical germarium. The bulbous base of the terminal filament contains spherical basiphilic nuclei approximately 2.5 μm in diameter and densely distributed in the cytoplasm. In Graphocephala there is no lumen as described in Heteroptera (Bonhag & Wick 1953; 1955). Mitotic division was not observed in the fully differentiated terminal filament.

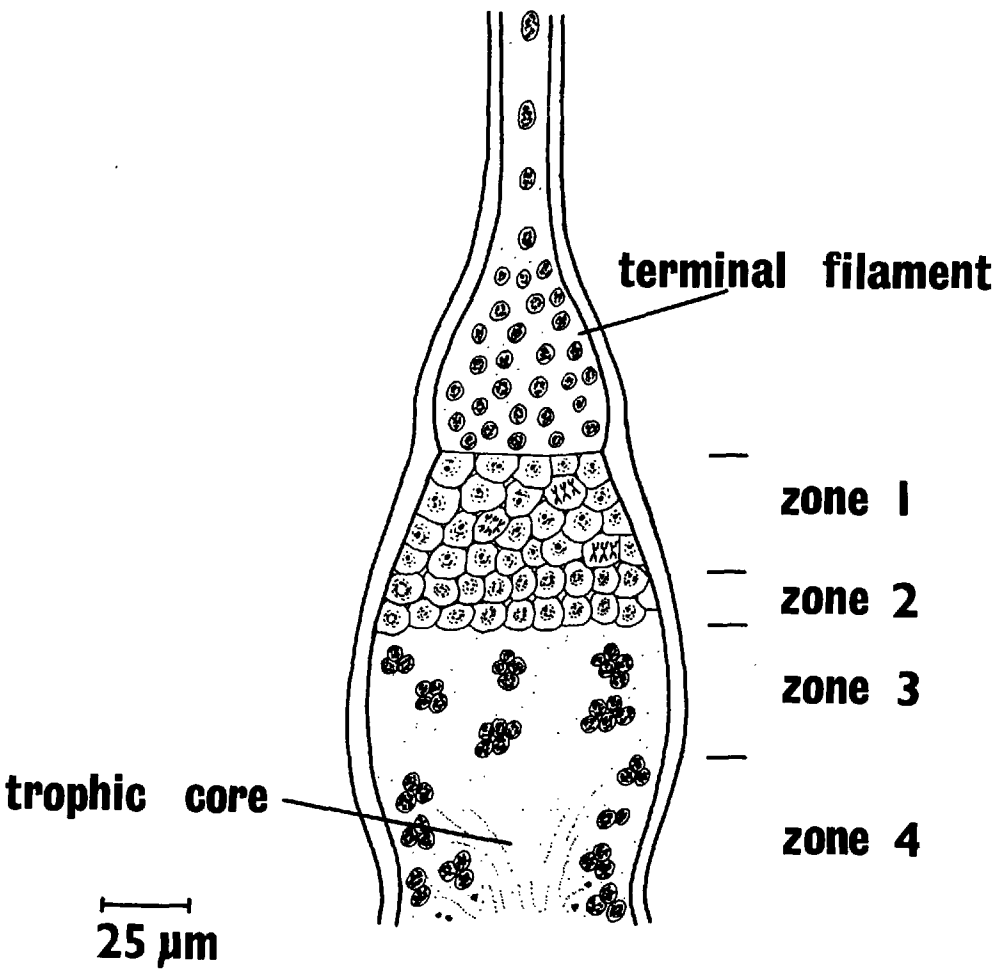
ii. The germarium

The germarium can be divided into four zones on the basis of its histology (fig. 3.10) and therefore differs from the Heteropteran germarium, in which only three zones have been recognised (Wick & Bonhag 1955; Ramamurty 1970; Wrightman 1973).

Zone 1. This occupies the apical quarter of the germarium and is characterised by considerable mitotic activity. The cells are small, isodiametric, approximately 10 μm diameter (fig. 3.11). The chromatin stains deeply and the

Fig 3.10

Diagrammatic section of the adult germarium



3.11

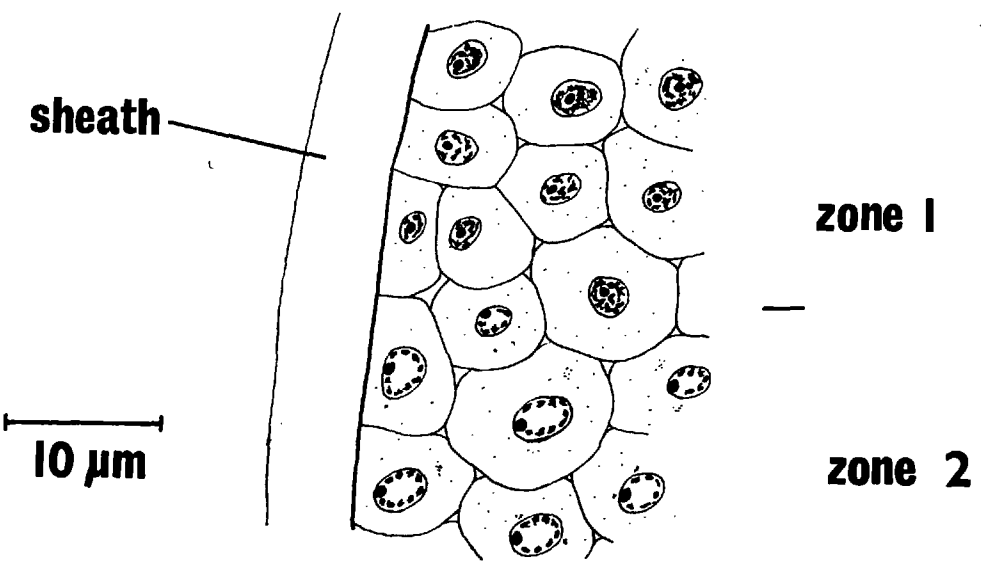
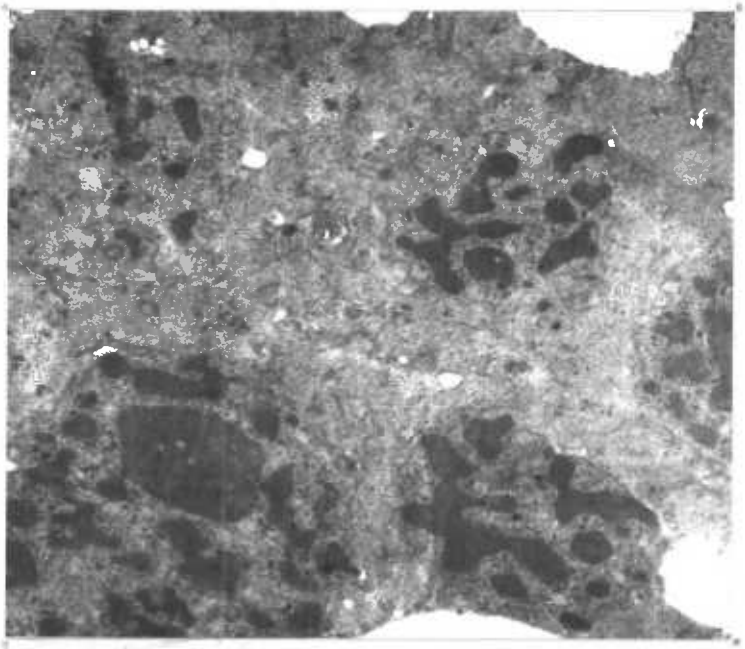


Fig. 3.11

An electron micrograph of zone 1 in the germarium of an adult female. The cells are isodiametric. G fennahi.



X 1300

cytoplasm only lightly so. Nucleoli are present and stain orange with Mallory's connective tissue stain. Cell boundaries are always present.

Zone II. This represents a narrow band of cells immediately posterior to Zone I. The nuclear dimensions increase posteriorly through this zone, initially being similar to that of zone I. Cell boundaries are still obvious, which contrasts with the findings of Shrader & Leuchtenberger (1952) working on Coreids. The zone is characterised by the distribution of the basiphilic chromatin material around the inner edge of the nuclear envelope, the nucleolus also being laterally displaced, leaving the nuclear centre devoid of all inclusions and stainable matter. Nuclear diameter in the posterior region of this zone is approximately 8.0 μm, double that in the anterior part of the zone. Mitosis was not observed in zone II.

Zone III. Cell boundaries are no longer present and the nuclei move together, forming small clumps of 3-5 nuclei. The nuclei are in close apposition and there is a marked tendency for nuclear size to increase through the zone. This is accompanied by a similar increase in the size of the nucleoli, which have adopted a more central position within the nucleus. This represents the transition zone of trophocyte differentiation.

Zone IV. This posterior zone occupies almost half of the germarium and contains the fully differentiated trophic tissue. Nuclear clusters, as described in zone III remain, but the number of nuclei in each group is reduced to two or three, and the nuclear size is increased. The major feature of this zone is the aggregation of the nuclei around a large central column of homogeneous cytoplasm, devoid of cellular structure, and termed the trophic core. The cytoplasm of the trophic core is continuous with that which surrounds the nuclear clusters.

The nuclei in the posterior region of zone IV extrude their DNA, which forms Feulgen positive globules in the cytoplasm (fig. 3.10). RNA is often present

within these globules, as demonstrated by Trevan & Sharrock's stain, and is suggested to be of nucleolar origin, since these nuclear inclusions can no longer be seen in this region. The globules show a tendency to merge more posteriorly and the nuclear envelopes, associated with the peripheral region of the globules, are now devoid of contents and are occasionally seen to disintegrate. As the trophocytes around the periphery of zone IV are used up they are replaced by cells from the preceding zones, the mitotic activity of zone I maintaining the nuclear population in all except senescent females when mitotic activity ceases in zone I.

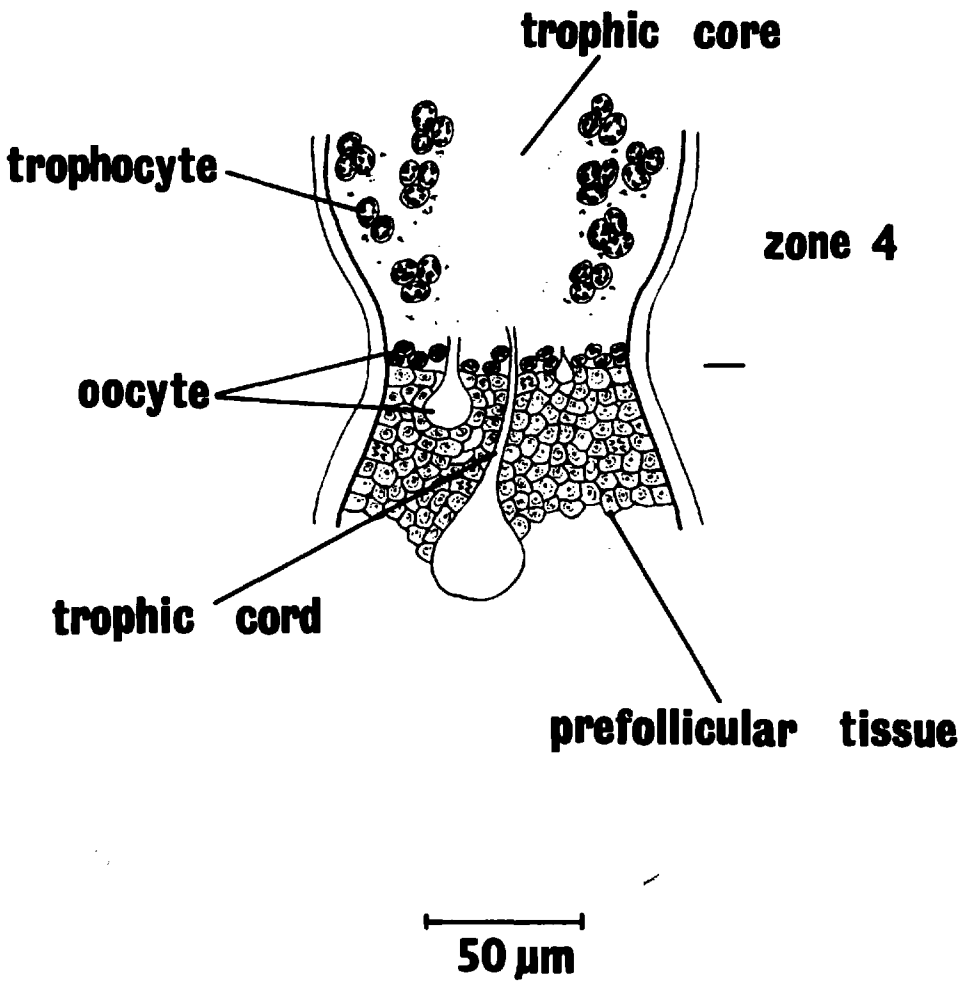
The posterior part of the trophic core is divided into a number of projections which are attached to developing oocytes within the extreme posterior part of the germarium (fig. 3.12). Similar projections extend into the vitellarium and are attached to the older oocytes. Clearly, these projections or trophic cords provide cytoplasmic continuity between the trophic layer and the oocytes. Sections stained with Mallory's phosphotungstic haemotoxylin clearly demonstrate lines streaming from the peripheral nuclear clusters of zone IV into the trophic core and posteriorly towards the trophic cords, thus suggesting the flow of material towards the developing oocytes. The whole of the trophic core is highly basiphilic in the mature adult, but showed only slight basiphilia in the late fifth instar nymph.

In an attempt to provide more evidence for the movement of material from the trophic layer to the oocytes, cytochemical tests for nucleic acids were used. The Feulgen reaction and Trevan & Sharrock's stain demonstrated that basiphilia were associated with accumulations of DNA and RNA and that the flow lines were, at least in part, rich in RNA derived from the trophic tissue, which underwent breakdown in zone IV.

Immediately posterior to the trophic tissue and, in principle, part of the germarium are the oogonia, which ultimately give rise to the oocytes that lie embedded in the pre-

Fig 3.12

Section through the distal end of the germarium



follicular and follicular tissue. The attachment of a trophic cord to an oocyte apparently induces its further development, marked by its sinking into the pre-follicular tissue. The oocytes then pass into the vitellarium, where they become surrounded by follicular epithelium to form a follicle. Graphocephala usually has three or four follicles per ovariole. The young oocytes can be distinguished by their lightly staining cytoplasm and large germinal vesicle. The prefollicular cells are small and undergo rapid mitotic division.

iii. The follicular epithelium

The importance of the follicle cells in vitellogenesis has been known for a considerable time. It is now generally accepted that they have three major functions: (a) Their primary role is to mediate the transfer of substances from the haemolymph into the oocyte. (b) They secrete the chorion. (c) They are able to resorb yolk proteins in abortive oocytes (Bonhag, 1959).

In recent years, several new approaches have been involved in the problems of insect vitellogenesis. Immunological techniques have demonstrated that blood proteins enter the oocyte via the intercellular spaces between the follicle cells and are incorporated by pinocytosis, (Telfer 1961). Numerous autoradiographic studies have demonstrated that tritiated amino acids are incorporated from the haemolymph and appear in the peripheral yolk spheres (Bier, 1962; Ramamurty 1964; Zinsmeister & Davenport 1971; Ullman 1973; Davenport 1975). In addition to these studies, electron microscopy has been able to add considerably to our understanding of the pinocytotic incorporation of haemolymph proteins (Telfer 1961; Anderson 1964; Bier & Ramamurty 1964; Huebner & Anderson 1972; Davenport 1974).

Despite this volume of research, there still remains much to be learnt of vitellogenesis, and in view of the importance of the follicle cells in yolk deposition

the present study describes the growth and structural differentiation of these cells in Graphocephala fennahi.

Prefollicular tissue, undifferentiated cells which display considerable mitotic activity, forms the anterior extreme of the vitellarium, and is present in the adult and later nymphal instars. In Graphocephala, this tissue possesses well-defined cell boundaries, unlike that recorded in some Heteroptera (Bonhag & Wick 1953; Masner 1968). Once stimulated by the growing oocytes that lie partially buried in prefollicular tissue, it undergoes dramatic changes which are presumably concerned with vitellogenesis. These changes will now be described.

The cell size of prefollicular tissue is small, approximately 8 - 10 μm diameter, and with a centrally placed nucleus containing dispersed chromatin and an obvious nucleolus.

As the oocyte enlarges it is gradually encompassed by a follicular epithelium, initially several cells thick. The trophic cord has established contact with the oocyte, ending freely on its dorsal surface and penetrating the follicular epithelium in this region. There is some indication from the work of King (1970) that contact is made much earlier, this will be considered further in the discussion. The follicular cells are columnar and form a layer approximately 35 μm thick. Numerous mitotic divisions were evident within the prefollicular zone, but once follicular tissue is established around the oocyte mitosis is rare, and it was never observed after the start of yolk deposition. In the youngest recognisable follicle the epithelium is a single layer of columnar cells surrounding the oocyte, the cells displaying an increase in gross size and nuclear size compared to the follicular epithelium in its earliest, many-layered form. (Changes in the follicular cells with posterior movement of the oocyte through the ovariole is shown in fig. 3.13). Trevan & Sharrock's stain indicates a concentration of RNA in the apical region of the follicle cells, though no evidence

Developmental changes in the follicular epithelium posteriorly through the vitellarium.

(a) Prefollicular epithelium. Note the spherical, centrally positioned nucleus with a distinct nucleolus and the presence of mitotic activity.

(b) Singled layered columnar epithelium surrounding the young oocyte.

Scale : ——— represents 10 μm.

(c) Follicular epithelium during late previtellogenesis characterized by the binucleate appearance of the cells.

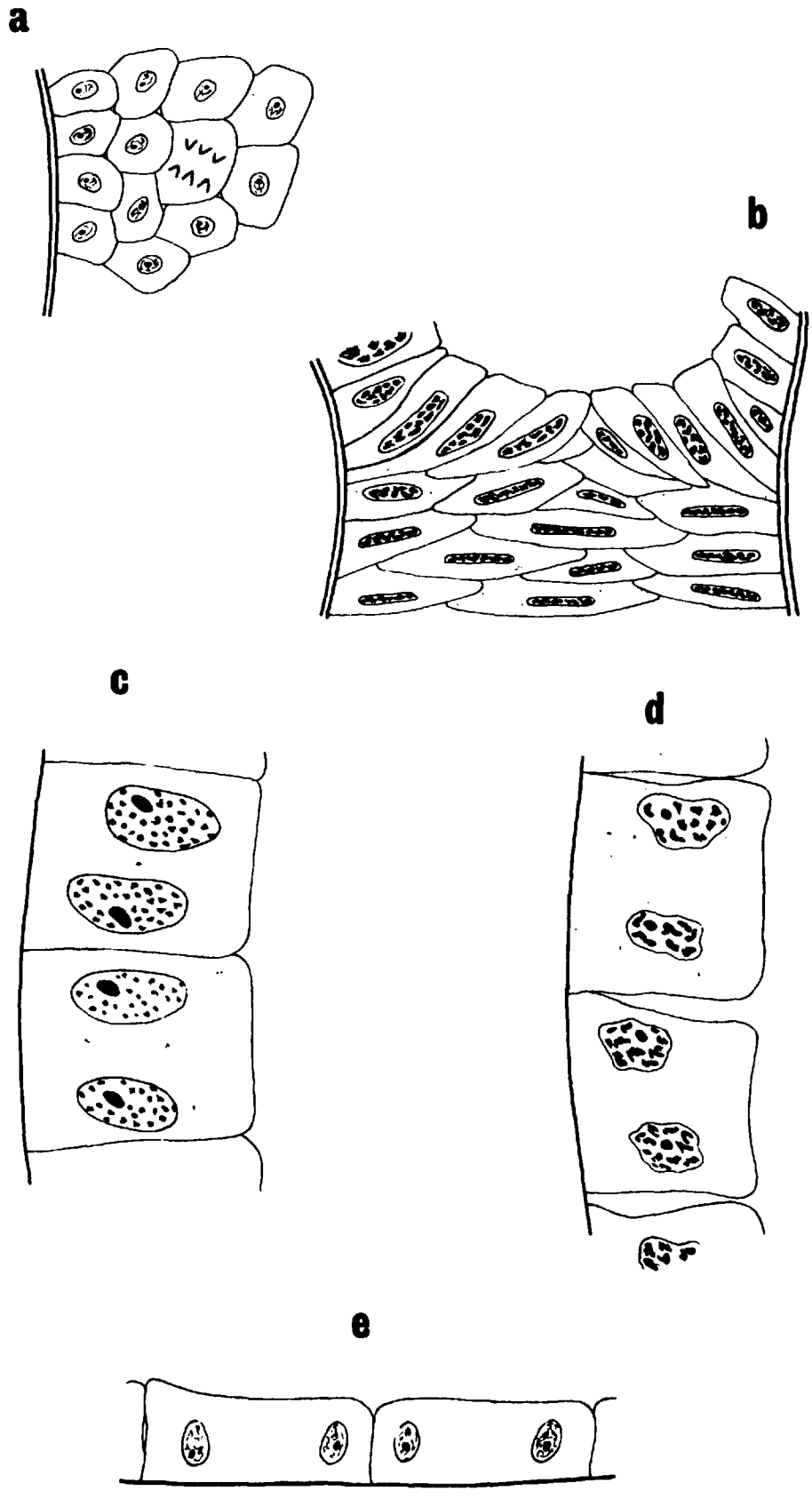
(d) During vitellogenesis spaces develop between the follicle cells which may be associated with the transmission of haemolymph proteins. The nuclear envelope of the follicle cell becomes much convoluted.

Scale : ——— represents 10 μm.

(e) The end of vitellogenesis is marked by the transition of the follicle cells to a squanous form, the binucleate condition being retained and the nuclear envelope is smooth in outline.

Scale : ——— represents 10 μm.

Fig 3.13



for its transport into the oocyte was obtained. The electron photomicrograph (fig. 3.14a) clearly demonstrates the abundance of rough endoplasmic reticulum in the follicle cell at this stage, suggesting considerable protein synthesis within the cell though no evidence for exocytosis or other means of transporting the protein out of the cell was detected.

The end of previtellogenesis is marked by the binucleate appearance of the follicle cells. It is suggested that this is brought about by the amitotic division of the nucleus, a conclusion arrived at due to the almost total absence of mitotic activity in the follicular tissue of this age, and that although a large number of sections were examined no indication of cell fusion could be found. Typically, immediately before the appearance of the binucleate condition, which usually occurred gradually over approximately 48 hours, the nucleolus split into two equal fractions which was then followed by nuclear division; the daughter nuclei then migrated to the two lateral walls of the cell. Amitosis has previously been reported in insect follicular epithelium (Masner 1966; Ramamurty 1972) and suggested by Bonhag & Wick (1953) to occur in Oncopeltus fasciatus. In the late previtellogenesis stage, intercellular spaces were irregularly present between the follicle cells, approximately 1-3 μm wide. Birefringence was apparent between the follicle cells and oocyte, which may indicate a brush-border, though this was not investigated with the electron microscope.

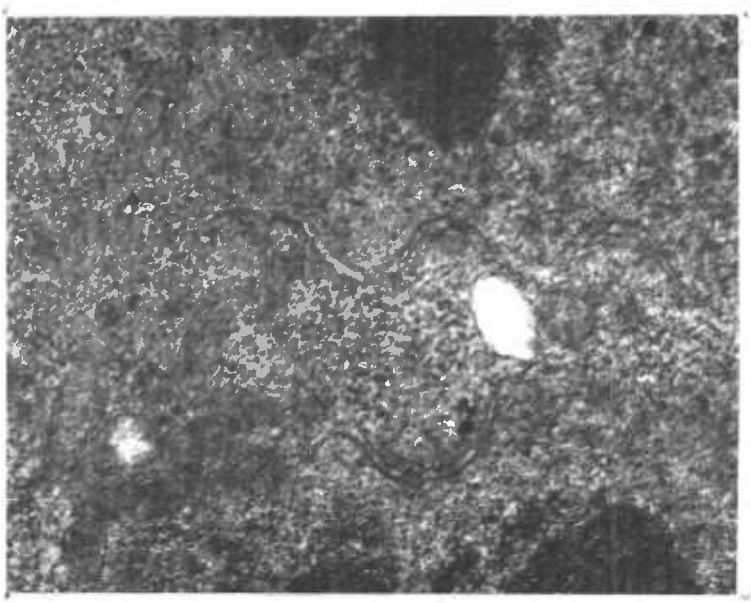
As vitellogenesis proceeds, morphological differences develop between the lateral and apical follicle cells. With the rapid increase in oocyte size the lateral cells become short, approximately 18-20 μm thick and broader and with occasional large spaces of 6-8 μm appearing between the cells their development could be traced as the follicle cells matured, indicating that they are real spaces and not artifacts. Rarely a single follicle cell may undergo break-down, leaving a considerable

Fig. 3.14

Follicular epithelmium during oogenesis in G. fennahi

a.

Numerous endophasmic reticulum in the columnar type follicle cells suggesting considerable protein synthesis x 2000



b.

During vitellogenesis the nuclear membrane of the follicle cells becomes considerably convoluted x 1800

gap in the follicular wall. The nuclear membrane of the lateral cells becomes considerably convoluted (fig. 3.14b) and the cytoplasm remains strongly basiphilic and stains positively for RNA. The cells in the apical region, around the trophic cord, retain their columnar appearance and are closely opposed to each other, as seen in the previtellogenic follicular tissue, and also in close contact with the oolemma.

During rapid yolk deposition (fig. 3.15) and growth of the oocyte, the follicular cells become cuboidal, approximately 15 μm across, and the end of vitellogenesis is marked by the transition of the cells to a squamous form as they become stretched to cover the enlarging oocyte. At this time the interfollicular spaces and follicular oocyte distance which were characteristic of yolk deposition are reduced (fig. 3.16).

The follicle cells remain binucleate, the nuclear envelope becomes less irregular and the chromatin is distributed peripherally around the nuclear membrane. This is the condition of the oocyte prior to insemination. Chorion synthesis and deposition by the follicle cells does not occur until a successful insemination. In old virgin females yolk resorption is initiated from the oldest oocyte.

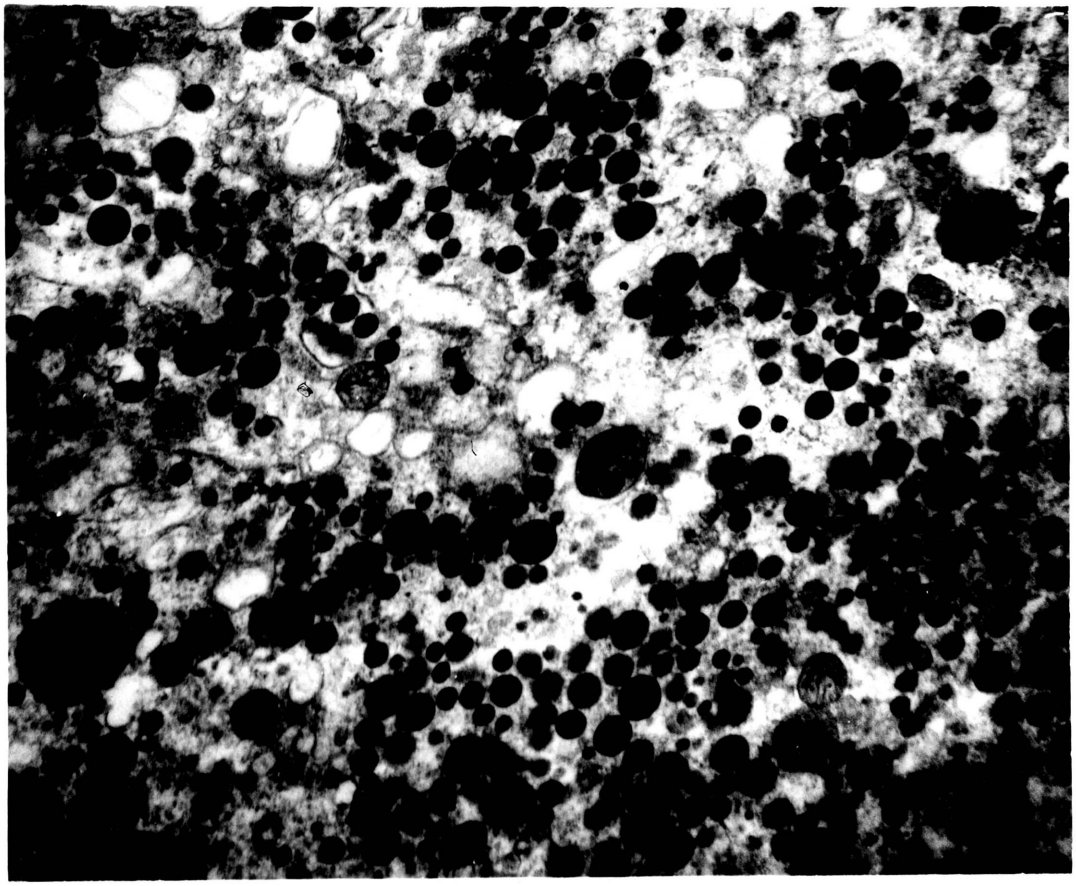
The chorion is an acellular layer deposited between the follicle cells and oolemma and in Graphocephala is not sculpured. During chorion formation, basiphilic particles were present in the follicular cytoplasm and released by exocytosis.

The interfollicular tissue between successive follicles arises from prefollicular tissue that becomes trapped during follicle formation. It retains the characters of prefollicular tissue, the cells being small and capable of mitotic activity. As the oocytes grow, the interfollicular plugs become compressed, causing their cells and nuclei to flatten. The tissue that seals the posterior end of the vitellarium is commonly referred

Rapid yolk deposition

Transverse section through a growing oocyte during the rapid accumulation of yolk droplets.

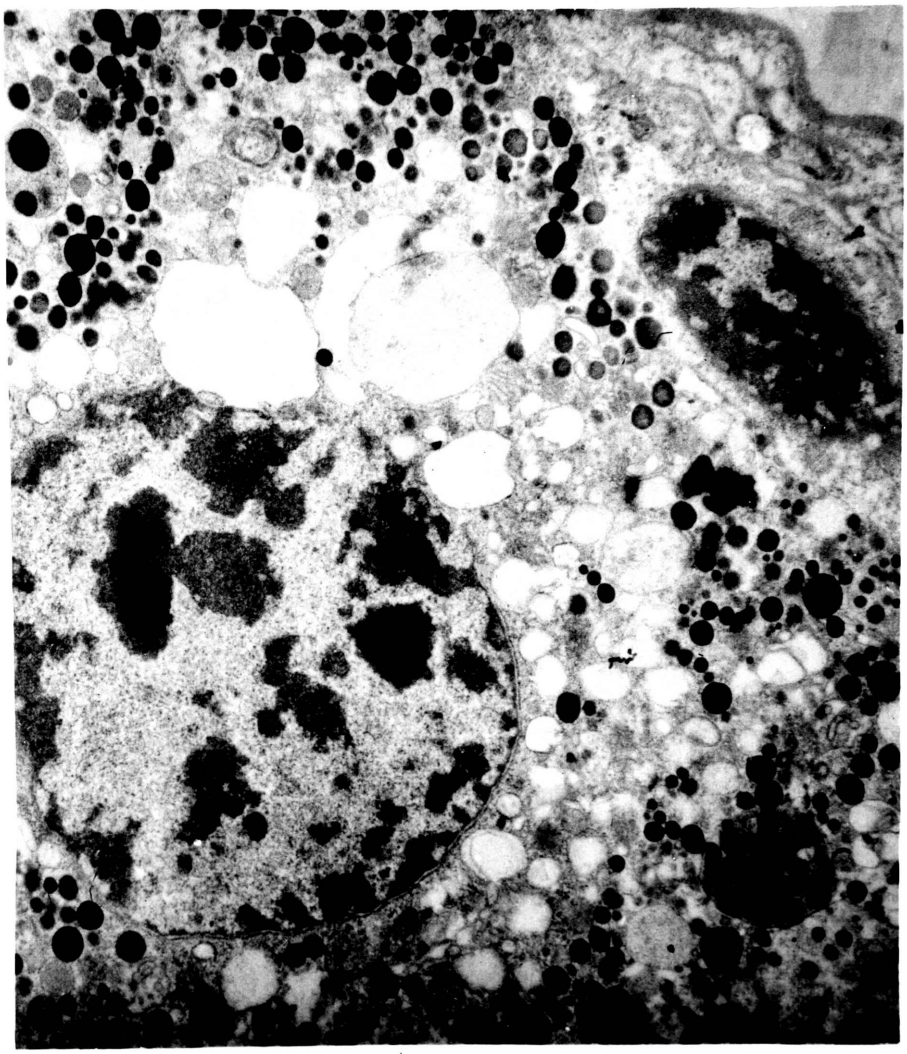
x 20,000.



Transverse section through an oocyte towards the end of vitellogenesis. Note the germinal vescicle and the squamous appearance of the follicular epithelium.

x 20,000.

3.16



to as the epithelial plug and pressure from the oldest oocyte causes its breakdown at ovulation. Collapse of the follicle after egg extrusion results in the formation of a corpus luteum formed by the disintegrating follicular epithelium at the base of the ovariole. The old follicle undergoes histolysis, the cells walls are no longer visible and the nuclei form intense basiphilic granules. This mass is extruded into the pedicel by the posterior movement of the next follicle where it appears to be resorbed.

One day after ovulation the corpus luteum has contracted to produce a highly vacuolated cytoplasm in which the regular arrangement of the nuclei is no longer present. Nucleic material is represented by scattered basiphilic granules often associated with the sides of the vacuoles. Within two days of ovulation this mass has become further reduced and vacuolization has increased. Pigmentation of the corpus luteum was not evident in Graphocephala, though it has been recorded in Locusta (Singh 1954).

Little work has been undertaken on the Hempiteran corpus luteum, the most notable studies being those of Wigglesworth (1936)in Rhodnius prolixus, and Bonhag and Wick (1953) in Oncopeltus fasciatus, but neither are detailed. Singh (1954) broadly described corpus luteum formation in insects to describe differences in its structure in the three types of ovaries.

iv. Ovariole sheath.

Comparatively few studies have been made of the insect ovariole sheath, the most thorough being that of Bonhag & Arnold (1961) on Periplaneta americana, in which they described three components: (a) the sheath tissue proper, (b) tracheal tissue, and (c) an intercellular matrix forming the tunica propria. The presence of muscle fibres has been reported in a number of insects by King (1960), Roth & Porter

(1964), King & Aggarwal (1965) and Cruikshank (1973).

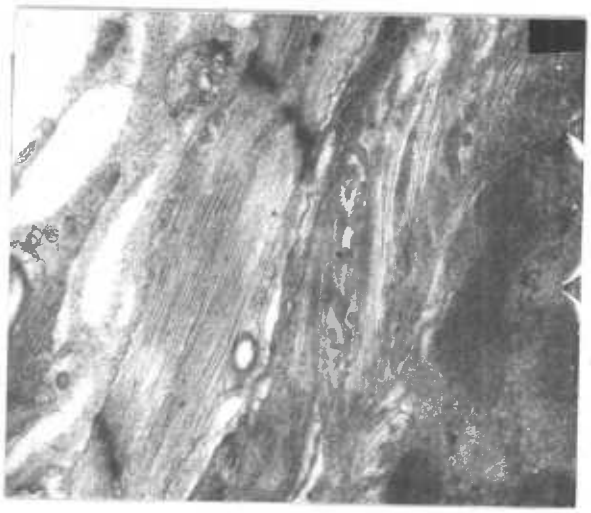
In Graphocephala, the sheath is continuous over each ovariole from the anterior extremity of the germarium posteriorly to the pedicel; it is a composite structure, composed of an outer layer of striated longitudinal muscle and a small inner layer of circular muscle, internal to which may be more, irregularly arranged, longitudinal muscle fibres. Striations are well defined with a mean sarcomere length of 4.44 μm (fig. 3.17a), which is comparable to that found by Cruikshank (1973) in the flour moth. No H band was discernible, probably due to the filaments not being superimposed. Mitochondria were frequently present, usually concentrated within the small layer of circular muscle, though also present around the periphery of the longitudinal muscle fibres. The sheath was richly supplied with tracheae, tracheoles penetrating the outer longitudinal muscle layer, though they were not observed to penetrate the whole sheath.

In the anterior half of the germarium the sheath possesses an outer layer of osmiophilic material 250 nm in thickness (fig. 3.17b), which gives way in the posterior part of the germarium to a less dense, more diffuse layer, which is continuous over the outer layer of longitudinal muscle.

The description of the ovariole given for Graphocephala differs from those provided for other groups in that it possesses a loose network of fibres inside of the outer longitudinal layer and in which the circular muscle is embedded. The outer layer is a well defined region of longitudinal muscle with an obvious sarcolemma. Inside this are small bundles of circular muscles interspersed in a loose network of longitudinal muscle fibres (fig. 3.17c), which do not form a discrete layer. The tunica propria separates the muscular component from the underlying trophic and follicular tissue. It is composed of two layers, between which is a diffuse, lightly staining, granular

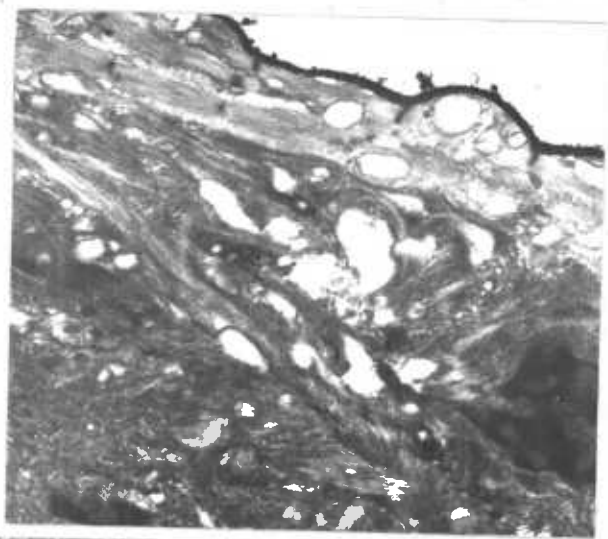
Fig. 3.17

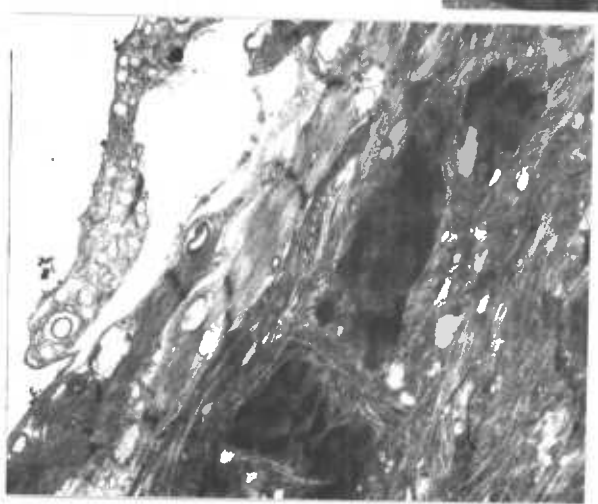
The ovariole wall



Striations on the longitudinal muscle of the ovariole sheath x 18,000

In the anterior germarium the sheath possesses an outer layer of osmiophilic material.





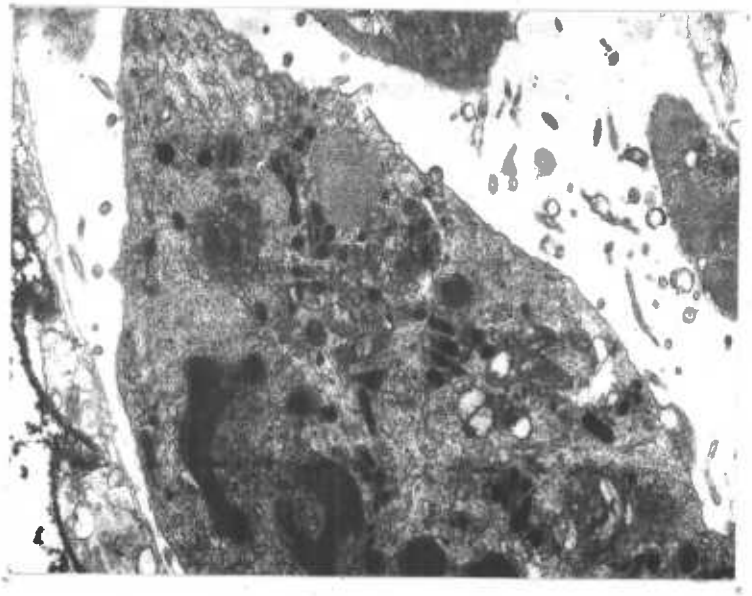
Electron micrograph to show the composition of the ovariole sheath, outer longitudinal muscle and inner circular muscle embedded in a loose network of longitudinal fibres. X 4300

material. Spaces were often present, either within the tunica propria, or between it and the muscular layers. In the vitellarium, the tunica propria is always closely applied to the follicle cells and remained as a discrete layer between the intercellular spaces of the follicle cells, so providing a diffusion barrier to haemolymph proteins. This differs from the condition described by Cruikshank (1972), where the tunica propria was discontinuous over the intercellular spaces of the follicle cells.

Cells were present within the matrix of the ovariole sheath, irregularly arranged throughout the sheath, either within the spaces of the tunica propria (fig. 3.18a), or more commonly within the loose network (3.17c) of contractile fibres. Such cells were also described by King & Aggarwal (1965) in Hyalophora cecropia, and termed lumen cells, which appears to have been adopted in the literature, though the term appears somewhat misleading as the cells are not associated with the lumen, but between the tunica propria and the sheath. It would appear more acceptable to term them haemocytes, especially if they can be demonstrated to be phagocytic, since Salt (1967), while discussing defense mechanisms, concluded that phagocytosis in insects is carried out exclusively by blood cells. These cells in Graphocephala commonly contain a large nucleus with an irregular outline and large quantities of rough endoplasmic reticulum. Mitochondria are also present. It is thought that these cells have a phagocytic function, since bacteria-like bodies in various stages of digestion and other, unidentified inclusions were present within the cell. In addition to this, the cells secrete a fine granular material into the sheath matrix. The electron photomicrograph (3.18b) shows a portion of one such cell, with dark asmiophilic granules present in the cytoplasm and considerable excytosis of material into the surrounding lumen. The composition or function of the secretion is not known, though in appearance it is

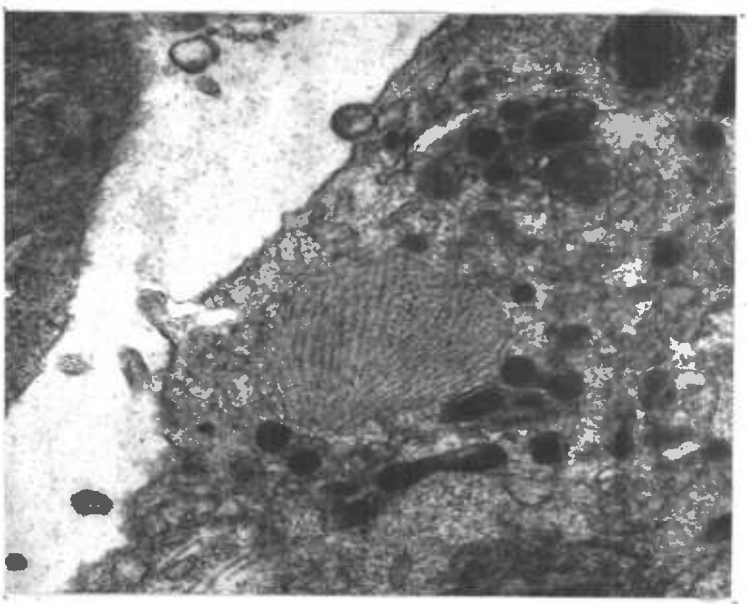
Fig 3.18

a.



Lumen cell within the tunica propria x 4300

b.



Lumen cell showing excytosis of material into the surrounding cavity. The function is not known though may be involved in tunica propria formation x 9800

similar to the homogeneous material within the tunica propria.

During ovulation, contraction of the sheath cells in the region of the vitellarium occurs and aids the rupture of the epithelial plug, so allowing extrusion of the oocyte, and also pulls the penultimate follicle down into the final position. In agreement with Cruikshank (1973) vivisection reveals that the oocytes can be moved within the ovariole sheath, probably through differential contraction of the sheath itself. Movement is greatest in the oldest follicle in which the trophic cord has been severed. This observation prompted Cruikshank to suggest that haemolymph proteins may be pumped mechanically through the follicle layer to the oolemma, through special pores in the sheath, by this action. However, in Graphocephala, the sheath, as viewed by the transmission electron microscope appeared to be continuous over the whole vitellarium, so it seems unlikely that Cruikshank's suggestion is possible in this species.

Gross staining of the ovariole demonstrates that the sheath cells contain RNA, which agrees with the work of Bonhag & Arnold (1961) for Periplaneta americana, and that of Cruikshank (1973) on Anagasta kühniella. Rough endoplasmic reticulum is not present in the sheath cells (only the lumen cells), so it is unlikely that any protein synthesised is released by the sheath cells or used in the formation of the tunica propria. The role and origin of this layer remains unclear, though it is continuous over the inter-follicular cell spaces, suggesting it may act as a mechanical barrier, which would prevent back diffusion when extra ovarian proteins have entered.

The presence of lumen cells has been recorded previously. Brunold (1957) described haemocytes in the space between the sheath and tunica propria, while King & Aggarwal (1965) described ameoboid cells occupying a similar position in Hyalophora cecropia. Cruikshank (1973) described phagocytes in this region. The cells described in the present study, measuring approximately 12 - 14 μm in their long axis, appear to serve phagocytic and secretary functions in Graphocephala, and may

be involved in formation of the tunica propria.

During the present study, symbionts were not detected within the body of the ovariole, though in other Cicadellid species they are known to be commonly present, and make a necessary contribution to the insect's metabolism. Hamon (1971) recorded the presence of Rickettsia-like organisms in the apical trophic tissue and the trophic cords of the telotrophic ovarioles of Ulopa reticulata, though few such organisms were detected in the oocytes.

The importance of the symbionts to certain Cicadellid species was recently demonstrated by Schwemmler (1973) in his work with Euscelis plebejus. He was able to show that symbionts are transmitted from generation to generation by intraovarian infection, and that partial elimination of the symbionts from nymphs and adults results in the slowing down of their vital functions while their complete elimination caused death.

v. Pedicel.

This forms the outlet duct of the ovariole, which leads directly into the calyx, the anterior extremity of the lateral oviduct. In the proximal region, that nearest to the epithelial plug, the ovariole sheath is continuous over the pedicel, though at its junction with the calyx the sheath tissue is disrupted and gives way to a thin muscular layer, similar in arrangement to that of the lateral oviduct, that is, a very thin outer layer of circular muscle and an inner layer of longitudinal muscle. The total muscle layer does not exceed 15 μm in thickness.

