STRUCTURE AND DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF SOME SPECIES OF NEMATOCERA (ORDER DIPTERA: SUBORDER NEMATOCERA)

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CONTENTS

The male and female reproductive systems in three typical species of Nematocera, order Diptera, representing the families of Chironomidae, Anisopidae and Mycetophilidae, have been studied and their development followed through the larva and pupa.

A description has been given of the parts in each case in the imaginal fly, and as seen in development and a nomenclature of parts adopted in accordance with the homologies ascertained.

In each case the basal plan is similar, but there is wide departure in the functional adaptations and form of the parts, so that without a study of the development the nature of some of the parts shown to be developmentally homologous would not be apparent.

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† No explanation of lettering abbreviations is given under the text-figures, and for this the list on p. 345 should be consulted.

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Briefly the reproductive system comprises in both sexes:

(*a*) A primary reproductive system consisting of a pair of gonads in the sixth segment with mesodermal strands forming the primative mesodermal oviducts and vasa efferentia and linking the gonads with the ectodermal efferent ducts.

(*b*) A secondary reproductive system of ectodermal origin which forms the greater part of both male and female efferent systems and which originates from hypodermal proliferative rudiments on the hypopygial segments and which may in various degrees replace by overgrowth the mesodermal ducts.

(*c*) Appendicular parts forming in the male clasping organs (gonocoxites) and gonapophysial organs (paramere lobes) and also intersegmental inflexions and sclerotizations such as the atrium and vaginal apodeme in the female and the theca, penis sheath and, when present, an eversible sac or endotheca in the male.

In the male recognizable homologous processes are the consecutive invaginations of the twin ejaculatory ducts and the median invagination of the penis tube, which later may be followed by a third intersegmental inflexion, that of the genital pouch excavating the penis and in *Anisopus* forming in its walls sclerotizations of the pompetta and piston apodeme.

In the female are three recognizable invaginations, that taking origin from the eighth sternite rudiments, which conjointly by their peripodial cavity form the vagina with the spermathecal and later the common oviduct invaginations, and that from the conjoined hypodermal buds on the ninth sternite forming the caecus (the accessory glands).

I. INTRODUCTION

The homologies and nomenclature of the parts of the reproductive system in the two sexes of the many forms of insects is a subject on which many different conclusions have been put forward by the many investigators who have dealt with it, so that it still forms one of the more controversial fields of research in biology. For the most part homologies have been assigned through study of the comparative anatomy of the parts, the alternative method of studying the post-embryonic development having been followed in relatively few cases, though in such cases in which this method has been followed it has formed a very helpful line of approach. In undertaking research on the reproductive system in the two sexes in Nematocera it was planned therefore to study in some representative forms not only the adult structures but to follow the development as seen in the larva and pupa. For this purpose both sexes of three species from different representative families of the suborder have been studied. These are:

(1) *Chironomus dorsalis*: family Chironomidae (Harlequin fly).
(2) *Anisopus fenestralis*: family Anisopidae.
(3) *Mycetophila cingulum*: family Mycetophilidae (Fungus gnat).

Two other species of the genus *Mycetophila* have been made use of for a special reason which is mentioned later; these are *M. lineola* and *M. fungorum*.

Each description starts with an account of the adult organs, as it was felt that this makes it easier to follow the development of the parts in the immature stages. The development of each sex is then followed through the larval and pupal stages. In each of the stages the organs are dealt with under two heads, the primary reproductive system and the secondary reproductive system. These definitions were first used in insects by Escherich (1892), then applied by Lowne (1895) and recently have come into general use. The primary reproductive system in insects includes the germ cells and their mesodermal coat with its backward extensions as the mesodermal strands. The secondary reproductive system includes those structures which arise from the hypoderm (ectoderm).

II. DEFINITIONS

The following definitions of named parts are applicable to all three forms studied, and their use will add greatly to precision in the descriptions of the parts given later. No special claim is made as to wide applicability of the terms used or their eventual suitability, those terms being employed which are now in more or less common use, such as gonocoxite, paramere, etc. The name theca has been used by Wesché (1906) for this structure in the Tipulidae, and as it was an early and appropriate name we have followed that author. For a recent review of the nomenclature of these parts, as based almost entirely on comparative studies, see Crampton (1942). For a recent account of the parts in *Panorpa*, order Mecoptera, see Ferris & Rees (1939), who figure two-jointed cerci and cercal base (our tenth tergite) as also present in some Diptera.

The Female

In the group dealt with the abdomen is composed of ten segments, the ninth and tenth of which are subjected to certain modifications. The ninth sternite is greatly reduced, and its only remnant is a small hairy and slightly sclerotized plate which corresponds to the *insula* of Christophers in mosquitoes. The tenth segment surrounds the anal opening in the shape of procts, the paraprocts (tenth tergite) and hypoproct (tenth sternite).

The atrium. The atrium is a depression, which corresponds to the genital cavity (pouch or chamber) in other insects. It occupies the interspaces between the eighth and ninth, and between the ninth and tenth sternites. This position of the atrium brings the insula (ninth sternite) to lie in the middle of the atrial cavity dividing it into two sections: an anterior or vaginal and a posterior or caecal section. The first consists of the short wide vagina receiving the opening of the common oviduct and of the spermatheca, while the second receives the opening of the caecus (corresponding to the accessory glands of many insects). The insula is so adjusted as to act as a valve between the two sections of the atrium, closing one and opening the other according to the different positions it takes.

The subgenital plate. This is the eighth sternite which covers the genital area ventrally, and by its posterior margin bounds the entrance of the vaginal section of the atrium.

The subgenital apodeme. This is an inflected plate at the posterior border of the eighth sternite which lies between the inner surface of this sternite and the vulvar opening to serve for the attachment of some hypopygial muscles.

The vagina and the vaginal apodeme. The vagina is a short intersegmental inflexion which is tucked in between the insula and the eighth sternite and forms a vestibule for both the vulvar and spermathecal openings. The vaginal apodeme is a special T-shaped sclerotization which is pushed forward from the wall of the vagina so that its body rests on the dorsal wall of the common oviduct and its arms rim the upper entrance to the vaginal section of the atrium.

The gonapophyses. These do not exist in any form in the studied species.

The ovarian investment. The outer sheath of the ovary is a non-cellular membrane which judged by its origin from the coelomic wall, I shall call in this work the peritoneal membrane. The wall of the ovariole tubes is here termed the *tunica propria*.

The mesodermal strands and the mesodermal oviducts. The first term is applied to the mesodermal cords which extend from the posterior ends of the gonads in the immature stages. The second term is applied to the ducts in the adult stage which are derived from the first-mentioned structures.

The common oviduct and the ectodermal oviducts. The first name is given to the duct which arises as a hypodermal invagination connecting the lateral oviducts to the exterior. The common oviduct may divide anteriorly before making connexion with the mesodermal oviducts, and in this case these branches are differentiated from the latter by the special term ectodermal oviducts.

The caecus. This is a term which was first applied by Christophers to signify the accessory glands in mosquitoes. It is a sac-shaped structure which may be slightly or deeply emarginated, or even divided into two equally sized terminal lobes. It has a single aperture which opens into the posterior section of the atrium. The caecus reaches a huge size in the aquatic midge *Chironomus*, is of medium size in the semi-aquatic *Anisopus*; and of small size in the terrestrial species of *Mycetophila* studied.

The male

The abdomen of the male fly is also composed of ten segments, the ninth forming the genital segment and attaining, more or less, an annular shape by the interpolation of the gonocoxites in the pleural region.

The subgenital plate. This is the ninth sternite or the hypandrium of some authors. It varies in size according to the development of the gonocoxites. It is a narrow strip in *Chironomus* with huge separate gonocoxites, of moderate size and partially amalgamated with the bases of the gonocoxites in *Anisopus* and of considerable size with relatively small gonocoxites in *Mycetophila*.

The gonapophyses. The interpretation of Pruthi (1925) concerning the origin of the gonapophyses in the male insect has proved in the present work to be the most satisfactory. The claspers or forceps of the nematocerous species studied are composed of basal parts which represent the gonocoxites and terminal parts which are the styli. There is a varying degree of amalgamation between the gonocoxites and the ninth sternite. The paramere lobes of Pruthi, in the species of Anisopidae and Mycetophilidae studied, divide to give rise to the penis valves (forming the *aedeagus*) and the parameres. In both cases the two pairs of appendages are small in size and do not take an effective part in the work of the intromittent organ. In the case of *Chironomus* the paramere lobes do not divide but form a large pair of club-shaped parameres. In this state of affairs I propose to use the term paramerophore for the undivided paramere lobes, a parallel term to that of aedeagophore of Pruthi.

The intromittent organ. In the present work two parts of the intromittent organ have been recognized according to their origin and mode of development. The distal section of the male efferent duct which is terminated by the gonopore is here termed the *penis tube*, and when it is dilated the *penis cavity*. This develops as a single median invagination posterior to the ejaculatory duct rudiments and quite independent of them. The posterior end therefore forms the gonopore, whilst the anterior end receives the two ejaculatory duct invaginations.

The outer surface of the intromittent organ is the cuticular coat which, surrounding the penis tube, forms a protrusive body in which the penis tube lies. This cuticular coat develops in different ways and from different structures, and hence the term applied to the protrusive organ must be related to the origin of its coat. When the coat is formed by the intersegmental membrane behind the ninth sternite we have termed the intromittent organ *theca*, following Wesché. The theca may be tucked inside the penis cavity, thus forming an *endotheca*, or it may be inflected into the body cavity forming a *penis sac*. Cuticular thickenings of thecal origin which may form the thickened coat of the protrusive organ (*Mycetophila*), or help in the eversion of the endotheca (*Chironomus*), or serve to guide the long filamentous penis at its exit from the penis sac (Anisopidae) we term the *penis sheath*.

Another origin of the intromittent organ coat is from the coalescence of the penis valves of the gonopophyses. With this appendicular coat the intromittent organ becomes the *aedeagus*.

It is then a matter of great importance to distinguish between the aedeagus, which is of appendicular nature, and the intromittent organ of thecal structure formed from intersegmental sclerotizations, as also to recognize the penis tube which is a special hypodermal invagination in its origin.

The testicular investments. The outer testicular sheath is here called, as in the female, the peritoneal membrane, a term which is preferred to the term basement membrane used by recent writers. When the testicular coat is of cellular structure it is called the peritoneal epithelium.

The mesodermal strands, vasa efferentia and vasa deferentia. The mesodermal cords which extend from the posterior ends of the male gonads are called, as in the case of the female, the mesodermal strands. When these strands change into tabular structures carrying the sperms from the gonads to the secondary reproductive efferent system they are then termed the vasa efferentia. On the other hand, the anterior branching of the ejaculatory ducts which join the mesodermal vasa efferentia are distinguished as the vasa deferentia.

III. MATERIAL AND TECHNIQUE

The specimens of the three nematocerous species were collected in the very young larval stage from their natural habitat, mostly from the vicinity of Cambridge, and reared throughout in the laboratory to provide the later stages up to the adult. Some of the anisopids were collected from the rotten beetroots of the sugar factory at Ely. The three mycetophilid species were all collected from the fungus *Polyporus squamosus*, the larger larvae of *Mycetophila fungorum* appearing during spring and throughout the summer, whilst the smaller larvae of *M. cingulum* and *M. lineola* appear later in autumn.

It was easy to identify the two familiar species of Chironomidae and Anisopidae, but the need for help was felt to identify and distinguish the three mycetophilid species, which was gratefully offered by Mr P. Freeman at the British Museum.

The material was dealt with in two ways:

(1) *Dissection.* An attempt was made to dissect as many different stages of each species as possible and to preserve a number of such dissections by permanent mounting. This was

achieved by carrying out the dissection on a piece of black rubber fixed on the waxy layer of the dissecting dish. Dissection was made with two very fine needles, a pair of fine scissors and the aid of a binocular microscope. The body wall of the animal, as well as the different organs of the dissection, were pinned carefully, and the piece of rubber which carried the fixed dissection removed from the dish to diluted acid fuchsin stain with a few drops of acetic acid. The tissues were stained in a few seconds and the excess stain then washed off. The dissection was dehydrated by carrying it with forceps through the alcohols to absolute and then cleared in benzene and mounted in canada balsam. Most of the specimens were dissected after being preserved in Pampel's fluid for a few days. This helps in showing the parts, especially the hypodermal buds, and makes it easier to remove the fat bodies.

(2) *Sectioning.* The greater part of the present work has been done through sectioning the different stages of each species. Owing to the difficulty of defining the exact instars of the species studied, an extra effort was made to cut sections through several specimens believed to be of the same age. These extra sections proved later to be of great help, as it came to be realized that the development of the organs does not run exactly the same in equally aged individuals.

The method used for cutting and staining sections was as follows:

Living specimens were killed in Pampel's fluid. This agent kills and fixes the tissues gradually and slowly and does not cause the severe shrinking observed when using strong fixatives such as Carnoy's. It also permits the animal to be stretched as required, which is very important, as any twisting would have spoilt the symmetry of the sections. Specimens were left in Pampel's fluid until needed, when the desired portion of the abdomen was cut and transferred to strong fixative, alcoholic Bouin, where it was left for 2 to 4 hr.

The material was then dehydrated and transferred to a solution of 1 % celloidin dissolved in methylbenzoate and left there until it became quite transparent.

Washing with benzene followed. Then thin flakes of wax, m.p. 52° C, were added to the benzene and left for 1 hr. The object was finally embedded after three successive baths of paraffins, the first a soft paraffin of m.p. 52° C, the second of 56° C and the third a mixture of new paraffin of 56° C and a hard old paraffin of 60° C m.p. This mixture gave very successful results.

The triple staining method of Ehrlich's acid haematoxylin, eosin and picric acid was used with good result, the cuticular wall being stained yellow, the cytoplasm of the cells red and their nuclei blue. Mallory's triple staining method was also used with some success, but its great defect is that it does not show cellular structure as does the first-mentioned method.

In spreading the ribbon on the slide distilled water with traces of albumen and diacetin was used and gave good results as a spreading and fixing agent. The slides were put on an electrical hot plate, and while the rows of ribbon were stretching on the slide they were adjusted to help them form straight lines with two needles. Diacetin to a great extent helps to stretch the folded parts of sections which might fail to do so without its use. No blotting paper was used to dry the sections on the slide, but excess spreading fluid was allowed to run to the edge of the slide and removed, the slide being then left to dry on a sheet of blotting paper laid on the hot plate.

IV. LETTERING OF THE DRAWINGS

a.sh.	anal shield
aed.	aedeagus
al.ca.	alimentary canal
ap.pl.	apodemal plate
at.	atrium
b.ap.	basal apodeme
c.od.	common oviduct
ca.	caecus
ca.l.	caecal lobe
ca.o.	caecal opening
cl.	claspette
c.ms.	circular muscles
cox.	gonocoxite
d.b.	dorsal bud
e.d.	ejaculatory duct
end.th.	endotheca
e.ep.	ectodermal epithelium
f.c.	fat cells
fol.c.	follicular cells
fol.ep.	follicular epithelium
g.	gonad
g.b.	genital bud
g.c.	germ cells
g.p.	genital pouch
gl.c.	glandular cells
hist.ep.	histolytic epithelium
hyp.	hypoderm
im.	imaginal
im.d.	imaginal disk
ins.	insula
l.b.	leg bud
lat.b.	lateral bud
m.od.	mesodermal oviduct
m.st.	mesodermal strand
ms.	muscles
my.c.	myoblastic cells
n.c.	nurse cells
o.d.	oviduct
ooc.	oocyte
oog.	oogonium
ov.	ovary
p.ap.	penis apodeme
p.c.	penis cavity
p.sac.	penis sac
p.sh.	penis sheath
p.t.	penis tube
p.v.	penis valve
pd.c.	peripodial cavity
pd.m.	peripodial membrane
pf.hyp.	proliferative hypoderm
pist.ap.	piston apodeme
pist.h.	piston hood
pp.	pompetta
pr.	paramere
pr.l.	paramere lobe
pt.ep.	peritoneal epithelium
pt.m.	peritoneal membrane
r.	rectum
s.	spermatheca
s.d.	spermathecal duct
s.g.ap.	subgenital apodeme
s.p.	spermatheca proper
sg.b.	segmental bud
sp.	spermatozoa
spc.	spermatocyte
spg.	spermatogonium
spr.	spiracle
sty.	style
t.	testis
t.b.	tegular bud
t.f.	terminal filament
th.cav.	thecal cavity
th.ap.	thecal apodeme
tu.pr.	tunica propria
v.	vagina
v.ap.	vaginal apodeme
v.b.	ventral bud
v.d.	vas deferens
v.e.	vas efferens
v.s.	vesicula seminalis
vl.o.	vulvar opening
w.b.	wing bud

N.B. Roman numbers are used to designate tergal sclerites and arabic numbers sternal sclerites. (*r.*) preceding the abbreviated lettering for any part signifies the rudiment of that part, and (*inv.*) following the abbreviated lettering signifies its invagination.

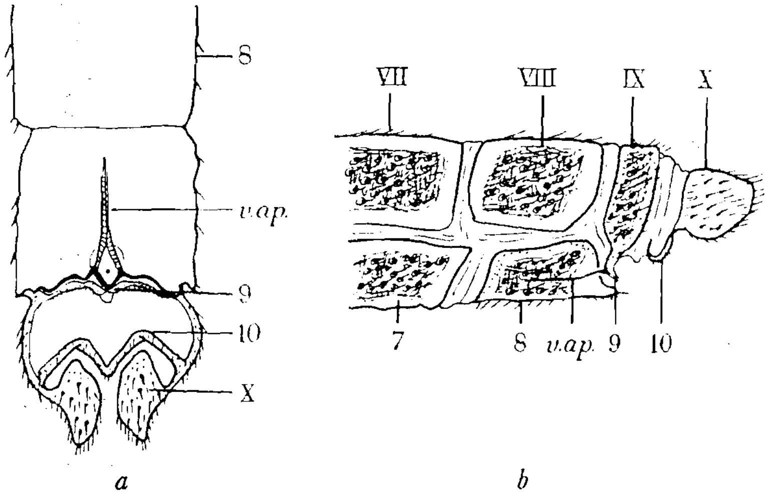
V. Family CHIRONOMIDAE (*Chironomus dorsalis*)

1. THE FEMALE

(*a*) *Structure of the reproductive system in the adult*

(i) *The segmental parts*

The hypopygium. The abdomen of the fly consists of ten segments, the tergal and sternal plates of which are easily recognized, except for the ninth and tenth sternites which have become greatly atrophied. The tendency of these sternites to become membranous excep for very narrow strips is associated with telescoping of the terminal portion of the abdomen The apparent tenth tergite terminates in two flap-like portions, which, as in related forms probably represent the cerci (see under §II, Definitions).



a *b*

FIGURE 1. The distal abdominal segments of the female adult *Chironomus dorsalis*: *a*, ventral view; *b*, lateral view.

The vaginal apodeme is of the shape and in the position described under definitions. Its arms, along with the insula, join the sides of the ninth tergite (figure 1, *v.ap.*). The vaginal and caecal sections of the atrium are separated by the median projection of the insula (ninth segment). At the entrance of the vulvar opening are two small hairy elevations which act as an egg guide.

(ii) *The primary reproductive system*

The ovaries. The two cigar-shaped ovaries (figure 2) extend from the fourth abdominal segment to the seventh segment. An old ovary appears as a thin membranous sac crammed with equally developed eggs. A cross-section in such an ovary shows that the eggs lie side by side with but very slight traces of follicular septa. In a younger ovary there is present in its middle a narrow cavity surrounded by a thin follicular layer. A longitudinal section of the ovary of a newly emerged fly shows very clearly this central cavity along the core of the ovary, and that follicular septa pass between the central tube and the peritoneal membrane enclosing the ova in separate ovarioles.

An ovarian egg is oval in shape and contains a large amount of yolk, which in the old egg occupies most of its volume, while the nurse cells are relatively small and externally situated. It is easy to distinguish the follicular coat around the young ovum before secretion of the chorion is completed.

The oviducts. It is very difficult to distinguish any tubular connexion between the ovary and the common oviduct such as could be called an oviduct (figure 2). Cross and longitudinal sections show that at the distal end of each ovary there is an aggregation of mesodermal epithelial cells which connect the ovarian sac with one side of the proximal end of the common oviduct. This aggregation of cells may represent the only remains of the mesodermal oviduct in the unfertilized adult, and it closes the passage between the ovary and the common oviduct until the time of oviposition.

(iii) *The secondary reproductive system*

The vagina and the common oviduct. The efferent system consists of the wide tube of the common oviduct which extends along the eighth abdominal segment and joins the short vestibule of the vagina at the posterior border of the eighth sternite. The epithelial wall of the common oviduct is thin in most of its course, and in some parts it lies very close to the body wall formed by the eighth sternite. Its most proximal portion is rather thick walled where the two ovaries join at its sides. This fact suggests that the cells at the proximal end retain their capacity to divide in order to fuse with the distal ends of the ovaries and to replace the last remnants of the mesodermal oviducts when these are removed to free passage for the eggs.

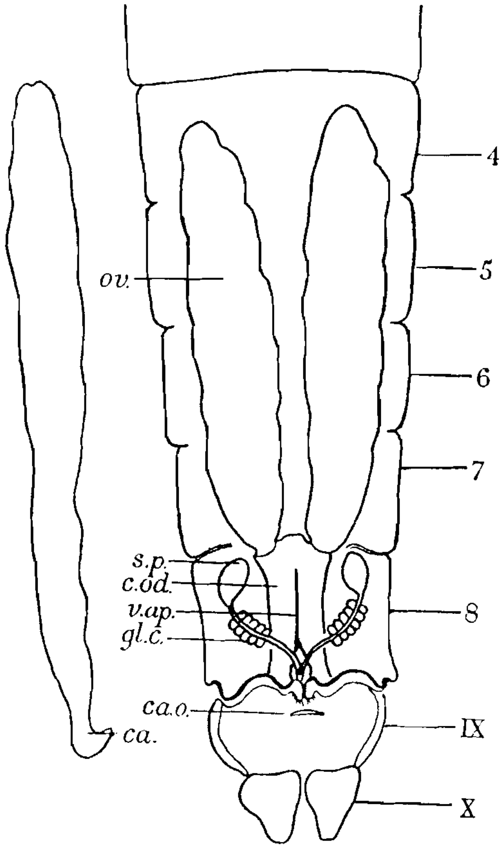


FIGURE 2. Showing dissection of the reproductive system in the female adult *Chironomus dorsalis*

The spermatheca. There are two spermathecae which are almost independent of each other (figure 2) except at the very distal end, where they discharge by one opening. This opening is situated on a little nipple-like protrusion on the dorsal wall of the vagina, slightly posterior to the vulvar opening and separated from it by the vaginal apodeme. The middle part of each of the spermathecal ducts is surrounded by a number of enormous glandular cells which cluster round the spermathecal duct in a mulberry-like fashion. It is obvious that these cells have a secretory function, as shown by the vacuoles at their inner ends and by their proportionally large nuclei, as also by the existence of secretion inside the duct where these cells embrace it.

The spermatheca proper is an oval-shaped sac with a thick inner cuticular layer surrounded by a thin degenerating epithelial layer. The secretion of the glandular part of the spermathecal duct is passed upwards to be stored in the sac of the spermatheca proper. No traces of this secretion could be detected in the basal non-glandular part of the duct before copulation has taken place.

The caecus. This is a long sac (figure 2) which extends between the fourth and ninth abdominal segments. It opens through a slot-like opening, which is wide laterally and narrow dorso-ventrally, into the posterior section of the atrial cavity. This shape of the caecal opening permits its excretion to flow in a film-like manner over the insula, which in turn allows it to spread evenly over successive eggs as they issue rhythmically from the vaginal section of the atrium.

Comparison of the structure of the caecus in two flies of different ages shows that this organ does not reach its highest secretory activity until some time after the emergence of the fly. In the young fly the wall of the caecus is very thick and the cavity is proportionately small, though containing some secreted substance. In an old fly the wall becomes thin following exhaustion of its glandular cells, leaving the space these have occupied to increase the cavity of the gland as this becomes increasingly filled with secretory substance.

(*b*) *Development in the larva*

(i) *The segmental parts*

The abdomen of the larva consists of nine tubular segments terminated by a small pseudo-segment which is continued into two abdominal feet resembling those of a caterpillar. The anal opening is surrounded by two pairs of anal lobes, and on the ventral surface of the eighth segment are two other pairs of long thin tubes which are believed to act as blood gills.

(ii) *The primary reproductive system*

The two gonads of the young larva are thin banana-shaped bodies (figure 3) which lie in the sixth abdominal segment at the sides of the alimentary canal. The terminal filaments are long and end at the posterior border of the third abdominal segment.

Oogenesis during the larval stage starts with small round undifferentiated germ cells. At a later stage the middle of the gonad becomes occupied by a smaller type of cell, while near the periphery round oogonia, each composed of several larger cells, are found. In the fully grown larva it can be seen that the small cells are becoming the follicular cells. They arrange themselves around a central cavity, linking this with the oogonia by short stalks. The true oocytes have not as yet become distinguishable from the other cells of the oogonia (figure 4).

The investment of the gonad at no stage has been observed to be formed of a definite cellular layer. In the young gonad it is even hard to distinguish any layer at all around the compact mass of germ cells. But as cavities appear among the cells a thin peritoneal membrane becomes apparent and is maintained to the end of development of the ovary as the peritoneal membrane.

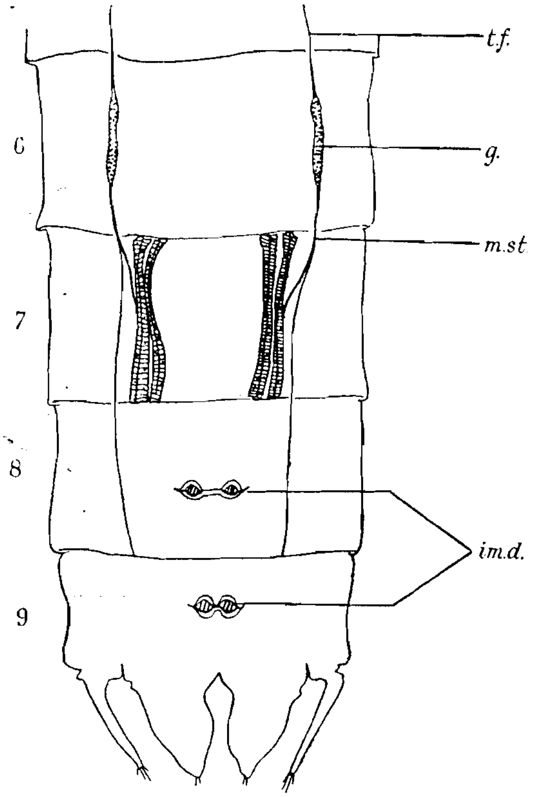


FIGURE 3. Dissection of the female larva of *Chironomus dorsalis* showing the gonads, the mesodermal strands and the genital buds.

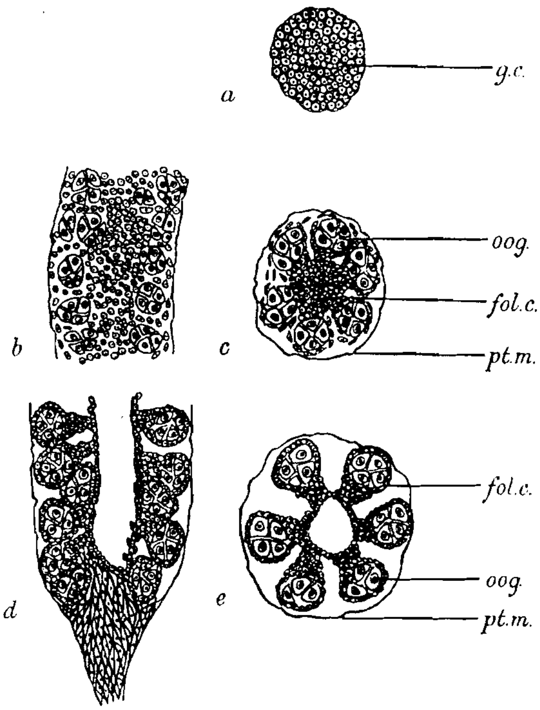


FIGURE 4. Sections through the gonad of the female larva of *Chironomus dorsalis*. *a*, transverse section through the gonad of a young larva; *b*, *c*, longitudinal and transverse sections through older larval gonads; *d*, *e*, longitudinal and transverse sections through the gonad of a fully grown larva.

The mesodermal oviducts. The mesodermal strands which extend from the posterior ends of the gonads show a feature of great interest. Each strand consists of a short solid cord, which ends in the middle of the seventh sternite at the side of the longitudinal ventral muscle bundles, and a long thin filament which extends to the anterior border of the ninth sternite (figure 3). This latter filament was overlooked in dissection and in sections until the time came to study the male. In this latter sex the branching of the mesodermal strands is more obvious, and on returning to examination of the female the branching condition was also disclosed. The long filament in the case of the female is very thin and quite inconspicuous. At the prepupal stage it perishes leaving only the short strand. This decreases in length as development of the gonad extends posteriorwards at the expense of the cord.

(iii) *The secondary reproductive system*

Dissection of a young female larva reveals two pairs of hypodermal buds on the mid-ventral area of the eighth and ninth abdominal segments (figure 3). Those on the eighth segment are the spermathecal rudiments, while those on the ninth segment are the rudiments of the caecus.

In their early stage the spermathecal buds appear as two small thickened spots of hypodermal cells quite separate from each other. In sections each bud shows as a dense solid elevation caused by proliferation of the hypodermal cells in the area (figure 5*a*). Then each bud acquires a peripodial cavity and approaches the mid-ventral line where the two cavities unite. Fusion of the two peripodial cavities takes place from before backwards, so that in one section the two cavities are still apparent, whilst in a section a few microns in front they are fused into one cavity. Each peripodial cavity carries on its dorsal aspect a small knob, which represents the first sign of the spermathecal invagination (figure 5*b*). In a more advanced larval stage all traces of partition between the two spermathecal buds disappear, and one cavity only exists on the dorsal wall of which are the two spermathecal invaginations which have now extended a little further anteriorwards.

Between the two spermathecal invaginations and very slightly in front of them a median invagination for the first time appears. This is the rudiment of the common oviduct.

In a longitudinal section through the larva at this stage it can be seen that the primitive peripodial cavity of the conjoined spermathecal buds is now acting as a vestibule into which open the rudiments of the spermathecae and of the common oviduct. This vestibule is the rudiment of the vagina (figure 6*a*).

In the fully grown larva the separate openings of the spermathecal invaginations join into one outlet, and each rudiment can be differentiated into a slightly swollen head and a stem. The common oviduct invagination by this time has increased considerably in length.

When the larva reaches the prepupal stage the common oviduct and the spermathecae have attained, to a considerable extent, the imaginal shape. The former has reached the border of the seventh segment and the latter have become completely differentiated into spermathecae proper and the spermathecal ducts. The peripodial cavity has now become modified to form the tubular-shaped vagina. This change in the shape of the peripodial cavity brings the vaginal opening more posteriorwards and nearer to the opening of the caecus (figure 6*b*). The dorsal wall of the vagina becomes thickened, giving the first indication of the formation of the vaginal apodeme.

The second pair of imaginal buds also appear in the young larva as two separate disks, but they are nearer to each other and to the mid-ventral line than are the spermathecal rudiments. Sections which show the spermathecal rudiments as solid knobs of hypodermal cells show, at the same time, the caecus rudiments with definite peripodial cavities, a sign of a later stage of development (figure 5*c*), this indicating that caecus rudiments appear

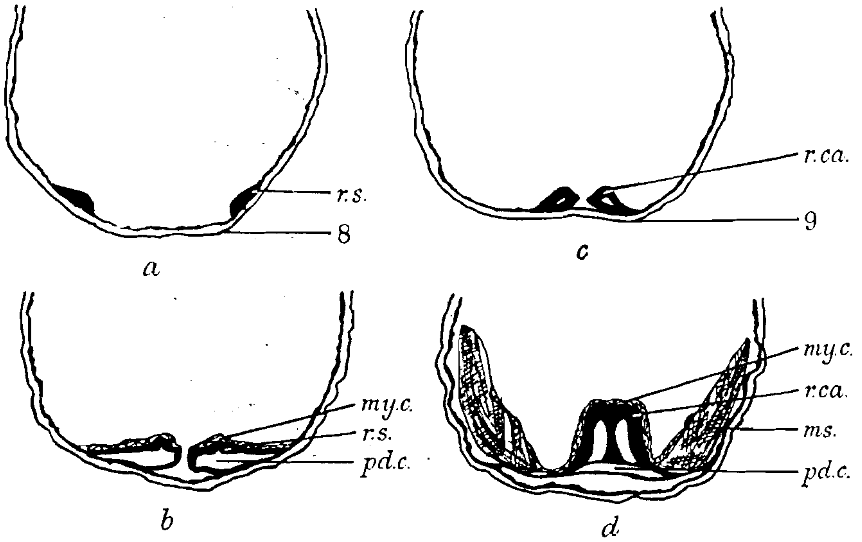


FIGURE 5. Transverse sections through the hypoderm of the eighth and ninth abdominal segments of the larva of *Chironomus dorsalis* showing: *a*, the rudiments of the spermathecae; *b*, the same rudiments in an older stage; *c*, the rudiments of the caecus; *d*, rudiments of the caecus in an older stage.

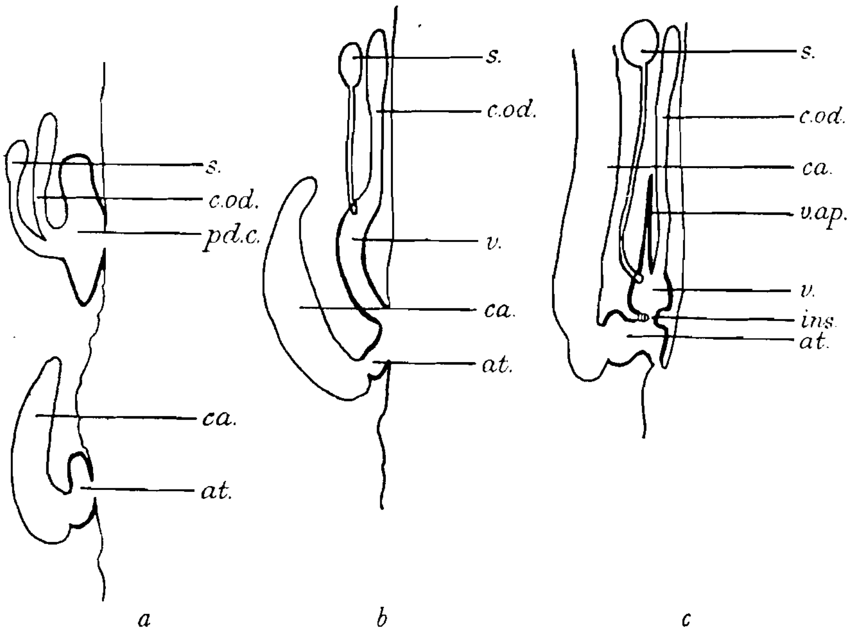


FIGURE 6. Diagrammatic scheme of the development of the female genital hypodermal rudiments in *Chironomus dorsalis*: *a*, in the larva; *b*, in the prepupa; *c* in the pupa. As seen in longitudinal median section.

earlier than those of the spermathecae, a fact confirmed by the very young female larva possessing the rudiments of the caecus on the ninth sternite and lacking any sign of spermathecal rudiments on the eighth sternite.

The two separate buds of the caecus unite in the middle line forming one cavity with a vertical division. In more advanced larvae all signs of double construction disappear, except near the opening of the invagination, which still shows a division in its cavity.

Little change later takes place in the shape and construction of the caecus, except that it becomes longer and its wall thicker. In some sections (figure 5 *d*) a mass of myoblastic cells can be seen running between the outer surface of the caecus rudiment and the nearby muscles. No traces of secretion are present in the caecal cavity during the larval stage. The peripodial cavity of the caecus rudiment changes in the prepupal stage into the caecal section of the atrium, which is separated from the vaginal portion as previously described by a hypodermal fold (formed by joining of the peripodial walls).

Other changes occur giving rise to appearance of segmental parts. The larval ninth sternite disappears completely, and the vestigial insula which will develop anew on the hypodermal fold between the two sections of the atrium does not as yet show in the larval stage. The tenth tergite develops from a pair of hypodermal proliferative spots which appear inside the rectum during the prepupal stage. The hypoproct also arises from a new proliferative area behind the caecus invagination.

(*c*) *Development in the pupa*

(i) *The primary reproductive system*

The following facts can be ascertained by following the development of the ovary in the pupa:

(1) The ovary has been continuously extending, mostly towards the anterior segments along the line of the terminal filament. In the old pupa it reaches to the fifth abdominal segment anteriorly and to the middle of the seventh segment posteriorly.

(2) The ova have arranged themselves around a central tube, which is formed by the compact arrangement of previously scattered follicular cells. These latter gradually cease to be dispersed among the ova and concentrate around the central tube.

(3) A definite follicular layer surrounds each oogonium which continues to be connected with the follicular central tube for some time and separates later in the old pupa.

(4) A special cell at the base of the oogonial group becomes distinguishable as the true oocyte. It has a clear round nucleus with a dark central nucleolus. The nucleus of the oocyte soon disappears when the yolk formation is initiated, transforming the body of the oocyte into a homogenous finely granulated substance. Eosinophile spheres begin to appear of different sizes and they increase in number as the oocyte increases in size.

(5) The cells which form the rest of the oogonium fail to show any sign of their previous cellular structure once the animal enters the pupal stage. They now come to form a nutritive mass which has the appearance of a huge cell, since it has a central deeply stained body which might be taken for a nucleus. As yolk formation starts in the oocyte the dark-staining central mass becomes pale and granular, giving it the appearance of newly formed yolk rather than of a nucleus. The explanation of these appearances would appear to be that the nutritive cells have joined into one syncytial mass and the yolk material formed

transferred to the oocyte, the nutritive mass decreasing in size simultaneously with increase of the oocyte yolk (figure 7).

The mesodermal oviducts. In the pupa the shortening of the oviducts continues with the extension of the ovaries backwards until the distal ends of the ovaries fuse with the proximal part of the ectodermal common oviduct. By this time the mesodermal oviducts have come to form merely the epithelial plugs which lie between the ovaries and the common oviduct.

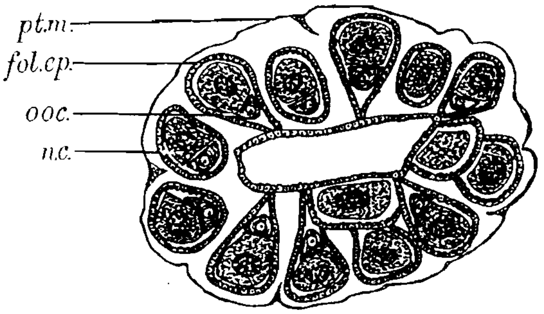


FIGURE 7. Transverse section through the ovary of the pupa of *Chironomus dorsalis*.

(ii) *The secondary reproductive system*

Owing to the short lifetime of the pupa the reproductive system reaches an advanced stage of development during the larval stage, so that in the short pupal stage the organs merely complete their change into the imaginal state.

The common oviduct. In the pupal stage the common oviduct extends along the whole length of the eighth sternite to the posterior part of the seventh sternite. The portion of duct running through the eighth segment is narrow laterally and rather wide dorsoventrally. This arrangement is reversed in the proximal part of the duct which lies in the seventh segment. This latter portion apparently takes the place of the two oviducts in other insects, receiving eggs from the ovaries of the two sides and passing them singly to the posterior part of the duct.