Each pedicel is approximately 290 μm in length and of mesodermal origin. The epithelium is composed of columnar cells which possess well defined cell boundaries, unlike the condition described in Adelphocoris by Masner (1966). The nuclei, approximately 4 μm in diameter, are situated towards the basal end of the

cell, away from the luman, and contain a single well defined nucleolus (fig. 3.19a). The cytoplasm is granular and faintly basiphilic. Masner indicated that, in Adelphocoris, the epithelium in the proximal region was modified in connection with the resorption of the histolysed follicle after ovulation. No such modifications could be seen in Graphocephala, its appearance being similar in the young immature female before ovulation and in the mature female.

(b) Lateral oviducts

The lateral oviducts are bulbous anteriorly, forming the calyx on either side and it is this region that the pedicels enter. Posteriorly, each pedicel fuses during development with a short branch of the median oviduct. Histologically the lateral oviducts are similar to the pedicel. The epithelium is columnar to cuboidal with centrally placed spherical nuclei. The duct lumen is approximately 35 µm wide, and contains a faintly basiphilic secretion. Eggs are not stored in any part of this lateral oviduct, a condition that contrasts with the Heteropteran species studied (Bonhag & Wick 1953; Davis 1961; Masner 1966). The muscular sheath is better developed than that of the pedicel (fig. 3.19b) and composed of a stout inner layer of longitudinal muscles approximately three times thicker than the outer circular muscle layer. The muscles possess obvious striations with a sarcomere length of 10 - 12 µm, very different from that of the ovarian sheath, suggesting a functional difference between the two regions. The muscle nuclei are peripherally positioned and strongly basiphilic.

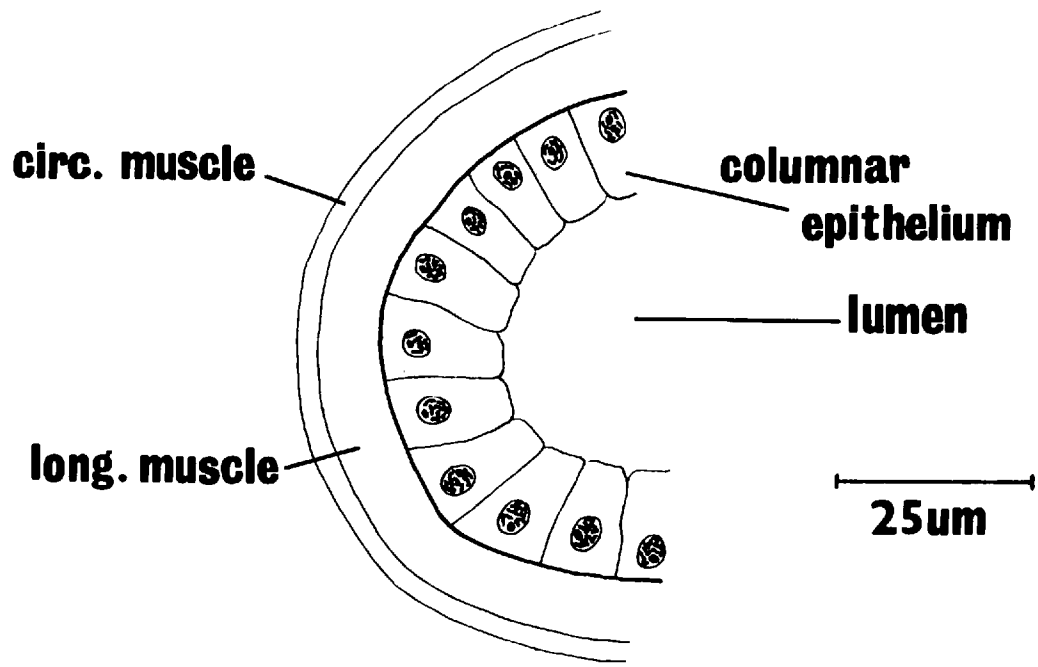
(e) Median oviduct and vagina.

Several clearly defined histological regions exist within this part of the reproductive tract. Though ectodermal in origin (ampullae) only the posterior part of the vagina possesses a chitinous intima.

The anterior fifth of the median oviduct is similar in all respects to the lateral

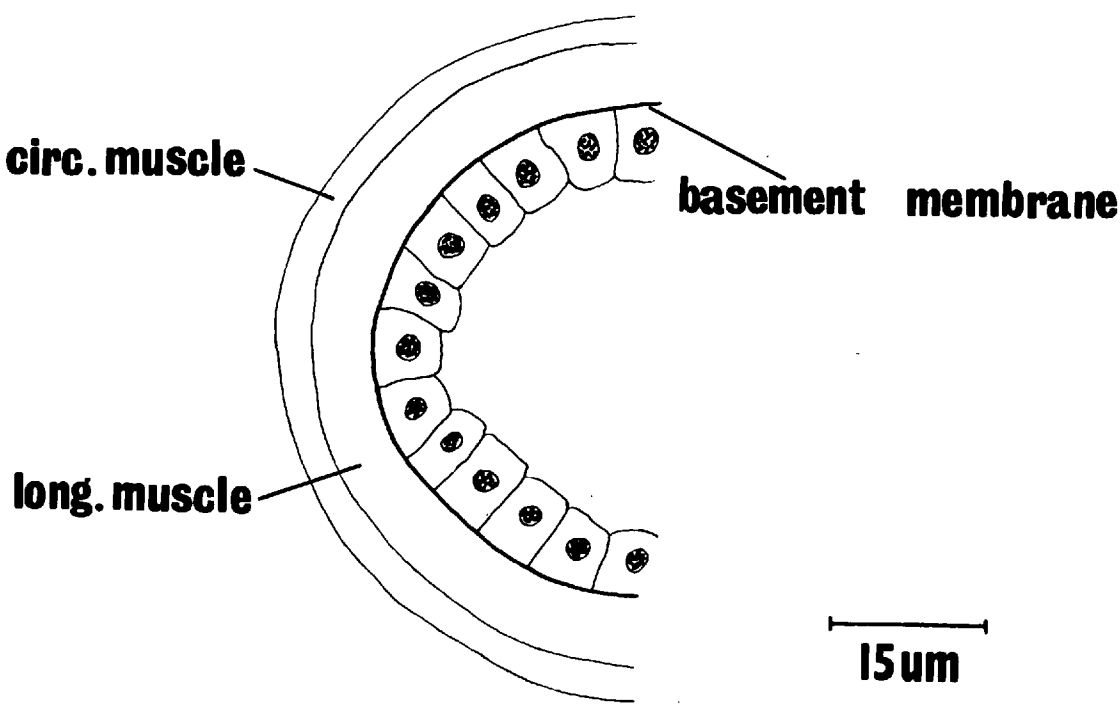
Fig 3.19a

Transverse section through the distal pedicel



b

Transverse section through the lateral oviduct

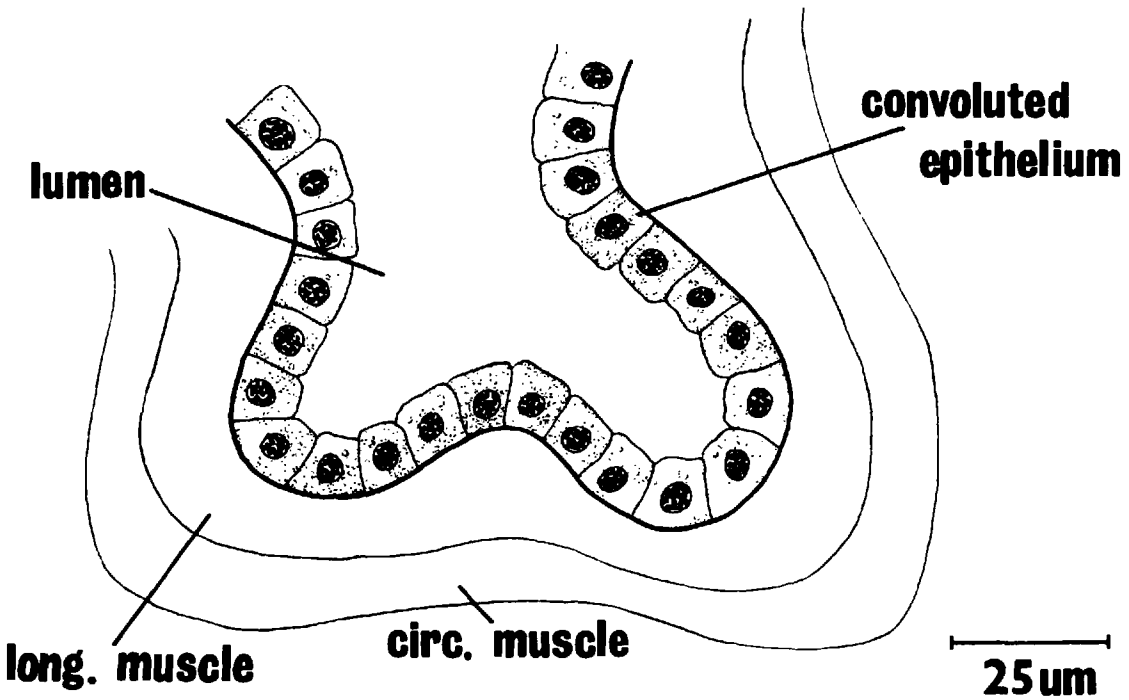


oviduct, the transition between the two regions being histologically difficult to define. The succeeding 300 μm is characterised externally as a thick, highly muscular, much convoluted tube, capable of considerable extension (fig. 3.20a). In transverse section the epithelium is composed of a single row of cuboidal cells which contain a highly granular, basiphilic cytoplasm and a very large densely basiphilic nucleus. The cells are involved in secretary activity, the products being released into the lumen via the irregular apical cell surface. The cells stained strongly for RNA, suggesting considerable protein synthesis. The epithelial cells rest upon a very fine basement membrane into which the longitudinal muscles insert. The muscular sheath is completed by an outer layer of circular muscle, the total sheath being some 25 μm in thickness. At the anterior and posterior ends of this region the circular muscle is somewhat reduced.

This muscular pouch passes into a third region, approximately 75 μm in length and 45 - 50 μm wide. This region lacks the extensive muscular sheath of the previous parts, and the lumen is less folded though considerably wider than that of the pouch. The epithelial cells are characteristically columnar in form with spherical nuclei 3.0 - 3.5 μm diameter situated at the basal end of the cell and containing a nucleolus. The cytoplasm stains only slightly for extra-nuclear RNA, so that these cells are probably not secretary in function (fig. 3.20b). This short duct passes into a fourth zone, the vagina, which forms a greatly distended, slightly muscular pouch, approximately 150 μm in length and 100 μm in diameter. It is thin walled, and the epithelial cells are cuboidal to squamous in form, with irregular flattened nuclei. The muscular sheath is considerably reduced, the longitudinal muscle forming the major component, outside which were occasional bundles of circular muscle fibres. The spermatheca enters this region dorsally by a narrow duct. This sac opens by a short, thin-walled duct between the gonapophyseal

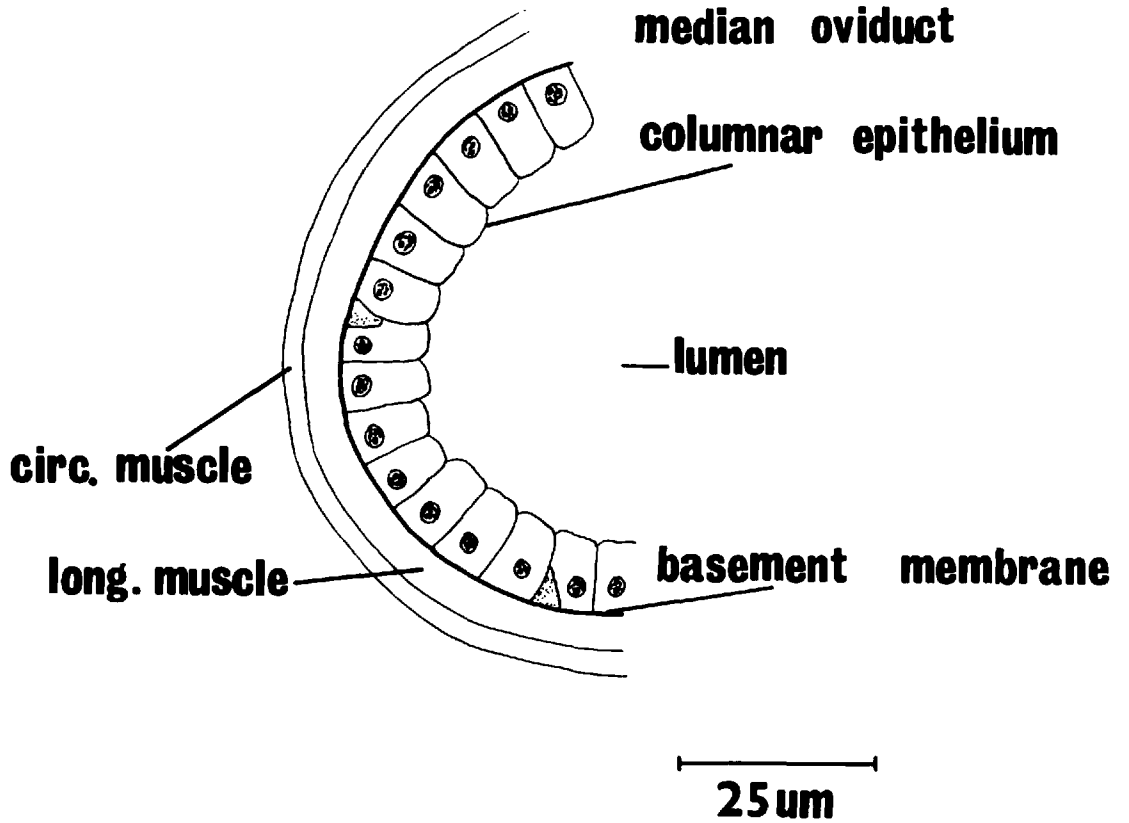
Fig 3.20a

Transverse section through the thickened region of the median oviduct



b

Transverse section through region 3 of the median oviduct



bases, longitudinal muscle fibres being absent from this extreme posterior region.

The oviduct can therefore be divided into four regions, of which one is probably secretary. The outer muscular sheath, though it varies considerably in the thickness of its constituent layers, always possesses an inner layer of longitudinal fibres and outer circular fibres.

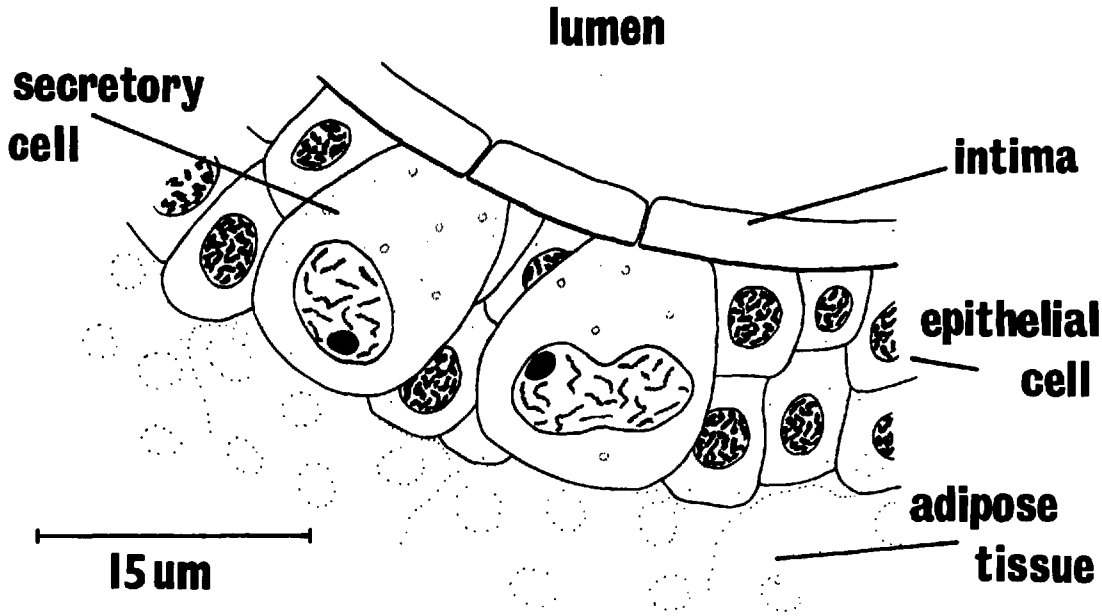
(d) Spermatheca

In Graphocephala the spermatheca retains its original function of sperm storage unlike that in some Heteroptera, e.g. Adelphocoris, Miridae as described by Masner (1966). The spermatheca is divided into two parts, the duct and the spermathecal gland, a large spherical organ, which is not only glandular, but also forms a sperm reservoir and which, in the natural position, lies partially hidden beneath the median oviduct, and is approximately 300 μm in diameter in the mature female. From this large bulb a short duct runs into the dorsal surface of the vagina. In the inseminated female a solid pink sphere, approximately 70 μm diameter, can be seen through the spermathecal wall and represents spermatozoa and other ejaculatory material. As described in the section on development (p. 102)the spermatheca is ectodermal in origin and both the duct and the gland possess a cuticular intima.

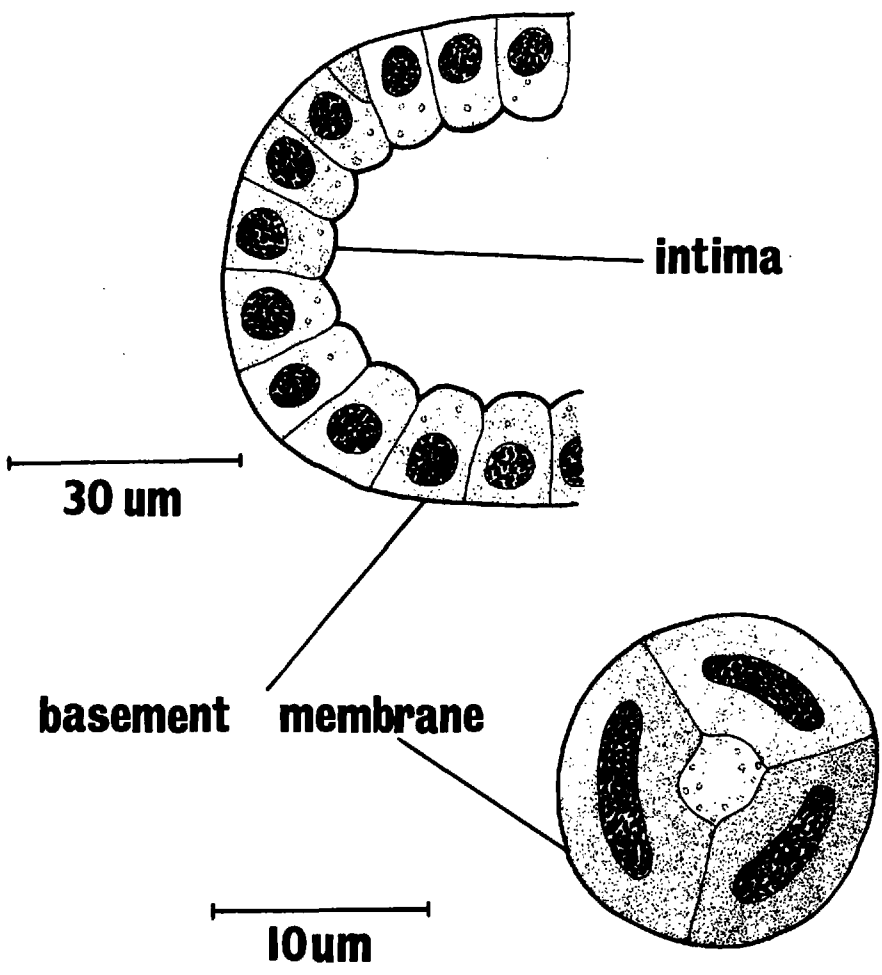
The wall of the spermatheca is produced into a complex array of folds and is composed of two cell types, the whole being covered by a thick intima (fig. 3.21a). No muscle fibres are associated with the spermatheca, the epithelial cells rest upon a tough, fibrous basement membrane and the whole gland is invested by an irregular deposit of adipose tissue. The main body of the gland is made up of small epithelial cells 10 - 12 μm in their greatest dimension and of irregular outline, though characteristically with their long axis at right angles to the wall. They contain large spherical nuclei 6 - 7 μm in diameter with diffuse chromatin, the nuclei being centrally placed within the cell. They produce a layer two or rarely three cells thick. Inter-

Fig 3.21a

Transverse section though the wall of a mature spermatheca



Transverse sections of the accessory gland



spersed among this tissue and particularly associated with the troughs of the folded epithelium are large secretory cells 15 - 18 μm in their longest axis and ovoid in form. They are irregularly grouped over the wall, never more than three per group and with a minimum distance of 25 μm between the groups. The large, densely basiphilic nuclei, approximately 10 - 11.5 μm diameter, are spherical or lobate in appearance, containing scattered chromatin and a nucleolus that was invariably associated with the nuclear envelope. The cytoplasm contains a fine granular material and stained heavily for RNA, suggesting a secretory function. Distributed throughout the cytoplasm are small vacuoles containing a basiphil material, which is apparently secreted into the gland lumen. No intracellular ductules were found, as reported by Bonhag & Wick (1953) in Oncopeltus.

The intima, secreted by the small epithelial cells, is continuous over the whole of the gland, interrupted only in the region of the secretory cells, where it was perforated by fine ducts, which presumably convey the secretory products into the gland lumen to nourish the stored sperm. The intima has a lamellar appearance, and is thickest on the ridges of the folds (7 - 8 μm) and thinnest in the troughs (4 - 5 μm) which is the region in which most of the secretory cells are concentrated. It is lightly pigmented and produces a slight fushinophil reaction.

The duct leading from the gland to the vagina is of simple construction, the epithelial wall is composed of a single layer of cuboidal cells, which rest on a basement membrane. The inner surface is lined by a thin intima, which is not folded or sculptured, and which encloses a small lumen of 8 - 10 μm diameter. Outside the basement membrane is a narrow zone of circular muscle fibres.

(e) Accessory gland

A single median accessory gland is present in Graphocephala fennahi, and can be divided into a proximal and distal region, according to its external appearance

and histology. The proximal half forms a wide duct with a lumen about 40 μm in diameter, and its single-layered epithelium is composed of columnar cells. The nuclei are large, 10 - 12 μm diameter, with a dense, basiphilic dispersed chromatin and situated basally in the cell. A nucleolus was not seen. The cytoplasm is highly granular, strongly basiphilic and with acidophilic vacuoles containing a homogeneous secretion (fig. 3.21b). The lumen is filled with a finely granular acidophil secretion which is absent in older females. Infrequently distributed between the apical region of the epithelial cells were small, angular cells, approximately 5 - 7 μm in their greatest dimension, each containing a large lobate nucleus with a thin coat of cytoplasm. In the large number of specimens sectioned, such cells were always present, numbering fifteen to twenty cells, and restricted to the thickened proximal region. The cells were not present in any of the nymphal stages, but were found in immature, mature and scenescent adult females. Their function is not known. Similar cells containing nuclei, triangular in section, were described in E. fabae by Helms (1968) and also along the lumina of several thoracic ectodermal glands in the Hemiptera described by Henrici (1940).

The distal region of this gland is extremely slender with a small lumen approximately 2 - 3 μm in diameter, and continuous with that of the proximal half. The single-layered epithelium is composed of small, flattened cells, approximately 8 - 9 μm in their long axis parallel to the lumen. The flattened nuclei are centrally situated and strongly basiphilic (fig. 3.21c). The cells are rich in extra nuclear RNA and contain acidophilic particles, similar to those present in the proximal epithelial cells. The lumen was densely filled with a granular substance that possessed similar staining properties.

A very fine intima was present only in the proximal third of the accessory gland, and no evidence for a muscular sheath could be found.

6. Discussion

The above study describes the development and differentiation of the female reproductive system in Graphocephala fennahi, and while several similar studies have been undertaken in the Heteroptera, principally by Gross (1901), Bonhag & Wick (1953), Wick & Bonhag (1955), Bonhag (1955), Ramamurty (1970) and Huebner & Anderson (1972), this is the first study of its kind in the Auchenorryncha. The work of Helms (1968) described the development in Empoasa fabae, and has been shown to differ in several aspects from that of the present species; he did not describe the histology of the adult reproductive system.

The origin of the prefollicular tissue is a controversial subject, though in Graphocephala it has been shown to arise from mesodermal tissue, and not the germinal tissue, as suggested by Ramamurty (1970). The present study agrees with the suggestion of Seidel (1924), Leutenshlager (1932) and Wick & Bonhag (1955), that only the trophocytes and oocytes arise from the germ-line, the prefollicular tissue being derived from the anterior region of the posterior strand, having a similar origin to the pedicel, which later becomes associated with the germ cells in the germarium.

Considerable variation occurs within the Hemiptera regarding the initial form of the prefollicular tissue. Gross (1903), Bonhag (1955) and Masner (1968) reported a syncytial arrangement, though this condition is not convincing until examined with the electron microscope, while in the present study and that of Huebner & Anderson (1972) on Rhodnius the prefollicular tissue was cellular. During previtellogenesis, mitosis occurred in the prefollicular zone and in the multi-layered follicular epithelium surrounding young oocytes. Cytoplasmic connections between the follicle cells or follicle cells and oocytes were not present and the previtellogenic phase of follicle cell differentiation is presumably due to activation by the oocyte and trophic tissue,

as suggested by Masner (1968), which may initially be the mechanical effect of growth.

As reported by Wick & Bonhag (1955) for Oncopeltus and Masner (1966, 1968) for Adephocoris and Pyrrhocoris, the follicular epithelium differentiates throughout adult life. In the adult, prefollicular tissue differentiates into the follicular epithelium in which a sequential development can be followed. However, the function of these cells remains relatively less clear than that of the trophocytes, though a wide variety of functions has been suggested (Raven 1961; Davidson 1968; Quattropani & Anderson 1969; Ramamurty 1970). While previous authors suggest the follicle cells function in the transport of RNA to the oocyte, there is little experimental evidence to support this. The present study showed the accumulation of RNA in the follicle cells, but its transport out of the cell could not be demonstrated. Some workers suggest that exogenously produced proteins are transmitted through or into the follicle cells prior to their appearance in the ooplasm (de Loof & Lagasse 1970). In Graphocephala, it seems more likely that such proteins reach the oocyte via intercellular spaces between the follicle cells, as has been shown to occur in Periplaneta (Anderson 1964 and 1969), Aedes (Roth & Porter 1964) and Tenebrio (Aggarwal 1968) In Graphocephala the lateral follicular epithelium undergoes changes to produce intercellular spaces during vitellogenesis. If proteins enter the oocyte by this route they still have to traverse the ovariole sheath and tunica propria. Though an endogenous protein is produced in the follicle cells of Hyalophora (Anderson & Telfer 1970) which is incorporated into the yolk, no histological or ultrastructural peculiarities which could be interpreted as indicating protein transport from the follicle cell was found in Graphocephala, and the histological evidence of increase in RNA and rough endoplasmic reticulum may be a preparation for the subsequent secretion of the chorion.

The apical epithelium tissue undergoes a less marked differentiation. Though derived from the same prefollicular tissue as the lateral epithelium, the apical follicle

follicle cells show less morphogenetic change, retaining a columnar form. Inter-follicular spaces were not observed in this region and the cells remain closely opposed to the underlying oolemma. The ooplasm beneath the apical cap is also distinct in the presence of only a small number of yolk droplets. This is similar to the condition reported in Hyalophora (Bier & Ramamurty 1964; Stay 1965).

The mechanism of DNA extrusion from the trophic tissue agrees broadly with the earlier work of Schrader & Leuchtenberger (1952) for Coreid bugs and Bonhag (1955) for Oncopeltus, though giant and migratory nuclei were not present. DNA release was observed only in zones III and IV of the germarium of Graphocephala, and resulted both from seepage of peripherally arranged material through the nuclear membrane and by the breakdown of the nucleus, which resulted in the release of droplets into the surrounding cytoplasm. This latter process occurred only in the posterior zone of the germarium. From these observations it seems likely that DNA contributed by the trophic tissue is transported to the developing oocytes. By the examination of a great number of sections the release of Feulgen-positive droplets from zone IV and their accumulation at the posterior end of the trophic region was shown to be cyclic. The Feulgen-positive droplets became Feulgen-negative, presumably by the conversion of DNA into a derivative form, and within a short time new nuclei in zone IV, derived from the mitotic activity of the cells in zone I, broke down to release a new generation of Feulgen-positive droplets. At the posterior end of the germarium the trophic core is extended into a number of trophic cords, each leading to a single oocyte. A Feulgen-positive reaction could not be obtained within these cords, and it is suggested that DNA derivatives are passed down the cords to the oocytes. In this way DNA in some form or other is supplied to the oocytes by the trophic tissue.

The present study demonstrated by the aid of Trevan & Sharrock's stain that

considerable amounts of RNA is contributed to the oocytes of Graphocephala by the trophic tissue. The cytoplasmic basiphilia around the posterior nuclear aggregates and that of the trophic core, posterior germarium and trophic cords is all due to accumulations of RNA. The streaming effect seen in haematoxylin-stained material was also shown to be associated with RNA. It can therefore be concluded that there is a continuity of RNA from the trophic nuclei of zone IV, through the trophic core and trophic cords, to the oocytes. RNA is contributed to the oocyte in this way until the trophic cord is severed at chorion formation. A gradation in basiphilia was evident, being most intense in the youngest previtellogenic oocytes, and it is suggested that this effect is due to the diluting of RNA by the deposition of yolk during vitellogenesis, and is not a reduction in RNA content.

Appreciable concentration of both DNA and RNA were present in the lateral follicle cells but no evidence can be offered to suggest that it is transported to the oocyte; it may be used within the follicle cells during chorion-formation. A small zone of basiphilia due to RNA was noted around the nuclei of the young oocyte, perhaps providing some evidence for RNA contribution by the oocyte itself.

The work of Zinsmeister & Davenport (1971) also suggests that RNA is derived from the trophic tissue and the oocyte nucleus but they were unable to demonstrate that DNA entered the oocyte from external sources.

The present study does not extend to consider the yolk protein synthesis that is found in the oocyte or detailed vitellogenesis. Considerable biochemical work has been directed to this field in the last decade. Evidence suggests that the protein content of insect egg yolk includes material from an ovarian source such as the follicle cells and trophic tissue (Anderson & Telfer 1969; Bell 1970; Wightman 1973), though the bulk of yolk protein is extra-ovarian in origin (Telfer 1961; Kessel & Beams 1963; Anderson 1964; Ramamurty 1964; King & Richards 1969; Wightman 1974;

Huebner et al 1975).

CHAPTER 4

ABDOMINAL MUSCULATURE

In common with other Cicadellids, Graphocephala possesses five nymphal instars, during which, as described in Chapter 2, the external genitalia develop to a form in the final juvenile instar very similar to that found in the adult. It would be expected that a development of the genital musculature, and indeed that of the whole abdomen, would accompany these changes in the sclerotized parts.

In the present study, the adult condition is described, and then myogenesis of the abdominal musculature from the basic condition, as seen in the early second instar, through to that of the adult is discussed, with particular attention being given to the muscles associated with the external genitalia and the processes involved in myogenesis. In the last decade the mechanisms of myogenesis have become of increasing interest to the histologist, particularly with the refined techniques of electron microscopy now available. Much of this work has been restricted to flight and general thoracic musculature both ultra-structure (Auber 1967; Termier & Lauge 1976), and the earlier light microscopy, (Tiegs 1955; Hinton 1959; Boettiger 1960; Pringle 1965). The development of abdominal musculature and that of the Auchenorryncha has been much neglected by previous authors. Vasvary (1966) gave a description of the generalized abdominal musculature of the Cicada Tibicen chloromera, though from a histological viewpoint it is inadequate and no attempt was made to describe its development. The few descriptions of myogenesis in the Cicadellids have invariably been concerned with the flight muscles. Tiegs (1955) has made the most notable study, describing myology and myogenesis in the leaf-hoppers Erythroneura, Stenocotis and Thymbris, but only the thoracic systems were considered. His observations of myogenesis were detailed, and while he notes that

it proceeds, in flight muscles, differently from that in other muscles, the differences were not described.

It is the aim, in the present chapter, to describe the adult musculature associated with the genitalia and of a generalized abdominal segment, together with a detailed account of myogenesis through the nymphal stages of a representative Cicadellid.

1. Abdominal musculature of the adult female

Maki (1938) described the pregenital segments of the adult Huechys sanguinea. Unfortunately, it is an incomplete study, and the muscle attachments were not described though they were illustrated. Kramer (1950) gave a detailed description of the abdominal musculature of the Membracid Ceresa bubalus, including the female genital region, which, though useful, does not permit a direct comparison with that of Graphocephala and it is felt by the present author that certain of the finer muscles were overlooked. Myers (1928), in his detailed description of the morphology of Cicadoidea, only briefly mentioned musculature and gave no descriptions of the abdominal muscles. Vasvary's (1966) description of the musculature in the fourth abdominal segment of Tibicen chloromera is also very brief and cannot be accepted as an exhaustive description.

A comprehensive study of abdominal musculature in the adult Cicadellid was not found in the literature, and it is hoped that the present study of Graphocephala will go some way to providing such an account.

(a) Musculature associated with the genital region of the adult female

Due to the segmental arrangement, the musculature of the first gonocoxa and gonapophysis is contained in segment eight, and that associated with the second gonocoxa and gonapophysis in segment nine (see figs. 4.1 and 4.1a). Numbering of the genital muscles (1 to 14) is consistent throughout the chapter. Muscle 1 in the

Fig 4.1

Dorsal dissection to display the genital musculature of G. fennahi

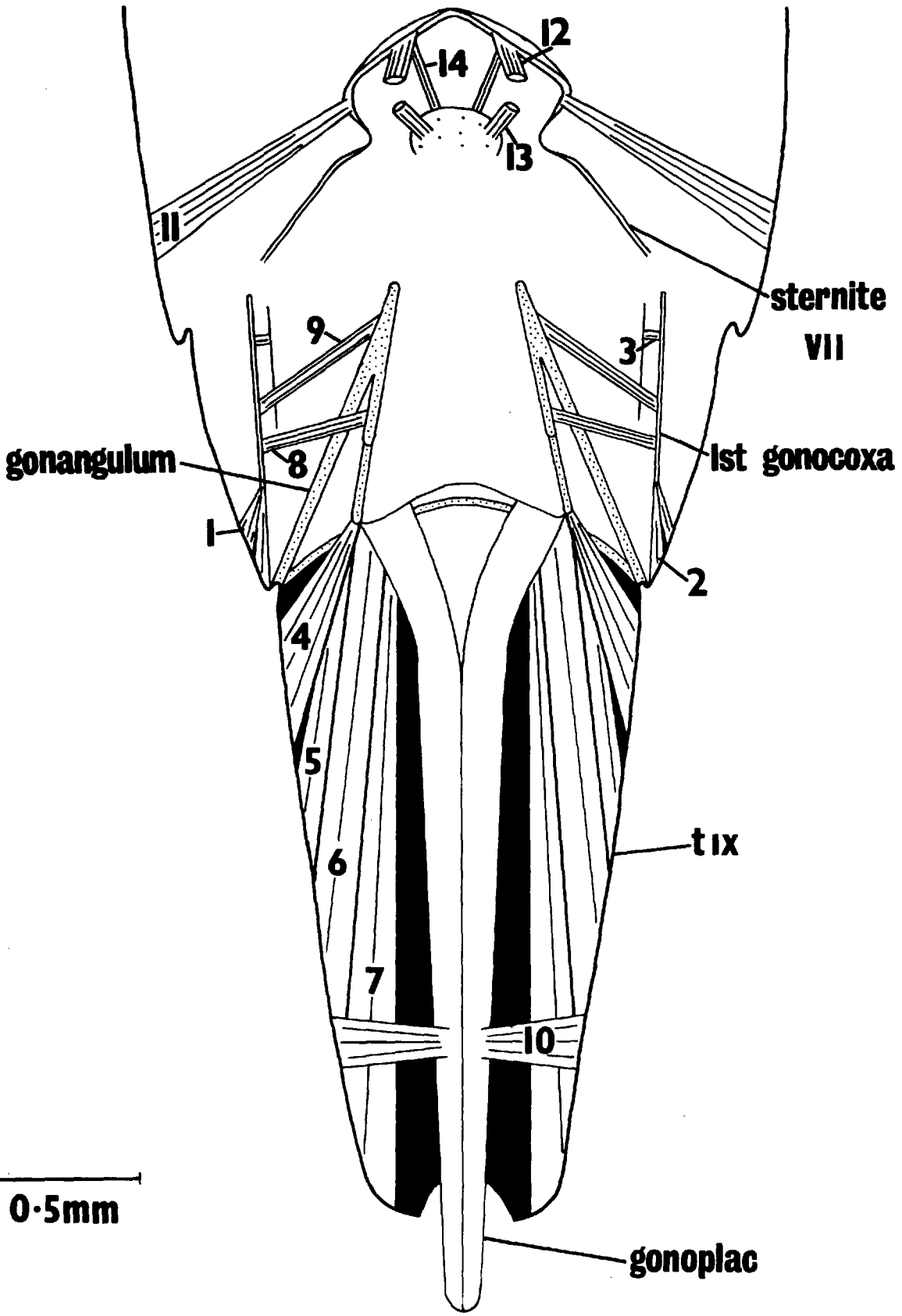
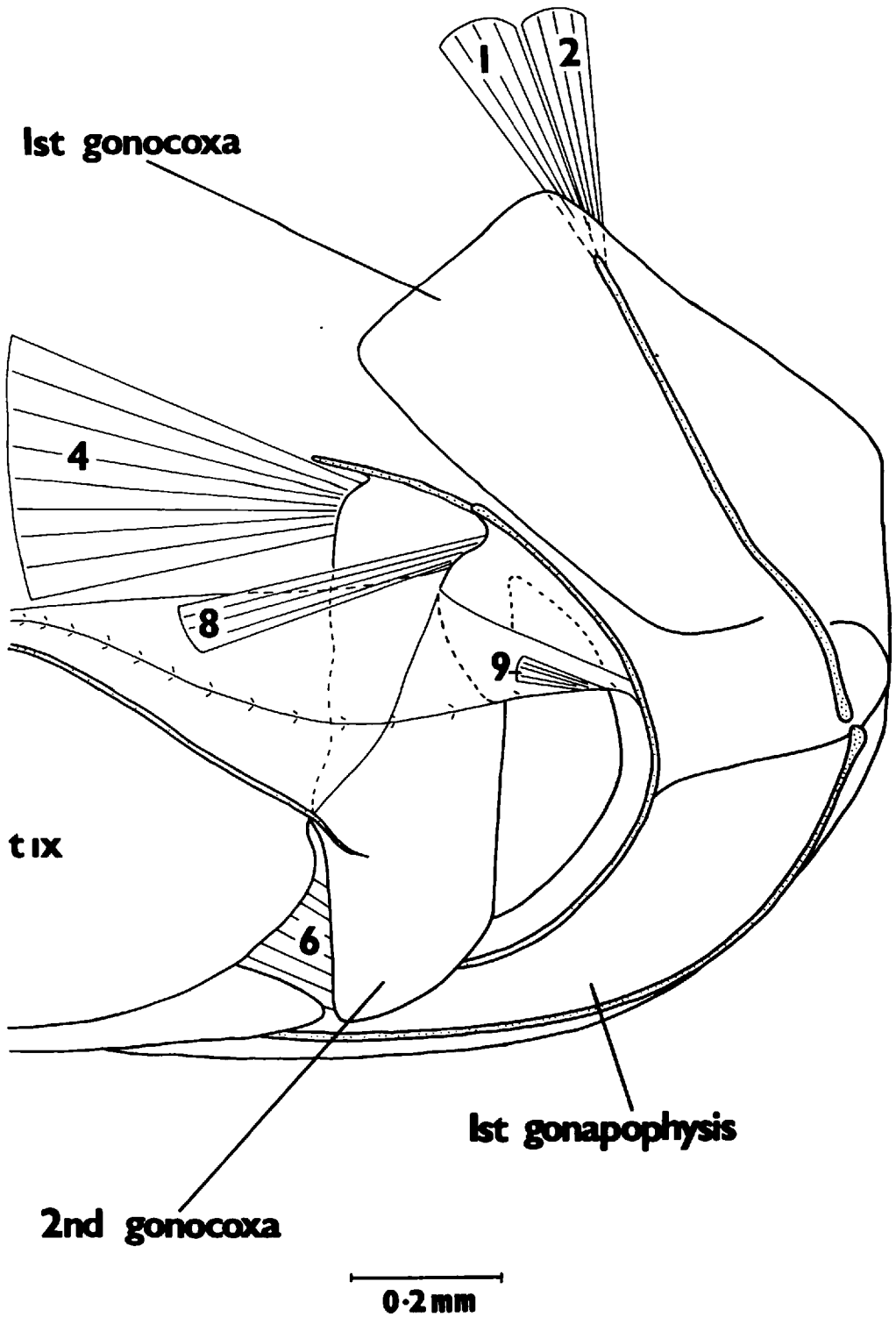


Fig 4.1 a

Musculature of the partially dissected genital region of G. fennahi. 1st gonocoxa displaced dorsally.



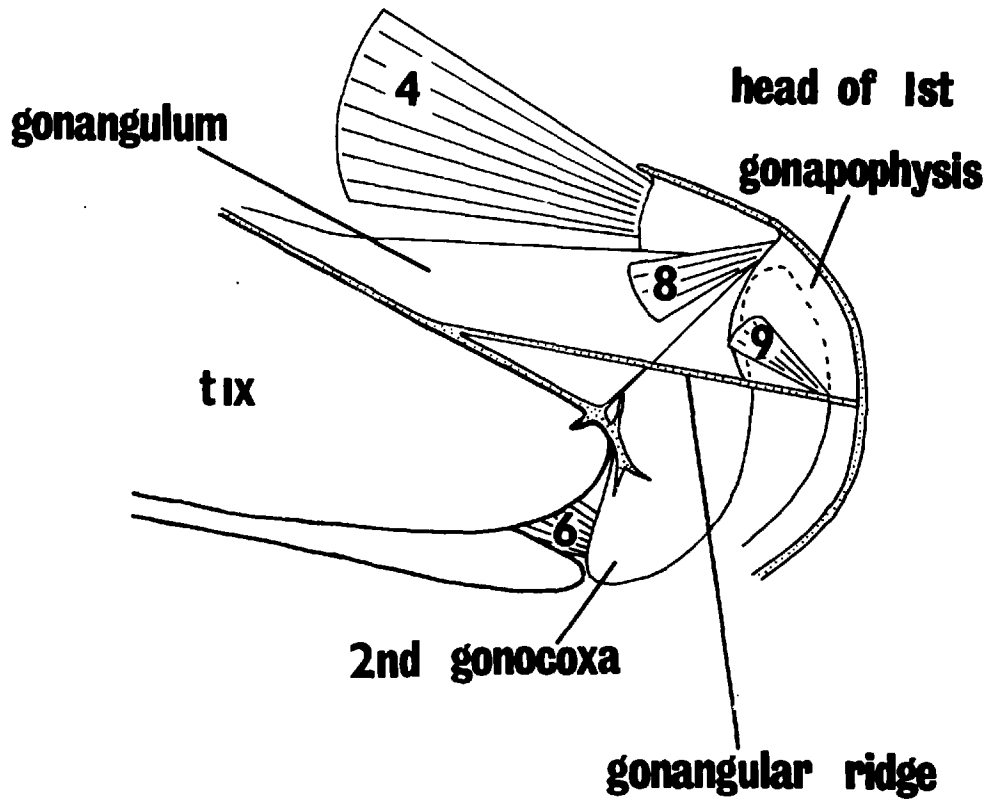
present section is equivalent to that in the nymph. The first gonocoxa has three muscles associated with it. Muscles 1 and 2 insert on the distal margin of the dorsal sclerotized rod of the first gonocoxa and run posteriorly to originate on the posterolateral region of tergite eight. Each muscle is composed of a single, very stout, fibre with numerous peripheral nuclei of 10 μm diameter, the muscle being obviously striated (fig. 4.2). The gonocoxa is made up of a flap, the inner part being small and largely membranous and running from the inner face of the sclerotized portion to the membranous part is a single, very slender muscle, designated muscle 3. The sclerotized rod, which forms the insertion for muscles 1 and 2, is fused at its proximal end to the outer ramus of the first gonapophysis, so that contraction of these two muscles causes a depression and slight anterior movement of the blade. These muscles, however, are not the main instigators of movement within the ovipositor.

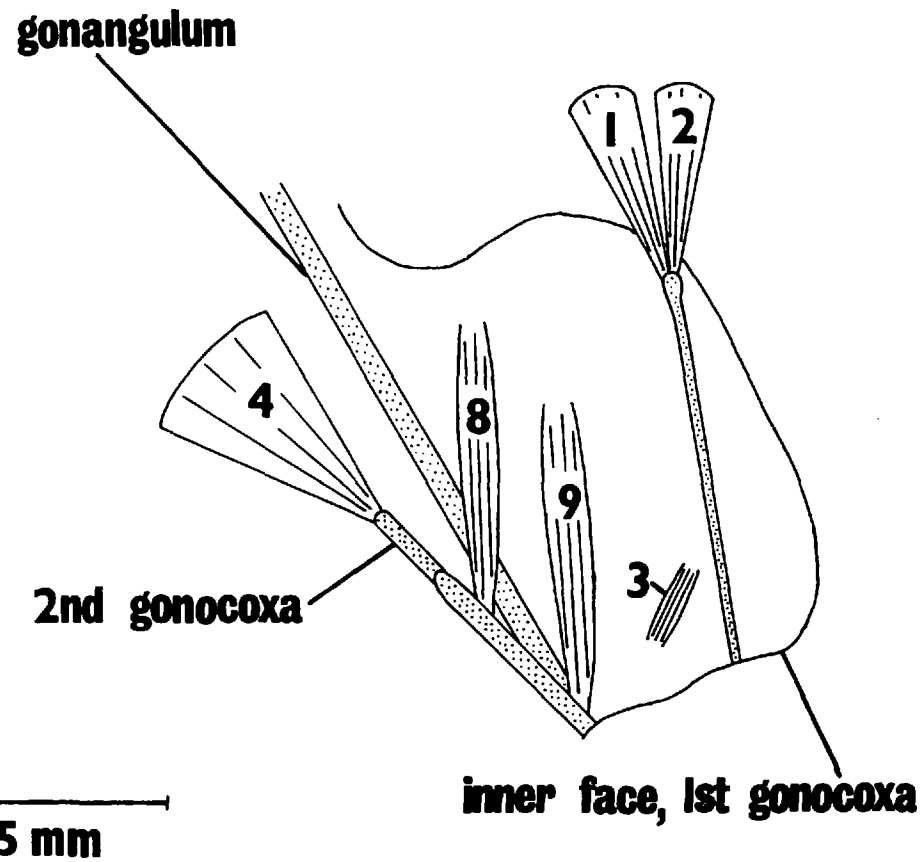
Two other muscles are associated with segment eight; both insert on the inner face of the first gonocoxa. Muscle 8 originates on the posterior part of the head at the proximal end of the first gonapophysis, (fig. 4.2) and runs posterior and transversely to insert on the first gonocoxa ventral to muscles 1 and 2. Contraction of this muscle would hold the first gonapophysis in position. It is composed of a single fibre which contains numerous dispersed nuclei which are not restricted to the peripheral zone. The average nuclear dimension was 8 μm in their long axis.