Posterior to the vulvar opening there lies the short vestibule of the vagina, on the ventral wall of which appear the two hairy elevations of the egg guide. Between the spermathecal and vulvar openings the wall of the vagina is tucked in to form an accessory pocket which lies over the dorsal wall of the common oviduct (figure 6 *c*, *v.ap.*). The wall of this pocket secretes the vaginal apodeme. The vaginal section of the atrium is limited posteriorly by the intervention of the fold of the insula. This latter is attached to the posterior border of the vaginal apodeme, having a median flap which hangs inside the atrial cavity.

The spermathecae. The changes occurring in the spermathecae during the pupal period are the appearance of muscles and of glandular cells around the ducts. The myoblastic cells have arranged themselves around the spermathecal ducts into a definite layer of longitudinal muscles. This layer of muscles seems to be disturbed in the middle section of the spermathecal tube where the huge glandular cells are attached. These latter are specialized epithelial cells which grow considerably in size and push their way out through the muscular layer.

The caecus. This organ grows greatly in length during the pupal period. In an old pupa it reaches the fourth abdominal segment and lies dorsal to and between the two ovaries. The glandular wall of the caecus increases in thickness considerably, so that it leaves only a very narrow central space. It is not until near the hatching of the fly that the fine elements of secretion can be detected at the inner periphery of the glandular cells.

2. THE MALE

(*a*) *Structure of the reproductive system in the adult*

(i) *The segmental parts*

The hypopygium. The ninth tergite is a triangular plate with its lateral angles curved ventrally and its pointed end directed backwards. The tenth tergite is firmly joined to the ninth tergite and has the form of a bird's beak. There are no indications of any structures suggesting cerci (figure 8 *a*). The ninth sternite is a narrow ribbon which joins the sides of the ninth tergite to form a complete annulus (figure 8 *b*). The tenth sternite is a frame-like plate which has three cuticular sides and a membranous middle area (figure 8 *c*).

The gonapophyses. There are two gonocoxites of considerable size with two sickle-shaped styles. At the inner side of each gonocoxite there is a very conspicuous rod-shaped paramerophore which has a tuft of strong hairs at its distal end (figure 8*b*). Beside these podial appendages there is a small pair of hook-shaped processes at the points where the gonocoxites, paramerophores and tenth sternite join together. I shall call these small hooks the claspettes.

The intromittent organ. The intromittent organ consists of a short tube, the penis cavity, into which a membranous endotheca is folded. At the sides of the endotheca there are two thin strips of elastic cuticle which, when the organ is at rest, are directed forwards and are jointed posteriorly with the claspettes of the gonocoxites. These cuticular strips, the penis sheath, when affected by muscular tension at their basal joints, cause the folded endotheca to be eversed (figure 8 *c*, *d*).

(ii) *The primary reproductive system*

The two spindle-shaped testes in the newly emerged fly lie in the sixth abdominal segment (figure 9). In the old fly they are dragged backwards, as a result of the shortening of the vasa efferentia, to lie between the seventh and eighth segments. Each testis is full of sperms which are dispersed at random inside the organ. Small round degenerate cells are present among the spermatozoa.

The vasa efferentia. In a young fly there extend from the posterior ends of the testes two thread-like vasa efferentia which reach to the posterior border of the eighth segment where they join the ends of the two vasa deferentia (figure 9). Gradually these long vasa efferentia decrease in length, dragging with them the two testes, until in the old fly these are brought back as far as the eighth segment. This shortening of the mesodermal vasa efferentia adds to the validity of the view that ectodermal replacement from the vasa deferentia takes place in the old fly. The wall of the vas efferens is thin, and it is difficult to detect any cellular structure, except for scattered fine dark spots which may represent nuclei of degenerate cells.

(iii) *The secondary reproductive system*

The ectodermal genital tract can be divided into five regions, according to their structure and function, as follows.

The penis cavity. This is the distal part of the genital duct and consists of a short wide tube coated by a layer of longitudinal muscles. Inverted into the penis cavity is the membranous endotheca with its penis sheath directed forwards.

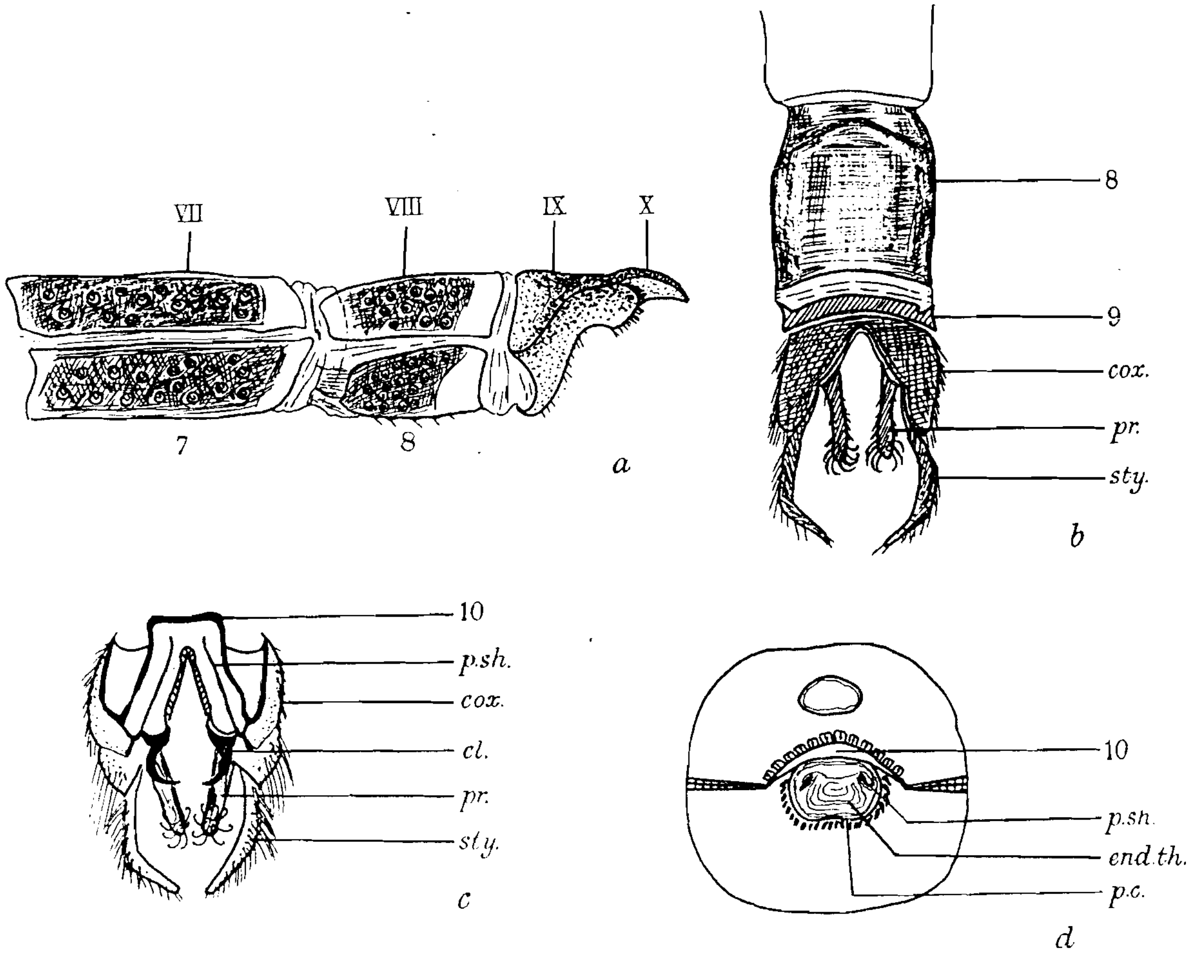


FIGURE 8. Hypopygium of male adult of *Chironomus dorsalis*: *a*, lateral view of the distal abdominal segments, excluding the ninth and tenth sternites and the genital appendages; *b*, ventral view of the eighth and ninth segments and the genital appendages; *c*, inner view of the gonocoxites with their appendages and the tenth sternite; *d*, transverse section through the ninth abdominal segment showing positions of the tenth sternite, penis cavity, endotheca and penis sheath.

The non-glandular ejaculatory ducts. These are two tubes bound together by a layer of circular muscles. They open into the proximal end of the penis cavity separately. Inside the muscular layer is an epithelial layer of well-defined cells which surrounds each lumen, now containing a small amount of secretion from the anterior portion of the duct.

The glandular ejaculatory ducts. These are continuous with the previous ducts with wider calibre and thicker glandular wall. They also are surrounded by an outer layer of circular muscles. They play the part of the separate accessory glands of the male reproductive system of many insects.

The vesiculae seminales. These are two ampoule-shaped sacs which join the ejaculatory ducts through narrow passages. The wall is thin and has no muscular layer but is covered by an adipose layer. Their cavity is filled with masses of sperms mixed with the secretion from the glandular ejaculatory ducts.

The vasa deferentia. These are the two ducts which run between the vesiculae seminales and the vasa efferentia. The similarity of their wall to that of the vesiculae and the continuation over their proximal portions of the adipose layer suggests an identity of origin of the two structures. Some of the secretion present in the vesiculae passes through the vasa deferentia, assisting in the movement of the sperms (figure 9; see also figure 12).

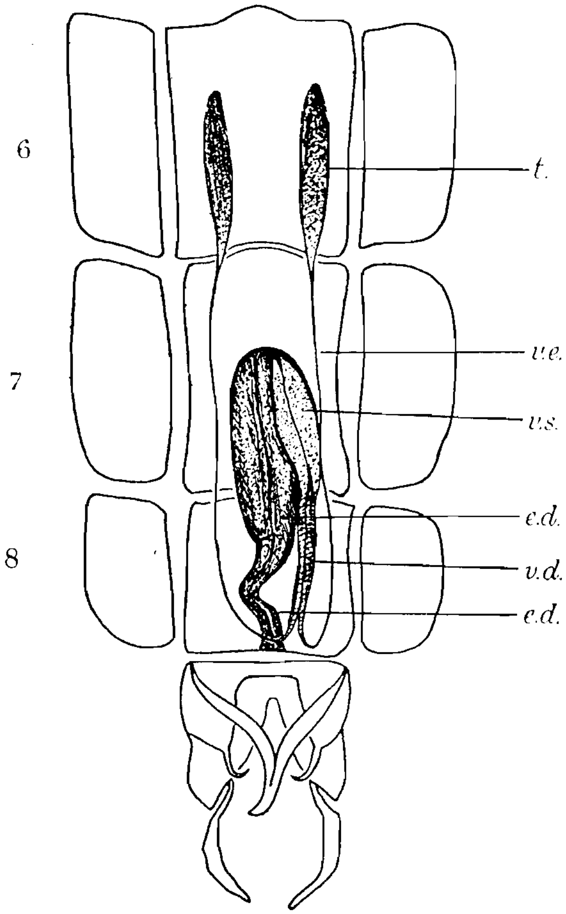


FIGURE 9. Dissection of the reproductive system in a young male adult of *Chironomus dorsalis*.

(*b*) *Development in the larva*

(i) *The primary reproductive system*

The gonads of a young larva are spindle-shaped with short terminal filaments. They lie in the sixth abdominal segment. The germ cells inside a young gonad are undifferentiated small round spermatogones (figure 10 *a*). They soon become slightly larger in size and aggregate in berry-shaped groups. These constitute the spermatocytes which in the fully grown larva are transformed into spermatozoa. These when freshly formed in the gonad retain for a while the stellate shape as in the spermatocytes, with their heads directed to the centre of the bundle and the tails directed outwards. But soon these spermatodesms disperse and the spermatozoa mix freely inside the testis.

In the young gonad some of the germ cells arrange themselves evenly inside the peritoneal coat and give it the appearance of a peritoneal epithelium, continuous with the terminal filament anteriorly and with the mesodermal strand posteriorly. This peritoneal epithelium during development of the gonad loses much of its cellular appearance and thickness. In the fully grown larva it has changed into a thin membrane with fine scattered nuclei (figure 10).

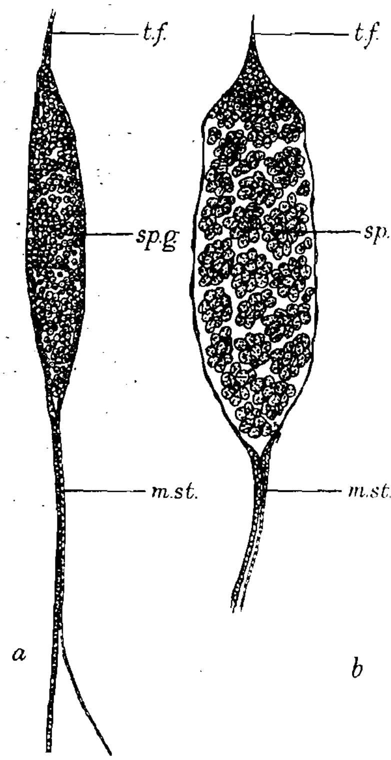


FIGURE 10. Longitudinal sections through the gonad of a male larva of *Chironomus dorsalis*: *a*, of a young larva; *b*, of a fully grown larva.

The vasa efferentia. As in the case of the female, the mesodermal strands are branched. Each consists of a long strand which extends up to the anterior border of the ninth sternite and a shorter one which ends at the middle of the seventh segment. In the case of the male it is the short strand which perishes at the prepupal stage and which is thinner than the long one. It is worth mentioning that the branched condition of the mesodermal strands is exactly the same in both sexes and differs only in the persistence of one or other of the branches according to the sex. The long strand in the male, which will give rise to the vas efferens, remains solid during all the larval period, and it is doubtful if junction of its distal end with the vas deferens of the secondary reproductive system takes place before the pupal stage (figure 10).

(ii) *The secondary reproductive system*

The gonapophyses. In a very young male larva the rudiments of the secondary reproductive system appear as two separate hypodermal buds on the mid-ventral surface of the ninth segment. Each bud acquires a peripodial cavity, and as both approach the mid-ventral line of the ninth sternite the dorsal part of the peripodial wall of each bud becomes thicker and the ventral wall thinner. When the two buds join, their inner walls form a median thin septum which for a while keeps their peripodial cavities apart. By this time the dorsal wall of each bud is moulded into a lobe-shaped body which protrudes into the peripodial cavity of its side.

The two lobe-shaped structures are the rudiments of the gonocoxites. Soon the distal ends are constricted giving rise to the telopodite portions which are the rudiments of the styles. This is followed by another division at the inner side of the bases of the gonocoxites which results in the appearance of the endites or the true gonapophyses (rudiments of the paramere lobes (figure 11).

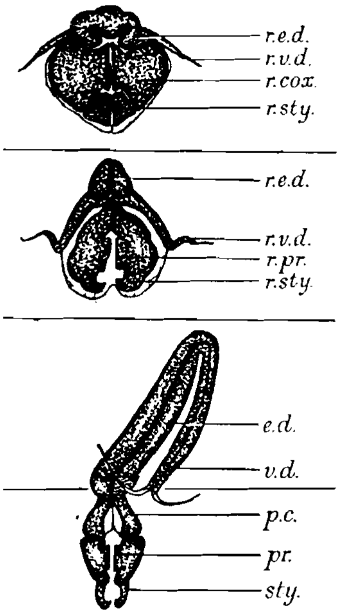


FIGURE 11. Dissections of the hypodermal genital rudiments in three successive stages beginning in the young male larva of *Chironomus dorsalis* and ending at its prepupal stage.

The efferent system. Two separate invaginations appear at the base of the gonocoxites. These are the rudiments of the double ejaculatory ducts which start at this stage quite independently of each other. In a more advanced larva a new shallow invagination takes place behind the ejaculatory duct rudiments forming an entrance to these, the rudiment of the penis cavity. This latter is therefore not merely the result of union of the distal ends of the ejaculatory invaginations, but is an entirely new and independent successive structure.

In the fully grown larva the two ejaculatory ducts run closely side by side and become of considerable length. They bend forming a U-shaped loop, and specialization of the different parts of the ducts takes place as development proceeds. The basal part becomes thick-walled but non-glandular, the middle section has a glandular wall with some secretion already in its lumen and the terminal portion is thinner and solid. The proximal thin part of the ducts form the vasa deferentia. These diverge and extend laterally to lie very close to the distal ends of the two long mesodermal strands at the anterior border of the ninth sternite (figure 11).

(c) *Development in the pupa*

(i) *The segmental parts*

The ninth and tenth tergites can be seen through the pupal skin occupying with the gonocoxites the ninth pupal segment. The tenth tergite overrides and becomes adherent to the ninth except for its terminal portion, which remains free as the beak-shaped structure in the adult. The ninth sternite forms a narrow region between the eighth sternite and the base of the gonocoxites. There is no external sign of the tenth sternite, but in cross-sections this can be seen lying over the penis cavity. The hypodermal cells of its fold secrete cuticular substance at the sides, while the middle part of the plate becomes membranous.

The gonocoxites grow quickly in size during the pupal period. The styles lengthen and reach their final shape before emergence of the fly. The paramere lobes do not divide secondarily but remain as one pair of parameraphores. This explains the reason for the absence of an aedeagus. The intersegmental membrane which in the larva existed between the ninth and tenth sternites has become folded and tucked inside the penis cavity to form the endotheca with its two cuticular strips.

(ii) *The primary reproductive system*

The testes in the pupa extend backwards to the anterior border of the seventh abdominal sternite. The whole contents of the testes have become transformed into spermatozoa, except for some germ cells which are carried over to the adult testes as small round degenerate cells. The peritoneal coat of the testes has become very thin except at the base, where it joins the mesodermal vas efferens. The vasa efferentia retain their solid condition until prior to emergence, when they acquire a lumen through which the sperms are poured from the testes to be stored in the vesiculae seminales.

(iii) *The secondary reproductive system*

The penis cavity. The shallow hypodermal invagination of the penis cavity deepens very slowly to form a short duct which is coated by a layer of longitudinal muscles. Cross-sections in the pupa show this with the intersegmental inflexion (endotheca) folded inside its cavity (figure 12).

The non-glandular ejaculatory ducts. The basal part of the double ejaculatory duct is carried deeper inside the body cavity and the two ducts become bound together by a layer of circular muscles, so that they look externally like one tube. The epithelial wall loses much of its thickness, so that this part of the efferent system is turned into a simple non-glandular double passage which ends in two separate openings on the front wall of the penis cavity (figure 12).

The glandular ejaculatory ducts. The proximal part of each ejaculatory duct becomes thicker due to thickening of its glandular wall. The lumen becomes filled with secretion poured out by the gland cells through small vacuoles at their inner ends.

The vesiculae seminales. The sperm stores do not appear until shortly before emergence of the fly. When the sperms start to flow down the vasa efferentia and through the vasa deferentia they are held up by blockage of secretion inside the lumen of the glandular ejaculatory ducts. Their aggregation in the anterior portions stretches the wall of the vasa deferentia which becomes thinner as accumulation of the sperms increases. For some reason this part of the duct which will be transformed into the vesiculae seminales is surrounded by a thick layer of fat body. This becomes much thinner in the fly.

The vasa deferentia. These are early differentiated in the larva as the divergent terminal portions of the ejaculatory duct invaginations. They exist before the junction of the two parts of the efferent system takes place. In the pupa the vasa deferentia are represented by those parts of the ectodermal ducts which lie between the vesiculae seminales and the mesodermal vasa efferentia. This means that the duct which links each testis with its vesicula seminalis is, in fact, partly ectodermal and partly mesodermal (figure 12 *a*).