Muscle 9 is located anterior to 8 and originates on the anterior gonangular ridge running posterodorsal to insert in the mid region of the first gonocoxa. It is a single stout fibre whose contraction would result in an anterolateral movement of the first gonocoxa, transmitted to the first gonapophysis as an anterior movement. Synchronous contraction of this pair of muscles, together with muscles 1 and 2, would

Fig 4.2

Musculature of the gonangulum and 1st gonocoxa





produce a significant movement in the first gonapophyses.

The muscles of segment nine are those most extensively modified by their relationship with the ovipositor. The principal muscles are all associated with the second gonocoxa, which is the main instigator of movement in the ovipositor blades. Movement imparted to the second gonapophyses is transmitted to the first by coupling mechanisms, the most important being the ball and socket joint described by Balduf (1933). The gonangulum is also a very important sclerite in this context, but these factors have been discussed in Chapter 1, pages 45 - 49.

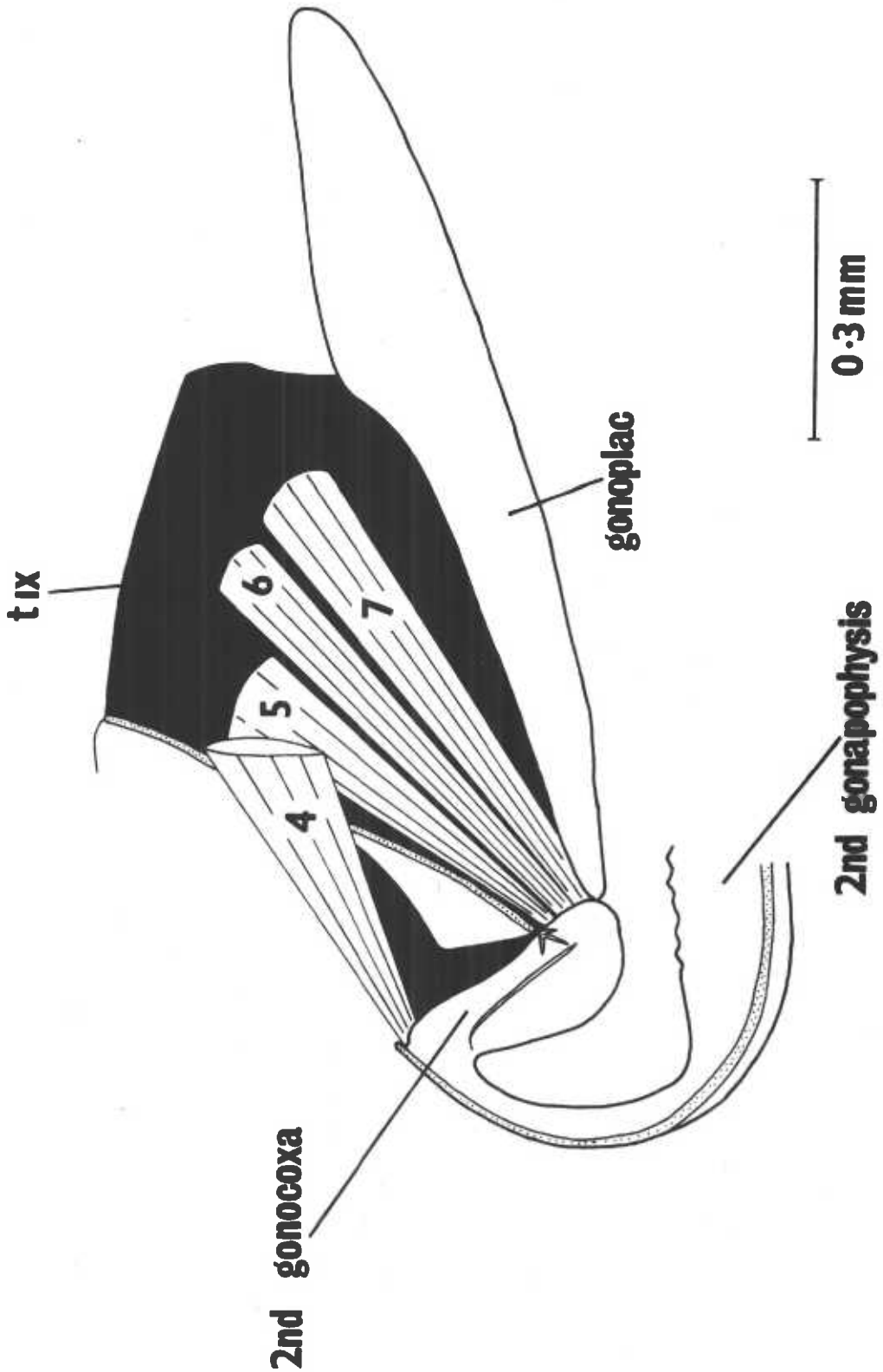
The musculature of the second gonocoxa comprises four pairs of muscles (fig. 4.3), each composed of a single fibre. Muscle 4 inserts on the dorsal edge of the second gonocoxa just ventral to the dorsal extension of the heavily sclerotized ramus of the second gonapophysis. This muscle runs posterolaterally to originate high on the anterior part of tergite nine. Contraction results in the rocking of the second gonocoxa on its fulcrum with the gonangulum and a strong anterior movement of the second gonapophysis.

Muscle 4 has three powerful antagonistic pairs, 5, 6 and 7, which all insert on the ventral posterior edge of the second gonocoxa. These muscles run posterodorsally to originate in sequence along the dorsal wall of tergite nine, muscle 7 having the most posterior origin, and fills the entire lateral region of segment nine. Contraction of this complex causes a powerful posterior movement of the second gonapophysis, and hence the thrusting action of considerable importance during oviposition.

The histology of these muscles is similar to that described for those of the first gonocoxa; they have large nuclei with deeply staining, though diffuse chromatin and obvious transverse striations in the fibres.

Fig 4.3

Musculature of the 2nd gonocoxa.



The genital musculature of segment nine is completed by muscle 10, a short but stout transverse muscle, that originates on tergite nine, approximately one quarter of the distance from the posterior edge and inserts on the reduced sternum nine which forms the lateral and median surface of the mid ventral groove. Contraction of this muscle opens the groove, allowing the ovipositor to be released and lowered.

No generalized arrangement of muscles remains in segment nine, and that of segment eight is considerably simplified when compared to that of preceding abdominal segments. Some of the general abdominal muscles of segment eight were modified during nymphal development in association with the ovipositor; what remains is complicated by the great reduction of sternum eight and its incorporation into the genital pouch. The dorsal muscles are composed of four fibres on each side, which run for only half the distance of the segment (fig. 4.4). Their insertions have been displaced slightly ventrally, so facilitating the raising of the segment. The laterals are composed of five, very stout fibres which arise from a strong posterior apodeme and run in an almost dorsoventral plane. A single fibre crosses the main group of laterals, inserting on a small apodeme on the lateral edge of tergite eight and originating on the large posterior apodeme.

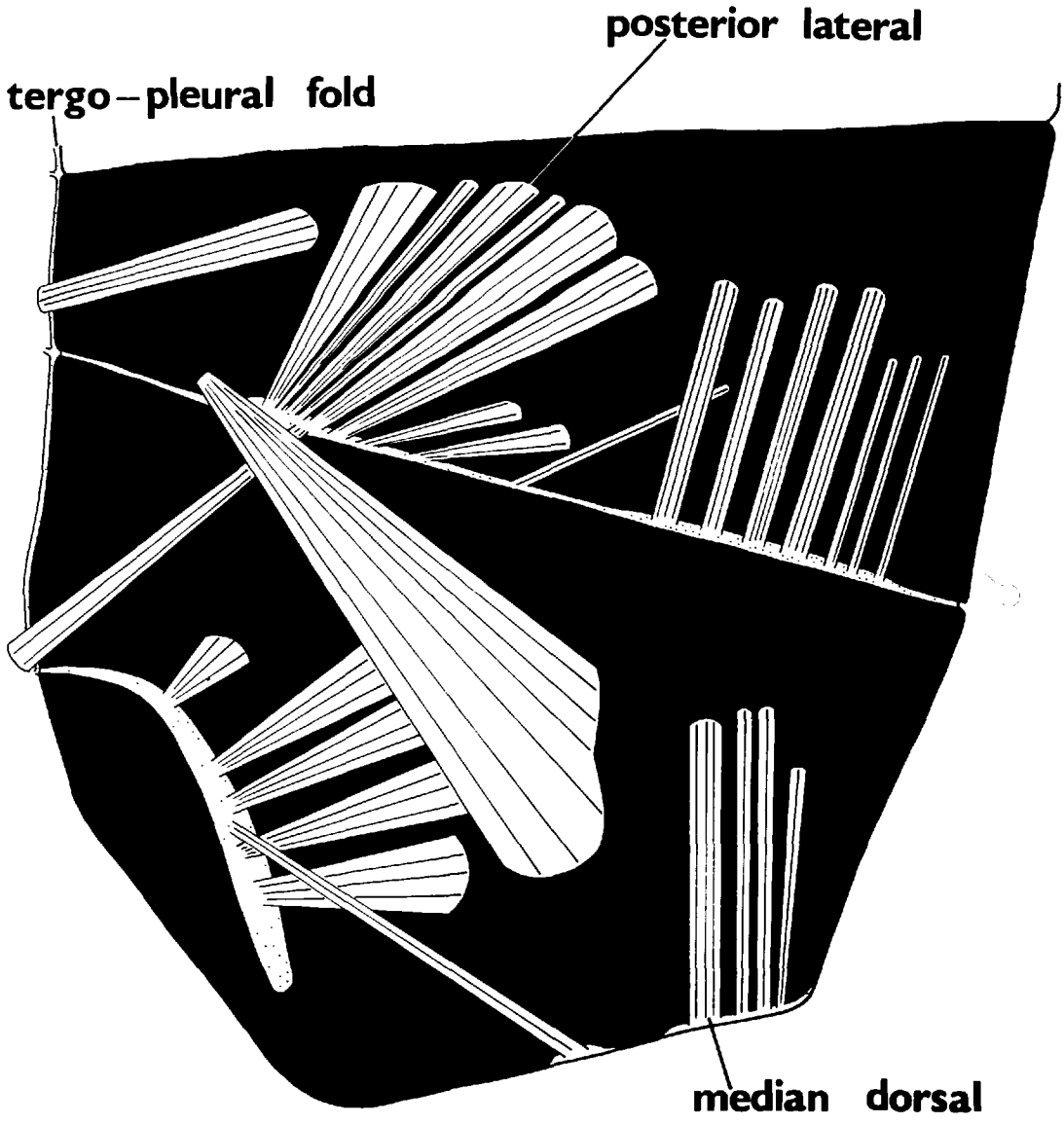
(b) Musculature of the pregenital segments in the adult female

(i) Segment seven will first be described, since it shows some modifications associated with the genital region.

The ventrals have been considerably disrupted by the free posterior edge of the seventh sternum and by the large apodeme in the mid-line immediately posterior to the intersegmental fold (see fig. 4.1). Four pairs of specialized muscles are present in this region, all, indirectly, having a genital function.

Fig 4.4

Musculature, other than genital, of segments VII and VIII



tergites folded out

0·2mm

11 and 12. Two pairs of depressor muscles that insert on the outer lip of sternum seven (fig. 4.1). Muscle 11 consists of a single, large fibre on each side that insert on the lateral anterior head of the sternum, anterior to the pivot with the preceding sternum, and runs laterally and slightly dorsally to originate low on tergite seven. Muscle 12, also a single fibre, inserts on the most anterior edge of the sternum, immediately lateral of the mid-line, and runs strongly dorsally and posteriorly to originate high on tergite seven.

13. This muscle is an antagonist to the preceding two, inserting on the sternal apodeme and running anteriorly to cross muscle 12 and originate on tergite seven. This muscle raises the sternum.

14. Runs longitudinally between the apodeme and the anterior base of the sternum to act as a holding muscle. It is composed of a single, slender fibre on each side, and lies just lateral to the mid-line.

This system is unusual in that it involves direct antagonistic sets of muscles, such systems being unusual in insect abdomens where muscles commonly rely upon the natural elasticity of the cuticle or resilin pads to return to or maintain the resting position.

15. The median dorsals consist of seven fibres, of which the median-most three are extremely fine, the remaining four being stout and identical in appearance to those of preceding segments.

21. The laterals. These can be subdivided :

21.1 The posterior lateral muscle complex is reduced in segment seven, and the points of muscle insertion are comparatively more dorsal than in preceding segments, so aiding the depression of the posterior part of the abdomen during oviposition. It is composed of six fibres, four of which are stout with very pronounced striations, the

remaining two are of smaller diameter (fig. 4.4). They originate on a slight thickening of the posterior inter segmental fold.

21.2 The dorsal posterior laterals are composed of three fibres, two very stout, originating on the posterior inter segmental fold immediately dorsal of the main laterals, and a third slender fibre, which originates slightly more dorsally and runs sharply dorsal and lateral to pass under the most lateral of the dorsal fibres to insert between this and the adjacent fibre.

The overall picture of the musculature in the seventh segment (fig. 4.4) is one of reduction, though several muscle blocks, principally the laterals, have increased in size, coupled with the need in the female for increased mobility of the posterior abdomen.

(ii) A typical pregenital segment. Segment three was selected and the musculature determined by dissection and sections (fig. 4.5).

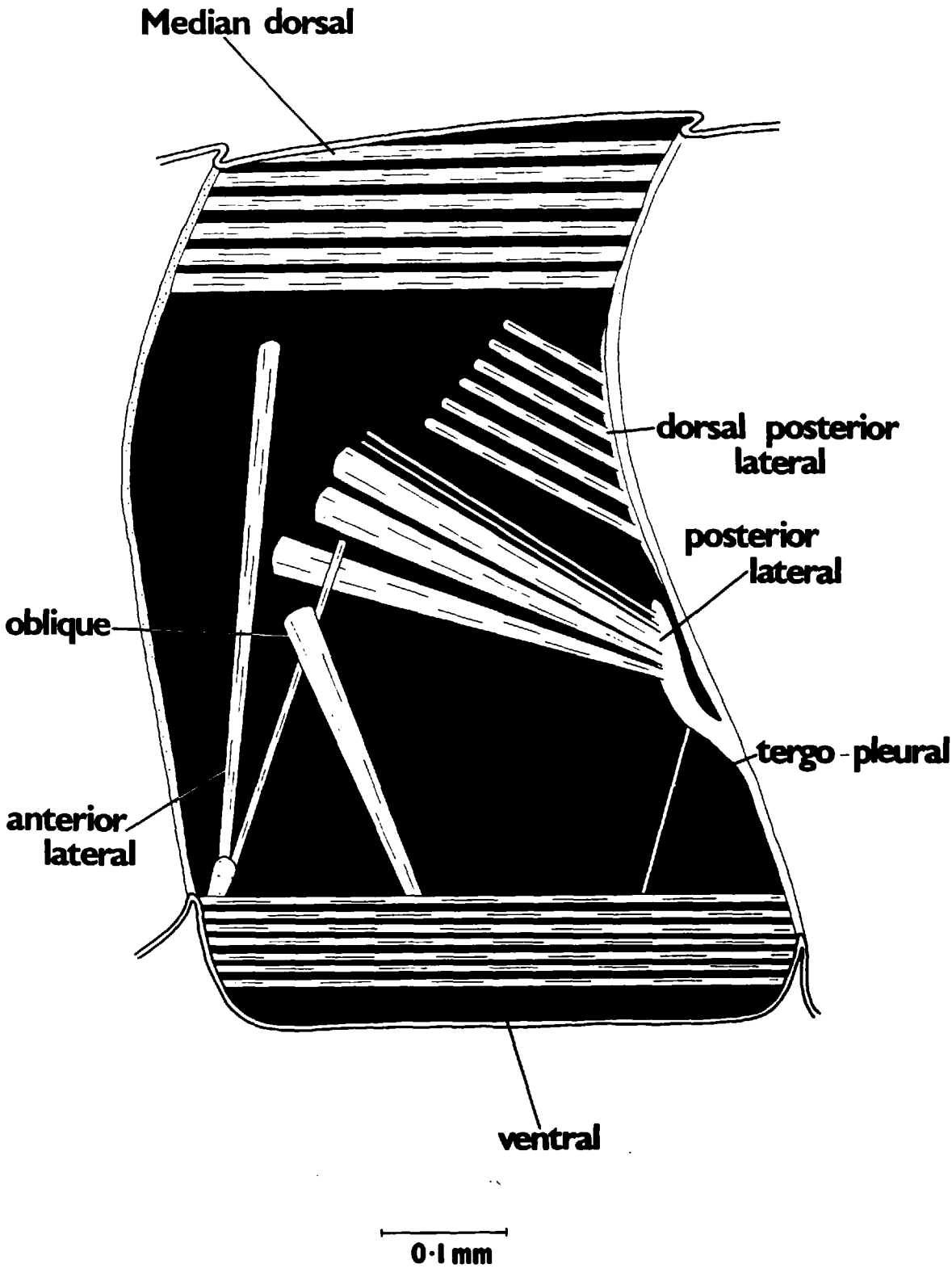
The musculature conforms with Snodgrass's conventions and can be divided into dorsals, ventrals and laterals as follows.

15. Median dorsals. This pair of muscles is composed of six fibres on each side, each being moderately stout and with their attachments on the anterior and posterior inter segmental fold. Nuclei are few, and when present ventrally displaced. These muscles are the abdominal retractors, allowing the segments to telescope within each other.

18. Ventrals. These consist of seven fibres; no sub-division into lateral and median groups can be made. The fibres are more slender than the dorsals, but run the entire length of the segment between the inter segmental folds. Nuclei are more numerous than in the dorsals and average 6.3 μ m with diffuse chromatin. The nuclei were peripheral, each coated with a thin layer of sarcoplasm. The internuclear distance

Fig 4.5

General adult abdominal musculature. Segment 3



was approximately 40 μm.

Laterals

21.1 Posterior laterals. These form a large group of five fibres on each side which originate upon an apodeme, arising from the posterior intersegmental fold approximately one quarter of the distance from its ventral end. These fibres fan out in an anterior direction (fig. 4.5). The three ventral most fibres of this group are very stout and insert just posterior to the first anterior lateral. The remaining two fibres run parallel to these but are considerably more slender.

21.2 Dorsal posterior laterals. These consist of six short, slender fibres on each side, originating on the dorsal half of the posterior intersegmental fold and running dorsally for a short distance to insert beneath and lateral to the median dorsals.

21.3 Anterior laterals. They originate ventrally on an apodeme which extends from the intersegmental fold running dorso-laterally to insert on the tergite ventral of the median dorsals. They comprise two muscles, each of a single fibre. The most anterior is the stouter of the two and inserts immediately beneath the median dorsals. The remaining muscle is very slender and runs more posteriorly to insert between the fibres of the posterior laterals.

Two other abdominal muscles are present. Both originate on the sterno-pleural sulcus.

21.4 An oblique muscle originating on the sterno-pleural sulcus, this runs dorsolaterally in an anterior direction to insert between the anterior laterals. It is composed of a single large fibre.

21.5 A tergo-pleural muscle originating on the posterior margin of the sterno-pleural sulcus running dorsolaterally to insert on the posterior apodeme.

This completes the abdominal musculature of the adult female Graphocephala

fennahi, and represents one of the most comprehensive studies of adult abdominal musculature in the Auchenorryncha. In his description of Ceresa bubalus, Kramer (1950) distinguished between median and lateral dorsals, but this was not possible in the above description. From his study, the laterals of the Membracids are apparently considerably simplified compared to that of Graphocephala. Kramer's description of the genital muscles is very superficial, though the major muscles are similar to those of Graphocephala. More detailed studies have been made of the abdominal muscles of the Heteroptera (Bonhag & Wick 1953 of Oncopeltus) but it varies considerably from that of Cicadellids, due to the different mechanisms of mating and oviposition.

2. Postembryonic development of the abdominal musculature

(a) Method

The methods used in this section were similar to those employed in the following section on myogenesis; the general method will be described.

It was necessary to examine a very large number of sections employing normal histological techniques, due to the small size of the insects. (Second instar Graphocephala fennahi measure some 1.5-2.0 mm in length). From these, reconstructions were made using serial sections. Only in the fifth instar, when the insects were approximately 4.0 mm long could sectioning be supplemented by accurate dissection.

Material for sectioning was fixed, after decapitation to facilitate rapid penetration, either in normal Bouins or formal saline prior to alcoholic dehydration and embedding in paraffin wax (M. pt. 56°C). The yellow coloration resulting from Bouin fixation aided accurate orientation of the specimen. Blocks were then cut at 6 or 8 μm in the transverse, parasagittal or horizontal plane, and stained with Heidenhain's Iron Haematoxylin or Mallory's Phosphotungstic Haematoxylin.

Only females were examined, these being readily identified from the mid third instar. Individuals maintained under constant temperature and light regimes (20°C/16 hours light) were consistent in the length of their nymphal instars. This permitted a number of stages to be used in the observation of myogenesis and the insects characterised according to their age in days. In all 15 stages were examined and this allowed a detailed examination of abdominal myogenesis. They were as follows :

1. 8 days. Early second instar approximate length 1.7 mm.
2. 11 days. Mid second instar. Genital disk well formed.
3. 14 days. Late second instar.
4. 17 days. Early third instar. First appearance of the external genitalia.
5. 22 days. Mid third instar.
6. 27 days. Late third instar.
7. 30 days. Early fourth instar.
8. 35 days. Mid fourth instar.
9. 39 days. Mid fourth instar.
10. 44 days. Late fourth instar.
11. 47 days. Early fifth instar.
12. 50 days. Early fifth instar.
13. 54 days. Mid fifth instar.
14. 59 days. Late fifth instar.
15. 64 days. Nymphal/Imago moult.

Reconstructions drawn from serial sections are presented for the middle of each instar to allow a rapid comparison of the developing abdominal musculature in the second through to the fifth instar (Figs. 4.6 to 4.9). Representative transverse

sections are also presented.

(b) Abdominal musculature of the second instar nymph

This is a comparatively simple system, the basic nymphal arrangement, showing considerable differences when compared to the adult musculature. The abdominal muscles of larval Cicadellids have not previously been described. In the following description the numbering of the muscles will correspond to that of the adult. In segments II through to VII the following symmetrically paired muscles occur.

15. Median dorsals (Fig. 4.6). Each muscle is made up of six moderately stout fibres on each side. Nuclei are few in number, but when present are located on the ventral, peripheral part of the fibre and contain diffuse chromatin. The fibres run the whole length of the segment, originating and inserting on the abdominal terga. This pair of muscles corresponds to the internal median dorsals of Snodgrass (1935).

16. Lateral dorsals. Each muscle contains a single fibre, lateral to the median dorsals but median to the intersegmental lateral. It runs parallel to the median dorsals.

17. Intersegmental lateral. Each segment contains one pair, each muscle made up of a single fibre. It originates on the lateral limit of the sternum near its junction with the preceding sternum and runs dorsally and laterally to insert on the dorsal region of the preceding pleuron and intersegmental fold.

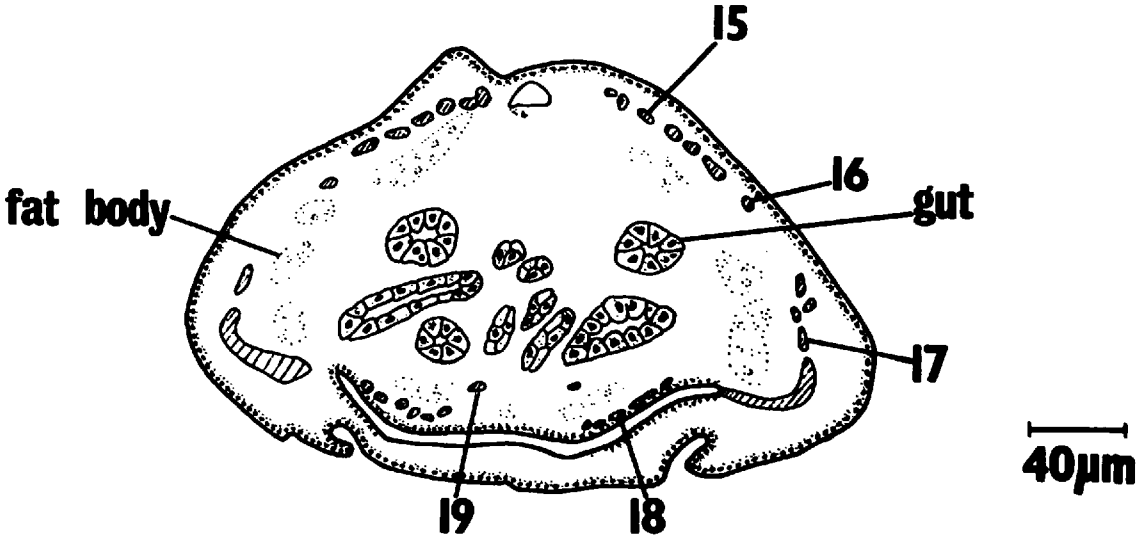
18. Ventrals. In a typical abdominal segment these are represented solely by lateral ventrals, which run the entire length of the segment between the intersegmental folds. They are composed of six or occasionally seven fibres. In segments VIII and IX they are supplemented by a median ventral on each side, which lies dorsal and internal to the lateral ventrals (Muscle 19 in fig. 4.6).

The intrasegmental laterals are represented by two pairs of muscles.

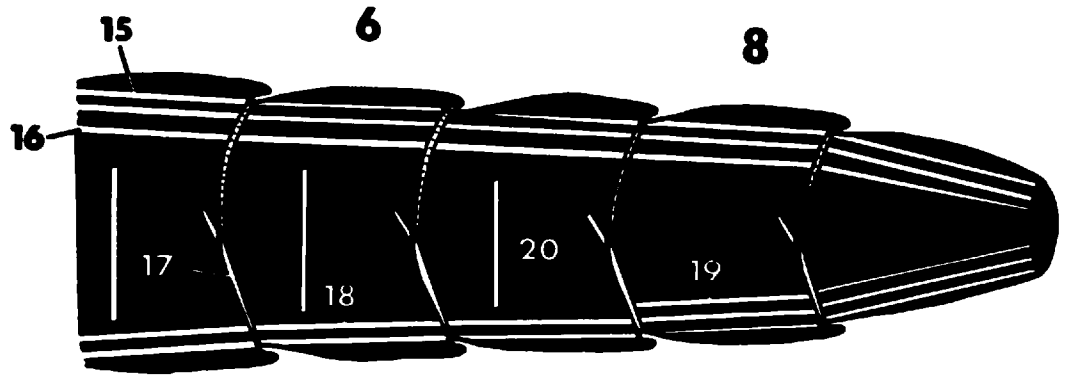
20. Tergo-sternal muscles. Present in the anterior part of each abdominal segment

Fig 4.6

Mid 2nd instar larva



Transverse section through the distal region of segment 8.



Abdominal musculature of the right side viewed from the sagittal plane. Reconstructed from serial sections. 6, 8, sixth and eighth abdominal segments

except VIII and IX. They originate lateral to the dorsals and run ventrally to insert lateral to the ventrals. Each muscle is composed of two or three stout fibres, each containing a large number of evenly distributed nuclei around the periphery of the fibre.

21. An extremely slender muscle composed of a single fibre which originates on the lower posterior tergite and runs ventroposteriorly for approximately 50 μm to insert on the intersegmental fold.

This completes the abdominal musculature of the second instar nymph. The muscles are metameric though the genital region differs due to the presence of median ventral fibres, the absence of anterior intra-segmentals and, in segment nine, the absence of inter-segmentals, due, in Cicadellids, to the reduction of segments X and XI. Lateral and medial proliferations of the sternal epidermis in segments VIII and IX represent the developing genital disk in the late second instar, though there are no indications of muscles or muscle rudiments associated with it. This contrasts with the considerable specialization seen in segments eight and nine of the adult female associated with the ovipositor.

(c) Abdominal musculature of the third instar nymph

The pregenital segments remain essentially as described for the second instar nymph. The genital musculature has undergone some modification and reduction as follows.

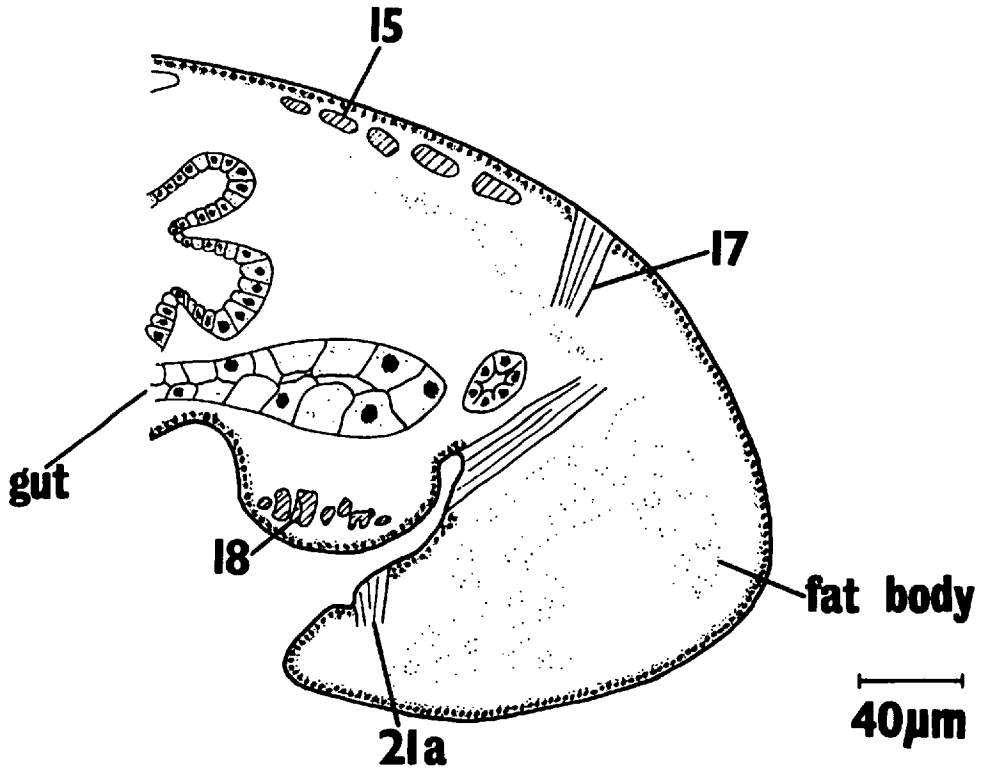
15. The dorsal muscles have undergone reduction in segment VIII. The median dorsals (fig. 4.7) are now composed of five fibres on each side, the origins and insertions of which have remained constant.

16. The lateral dorsal, which in the second instar nymph was represented by a single fibre, has been lost as will be described under myogenesis.

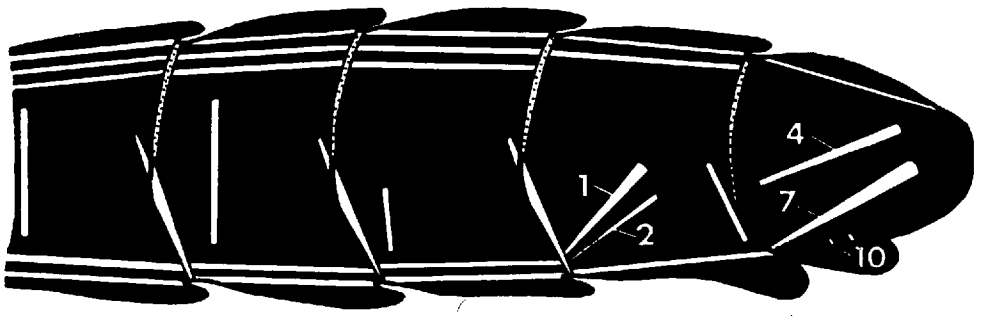
17. The intersegmental muscle. This has undergone a shift dorsally and anteriorly

Fig 4.7

Mid 3rd instar larva



Transverse section through the distal region of segment 7.



Serial reconstruction of abdominal musculature on the right side, viewed from the sagittal plane.

to a point in front of the intersegmental fold dorsal of the genital rudiment to become an intra-segmental muscle of segment seven.

18. Ventrals. The median ventral has been lost, the lateral ventrals each consist of four fibres.

21. The posterior dorsal intra-segmentals have been lost.

The basic musculature has been further reduced in segment IX. The median dorsals have been reduced to two fibres and a reduction in fibre length results in a shift of the insertion so that the fibres no longer run the whole length of the segment. Ventrals are not represented in segment IX.

Associated with the development of the external genitalia five new muscles have arisen in the genital region.

1 and 2. Rudiments of the definitive first gonocoxal muscles which, during the third instar, are still undergoing myogenesis. They are derived from the ventral muscles of segment eight as will be described under myogenesis. By mid third instar, these muscles insert on the anterior lateral edge of sternum eight and run dorsoposteriorly to originate low on tergite eight.

Three new pairs of muscles have arisen in segment nine.

4. Rudiment of the dorsal muscle of the second gonocoxa developed in the late second instar from free myoblasts. By the mid third instar, the muscle inserts on the anterior tergo-pleural fold and originates posteriorly on tergite nine.

7. Rudiment of the ventral muscle of the second gonocoxa. This inserts antero-laterally on sternum nine and originates posteriorly on tergite nine.

10. Rudiment of the definitive tergo-sternal muscle of segment nine. During the mid third instar it is composed of two fibres.

(d) Abdominal musculature of the fourth instar nymph

The changes that occur between the third and fourth nymphal instars in abdominal segments II through to VII are slight, involving the formation of no new muscles. The insertions of the extreme lateral fibres of the dorsal muscles are shifted posteriorly, so that the fibre runs for approximately three quarters of the segment. This is a further trend towards the adult condition. In the genital region, segments eight and nine, there is further reduction of both the dorsal and ventral muscles.

By the mid fourth instar the musculature of the genital region already closely resembles that of the adult (Fig. 4.8). That of segment VIII is as follows :

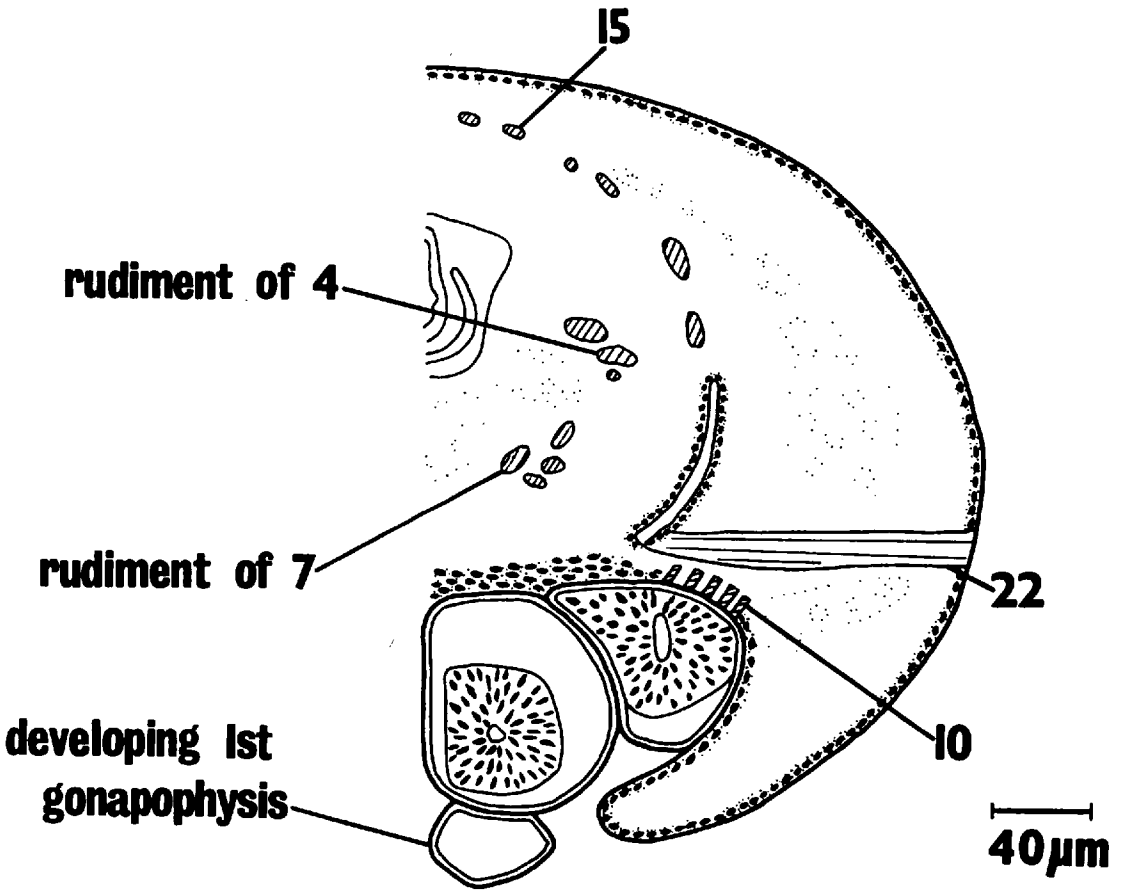
1 and 2. Rudiments of these first gonocoxal muscles were already well formed by the mid third instar, though myoblast incorporation continued until the late third/early fourth instar. They are the major definitive muscles of the first gonocoxa and by the mid fourth instar they are both moderately well developed fibres inserting on the anterior end of the genital disk in the latero anterior corner of sternum VIII, and originating on the tergo-pleural region in the posterior region of that segment. By the mid fourth instar, no undifferentiated myoblasts are associated with the fibres which bear obvious cross-striations. Nuclei are evenly distributed along the fibre and measure approximately 6.0 μm in their longest axis. The nuclei are coated with a very thin layer of sarcoplasm and form bulges along the fibre, the nuclei not having sunk into the fibre.

23. This muscle inserts on the proximal end of the rudimentary first goapophysis and runs laterally to originate on the pleuro-sternal region. It is an extremely short, fine fibre.

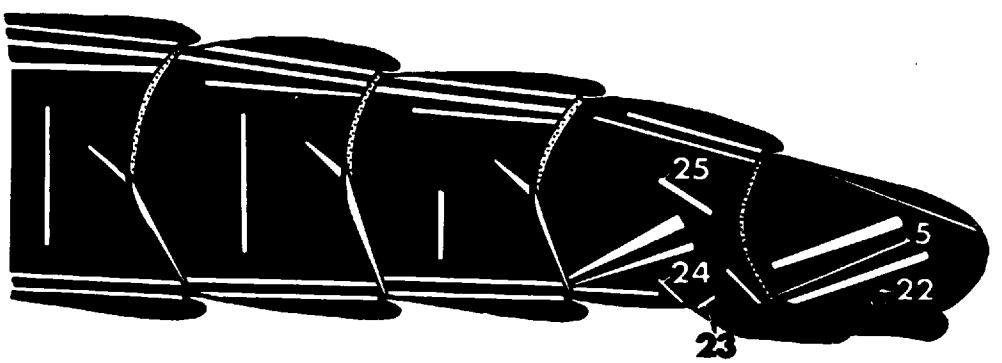
24. This muscle inserts anterior to muscle 23, near the mid-line, and runs laterally

Fig 4.8

Mid 4th instar larva



Transverse section through the mid region of segment 9.



Serial reconstruction of abdominal musculature of the right side, viewed from the sagittal plane.

to originate above the ventrals. It is also a very short, fine muscle, composed of a single fibre.

25. A lateral muscle, formed early in the fourth instar, which, by the mid fourth, is composed of approximately three very closely applied fibres. This muscle is the rudiment of the complex of lateral muscles which is characteristically present in all adult abdominal segments, though a system poorly developed in the nymphs perhaps because of the natural elasticity afforded by their limited sclerotization.

The dorsals, ventrals and intersegmental muscle undergo a gradual reduction towards the definitive adult condition. The ventrals, composed of three fibres on each side, extend from in front of muscle 24 to the anterior intersegmental fold and undergo further degeneration until they are totally histolysed at the nymphal/adult moult. A similar process occurs in the dorsals, when at the adult moult four very slender fibres remain, which traverse approximately half of the segment.

In segment nine, the second gonocoxal muscles have undergone considerable development and begin to approach the adult condition.

4 and 7. The dorsal and ventral second gonocoxal muscle respectively are now represented by very stout fibres that insert above the rudiments of the second gonapophysis in the anterolateral corner of segment IX, originating upon the posterior tergum. They already occupy a considerable volume of the segment.

5. The second ventral muscle of the second gonocoxa is produced early in the fourth instar by a longitudinal cleavage of muscle 7. The origins and insertions of this muscle are similar to those of 7.

10. A pair of sterno-tergal muscles, each muscle being composed of five fibres which originate on the ventrolateral region, mid-way along tergite nine and insert on the sternum over the lateral region of the developing gonoplac (fig. 4.8). At this stage

of nymphal development, sternum nine has inverted to form a groove in which the developing external genitalia can partially lie. This will, in the adult, form the genital groove, which will partially fuse with the gonoplac. By this stage, muscle 10 is well formed, each fibre being very slender, and is carried over almost unchanged into the adult body.

22. This is a transverse muscle, occupying a similar position to muscle 10, and runs between the mid ventrolateral region of tergite IX and the dorsal surface of the rapidly developing genital disk. In the fourth instar it is a stout muscle, composed of a single fibre. Though only developed during the late third instar, it undergoes phagocytosis during the late fourth instar and has been completely removed by the mid fifth instar. The function of this muscle is not known.

The only remaining muscle in segment IX is the median dorsal, which is undergoing progressive histolysis. At the mid third instar the fibres are of approximately 20 μm diameter; by the mid fourth instar this is reduced to 15 μm and the sarcolemma appear to be slightly blistered and puckered.

(e) Abdominal musculature of the fifth instar nymph

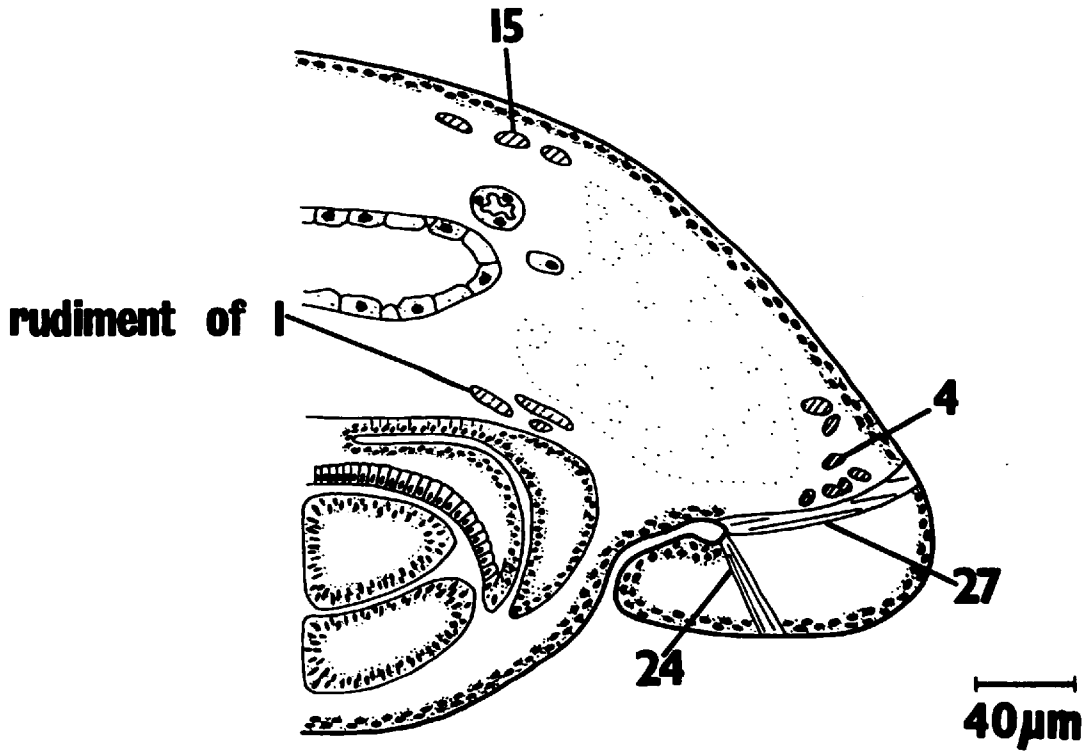
(i) Segment six, a generalized pregenital segment (fig. 4.9).

15. Median dorsals. Each muscle is composed of three delicate fibres, each of which later undergoes longitudinal cleavage to produce the six definitive adult fibres. The two median-most fibres run the entire length of the segment between the intersegmental boundaries. The outermost fibre has been shortened and runs anteriorly for two thirds of the segment to insert on the tergal epidermis. The fibres are approximately 25 μm in diameter with peripheral, darkly staining nuclei. The fibrillar structure is rather indistinct.

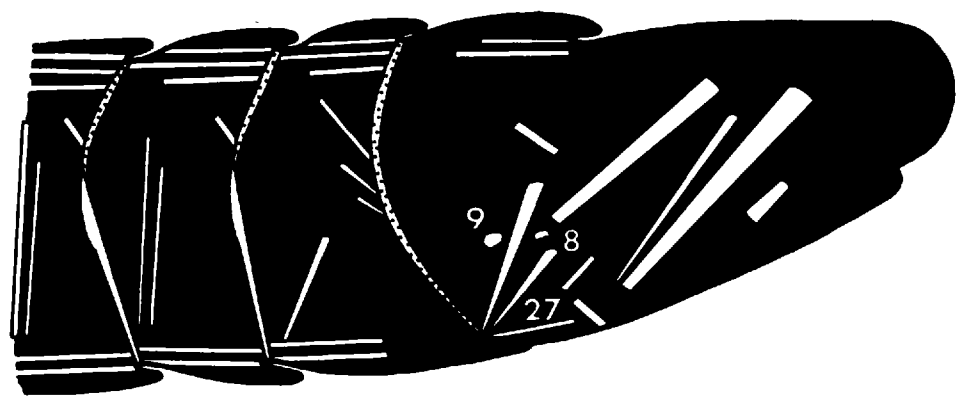
17. Intersegmental lateral. A very stout and well defined muscle, composed of three

Fig 4.9

Mid 5th instar larva



Oblique transverse section through segment 8



Serial reconstruction of abdominal musculature of the right side, viewed from the sagittal plane

or occasionally four fibres that originate anteriorly in the segment immediately lateral to the insertions of the median ventrals. It runs dorsolaterally to insert over a wide region of the intersegmental field in the tergal region, where it forms very strong attachments. The nuclei are scattered and peripheral. This muscle, though present in all nymphal instars, is not carried over into the adult and undergoes progressive histolysis in the late fifth instar; remnants remain in the early adult but the muscle is totally lost, due to the action of phagocytes, within three days of the adult moult.