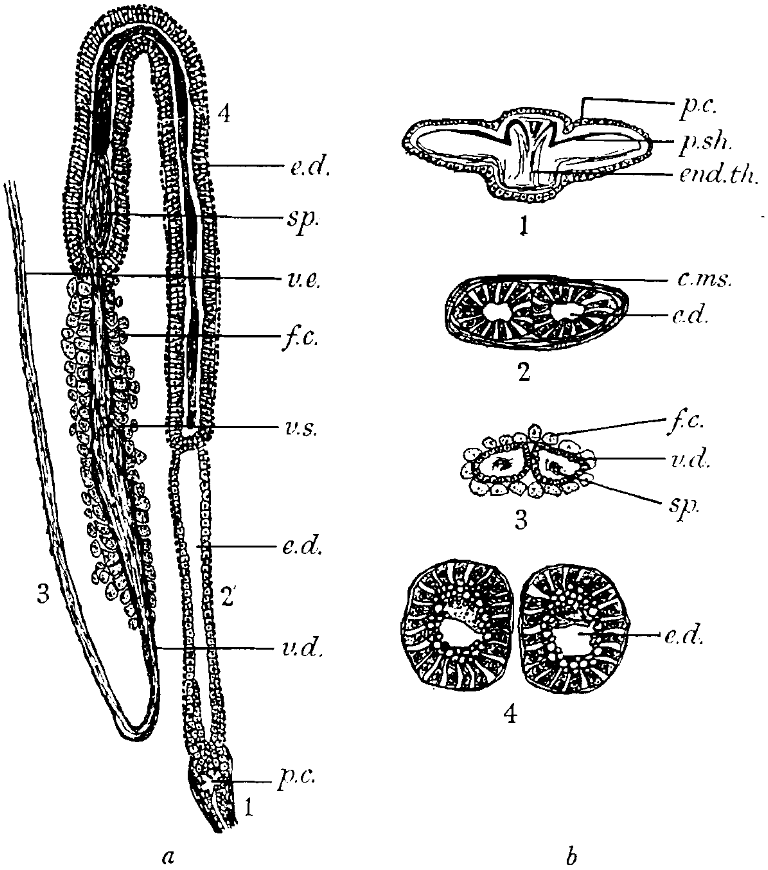


FIGURE 12. The efferent system in the male pupa of *Chironomus dorsalis*: *a*, reconstructed longitudinal section; *b*, transverse sections through the different parts.

VI. Family ANISOPIDAE (*Anisopus fenestralis*)

1. THE FEMALE

(*a*) *Structure of the reproductive system in the adult*

(i) *The segmental parts*

The hypopygium. The hypopygium of the fly in this species is essentially the same as that of *Chironomus*, except that the ninth tergite is mostly membranous, that the tenth tergite is composed of a basal portion with two lobed structures (cerci) and that the tenth sternite is more prominent. In addition, there is a shield-shaped plate which is inflected at the posterior edge of the eighth sternite and plays the part of a subgenital apodeme for the attachment of the hypopygial muscles (figure 13).

The atrium. This has exactly the same arrangement as in *Chironomus*.

(ii) *The primary reproductive system*

There are two lemon-shaped ovaries lying in the sixth abdominal segment, each terminating in a short filament attached to the anterior border of the same segment. The germarium area is small and terminal. It contains oogonia in different stages of development. No traces of ovarioles can be detected in this ovary which looks from the outside like a sac filled with oval-shaped eggs (figure 14). The peritoneal coat shows cellular structure in the young fly and loses this gradually with age.

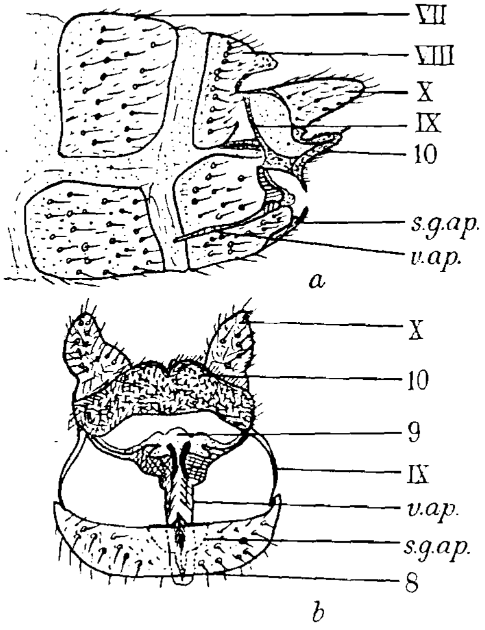


FIGURE 13. The distal end of the abdomen of the adult female of *Anisopus fenestralis*: *a*, lateral view; *b*, posterior view.

In the young fly ovary the eggs are embedded in a follicular epithelium which surrounds and encloses them in separate follicles. The follicular epithelium forms an epithelial plug at the base of the ovary with extensive tapering anteriorly. It would appear that this epithelium also does not merely play the part of secreting the egg chorion, but is important as a nourishing factor. This conclusion is supported by the fact that histolysis takes place among the follicular cells of the basal plug. Before passing into the histolytic stage these cells show great activity having large nuclei and clearly displayed nucleoli. The bodies of the histolytic cells later fuse together into one mass surrounded by a layer of follicular cells which secrete a spherical membrane around it. In one case it was found that histolysis had occurred in more than one place and consequently more than one mass had appeared. The most outstanding feature of the histolysis is that the resultant bodies acquire a resemblance to cellular structure appearing as giant cells with definite outer wall and containing a nuclear-like body (see figure 18, which shows the condition in the pupa). In the old fly ovary all giant and follicular cells disappear, except for traces of the latter which are scattered in the small spaces among the ova, the ova having increased in size at the expense of the digested follicular epithelium.

The oviducts. The oviducts extend from the posterior ends of the ovaries to converge towards the mid-ventral line of the seventh abdominal segment, where they join the proximal end of the common oviduct (figure 14). In the young fly the wall of the oviduct consists of two distinctive layers: an outer layer which, I believe, originates as an extension from the ectodermal layer of the common oviduct and an inner layer which represents the original mesodermal oviduct. The latter is continuous with the follicular epithelium of the plug at the base of the ovary. In the old fly when the oviduct acquires a large lumen its wall is composed of one layer of cells only, the previous outer layer. The proximal part of the oviduct, however, still shows a partly degenerate inner layer. The oviducts are coated with a layer of longitudinal muscle.

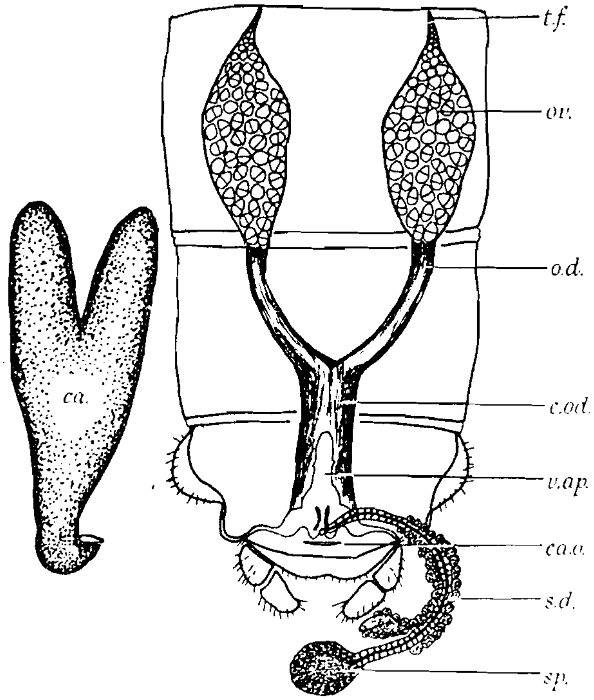


FIGURE 14. Dissection of the reproductive system in the female adult of *Anisopus fenestralis*.

(iii) *The secondary reproductive system*

The common oviduct. This is a wide flat tube which starts at the middle of the seventh segment to end at the posterior border of the eighth sternite where the vulvar opening lies (figure 14). The wall of the common oviduct resembles to a great extent the outer layer of the paired oviducts and is similarly coated with a layer of longitudinal muscles.

The spermatheca. This has a long spermathecal duct with a terminal functional spermatheca proper and a membranous non-functional one which branches off from one side of the spermathecal duct. The middle portion of the spermathecal duct, as well as the cuticular sac of the functional spermatheca, is surrounded by a layer of glandular cells which form an additional layer outside that of the epithelium. The spermathecal duct is coated by a layer of longitudinal muscle. Its opening lies between two small heavily chitinized rods at the distal end of the vaginal apodeme and slightly posterior to and a little dorsal to the vulvar opening (figure 14).

The caecus. In this species the caecus is divided anteriorly into two lobes (figure 14) which join in one common lumen which opens into the caecal section of the atrium. The glandular wall of the caecus is thrown into furrows and ridges surrounding the lumen which is filled with secretion. Outside the glandular wall is a layer of circular muscle which is very thin around the two lobes but becomes much thicker around the common lumen near its outlet.

(*b*) *Development in the larva*

(i) *The segmental parts*

The abdomen of the larva consists of eight segments of the tubular type with intercalary rings which increase the number of superficially apparent segments. The last segment, the apparent eighth, is divided into four subdivisions and the anal opening is a ventral transverse slit between the first and second subdivisions. Surrounding the anal opening is the anal shield of Keilin & Tate (1940), which is composed of large hexagonal hypodermal cells with clear nuclei and distinctly granular cytoplasm. This shield is continuous with the hypoderm of the rectum.

The larva is metapneustic with two spiracles opening at the termination of the abdomen surrounded by five small terminal lobes. At the ventral junction between the seventh segment and the anal shield is a hypodermal fold. On the median portion of this fold originate the rudiments of the secondary reproductive system.

(ii) *The primary reproductive system*

The ovaries are spindle-shaped in the young larva and become pear-shaped later. They are embedded in the fat body in the sixth abdominal segment. In the early stage the germ cells are undifferentiated small cells which are packed inside the solid gonad without any intercellular spaces and covered by a thin peritoneal membrane. Gradually the ovarian cells arrange themselves in longitudinal rows which run between the outer wall and the base of the gonad. The cells of these rows are still undifferentiated, but a special aggregation of cells is distinguishable as a cork-shaped plug at the base of the gonad.

A secondary thin layer of fat cells appears outside the peritoneal membrane, and gradually the two layers adhere to each other giving the appearance of a single layer (figure 15 *a*).

In the prepupal stage the first sign of differentiation among the ovarian cells takes place. The proximal cells of the rows which lie underneath the peritoneal coat become larger and form small globular masses, while the rest of the row is transformed into smaller follicular cells. The globular masses are the differentiated oogonia, each of which is surrounded by a thin layer of follicular epithelium.

Beside the arrangement in rows of the ovarian cells some follicular cells form intermediate septa between the rows dividing the ovarian space into separate columns. This means that the ovary in this early stage of its development shows an ovariole-like arrangement, but the rows, instead of showing the usual succession of oogonia, have one terminal oogonium for each row while the rest of the cells become follicular cells (figure 15 *b*).

The mesodermal oviducts. The mesodermal strands extend to the posterior border of the seventh abdominal sternite. In dissection the basal ends of the mesodermal strands appear to converge and join in an area in front of the imaginal hypodermal disk. This area is not formed, however, by the amalgamation of the two mesodermal strands, but consists of a connective tissue of myoblastic cells derived from the nearby muscles. The mesodermal strands of this species are solid cords of considerable thickness which makes it easy to trace them through serial sections.

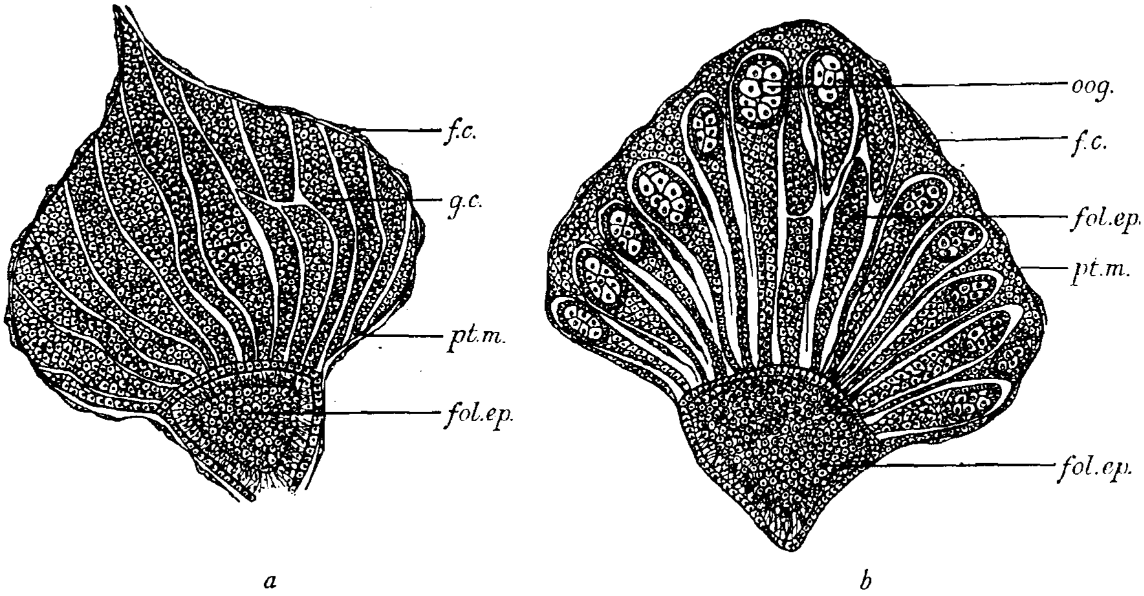


FIGURE 15. Longitudinal sections through the gonad of the female *Anisopus fenestralis*: *a*, of the larva; *b*, of the prepupa.

(iii) *The secondary reproductive system*

The normal arrangement of the genital imaginal disks in the female on the eighth and ninth abdominal segments is, in this species, obscured owing to the peculiar segmentation and the ventral position of the anal opening. At the junction of the seventh sternite with the anal shield there is a posteriorly directed hypodermal fold. The upper layer of this fold gives rise eventually to the eighth and ninth imaginal sternites, whilst the lower layer forms an intersegmental membrane. The imaginal disk from which the rudiments of the secondary reproductive system develop appears at the middle point of the summit of the fold (figure 16 *a*).

The imaginal disk of a very young larva appears as a thickened area of hypoderm covered by a paler layer formed of myoblastic cells extending from the nearby muscles. This thickening then acquires two small heads which represent the first rudiments of the caecus. Soon the two heads approach medially and fuse, giving rise to single invagination, the caecal invagination. Two other invaginations appear successively in front of the caecus invagination. The spermathecal invagination appears first and entirely independent from the common oviduct invagination which follows and lies slightly in front (figure 16 *b*).

The double nature of the caecus disappears completely, leaving no trace other than that of the anterior notch which gives this structure its bilobed character.

In a fully grown larva different invaginations become clearly defined, the caecus as a small emarginated sac, the spermatheca as a narrow tube, and the common oviduct as a shallow depression.

Linkage between the ectodermal common oviduct and the mesodermal strands starts in the fully grown larva. Longitudinal sections show that this linkage occurs at the posterior border of the seventh sternite by junction of the two mesodermal strands with the sides of the proximal end of the extended ectodermal common oviduct.

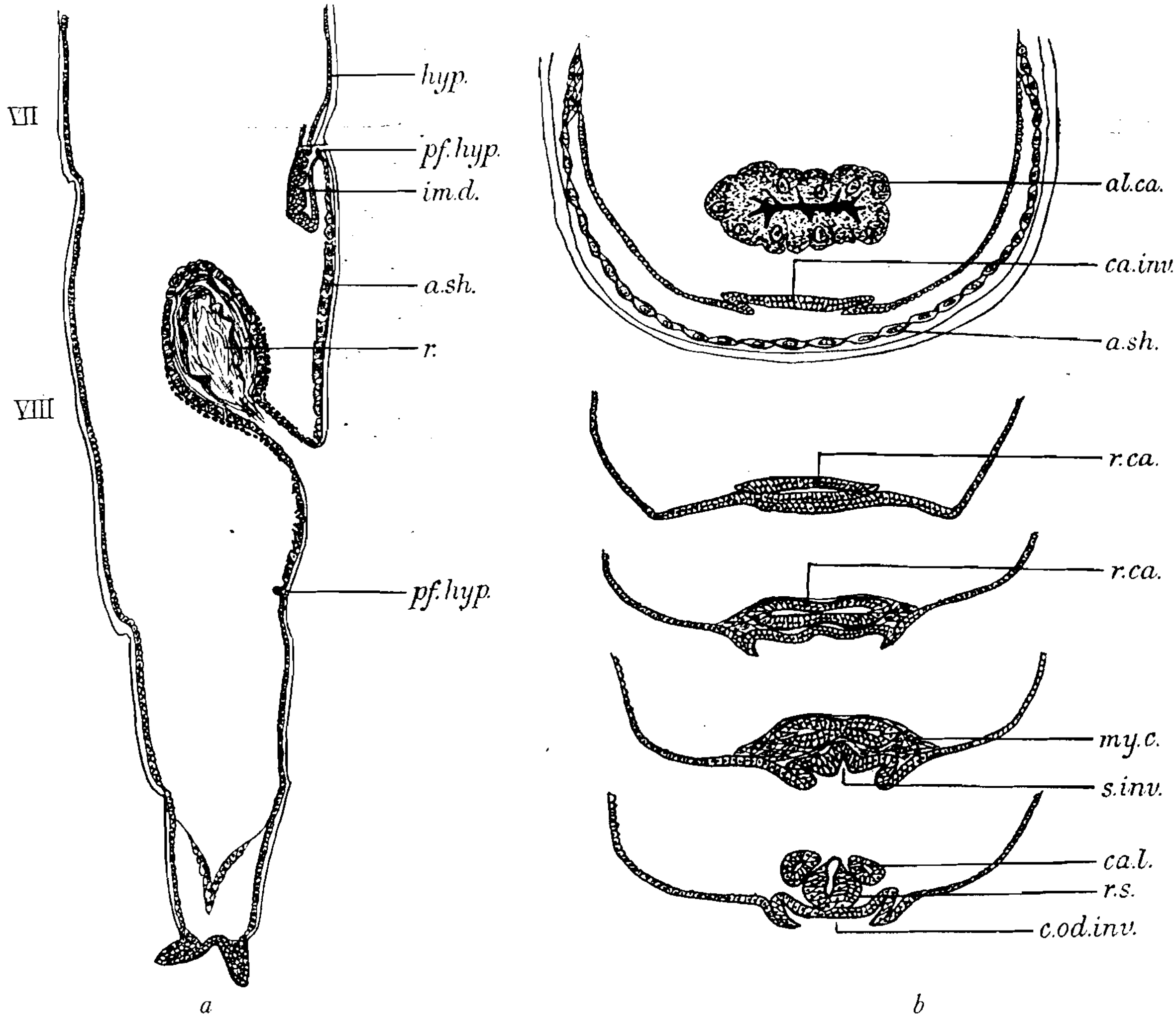


FIGURE 16. The genital hypodermal rudiments in the female larva of *Anisopus fenestralis* shown by: *a*, longitudinal section through the distal end of the larva; *b*, serial transverse sections through the area of the genital imaginal disk.

The lower layer of the posteriorly directed hypodermal fold is thin, and it eventually forms the intersegmental membrane between the ninth and tenth sternites. Between the lower layer of the fold and the anal shield there is a ridge of proliferative hypoderm, the rudiment of the tenth sternite. Another similar ridge exists at the posterior border of the anal shield, which, after the engulfment of the anal shield as described later, develops into the tenth tergite (figure 16 *a*).

(*c*) *Development in the pupa*

(i) *The segmental parts*

In the prepupal stage the anal shield begins to be withdrawn through the anal opening to lie inside the body cavity, forming a separate sac underneath the rectum. Keilin & Tate, who have studied the immature stages in Anisopidae, were unable to explain the reason for the existence of the anal shield, except that they thought that its huge cells might contain symbionts. The hypodermal fold, which exists in the larva between the seventh sternite and the anal shield, assumes a different position after the engulfment of the latter through the anal opening. The upper surface, which has now become of considerable width, develops into the eighth sternite. In connexion with its posterior border two hypodermal inflexions now become evident. The first is of apodemal character and eventually gives rise to the subgenital apodeme. The other is the insular fold between the caecal and vaginal openings, the middle part of which becomes the insula. The lower surface of the larval hypodermal fold forms a membranous area between the caecal opening and tenth sternite and later forms the caecal section of the atrium.