18. Median ventrals. A single muscle on each side composed of two to four fibres which run the whole length of the segment, inserting on the intersegmental fold at the anterior and in front of the intrasegmental laterals. Histologically they resemble the median dorsals.

20. Two slender muscles, each composed of a single fibre, in the anterior part of the segment. They are parallel (fig. 4.9) and run from the tergite, lateral and ventral, to the median dorsals and insert on the sternum near the sterno-pleural fold lateral to the median ventrals.

21. A short, slender intra-segmental muscle composed of one or two very fine fibres that arise posteriorly on the intersegmental fold dorsal of the intersegmental muscle (17). It runs dorsally and laterally a short distance to insert beneath the median dorsals. This muscle shows little differentiation, the nuclei lying deeply in the muscle cytoplasm.

(ii) The musculature of the genital region is somewhat more complex. The clear boundary between segments eight and nine is no longer apparent, due to the reduction and inversion of sternum eight and the anterior elongation of tergite nine.

1 and 2. The major dorsal muscles of the first gonocoxa insert upon the posterior

extremity of the dorsal sclerotized rod of the first gonocoxa and originate posteriorly on tergite eight. By the mid fifth instar they are well formed muscles, each composed of a single, moderately stout fibre with well developed fibrillae and peripheral nuclei. The definitive position of these muscles is achieved during the final moult, and is brought about by an increased sclerotization of the dorsal rod and the relative posterior movement of the first gonocoxa.

4. The dorsal muscle of the second gonocoxa inserts on the dorsal anterior edge of that sclerite and runs dorsolaterally to originate half way along tergite nine. It is composed of two to three well developed, tightly bound fibres.

5 and 7. The ventral muscles of the second gonocoxa, both insert on the ventral posterior edge of the sclerite and originate high on tergite nine, posterior to that of muscle 4 (fig. 4.9). Muscle 5 is a single slender fibre, while muscle 7, also a single fibre, is considerably more stout. During the final moult, muscle 7 undergoes a further longitudinal cleavage to produce the definitive adult muscles 6 and 7.

In the fifth instar nymph muscles 4, 5 and 7 occupy most of segment nine as the second gonocoxal muscles do in the adult.

8 and 9. Transverse muscles of the first gonocoxa which run in a slight anteroventral plane between the anterior lobes of the developing genitalia. In the adult they insert on the inner face of the first gonocoxa, originating on the head of the first gonapophysis and gonangular ridge respectively.

10. This tergo-sternal muscle originates half-way along tergum nine, running transversely to insert on sternum nine along the mid-ventral groove. It is composed of three, short, stout fibres which permit the opening of the ventral groove in the adult, and pass unchanged through the final moult.

Of the basic abdominal musculature remaining in the genital region that of segment eight is considerably reduced and simplified, while all the muscles of segment

nine are associated with the developing external genitalia.

Musculature, not associated with the genitalia, in segment eight.

15. The median dorsals have been further reduced to two fibres on each side which run from the posterior intersegmental fold anteriorly for approximately half the length of the segment before inserting onto the tergal epidermis.

18. The median ventrals are reduced to a single fibre on each side extending from the anterior intersegmental fold posteriorly for some 300 μm terminating close to the mid-line, between the bases of the gonapophyseal rudiments. This muscle is lost during the final moult.

24. A ventrolateral muscle composed of a single, very slender fibre. It remains undifferentiated with no apparent division into a fibrillar structure. It undergoes rapid histolysis in the late fifth instar, due to the posterior development of the first gonocoxa.

25. Lateral intrasegmental. Due to changes in tergite eight during the early fifth instar this muscle became reduced in length as indicated by a decrease in average inter-nuclear distance. This muscle originates on the lateral apodeme formed by an infolding of the epidermis anterior to the posterior intersegmental fold, and runs dorsally and laterally to insert between muscle 1 and the median dorsals. The nuclei are peripheral.

27. As for muscle 24.

This completes the abdominal musculature of the fifth instar nymph. See Table 4.1 for a comparison of the nymphal musculature and for a comparison of the total nymphal muscles with those of the adult.

3. Myogenesis of the abdominal muscles

None of the basic nymphal muscles are retained unchanged by the adult in the form seen during the second instar. All muscles associated with the external

Table 4.1

The total number of abdominal muscles (not fibres) in each nymphal instar and the adult together with the number of muscles peculiar to the nymph and adult stages.

	Total Muscles				
	Stage				
Region	II	III	IV	V	Adult
Abdominal segments 1 - 7	35	35	35	34	48
Abdominal segments 8 and 9	6	9	14	13	13

Tabulation of data into three categories

A - Muscles present in both nymph and adult

B - Muscles peculiar to the adult

C - Muscles peculiar to the nymph

	Category		
Region	A	B	C
Abdominal segments 1 - 7	27	21	8
Abdominal segments 8 and 9	10	3	4

genitalia are either totally new formations which arise during later nymphal instars or highly modified nymphal muscles. The major genital muscles have been laid down by the mid third instar, though they may undergo later changes in the position of their origins and/or insertions. This observation is in agreement with other workers, who have suggested that in Exopterygotes the adult muscles have characteristically been laid down early in the nymphal development, though complete differentiation may not occur until a later instar. This contrasts with Enderopterygote development, where adult muscles are often of a relatively late post-embryonic origin (Tiegs 1955; Wittig 1955).

(a) Myogenesis during the second and third instar

During the second instar five new muscles develop in the genital region, three by the gross reconstruction of pre-existing muscles, while the remaining two are completely novel structures. The myogenesis of muscles 1, 2 and 7 by the partial histolysis and reconstruction of the ventral muscles in segments eight and nine will now be described. Myogenesis was similar in all three muscles, so only changes recorded in muscle 7, the precursor of the ventral second gonocoxal muscle of the adult, will be noted.

In the mid second instar the lateral and median ventral muscles are composed of well differentiated fibres, which extend between the intersegmental folds. The approximate diameter of the fibres is 9 μm with basiphilic nuclei 3.0 μm x 2.5 μm in size distributed evenly along the fibres.

During the late second instar the nuclei surrounding the median and lateral ventral muscles of segment nine increase considerably in number, though mitoses were not observed; they become strongly basiphilic and almost spherical, measuring approximately 3.2 μm x 3.0 μm. By the early third instar the median ventrals and innermost four fibres of the lateral ventrals have been reduced to fine threads, though

they still retain their original staining properties. The number of nuclei associated with these histolysed muscle fibres are greatly reduced and by the mid third instar the fibres have undergone total histolysis. Though histolysis was rapid, it followed an orderly process, relatively unspectacular and without evidence of accompanying phagocytosis.

In the outer two lateral ventral fibres histolysis progresses as outlined above, but at the early third instar the nymphal muscle nuclei accumulate in the vicinity of their origins. Free cells, present in the haemocoel, which will be termed myoblasts, aggregate to form a fine strand. In transverse section it can be seen that the strand is hollow, the myoblasts lining up in a spiral configuration running out from the origin of the ventrals posterior laterally. The nymphal muscle nuclei then come to lie evenly distributed along and within the myoblast strand, each nucleus being enveloped in a thin coat of cytoplasm. The new muscle contains nuclei derived from both myoblasts and the original nymphal muscles. At this stage, the myoblast and nymphal muscle nuclei can be distinguished by the very deeply basiphilic nuclei and highly granular appearance of the myoblasts, the myoblast nuclei also being slightly larger than those of the nymphal muscle. The muscle nuclei move towards the periphery of the rudiment and mitotic divisions of the myoblasts produce a pronounced change in the appearance of the tissue with a rapid increase of nuclear material. Initially, much of this is typical myoblast nuclear material, which forms a sheath around the rudiment proper. Gradually myoblast nuclei penetrate the rudiment in increasingly large numbers and become indistinguishable from the muscle nuclei. Incorporation of myoblast nuclei and cytoplasm, and hence growth of the rudiment, continues with the late third instar, when no undifferentiated myoblasts are left surrounding the rudiment. The increase in muscle size to the definitive

adult stage, which occurs largely in the late fifth instar and nymphal/adult moult, is brought about by an increase in the number of fibrils and sarcoplasm. There is no appreciable cleavage into daughter fibres, so that the overall effect is an increase in the size of the muscle block.

Muscles 1 and 2 of the first gonocoxa develop in essentially the same manner by the massive reconstruction of the ventrals in segment eight. The overall picture is one of partial histolysis of the nymphal muscle into its constituent nuclei, each with a thin coat of sarcoplasm, and the incorporation of free myoblasts, which provide a "template" for the developing muscle and a sheath around the rudiment. The myoblasts undergo rapid division and become closely applied to the rudiment where they grow by bipolar elongation. Their incorporation into the fibre provides additional nuclei and fibrils, permitting a gradual increase in the size of the rudiment, both in length and diameter. Accompanying this is a migration of nuclei to the periphery of the fibre so that during active myogenesis the surface appears very rich in nuclear material. Each myoblast provides a single fibril, further growth of the muscle block occurring by the longitudinal division of the fibrils or cleavage of the fibre.

At the stage demonstrated by the reconstruction of the third instar nymph (fig. 4.7), muscles 1, 2 and 7 had not completed myogenesis, and myoblasts were still present around the periphery of the fibre, though the strongly basiphilic nuclei of the myoblasts were out-numbered by the lighter and less granular muscle nuclei. The muscles were only weakly attached to the epidermis.

Muscles 4 and 10 originate, not from pre-existing nymphal muscles, as above, but from clusters of free myoblasts. The description given here is for muscle 4, that of muscle 10 is fundamentally similar, though the myoblasts, present during the mid second instar and derived from cells in the ventral epithelium of the developing

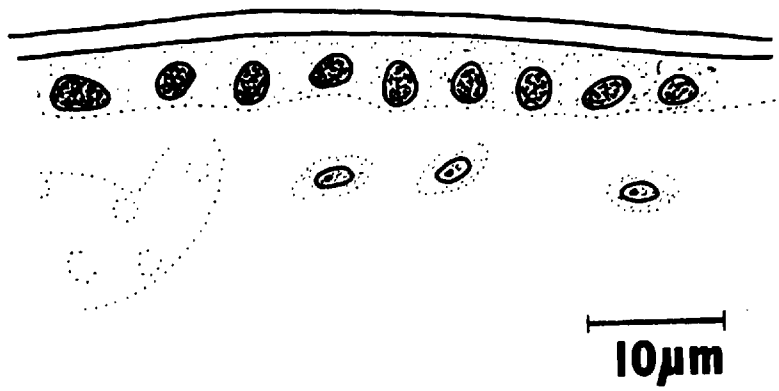
genital disk, are three in number.

In the mid second instar, six to eight spindle-shaped cells are present in the anterior dorsal region of the haemocoel in segment nine (fig. 4.10). The nuclei are basiphilic and measure approximately 6 μm x 3.2 μm; the cytoplasm is densely granular. These myoblasts gradually increase in number by mitosis and assume the approximate position of the definitive muscle they are to form, extending from the anterior pleural region of segment nine and spreading posterolaterally to end freely on tergite nine, two thirds of the way along the segment. The form of the muscle is determined by the elongation of a small number of myoblasts along the axis of the future muscle. These will be referred to as the pioneer myoblasts and form the basis of the definitive muscle. Pioneer myoblasts provide nuclei and sarcoplasm; during elongation, the cytoplasmic granules are pulled out along the longitudinal axis of the cell and finally coalesce to form a single fibril. This unit forms the basic muscle rudiment and is surrounded by a mass of free myoblasts. A rewarding extension to this study would be the electron microscopic examination of fibril formation to determine the mechanism of elongation and the coalescence of cytoplasmic granules.

The free myoblasts undergo rapid mitosis, so that the sheath around the rudiment becomes increasingly thick. During this process it becomes obvious that the number of fibrils and muscle nuclei, within the rudiment, are increasing, though no evidence of mitosis within the muscle could be found. With continued myogenesis, it became clear that myoblasts from the outer sheath were being incorporated into the rudiment and gradually, with a reduction in the rate of myoblast division, the zone of free myoblasts became reduced. By the late third instar no free myoblasts were present, mitosis having ceased, and the incorporation of the products complete.

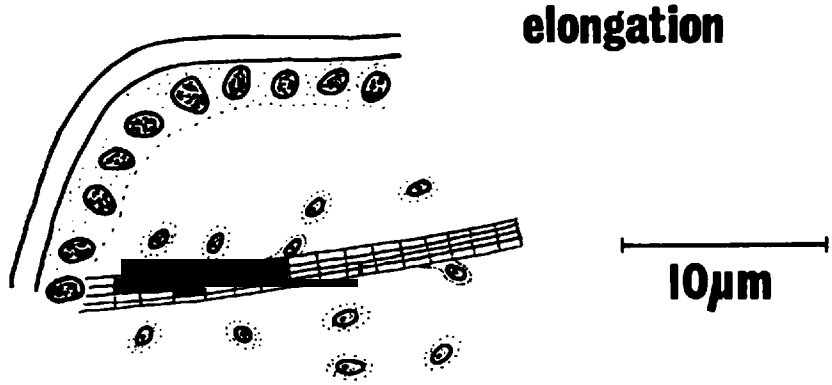
Fig 4.10

Free myoblasts



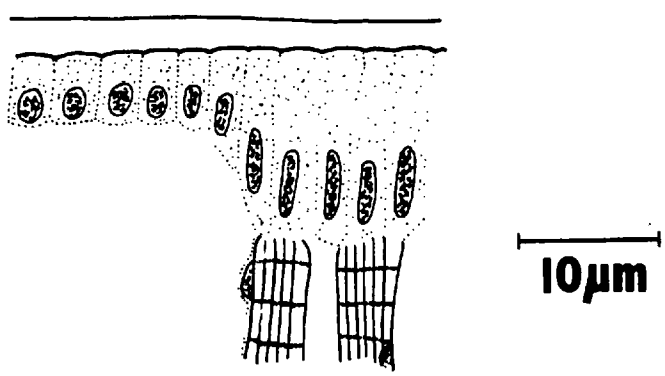
4.11

Myoblast incorporation by bipolar elongation



4.12

Recruitment of epidermal cells into a muscle attachment



Difficulty was experienced in observing myoblast incorporation, largely due to the rapid mitosis and the crowding effect of the mitotic products around the muscle rudiment. However, by combining transverse, parasagittal and horizontal sections, coupled with considerable searching under high power and oil immersion objectives, it was possible to clarify some aspects of this process.

The pioneer myoblast undergoes bipolar elongation to produce a long spindle-shaped cell, the nucleus remaining in the middle of that greatly lengthened cell. This single cell is the basic muscle rudiment. Free myoblasts that are to become incorporated into the rudiment become very closely applied to its surface. At this stage the free myoblasts, surrounding the elongate pioneer myoblast, are still short spindle-shaped cells of approximately 8.5 μm x 4.0 μm with a deeply staining ovoid nucleus 3.5 μ m long, the cytoplasm is densely granular. The closely applied myoblasts then undergo longitudinal elongation in the fashion of the pioneer along the axis of the rudiment, becoming extremely long and filamentous in appearance. As can be appreciated, it is extremely difficult to follow a single cell in one section, but by examining serial sections it was determined that the cell elongated, without division, to a length of approximately 75 μm. After elongation, the separating cell walls break down, allowing the myoblast contents to mix with those of the rudiments (fig. 4.11).

Crossley (1972), in an electron-microscope study of myogenesis in Calliphora erythrocephala, was able to demonstrate in this species three modes of contact between presumptive myoblasts and rudimentary muscle fibres. He suggested that this may represent a temporal sequence during myoblast fusion. At the third stage, cytoplasmic continuity between the cell systems produces fusion. An important character that Crossley detected at the time of fusion was the strict relative orientation of the micro-

tubule array in each system, so that the long axis of the myoblast was always parallel to that of the fibre. The development of microtubule orientation prior to or immediately after the formation of the initial cytoplasmic bridges suggests that the microtubule array in the rudiment develops in response to the pre-existing microtubule arrangement of the myoblast. The microtubules of the rudiment have no regular orientation until myoblast fusion occurs. Crossley (1972a) obtained no evidence that mitosis or amitosis occurred either in the muscle nuclei or those of the myoblasts. In the present study, occasional mitoses were recorded in the myoblasts.

During light microscopic observations in the present study, it was obvious that the cross striations of the contractile element developed at a very early stage and faint striations could be resolved even at the four to five fibril stage. The fate of the myoblast plasma membrane and its possible inclusion into the sarcolemma was not determined, so it is not known whether at fusion the plasma membrane breaks down or becomes continuous with the sarcolemma.

(b) Myogenesis during the fourth and fifth instars

Five new muscle rudiments have formed in the genital region by the mid fourth instar, accompanied by a further reduction of both the dorsal and ventral muscles. Three new pairs of muscles have arisen in segment eight, all derived from free myoblasts and not by the reconstruction of existing nymphal muscles. Two new pairs of muscles have arisen in segment nine (fig. 4.8). A transverse muscle (22) derived from myoblasts in the region of the genital disk and a further ventral muscle of the second gonocoxa by a longitudinal division of muscle 7.

The reduction seen in the dorsal and ventral muscle fibres was brought about by non-phagocytic histolysis and accompanied by a general reduction in muscle material to produce finer muscle strands. The reduction of sarcoplasm was indicated by a considerable reduction in the average nuclear spacing, producing the appearance of

increased nuclear material. During the late third and early fourth instar, an increase in the number of vacuolated cells and free haemocytes were noted in the vicinity of these muscles. It is possible that these cells were taking up extruded muscle material, though conclusive evidence, at the level of the light microscope, would be difficult to obtain. At no time were phagocytic haemocytes seen to attack the muscles.

Prior to the partial histolysis of the muscle in preparation for a change in the position of the insertion, an increased vacuolization of the muscle fibre occurred, accompanied by a reduction and then loss of the cross striations. There was no aggregation of haemocytes around the muscle insertion during this phase. The muscle then underwent partial disruption with constituents being released into the haemocoel. Breakdown of the muscle nuclei in the region of the insertion occurred, and a new insertion was formed by the attachment of muscle cells to the epidermis, approximately 200 μm from the anterior edge of the segment. In the region of the new insertion the number of nuclei increased, each possessing dense chromatin material.

The musculature of the genital region undergoes considerable modification between the mid fourth and fifth instar. The developing external genitalia are sufficiently advanced to permit the formation of definitive origins and insertions, which in many cases requires the partial histolysis of the muscles. This process continues through the fifth instar into the final moult, so that the newly emerged adult possesses its full complement of muscles, though not all are immediately functional.

This process of histolysis and reconstruction will only be described in muscle 1. The first visible indication of muscle degeneration is the formation of large vacuoles in the peripheral sarcoplasm, which gradually extend between the fibrils, causing their partial separation. Within twelve hours of the vacuoles' appearance, large

numbers of haemocytes aggregate around the muscle. These cells, almost spherical in form, measure approximately 10 μm diameter, the nuclei are small, 2.5 μm diameter, and stain only moderately with haematoxylin and the cytoplasm contains large numbers of darkly staining granules. These cells were seen to invade the muscle and aid its disintegration by phagocytosis.

Considerable speculation has been applied to the origin of haemocytes and phagocytes. Viallanes (1833) incorrectly ascribed a muscular origin to phagocytes, though it is now a well established fact that haemocytes arise in the embryo from undifferentiated mesodermal tissue (Wheeler 1892), which are capable of mitotic division throughout the life of the insect; some of these cells may become phagocytic. They are capable of ingesting foreign particles within the haemocoel (Hollande 1909) or histolysing tissues (Wigglesworth 1933). Considerable debate has arisen concerning the state of the tissue at the onset of phagocytosis. It is now thought that phagocytes ingest only dead cells already in the process of autolysis, though such cells may show few histological changes (Boehm 1961). Whitten (1964) described the phagocytic haemocytes of Sarcophaga bullata and the process by which they engulfed fragments of the disintegrating muscle. In the pupa of Calliphora, haemocytes filled with granules of disintegrating tissue, the Körnchenkugeln of Weismann, are abundant in the blood (Pardi 1939).

One of the first comprehensive studies of endopterygote metamorphosis was that of Perez (1910), who described phagocytosis in Calliphora erythrocephala by haemocytes possessing psuedopodia. There are various processes by which histolysis of tissue can be brought about within the insect body, Boehm (1961) indicated that chemical autolysis and phagocytosis can occur side by side.

Wigglesworth (1972) recognised five types of haemocytes in insects, not all of which are phagocytic. Walters (1970) working on Hyalophora, described two

dominant types, plasmocytes, which were phagocytic, and granulocytes, which were not phagocytic. The phagocytic forms involved in muscle break-down have sometimes been termed Type F haemocytes, the cytoplasm of which is usually filled with vacuoles, which give a strongly positive acid phosphatase reaction, typical of lysosomes (Essner & Novikoff 1961). It is not known whether all the hydrolytic enzymes involved in muscle break-down originate within the haemocytes. It is generally considered that, prior to haemocyte invasion, the muscle cytoplasm contains few vacuoles of the lysosome type which contrasts strongly with that of the haemocytes. However, the changes described above in muscle preceding haemocyte invasion and the frequently different behaviour of adjacent muscles in the same haemocyte environment suggests that the initial stimulus for muscle degeneration must originate within the muscle itself.

Muscles 22, 23 and 17 of segment 8 all underwent active phagocytosis, as described above. In these muscles degeneration was complete, and they were not carried through into the adult. In muscle 1, that underwent partial degeneration, phagocytosis was restricted to the origin and insertion. Vacuolation and sub-division into fibrillae was more general within the muscle, the cross-striations disappeared but at no time did the whole muscle fragment, and the muscle nuclei remained.

When the histolysis of the origin and insertion was complete numerous myoblasts approximately 8.5 μm in their long axis appeared in the haemocoel. By the early fifth instar, these had aggregated around the disrupted muscle. They were the typical spindle-shaped cells as previously described, and they come to be very closely applied to the muscle surface. Quite suddenly myoblast cell nuclei appeared, deeply embedded within the sarcoplasm, intermingled with the nymphal muscle nuclei. As previously described, the process of invasion continued until the whole population of undifferentiated cells surrounding the muscle had been incorporated.

The fibrillar nature of the muscle was rapidly reformed with the nuclei forming up in chains along the fibres. The muscle ends establish contact with the epidermal cells, and new origins and insertions are formed.

By the late fifth instar, almost all of the definitive muscles are present, though they are considerably more slender than those of the adult. The increase in fibre size continues for the first two days of adult life. During the mid to late fifth instar, the major musculature of the first gonocoxa is completed, though some of the insertions are still in the process of formation. Movement of the insertions, and to a lesser extent origins, may also be affected by the developing basal genitalia. This is particularly the case in the first gonocoxa, where the insertions of muscles 1 and 2 on the posterior extremity of the dorsal sclerotized rod are formed by the early to mid fifth instar. The definitive, more posterior, location of these insertions is then brought about by the posterior growth of the sclerotized rod as the first gonocoxa undergoes further development. Muscle 3, a very slender structure, is formed during the final moult and its position has been described under the adult system.

This shift in the position of the origins and insertions would produce a change in the mechanical advantage of the muscles between the nymphal and adult stage.

Considerable changes occur in the pregenital segments between the fourth and fifth instar so that the adult condition is effectively assumed prior to the final moult. No major alterations occur in the dorsal and ventral muscles, and tergosternal muscle 20 (fig. 4.9), that arises from an anterior ventral apodeme formed by an unfolding of the epidermis near the sterno-pleural ridge, merely undergoes longitudinal cleavage to produce two daughter fibres as in the definitive condition.

Most development occurs in segment seven, where further differentiation of the segmental laterals occurs. These, in the adult, form a complex group of fibres. By the mid fifth instar, three pairs of fibres originate on the posterior intersegmental fold towards the ventral region of tergite seven. These run anteriorly in a dorso-lateral direction to insert on the epidermis of tergite seven ventral and lateral of the median dorsals.

Further differentiation of the pregenital musculature occurs during the final moult. The segmental laterals develop further, the definitive condition being assumed by the longitudinal cleavage of the fibres and the further incorporation of free myoblasts. During the late fifth instar all muscles undergo growth, and the nuclei migrate towards the periphery of the fibres.

The adult condition is finally achieved after further muscle growth, the development of definitive striations on the fibres and of the deeply basiphilic nature of the muscle nuclei. In the more massive muscles such as those of the second gonocoxa, the attachments to the epidermis are strengthened by the shortening of the muscle fibre and the pulling out of the epidermal cells into short, powerful, tendon-like apodemes. (fig. 4.12).

4. Discussion

The preceding section indicates that muscle metamorphosis, to the definitive condition, in Graphocephala fennahi proceeds via a number of different pathways : (a) The massive reconstruction by partial autolytic histolysis with the retention of nymphal muscle nuclei and the incorporation of free myoblasts. (b) New formations that arise through the aggregation and differentiation of free myoblasts. (c) The longitudinal cleavage of nymphal muscles, the products of which are carried over into the adult with little or no change. (d) The phagocytosis of muscles, principally at the

origins, and insertions and reconstruction incorporating nymphal muscle nuclei and free myoblasts. In addition to these changes, the metamorphosis of the muscles may require the histolysis of a few peculiarly nymphal muscles brought about either by autolysis or phagocytosis.

It has been demonstrated in the present study that phagocytosis is always preceded by preliminary changes in the muscle fibre. There are two sources of evidence for this claim; the first being histological, vacuolation and separation of the fibrils occur in the reactive fibre prior to haemocyte invasion. The second is that different muscles, though often adjacent, react in different ways, some undergoing histolysis, while close neighbours are unaffected.

During the histolysis of certain muscles, the production of sarcolytes (muscle fragments) is independent of haemocyte activity. This agrees with the findings of de Bruyne (1898) on Calliphora, Hollande (1909) and Evans (1936) on Lucilia and Robertson (1936) in Drosophila. In this process, haemocytes only enter the muscles at a later stage after fragmentation and are only concerned with the ultimate digestion of the muscle fragments.

The extent of molecular disintegration that the myofilaments undergo during metamorphosis is unknown, though it would appear likely that most protein molecules are retained in an intact form. Lysosomes and lysosomal acid hydrolases are only sparsely distributed in normal muscle (Weinstock & Iodice 1969). Recent electron-microscopic studies have not produced evidence that the myofilaments are isolated within a vacuolar apparatus during autolysis (Stegwee et al 1963; Lockshin & Williams 1964; Lockshin 1969b; Crossley 1972). Lockshin and Williams (1965) suggested the release of lysosomal enzymes directly into the muscle cytoplasm by the rupture of vacuole membranes. This is contrary to the normal arrangement seen in cells, where

enclosure in a vacuole is a prelude to catabolism (de Duve & Walliaux 1966), though it is possible that enzymes specific for muscle protein hydrolysis could be released directly into muscle cytoplasm. Such enzymes might not be detected by standard techniques for lysosomal enzymes, and so would account for the absence of Gomori-positive vacuoles in degenerating muscles.

Lockshin (1969a) demonstrated that protein synthesis was necessary for the initial breakdown of muscles in Saturniid moths, suggesting that this protein then activated lysosomal enzymes. However, as cytolysomes have not been reported from normal insect muscle, the morphology of active lysosomes in insect muscle has not yet been described. Randall (1970) reported acid-phosphatase positive granules in denervated Galleria muscle, but it is not known if they are present in normal metamorphosing muscle.

One aspect of myogenesis that always presents a difficult problem is the origin of myoblasts. Two types of accumulation were recorded in the present study : (a) The aggregation of myoblasts around preformed nymphal muscle rudiments, and (b) their accumulation in sites where no nymphal muscle was present, and no previous rudiment could be detected. Conclusive evidence as to the origin of myoblasts was not determined in the present study, they may be derived from circulating haemocytes or undifferentiated mesodermal tissue, as is present in the genital disk. This problem requires considerably more attention and the use of specific labelling techniques may go some way to providing an answer, though finding suitable labelling material is in itself a major problem. Arvy (1953) recorded accumulations of haemocytes in the second instar of Musca, and Crossley (1964) was able to demonstrate that similar groups formed important haemopoietic centres. He concluded that the increase in the population of phagocytic (Type F) haemocytes circulating in the body cavity at the time of puparium formation, in

Calliphora, was traceable to a change in the differentiation pathway of stem cells within these centres. Crossley (1965), using crystalline crustecdyson (β ecdysone), was able to demonstrate that these haemocyte alterations are probably under humoral control.

Wigglesworth (1959) reviewed the early work on haemopoietic organs. Hoffman (1970) was able to demonstrate the presence of a haemopoietic organ along the dorsal vessel of Orthoptera, made up of reticular cells of mesodermal origin with phagocytic activity.

It was shown in this study that certain muscles (i.e. 22 and 23) are liable to phagocytosis by Type F haemocytes, phagocytosis frequently being heralded by the appearance of abnormal elongated vacuoles between the fibrils. These changes that precede haemocyte invasion are restricted to the sarcoplasm, the fibrils are not disrupted, and, at this stage, there was no loss of striation. There is also evidence to suggest that the sarcolemma does not break down prior to haemocyte invasion.

In Graphocephala, no known histoblasts or similar aggregations of cells, such as are found in Calliphora, were found, and it is suggested that presumptive myoblasts are derived from the irregularly distributed haemocytes. They differ from Type F haemocytes both in size and gross histology, as described above, but are thought to have arisen by the differentiation of residual mesoderm cells, which move freely in the haemolymph and which in the eighth and ninth abdominal segments may be associated with the genital disk. Certainly, undifferentiated haemocytes cannot be distinguished using light microscopy, from the pioneer myoblasts and clusters of myoblasts that form around the earliest muscle strands.

The myogenesis of Graphocephala differs from that described in other species

in the diversity of the mechanisms. Davies (1969) described three major types in Limothrips cerealium; (a) Those muscles that pass into the adult from the nymph with relatively few histological changes. (b) Those which are essentially new formations arising through the aggregation and differentiation of free myoblasts. (c) Those that involve the incorporation of myoblasts into pre-existing nymphal muscles, which then undergo extensive differentiation into the adult condition. Phagocytosis was not recorded.

In Calliphora, no nymphal abdominal muscles pass unchanged into the adult (Crossley 1965); the degenerated muscle undergoes complete reconstruction with the imaginal muscle nuclei having their origin in histoblast tissue.

Two distinct forms of myoblast incorporation were detected in the present study. Firstly, when a muscle differentiates from free myoblasts with no remnant of a nymphal muscle present, the precursor cells initially appear free in the haemolymph as typical myoblasts, short, spindle-shaped cells with a dense granular cytoplasm. These cells increase in number to form a small cluster in the position of the future muscle. A small number of myoblasts then undergo growth in the longitudinal axis to produce a long filamentous cell, here termed the pioneer myoblast, the first fibril being formed by the coalescence of the cytoplasmic granules. Free myoblasts then aggregate around the presumptive rudiment, multiply by mitotic division, and gradually undergo bipolar elongation and incorporation into the muscle rudiment, providing another nucleus and fibril, together with additional sarcoplasm.

The second type occurs when free myoblasts are incorporated into nymphal muscle that is undergoing major reconstruction. Here, myoblast cells aggregate around the nymphal muscle and become very closely applied to it. Quite suddenly myoblast nuclei appear deep within the muscle, mixed with nymphal muscle nuclei, and supposedly also incorporating additional cytoplasm. The two nuclear populations

are clearly distinguishable by differing staining properties, those of the myoblast being more strongly basiphilic. In this second method, no bipolar elongation of the myoblast occurred, and there appeared to be a general mixing of larval muscle nuclei and sarcoplasm with that of the myoblasts. For Graphocephala, no evidence was obtained to suggest the disintegration of nymphal muscle nuclei, as observed by Crossley (1965) in Calliphora; instead, nymphal muscle nuclei were carried over into the adult.

CHAPTER 5

STRUCTURE OF THE OVIPOSITOR RELATED TO OVIPOSITION SITE

1. Introduction

There are several works which adequately describe the morphology and terminology of the female genitalia of the Cicadellidae, but no comparative studies have been made to link structure with oviposition behaviour and habitat. Snodgrass (1933), in his study of Amblydisca gigas, probably represents one of the most important works on the morphology and much of the terminology used in recent papers on females of Auchenorrhynchan Homoptera follow this work (Cunningham & Ross 1965; Nielson 1965; Helms 1968). Scudder (1957; 1958; 1959; 1961a; 1961b; 1964) has questioned some aspects of the work of Snodgrass in a series of papers and developed a new theory of the ovipositor, which he calls the "gonangulum theory", as he considers this to be one of the most important sclerites of the ovipositor (Scudder 1961a), because it affects the movement of both the first and second gonapophyses. This is a point of view that will be developed further in the present chapter.

Though it is known that the type of ovipositor is largely dependent upon the oviposition site, few comparative studies of species have been made to demonstrate this character. Scudder (1959) in his classification of the Heteroptera was able to show that those families that oviposit in plants possess a laciniate type of ovipositor with lanceolate gonapophyses and a heavily sclerotised anterior bar to the gonangulum. Where oviposition is on the surface of the substrate, the ovipositor is plate-shaped with flap-like gonapophyses and the gonangulum is reduced or absent. Some work has also been undertaken in the Sawflies and Thysanoptera, but this point will be raised further in the discussion.

Whether differences at lower taxonomic levels depend on the density of the

oviposition substrate has not been considered in the literature and forms the object of the present study. Three species of Cicadellidae were considered: Ulopa reticulata (Fabricius), Macropsis scutellata (Boheman), and Graphocephala fennahi (Young). The oviposition sites range from woody stems in the first species to the sub-epidermal tissues of over-wintering buds in the last species. The effect of this on ovipositor structure was examined.

2. Method

The precise oviposition site of the three species had first to be determined. This was achieved by field observations of ovipositing females and dissection of the plant material to expose the eggs.

The external genitalia were examined with the light microscopic and the scanning electron microscope. Specimen preparation for the latter technique is described in detail under Chapter 2. Specimens of Ulopa and Macropsis were cleaned prior to dehydration, using ultrasonic vibrations. The basal genitalia and genital musculature were examined by dissection. From this the genitalia and associated musculature could be described for the three species and the variation in structure determined.

Where necessary specimens were sectioned for light microscopy and stained with Mallory's Phosphotungstic Haematoxylin.

3. The external genitalia and associated musculature of the three species

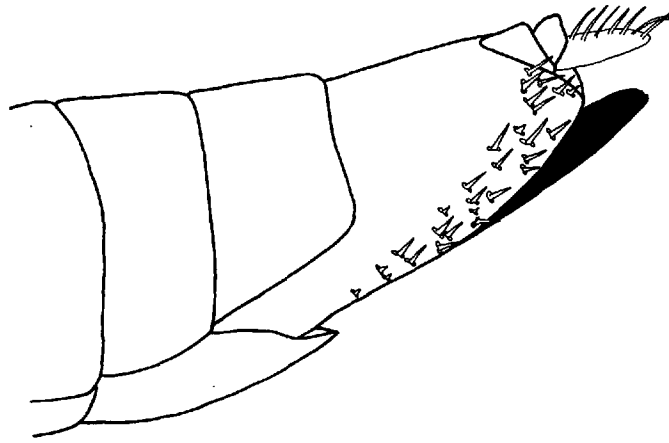
Gross differences could be seen in the form of the abdomen (fig. 5.1) and in the size of the species, Graphocephala and Macropsis being the largest (8.5 - 9.4 mm and 5.2 - 5.5 mm respectively in total length of the female). Ulopa was the smallest of the three species, the females measuring 3.3 - 3.7 mm total length.

(a) Graphocephala fennahi

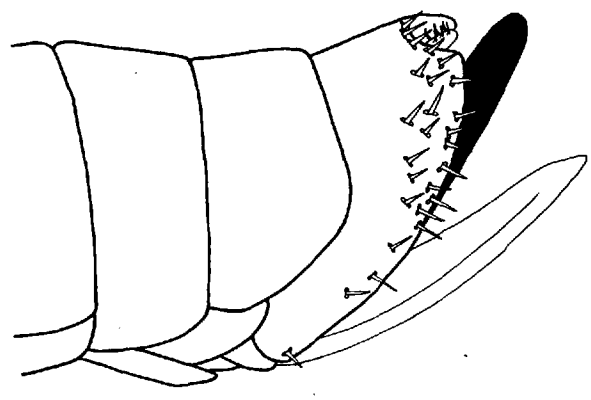
This was described fully in Chapter 1 and 4 and will not be repeated here.

Fig 5.1

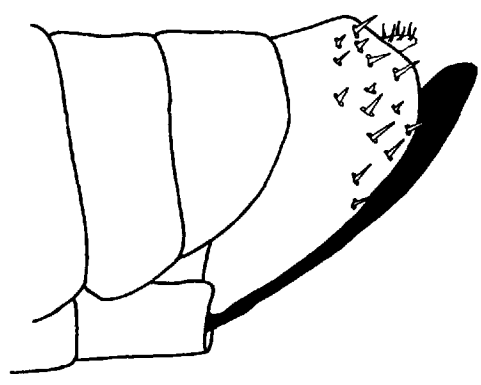
The genital region



G. fennahi



M. scutellata



U. reticulata

0·8mm

(b) Ulopa reticulata

Observations on oviposition behaviour showed that the species oviposits in the woody stems of Erica and Calluna, and that the gonapophyses are used to make an incision into this material. In the present study this was considered a very hard substrate to penetrate, which may be indicated by the structure of the external genitalia.

The structure of the first gonocoxa is essentially similar in form and musculature in the three species studied, so that the description of that for G. fennahi on p. 21 and p. 146 will suffice.

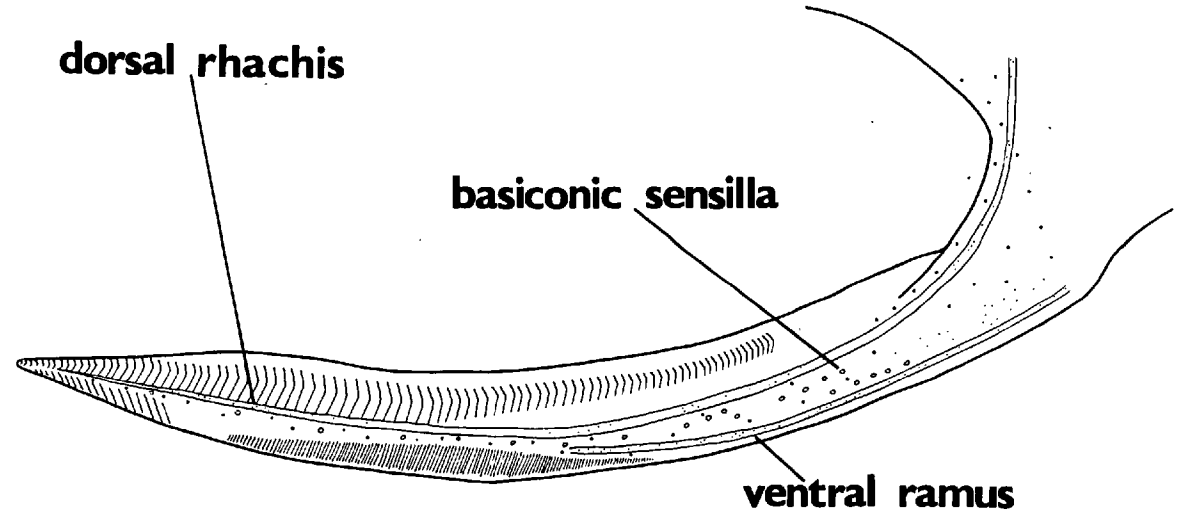
i. First gonapophyses

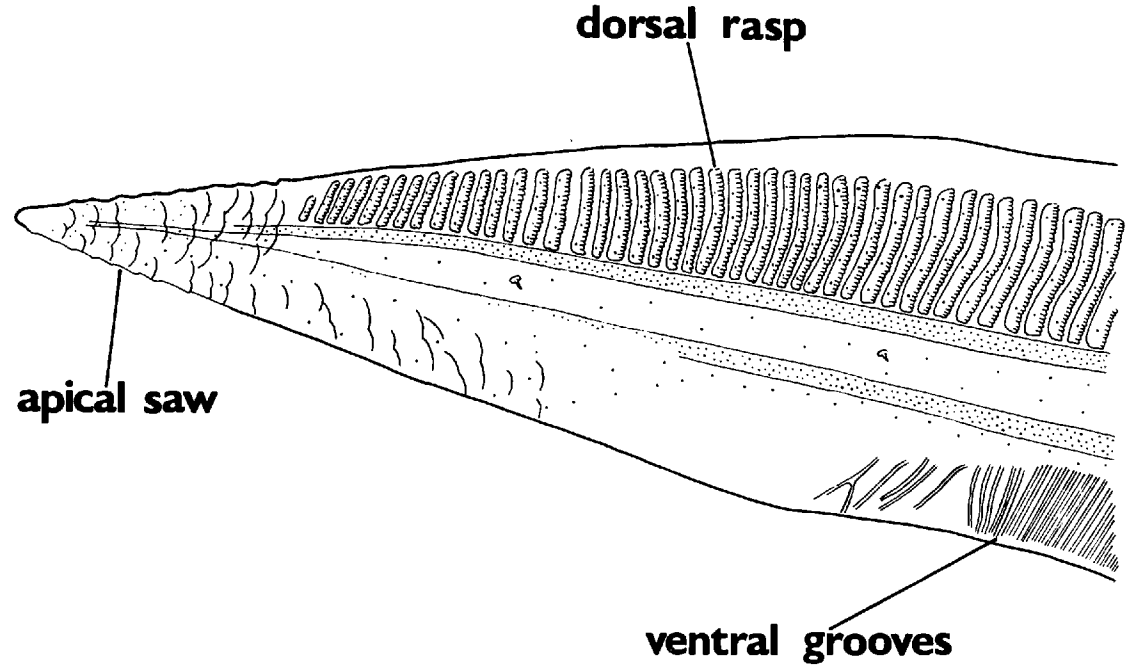
This is similar in gross form to that of Graphocephala (fig. 5.2) in that the blades are lanceolate and lie in the vertical plane. They are strongly sclerotized structures and laterally compressed. Transverse section reveals the presence of intra-gonapophyseal spaces which are reduced, compared to those of Graphocephala, permitting a greater thickness of sclerotization on the lateral and dorsal walls. The spaces are restricted to immediately beneath the dorsal edge and around the median inter-locking mechanism. The blades are considerably more sclerotised than those of Empoasca, as shown by Balduf (1933). The reduction of intra-gonapophyseal spaces in Ulopa might be expected to result in a stronger though more brittle system. The latter character may be partially alleviated by the increased ratio of gonapophyseal length: total body length (3.67 in U. reticulata, 2.96 in G. fennahi and 3.03 in M. scutellata).

The first gonapophyses possess a dorsal curvature throughout their length (fig. 5.2) and taper distally to a fine point (fig. 5.3). More proximally the broad thin form is apparent and bears a prominent dorsal and ventral rasp on the outer wall. The dorsal rasp (fig. 5.4a) extends from within 50 μm of the distal tip almost to the

Fig 5.2

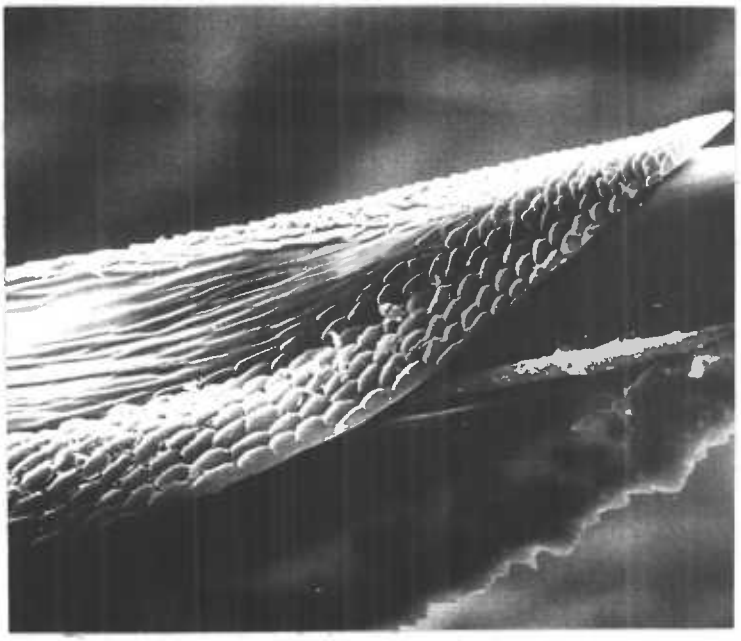
Ist Gonapophysis U.reticulata





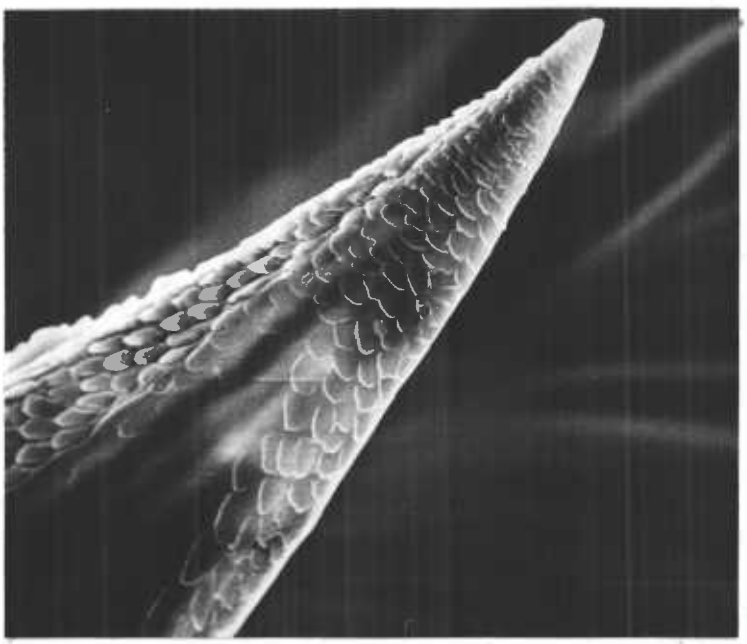
Distal 1st. gonapophysis of U. reticulata.

a.



N. B. strongly pointed tip. x 550 KV 20

b.

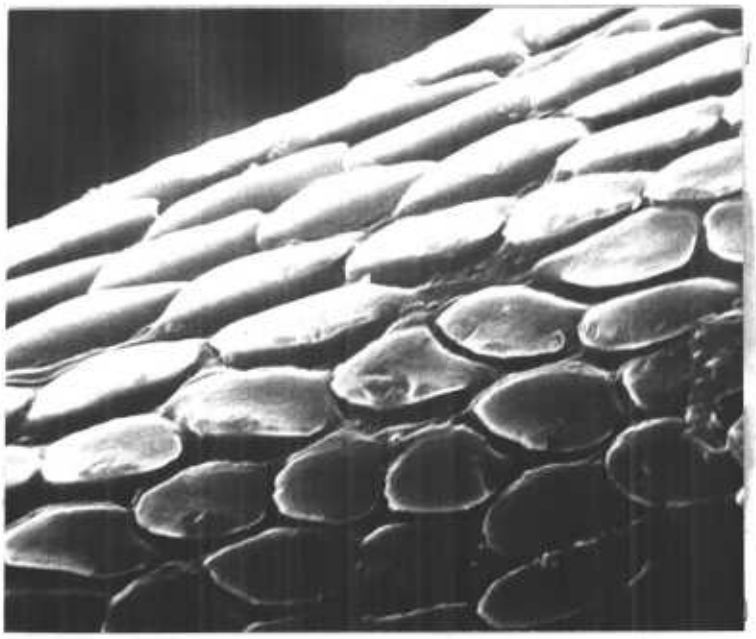


The distal rasp x 800 K.V. 20

Fig. 5.4

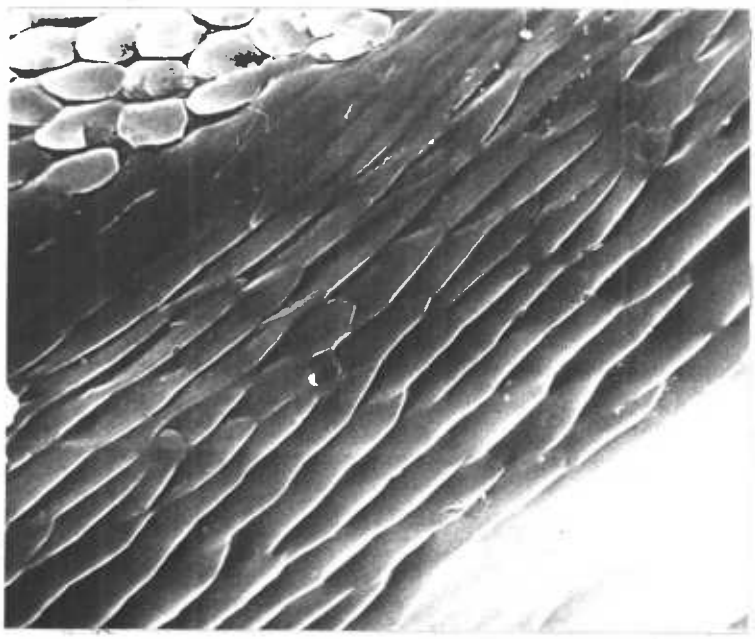
Sculpturing of the outer wall in the distal half of the 1st. gonapophysis of U. reticulata.

a.



Dorsal portion of the wall. x 2400

b.



Ventral portion of the wall. x 1200

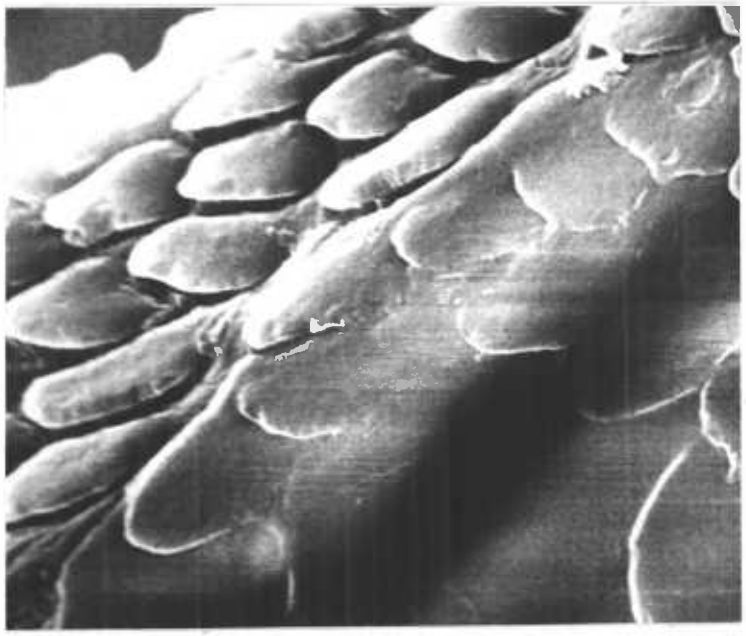
base of the first gonapophyses though it is best developed in the region 0.5 mm - 0.9 mm from the proximal end. It extends ventrally as far as the rhachis, and can be divided into two regions: (a) a narrow dorsal strip, approximately 15.0 μm wide, is composed of elongate, rounded blocks of sclerotized cuticle and angled slightly towards the blade apex. These blocks measure approximately 12.7 x 2.8 μm, and are raised above the outer surface of the first gonapophyses and closely opposed, the channels between the blocks being formed by the angled edges of the blocks themselves. (b) The remainder of the dorsal rasp is approximately 40 μm wide in the well developed region and composed of discrete circular plates of sclerotized cuticle approximately 9 μm in diameter and separated from each other by a channel 0.7 - 1.1 μm wide. The dorsal rasp gives way ventrally to a zone of poorly defined overlapping sclerotized scales (fig. 5.5).

A considerable difference was noted in the structure of the ventral rasp, which was much more restricted in extent, extending from 400 μm distally for 350 μm, and so ending some 200 μm proximal of the tip. This rasp is composed of an area of slightly thicker sclerotized cuticle standing just proud of the surrounding region and traversed by numerous fine channels (fig. 5.4b), which are directed at an angle of about 60° towards the distal end of the blade. These rasping areas are considerably more extensive than those of Graphocephala and their more complex form suggests they may aid in the formation of the oviposition cavity. Proximally the dorsal rasp gives way to lightly sclerotized scales, the anterior edges of which are drawn out into a fine fringe, which is directed towards the ovipositor base. These scales are similar to those present in Graphocephala, and are assumed to serve the same function, that of anchoring the ovipositor blades in the incision during oviposition.

A well developed, heavily sclerotised groove is present on the inner surface

Fig. 5.5

To show the transition from the dorsal rasp to a region of ill-defined overlapping scales on the distal region of the 1st. gonapophysis of U. reticulata.



x 2400

of the first gonapophyses and extends the whole length of the blade to within 25 μm of the tip. This forms part of the interlocking system between the first and second gonapophyses. As in Graphocephala, the first gonapophyses of each side are united along their proximal two thirds by a membranous extension of their ventral edges, which are connected in the mid-line by a heavily sclerotised interlocking device.

The inner wall of the proximal half is developed into scales some 10 x 7 μm, the anterior edges of which are drawn out into a fine fringe approximately 2.5 μm long, directed towards the ovipositor base and similar to the condition seen in Graphocephala.

Proximally two rami are present on each side, which represent sclerotized edges of the blades, the region between them being strongly sclerotised. The ventral ramus articulates with the anterior extremity of the first gonocoxa, while the dorsal ramus passes dorsally and is expanded into a small head that is fused with the gonangulum.

Only sensilla basiconica are present on the first gonapophyses. They can be divided into Type I and Type II, as in Graphocephala. The former are distributed along the whole length of the blade, immediately ventral of the rhachis, except in the proximal 100 μm and were not observed in the distal 150 μm. The total number per blade is 17 - 20 and in the distal half they are placed singly and regularly spaced every 70 - 80 μm. In structure they are essentially similar to those of Graphocephala. The basal pit measures 2.2 μm in diameter, and is sunk approximately 1 μm beneath the surface, the peg length is 1.8 - 2.0 μm and diameter 1.1 μm at the base, tapering to 0.36 μm at the tip. A single distal perforation is present.

Type II sensilla basiconica are concentrated proximally, about 100 μm from the ovipositor base. They number 10 - 12 per blade, and are of the blunt, straight

type with no visible perforations of the peg. This type was somewhat larger than the previous one, the pit diameter measures 2.5 μm, and the peg length 3.3 - 3.8 μm, with a basal diameter 1.3 μm, tapering to 0.34 μm at the distal end.

The distal perforation of the type I sensilla suggests a chemoreceptor function. Sensilla basiconica have rarely been described from the ovipositors of other insects usually being more associated with the labium (Norris & Chu 1974; Mustaparta 1975), where they have been ascribed a gustatory function.

The musculature associated with the first gonapophyses and first gonocoxa is essentially similar to that of Graphocephala, the muscle complement being the same, as are the positions of this origin and insertions (see that for Graphocephala, fig. 4.2). Differences noted in Ulopa were that muscle 3, which runs between the two faces of the first gonocoxa, is considerably reduced to a single, very fine fibre that could very easily be overlooked. Muscle 9, originating on the anterior gonangular ridge, is represented by a single fibre, but is thicker in Ulopa. Of the major first gonocoxal muscles, muscle 1, originating on tergite eight, shows sub-division into two fibres, closely bound together and inserting on the dorsal sclerotized bar of the first gonocoxa. The origins of these major first gonocoxal muscles, designated 1 and 2 in the description of Graphocephala, are displaced ventrally in Ulopa to the posteroventral corner of tergite eight. This would allow increased efficiency in that there would be greater displacement of the first gonocoxa in the anteroposterior direction which would be transmitted to the first gonapophyses.

ii. Second gonocoxae

In gross form the second gonocoxae are similar to those described for Graphocephala, being heavily sclerotized, elongate plates lying in the vertical plane beneath the anterior extremity of tergite nine. The second gonapophyses are fused to the anterodorsal margin of the second gonocoxa, and the gonoplacs are

membranously connected to its posteroventral edge (fig. 5.6). The second gonocoxa also articulates with the ventral region of the gonangulum over a broad area, about its mid-point on the posterior edge, to provide the fulcrum which allows considerable rocking motion of the second gonocoxa, whose movement is then transmitted to the second gonapophyses. This is one of the most important articulations of the Cicadellid ovipositor and in Graphocephala it is responsible for controlling the movement in the gonapophyses.

The second gonocoxae of Ulopa are more elongate, in the dorsoventral plane, and more slender than those of Graphocephala. Immediately anterior to the fulcrum, enclosed dorsally and ventrally by ridges of increased sclerotization (fig. 5.7), is an oval area of sclerotized cuticle, richly supplied with basiconic or short trichoid sensilla, which were grouped into a major cluster immediately anterior to the fulcrum. This group comprised 12 - 18 sensillae (fig. 5.8a) which were similar to those of Graphocephala in all respects. Their position implies a mechanoreceptor function.

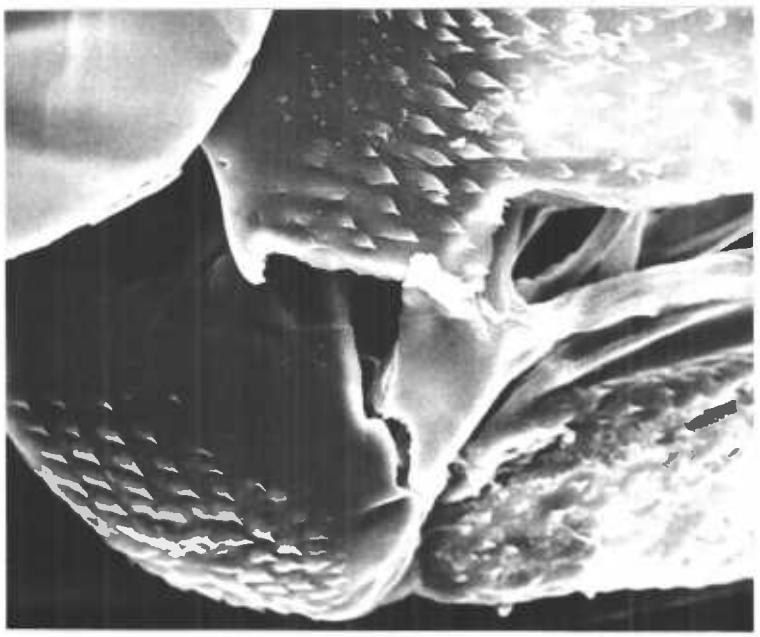
No sensilla are present at the posteroventral corner of the second gonocoxa, as was recorded in Graphocephala. Large companiform sensilla are present on the thickened ventral edge of the second gonocoxa of Ulopa and Macropsis (fig. 5.8b). These comprise circular pits, approximately 8 μm in diameter, the aperture of which is covered by a membrane leaving a central perforation 2 - 3 μm diameter through which a peg, 1.5 - 1.9 μm in length with two to three distal perforations could be seen.

Whether these are mechanoreceptors, as suggested above, or an unusual type of chemoreceptor, could not be resolved with scanning electron microscopy. Sections through the bases of the sensilla to determine the number of associated neurons and electrophysiological data would be necessary in order to make valid conclusions as to their function.

Fig. 5.6

2nd. gonocoxa of U. reticulata obscured at the top left by the posterior extreme of the 1st. genocoxa. The gonoplacs join the 2nd. gonocoxa ventrally.

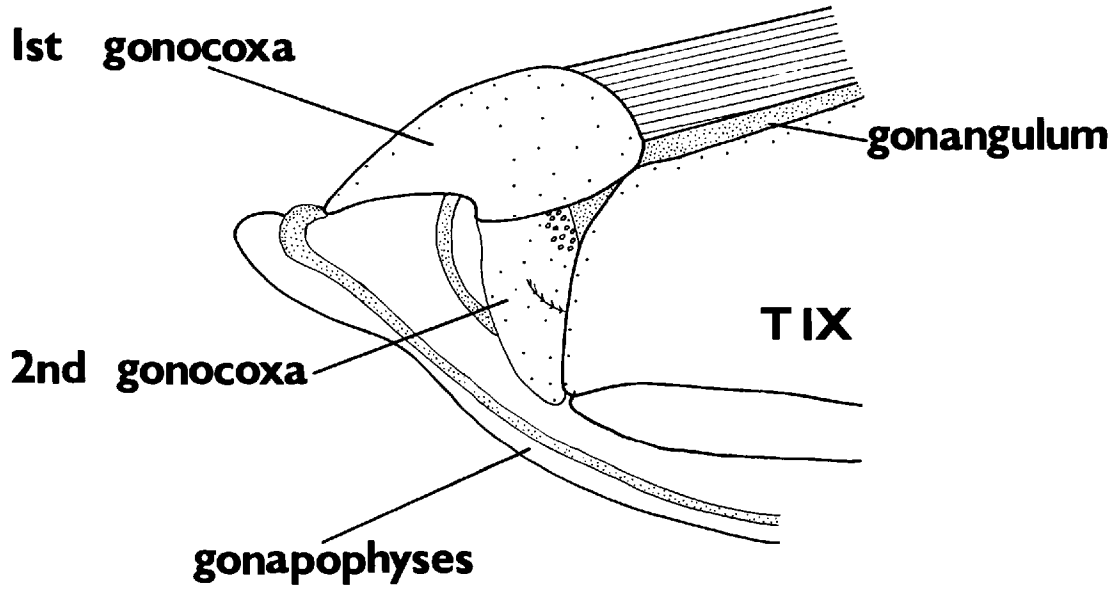
U. reticulata.

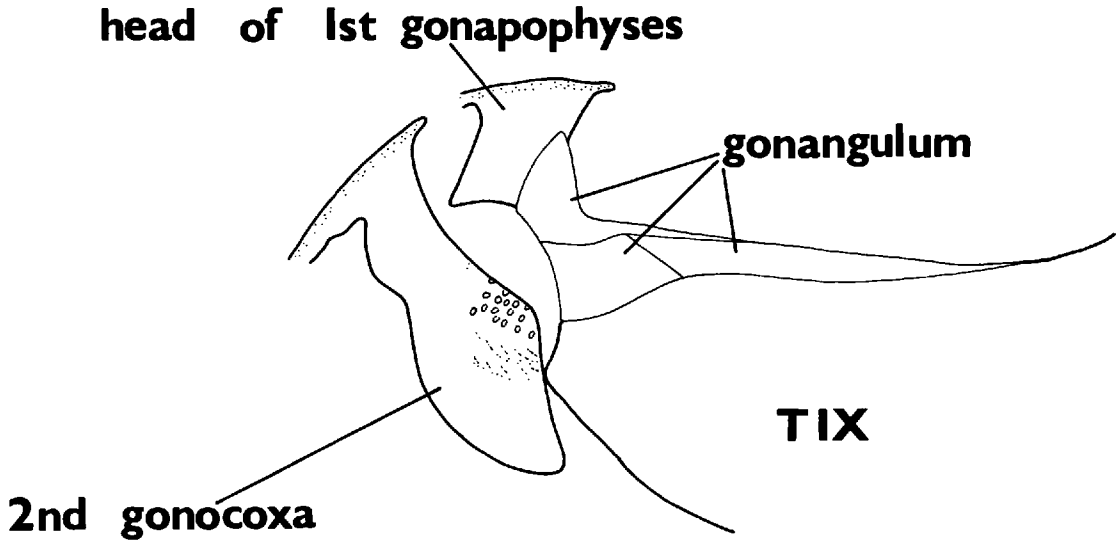


Tergite IX, dorsal to the genoplacs in the photomicrograph is damaged. x 240

Fig 5.7

Basal genitalia of Ulopa





1st gonocoxa removed

0·6mm

Fig. 5.8

Sensory structures on the 2nd. gonocoxa of U. retuculata

a.

Mechanoreceptors anterior of the fulcrum with the gonangulum. x 900

b.

Campaniform sensilla on the ventral edge, x 500

The musculature is similar to that of Graphocephala and composed of a single, large dorsal second gonocoxal fibre, that originates dorsally on the anterior part of tergum nine. Three ventral second gonocoxal muscles are present which originate over a large area of tergum nine, each of the three origins covering the median and lateral regions of this tergite, so effectively filling segment nine except for a very narrow median cavity separating the muscle blocks of each side. The second gonocoxal muscles in Ulopa are relatively larger than those of Graphocephala, though the fibre length is shorter, compared to the thickness of the fibre, providing a relatively more powerful unit.

iii. Second gonapophyses

These are lanceolate blades, similar in structure to the first gonapophyses against which they are applied, though the dorsal serrated edge projects above that of the first gonapophyses. Distally the inner and outer wall is heavily sclerotized, as is the dorsal edge along the whole length of the blades (fig. 5.9). The outer ventral wall is composed of lightly sclerotized scales with free, overlapping edges directed anteriorly. The inner ventral wall is similarly composed of lightly sclerotized scales, some 4 μm by 7 μm, and the free anterior edge is drawn out into a fine fringe, approximately 1.6 μm in length. This is similar to the fringe found in Graphocephala, where if formed a soft-textured buffer surface, over which the eggs pass during oviposition.

The distal end of the second gonapophysis is roughly pointed (fig. 5.10), though not as angular as that of Graphocephala, the distal region being narrow with no well developed ventral cutting edge. The main cutting regions of the second gonapophyses are the dorsal asymmetrical teeth, which extend from the distal extremity to mid-way along the blade, when they become much reduced and give way to a smoothly rounded

Fig 5.9

2nd gonapophysis of Ulopa

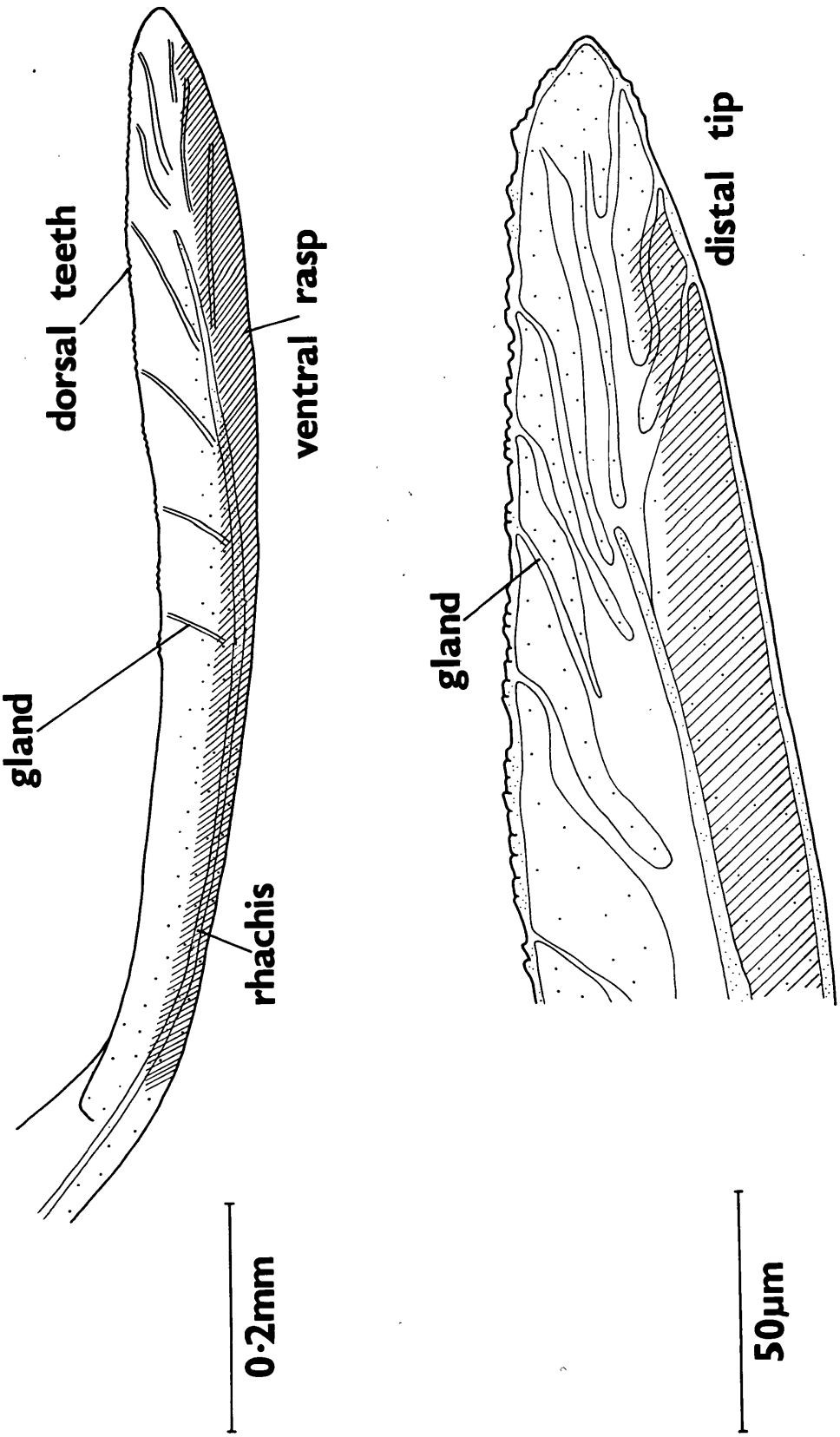
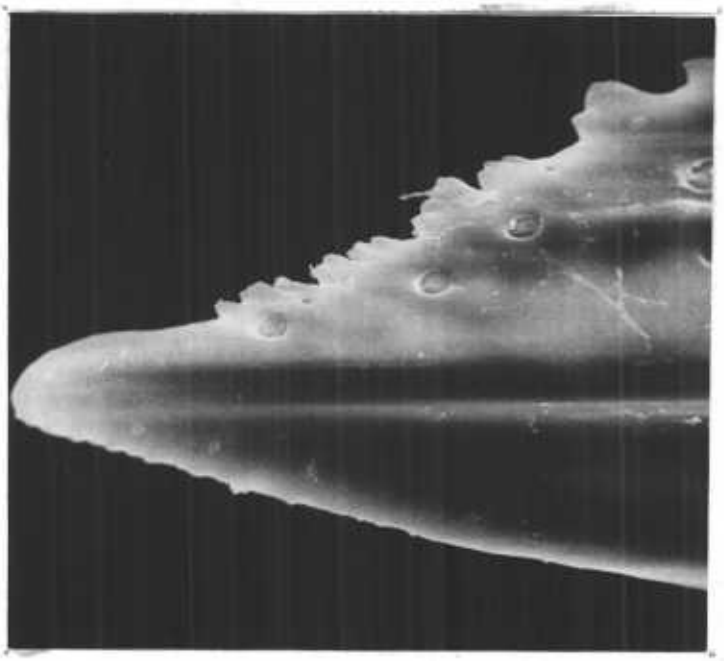


Fig. 5.10

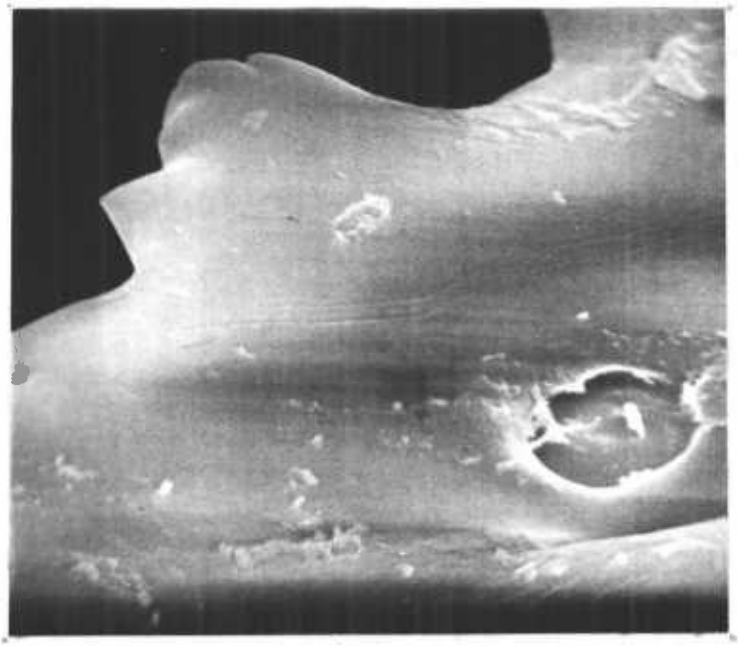
Distal end of the 2nd. gonapophysis of U. reticulata

a.



The distal end. x 1100

b.



Highly elevated, serrated dorsal tooth in the distal region. x 4700

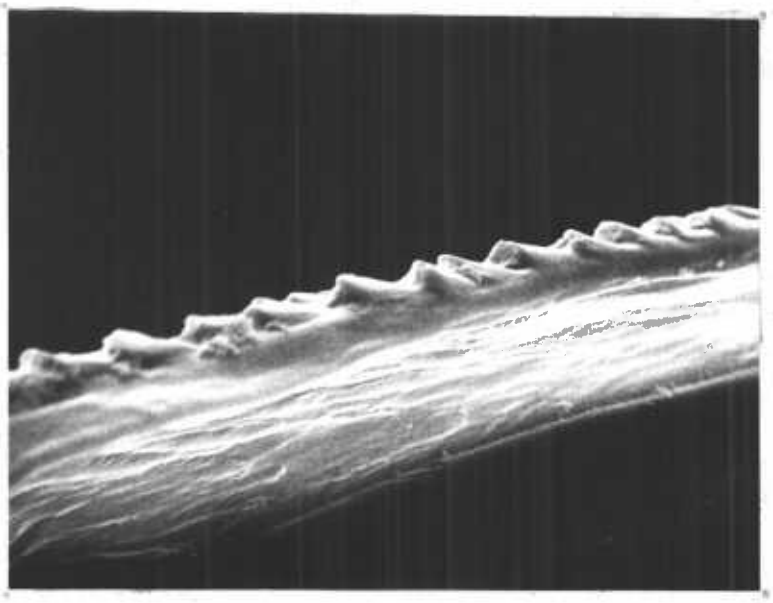
dorsal surface. The teeth are considerably more complex than the asymmetrical teeth of Graphocephala. In the distal 100 μm they are highly elevated (fig. 5.10b) above the dorsal edge of the gonapophysis, the anterior edge of the tooth forming a gentle slope, the apex is serrated and the posterior edge bears a single, large serration. The whole tooth, as seen in transverse section, is completely sclerotized. For the remaining length of the dorsal saw, the teeth are less elevated and possess multiple crowns, similar in appearance to the mammalian molar (fig. 5.11). These teeth are numerous, closely packed and the crowns pointed, thus forming an efficient rasp that would function on both the anterior and posterior strokes. The ventral edge of the second gonapophyses is in no way modified for cutting, which contrasts with the specialized and diverse cutting areas of the second gonapophysis in Graphocephala. That of Ulopa, though of simpler construction, provides a much stronger, though less delicate, system.

Transverse sections indicate that the second gonapophyses are less heavily sclerotized than the first gonapophyses, and that intra-gonapophyseal spaces are more widespread in the second pair, though large cavities are associated dorsally and ventrally with the main interlocking mechanism. The smaller cavities, particularly those associated with the dorsal edge, are not continuous throughout the length of the blade, but form a network of small chambers with sclerotized walls. These cavities probably aid the development of a lighter, though almost equally strong, structure, and the more heavily sclerotized first gonapophyses form a shaft round the median pair, thus providing additional support.

Each second gonapophysis possesses a ventral rhachis on its lateral surface, approximately one third dorsal of the ventral edge. This forms the linkage mechanism with the dorsal rhachis on the median wall of the first gonapophyses, and is the T-

Fig. 5.11

Dorsal teeth of the 2nd. gonapophysis of U. reticulata just proximal of the distal extreme.



Note their molariform appearance. x 2000

shaped process described by Balduf (1933). The direction of the T-shaped process is slightly dorsalwards throughout its length and there is no rotation in the direction of the linkage mechanism, as observed in Graphocephala, so allowing considerable freedom of the first and second gonapophyses to slide upon each other.

The ventral rhachis extends anteriorly as a single basal ramus which curves dorsally to fuse with the dorsoanterior edge of the second gonocoxa. This region was sclerotized in Ulopa and devoid of the overlapping fringed scales present in Graphocephala.

The second gonapophyses of Ulopa are less well supplied with sensory receptors than those of Graphocephala; only a single type is present and they are associated with the base of the dorsal teeth and restricted to the distal 100 μm, no sensilla being associated with the multiple crowned teeth. The sensilla comprise a circular pit 0.95 - 1.13 μm diameter, containing a tightly sclerotized plug 0.6 - 0.73 μm diameter. There are approximately 10 - 12 such structures per blade and they are thought to be mechanoreceptors of the campaniform type (figs. 5.10a and b). They are the only sensilla present on the second gonapophyses and may be concerned with monitoring cuticular stresses as the oviposition incision is made.

iv. Gonangulum

This sclerite is well developed in Ulopa, heavily sclerotized and pigmented a deep brown to almost black. It is fused over a wide area to the expanded head of the first gonapophyses (fig. 5.7), and can readily be divided into three regions defined by their pigmentation. Region 1 is fused to the first gonapophysis. Region 2 is fused with the anterior part of tergum nine and forms the fulcrum with the second gonocoxa; region 3 is slender, elongate, and fused over a large area to the antero-dorsal edge of tergite nine. These divisions do not correspond to those given by

Snodgrass (1935) for the second valvifer of Amblydisca gigas.

The edges of tergum nine at the points of fusion were also moderately sclerotized, a condition not seen in Graphocephala. The fulcrum is formed by the broad, rounded ventral part of region 2 with which a knob on the posterior edge of the second gonocoxa articulates, allowing the antagonistic action of the muscles of the second gonocoxa to rock this structure about the fulcrum.

v. Linkage mechanisms

Two linkage systems are present in Ulopa, joining the first gonapophyses of opposite sides, and the first and second gonapophyses of each side. The two second gonapophyses are not linked proximally by a membrane, as was demonstrated in Graphocephala, so allowing limited independent movement between these two blades.

The first and second gonapophyses of each side are linked by the usual tongue and groove mechanism, which was first described in Cicadellids by Balduf (1933) and appears to be general in Cicadellids; it is the structure common in other insect orders that possess an ovipositor. In Ulopa, the heavily sclerotized tongue is carried on the outer wall of the second gonapophysis and is similar in construction to that of Graphocephala, being composed of a sclerotized rod, made up of units 14.7 - 15.3 μm in length, the dorsal and ventral edges of which are flanged to prevent it being pulled out of the groove by laterally directed stresses. Large intra-gonapophyseal spaces are present at the base of this structure which lighten sthe structure.

The groove lies on the medial wall of the first gonapophysis and represents the sclerotized rhachis. Separation of the two blades is possible only by sliding one along the other; they cannot be pulled apart laterally. When joined, the dorsal cutting edge of the second gonapophysis projects above that of the first gonapophysis,

so exposing the dorsal teeth. The tongue and groove system is not supplemented, as in Graphocephala, so permitting movement of the second with the first gonapophyses. Transverse sections through the ovipositor showed that the interlocking mechanism was functional throughout the length of the blades, except in the distal 50 - 70 μm, where the tongue and groove is insufficiently developed to hold the two blades together. As previously stated, there is no rotation of the tongue in Ulopa, thus permitting the sliding of the two blades upon each other.

The first gonapophyses are united for the proximal two thirds of their length by a modified tongue and groove mechanism supplemented by interlocking spines. In this region the ventral edge of the first gonapophysis is extended into a short, lightly sclerotized flap, which is flexed dorsally to meet that of the opposite side in the mid-line. The distal quarter of each flap is highly sclerotized, and that of the right first gonapophysis is modified into a tongue and that of the left into a modified groove, so that the two sides are inseparably joined on a short ventral flap. The region surrounding the linking mechanism is heavily sclerotized and supplemented by spines and sockets which surround the primary mechanism. In the distal third of the blades sclerotization is considerably reduced, the tongue and groove become disarticulated, and the flap reduced. This structure is absent from the distal extreme of the blade, which is very heavily sclerotized.

Such a mechanism would require the first gonapophyses to move in unison, while allowing the possibility of independent movement of the second gonapophyses with respect to each other and to the first gonapophysis of their own side.

(c) Macropsis scutellata

Observations confirmed that this species oviposits deeply in the square stem of Urtica dioica within the collenchyma buttresses and central parenchyma tissue. This substrate was considered intermediate in hardness between the woody stems of Erica

and the epidermal tissue of Rhododendron buds.

In gross appearance the ovipositor of Macropsis is similar to that of the other two species studied, and the description of the first gonocoxa given for Graphocephala will suffice for this species, except that the median membranous flap present on the first gonocoxa of Graphocephala is absent in Macropsis as is muscle 3. The dorsal sclerotized rod is well developed along the dorsal margin of the first gonocoxa and bears distally the insertions of muscles 1 and 2, the two major muscles of this sclerite. Muscles 8 and 9 are as described for Graphocephala.

1. First gonapophyses

These are lanceolate blades with considerably less curvature than in Ulopa (fig. 5.12) and distinctly pointed distal parts (fig. 5.13). The first gonapophysis is dark brown and strongly sclerotized. Transverse sections indicate large intragonapophyseal spaces, which are discontinuous and divided into elongate chambers. Distally, spaces of small diameter occur throughout the depth of the blade, except in the dorsal edge and are noticably concentrated in the mid region and associated with the ventral edge. Half way along the blade the intragonapophyseal spaces have undergone fusion to produce large chambers ventral of the interlocking device and associated with the ventral edge, the dorsal edge being highly sclerotized. In the extreme proximal zone of the first gonapophyses, the spaces are considerably reduced and sclerotization increased.

The gonapophyses broaden anteriorly from the fine distal tip to produce the characteristic, laterally much compressed blade with a prominent dorsal rasp along the whole of the dorsal surface of the outer wall except in the proximal quarter and distal 70 - 80 μm, and extending ventrally to the edge of the rhachis. It cannot be divided into regions as in Ulopa, the whole rasp bearing strongly sclerotized semi-

Fig 5.12

Ist gonapophysis of Macropsis

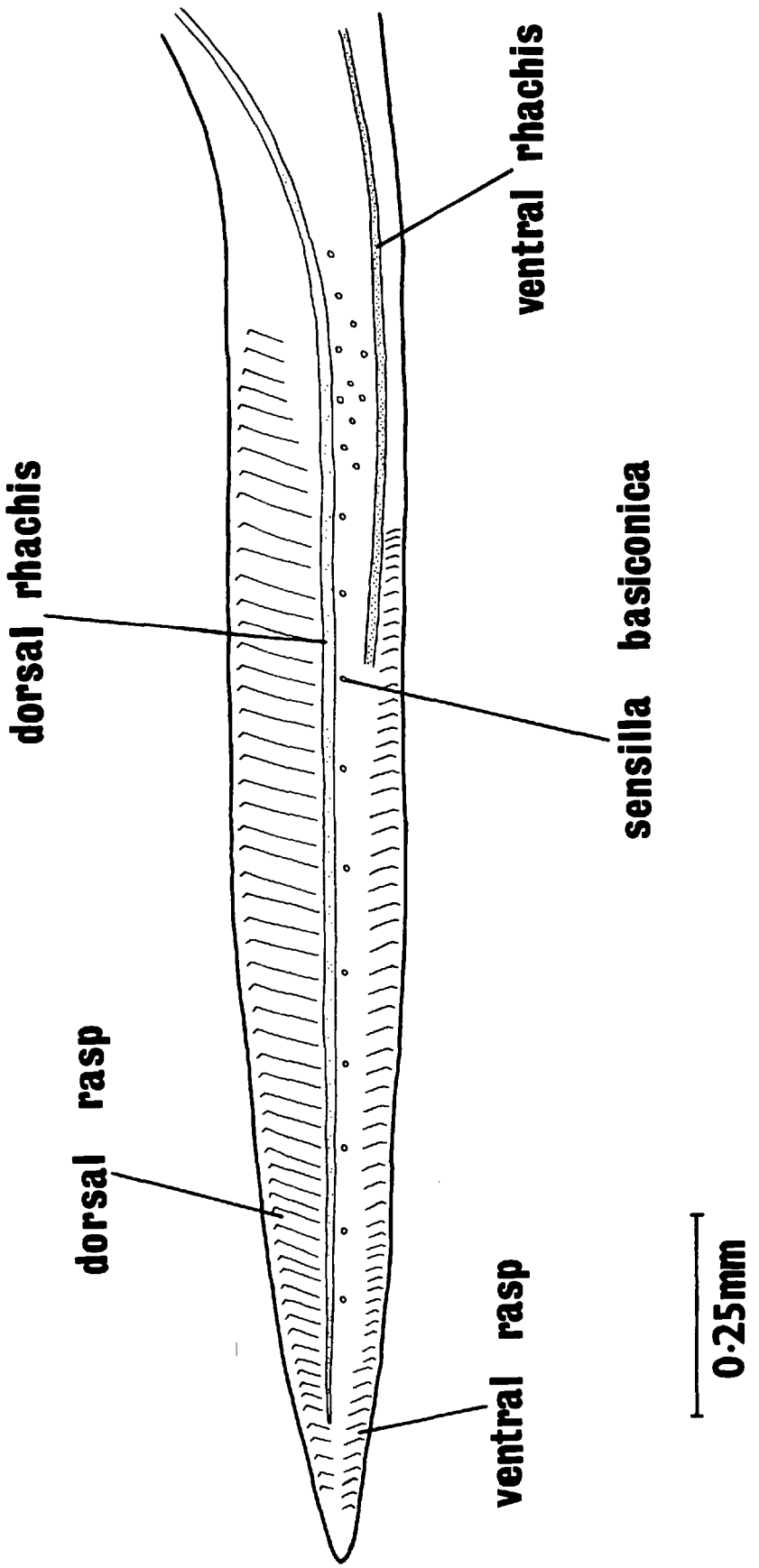
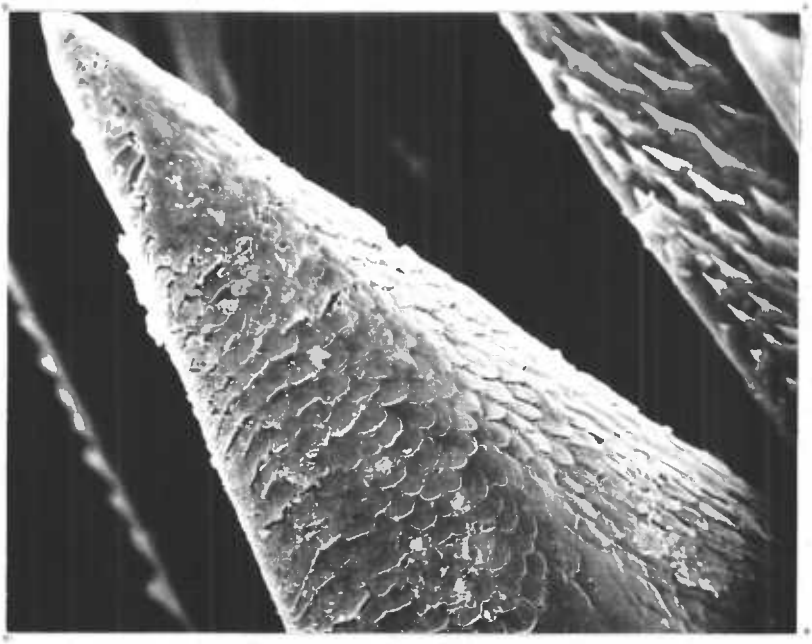


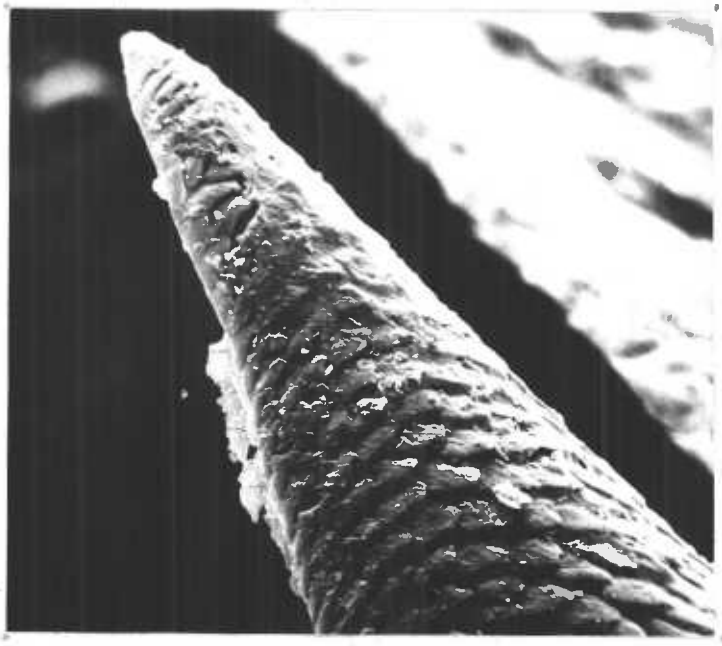
Fig. 5.13

The distal end of the first gonapophysis of M. scutellata.



a.

To show the general rasping areas in the distal extreme
X 570



b.

Highly pointed distal tip. x 1000

circular plates of 8.5 - 8.8 μm diameter. They do not overlap, but are thickened so that the semicircular edge projects slightly from the wall of the blade, this face being directed towards the distal end. Towards the distal edge, the plates become elongate and oval with a greatest length of up to 10.2 μm (blades from 28 specimens were measured). This area of the rasp cannot be considered a distinct zone since the transition to the elongate form is gradual and slight. Ventrally in the dorsal rasp the form of the scales is retained, but the elevation of the rounded edge decreased until immediately dorsal of the rhachis the scales are flush with the surface of the blade.

The ventral rasp is composed, for its entire length, of overlapping scales, the rounded edge directed distally and approximately 10.4 by 6.1 μm in size. The overlapping edge of these scales did not project considerably above the surface of the blade.

In the extreme distal region where discrete rasps are not evident the highly sclerotized walls possess small denticles, approximately 4.0 μm in their greatest dimension, set in concentric lines with their rounded apices directed distally (fig. 5.13 bottom).

The inner wall of the proximal two thirds is produced into scales 7 by 5 μm in size, and the anterior edge is irregularly drawn out into a short fringe 1.0 to 1.5 μm long. This is similar to the condition seen in the previous two species and appears to act as a soft textured buffer zone between the first and second gonapophyses of each side.

A well developed sclerotized groove is present on the inner wall, extending distally to within 70 to 90 μm of the tip, and forming part of the interlocking device with the second gonapophyses. This thickened region extends proximally

on the dorsal ramus which is extended into the dorsal head of the first gonapophyses by which it is fused to the gonangulum. The ventral ramus, as in other Cicadellids, articulates with the anterior extreme of the first gonocoxa.

As in Graphocephala and Ulopa, only sensilla basiconica are present on the outer wall of the first gonapophysis of Macropsis just ventral of the rhachis. No sensilla were recorded from the inner wall. The sensilla are similar to those of Graphocephala (fig. 5.14) and are divisible into Type I and Type II. Type I sensilla are most common, being distributed along the length of the blade to within 120 μm of the distal end. They differ from those of Graphocephala in that the basal plate forms a shallow pit from which the peg protrudes. The diameter of the basal pit is 2.0 - 2.1 μm and it is approximately 1.0 - 1.3 μm deep. The peg is 1.6 - 1.9 μm long and tapers to a very fine point, which bears a single perforation on a small papilla. Both the right and left flexed type are present, as in Graphocephala (p. 277).