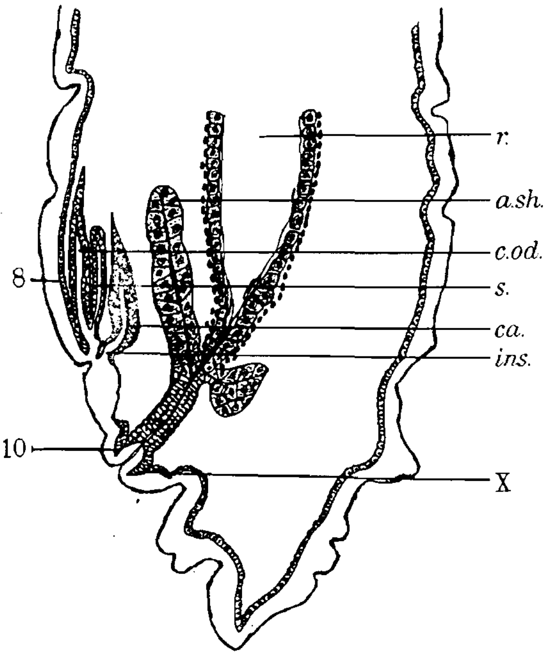


FIGURE 17. Longitudinal section through the abdomen of the female pupa of *Anisopus fenestralis*.

The anal segment now surrounds the anal opening, and its plates arise as previously described from hypodermal ridges bounding the anterior and posterior margins of the anal shield (figure 17).

The vagina and the vaginal apodeme. The vestibule which has appeared in the larval stage as a shallow inflexion now forms the short vaginal duct which extends between the insula and the vulva opening. The vaginal apodeme arises exactly as in *Chironomus*.

(ii) *The primary reproductive system*

The main changes in the structure of the gonads during the pupal stage are as follows:

(1) The fat cells, which have formed a secondary envelope outside the peritoneal membrane in the larval stage, have combined completely with the latter, forming a peritoneal epithelium.

(2) The oogonia can now be easily distinguished from the follicular epithelium. Each oogonium consists of a group of large cells with darkly staining cytoplasm, large nuclei and clearly defined nucleoli, while the follicular epithelium consists of narrow faintly stained cells with degenerate nuclei.

(3) The formation of the yolk does not start during the pupal stage, and hence differentiation of the oocyte from the rest of the oogonial cells is not possible. Each cell group of the oogonia is surrounded by a thin layer of follicular cells.

(4) The follicular rows between the oogonia and the basal epithelial plug have amalgamated and become continuous with the latter in the shape of a central core with short stalks which keep the oogonia in connexion with the central follicular epithelium.

(5) In the old pupa the follicular epithelium goes through the histolytic phases previously described. This process is not simultaneous in the two ovaries. In the same pupa one gonad may contain two giant cell-like bodies in the place of the basal plug, while in the other gonad the follicular epithelium forms a continuous central core (figure 18).

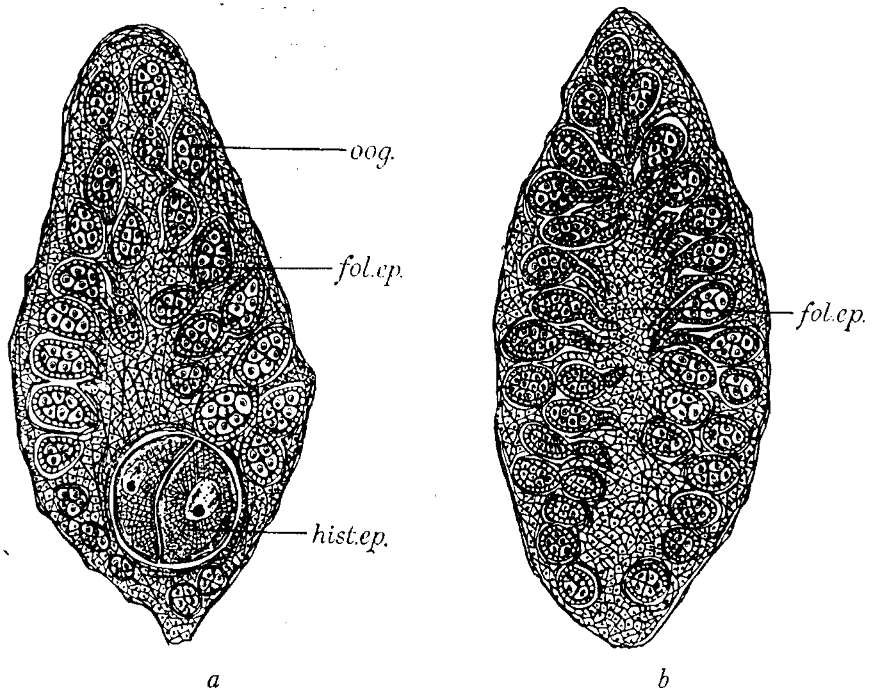


FIGURE 18. Longitudinal sections through the gonads of the female pupa of *Anisopus fenestralis*: *a*, of the right gonad; *b*, of the left gonad.

The oviducts. In the early pupa the mesodermal oviducts are still solid and of the syncytial type. At the distal ends where they join the common oviduct the nuclei arrange themselves near the periphery and around a central pale cytoplasm. Later the mesodermal oviducts acquire a central lumen, the formation of which proceeds from behind forwards. The wall of the oviducts during the pupal stage is composed solely of the mesodermal layer, but the ectodermal encroachment can be detected at the two places of junction. Here the ectodermal wall of the common oviduct encloses the two ends of the mesodermal oviducts and is beginning to form an outer layer.

(iii) *The secondary reproductive system*

The common oviduct. During the pupal stage the common oviduct extends into the seventh abdominal segment and is provided with its muscular coat which spreads over the basal ends of the oviducts as the common oviduct creeps over their mesodermal tissue.

The spermatheca. In the pupa the spermathecal invagination, after being greatly lengthened, is differentiated into a spermathecal duct and two saccular structures. The first is terminal and represents the functional spermatheca proper and the second subterminal. The two spermathecal sacs at first look identical, but soon the wall of the terminal sac becomes thicker and provided with glandular cells near the outer periphery, while the wall of the subterminal sac becomes thinner and its cells regress.

The glandular cells of the functional spermatheca secrete a fluid into its cavity, and the other epithelial cells lay down the inner cuticular lining. Similar glandular cells to those of the spermatheca proper appear on the middle portion of the spermathecal duct. Owing to the fact that the duct is surrounded by a layer of longitudinal muscle these glandular cells are separated from the lining epithelial cells as the mulberry-like structure described in the adult.

The caecus. The caecal invagination extends deeply during the pupal stage until its lobes reach the sixth abdominal segments. Its glandular wall attains its greatest thickness in the old pupa, but the release of its secretion does not start until after the emergence of the fly. It is also at this time that the caecus is provided with its muscular coat.

2. THE MALE

(*a*) *Structure of the reproductive system in the adult*

(i) *The segmental parts*

The hypopygium. The ninth abdominal segment is a heavily sclerotized ring which is narrow dorsally and widening laterally to join the triangular ventral plate of the ninth sternite which acts as the subgenital plate (figure 20 *a*). The anal segment is similar to that in the female. A long genital pouch exists between the ninth and tenth sternites, and its development during the pupal stage is so peculiar that recognition in the adult of its true homologies is only possible after following its development. Briefly anticipating what will be said later on this subject, it may, to simplify its description, be said to appear during development as a special intersegmental inflexion, forming in this case a huge sac (thecal pouch) which becomes divided by a horizontal constriction into dorsal and ventral pockets (figure 19). The dorsal pocket lays down a large hood-like apodeme, while the ventral pocket keeps its saccular appearance forming a long narrow membranous chamber, the penis sac, in which the long flagella-like penis tube (penis) is spirally wound. At the entrance of the penis sac there is a special sclerotization of its wall, which forms a semi-tubular channel, the penis sheath. Outside the sac, at its mouth, are the appendicular penis valves (aedeagus), between which and the penis sheath the long filamentous penis slides during its extrusion and retraction (figures 19, 20). The parts are very similar to, and closely homologous with, the complicated structures seen in the Tipulidae and regarding which up to the present no adequate description of the essential morphology has been given.

The gonapophyses. The gonocoxites join the sides of the ninth sternite except for their distal ends, where they carry dark spear-shaped styli. The true gonapophyses consist of two pairs of processes which lie at the inner side of the gonocoxites beneath the mouth of the penis sac. The first pair is shapeless and heavily sclerotized, so that it looks almost black, and is studded with short spines. At first sight this pair of processes appears as one piece, but there is a median suture which allows separation into two parts by slight pressure with a needle. These processes correspond to the true parameres in other insects. The second pair of gonapophyses are two narrow cuticular structures which lie over the first pair of gonapophyses attached below the opening of the penis sac. These latter processes correspond to the penis valves in other insects and represent an incomplete aedeagus, most of whose work has been taken over by the apodemal structure, the penis sheath (figure 20).

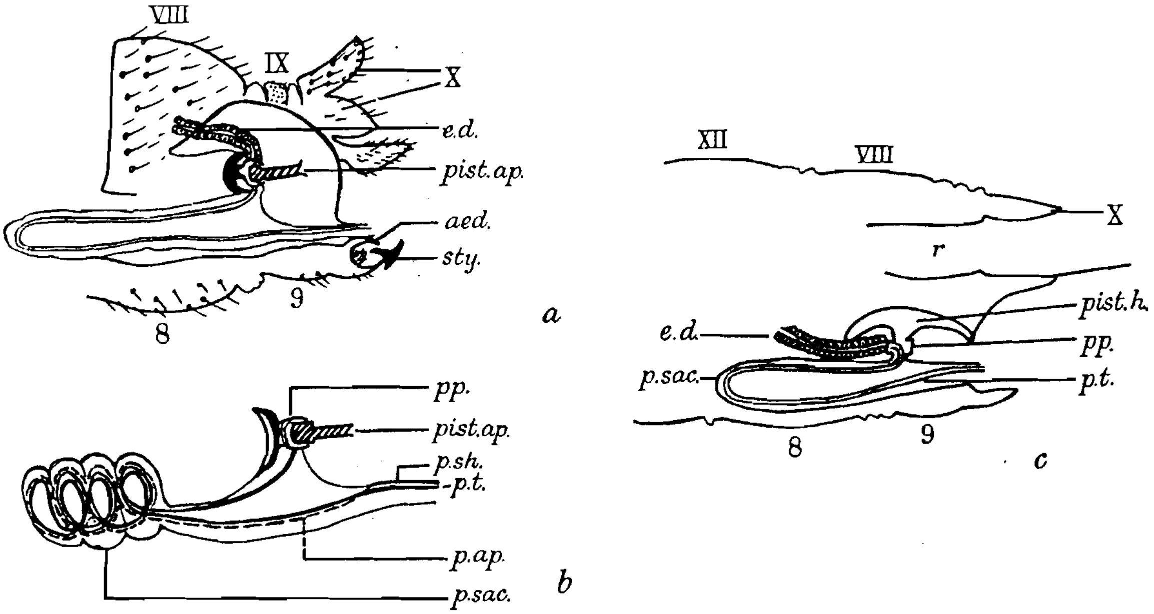


FIGURE 19. The hypopygium, thecal structures and genital appendages of the male adult *Anisopus fenestralis*: *a*, lateral view of the distal end of the abdomen with part of the sclerites removed to show the inner structures; *b*, the course of the penis tube between the pompetta and penis sheath; *c*, schematic longitudinal section as *a*.

The intromittent organ. The intromittent organ (which will here be termed penis) is not aedeagus but, as will be clear later, the excavated penis tube. This a long cuticular flagellum which arises at the distal end of the ejaculatory duct. Contained within the penis sac, it first runs forwards as far as the anterior border of the seventh segment and then passes backwards, forming along its course spiral loops, until it reaches the mouth of the sac. Running along and underneath the penis is a thread-like apodeme which forms a tail-like continuation from the penis sheath, the penis apodeme. The penis apodeme accompanies the penis very closely up to the point where the latter begins to change its course, i.e. to pass backwards. In dissection the three threads, i.e. the double-running penis and the penis apodome, look like one thread. With much patience one can separate the actual penis from its apodeme. Moreover, sections show three transversely cut bodies at the top and bottom of each spiral loop of the penis sac; the inner two, which are sections of the penis, have a minute central lumen, while the outermost, which is the apodeme is solid.

The penis apodeme, though in dissection it looks like a fine filament, is in reality a fixed rail-like thickened ridge of the otherwise thin-walled penis sac. The penis sac encloses the penis and follows its loops. It is easy to imagine that first the penis sac was a plain bag-like structure with smooth walls and the penis running inside in a simple loop (figure 19 *a*, *c*).

The upper (dorsal) part of the loop continued from the termination of the ejaculatory duct in the roof of the sac and the lower part lying along the floor of the sac to end at the penis sheath. The two portions of the loop then come close together and are twisted in a close spiral. The wall of the penis sac collapses on to and between these spirals, giving the whole structure the appearance of an accordion (figure 19 *b*).

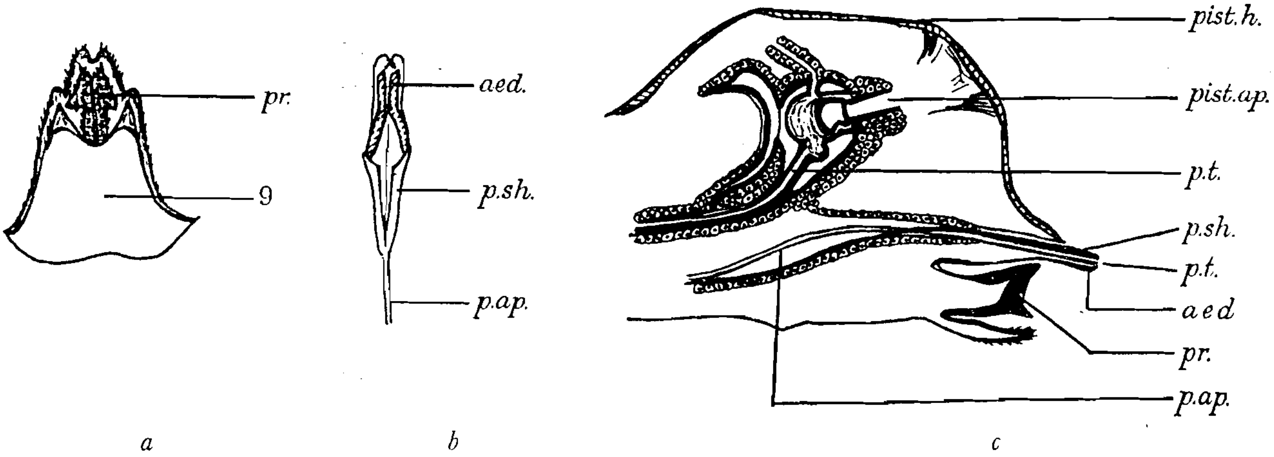


FIGURE 20. *Anisopus fenestralis* male adult: *a*, inner view of the ninth sternite with parameres attached; *b*, vertical view of the penis sheath and the aedeagus; *c*, longitudinal section through the piston hood passing through the pompetta.

At the junction of the penis with the ejaculatory duct there is an organ clearly having the function of pumping the seminal fluid through the extremely fine capillary duct of the penis, the pompetta. The cylinder of the pompetta is a small cuticular box with two lateral alate apodemes. The piston is a rod-like piece with a round head which fits exactly in a hole in one side of the cylinder. This hole is closed inside by a thin elastic tissue which allows a certain amount of movement of the head of the piston. The piston rod springs from a large cuticular structure in the shape of a bird's sternum without a keel. This serves as a hood over the pompetta and provides a suitable surface for the attachment of the muscles responsible for the movements of the piston. I intended first to call this part the ejaculatory hood, until I realized that this term had been applied to a different part by other authors, viz. to a structure which overhangs the gonopore. Snodgrass (1904) gave the name central vesicle to a very similar structure in Tipulidae without discriminating between the real pompetta and the outer apodemal structure. I propose to call this apodemal structure the piston hood, since the piston rod is, in fact, a part of it shaped as such to serve the pumping function.

The ejaculatory duct passes underneath the piston hood and between the horns of its deeply emarginate anterior border and under its arch until it reaches the small box of the pompetta which it enters through a hole in the roof.

The opening in the pompetta leading to the penis tube lies in front of a deep pit in the floor of the box and somewhat anterior to the opening of the ejaculatory duct. The pushing in of the piston head closes the opening of the ejaculatory duct, while the passage to the penis tube is still free, and so the compressed seminal fluid in the pompetta has no choice but to pass into the capillary tube of the penis (figure 20 *c*). I failed to find any musculature which might be responsible for the extension and protrusion of the twisted penis filament, and it would appear that the power produced by the pumping force is the only factor in such action. The penis apodeme works merely as a rail on which the penis slides on its way to the penis sheath and which assists it in returning to its previous position after cessation of the pumping force.

The torsion of the hypopygium. In this species the hypopygium is twisted gradually during the 24 hr. following emergence of the fly. The twist includes the region of the three terminal segments and is of 180° about the main axis of the body, so that after rotation the two spear-shaped styles are situated dorsally, and slightly beneath them protrudes the end of the penis sheath. The tenth segment, which surrounds the anal opening, lies ventrally.

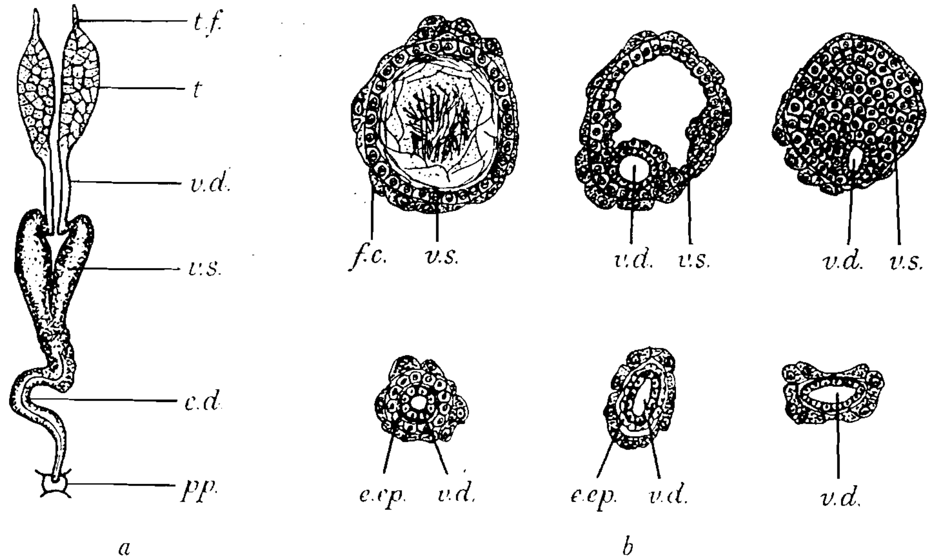


FIGURE 21. *a*, dissection of the reproductive system of the male adult of *Anisopus fenestralis*; *b*, several transitional sections at the junction between the vescicula seminalis and the vas deferens.

(ii) *The primary reproductive system*

The pear-shaped testes (figure 21 *a*) lie in the sixth abdominal segment with short anterior terminal filaments attached to the anterior border of the same segment. Each testis is divided internally into separate loculi, which are bounded by a thin membrane continuous with the surface peritoneal membrane. Some epithelial cells are scattered in the septa, especially at the corners of the loculi. The loculi are mainly occupied by masses of spermatozoa, except for some degenerate cells which are to be found scattered between the sperm masses. Externally the testes are brown in colour, apparently due to a special kind of pigmented fat tissue which envelops them and which can be easily separated by dissection.

The vasa deferentia are rather short ducts which run posteriorly to the hind border of the seventh segment. They are surrounded by the same kind of pigmented fat tissue as invests the testes. Serial cross-sections show that the vas deferens consists of one layer throughout its whole length, except near the place where it joins the vesicula seminalis. If the structure of the seminal tract be examined at this place it shows the replacement phenomenon of the mesodermal tissue of the vas deferens by ectodermal tissue from the anterior part of the vesicula seminalis. This latter is composed of a single layer of glandular cells which at the distal end of the vasa deferentia becomes suddenly thicker so that the lumen is blocked by a solid mass of cells. Some of these cells arrange themselves around the vas deferens, providing a second external coat of ectodermal origin. This extends for a short distance, thinning gradually until the tube of the vas deferens is composed only of the original mesodermal epithelium, a condition which continues until it links with the peritoneal membrane of the testis (figure 21 *b*).