Type II sensilla are longer and concentrated within an area approximately 300 μm in length and 150 - 200 μm from the proximal end between the dorsal and ventral rhachi. The basal plate is 2.3 - 2.6 μm across and not developed into a pit; the peg is 3.2 μm long, unsculptured and with no evidence of perforations.

ii. Second gonocoxae

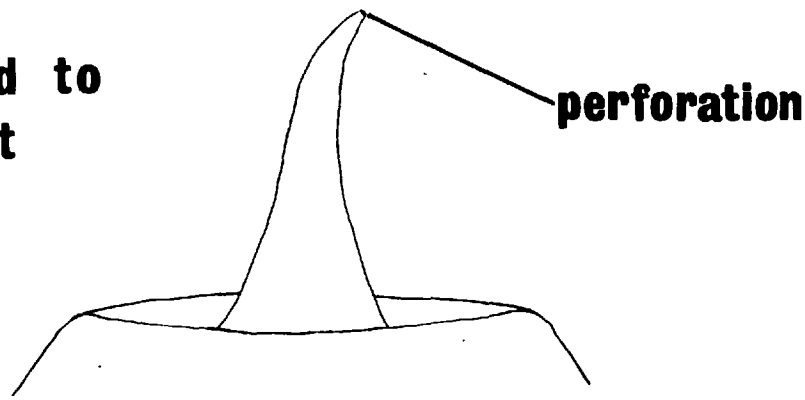
These are elongate, slender plates which lie beneath tergum eight and are concealed by the first gonocoxa (fig. 5.15). They are strongly sclerotized, and the anterodorsal margin is fused to the second gonapophyses while the posteroventral corner is membranously connected to the gonoplac. The second gonocoxae are articulated to the ventral angle of the gonangulum slightly ventral to the mid point to form a fulcrum about which the antagonistic muscles of the second gonocoxa can

Fig 5.14

Sensilla basiconica from the 1st gonapophysis of Macropsis

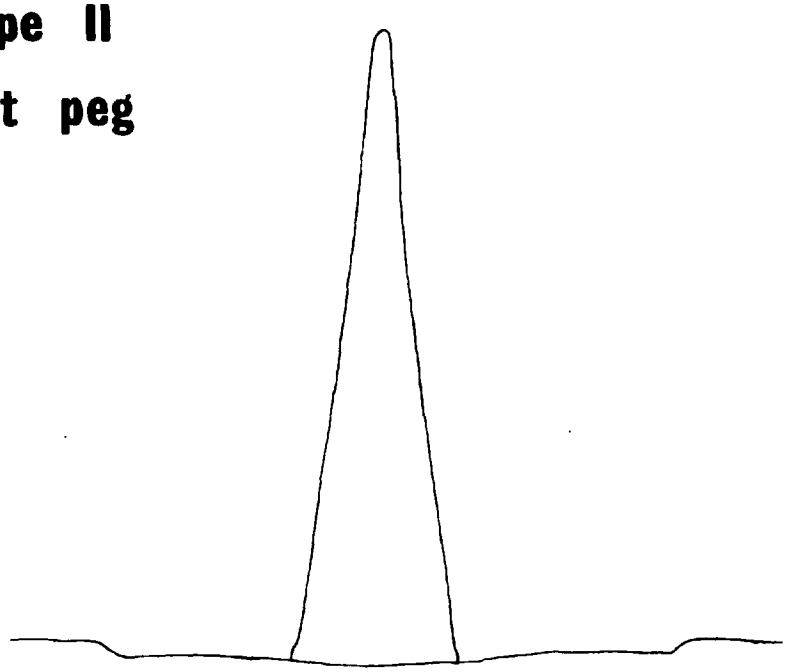
Type I

peg curved to the right



Type II

straight peg



1µm

Fig. 5.15

Basal genitalia of M. scutellata. The anterior part of tergite IX being in the top left of the photomicrograph. Note the sensory pits on the ventral edge of the 2nd. gonocoxa.

X 2000

function (fig. 5.16).

Pores of 3 - 3.5 μm diameter are present on the ventral edge of the second gonocoxa (fig. 5.15), but, unlike those of Ulopa, no internal structure could be resolved and their function is unknown.

Ventral to the fulcral region the outer wall of the second gonocoxa is developed into a very strong ridge, within which are a group of 25 - 28 sensilla basiconica (fig. 5.16). They measure 20 - 24 μm in length, and taper to a fine point. Those of Graphocephala were considerably shorter, 7.5 - 10.0 μm in length, with a more rounded apex (fig. 5.17).

The general shape of the second gonocoxa is more slender than that of Graphocephala, though between the species and Macropsis, which are more comparable in size, there is no real difference in distance between fulcrum and muscle insertions of either groups of antagonistic muscles, so there is little structural evidence of a difference in the efficiency of the two systems.

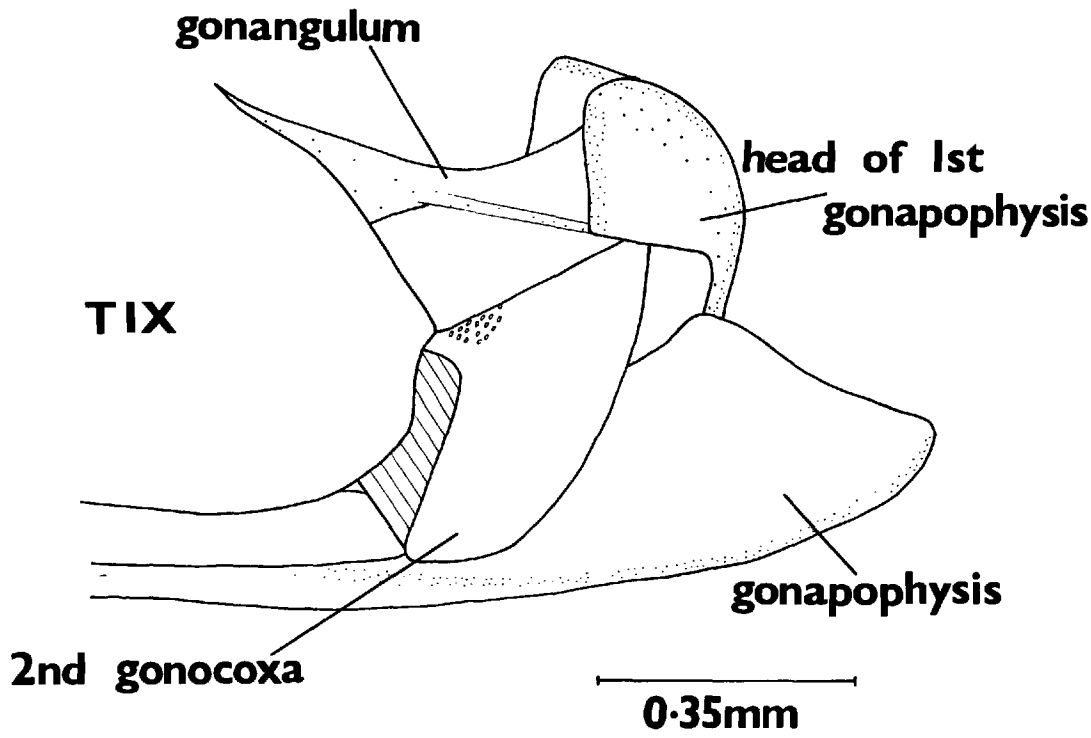
The musculature of the second gonocoxa of Macropsis is similar in all respects to that of Graphocephala.

iii. Second gonapophysis

This lies medial to the first gonapophysis, and its dorsal serrated edge projects beyond that of the first gonapophyses. The inner and outer walls in the distal region are highly sclerotized and darkly pigmented, as is the dorsal edge (fig. 5.18). Throughout the rest of its length the outer wall is composed of slightly corrugated sclerotized cuticle, the ventral region being more corrugated than the dorsal, though there was no evidence of scales, as observed in Ulopa. The proximal two thirds of the inner wall is developed, except in the dorsal 20 - 25 μm and the extreme ventral edge, into a dense system of overlapping scales measuring approximately 9.0 x 5.0 μm and lightly sclerotized. Their anterior border is drawn out into a fringe some 2 μm in

Fig 5.16

Basal genitalia of Macropsis 1st gonocoxa removed



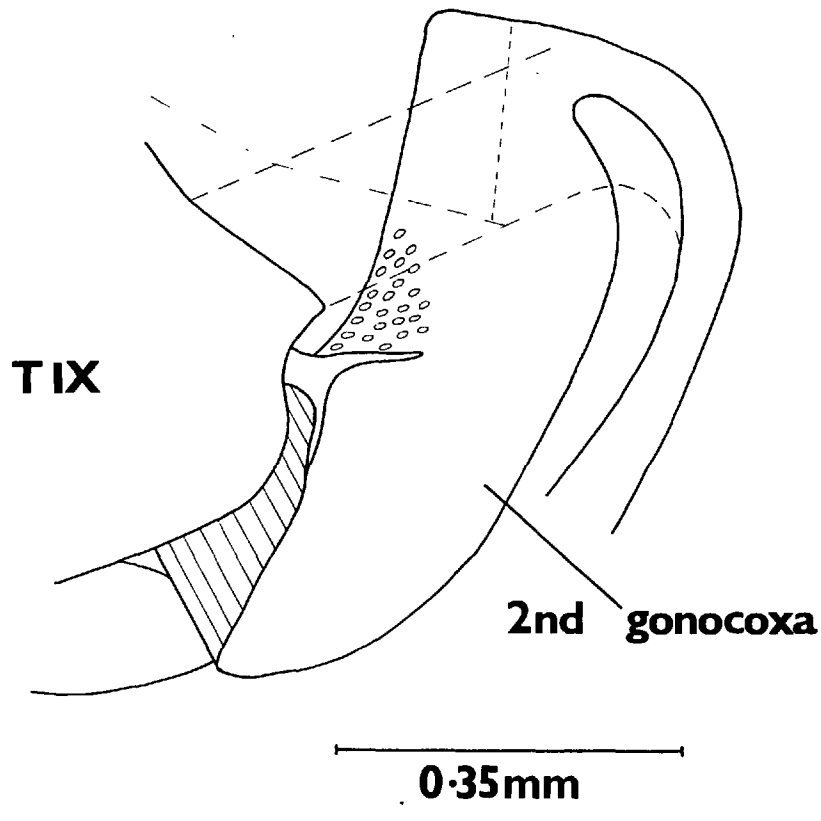
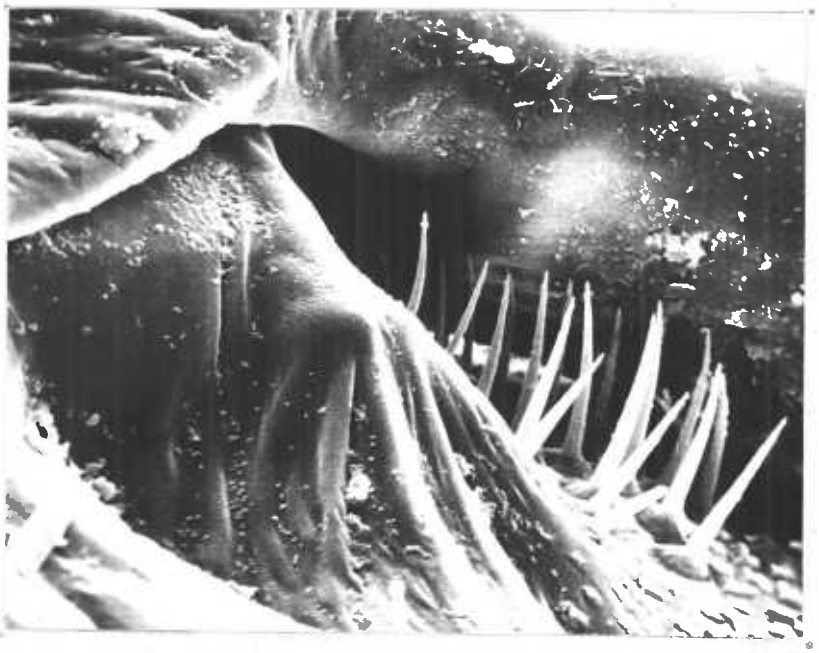


Fig. 5.16

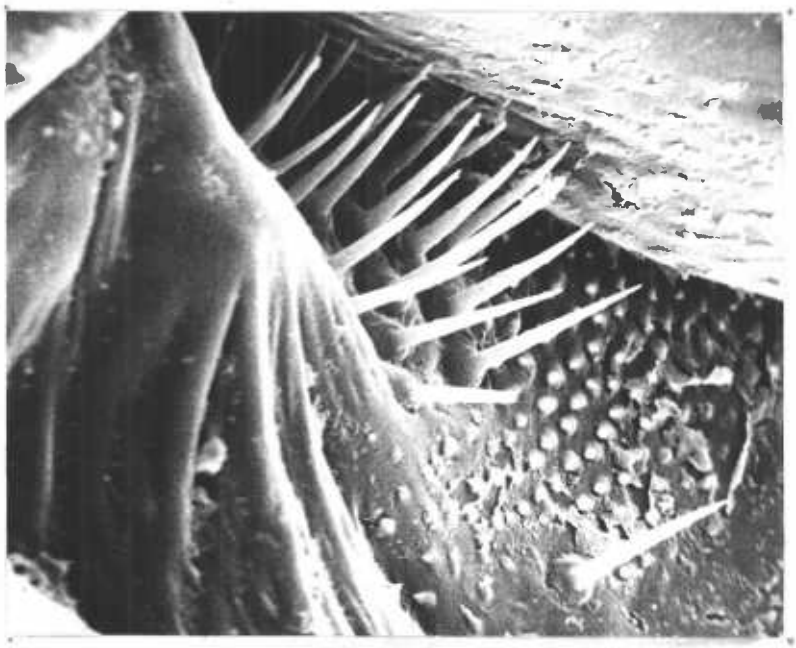
Mechanoreceptors on the 2nd. gonocoxa of M. scutellata

a.



Lateral view of the sensilla x 1200

b.

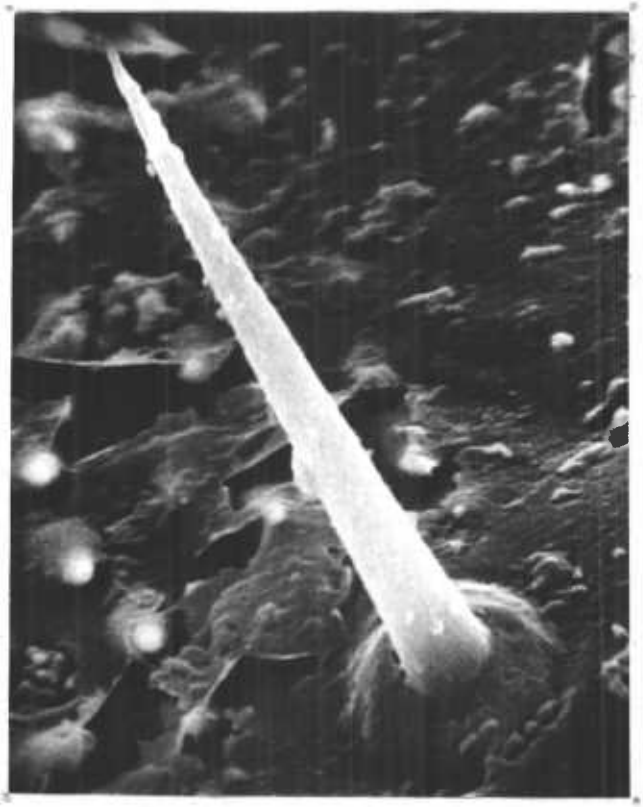


The sensilla are considerably longer than those of Ulopa. x 1400

Fig. 5.17

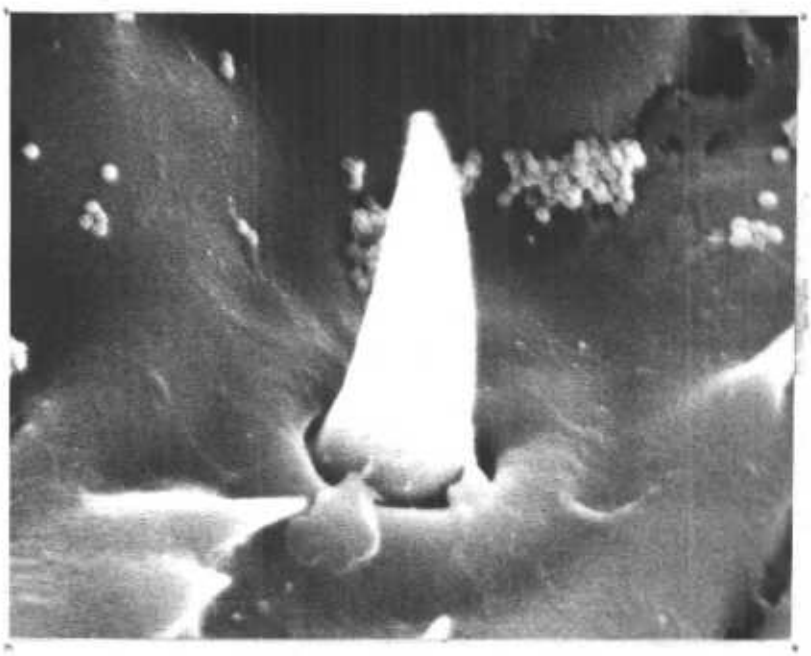
Comparison between the mechanoreceptors of the 2nd. gonocoxa of *Macropsis* and *Graphocephala*.

a.



Single sensillum of *M. scutellata*. x 3300

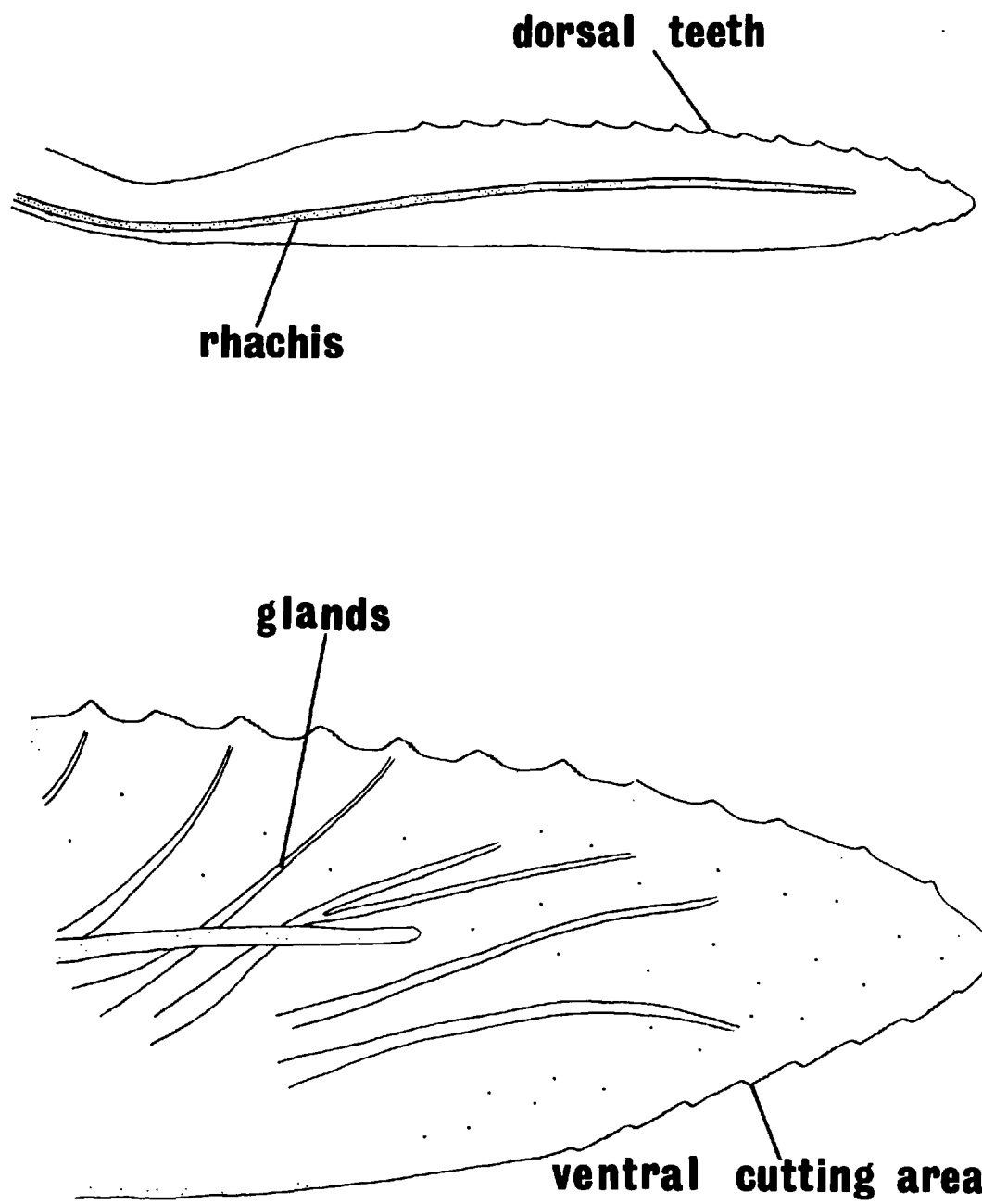
b.



Single sensillum of *G. fennahi*. x 5500

Fig 5.18

2nd gonapophysis of Macropsis



extent. This was present in the other two species and appears to line the egg passage with the fringed border, permitting better control of the egg movement. The dorsal edge, for three quarters the length of the blade from the distal end possesses regular serrated teeth, which provide the main cutting area of the second gonapophyses. The teeth are well developed throughout their range and similar to those of Graphocephala, possessing a short anterior edge, which is not serrated and a long posterior edge (5.18) which is divided into two or three small teeth. This structure suggests that the effective stroke is on the posterior thrust. The teeth are slender structures, differing considerably from the crowned teeth of Ulopa. At the proximal extremity of the dorsal cutting zone, the teeth become more rounded and rapidly give way to a smooth, rounded dorsal edge. The dorsal teeth continue to the distal extremity and a short ventral cutting area is also present in this species, though it is not as elaborate as that seen in Graphocephala. In Macropsis, it consisted of short teeth, similar to, though smaller than, the dorsal ones which extend along the ventral edge for 30 - 40 μm.

In transverse section, the intragonapophyseal spaces are more numerous than in the first gonapophyses, though they are concentrated dorsal and ventral of the interlocking device and in the ventral region of the blade. They consist of large cavities which form a system that runs throughout the length of the blade. Sclerotization is reduced in comparison with the first gonapophyses and those of Ulopa, and is best developed in the dorsal region, where it is associated with the teeth, and on the outer lateral wall around the interlocking mechanism.

On the outer wall approximately one third dorsal of the ventral edge, each second gonapophysis possesses a ventral rhachis, composed of solid, sclerotized cuticle and forming the T-shaped process of the interlocking system. It is essentially similar in all aspects to that described for Graphocephala, except that there is no

change in its angle with the main gonapophyseal wall. It projects from the wall in a slightly dorsal direction, and this permits freedom of movement of the first and second gonapophyses of each side relative to each other.

This interlocking device extends the length of the blade, except in the apical fifth, and is extended anteriorly as a sclerotized basal ramus, which is fused to the anterodorsal corner of the second gonocoxa.

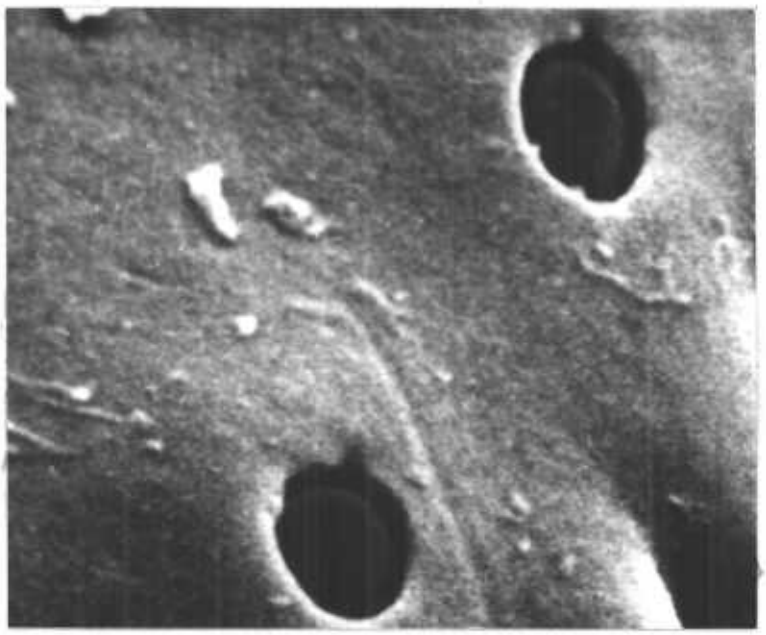
A single type of sensory structure occurs on the outer wall of the second gonapophyses and is associated along the length of the blades with the base of the dorsal teeth. The sensilla are similar to the campaniform sensilla described in Graphocephala, containing a central plug communicated with the rim of the pit via two sclerotized bars (fig. 5.19). The pit is 1.2 μm in diameter with a central plug of approximately 1.0 μm across. These structures, of which there are 18 - 22 per blade, are thought to be mechanoreceptors, which monitor local cuticular stresses. Glands open at the base of the dorsal teeth on the inner wall and in transverse section are essentially similar to those present in the second gonapophyses of Graphocephala. They are multi-cellular dermal glands, each composed of three to five cells, containing large, densely basiphilic nuclei and a granular cytoplasm. No discrete gland reservoir is present, the cells forming a compact group, which leads directly into the enlarged base of the duct which opens at the base of the dorsal teeth. The type of secretion and its function is not known though from the distribution of the glands it may serve as a lubricant to the ovipositor parts or may coat the egg and provide some sort of protection.

iv. Gonangulum

This is a highly developed and strongly sclerotized structure in Macropsis; it is fused over the entire posterior face of the head of the first gonapophysis and also

Fig. 5.19

Mechanoreceptors on the 2nd. gonapophysis of M. scutellata associated with the bases of the dorsal teeth.
Note the two cuticular bars communicating with the central disk.



x 10,000

fused broadly with the anterodorsal edge of tergum nine. It possesses a prominent anterior gonangular ridge, upon which muscle 9 originates, and a well developed articulation with the second gonocoxa. It is broader and more heavily sclerotized than in Ulopa (fig. 5.16), with region 2 highly developed.

v. Linkage mechanisms

Two linkage systems are present in Macropsis, joining the first gonapophyses of opposite sides and the first and second gonapophyses of each side. In addition to this the second gonapophyses are united in their extreme proximal region by membrane which limits their independent movement as in Graphocephala, though not in Ulopa.

The interlocking device between the first and second gonapophyses of each side is the typical tongue and groove mechanism previously described. It runs the length of the two blades to within one fifth of the distance to the distal extremity of the second gonapophysis. It is functional throughout its length and is uniform in structure, except at its extreme distal end. The heavily sclerotized tongue is present on the outer wall of the second gonapophysis, one third dorsal of the ventral edge, and composed of a sclerotized rod, made up of units 18.8 - 19.3 μm in length, essentially similar to that described for Graphocephala and Ulopa. The mechanism is increased in mechanical efficiency by basal intra-gonapophyseal spaces. The corresponding groove is present on the inner wall of the first gonapophyses and the arrangement of the interlocking device is such that the first and second gonapophyses of each side can slide along each other.

The two first gonapophyses are connected ventrally along their proximal two thirds by a modified tongue-and-groove system similar to that described for Ulopa. The tongue is carried on the ventral extension of the right first gonapophyses, and the groove on the left first gonapophyses. The system is supplemented by spines and

sockets and sclerotized to a similar degree as in Graphocephala, which is less than the condition described in Ulopa.

4. Discussion

In the three species examined the gross appearance of the ovipositor and basal parts of the genitalia are similar. The ovipositor is a well developed, sclerotized structure and the blades are lanceolate. Only differences in detail are apparent in the musculature of the three species, except for that of the first gonocoxa in Ulopa, and the major distinctions occurred in the extent and development of the cutting and rasping regions of the gonapophyses and the size and degree of sclerotization of the gonangulum, the sclerite maintained by Scudder (1957) to be of considerable importance in the functioning of the ovipositor and an indicator to the type of ovipositor site.

Considerable differences between the three species were present in the form and extent of the rasping areas present on the first gonapophysis. These were considered by Balduf (1933), Ross & Moore (1957) and Cunningham & Ross (1965) studying Empoasca to be of importance in cutting the oviposition cavity, though the present author was able to demonstrate (p. 284) with electron microscopic and video recording techniques that in Graphocephala the so-called dorsal and ventral rasping areas are regions of lightly sclerotized fringed scales which behavioural observations indicated are primarily of importance in clearing plant debris from the oviposition site and anchoring the ovipositor during egg-laying. They play only a minor role in opening up the oviposition chamber. On the other hand observations on the structure of the dorsal and ventral rasps of the first gonapophyses of Macropsis and Ulopa, in particular those of the latter, suggest that in species ovipositing within hard substrates, these rasps are of considerable importance in the initial cutting of the oviposition cavity. Their structure, combined with the type of linking mechanism between the first and second gonapophyses, provides strong circumstantial evidence

for this. Direct behavioural proof would be very desirable.

The degree of sclerotization of the first gonapophysis in Ulopa exceeds that of the other two species examined and also the species of Empoasca studied by Balduf (1933; 1934) and Helms (1968). The specialization of the dorsal rasp into two areas is not found in Graphocephala or Macropsis. The dorsal rasp is considerably more extensive in Ulopa, extending the whole length of the blade except for the extreme proximal region and the extreme, highly sclerotized, distal tip.

Similarly, the extensive dorsal rasp of Macropsis, composed of angled semi-circular scales projecting from the gonapophyseal surface, indicates a cutting function as opposed to the more passive role as proposed for the dorsal rasp in Graphocephala.

The sensory equipment of the gonapophyses in Ulopa and Macropsis is poorly developed compared to the chemo- and mechanoreceptors of Graphocephala. Only sensilla basiconica are present on the first gonapophyses of the former two species, and these are sunk within pits with only the extreme distal end of the sensory peg projecting. The sensory structures on the ovipositor of Graphocephala suggest a more precise selection of site in which the ovipositor serves an important function.

Considerable variation is displayed between the three species in the cutting surfaces of the second gonapophyses. They are best developed, though most delicate, in Graphocephala and strongest in Ulopa. Three major cutting areas are recognized in the present study on the second gonapophysis of Graphocephala, the principal one being the dorsal teeth, which are large, asymmetrical and serrated. This is supplemented by two ventral cutting areas on the broader distal face. In the other two species the distal extremity is considerably more pointed and better developed for a stabbing action, and a ventral cutting region is present only in Macropsis, where it

is short and similar in structure to the dorsal region.

The powerful dorsal teeth of Ulopa are divided into two types; (a) a distal very heavily sclerotized type similar, though with deeper serrations, to those of Graphocephala, and (b) more proximally the character of the teeth differs from those of the other two species studied, being molariform in appearance with multiple sclerotic crowns, which represent formidable cutting tools.

Associated with the more powerful cutting areas of the ovipositor in Ulopa is the more strongly developed musculature of the first gonocoxa which permits a greater degree of independent movement of the first gonapophysis and which, aided by the simplified interlocking mechanism between the first and second gonapophyses, would seem to allow alternate thrusts of the two pairs of blades, both sets independently achieving an efficient cutting stroke. The rotation of the tongue and groove in the ovipositor of Graphocephala effectively reduces a sliding action between the two blades, so that the ovipositor acts as a single unit, effective during the posterior thrust. The ovipositor of Macropsis displays an intermediate condition, whereby the tongue and groove mechanisms allow the free sliding of the first and second gonapophyses, of each side, over each other but the two first gonapophyses are connected via a modified tongue and groove linkage. As in Graphocephala, the two second gonapophyses are membranously connected in their proximal region, so restricting their independent movement. The two first gonapophyses therefore act as a single unit and the two second gonapophyses as a unit within the first pair. The maximum freedom of movement of the parts, seen in Ulopa, suggests that in this species the preparation of the oviposition site is brought about by the sawing action of the gonapophyses while posterior thrusts of the whole abdomen, as seen in Graphocephala, are of less importance. Satisfactory behavioural evidence is needed in support of this.

The present work extends Scudders's (1959) suggestion that the size and degree of sclerotization of the gonangulum can be used as an indicator of the type of oviposition substrate to lower taxonomic levels. In Cicadellidae, however, the cutting areas of the second gonapophyses and the characteristics of the rasping areas of the first gonapophyses provide more information on the oviposition behaviour of the species. The range of oviposition sites indicated by the above three species suggests considerable variability in ovipositor structure, which may be of taxonomic importance (Balduf 1934; Cunningham & Ross 1965) and of use in future studies of reproductive habits in the field.

CHAPTER 6

SEXUAL BEHAVIOUR OF GRAPHOCEPHALA FENNAHI

The sexual behaviour of Cicadellids is a field which has received little attention in recent years and morphological studies of the ovipositor and genital region have not been combined with a more functional approach. New and alternative methods for the control of leaf-hoppers are now required, and while suppression of their reproduction may be a rewarding area for such research the basic knowledge of their sexual behaviour, a prerequisite for such work, is lacking. The development of alternative control methods to replace or supplement conventional insecticides and pest management requires detailed, basic research in many entomological disciplines, including histology and insect behaviour.

Work in this field has largely been concerned with oviposition behaviour though Carlson (1967) studied mating of Empoasca fabae, recording age at sexual maturity and the frequency of mating. Selective oviposition has been observed by direct egg counts in E. fabae (Carlson & Hibbs 1962). Descriptions of oviposition have been given for the Membracids (Funkhouser 1917), Typhlocybids (Readio 1922), Draeculacephala (Balduf 1933), the bramble leaf-hoppers more recently by Raine (1960) and for Circulifer tenellus (Maramorosch 1974). The accounts are, however, scattered and often superficial, so that there is a need for a detailed study of sexual behaviour to resolve what appears to be a complex sequence. Raine (1960) working with E. fabae suggested that behaviour at oviposition followed a well defined order. If this is true, any disturbance that disrupts the pattern may provide an insight into host selectivity and the factors necessary to elicit the initiation and release of oviposition. Reference was made to the occurrence of pre-copulatory behaviour in the beet leaf-hopper by Severin (1919) and in the rice Delphacid Sogata orizicola by McMillian (1960) but no detailed descriptions were given.

The aim of the present study is to provide detailed descriptions of sexual behaviour in Graphocephala fennahi and to investigate some physical factors that may influence mating and oviposition. Thorough descriptions of this behaviour in Cicadellids do not occur in the literature. It is felt by the present author that a knowledge of the physical and biological factors that affect sexual behaviour could be of use in control techniques and findings from this study may be applicable to other, more economically important Cicadellid pests.

(a) Behaviour prior to mating

1. The courtship dance

During the two summer seasons when this work was undertaken some three to four hundred courtship displays were observed in the field. A further one hundred observations, approximately, were made under laboratory conditions during which displays were recorded on video equipment, the details of the display then being determined by an examination of the tape.

The above observations indicated that the courtship dance of G. fennahi was a stereotyped sequence, which always preceded and was a prerequisite for copulation. As described by Perkes (1970) in E. fabae, courtship is initiated by the male, the female making no attempt to orientate towards him. Courtship occurs on the upper surface of the Rhododendron leaf, and, except for abdominal vibrations, as described below, the female maintains her normal behaviour of remaining stationary or spontaneously moving away a short distance, until physical contact is established by the male. During courtship the stylets of both the male and female are retracted from the plant.

The first visual sign of pre-mating behaviour occurred when a sexually active male landed on the same leaf as a female or approached to within 8 cms, (the maximum

distance, more usually an initial reaction occurs when the male approaches within 4-6 cms). The first response, by the male, after orientating towards the female, was a rapid fluttering of the fore-wings while remaining stationary. This occurred in bursts of 1.7 ± 0.4 seconds separated by 2.8 ± 0.8 seconds of inactivity. This behaviour lasted 15 ± 3.5 seconds, i.e. approximately three bursts of wing fluttering. The duration of these activities was determined from 19 observations recorded on video film. The male then exhibited rhythmic up and down movements of its entire abdomen at a frequency of approximately 3 per second. If the female was receptive she responded by a slow dorsoventral movement of the abdomen, one complete pulse every 2 seconds $\pm$ 0.25 seconds, at the same time depressing sternite seven and unsheathing the distal ends of the gonapophyses. The female still made no attempt to orientate towards the male. The next sequence in the male's display consisted of rapid darting runs to within 0.7 ± 0.4 cms of the female, simultaneously fluttering the fore-wings. The male then moved away for a distance of 3 - 5 cms, and at the end of each dart the male displayed abdominal bobbing, as described above. No physical contact was made between the individuals during this period, which, over 50 timed observations, both in the field and laboratory, averaged 2.84 minutes with a maximum of seven minutes. Only if the female tolerated this part of the courtship did the male display continue. (Table 6.1)

Immediately prior to mating, the male placed one of his front tarsi on the back of the female, immediately posterior to the scutellum in the mid-line. It is not clear whether this action has a role in sexual communication. Except for slow abdominal movements, receptive females play no visual part in active courtship. If courtship of an unreceptive female begins she responds, during the male darts, either by leaving the leaf or by striking the male with her wings and/or legs.

Table 6.1 To determine the length of a courtship dance in G. fennahi from the on-set of male wing fluttering to coupling. Sample size 25 pairs.

Pair Number	Time taken (mins.)	Pair Number	Time taken (mins.)
1	4 mins 39 secs	14	3 mins 57 secs
2	7 mins 10 secs	15	5 mins 12 secs
3	3 mins 57 secs	16	6 mins 23 secs
4	3 mins 40 secs	17	5 mins 17 secs
5	5 mins 15 secs	18	3 mins 59 secs
6	6 mins 10 secs	19	7 mins 11 secs
7	6 mins 23 secs	20	3 mins 14 secs
8	5 mins 50 secs	21	6 mins 31 secs
9	3 mins 05 secs	22	5 mins 49 secs
10	7 mins 15 secs	23	6 mins 17 secs
11	4 mins 37 secs	24	4 mins 12 secs
12	4 mins 16 secs	25	4 mins 23 secs
13	5 mins 00 secs		

Average length of the male courtship dance was 5 mins 01 secs.

2. Sexual Communication

From the above it appears likely that there is some form of communication between sexually active males and females. Though the observer could detect no signals, the female presumably communicated her presence and perhaps sexual state to a prospective mate. It is possible that the maintenance of a stationary posture itself may be regarded as a response to the presence of a courting male. However, this is considered normal behaviour and the occasional apparently spontaneous movements by the female suggests that it was not, in itself, a response.

Many workers have been interested in the field of insect communication, some concentrating on the role of chemicals and their possible use in pest control. Wright (1964) considered insect sex pheromones as amongst the most biologically active substances, minute quantities eliciting a behavioural response (Shorey 1964; Guerra 1968; Tashiro & Chambers 1968; Oloumi-Sadeghi 1974; Ritter et al 1975). Several reviews of insect sex pheromones are available in the literature, (Jacobson 1972 ; Leonard & Ehrman 1974; Wheeler 1976; Baker & Evans 1977). Little work has been done in the Auchenorryncha, though the reactions in Aphids has been studied, (Pettersson 1971; Tamaki et al 1970; Ishiwatari 1976; Brennan et al 1977). It is known that in some female Homoptera, i.e. sexually mature virgin females of Aonidiella aurantii the California red scale, that a sex pheromone is continually present which may be released or withheld. The female, however, becomes unattractive within 24 hours after insemination (Tashiro & Moffitt 1968).

A detailed investigation of the possible presence of a sex pheromone in Graphocephala was not attempted. As a point of interest, however, during a general scanning electron microscope examination of adults, sensilla coeloconica of the corrugated fence type were located on tergum nine. It is the first time such sensilla have been located on the genital region of Homoptera, and from work carried

out in other groups where these sensilla were found on the antennae, (Callahan & Lee 1974; Chu-Wang et al 1974; McIver & Siemick 1975), they have been associated with the detection of pheromones. (This work was largely performed on Lepidoptera and Diptera). Their presence in the Homoptera may warrant further pheromone work within this group. Perkes (1970) conducted simple squash experiments and attempted, using various solvents, to extract sex pheromones from virgin females of E. fabae, but he was unable to elicit sexual activity in males as a response to the extracts.

Similar, simple experiments were conducted using Graphocephala fennahi Males did not orientate to, or attempt to court filter paper discs onto which virgin females had been squashed. Female extracts made up in alcohol, acetone, benzene, chloroform, methonol and xylene applied to filter paper discs and allowed to dry also failed to elicit male sexual behaviour.

Several other forms of communication are possible.

(a) Vision. A preliminary experiment was devised at the end of the 1977 season to determine the possible role of vision in sexual encounters. Only three virgin pairs were available, (age 14 ± 1 week old), of which one was used as a control. Previous observations indicated that in a sexually active pair the male approached the female; in the present experiment the eyes of the male were occluded using a black photographic paint, so effectively blinding the male. The paint was allowed to dry for one hour prior to the introduction of the pair.

Pairs were individually housed in cylindrical cages at 22°C and observed for 1.5 hours. In the control pair the eyes of the male were not painted out, and here courtship was initiated within 8 minutes of the introduction, and was terminated by coupling. In the experimental pairs, both males showed an initial increase in

leaping frequency compared to normal insects. Male A continued this behaviour for 56 minutes and male B for 42 minutes. In both cases it was followed by a short period of inactivity of less than four minutes. Both males then began walking over the Rhododendron leaf, male A reached the female after a further 2.74 minutes and initiated courtship, and male B after 3.5 minutes.

This experiment proved unsatisfactory, due to the small sample size and apparent distressed behaviour displayed by the males. A further experiment was conducted into the role of vision during May 1978, when 14 virgin pairs of 3 weeks $\pm$ 4 day old insects were assembled.

A female was penned, in a paper cell measuring 1.5 x 1.0 cms, and 0.7 cms deep, onto the bottom most leaf of a Rhododendron branch 1 metre $\pm$ 5 cms long. A male was then introduced to the upper most leaf and observed for one hour. The linear distance between the pair was 94 cms $\pm$ 6 cms. This was repeated for 13 pairs. Pair 14 was a control in which the female was placed freely on the lower most leaf and not visually hidden. This experiment was conducted at 22°C. Results are given in Table 6.2.

It is demonstrated that the male approaches the female without visual cues and the time taken to reach the female in the control is the same as that in the experimental pairs, suggesting that a stimulus other than vision is of importance in bringing a sexually active pair together. It was also observed in the field by the author that males would court other males or fifth instar nymphs which had settled in the vicinity, thus reinforcing the suggestion of the male's inability to distinguish visually males or large nymphs from females of the same species.

General observations both in the field and laboratory indicated that it was unusual for male G. fennahi to walk such large distances displaying no inclination to leap. During the control experiment above, the female was on the upper surface

Table 6.2

Time taken for a male G. fennahi to locate a visually hidden female from a distance of 94 cms $\pm$ 6 cms on Rhododendron.

Pair no.	Time taken (minutes)
1	25.4
2	18.7
3	23.8
4	31.2
5	40.5
6	26.3
7	19.2
8	25.7
9	23.2
10	21.8
11	29.1
12	27.3
13	26.9
Control	23.3

Average of experimental pair 26.1

of the leaf and remained stationary with no indication of feeding throughout the experiment.

(b) Acoustic Stimuli. It is well documented that sounds can be produced by some Auchenorryncha. Ossiannilsson (1949) gave preliminary results of sound production in a wide range of adult leaf-hoppers and was able to divide them into stress, sexual and social calls. He demonstrated the existence in all families studied of a tymbal mechanism similar to that responsible for sound production in the related cicadas (Pringle 1954). The known songs of Cicadellids are all of a much lower intensity than those of cicadas. Strübing (1958 and 1959) established sound production in Delphacids and in 1962 studied the songs of 14 species of Delphacids using tape recordings and oscillograms. Moore (1961) produced sound spectrograms for a number of North American species. There would appear to be little doubt that most Auchenorrhyncha are capable of definite songs by means of the tymbal apparatus and most species studied appear to possess a repertoire of song patterns that may serve different functions. More recently, Claridge & Howse (1968) demonstrated vocalization in three species of British Oncopsis and a potential receptor in the form of Johnston's organ at the base of the antennal flagellum of Oncopsis flavicollis (Howse & Claridge 1970). Most Cicadellid songs appear to be differentiated in two ways, by the pulse repetition frequency and the carrier frequency. It is the first that is often a specific character and is the parameter to which tympanal organs, subgenual organs and antennal receptors appear to respond in insects (Haskell 1961 ; Howse 1967). Most studies agree that the pulse repetition frequency for Cicadellids lies between 30 - 1000 cycles per second.

Preliminary sound experiments were conducted using G. fennahi. Virgin pairs were enclosed in standard 25 mm diameter boiling tubes containing a small

amount of food material, the mouth was closed by the sensitive end of a crystal microphone. The output from the microphone was fed into a Revox 77A recorder and Kay Elemetric's Corporation Sona-Graph 6061 B and monitored over a frequency range of 20 - 1500 cycles/second for three periods, at various times of the day, each of 4 hours duration. No calls were detected except for very infrequent clicks, the source of which was not convincingly demonstrated, and which were not thought to be communicatory. In view of other studies, as outlined above, the frequency range selected was thought to be satisfactory.

A scanning electron microscopic examination confirmed the presence of a tymbal apparatus on the first abdominal segment of both sexes, though much reduced in the female. The tymbal musculature, as revealed in transverse sections, was composed of very slender muscles. As no sound was detected between sexually active pairs, it is suggested that the tymbal apparatus is non-functional in G. fennahi.

(c) Substrate borne vibrations. Such a system would confer a number of advantages over airborne sound waves in that :

(i) attenuation in a solid substrate i.e. leaf or woody stem, is less than in air, so permitting communication over greater distances. Ossiannilsson (1949), commenting upon air-borne sound waves produced by Auchenorryncha, suggested that if they were to be heard at distances of more than a few centimetres, a very sensitive auditory system would be necessary. The transmission would obey a modified inverse-square law, whereby the intensity from a point source is inversely proportional to the square of the distance from that source corrected by the attenuation factor for the substrate.

An important difference between cicadas and leaf-hoppers is that there are no well developed tymbal air sacs in the latter. Pringle (1954) was able to demonstrate

that the air sacs of cicadas possess a resonant frequency approximating to the carrier frequencies of the song (4 - 8 kilocycles/second). If a similar mechanism was to operate in leaf-hoppers tymbal air sacs of appropriate dimensions would have extremely high resonant frequencies. No tympanic membrane is present in the cicada Tettigarcta tomentosa (Pringle 1957), where chordotonal organs are present at the base of the abdomen. Other chordotonal organs have been found at the tibio-tarsal joint, the wing base (Fudalewicz 1949) and in the antenna (Johnston's organ, Roth 1948; Howse & Claridge 1970). While the cicada typanum is tuned to the carrier frequency of the song by the involvement of resonant air sacs that it shares with the tymbal (Pringle 1954) it appears likely that the leaf-hopper receptor is tuned to the modulation frequency of the song, which may be detected by chordotonal organs, perhaps situated on the leg (Ichikawa 1977).

(ii) The second advantage of substrate transmission compared to air is its relative speed. The speed of sound at room temperature in air is 331.3 ms^{-1}. No comparable figures are available for Rhododendron leaves and stems, but the transmission rate in Oak, along the fibre, is 3850 ms^{-1}.

(iii) A substrate such as a plant would serve to channel signals rather than radiate them as in air.

More workers are now becoming interested in substrate-borne vibratory stimuli among insects (Busnel 1956; Rupprecht 1974; Ichikawa et al 1975; Ichikawa 1976) and more work is being conducted to investigate vibration-sensitive organs (Alexander 1967). Orchard (1975) investigated the structure and properties of abdominal chordotonal organs in Carausius morosus and Blaberus discoidalis, and their role as vibratory receptors and provided a general review of abdominal chordotonal organs of insects generally. Rupprecht (1975) indicated that Sialis

possesses vibratory receptors on the legs, while Dambach (1976) describes subgenual organs on the legs of Gryllids. Wigglesworth (1972) reviews the older work.