(iii) *The secondary reproductive system*

Each vas deferens joins a long seminal vesicle after being twisted into a short V-shaped bend. The pigmented fat tissue is continued on to and covers the whole vesiculae. A newly emerged fly already contains large masses of sperms, which descend shortly after emergence and are stored in the vesiculae. The glandular cells of the vesicular wall produce a secretion which surrounds the spermathecal mass separating it from the wall. The vesiculae are coated by a very thin layer of circular muscles which extend over the proximal part of the ejaculatory duct.

The ejaculatory duct is a long convoluted tube which starts at the base of the vesiculae seminales as a thick tube and tends to become thinner as it approaches the pompetta. The proximal end of the tube has the same structure as the vesiculae and has a large lumen. The remainder of the duct has a very narrow lumen and a thick wall with cuticular lining. At the middle part of the duct the wall becomes markedly glandular. The cells are set radially around the narrow lumen of the cuticular tube, and each cell has a large vacuole pushing the cytoplasm and nucleus to the outer corner of the cell. Approaching the distal end the cells lose their vacuoles and gradually the tube becomes non-glandular (figure 21 *a*).

(*b*) *Development in the larva*

(i) *The primary reproductive system*

The larval gonad is a miniature of that of the fly in the arrangement of the loculi, as well as in structure of the peritoneal membrane and the progress of spermatogenesis. The only difference is that the complete transformation of the spermatozoa does not take place except in the basal part of the loculi and proceeds forwards as development progresses. The young gonad is surrounded by a large mass of adipose tissue.

The mesodermal strands are solid syncytial cords which extend to the posterior border of the seventh abdominal sternite to lie at the sides and slightly in front of the genital disk. In sections the distal ends of the mesodermal strands are seen to be continued as myoblastic tissue which extends medially to cover the surface of the imaginal disk.

The imaginal disk is a centre towards which migrate a large number of myoblastic cells which detach themselves from the larval muscles. These groups of myoblasts are the rudiments from which originates the hypopygial musculature of the adult.

(ii) *The secondary reproductive system*

The genital imaginal disk of a young male larva looks strikingly similar to that of the female. It begins as a hypodermal thickening on the upper layer of the posteriorly directed hypodermal fold situated between the hind border of the seventh segment and the anal shield. This thickened hypodermal area later becomes differentiated into two outer slight elevations and two inner solid swellings. The former are the first sign of the gonocoxite rudiments and the latter are the rudiments of the ejaculatory ducts. A thin layer of myoblastic tissue covers the rudiments of the ejaculatory ducts at this early stage and extends to link up with the distal ends of the mesodermal strands. This may wrongly suggest that this myoblastic tissue is a continuation of the mesodermal strands, since it may appear as if these structures ended in two hypodermal swellings. The myoblastic tissue, however, is not in fact a strand, but spreads broadly to cover the area between the hypodermal rudiments and the nearby muscles.

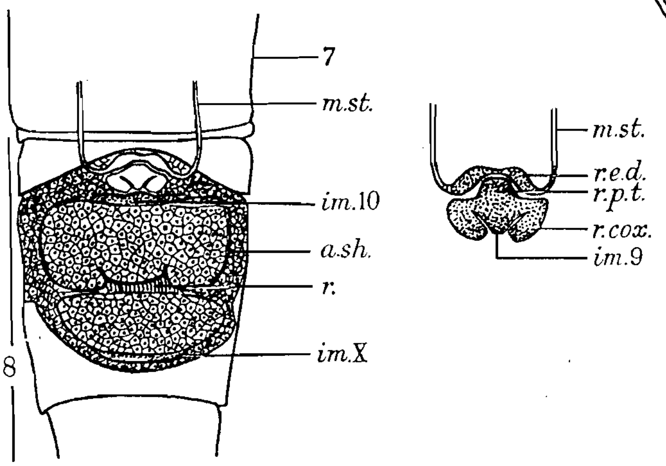


FIGURE 22. Dissection of the male larva of *Anisopus fenestralis* showing the anal shield with the two hypodermal proliferations and the hypodermal genital rudiments joined with the mesodermal strands. At the right side of the dissection the genital rudiments are drawn enlarged and named.

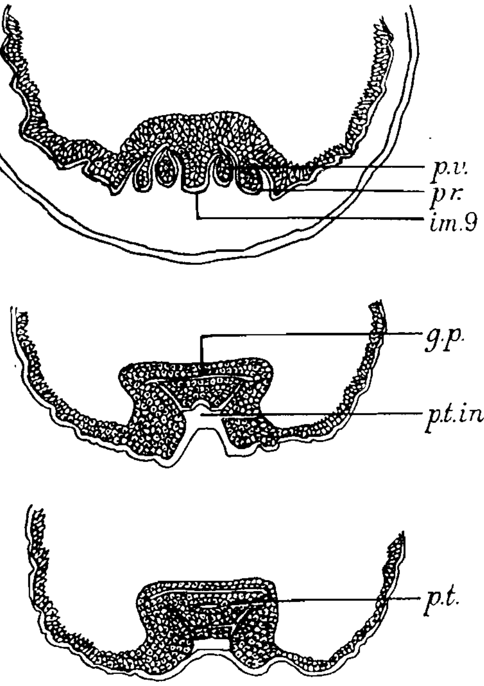


FIGURE 23. Successive transverse sections through the hypoderm of the male larva of *Anisopus fenestralis* in the region of the hypodermal genital rudiments.

Later differentiation of the gonocoxite rudiments becomes clearer and the rudiment of the ninth sternite appears between them. The two invaginations of the ejaculatory ducts extend deeper still as solid structures. A new single invagination with a definite cavity then appears behind the double invaginations of the ejaculatory duct and between the new ninth sternite and the bases of the gonocoxite rudiments. This single invagination is that of the penis tube which appears shortly after and behind those of the ejaculatory ducts and is a quite separate structure (figure 22).

Later the penis-tube invagination extends deeper and lies between the two ejaculatory duct rudiments which still keep their connexion with the hypoderm. It is at this stage in the larva that a pair of small separate bodies is differentiated at the inner side of the gonocoxite rudiments, the paramere lobes. Later these lobes have divided secondarily into a median pair of penis valves and an outer pair of parameres.

Around the penis invagination there appears a splitting in the hypoderm, one above and the other below the invagination. This is the beginning of the genitial cavity (figure 23).

The two ejaculatory duct rudiments acquire a central lumen and their posterior portions unite into a single duct, which joins the anterior end of the penis-tube invagination, while the anterior parts keep their double structure.

(c) *Development in the pupa*

(i) *The segmental parts*

The upper layer of the posteriorly directed hypodermal fold between the seventh segment and the anal shield is differentiated into the hypoderm of the imaginal eighth and ninth sternites. The two hypodermal ridges at the edges of the anal shield come now to surround the anal opening forming the anal or tenth segment.

The two hypodermal splittings, already described in the larval stage as dorsal and ventral to the penis tube, come into communication forming one genital pouch.

(ii) *The primary reproductive system*

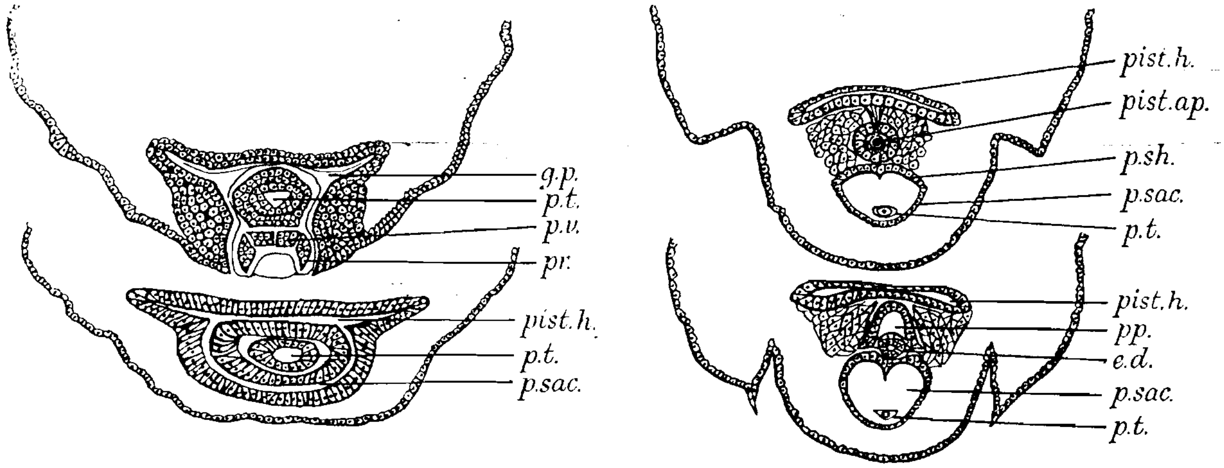
The gonads develop gradually during the pupal period to reach the imaginal condition, spermatogenesis proceeding posteriorwards so that most of the loculi contain fully developed spermatozoa. The pigmented investment of the gonad for the first time now appears.

The vasa efferentia of the pupa are derived from the larval mesodermal strands. At either end the two strands keep for some time their solid condition, the proximal end to prevent the sperms from the gonad flowing down and the distal end to facilitate the joining up of the vas efferens with the anterior part of the ectodermal vesicula seminalis. The median part of the vas efferens meanwhile acquires a narrow lumen. In the old pupa the vasa efferentia become tubular throughout and the sperms pour down through them. At their distal ends, where they join the vesiculae seminales, the mesodermal epithelium of the vasa efferentia receives an outer coating from the vesicular epithelium. A replacement process takes place after emergence as noted when describing the parts in the fly.

(iii) *The secondary reproductive system*

In the young pupa the two pairs of gonapophyses become well separated, the median pair now lying immediately beneath the penis-tube opening. Later in the pupal stage the gonocoxites become of considerable size and are attached more closely to the ninth sternite. At their distal ends the styles appear as claw-shaped constricted off-portions.

During the pupal period the genital pouch enlarges, and changes take place which bring the structures close to that of the imaginal stage showing very clearly the essential homologies of the very complex arrangement in the adult. The splitting of the hypoderm which began around the mouth of the penis-tube invagination has by extension forward formed the rudiment of the genital pouch, and at the same time by excavation of the parts around the penis tube converted this latter into a tubular projection, the rudiment of the penis. During the pupal period the upper part of the pouch becomes separated from the lower by a horizontal constriction. The upper part giving rise to cuticular secretion forms the piston hood. The lower part continues as the penis sac, the walls of which become thin and membranous, except where there forms a thickened ridge, the rudiment of the penis apodeme (figure 24 *a*).



a *b*

FIGURE 24. Successive transverse sections in the male pupa of *Anisopus fenestralis*, showing the formation of the genital pouch, penis sac and pompetta: *a*, in the young pupa; *b*, in an old pupa.

At the entrance of the penis sac sclerotization in its wall gives rise to the penis sheath. The penis tube with the excavated tissues around it (penis) loses much of its thickness so that it becomes loose inside the penis sac. As it further lengthens it forms an anteriorly directed loop and the spiral arrangement already described.

At the point where the penis tube arises the walls of the area lying between and separating the piston hood from the penis sac become thickened and sclerotized to form the pompetta (figure 24 *b*).

The single ejaculatory duct, formed by fusing of the distal portions of the double ducts, increases considerably in length and becomes differentiated into a distal thin non-glandular part and a proximal wide glandular part with thick-ridged wall and large central lumen. Both parts of the duct are surrounded by a layer of circular muscle which is more prominent on the proximal part.

The double ejaculatory ducts, or anterior ununited portions, develop a thick glandular wall which gives them a saccular appearance, the first sign of their transformation to serve as vesiculae seminales. They are coated by two separate layers, an inner layer of muscles and an outer one of pigmented fat cells, of the same nature as those which surround the gonads and the vasa efferentia.

In the old pupa the different structures described attain the imaginal condition. The gonocoxites join the sides of the ninth sternite for most of their extent. The styles are provided with a thick cuticular layer and become sickle-shaped. The penis valves are fixed beneath the penis sheath. The parameres are provided with short spines and come to lie nearer to the inner side of the ninth sternite. The penis sac extends to the anterior border of the seventh segment. At its distal portion it keeps its original cylindrical shape, but anteriorly the wall is deeply creased in ridges in accord with the spirals of the long penis.

The penis sheath attains a tubular shape with a dorsal furrow where the distal end of the penis tube rests.

At the posterior end of the piston hood the rod-like piston apodeme comes to be arranged so that its head is fitted into a hole in the pompetta and the latter becomes provided with its alate apodemes.

The penis becomes of great length and is composed of a filamentous cuticular tube which is devoid of cellular coat except at its most proximal part, where the penis tube is surrounded by the pompetta. At the point where the filamentous rigid penis tube loops on itself the two portions come very close together and are wound together in spirals inside and together with the penis sac.

The vesiculae seminales contain no sperms during the pupal stage but a finely granular secretion. The single ejaculatory duct becomes twisted on itself, and the lumen of its anterior glandular part filled with secretion, whilst the distal non-glandular part acquires an inner narrow cuticular tube which is similar to that of the penis.

VII. Family MYCETOPHILIDAE (*Mycetophila cingulum*)

1. The female

(*a*) *Structure of the reproductive system in the adult*

(i) *The segmental parts*

The hypopygium. The three terminal segments of the abdomen are telescoped inside the seventh segment so that only the distal ends of the tenth tergite and eighth sternite are showing, the intersegmental membrane between the seventh and eighth segments being extensively developed to admit of this. The eighth sternite is a large concave plate,

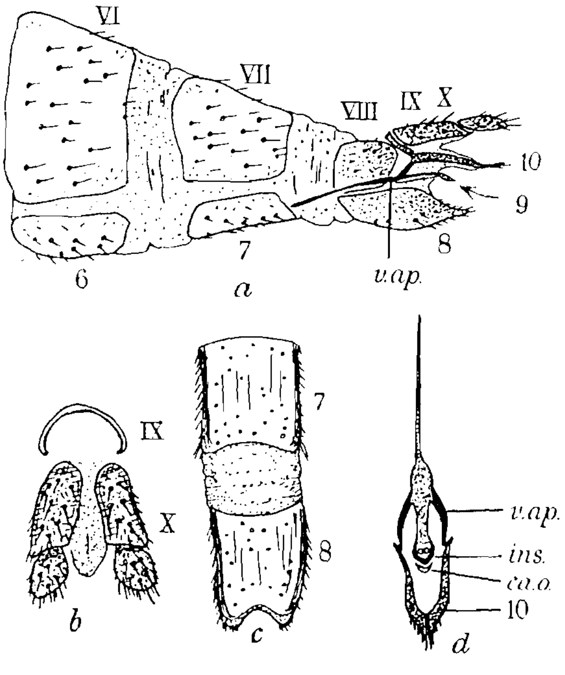


FIGURE 25. The hypopygium of the female fly of *Mycetophila cingulum*: *a*, lateral view of the abdomen terminalia; *b*, dorsal view of the ninth and tenth tergites; *c*, inner view of the seventh and eighth sternites; *d*, inner view of the vaginal apodeme and tenth sternite.

emarginated distally, which plays the part of a subgenital plate. The ninth tergite is a convex narrow strip connected at each side with the angle formed by the meeting of the tenth sternite with an arm of the vaginal apodeme. This apodeme is composed of a long slender body which lies over the common oviduct, and two arms at its posterior end which rise up to meet the tenth sternite and the ninth tergite in a triple joint at each side. The ninth sternite is the small insula which lies just behind the distal end of the vaginal apodeme and is connected with the tenth sternite by a thin intersegmental membrane (figure 25*a*).

The tenth tergite is composed of two lobe-shaped paraprocts, each of which is constricted distally giving the appearance of a terminal and basal joint. This division occurs late in the pupal stage, and no such division occurs in the case of the male. It is therefore uncertain how far these two joints are to be considered both cerci, or both portions of a divided tergite or tergite and cercus respectively (figure 25*b*).

The tenth sternite is a V-shaped frame pointing backwards and with the arms linking with those of the vaginal apodeme (figure 25*d*).

The atrium. The atrium is in exactly the same position as described in the other two species, except that the intersegmental membranes between the insula and the eighth and tenth sternites are so short that they bring these parts together and make it difficult to recognize any proper atrial cavity.

(ii) *The primary reproductive system*

There are two huge ovaries, which, when they are fully matured, fill most of the abdominal cavity. Each is composed of twelve ovarioles which are at first enclosed in a very thin peritoneal membrane. This, however, soon perishes, leaving the tubular ovarioles to hang freely in the haemocoele. The tunica propria which forms the wall of the ovarioles is also thin. The proximal ends of the ovarioles form a terminal filament which is fixed to the body wall, the ova following successively according to their degree of maturity (figure 26).

The lateral oviducts are two wide tubes of considerable length, which run along the inner sides of the two ovaries and extend posteriorly to the middle of the seventh abdominal segment, where they converge and join the common oviduct. Each oviduct gives off on its outer side twelve short ducts in two rows which form the bases of the ovariole tubes linking their cavities with the oviduct lumen (figure 26).

(iii) *The secondary reproductive system*

The vagina and common oviduct. The vagina is a very short and wide intersegmental inflexion at the posterior end of the eighth sternite. Its wall is membranous and is surrounded by longitudinal muscular fibres, except at its entrance. The common oviduct is a wide and rather short tube, the wall of which is coated by a thick layer of longitudinal muscles distally, whilst more anteriorly another layer of circular muscles is added.

The spermathecae. There are two spermathecae which are entirely independent from each other. The two spermathecal openings lie very slightly in front of the insula and lead to two straight spermathecal ducts which run forward for most of their length and then bend backwards abruptly for a short distance to end in two teat-shaped spermathecae proper. The straight part of the spermathecal duct acts as a conductive canal. It is lined by a cuticular layer and coated by a thin layer of longitudinal muscles. The short proximal portion which is bent backwards is thicker in diameter owing to the thick coat of glandular cells and is nearly devoid of any muscular investment. The basal part of the spermatheca proper, which may be termed the intermediate chamber, is lined by what look like fine bristles. These may be a special adaptation for the transference of the sperms from this chamber to the saccular part of the spermatheca proper. This latter has a thick glandular wall (figure 26).

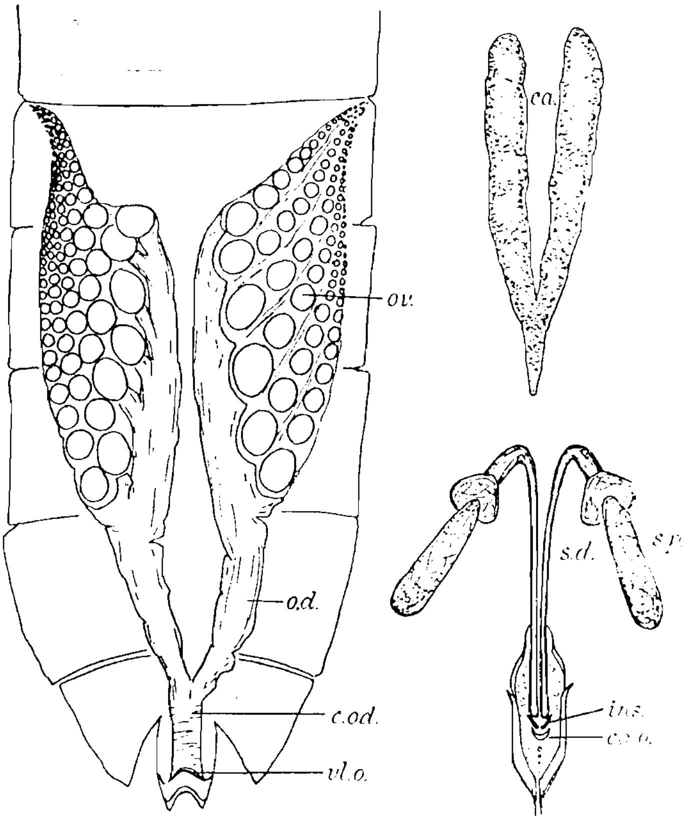


FIGURE 26. Dissection of the reproductive system of the female adult *Mycetophila cingulum*.