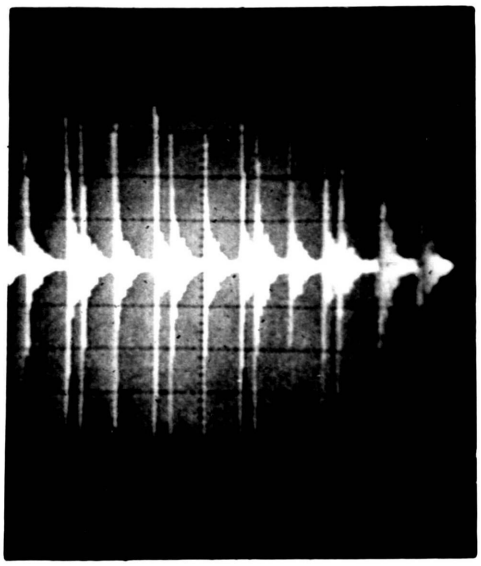
The location and structure of any such organs were not included in this study though experiments were performed to determine whether substrate-borne vibrations may be used for communication by Graphocephala.

A system was devised whereby vibrations produced in a Rhododendron leaf by G. fennahi could be monitored. This requires the construction of a very sensitive system. The final design consisted of a piezo-resistive strain gauge, acting as a pick-up system, and mounted on a perspex block clamped to the upper surface of the leaf. The output was connected, via a small-gauge coaxial cable, to a Telequipment storage oscilloscope DM 64. Careful and complete earthing of the apparatus into the oscilloscope considerably reduced background signal noise. Due to the sensitivity of the apparatus the experiments were conducted on a solid marble balance slab, thus eliminating background vibrations. The leaf clamped at the petiole, together with the pick-up, were enclosed within a clear plastic (dexroid) cage to eliminate air vibrations and the escape of the insects. This produced a control trace on the oscilloscope of very small amplitude and permitted a noise to signal ratio of approximately 1 : 20. Insects were introduced onto the leaf via a window in the side of the cage which was then closed.

The results are presented as oscilloscope traces (fig. 6.1 and 6.2). The equipment was monitored for possible vibrations during four conditions as follows :- (a) Solitary virgin female (b) Solitary virgin male (c) Virgin pair (d) a pair, of which the female had already mated. In all cases, insects were sexually mature. In all cases the trials were repeated ten times with different individuals or pairs, and each trial continued for one hour or until a signal was monitored.

Fig 6.1

Oscilloscope traces of substrate-borne vibrations made by the female of *G. fennahi* when calling to males prior to courtship.



Scale: 50mV/div. & 50msec/div.

Fig 6.2

Oscilloscope traces of substrate-borne vibrations made by the female of *G. fennahi* during courtship.

Scale: 50mV/div. & 50msec/div.

Scale: 50mV/div. & 20msec/div.

No vibrations were detected in (b), suggesting the male does not produce substrate-borne vibrations. This contrasts with the findings of Ichikawa (1977), working on three species of Delphacids, who detected male-produced signals. In (a) a solitary virgin female, a vibratory pattern was detected (see fig. 6.1). During monitoring, the female was on the upper surface of the leaf, over the main rib; her stylets were not in contact with the leaf surface. The signal was produced in irregular bursts of 4 ± 1.6 seconds duration at intervals of 8.7 ± 2.9 seconds at a frequency of 30 cycles/second. Each pulse possessed a characteristic high peak and rapid attenuation and a pattern of two rapid pulses followed by a single pulse of smaller amplitude was established. The amplitudes given in this study are of no direct quantitative significance, as they are a product of calibration. However, they are comparative within the study. The rapid pulses had an amplitude of 160 mV $\pm$ 6.5 while the single pulse was of 150 mV $\pm$ 5.0. The characteristics of these pulses are likely to aid transmission and reduce attenuation.

The pattern characteristics changed when a virgin pair were in close proximity (see fig. 6.2). The sequence then consisted of three individual peaks followed by a wide signal band of 80 - 100 m. sec. duration. The peak characters differ from those produced by a solitary female, producing an amplitude of 200 mV $\pm$ 10 and gradual attenuation. The wide signal band possessed an initial peak of 100 mV $\pm$ 5.0 amplitude, which rose to the main peak of 150 mV $\pm$ 7 amplitude. The frequency of this sequence was 20 cycles/second, and the characteristics suggest short distance transmission. In G. fennahi, vibrations were produced by drumming of one or other hind tibia. No signals were detected from mated females.

Little direct evidence for substrate-borne vibrations in the Hemiptera has previously been cited in the literature. Ichikawa (1977), working on three species

of Delphacids was able to demonstrate that in this superfamily substrate-borne vibrations are emitted by the male and female produced by tymbal organs and abdominal vibrations respectively. The possibility of substrate-borne vibrations in the Cicadellids has previously been suggested by Claridge & Howse (1968), Takeda (1974), Ishii & Ichikawa (1975), and Ichikawa (1976). The present study clearly suggests the function of such a system in the communication of G. fennahi with song characters when a female is calling to prospective mates, distinct from that used during courtship. This work allowed females to be put into three groups, (a) unreceptive virgin females, within a few days of emergence, which failed to display drumming behaviour, (b) receptive females which produced a signal in the presence or absence of a sexual partner, (c) unreceptive mated females which did not show calling behaviour and which avoided male attention by actively moving from the area. The behavioural changes associated with the cessation of calling by mated females appears to coincide with insemination. Females that were not inseminated during mating resumed calling behaviour within 24 hours (data based on five observations), the spermatheca being examined at the end of the experiment. It is concluded that in Graphocephala vibrations produced by the female produce a searching response and the initiation of courtship by the male. Maintenance of courtship is probably brought about in the male by the female vibratory stimulus, and in the female by the visual stimulus of a courting male.

The natural frequency of live Rhododendron leaves, over the whole size range was then calculated to determine the resonant frequency, which, if it coincided with the frequency produced by the insect, would facilitate transmission.

The natural frequency of a leaf depends upon its stiffness and mass distribution, thus water content, though having no direct effect on sound transmission, would affect the mass distribution.

The equation to determine natural frequency is :

$$f = \frac{1}{2\pi}\sqrt{\frac{Kg}{m}}$$

where f = frequency

π = 3.142

K = stiffness of the leaf or the slope of a load (Newtons)/deflection (metres) curve

g = acceleration due to gravity (9.81 m/sec^2)

m = mass of the leaf (Kg)

Slope/deflection graphs for a large and small leaf are produced (fig. 6.3). Substitution into the above formula gave the results :

Natural frequency of a large leaf = 25.6 cycles/second.

Natural frequency of a small leaf = 48.7 cycles/second.

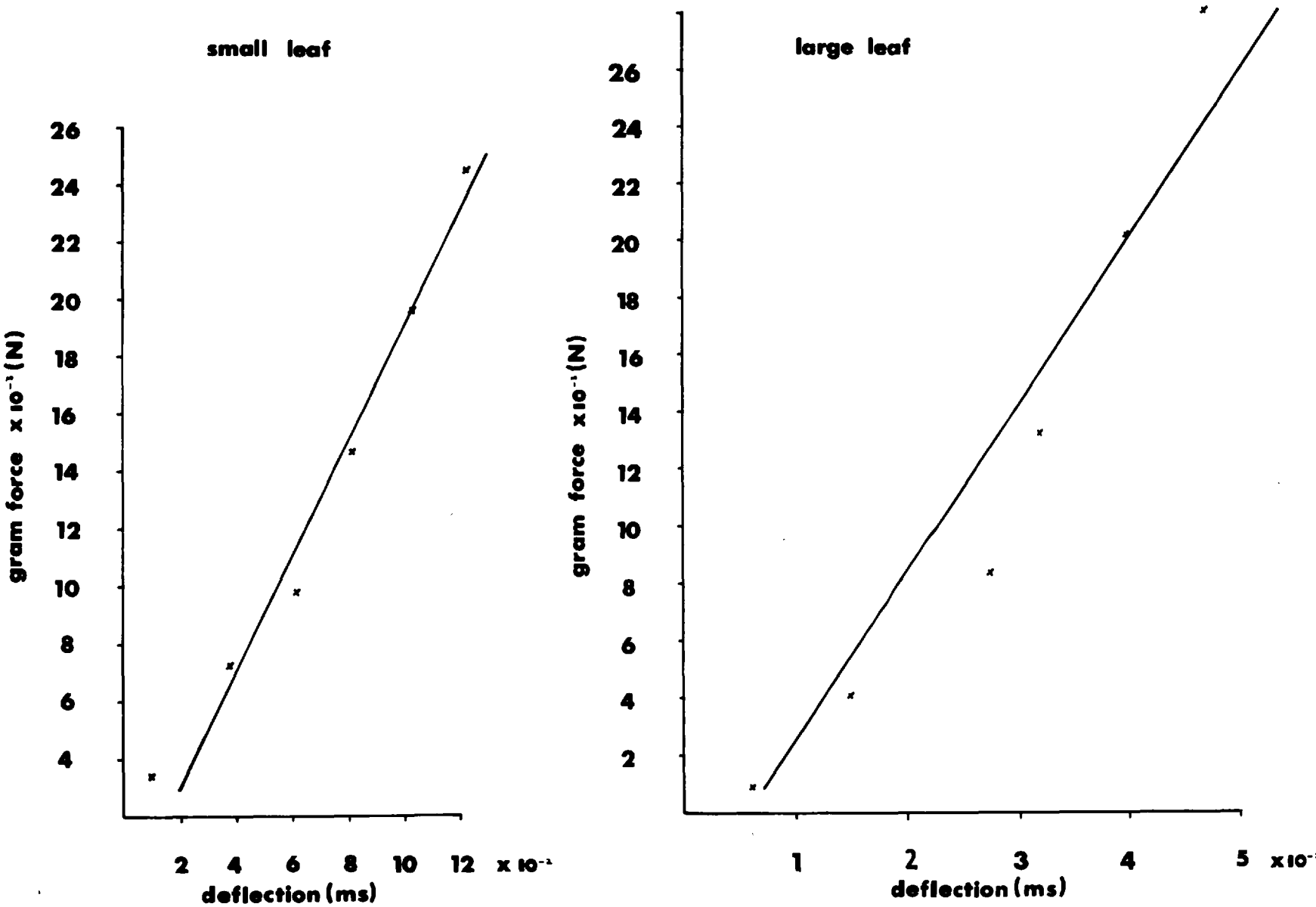
These results approximate to those experimentally obtained for the insect vibrations, suggesting transmission would result and that a potential communication system could be established.

Discussion on sexual communication

The emission of sounds during courtship has been well documented in the Auchenorryncha other than Cicadidae (Claridge & Reynolds 1973; Ossiannilsson 1953; Smith 1971 and Strübing 1962), though sound waves were not recorded from G. fennahi in the present study. Moore (1961) assumed auditory stimuli to be close-range signalling devices, suggesting that olfaction or visual stimuli play a prime role in intraspecific communication. The emission of a sex pheromone by males has been reported in Nezara viridula (Mitchell & Mau 1971). Though the emission of sex

Fig 6.3

Weight/Deflection graphs for Rhododendron leaves



pheromones has been reported in other Heteroptera (Scales 1968; Baldwin et al 1971) and Homoptera Sternorrhyncha (March 1975) it has not been proposed in Auchenorryncha. Visual and olfactory factors have been excluded from the possible sign stimuli involved in the sexual behaviour of G. fennahi in the present study, and similarly were dismissed by Ichikawa (1977) in Delphacids. Claridge and Reynolds (1973) observed males of Oncopsis courting other males, suggesting a limitation of visual ability in that genus.

Ossiannilsson (1949) suggested very limited transmission distances for air-borne sounds, and Ichikawa (1976) demonstrated that Callygypona lugubrina, C. adela and Euidella speciosa never responded in mating behaviour when their songs were played as air-borne stimuli, even over 1 or 2 cms, but only when they were produced as substrate-borne vibrations. It has been shown in the present study that vibrations can be perceived by the male of G. fennahi at distances of at least 95 cms. Species specific substrate vibrations are also known in two species of Cydnidae (Gogala et al 1974) and Gerridae (Wilcox 1972).

3. Physical factors affecting pre-mating behaviour

(i) The effect of time of day

Preliminary experiments were conducted during June, July and August 1976, on two captive groups, each of twenty pairs. The groups were each sub-divided into four groups, each of five pairs, sub-groups being individually housed in a large cylindrical cage. This allowed more accurate and quicker observations. Group I were maintained in a constant temperature room on a light/dark regime of 16 hrs/8 hrs and temperature range 21 - 25°C. This regime had been maintained since early fourth instar. Group II were kept on the roof of the Zoology department under natural conditions of light and temperature. (Minimum night temperature of 17°C; day time maximum of 29°C). Insects were observed over an 8-day period. Records of the

presence or absence of sexual behaviour, either courtship or mating, were made for 5 minutes each hour, on the hour (see Table 6.3). The results suggest sexual activity in G. fennahi occupy a morning and evening period.

Table 6.3

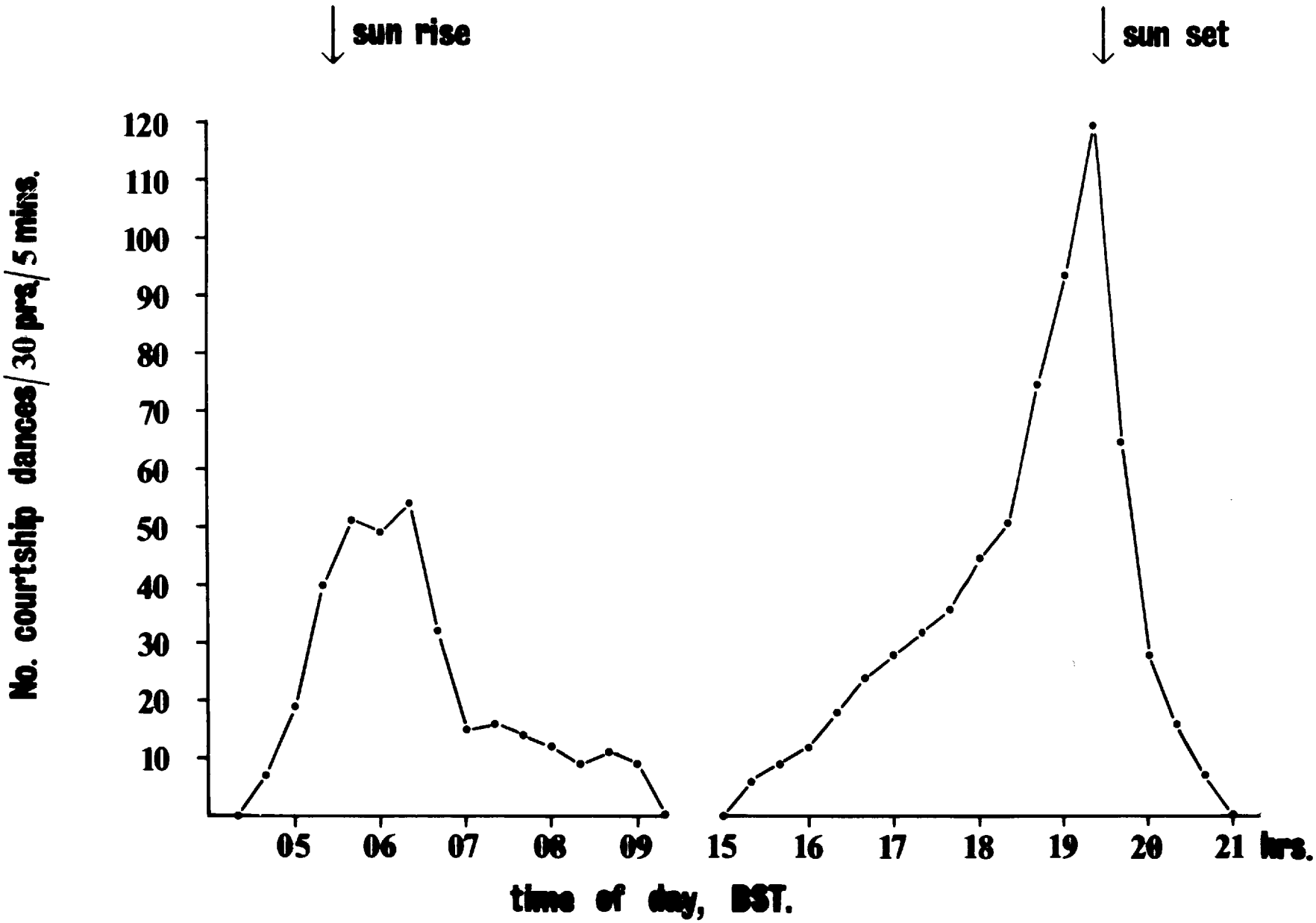
Periodicity of sexual activity as determined by the presence of courtship dances in G. fennahi. The experiment was conducted with two groups, each of 20 pairs of insects. Group I in a constant temperature room, Group II caged under natural environmental conditions. x denotes the presence of activity.

Date	Time of day (hours)																		
			sun↓rise															sun↓set	
	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
August 10th (Group 1)			x	x	x	x	x						x	x	x	x	x	x	
12th (Group 2)				x	x	x	x							x	x	x	x	x	x
14th (Group 1)				x	x	x	x	x						x	x	x	x	x	x
16th (Group 2)			x	x	x	x	x	x						x	x	x	x	x	
18th (Group 1)			x	x	x	x	x						x	x	x	x	x	x	x
20th (Group 2)			x	x	x	x	x	x						x	x	x	x	x	x
22nd (Group 1)				x	x	x	x							x	x	x	x	x	x
24th (Group 2)			x	x	x	x	x							x	x	x	x	x	x

This prompted a 3-day experiment, conducted at the end of August 1977, to determine more precisely the limits and characters of the bimodal distribution of sexual activity. 30 pairs of 14-day old virgins were assembled and housed in a constant temperature room as above, 10 pairs were placed in each cage, 3 cages in total. The total number of dances (forward darts) performed in a 5 minute period were recorded every 20 minutes (see fig. 6.4).

Daily distribution of sexual activity in G. fennahi

Fig 6.4



The graph demonstrates a strongly bimodal distribution of sexual behaviour in the males of G. fennahi with peaks centred around sunrise and sunset; no activity was recorded during the middle part of the day or the hours of darkness. Mating occurred throughout the active periods and was only observed once at other times of the day. The evening peak rose to a greater maximum than that of the morning, and morning activity rises rapidly to its peak and falls away gradually while the evening activity gradually approaches its peak and falls rapidly away after sunset.

During a total of 40 days field observations a similar distribution for sexual activity was not always found to be present, though when present sexual activity always occurred during the periods demonstrated above, suggesting that a minimum temperature threshold may operate. The time of maximum activity suggests that the effect may be related to light intensity. Normal activities of leaf-hoppers, such as feeding and resting, occur on the underside of the leaf, and only comparatively rarely does the insect appear on the upper surface, where its red, black and yellow markings would make it conspicuous. The dawn and dusk appearance of activities on the upper surface of the leaf may be a mechanism to reduce predation. Tay (1972) while studying the bionomics of Graphocephala recorded its main predators to be the spider Linyphia triangularis, the earwig Forficula auricularia, the harvestmen Oligiophus agrestris, and Liobunum rotundum, the Mirid Dicyphus sp. together with sparrows, Great tits and Blue tits.

(ii) The effect of temperature

As shown above, the occurrence of male courtship dances had a bimodal distribution under normal laboratory conditions while in the field, when the weather was particularly cold, this was not found to be the case (Table 6.4).

Table 6.4

The effect of temperature on sexual activity and the suppression of its bimodal distribution by subliminal temperatures in the field. No activity recorded between 10.00 and 15.00 hours. Sample of 20 pairs in each group. + denotes activity.

	Laboratory		Field			Laboratory		Field	
Time	Temp°C	Sex Activity	Temp°C	Sex Activity	Time	Temp°C	Sex Activity	Temp°C	Sex Activity
04.00	21.0	-	10.0	-	15.00	27.0	-	24.5	-
04.30	23.0	+	10.0	-	15.30	26.5	+	24.0	-
05.00	23.0	+	10.5	-	16.00	26.5	+	24.0	+
05.30	23.5	+	10.0	-	16.30	25.5	+	22.0	+
06.00	23.5	+	10.5	-	17.00	25.5	+	22.0	+
06.30	23.5	+	10.0	-	17.30	25.5	+	21.0	+
07.00	23.5	+	11.0	-	18.00	24.5	+	20.0	+
07.30	23.5	+	11.0	-	18.30	24.5	+	19.5	+
08.00	24.0	+	11.0	-	19.00	24.5	+	18.0	+
08.30	24.5	+	11.5	-	19.30	24.5	+	17.0	+
09.00	25.0	+	13.5	-	20.00	23.0	+	15.5	+
09.30	24.5	+	14.0	-	20.30	23.5	+	15.5	+
09.45	24.5	-	15.0	+	21.00	23.0	-	15.0	-
10.00	24.5	-	18.0	-	21.30	22.0	-	15.0	-

To investigate whether sexual activity occurred between certain thresholds the following experiment was conducted. Preliminary observations suggested a lower threshold of approximately 15°C.

A wide range of temperatures was used, and tests performed in phials (5 cms

long by 2 cms diameter), each containing one pair of insects. Only 10-day old males and 15-day old virgin females were used, 10 pairs at each temperature. Pairs were allowed to acclimatize for one hour before being introduced, and then allowed 10 minutes to settle down. The number of forward darts by the male that occurred in a 5 minute period were then recorded. One control was run at each temperature where a female was not placed in the phial. Results are given in Table 6.5 and indicate that courtship was not initiated below 14°C, courtship did not go to completion, and mating did not occur below 16°C, indicating separate thresholds for courtship and mating. The lower threshold at which courtship occurs in Endria inimica (15.6°C) as determined by Coupe & Schulz (1968) was above that of Graphocephala and they did not distinguish separate lower thresholds for courtship and mating.

Table 6.5

Dance activity, at various temperatures, of 10-day old virgin males to 15-day old virgin females, during a 5 minute test period. Sample size 10 pairs per temperature. No female present in the control.

Temp°C	Female present		Control	Temp°C	Female present		Control
	Number of males dancing	Total dances	Total dances		Number of males dancing	Total dances	Total dances
10	0	0	0	17	7	30	0
12	0	0	0	20	10	38	1
13	0	0	0	25	10	40	0
14	3	13	0	30	9	36	0
15	4	21	0	35	10	40	0
16	6	27	0	40	3	11	0

iii) The effect of day length

It was shown that day length can affect the periodicity of sexual behaviour. During the course of this work, several hundred insects were reared from the early fourth instar under a light/dark regime of 16 hrs/8 hrs, and they displayed, as adults, the normal rhythm of sexual activity as described above. Forty insects of mixed sex were reared from the fourth instar in constant light, other factors also remaining constant. As adults, these insects displayed no rhythmical behaviour and remained sexually active 24 hours a day, though slight increases in the frequency did correspond to peaks found in normal insects. This phenomenon has not been reported in Homoptera previously, though Shorey et al (1965) described a similar effect on the mating behaviour of Drosophila melanogaster when reared in constant light.

iv) The effect of wind

No quantitative study was made of this phenomenon, though it was noted at times in the field that wind above a slight breeze inhibited courtship and hence mating. Carter (1930) said that Empoasa fabae usually seeks protection from wind, but when settled on a plant can tolerate considerable air movement. If disturbed into flight they are easily carried along. Lawson (1951) studying migration of Cicadellids, confirmed this and demonstrated in a wind tunnel that leaf-hoppers could not make progress against a wind of more than 2 miles per hour.

(b) Mating Behaviour

There are no detailed descriptions of mating behaviour of Cicadellids in the literature. Previous investigators have only described a limited part of the behaviour (Claridge & Reynolds 1973; Ichikawa 1976; Coupe & Schulz 1968), rarely studying the effect of other biological factors, e.g. age. One of the most complete studies

available is that of Nielson & Toles (1968) on Acinopterus angulatus and Aceratagallia curvata. Courtship is not described, and the study is restricted to seven observations on A. angulatus and one on A. curvata. A brief description of mating was given by Raine (1960) for the bramble leaf-hopper.

In the present investigation a detailed study was made of the biological factors which affect mating in G. fennahi and a thorough investigation of its mating behaviour.

(i) The effect of duration and frequency of mating.

In many insect species the female requires a single mating to produce fertile eggs throughout her life and once mated is often unreceptive to further male advances. This phenomenon has been described in Auchenorryncha by Harries & Douglass (1948), McMillian (1963). Nielson & Toles (1968) were able to show that once mated females of Aceratagallia curvata live longer and produce more progeny than multi-mated females of the same brood. An experiment was devised to determine whether females of G. fennahi remain fertile for an extended period after a single mating, and whether this affected their attractiveness to males.

Tests were conducted in standard 24 cms diameter cylindrical cages. Twelve 10-day old virgin females were presented to 14-day old virgin males repeatedly over a 28-day period. The procedure was as follows : females were individually caged on Rhododendron and a virgin male introduced to each female. By recording the duration of copulation the coition time necessary for insemination could be determined by an examination of the spermatheca. After copulation, males were removed and the fecundity of the female determined after 7 days by dissecting the Rhododendron bud and examining the eggs, the presence of red eye spots indicating fertile eggs. The pre-oviposition period for G. fennahi, in this study, averaged 5.8 days. Direct

evidence for sperm transfer by dissection of spermatheca was established. After the seven days females were presented to another 14-day old virgin male, and if courtship resulted the pair were separated prior to mating and penned on Rhododendron for a further 7 days when the trial was repeated. Pairs in which the male was not stimulated to dance were left together for 2 hours to determine if a second mating would occur. The female was then replaced on Rhododendron for a further 7 days when the trial was repeated. The results are presented in Table 6.6.

Table 6.6

To determine the duration of mating and attractancy of mated females to males by the repeated presentation of females to males at 7-day intervals. Sample size twelve pairs. At the start of the test, all females were 10-day old virgins. 14-day old males used throughout. Fecundity of the females determined at the end of each 7-day period.

Female Number	Initial Coition Time	State of female: 7 Days I*	7 Days A*	14 Days I*	14 Days A*	21 Days I*	21 Days A*	28 Days I*	28 Days A*
1	1 min 52 secs	+	-	+	-	+	-	+	-
2	1 min 37 secs	+	-	+	-	+	-	+	-
3	2 min 20 secs	+	-	+	-	+	-	+	-
4	0 min 57 secs	-	+	-	+	-	+	-	+
5	4 min 39 secs	+	-	+	-	+	-	+	-
6	7 min 09 secs	+	-	+	-	+	-	+	-
7	0 min 21 secs	-	+	-	+	-	+	-	+
8	6 min 03 secs	+	-	+	-	+	-	+	-
9	3 min 15 secs	+	-	+	-	+	-	+	-
10	1 min 53 secs	+	-	+	-	+	-	+	-
11	0 min 36 secs	-	+	-	+	-	+	-	+
12	3 min 23 secs	+	-	+	-	+	-	+	-

I* = inseminated A* = attractive

It is clearly indicated that females inseminated during the first mating failed to elicit courtship dances in males on future introductions, while mated females that were not inseminated remained attractive throughout the 28 day test period. This implies the female's reproductive state is communicated to the male. As determined in an earlier experiment, a sexually receptive female signals to the male via substrate-borne vibrations, insemination appears to bring about a change in the female's behaviour, resulting in their ceasing to vibrate the substrate and carry out an active response against male courtship. Females that failed to oviposit after mating all had coupling times of less than one minute and remained sexually active, suggesting insemination is a necessary component for the loss of female attractiveness. The 3 females that remained attractive during this experiment were dissected at the end of the 28 day period; their spermathecae were small in size, similar to that of immature females, with no trace of sperm in them. It is probable that seminal fluid or accessory gland secretions introduced during insemination are responsible for this female change.

Recently, much work has been conducted upon the nature of reinsemination barriers, and the literature has been reviewed by Hinton (1974) and Leopold (1976). Much of the work has been carried out on mosquitoes, where in many species the female mates only once, (Abdel-Malek et al 1967; Craig 1967). The work of Spielman et al (1969) suggested that in Aedes aegypti this was due to the semen from subsequent matings being expelled. More recent work, on the same species, by Gwadz (1972) indicated that the reinsemination barrier was due to a post-copulatory change in the female's mating behaviour, which is also suggested by the present author for G. fennahi, as indicated by the cessation of the female's vibratory stimuli once inseminated, and her response against courting males. In Diptera and some Lepidoptera it is known that mating plugs are the principal barrier to multiple matings. There is no

evidence for this in Auchenorynncha.

Recent work has indicated that at least in some species, male accessory gland secretions inhibit remating by altering the female's mating behaviour. The effect is initiated chemically through the central nervous system and may be characterised by an active display of resistance by the female to subsequent copulatory attempts. Much of this work has been performed in Diptera (Baumann 1974; Burnet et al 1973; Hiss 1972, and Terranova & Leopold 1971).

The role of neuro-humoral factors in the control and modification of mating behaviour is a large and rapidly developing field. Truman & Riddiford (1974), working on Diptera, were able to link the release of juvenile hormone from the corpora allata with the development of receptivity though it would appear unlikely that a termination of receptivity is simply due to the inactivation of the corpora allata in mated females, since it has been demonstrated that the corpora allata, in some mated females, may be directly or indirectly involved in egg maturation (Wiggleworth 1964; Engelmann 1970; de Wilde & de Loof 1973). The conclusion that mating refusal involves a neuro-humoral factor is based largely on evidence provided by Leopold (1971) and Degrugillier (1972) on Musca and Gwadz (1972) on Aedes.

It is not thought that the mode of action is the same in all groups. In Drosophila the barrier to reinsemination is not an accessory gland secretion, but due to the presence of sperm in the spermatheca (Burnet et al 1973). It is generally agreed that in species that avoid reinsemination it is a behavioural reaction and not a permanent mechanical one.

(ii) The mating frequency of males

Preliminary tests suggested that males could mate with more than one female.

This is the usual situation in insect species that exhibit sexual reproduction. Two tests were performed. In the first, ten 14-day old virgin males were caged individually in a constant temperature room at 25°C. A virgin female between 10 and 18 days old was caged with each male and left for 36 hours. At the end of this time the female was removed and caged individually on Rhododendron. The bud was dissected 21 days later, and any eggs examined as above to determine female fecundity. A new female was placed with each male every 36 hours for 15 days and its fertility monitored as above.

The following results were obtained :

No. Males/Test	No. Females/Test	No. Females Fertilized	Average No. females fertilized/ male
10	100	81	8.1

This demonstrates that males are polygamous and capable of multiple fertilizations.

In the second test, one virgin 14-day old male was placed in a cylindrical cage with fifteen 10 - 20 day old virgin females for 36 hours. The females were then removed and individually caged on Rhododendron, and their fertility determined as above. Eight males were used in total, and the results are shown in Table 6.7.

Table 6.7

The total number of females inseminated by a single 14-day old virgin male in a 36 hour period. Sample size 8 males.

Male	Number Females/cage	Number of Inseminated Females
1	15	2
2	15	2
3	15	3
4	15	5
5	15	1
6	15	3
7	15	2
8	15	4

Average number of females inseminated per male = 2.8

The results of these two experiments indicate that although males are capable of multiple matings they are not capable of fertilizing large numbers of females in a short time. This agrees with the findings of Leopold (1970) on Musca, where at successive matings the time spent in copula increased greatly. He suggested that this was due to a reduced production of accessory gland secretions in males that had recently mated, and demonstrated this by injecting males with cycloheximide, a protein synthesis inhibitor, when mating took 3 hours compared to the normal 72 minutes.

(iii) The effect of temperature on fertilization

14-day old virgin males and females were caged in pairs on Rhododendron at various temperatures for a period of 36 hours. The insects had previously been conditioned to the test temperature for 5 days. The tests were carried out in constant temperature rooms and incubators on a light regime of 16 hours light/8 hours dark, but, due to the range of temperatures used, the experiment was conducted over a period of 30 days. At the end of the test period, males were removed and the females left at the same temperature for a further 21 days, after which the Rhododendron buds were dissected and the eggs counted to provide an estimate of fecundity. 10 pairs were used at each temperature (see Table 6.8).

It can be seen that the optimum range for mating is between 21 - 35°C. Successful copulation did not occur below 16°C.

(iv) The effect of age on mating

No quantitative description of the age of leaf-hoppers at mating has been given in the literature. Stahl (1920) suggested that E. fabae mated within a few days of the adult moult in the summer generation, though the interval between adult emergence and mating in the autumn generation is, he thought, considerable.

Table 6.8

To determine the effect of temperature on mating. 14-day old virgin pairs were caged together for 36 hours at various temperatures. The fecundity of the females was then determined after a further 21 days. 10 pairs used at each temperature. 3 females died during the 21 days at 44°C.

Temperature (°C)	Total number of females inseminated
10	0
12	0
15	0
16	1
18	3
21	8
24	9
27	8
30	10
35	9
38	3
40	1
44	0

Harries & Douglass (1948), working on the same species, suggested that mating occurred within the first 3 days after adult emergence. It is important from an economic viewpoint to know at what age the adults attain sexual maturity, and whether virgin females can be fertilized when quite old.

It was noted during the rearing programme of G. fennahi that adult males emerge some 2-3 days prior to the females. This trait can be traced back into the nymphal instar, and is indicative of the greater development time required by the females of this species. It also ensures that sexually mature males will be available immediately upon the maturity of the females.

In the first experiment, sexual activity was determined in relation to age, the effect of rearing temperature also being investigated. Two groups of leafhoppers were used, the individuals having been sexed in the late third instar, males and females being kept separate. One group was reared from the third instar at 20°C and the second at 28°C. When ready for the final moult, cages were checked at 09.00 hours and 17.00 hours, and all newly emerged adults removed and caged according to age.

Sexual attractiveness was determined by the presence or absence of a prolonged courtship dance by the male, when pairs were caged together and observed over an 8 minute period. For each age combination 20 pairs were used. Males, however, were repeatedly used and females re-used if they had not elicited courtship. This action, though not satisfactory, was deemed to have no adverse effect on the results, and was necessary, due to the difficulty in rearing large numbers of adults of known age.

The results (see Table 6.9) show that no insect, irrespective of sex, less than 24 hours old was sexually attractive. As expected, due to developmental and

Table 6.9

To determine the age of sexual maturity in both males and females reared at 20°C and 28°C during an 8 minute test period. Sample size 20 pairs per combination. Age in days.

		20°c		28°c				20°c		28°c	
Age males	Age females	No. Dances	No. Matings	No. Dances	No. Matings	Age Males	Age Females	No. Dances	No. Matings	No. Dances	No. Matings
1	1	0	0	0	0	1.5	7	11	10	14	12
1	1.5	0	0	0	0	1.5	10	15	15	17	16
1	3.0	0	0	3	0	5	1.5	0	0	0	0
1	7.0	9	5	11	10	10	3	0	0	0	0
1	10.0	16	15	18	18	20	5	7	6	10	9
1.5	1	0	0	0	0	30	7	14	13	15	15
3.0	1	0	0	0	0	30	10	18	18	17	16
7.0	1	0	0	0	0	10	10	17	15	18	18
10.0	1	0	0	0	0	20	20	19	19	20	19
1.5	1.5	0	0	0	0	30	30	20	19	20	20
1.5	3	0	0	1	0						

maturational differences, there was a difference in the attainment of sexual maturity between males and females. In G. fennahi, males became sexually active and capable of fertile matings at between 24-30 hours, females, however, will not accept a male until they are approximately 5 days old. As stated above, this, linked with differences in the emergence times, ensures that when females are receptive, all males are sexually active. If a male attempted to court an unreceptive female, he was either pushed away or the female left the leaf. The results indicate that rearing temperature has little effect on the rate of sexual maturation, though development was very slightly faster at 28°C. In the above experiment, the age at sexual maturity in G. fennahi is clearly demonstrated; quantitative experiments of this sort have not previously been conducted on Cicadellids.

(v) Mating response in the older leaf-hopper

Because of mortality, particularly amongst the males, only seven virgin pairs were available that were more than 60 days old. Prior to the test, the insects had been maintained under natural conditions on the roof of the Zoology Department, but the experiment was conducted at 22°C in a constant temperature room. Pairs were caged together and observed for one hour, the females then placed on Rhododendron and the buds dissected after 10 days; the fertility of the eggs and hence fecundity of the females was then determined in the usual manner.

The results (see Table 6.10) indicate that all pairs were sexually active and 6 out of 7 fertile matings occurred, suggesting that sexual responsiveness and potential does not decrease with age in a virgin, and that the insects are sexually responsive for much of their life. This experiment, combined with the results of light microscopic examination of ovarioles from various females as described in Chapter 3 (p.107). indicates that mating is a contributory factor in eliciting egg maturation. This is an unusual effect, not previously described in the Auchenorryncha, though it has been

Table 6.10

Sexual activity in leafhoppers of 60 + days. Insemination determined by the oviposition of fertile eggs after 10 days. Sample size 7 pairs, which were observed for one hour.

Pair Number	Presence of Courtship Dance	Successful Insemination
1	+	+
2	+	+
3	+	+
4	+	+
5	+	-
6	+	+
7	+	+

demonstrated occasionally in other insects (Engelmann 1970; Labeyrie 1970), and it has been shown in some Acridids that it is an accessory gland secretion that stimulates egg production.

(c) Description of the act of mating

As already stated, mating is always preceded by a stereotyped courtship dance, which ends with the male placing the front tarsi on the back of the female so that he is positioned parallel to her, with heads pointing in the same direction. The behaviour of both courtship and mating was determined by the use of video tape recording, and, where necessary, playing back the tape frame by frame. The male, when alongside the female, still with one tarsus on her back, flexed the distal portion of his abdomen downwards and laterally, curving around the ventral part of the female's abdomen. At the same time, the female lowered sternite VII but not the gonapophyses. The aedeagus entered the female by a series of short thrusts, the final mating position being achieved when the male released the female with his front tarsus and turned through 180°, until the heads of the two insects were pointing in opposite directions. This position was maintained for a variable time, but a fertile mating was not observed with a coupling time of less than one minute. The maximum coupling time observed in the field was 11.5 minutes, during which the larger female walked around, dragging the male behind. Disengagement was brought about rapidly without a return to the side to side position. Nielson & Toles (1968) reported a different mating position in Aceratagallia curvata, where the final coition position is achieved with the male parallel to the female, with their heads pointing in the same direction. Copulation in this species took approximately 17 minutes, but detailed observations of the behaviour were not recorded.

Alexander (1964) lists 6 mating positions in the Homoptera, but unfortunately provides no details of species or if Sternorrhyncha were included.

Discussion

The present study describes in detail mating and pre-mating behaviour of G. fennahi, and some of the factors that govern it. The observations and data described help considerably to provide a more complete picture of Cicadellid sexual behaviour. Much of the information described was not previously available in the literature for any species.

The view of McMillian (1960) that Cicadellids have a generalized courtship dance is confirmed, though the dance described here for G. fennahi differs considerably in detail from that of the rice Delphacid. The visual close quarter courtship is always initiated by the male, and the female may be considered to take a passive role. Here novel recordings from Rhododendron leaves revealed that substrate-borne vibrations of a low frequency produced by the female are of considerable importance in attracting the male and maintaining courtship. During the courtship dance the vibrations produced by the female are of lower frequency, and the frequency pattern becomes more complex, thus indicating to the male that the female is receptive. It was further demonstrated that mated females fail to produce these vibrations and so do not attract males. This cessation of communication, i.e. a behavioural change in the female, may be due to accessory gland secretions from the male (Gwadz 1972; Baumann 1974 in Diptera). The functional range of the vibrations was not examined in detail, though the effective distance of the female signal was at least 97 cms on Rhododendron. There are few other comparative experiments on the function of substrate-borne vibrations in Auchenorryncha, the only other definitive experiments being those of Ichikawa (1977). Perkes (1970),

using artificial substrates, established the presence of substrate-borne vibrations in Empoasca fabae, but did not develop the experiment.

Courtship and mating are governed by temperature thresholds, and in wild populations the strongly bimodal distribution of sexual activity may be suppressed by low ambient temperatures. Coition time in G. fennahi is short compared to other Cicadellids for which there is data available. Nielson & Toles (1968) reported that successful matings in Acinopterus angulatus had a mean duration of 75.2 minutes, and that of Aceratagallia curvata of 17 minutes. Successful matings in G. fennahi were accomplished in a minimum of 1 minute, though as suggested by Leopold (1970) the coupling time required for successful matings increased with an increase in the number and frequency of females fertilized by a particular male. Sevin & Klostermeyer (1950) in Colladonus geminatus, recorded a maximum coition time of 6 days.

Vision, olfaction and acoustic reception were shown to play very little part in intraspecific communication and the initiation of courtship, substrate-borne vibrations, as discussed above, being of prime importance. In other Cicadellids (Strübing 1958; 1959; 1960; Claridge & Howse 1968; Claridge & Reynolds 1973) acoustics stimuli, perhaps in association with substrate-borne vibrations, have been put forward as the main means of communication. The findings of this study and those of Ichikawa (1977) suggest the need for further investigations into the use of substrate-borne vibrations.

Oviposition and Site Selection

(a) Characters used in site selection

The eggs of Graphocephala are laid sub-epidermally in the overwintering flower buds of Rhododendron. Prior to oviposition the insect positions itself on the bud, head pointing towards the apex. The bud is then examined, both by numerous short probings with the stylets, and then by passing the distal end of the labium over the surface. A

detailed examination of this region was not made with electron microscopy, but behavioural observations suggest the importance of sensory receptors present on the mouthparts in the location of potential oviposition sites. The presence of receptors on the labium of phytophagous insects and their role in gustation is well documented (Boeckh et al 1965), and various workers have suggested the importance of contact chemoreceptors in the location of oviposition sites (Dethier 1963; Hawke 1973). Behavioural observations on G. fennahi suggest sensory receptors on the mouthparts, particularly the labium, do have a function in oviposition. After egg-laying, the area is again examined by the mouthparts; it is probable that sensory fields on the labium and/or information gained by the stylets provide primary data on site selection.

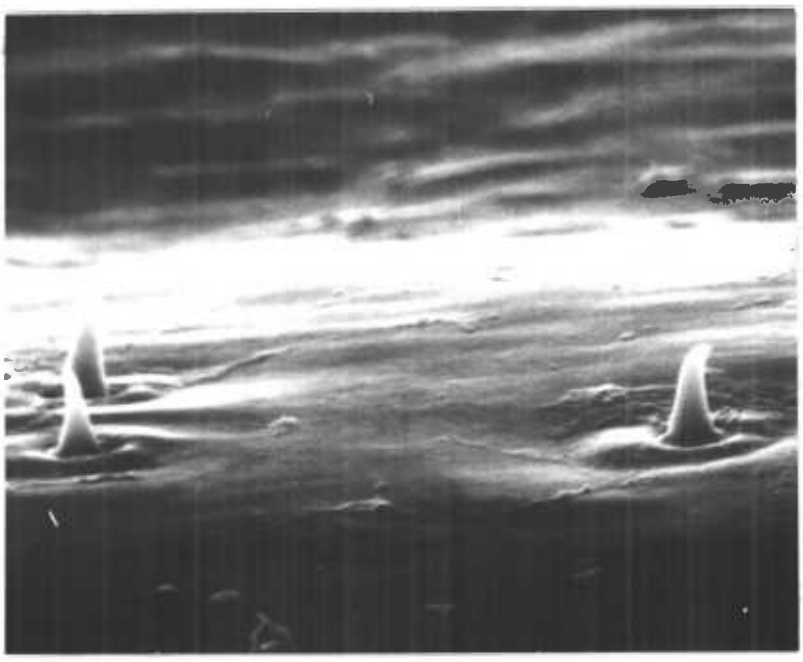
During the present study, scanning electron micrographs revealed cuticular structures located on the ovipositor blades and basal region of the genitalia, which may function in the control of oviposition. In the literature descriptions of Cicadellid oviposition are extremely scarce and superficial. Raine (1960) gave a description of oviposition on the bramble leaf-hopper. Studies of sensory structures have not been made on Cicadellid genitalia, and only rarely on those of other groups. Hooper et al (1972) described sensory structures on the ovipositor of the face fly, Musca autumnalis, indicating that the ovipositor possesses both tactile and olfactory sense organs, including campaniform receptors. Most of the work conducted on the sensory structures of ovipositors has been in Hymenoptera. Behavioural studies indicating that the ovipositor is used as a sense organ in host selection (Greany & Oatman 1972, and Vinson 1972) Hawke et al 1973 indicated that the Braconid Orgilus lepidus could distinguish, after inserting the ovipositor into the host, whether the host was healthy or previously parasitized. They concluded that contact chemoreceptors on the medial and lateral

stylets were responsible. In the Cynipid Charips victrix, a hyperparasite of aphids, sensilla at the tip of the ovipositor allowed discrimination between parasitic larvae lying in the aphid haemocoel (Gutierrez 1970).

For most insects, oviposition is the culmination of a complex chain of behavioural and physiological events, and largely because of this complexity in the sequence of sensory events leading to oviposition, its study has been neglected in insects, except for behavioural observations (Miller & Treece 1968; Carlson & Hibbs 1970). Wallis (1962) demonstrated in Phormia regina that tactile receptors function in the final selection of a suitable oviposition site, Barton Browne (1960) working on the same species had indicated that olfactory receptors on the ovipositor also function in oviposition in addition to those located on the antennae, palps and labellum.

Four types of sensilla were found on the gonapophyses of Graphocephala fennahi. The sensilla on the gonapophyses of Cicadellids have not previously been studied. The first gonapophysis, outermost of the pair, possess only sensilla basiconica which will be designated Type I and Type II (fig. 6.5).

Type I sensilla are the commonest, there being approximately 28 per blade. They are located immediately ventral to the dorsal rhachis and extend the whole length of the blade to within 200 μm of the distal end. Type II sensilla are concentrated in the proximal 500 μm of the blade between the dorsal and ventral rhachi, there being approximately 12 per blade. Reports of sensilla basiconica on the ovipositor are rare (Hooper et al 1972 on Musca) but when present they have usually been ascribed a chemo-receptive function. The morphology of the major types of sensilla have now been described for several groups, which may allow certain sensilla to be linked with particular functions though one must always be wary of assigning functions to sensilla on structural evidence. A particular



Type I and Type II sensilla basonica on the proximal outer face of the 1st gonapophyses.

x 2,300 10 KV Gamma 1

sensillum may subserve one function in one species and a different one in another. Also, a single sensillum may consist of more than one type of receptor, e.g. in Phormia, the labellar hairs have two chemoreceptors and a mechanoreceptor associated with them (Dethier 1955).

Type I sensilla basiconica range in length from 2.5 - 3.2 μm with a mean of 2.7μm. The diameter ranges from 1.2 μm at the base and tapers to 0.3 μm at the tip, and they are of the blunt, curved type (curved both left and right). The basal pit has a diameter of 2.5 μm. They are all of the thick walled type with no surface sculpturing or pitting evident. A single distal perforation of very small aperture was noted.

Type II sensilla range from 3.7μm - 4.2 μm in length with a mean of 4.0 μm and a diameter of 1.5 μm at the base, tapering to 0.3 μm at the tip. Basal pit diameter is 2.5 μm. They are of the blunt straight type, and the tip displayed a cap of slight darkening, possibly indicating thickening of the cuticle in this region.

Sensilla basiconica, rarely described from the ovipositor, are more commonly found on insect mouthparts and antenna where ultra-structural examination, combined with electrophysiological and behavioural tests, suggest a gustatory function (e.g. Mustaparta 1973 and 1975; Norris & Chu 1974; Payne et al 1973). The external structure and distal perforation of the Type I sensilla suggest a chemoreceptive function.

Three types of sensilla are present on the second gonapophyses. The first is associated with the base of the dorsal teeth on the outer surface. Their structure is interesting and unusual. They comprise a pit 0.83μm by 1.1 μm, elliptical in form and containing a disk or plug of 0.53 μm x 0.9 μm. This disk is in communication with the pit edges nearest to the distal end of the gonapophysis by two slender cuticular bridges. Similar structures have been described on the Hymenopteran ovipositor by Hawke (1973). It is thought that these structures are mechanoreceptors of the campani-

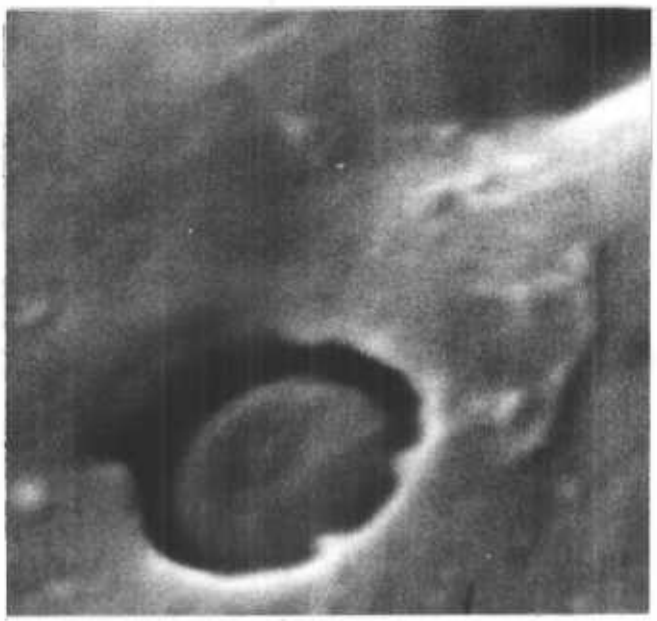
form type and might monitor cuticular stresses as the ovipositor made an incision into the host plant. Glands are also present in this region, but open onto the inner surface (fig. 6.6).

The second type of sensillum is found on the distal ventral cutting edge of the second gonapophysis, set back from the edge and of a sensillum coelonconium type with a short central peg 0.75μm long, set in a shallow depression. The diameter of this external cavity is 1.2 μm by 2.0 μm. These sensilla are restricted to this region of the blade, and number approximately 7 per blade. The central peg has a knobbly surface sculpturing and a distal aperture 0.15 μm diameter. The structure of this sensillum suggests a chemo-receptive function, and they might respond to oviposition-inducing stimuli or to oviposition deterrents, which may be alkaloids or other plant constituents present in the bud (Dahlman & Hibbs 1967) (see fig. 6.7).

Scanning electron microscopy also revealed a third type of receptor, which is associated with shallow depressions in the cuticle surface. The pits are 1.2μm diameter and approximately 0.2μm in depth, and restricted to the proximal third of the second gonapophyses, they are thought to be a mechanoreceptor concerned with cuticular stresses.

In most previous studies, the fine structure and function of the sensilla associated with various parts of the genital area have been limited to those on the cerci of cockroaches (Nicklaus et al 1967; Roth & Slifer 1973), crickets (Schmidt & Gnatzy 1972), Acridids (Thomas 1971), and on the ovipositor of parasitic Hymenoptera (Hawke et al 1973). Rossignol & McIver (1977) described the genital sensilla of Aedes aegypti and their role in the sexual behaviour of the insect. They found, in agreement with Provost & Haeger (1967), also working on mosquitoes, that all the sensilla on the genital region of both sexes were mechanoreceptors, and, as such, were probably only involved in the

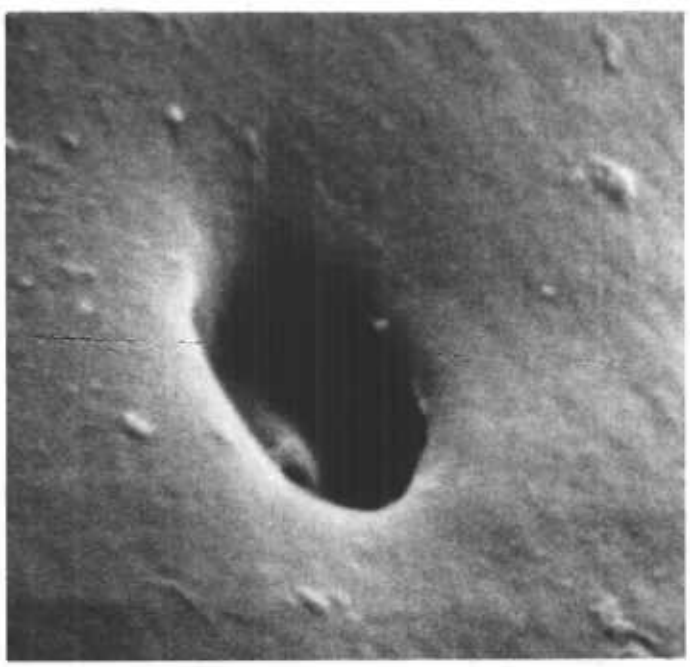
Fig. 6.6



Mechanoreceptor, concentrated beneath the dorsal teeth and distal region of the 2nd gonapophyses. Note the two cuticular bridges.

x 20,300 10 KV Gamma 1

Fig. 6.7



Chemoreceptor from the sub-apical face of 2nd gonapophyses.

x 20,000 20 KV Gamma 2

act of copulation and not in prior courtship. Copulation therefore seems to be mediated largely, if not entirely, by mechanical stimulation. The present study has indicated that the ovipositor of Graphocephala possesses an array of sense organs which could perhaps provide information about the position, movement and the chemical environment of the ovipositor and hence aid in the location of suitable oviposition sites.

(b) Oviposition and the method of incision by Graphocephala

The act of oviposition has been observed several times in the Auchenorryncha (Readio 1922; Balduf 1933; Raine 1960; Jay 1972 and Maramorosch 1974), but most descriptions are brief and incomplete. Carlson & Hibbs (1970), following the work of Raine (1960), attempted to analyse some of the plant characters necessary to elicit oviposition in E. fabae by varying several plant stimuli and observing the behavioural effect on the insect.

In Graphocephala, the behavioural sequence culminating in oviposition was observed in the field and analysed in the laboratory, using a Sony television camera and Sony videocorder CV - 2100 ACE. With the use of extension tubes and fibre optic light source it was possible to obtain a focal distance of 6 cms. The depth of field was critical at this range, but careful focussing and high light intensity permitted detailed, accurate photography. During recording, the insects were not enclosed but placed loose on a Rhododendron bud. This allowed a complete analysis of oviposition until the female left the site after egg-laying. Observations were supplemented by light microscopy sections, where further information, particularly regarding passage of the eggs along the ovipositor, was required. Such a complete study has not previously been reported in the literature.

The first visible action associated with oviposition occurred when the female

stopped feeding on the underside of the leaf, this being the usual female activity, and became restless, walking over the plant until a bud was located. The female then walked over the bud and finally settled near the bud base, where it was at its greatest diameter with the head of the insect pointing towards the bud apex. Probing of the bud with the stylets then began, with the female moving slowly up and down the bud, frequently inserting the stylets and passing the distal tip of the labium over the bud scales. When an apparently suitable site had been located with the mouthparts, the insect moved towards the bud apex until the genital region was over that sampled by the stylets. Only at this time were movements initiated in the genital region. The length of time spent in searching behaviour, i.e. from the cessation of feeding to the location of an oviposition site varied from 3 minutes to 18 minutes and averaged 7 minutes (65 observations, of which 15 were in the laboratory).

The ovipositor blades were then moved in an anteroposterior manner in short thrusts, while the distal ends remained within the gonoplacs though the basal region of the ovipositor was exposed. The first and second gonapophyses worked in unison with a thrust frequency of approximately two per second. These movements were discontinuous, the leaf-hopper remaining stationary between bouts and this sequence lasted some 15 seconds. At this time the stylets were not inserted into the plant tissue and the labium was not in contact with the plant surface.

The ovipositor blade was then unsheathed, partially by movement of its basal parts and in part by an opening of the gonoplac groove, brought about by contraction of intrasegmental muscles in tergite IX. Sternite VII was depressed with the insect standing well above the plant. The ovipositor was then lowered to form an angle of approximately 40° with tergite IX. The distal part of the abdomen was

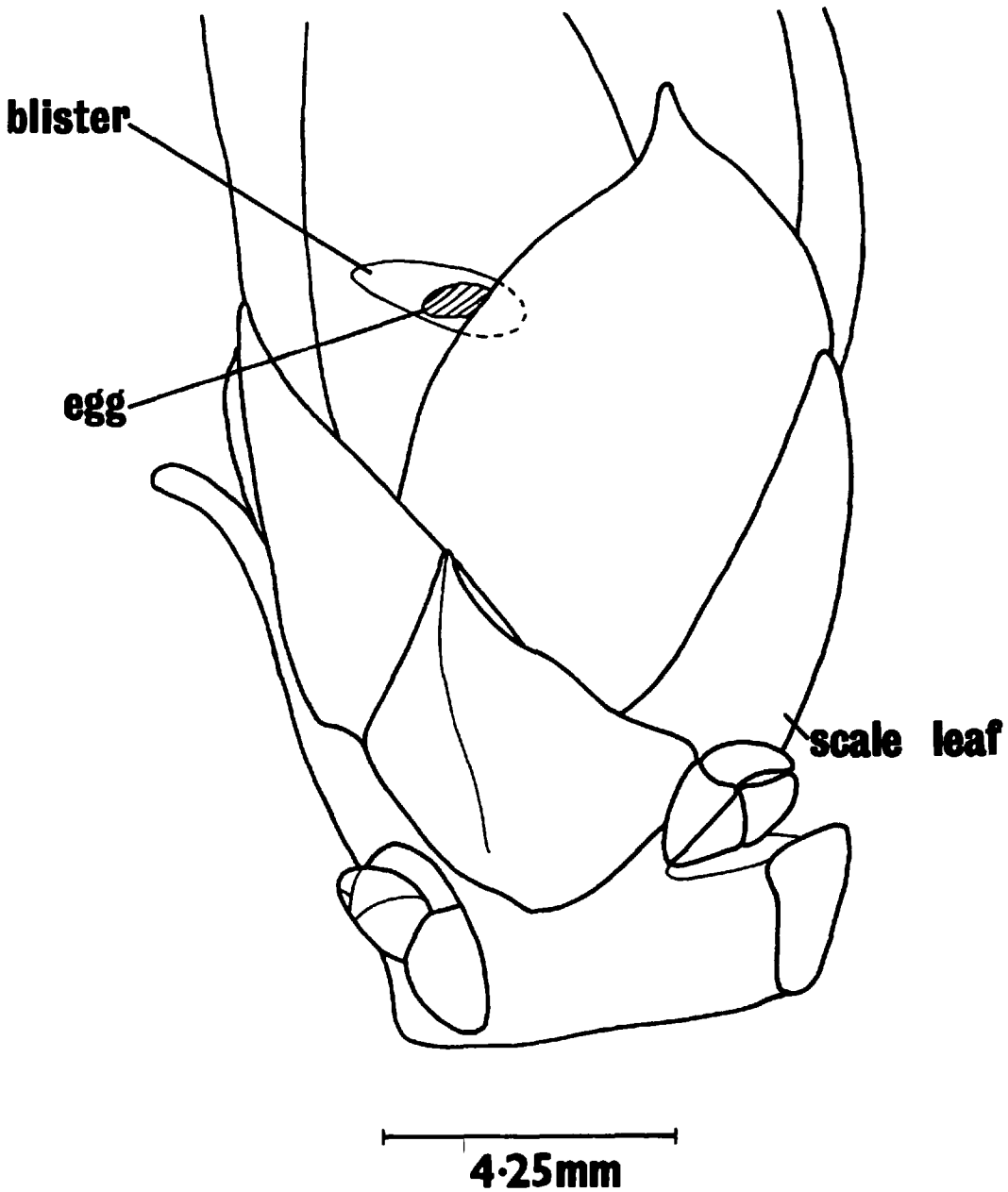
also flexed downwards at the junction of tergites VII and VIII forming an angle of approximately 30° to its resting position. The distal part of tergite IX rested upon the bud scale in this position. The animal then lowered itself until the ovipositor tip made contact with the plant surface at an angle of approximately 70°.

The overwintering bud of Rhododendron is made up of approximately 9 whorls of scale leaves. Whorl 1 consists of those at the base of the bud and are too small to allow oviposition and protection of the eggs. In successive whorls the scales abut more distally and are larger. Oviposition by Graphocephala is usually in whorls 4 and 5 where the eggs are afforded greatest protection. (fig. 6.8). An incision is made when the ovipositor tip is placed against the bud scale epidermis. The insect then walks backwards with the blade running along the scale surface until it meets the edge of the preceding scale. The angle of the ovipositor to the scale surface is then increased to approximately 80° by further depression of the gonapophyses and the initial penetration of the plant epidermis is made by several backward thrusts of the ovipositor and whole abdomen. Further penetration is brought about by abdominal thrusts and alternate anteroposterior movements of the first and second gonapophyses in a sawing action. The dorsal edge of the second gonapophysis stands proud above that of the first, and this action brings into effect the rigid, heavily sclerotised, toothed dorsal edge of the second. During this activity an anteroposterior rocking action was also made by the gonoplacs within the ventral groove of segment IX.

Once the epidermis had been penetrated the blade angle was reduced and the ovipositor made a shallow penetration sub-epidermally for approximately $\frac{3}{4}$ of its length. This resulted in a single small point of entry and the plant epidermis being separated from its cortex along a straight incision approximately 2 mm long.

Fig 6.8

Basal part of a rhododendron bud, superficial scales removed, to show the egg position



Eggs were passed along the ovipositor into the incision and forced out under the epidermis at an angle to the original line of incision, so enlarging the blister. Four or five eggs are frequently laid in the same oviposition site, producing a bottle-shaped blister with the eggs radiating out from the original point of penetration. The cephalic end of the egg (evident by the prominent red eye spot that is present early in development) is towards the aperture (see fig. 6.8).

From behavioural observations, with the use of video recording, and from the structure of the first gonapophyses, it is suggested that their principal role is one of supporting and strengthening the second gonapophyses which operate between them. The lateral rasps, reported by others (Balduf 1933) to be of primary importance in the penetration of the plant tissue, are here thought not to function in this manner. Scanning electron microscopy indicates that they are only moderately sclerotized, and the orientation of the fringe, pointing in a proximal direction suggests that they function in the retention of the blade within the plant tissue during egg expulsion without rendering the blade inextricable. Scales on the distal most part of the first gonapophyses are not fringed and probably aid in the removal of plant fragments from the incision.

As described in an earlier chapter, a tongue and groove system extends from the proximal end of the ovipositor and serves to unite the first and second gonapophyses of each side, while still allowing alternate anteroposterior movements. This also provides for a strong shaft, which would be more efficient in the penetration of plant material. In the proximal region the first gonapophyses are united ventrally by a ball and socket joint for approximately $\frac{1}{3}$ of the distance along the blades. Where this ball and socket mechanism ends, approximately half way along the blade, the ventral margins of the first gonapophyses are extended into membranous flaps (fig. 1.5).

These are not fused in the mid-line but serve to guide the egg along the blade until it is expelled from the ventral surface approximately $\frac{2}{3}$ of the distance along the ovipositor. In the proximal 200 μm the second gonapophyses are joined by a dorsal membrane, thus keeping the two halves of the ovipositor together, and limiting the possible separation of the blades in the proximal region during the passage of an egg.

Proximally, the inner faces of both the first and second gonapophyses are covered by scales, the distal edges of which (that nearest the tip of the ovipositor) are produced into a fine fringe. This would aid passage of the egg along the ovipositor during the rapid opposing movements of the gonapophyses and allow a better control and positioning of the egg. During its passage along the blade, the egg is exposed to considerable compression. Light microscopy revealed that this stress is absorbed by deep convolutions of the elastic chorion and as the egg progresses posteriorly the two halves of the blade would be forced apart as the egg assumes its normal turgid ovoidal form.

When multiple oviposition occurs within the same blister, the ovipositor is partially retracted and then re-positioned within the blister. When oviposition is complete the blade is retracted out of the incision, Tergite VIII and IX then return to their normal position. The ovipositor remained fully exposed at an angle of approximately 25° to the gonoplace for 4-6 seconds. During this time, both gonoplac and ovipositor were rocked anteroposteriorly with a frequency of 2-3 strokes per second. The ovipositor was then re-sheathed in the gonoplacs but the rocking motion continued for a further 2 seconds. The insect then moved backwards for a few millimetres until the head was over the oviposition site and the region was again tested with the labium for 3-5 seconds. The insect then moved off. The sequence of oviposition (of a single egg) lasted approximately 50 seconds.

Oviposition by Graphocephala differs from the preliminary description for E. fabae given by Carlson & Hibbs (1970). In the latter oviposition required 10 - 40 minutes. A detailed description which linked behaviour with ovipositor structure had not previously been given for Cicadellids. It is not improbable that plant substances with specific attractant or deterrant properties are detected by the labium and stylet probing, and that the contact chemoreceptors of the mouth-parts play an important role in initial site selection, the precise location then being determined by sensory receptors on the ovipositor. Dahlman & Hibbs (1967) were able to demonstrate the inhibitory effect of certain plant alkaloids upon leaf-hopper feeding and suggested that alkaloids may indirectly deflect oviposition away from plant tissues containing such chemicals above a certain threshold.

GENERAL DISCUSSION

In the preceding account of the developmental and functional morphology of the female reproductive system of G. fennahi a discussion has already been provided for many topics, though several arose which require further discussion in the light of other work. Six subjects are of particular interest and will be discussed here. They are: (a) The linkage mechanisms of the gonapophyses. (b) The absence of an intima on the median oviduct. (c) The origin of the ovariole prefollicular tissue and its relationship with the germ tissue. (d) Mechanisms involved in myogenesis and myoblast incorporation. (e) The extent to which taxonomic differences in the gonapophyses are adaptive. (f) Sexual communication and the role of substrate-borne vibrations within the Auchenorryncha.

(a) Linkage mechanisms. Prior to this study the only comprehensive description of the linkage mechanisms of the ovipositor valves in the Auchenorryncha was that of Balduf (1933) for Draeculacephala. Smith (1968) developed a new terminology for the linking apparatus based upon the Hymenoptera but his system is not followed in the present study. The linkage mechanism shows little variation within the Cicadellidae except in minor detail. In the present study a scanning electron microscope study of the tongue and groove joint connecting the first and second gonapophyses in Graphocephala revealed that in this species it is somewhat simpler than that described by Balduf. The tongue is a solid cylinder made up of units 17 μm in length which slots into the heavily sclerotized groove situated on the inner wall of the first gonapophyses.

Previous descriptions of gonapophyses that have been given in taxonomic works have not included the linkage mechanisms. Three species, from separate genera that were examined in the present study all possessed the same basic mechanism. A tongue and groove between the first and second gonapophyses of either side and a modified

tongue and groove linking the two first gonapophyses. The differences that were apparent in the degree of freedom of the four gonapophyses relative to each other are associated with variable sclerotization of the parts and, in Graphocephala, with rotation of the tongue and groove along the length of the ovipositor (figs. 1.4 and 1.5). These characters have not previously been discussed in the literature. In the three species studied, reduction in the freedom of movement of the gonapophyses is brought about in a number of ways : (i) A membranous connection between the two second gonapophyses in their proximal parts. (ii) The degree of sclerotization and hence rigidity of the connection between the two first gonapophyses. (iii) The rotation of the tongue and groove linking the first and second gonapophyses of each side, so severely limiting movement between the gonapophyses of each side. How widespread this phenomenon is in the Cicadellidae is not known; it is reported here for the first time in Graphocephala. (iv) The primary linkage mechanisms may be supplemented by accessory spines as reported by Balduf (1933).

It was demonstrated, for the three species studied, that the greatest freedom of movement between the gonapophyses is found in Ulopa, which oviposits in a very hard substrate. It is suggested that this is associated with the need for individual sawing actions of the first and second gonapophyses as well as movement between each pair of gonapophyses. This suggestion is reinforced by the structure and extent of the possible rasping areas. In Graphocephala, which oviposits into soft material, there is only limited movement between the first and second gonapophyses of each side and the proximal regions of the second gonapophyses are membranously connected. Observations of oviposition behaviour, aided by video recording, indicated that the oviposition incision was opened by posterior thrusts of the whole abdomen which would require the ovipositor to function as a single, rigid unit. The weak structure of the "rasps" in Graphocephala also suggests they are of little importance in making the

incision.

The linkage mechanisms present in the Cicadellids differ from those reported in the Cicadidae (Kramer 1950; Snodgrass 1935), where the second gonapophyses are fused along their length in the mid-line and hence function as a single unit within the lateral first gonapophyses. This may be an adaptation for oviposition into soil. This difference between the Cicadidae and Cicadellidae has not previously been discussed in the literature. Similar information on the gonapophyseal linkage mechanisms in the Cercopidae and Membracidae is not available in the literature. Such data is clearly of interest and may provide useful taxonomic information.

(b) The absence of an intima on the common oviduct. Little reference is made in the available literature, much of which is very old, to the presence or absence of a sclerotized intima on the common oviduct of insects, though it is generally assumed that the structure, being derived from an ectodermal invagination, possesses an intima (Snodgrass 1935). Much of the work on the histology and development of the efferent reproductive ducts in the major insect orders, was carried out at the beginning of the century, and though often superficial and incomplete, has not been repeated.

Historically there has been much debate on the origin of the common oviduct, Herold (1815) and Balbiani (1870) both stated that it was formed by the fusion of the lateral oviducts and hence of mesodermal origin. Verson & Bisson (1896), working on Lepidoptera, suggest that the common oviduct is derived from paired ectodermal vesicles which fuse later in postembryonic development to form a median duct. This is in general agreement with Nussbaum (1882). More recent opinion (George 1928; Nel 1929; Singh Pruthi 1924) is that the common oviduct is unpaired in origin and ectodermal. This opinion is not disagreed with in the present study.

Several accounts of the development of the efferent system in the major orders

are available. The works of Abul-Nasr (1950), Bodenstein (1946), Dean (1943) and Metcalfe (1933) are all concerned with development in the Diptera. It is generally agreed that here the lateral oviducts are partly of mesodermal and partly of ectodermal origin, as has also been demonstrated in the Coleoptera (Singh Pruthi 1924; Metcalfe 1932) and Hemiptera (Helms 1968; Metcalfe 1932 and the present study), while the rest of the efferent system is ectodermal. This has been shown in the Diptera by Christophers & Barraud (1926), Koch (1929) and Lowne (1890) and confirmed in other orders by many workers. A possible exception to this occurs in the work of George (1928) who suggested that the anterior part of the common oviduct in Agrion is derived by the fusion of the mesodermal lateral oviducts. However, despite considerable debate regarding the position of the opening of the efferent system it is now generally agreed that the common oviduct arises as an invagination of the ectodermal wall and as such would be expected to possess an intima. Metcalfe (1933), during her studies of the Diptera, did not record the presence of an intima while in the Coleoptera (Metcalfe 1932) she states that the intima is not secreted until the mature pupal stage (i.e. presumably the pharate adult).

Nel (1930) brings together a large amount of the earlier data and concludes that except for the doubtful case of the Odonata the common oviduct has a solely ectodermal origin in a number of insect orders, e.g. Mallophaga (Strindberg 1916), Homoptera (George 1928), Lepidoptera (Jackson 1890; Verson & Bisson 1896), Diptera (Christophers 1923; Christophers & Barraud 1926), Hymenoptera (Seurat 1899) and Coleoptera (Singh Pruthi 1924).

It would appear a general rule that the common oviduct is of ectodermal origin. Though Helms (1968) asserts that the absence of an intima in Empoasca is due to the ampullar origin of the common oviduct, embryologically the ampullar was presumably

derived by ectodermal invagination, so that the ampullar origin does not in itself preclude the presence of an intima. The intima of Cicadellids may be too thin for resolution with the light microscope or classical staining techniques and further work with the electron microscope may permit a more satisfactory explanation to this problem.

(c) The origin of the ovariole prefollicular tissue. In Graphocephala the posterior strand of the ovariole rudiment differentiates during the early third instar to form the pedicel and prefollicular tissue. The origin of the insect ovarian tissues has been a point of controversy for many years, the early insect histologists being divided as to which tissues were of germ cell origin and which were of mesodermal origin. There were two points of view, one suggested that the germ cell line produced only the oocytes and trophocytes, while the more widely held view was that the germ cells produced oocytes, trophocytes and follicular epithelium. Gross (1903) reviews the literature to that date. More recent work supports the view that the germ cells produce only the oocytes and trophocytes (Huebner & Anderson 1972; Lautenschlager 1932; Nelsen 1934; Ramamurty 1970; Wick & Bonhag 1955). Aboim (1945) was among the first to provide clear experimental evidence that the prefollicular tissue was of mesodermal origin using ultraviolet light irradiation to damage germ cell tissue while prefollicular tissue remained healthy.

Few detailed developmental studies have been made within the Auchenorryncha which have allowed the development of the follicular tissue to be followed. Detailed investigations have been made in the Heteroptera by Bonhag & Wick (1953), who also concluded that the prefollicular tissue was mesodermal in origin. Some reports suggest that prefollicular tissue is syncytial and cell membranes only form during the early stages of oogenesis to produce a layer of follicle cells surrounding the developing

oocytes (Bonhag 1955a; Masner 1966, 1968). Though these studies are not conclusive they suggest an interesting case of cellular induction within the Heteroptera, which would require further investigation. It is, however, more likely that electron microscopy would reveal cell boundaries previously unresolved by the light microscope.

The early ovariole rudiment can therefore be divided into three types of tissue, the germ cells which give rise to the oocytes and trophocytes, the anterior strand which forms the terminal filament, and the posterior strand which gives rise to the prefollicular tissue and pedicel. The prefollicular tissue is usually thought to represent the posterior part of the germarium (Snodgrass 1935; Wigglesworth 1950). De Wilde (1964) suggested that the vitellarium was represented solely by the section containing oocytes surrounded by follicular tissue. Since the transition of prefollicular tissue to follicular epithelium is gradual, no clear boundary can be discerned between the germarium and vitellarium. The prefollicular tissue has been considered the boundary between the two zones (Schlottman & Bonhag 1956). Developmentally the germarium, composed of trophocytes and unfounded oocytes, is of germ cell origin, while the vitellarium, composed of follicular tissue into which oocytes have migrated, is of mesoderm origin. In the telotrophic ovariole, as found in Hemiptera, an alternative suggestion can be made; the germarium is apical and represents the zone of trophocyte development containing posteriorly a narrow zone of oocytes prior to any development. If the vitellarium is the zone of oocyte development it is delimited anteriorly by the prefollicular tissue since this is where oocyte development begins and hence in the telotrophic ovariole the prefollicular tissue can be considered as being part of the vitellarium. This is in agreement with Masner (1966).

Once development of the oocyte begins prefollicular tissue undergoes considerable morphogenetic changes accompanied by oocyte growth. During the vitellogenic stage the follicle cells appear to have an important role in regulating the flow of metabolites and yolk precursors and in some species the production of protein for the

oocyte (Anderson & Telfer 1969), though this latter function has not been recorded in the Auchenorryncha. The follicle cells are also responsible for secreting the chorion prior to ovulation and for resorbing the yolk of aborted follicles. Associated with these varied functions is the differentiation of the follicle cell as described in some Heteroptera (Huebner & Anderson 1972; Masner 1966; Ramamurty 1970) and in the Auchenorryncha as reported in the present study. However the functions of these cells are still not clear (Davidson 1968; Raven 1963; Telfer 1964) and throughout the insect orders a wide range of functions have been suggested for them (Quattropani & Anderson 1969; Ramamurty 1970). In Graphocephala exogenously produced proteins may reach the oolemna via the extracellular spaces as reported in Periplaneta (Anderson 1964, 1969), Aedes (Roth & Porter 1964), Hyalophora (King & Aggarwal 1965; Stay 1965; Telfer & Anderson 1968) and Tenebrio (Aggarwal 1968). There is some suggestion that exogenously produced proteins pass through the follicle cells before being taken up by the oocytes (de Loof & Lagasse 1970). In Graphocephala intercellular spaces develop between the follicle cells during vitellogenesis. The absence of evidence for the release of protein from the follicle cells suggests that the mechanism of yolk incorporation is via these intercellular spaces and not by the mechanism proposed by de Loof & Lagasse (1970) for Leptinotarsa. However, the mechanism involved in traversing the tunica propria and the process by which yolk precursors pass between the follicle cells has not been ascertained. A selective concentration of yolk protein within the intercellular spaces of the follicle was demonstrated by Anderson & Telfer (1970), who suggested the production of excellular binding agents by the follicle cells, though this has not been confirmed.

(d) Mechanisms of myogenesis and myoblast incorporation

The present study indicates that the adult muscles of G. fennahi include three major types : (i) Those that pass from the nymph into the adult with little, if any,

histological change. (ii) Those adult muscles which develop by incorporation of myoblasts into pre-existing nymphal muscles, the nymphal muscle being the template which undergoes extensive differentiation to produce the adult structure. (iii) New muscles which arise late in the nymphal instars by the activity of free myoblasts.

There is apparently a clear distinction between the myogenesis of Endopterygotes and Exopterygotes, since in the former adult muscles are often of fairly late postembryonic origin, while in the latter the adult muscles are recognisable from an early stage, though they may be poorly developed, and differentiation may not be complete until the final instar (Maloeuf 1935; Tiegs 1955; Wittig 1955). There is also some evidence to suggest that in some more primitive Endopterygotes few completely novel muscles are formed at metamorphosis; most adult muscles arising from the redifferentiation of pre-existing larval muscles (Korn 1943). Davies (1969) found the same in the holometabolous Exopterygotes (Thysanoptera), but in specialized Endopterygotes adult muscles are largely formed at metamorphosis.

Despite recent ultrastructural studies, the histological processes involved in myogenesis and their physiological background still remains unclear. The work of Crossley (1965) and Hoffman (1970) on the distribution and functioning of haemopoietic centres has provided some information on the origin of haemocytes and phagocytes, though these centres appear not to be general throughout the insect orders, and so the origin of these cells and myoblasts remains unclear. Crossley (1964) demonstrated that an increase in phagocytic (Type F) haemocytes, often associated with muscle histolysis, at the time of puporium formation in Calliphora was traceable to changes in the differentiation pathway of the stem cells of the haemopoietic centres. No such accumulations of haemocytes have been found in the Auchenorryncha or could be detected in the present study.

In most cases the presumptive myoblasts accumulate in sites where no previous muscle rudiment could be detected, and in these cases it is tempting to suggest that they provide an example of the differentiation of haemocytes, which are acting as residual mesodermal cells, into a specialized muscular tissue. This would agree with the suggestion of Berlese (1901), that of Shatoury & Waddington (1957) working on Drosophila, and Wigglesworth (1959). In any event, the abdominal haemocoel of Graphocephala contains irregularly distributed haemocytes which, with the light microscope, cannot be distinguished from the myoblasts which aggregate around the earliest muscle strands.

In the present study two major types of myoblast incorporation are described (p. 190)which differ somewhat from descriptions in other insect groups. Incorporation by bipolar elongation is associated with the creation of a new muscle, while the second method occurs during the reconstruction of nymphal muscles. In the latter case the nymphal muscle nuclei are carried over into the adult muscle. This differs from the situation in Calliphera, as described by Crossley (1965), in which the larval muscle nuclei clearly undergo histolysis.

(e) The extent to which taxonomic differences in the gonapophyses are adaptive

Several studies have been made of the female genitalia of leaf-hoppers, of which the more important are the following: Kershaw & Muir (1922) and Brittain (1923) tried to determine the homologies of the genital structures between the male and female Auchenorryncha. In a detailed investigation of the genital structures in female leaf-hoppers, Readio (1922) examined 48 genera and concluded that characters of the second gonapophyses were among the most constant and useful for specific diagnosis. Balduf (1934) examined the distal region of the first and second gonapophyses in thirteen species of Empoasca and used the dorsal teeth of the second gonapophysis as one of his major characters. He described three types of dentition, the first as in E.

maligna, where the teeth are coarse and arranged in widely separated groups. The other extreme is shown by E. bifurcata and E. abrupta, where the teeth are similar in form and are usually small, though occasional large teeth become more numerous towards the distal end. The third type is commoner and intermediate. The dorsal edge of the second gonapophyses bears a continuous series of coarse teeth, the teeth themselves being serrated as found in Graphocephala. Unfortunately there are few behavioural studies that link structure with oviposition habits.

Wagner (1950, 1953) produced a similar study where he described the distal region of the gonapophyses in ten European species of Macropsis. Ribaut (1952), Young & Beirne (1958) and Nielson (1965) have all used gonapophyseal structures as taxonomic characters. Le Quesne (1965) used characters of the second gonapophyses to separate some species of Macropsis, though, as in most of these studies, the descriptions are brief and the diagrams only outline. Cheng (1964) also produced a study of the gonapophyses. Cunningham & Ross (1965) described several characters at the bases of the first gonapophyses and in particular they found that the rami was constant in shape for a particular species of Empoasca.

The external female genitalia have been used as a source of taxonomic characters in other groups where these structures have been described. In the Terebrantian Thysanoptera the ovipositor is well developed and saw-like, and though there are variations in the ovipositor structure it can not be linked to behaviour, however it is known that most species oviposit in plant material.

Limited functional information has been gained within the Odonata. The typical female genitalia, as seen in the Zygoptera, consists of three pairs of appendages which make up the ovipositor. The first and second gonapophyses are adapted for cutting, and together constitute the terebra, the third pair of appendages represents the gonoplac.

A similar type of ovipositor is found in the Anisoptera in the Aeshnidae and Petaluridae. St. Quentin (1962) was able to demonstrate that the different types of ovipositor are correlated with different modes of oviposition, and where the ovipositor is vestigial, eggs are laid on the water surface, while in those types which possess a well developed ovipositor eggs are deposited endophytically beneath the water surface.

Most work on the functional significance of ovipositor structure is available for the Symphata where Benson (1958) has used saw structure as important taxonomic characters. Though his approach is not functional, he provides information that permits structure to be linked to function. An examination of his data suggests that a simple saw pattern is associated with species that oviposit superficially or in comparatively soft material. Nematus umbratus has a weakly developed saw with poorly defined dorsal teeth and is thought to oviposit in Ribes. N. salicis, which possesses a well developed saw pattern and dorsal teeth, the oviposition site in this species is thought to be Salix.

In the present study the ovipositor structure of three species of leafhopper know to oviposit in substrates of differing hardnesses, were examined in some detail and shown that structural differences, from which useful taxonomic characters might be derived, could be associated with differences in oviposition behaviour, suggesting the adaptive value of taxonomic differences at or near the generic level. The most obvious characters were associated with the dorsal teeth of the second gonapophyses though the lateral rasps of the first gonapophyses also provided diagnostic characters which are indicative of the oviposition substrate.

(f) Sexual Communication and the role of substrate-borne vibrations within the Auchenorryncha. It is known that sounds are produced during the mating behaviour of some Heteroptera and Auchenorryncha other than Cicadidae (Claridge

and Reynolds 1973; Gogala 1970; Ossiannilsson 1949; Strübing 1958). It is known that Heteroptera produce the sounds by moving a moveable scraper over a stationary file while the Homoptera produce sounds by vibrating tergal abdominal timbals. It was suggested by Ossiannilsson that sounds were produced largely by the male of a species, though this has now been shown to be incorrect. He probably arrived at this conclusion because his material was captured in the wild, and hence receptivity of the females was not known. The present study and that of Ichikawa (1976; 1977) on Delphacids indicate that females emit signals only when receptive.

From the extensive sound recording made by Moore (1961) from Heteroptera and representatives of all families of Homoptera Auchenorryncha other than Cicadidae he suggested that auditory stimuli functioned over a short range, and he considered that visual or olfactory stimuli are of prime importance in bringing a sexually receptive pair together. While the release of a sex pheromone has been demonstrated in males of Nezara viridula (Mitchell & Mau 1971) and in other Heteroptera (Scales 1968) and Sternorrhyncha (Marsh 1975), sex pheromones have not been detected in the Auchenorryncha. The present study has indicated that visual and olfactory stimuli do not function in the sexual behaviour of G. fennahi. The observations of Claridge & Reynolds (1973) that males of Oncopsis will court other males suggests that males of this genus are unable to distinguish males from females visually. A similar observation was also recorded in Graphocephala.

The Cicadidae (except Tettigarcta) have well developed auditory organs while in the other Homoptera and Heteroptera possible receptors have not been reported (Leston & Pringle 1963; Pringle 1957). It was often assumed that sounds were transmitted either through air or water, though some early authors suggested transmission through a solid substrate (Autrum & Schneider 1948; Ossiannilsson 1949; Schneider

1950). It is possible that Johnston's organ in the second antennal segment may function as a sound receptor of air-borne sounds in the Auchenorryncha (Howse & Claridge 1970; Moore 1961).

In some species of Hemiptera faint sounds which are within the audio-frequency range are produced during the emission of vibratory signals (Claridge & Howse 1968; Ichikawa 1976), though in the present study acoustic signals were never detected from Graphocephala during sexual encounters. Ichikawa (1977), working on Nilaparvata lugens (Delphacidae) was able to demonstrate that sexually receptive individuals only responded to species-specific vibratory signals emitted by the opposite sex. A similar situation has been demonstrated in two species of Cydnidae (Gogala et al 1974) which also communicate via substrate borne vibrations. Wilcox (1972) has shown that sexual communication in the aquatic Gerridae is brought about through characteristic water-surface waves which are produced by leg movements in essentially the same manner that substrate borne vibrations are produced on Rhododendron by Graphocephala.

Considerably more work is required on the emission and reception of substrate-borne vibrations since it appears to be a widespread means of communication within the Hemiptera. In the isolated cases where such communication has been described there appears to be considerable variation in the mode of production, which individually produce the vibrations, and their sexual state during signal emission. It seems likely that this would be a rewarding field for future research, particularly if vibration emission could be linked to receptor sites and its role in epigamic behaviour analysed experimentally.

SUMMARY

1. A detailed morphological study was made of the external genitalia in G. fennahi, including a scanning electron microscopic study of the gonapophyses. The linkage mechanisms between the gonapophyses are described and rotation of the tongue and groove linking the first and second gonapophyses of each side is shown to reduce the freedom of movement between the pairs to a minimum.

2. An examination of the sensory structures of the gonapophyses and basal genitalia components was made, and it is suggested that the second gonapophyses possess both mechano- and chemoreceptors. Mechanoreceptors of the basiconic or trichoid type are also present on the second gonocoxa. It is probable that the sensory structures of the ovipositor function during oviposition site selection.

3. The gonangulum is shown to be an important sclerite in controlling ovipositor movement and is well developed in G. fennahi.

4. The external genital rudiments of the female are first apparent on the ventral surfaces of segments eight and nine during the early third instar. The rudiments of the second gonapophyses and gonoplacs arise by longitudinal division of the primary genital rudiment in the mid fourth instar (approximately 36 days old).

5. The gonocoxae arise anterodorsally to the bases of the anterior and posterior genital rudiment during the third instar, the rudiment of the second gonocoxa arising on the anterodorsal edge of sternum nine. In G. fennahi, the gonangulum was shown to originate on the anterodorsal edge of sternum nine immediately posterior to the second gonocoxal rudiment. Fusion of the gonangulum with the other basal genitalia occurred during the fifth instar.

6. The gross anatomy of the adult female reproductive system is described.

7. The posterior strands of the ovarioles of either side fuse during the second instar to produce the calyx and lateral oviducts which are therefore of mesodermal

origin. During the third instar the anterior portion of the posterior strand differentiates into the pedicel and prefollicular tissue. The median efferent duct is of ectodermal origin. The anterior extremity of the common oviduct rudiment divides during development and fuses with the lateral oviducts, the posterior extremities of which are therefore ectodermal. The common oviduct originates postembryonically from ampullar tissue and with the light microscope no intima could be detected in the adult female.

8. The germarium of the adult ovariole is divisible into four zones, Zone 1 being characterised by considerable mitotic division, while Zone 4 represents fully differentiated trophic tissue and the trophic core. Nucleic acids are lost to the trophic core by extrusion through the nuclear membrane and by nuclear decomposition.

9. Though histological evidence was obtained for active protein synthesis within the follicular epithelium, there was no indication that material is transported from the follicle cells to the oolemma.

10. Histological evidence suggests that blood proteins may be transported to the oolemma via the interfollicular spaces.

11. The ovarioles of G. fennahi possess an ovariole sheath composed of an outer layer of longitudinal muscle fibres with well pronounced striations. Internal to this is a loose network of longitudinal fibres in which are dispersed circular muscle fibres. In the anterior half of the germarium the sheath possesses an outer osmiophilic layer 250 nm in thickness. "Lumen cells" were present either within the tunic propria or the loose reticulum of fibres. They may be phagocytic in function. Considerable exocytosis of material was noted from these cells; the composition of these secretions is not known, but they may be involved in tunica propria formation.

12. The genital musculature preserves its segmental arrangement; that associated

with the first gonocoxa and gonapophyses is located within abdominal segment eight and the musculature of the second gonocoxa and second gonapophyses is restricted to segment nine. Little trace of the generalized abdominal musculature is present in segment eight, and segment nine contains only genital musculature. The massive musculature of segment nine indicates that the second gonocoxa is of major importance in initiating movement in the ovipositor.

13. The pregenital musculature can broadly be divided into dorsals, ventrals, laterals and oblique muscles. This pattern cannot be recognised within the adult genital region.

14. The basic nymphal musculature is already represented in the second instar and is composed of median dorsals, lateral dorsals, intersegmental laterals, ventrals and intrasegmental muscles. The musculature is metameric throughout the first eight abdominal segments. That of segment nine differs somewhat, dur to the reduction of segments ten and eleven. The nymphal muscles, particularly in the genital region, undergo a gradual change until, by the late fifth instar, a condition very similar to that of the adult is formed.

15. Myogenesis proceeds via four major pathways : (i) The massive reconstruction of an existing muscle by partial autolytic histolysis, the nymphal muscle nuclei being retained and free myoblasts incorporated. (ii) Muscles arise as new formations through the aggregation and differentiation of free myoblasts. (iii) Existing nymphal muscles may undergo longitudinal cleavage, the products being carried over, with little further change, into the adult. (iv) Muscles may undergo phagocytosis followed by reconstruction involving the incorporation of the nymphal muscle nuclei and free myoblasts. Myoblast incorporation may occur by the bipolar elongation of the cell and fusion with the muscle rudiment; this method is associated with the development of a completely new muscle. Myoblast nuclei and probably also the accompanying

cytoplasm may invade an existing muscle fibre.

16. The origin of the myoblasts remains unresolved by the present study, although it is suggested that they differentiate from circulating haemocytes or residual mesodermal cells, such as are present in association with the genital disks. No discrete haemopoietic centres were found in G. fennahi.

17. A morphological study of the ovipositor in three species of British Cicadellidae, Graphocephala fennahi, Ulopa reticulata and Macropsis scutellata, revealed that potentially useful taxonomic characters can be of adaptive significance. It is shown that the degree of sclerotization, the linkage mechanisms of the gonapophyses, the structure of the lateral rasps of the first gonapophyses and the dorsal teeth of the second can be both characteristic of the species or genus and also provide precise information regarding the oviposition habits of the insect. In G. fennahi, which oviposits in soft material, the "lateral rasps" are formed from weakly sclerotized fringed scales, which serve two functions : (a) the removal of plant debris during the cutting of the oviposition chamber; (b) the retention of the ovipositor within the oviposition chamber during egg-laying. In U. reticulata, which oviposits in very dense woody material, the rasps are heavily sclerotized and the ovipositor functions as a saw. The linkage mechanisms determine the freedom of movement of the respective blades and hence mode of action of the ovipositor. The reduced freedom of movement in the ovipositor of G. fennahi dictates that it functions as a single unit, the effective strokes resulting from posterior thrusts of the whole abdomen. Considerable freedom of movement occurs between the four blades in Ulopa, and the ovipositor functions by a sawing action.

18. G. fennahi displays a stereotyped courtship dance which always proceeds mating; the dance sequences are performed solely by the male. Sexual activity

shows a strong bimodal distribution over the day, linked to light intensity and temperature-dependent.

19. Sexual communication in Graphocephala is via substrate-borne vibrations which are emitted by receptive females. The signals can be divided into pre-courtship calling, which occurs in the absence of a male. This signal is produced in irregular bursts with a frequency of 30 cycles per second, and displays rapid attenuation. When a sexually active male is in close proximity and courtship has been initiated, the frequency of the signal falls to 20 cycles per second, and the peak characteristics change. This signal is produced throughout the courtship and may encourage the male to complete the dance successfully. Video-recording indicates that the vibrations are produced by drumming of the hind legs. The stylets are withdrawn from the plant during this process. This mode of communication has an effective range of at least 96 cms. on Rhododendron (leaf to leaf transmission via the stem).

20. The minimum duration of copulation required for a fertile mating is 1 minute. Inseminated females mate only once; after insemination vibrational signals are no longer produced and the female loses her sexual attractiveness. In the event of an unsuccessful mating, calling is resumed within 24 hours when the female again becomes sexually attractive. Virgin individuals of both sexes remain sexually potent throughout their life. Males are capable of multiple insemination.

21. Mating is initiated when the male and female are parallel and pointing in the same direction. The aedeagus enters the female by short thrusts, and the final coition position is achieved by the male rotating through 180° until the heads of the two insects point in opposite directions.

22. Oviposition site is selected by sensory structures on the labium and ovipositor.

A scanning electron microscopic study of the ovipositor in G. fennahi suggests the presence of both chemo- and mechanoreceptors on the gonapophyses.

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