(*b*) *Development in the larva*

(i) *The segmental parts*

The abdomen of the larva consists of nine proper segments and an extra terminal division around the anal opening. On the ventral aspect there are nine intersegmental oval-shaped protrusions, each provided with two rows of small hooks besides being studded all over its surface with very fine bristles. These protrusions act as abdominal feet.

(ii) *The primary reproductive system*

The gonads in the larva are spindle-shaped and situated in the sixth abdominal segment with long terminal filaments which extend to the third segment. Transverse and longitudinal sections of the gonad in successive stages of development show that a young gonad is composed of similar small round cells covered by a very thin non-cellular peritoneal membrane. Soon some of the inner cells arrange themselves into a one-cell layer very close inside the peritoneal membrane. This is the first sign of the formation of the peritoneal epithelium, the thickness of which increases gradually more on one side of the gonad than on the other until, during the prepupal stage, the epithelium on the inner thickened side becomes continuous with the mesodermal strand of the gonad. The rest of the germ cells are divided into several groups by the penetration of fine septa from the peritoneal epithelium. In the prepupa several groups of germ cells are enclosed in definite tubes, the walls of which are derived from the peritoneal epithelium and are the origin of the tunica propria of the ovarioles. The mesodermal strands are syncytial cords which extend from the gonads posteriorly to reach the middle of the seventh abdominal segment (figure 27).

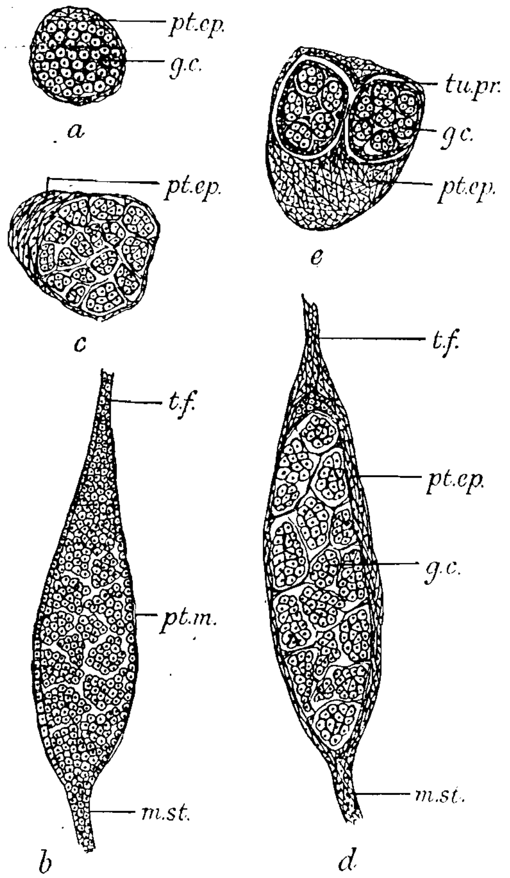


FIGURE 27. Sections through the gonad of the female larva of *Mycetophila cingulum*: *a*, transverse section in a young larva; *b*, longitudinal section of the same; *c*, transverse section in a fully grown larva; *d*, longitudinal section of the same; *e*, transverse section in a prepupa.

(iii) *The secondary reproductive system*

Before proceeding to discuss the development of the secondary reproductive system there is a point of special interest which I came across when studying this species that should be recorded. When dissecting the larval skin of *Mycetophila cingulum* to study the genital imaginal disks on the eighth and ninth abdominal segments my attention was drawn to other hypodermal buds in the same area. When the two segments had been cleared completely from their muscles I found that there were two other pairs of buds on each genital segment besides the middle pair of genital buds. On cleaning the skin of the whole larva it was found that on each segment of the body there are three pairs of hypodermal buds situated, respectively, dorsally, laterally and ventrally. The lateral pair of buds is clearly to be distinguished from the spiracle invaginations. Sections through different parts of the larval body shows very explicitly that all these buds are true proliferative hypodermal elevations which retain their character up to the pupal stage. At this time, all of them, except those which develop into imaginal structures, disappear, taking part in the formation of the body-wall sclerites. This species would appear to be the first case in insects recorded in the literature in which such presence of three pairs of hypodermal buds are present on every segment of the body (thorax and abdomen) (figure 28 *a*).

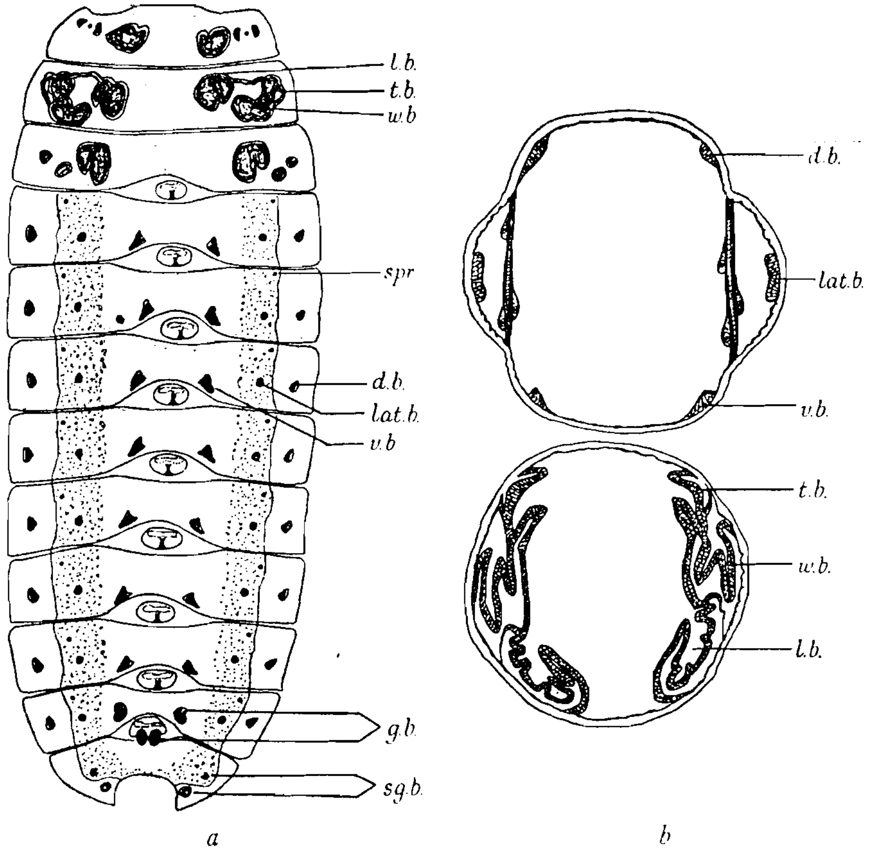


FIGURE 28. *a*, dissection of the skin of a female larva of *Mycetophila cingulum* showing the segmental hypodermal buds; *b*, two reconstructed transverse sections, the upper one through the abdomen, the lower one through the mesothorax.

Two other species of *Mycetophila* were examined to find out how far this phenomenon occurs in other species of the same genus. In *M. fungorum* no traces were found of extra buds other than the usual wing and leg buds in the thorax and the genital buds in the abdomen. In *M. lineola* the condition is intermediate, i.e. three pairs of very faintly indicated buds, which might easily be overlooked, were found on each segment.

Comparing the buds on the abdominal segments with those on the thorax the following conclusions are to be drawn:

(1) The genital buds correspond to the other abdominal ventral buds and to the leg buds on the thorax. From the appearances shown in transverse sections it would be reasonable to consider that the thoracic ventral buds give rise both to the legs and to the imaginal sternites. The ventral buds of the abdominal segments may similarly take part in the formation of their respective sternites before they apparently disappear.

(2) The wing buds on the mesothorax and the halteres buds on the metathorax correspond to the lateral buds on the abdominal segments. This would seem to indicate that the wings are lateral and not dorsal structures.

(3) The dorsal pair of buds is more developed on the mesothorax and the ninth abdominal segment. In the first case they grow to a certain extent and suddenly flatten down on reaching the prepupal stage. They recall to mind the tegulae found at the base of the anterior wings in Lepidoptera, Trichoptera and Hymenoptera, and I have applied the term tegular buds and tegular plates to the dorsal buds of the mesothorax during the larval and prepupal stages respectively (figure 28 *b*).

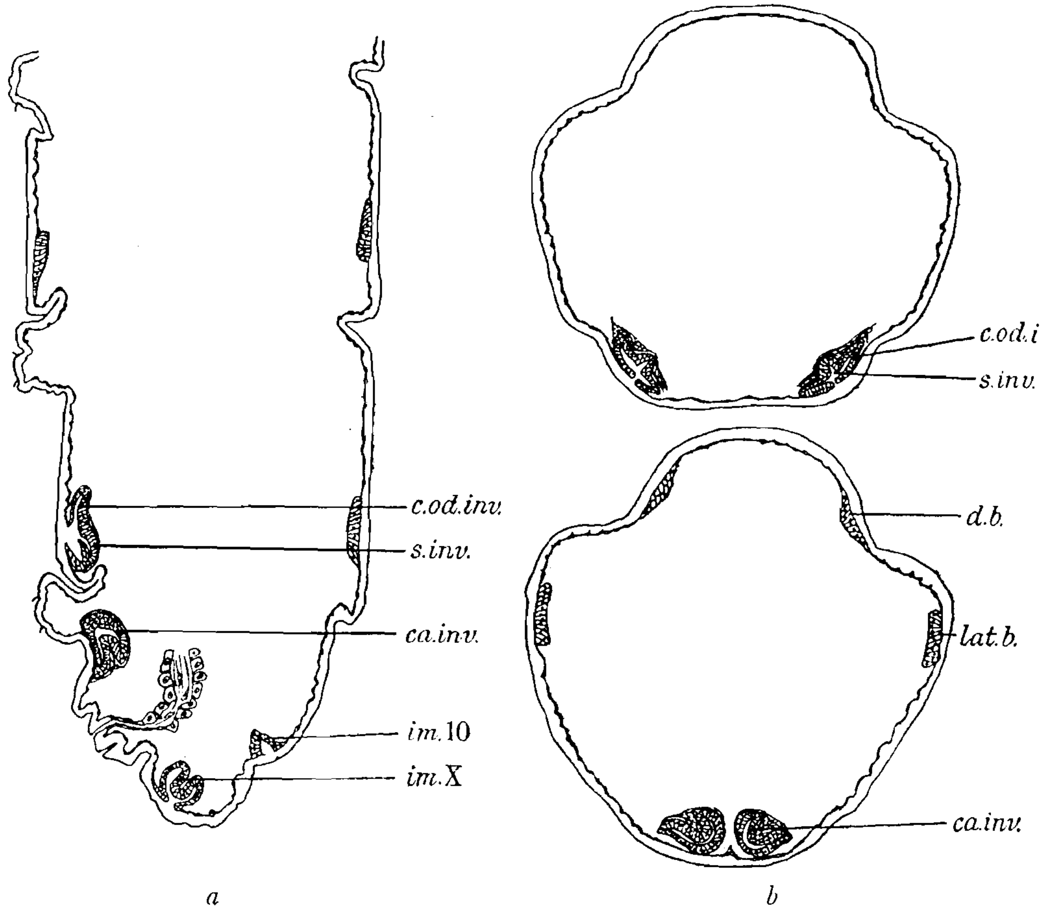


FIGURE 29. *a*, longitudinal section through the distal end of the abdomen of a female larva of *Mycetophila cingulum*; *b*, two transverse sections through the same larva, the upper one through the area of the spermathecal buds and the lower one through the area of the caecal buds.

On the ninth abdominal segment the dorsal buds form the paraprocts which are considered to be the tenth tergite. This suggests also that in other segments dorsal buds may take part in the formation of the tergal sclerites (figure 29 *a*).

In the female larva the ventral pair of buds on the eighth abdominal sternite form the rudiments of the spermathecae and the common oviduct, while that on the ninth segment develops into the rudiment of the caecus. Development begins with the buds acquiring a peripodial cavity. This takes place in the caecal rudiment on the ninth segment earlier than in the buds on the eighth segment. Then each bud of the same pair moves medially so that the two buds eventually join in the mid-ventral line. This takes place in the caecal buds when the larva reaches maturity. The buds on the eighth segment remain separate until the animal enters the pupal stage (figure 29 *b*). This clear origin of the caecal and spermatheco-common oviduct rudiments leaves no doubt of their double nature.

In the fully grown larva the spermathecal invagination appears on the dorsal wall of the peripodial cavity of each ventral bud on the eighth sternite. This happens while the two buds are still quite apart. A little later a sign of another invagination on the anterior wall of the peripodial cavity appears, presenting the double invaginations on the buds of the common oviduct. Both the hypodermal rudiments on the eighth and ninth segment become covered by myoblastic cells coming from the nearby muscles (figure 30 *a*).

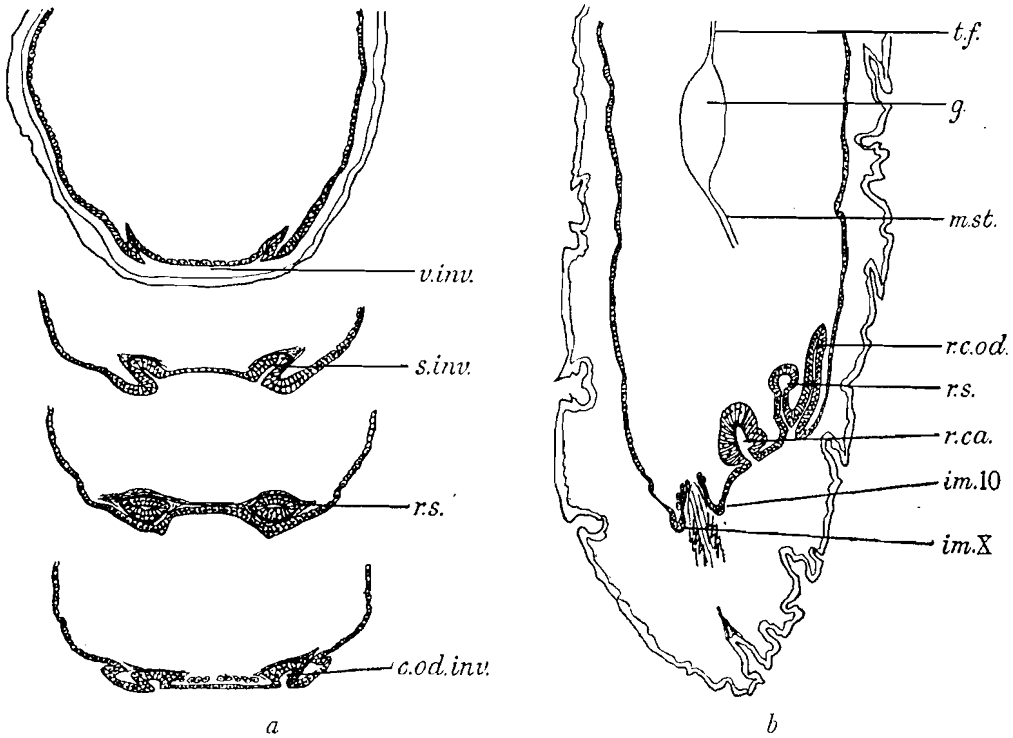


FIGURE 30. *a*, serial transverse sections through the hypoderm of the female larva of *Mycetophila cingulum* in the area of the hypodermal genital rudiments; *b*, reconstructed longitudinal section through the abdomen of the female prepupa of *M. cingulum* showing the position of the gonad and the hypodermal genital rudiments.

In the prepupal stage the fate of the developing structures becomes clearer. The two dorsal buds of the ninth larval abdominal segment develop into the rudiments of the imaginal tergite. The two lateral buds of the same segment move ventrally and come to lie beneath the rectum where they develop into the imaginal tenth sternite.

The two rudiments of the caecus join at the mid-ventral line, their peripodial cavities forming one short distal duct and two small lobes anteriorly.

The ventral pair of buds on the eighth segment which are the rudiments of the spermathecae and common oviduct have also approached very closely to the mid-ventral line so that their peripodial cavities join into one which is the rudiment of the vagina. On the dorsal wall of this cavity arise the two separate invaginations of the spermathecae, and slightly in front of them follow the two invaginations of the common oviduct (figure 30 *b*).

(c) *Development in the pupa*

(i) *The segmental parts*

In the pupal stage the segmental structures begin to arrange themselves according to the imaginal plan. The new tenth abdominal segment surrounds the anal opening. The eighth sternite forms the subgenital plate after the disappearance of the ninth sternite, which does not show itself until late in the old pupa.

(ii) *The primary reproductive system*

In the young pupa each ovary is already divided into the final number of ovarioles, each of which is enclosed by the epithelium of the tunica propria, which is now continuous with the epithelium of the oviduct. This epithelium thins out as the growth of the contained ova proceeds until it becomes nearly as thin as the outer peritoneal membrane.

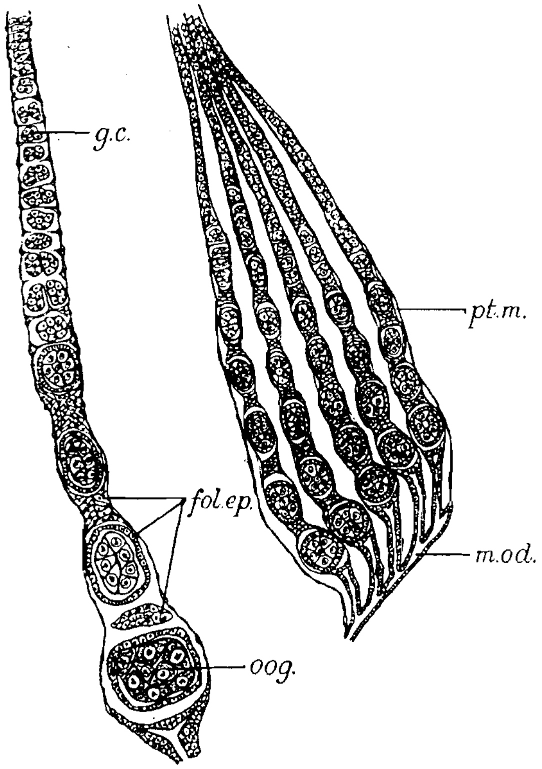


FIGURE 31. Longitudinal section through the ovary of the pupa of *Mycetophila cingulum* with an enlarged ovariole on the left side.

The group of germ cells inside the ovariole wall play different parts as they come nearer and nearer to the base of the ovariole. Some are transformed into follicular epithelium, others grow larger into the oogonial cells which lie encased inside the follicles. Some of the follicular epithelium surrounds the oogonial cells in the form of a definite layer, while that which lies between the oogonia is gradually used up (figure 31).

(iii) *The secondary reproductive system*

The vagina and common oviduct. In the young pupa the vaginal cavity forms a short vestibule at the posterior border of the eighth sternite enclosing the vulvar opening anteriorly and the spermathecal openings dorsally. Soon the wall of the vagina between the spermathecal and vulvar openings is tucked forward in the form of an apodemal pocket, the rudiment of the vaginal apodeme.

The two invaginations of the common oviduct coalesce at their distal portions into one common oviduct, while their anterior portions remain as separate ducts. These latter very quickly extend and soon join the distal ends of the mesodermal oviducts. The myoblastic tissue around the growing ducts is transformed into their muscular coat.

The spermathecae. The spermathecal invaginations which arise independently remain so until they reach the imaginal state. During pupal life the distal part of the invagination lengthens, and its wall becomes non-glandular and coated with a layer of longitudinal muscle. Proximal to this is a portion of the invagination where the wall is more than one cell in thickness. In the old pupa the different parts of the spermatheca become distinguishable. The thick-walled portion has developed an additional outer layer of glandular cells. The proximal part has become the glandular lobe of the spermatheca proper.

The caecus. During the pupal stage the caecal invaginations, which during the prepupal stage have joined into one common duct and two lobes, grow continuously to reach the imaginal condition. The common duct increases in length and becomes surrounded by a thick layer of circular muscle. Towards its anterior portion the cells forming its wall begin to show a glandular appearance which is clearly displayed in the wall of the two caecal lobes. In the old pupa the wall of the lobes is composed of large glandular cells whose inner ends seem to be on the point of bursting to release their secretion into the lumen.

2. THE MALE

(*a*) *Structure of the reproductive system in the adult*

(i) *The segmental parts*

The hypopygium. As in the female the terminal segments of the abdomen are telescoped, but in the case of the male inside the sixth abdominal segment. The seventh and eighth tergites are mostly membranous, except for two narrow semi-circular cuticular streaks. Both seventh and eighth sternites are divided into shield-shaped plates and extra-intersegmental small triangular sclerites which stand at an angle to facilitate the telescoping. The ninth tergite is a small slightly arched plate, lightly emarginated at its posterior border. The ninth sternite is heavily sclerotized, deeply emarginated and markedly curved at the sides to reach the ninth tergite which forms the roof of a complete ring. The tenth segment consists of the usual paraprocts and hypoproct, the former of which may represent tenth tergite and cerci (figure 32 *a*, *b*, *c*).

The gonapophyses. The gonocoxites are two small rectangular plates fringed at their inner sides with rows of strong bristles. Each gonocoxite is jointed to the ninth sternite at two points, allowing movement in a horizontal plane. The styles are sickle-shaped, terminating in two long claws and at the inner side of their bases with rows of strong bristles similar to those on the gonocoxites. Each style is attached to its gonocoxite by a small flexible joint which permits a large range of movement. When the styles are at rest they are directed forward so that their points lie very near to the gonopore (figure 32 *d*, *e*).

The intromittent organ. Before describing the intromittent organ I shall first refer to two apodemal structures which lie around it and play an important part in the musculature of the hypopygium. These apodemes are sclerotizations in the intersegmental membrane which lies between the posterior edge of the ninth sternite and the intromittent organ. The first of these I have termed the basal apodeme, since it lies beneath the intromittent organ with its ends joining the anterior fixation points of the gonocoxites (figure 32 *e*). The second of these hypopygial apodemes consists of two large triangular plates lying at the inner side of the ninth sternite and ending anteriorly in two narrow strips which run medially to join the intromittent organ sheath. They are here termed the thecal apodemes (figure 32 *d*).

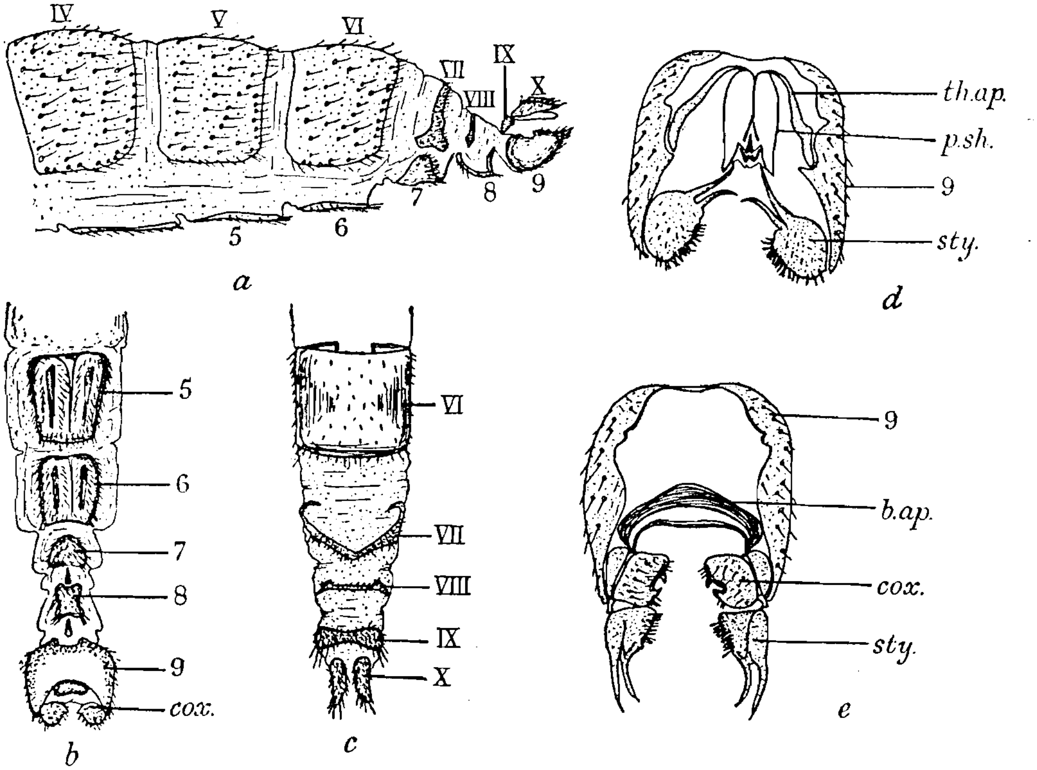


FIGURE 32. *a*, lateral view of the distal part of the abdomen of the male adult of *Mycetophila cingulum*; *b*, ventral view of the same; *c*, dorsal view of the same; *d*, inner view of ninth sternite with intromittent organ and styles at rest; *e*, same as *d* with styles extended.

The rest of the intersegmental area is membranous, and the region which lies between the basal apodeme and the intromittent organ is bulged outwardly in the shape of two round prominences whose cuticle is finely wrinkled. These prominences contain large fat cells which give to the structures the character of soft cushions.

The protrusive body of the intromittent organ consists of four parts, viz. the penis sheath, the apodemal plate, the aedeagus and the parameres.

The penis sheath. This is a sclerotized theca excavated from the intersegmental membrane. It consists of two halves which join medially in the form of a cylinder enclosing the penis tube and acting as attachment for the hypopygial muscles. At the proximal end is a large hole through which the penis tube passes to join the ejaculatory duct (figure 33 *a*).

The apodemal plate. This is a small triangular plate which lies inside the ventral wall of the penis sheath and just above the mid-ventral junction line of the two halves. This plate is permitted a to-and-fro movement by means of a membranous fold which links it with the ventral wall of the penis sheath. The distal broad end of the plate lies beneath the gonopore and causes a bend in the course of the penis tube. It would appear that this plate, by its situation, can easily control the use of the gonopore (figure 33 *b*,*c*).

The aedeagus. This is a very small inconspicuous cuticular arch which overhangs the gonopore. Its recognition as the real aedeagus is not possible unless its development has been followed through the pupal stage (figure 33 *b*).

The parameres. These are also small and together form another, but larger, arch than that of the aedeagus lying underneath the gonopore. The parameres, nevertheless, show their double structure (figure 33 *b*).

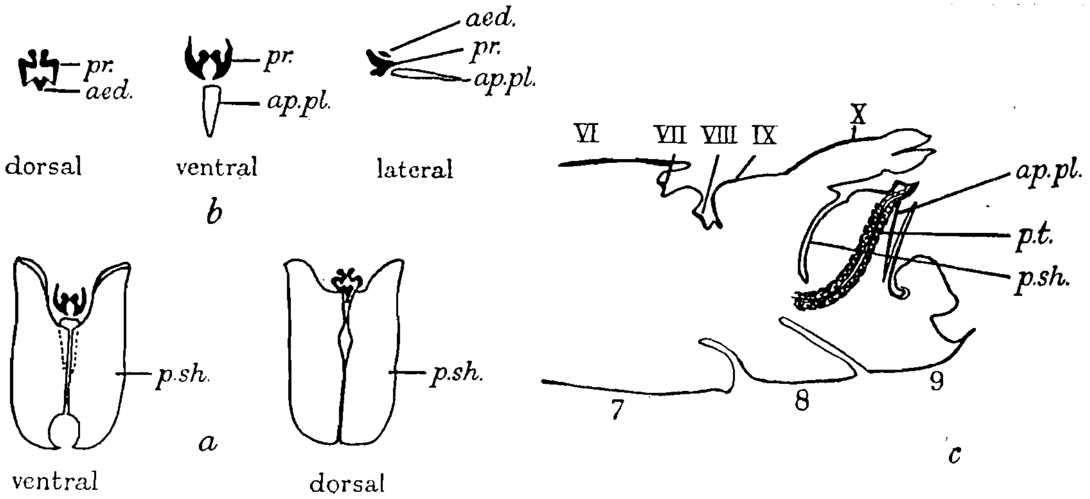


FIGURE 33. Male hypopygium of *Mycetophila cingulum*. *a*, relation of the appendicular appendages to the penis sheath; *b*, relation of the rudimentary aedeagus and parameres to the apodemal plate; *c*, longitudinal section through the distal part of the abdomen showing relation of the apodemal plate to the gonopore.

(ii) *The primary reproductive system*

There are two lemon-shaped testes lying between the fifth and sixth abdominal segments. Each testis is composed of an outer layer of peritoneal epithelium enclosing masses of sperms with a wavy appearance, which I believe is the effect of the mixing of secretion received from the vesiculae seminales (figure 34).

(iii) *The secondary reproductive system*

The two vasa deferentia are rather short ducts which, before joining the two vesiculae seminales, bend back on themselves. They are coated by a thin layer of circular muscles and contain sperms in their lumen. The vesiculae seminales are two long sacs which extend anteriorly to join two globular swellings at the proximal end of the ejaculatory duct. They have thick glandular walls which secrete a viscous finely granular substance into the lumen. The thickness of the vesicular wall decreases and the lumen increases as the vesiculae extend anteriorly. Outside the glandular wall is a thin layer of circular muscles.

The ejaculatory duct starts from the anterior ends of the vesiculae seminales and runs backwards until it joins the penis tube. Along most of its length it is composed of two epithelial lined narrow tubes which are bound together by a thick layer of circular muscles. At its distal portion the duct becomes single.

The penis tube is the last section ~~of~~ the efferent tract lying between the distal end of the ejaculatory duct and the gonopore. It is coated by a layer of circular muscles and passes through the penis sheath to open between the aedeagus and the parameres (figure 34).

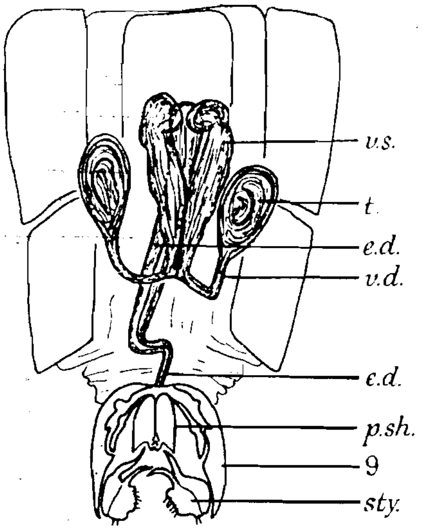


FIGURE 34. Dissection of the reproductive system of the male adult *Mycetophila cingulum*.

(*b*) *Development in the larva*

(i) *The primary reproductive system*

The two gonads are lemon-shaped and lie in the sixth abdominal segment. Each gonad is covered by a thin peritoneal membrane terminating at both ends of the gonad in the surrounding fat body. This means that the gonads in this species have neither terminal filaments nor mesodermal strands of any length. The germ cells are small round cells with clear nuclei, and they do not go through any differentiation in the larval stage. In the prepupa the germ cells become larger and their nuclei are divided into a number of nuclear particles. Inside the peritoneal membrane there can be seen in this stage an even layer of germ cells which lie very close to the outer membrane.

(ii) *The secondary reproductive system*

The three pairs of hypodermal buds which exist on the ninth segment of the larva determine between them the future arrangement of the hypoderm up to the imaginal state. The dorsal pair form the rudiment of the tenth tergite, the lateral pair that of the tenth sternite, while the ventral pair are the genital buds. These latter, as previously noted, do not only give rise to the genital appendages and ducts, but their main part develops into the ninth imaginal sternite.

The genital buds acquire peripodial cavities and approach towards the median ventral line until their inner peripodial walls join into one septum. This latter quickly thins, leaving the two lateral podial projections protruding into one cavity. The podial projections, which are the rudiments of the ninth sternite, grow continuously in a limited space inside the larval skin, so that in the prepupal stage their surface is seen to be wrinkled into small folds (figure 35). The first sign of coxal lobes appears on the distal inner side of the rudiments of the ninth sternite during the prepupal stage.

At the base of the podial projections there appear two invaginations, which are the rudiments of the ejaculatory ducts. This happens whilst there is, as yet, no sign of thecal or penial invaginations.

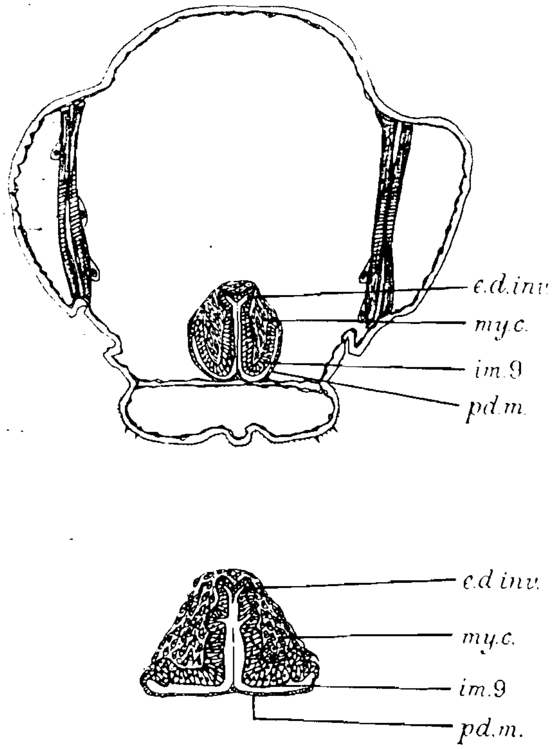


FIGURE 35. Transverse section through the imaginal bud on the hypoderm of the ninth abdominal segment of the male larva of *Mycetophila cingulum*; beneath is shown the imaginal bud in a more advanced stage (slightly larger scale).

On reaching the prepupal stage there appears between the coxal lobes a shallow intersegmental inflexion which I have called the thecal invagination. At the same time a new hypodermal invagination appears distal to the two invaginations of the ejaculatory ducts which carries their openings deeper. This is the penis-tube invagination.

At this stage the ejaculatory duct invaginations have developed into two tubes which become thin and widely separated. Each tube becomes surrounded by a coat of myoblastic cells.

(*c*) *Development in the pupa*

(i) *The primary reproductive system*

The layer of germ cells lying close to the peritoneal membrane of the gonad joins the latter so that the gonadial investment now appears as a peritoneal epithelium. Spermatogenesis has, in the old pupa, brought the contents of the gonads to the spermatozoal stage. Sperms are found in wavy bundles intermingled with secretion.

(ii) *The secondary reproductive system*

During the pupal stage the two halves of the ninth sternite rudiment grow considerably in size and join anteriorly, while their distal parts, which carry the gonocoxites, become bent upwards. Eventually each coxal lobe divides into a basal gonocoxite and a terminal style.

The median part of the intersegmental thecal inflexion begins to project, forming a wide short eminence which carries outwards the opening of the penis tube, the space between the thecal wall and the penis tube finally becoming filled with myoblastic cells which develop gradually into muscular bands.

At the opening of the penis tube on the thecal eminence there appear the second pair of genital appendage rudiments, the paramere lobes (figure 36 *a*). The paramere lobes divide into the two small lobes of the parameres and the median lobes which form the aedeagus.

In the old pupa a cuticular layer is laid down outside the thecal eminence in the form of a cylinder whose sides are continuous with the rest of the intersegmental membrane. This cuticular cylinder forms in the adult the penis sheath (figure 36 *b*).

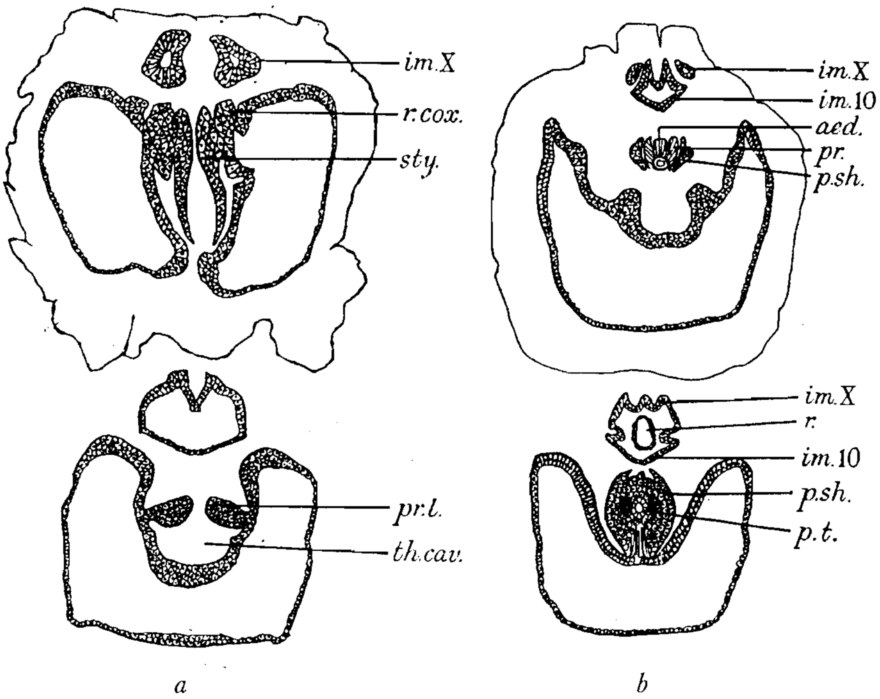


FIGURE 36. *a*, two successive transverse sections through the distal end of the abdomen of a young male pupa of *Mycetophila cingulum* showing formation of the gonocoxites and their styles, the thecal cavity and the paramere lobes; *b*, the same in an old pupa showing aedeagus, parameres and penis sheath.

The efferent tract. The penis tube lengthens. The distal ends of the double ejaculatory duct amalgamate into a single duct for a short distance in front of the penis tube, but otherwise they keep their double structure until they reach the testes. The wall of the middle portion of the duct becomes thickened and its cells develop a glandular character. The duct lengthens very quickly and becomes bent upon itself. The posteriorly directed limb of the bent duct gradually shows a vesicular appearance. The lumen receives secretion from the glandular wall, and this ascends through the vasa deferentia and can be detected at the basal part of the gonads. The two ducts run parallel to each other for most of their course and eventually are bound together by a thick coat of myoblastic tissue which is later transformed into bands of circular muscles.

The proximal ends of the ejaculatory ducts remain separate as thin tubes with narrow lumen and a thin layer of circular muscles. It is only in the old pupa that these narrow terminal portions, which in the adult play the part of vasa deferentia, come into communication with the thick peritoneal epithelium at the base of the two gonads.

VIII. CONCLUSIONS

(*a*) *The abdominal segments*

In all cases the last abdominal segment of the larva gives birth to the additional imaginal segment or segments, bringing the number of segments of the fly's abdomen to ten. This is accomplished through hypodermal proliferative regions (Chironomidae), or proliferative ridges (Anisopidae), or hypodermal buds (Mycetophilidae). The tenth, or anal, segment is composed of three lamellae anales or procts, viz. two paraprocts, representing lateral portions of the tergite or cerci or both, and one hypoproct, representing the sternite.

Torsion of the hypopygium, which is recorded to take place in some species of Culicidae, Psychodidae, Dixidae and Tipulidae, also occurs in *Anisopus fenestralis* (Anisopidae). The rotated parts include the eighth abdominal segment.

(*b*) *The abdominal appendages*

In *Mycetophila cingulum* larva three pairs of buds are present on all the abdominal segments corresponding to those on the thoracic segments, viz. dorsal, lateral and ventral pairs, the last of which in the thorax give rise to the thoracic legs. On entering the pupal stage these buds disappear. A less distinct but similar presence of hypodermal buds occurs in *M. lineola.* The presence of such a complete series is of exceptional interest and gives interesting leads in respect to homologies as discussed in the text. The genital buds correspond to the ventral series in the other abdominal segments and to those of the thoracic legs. On the ninth segment the dorsal buds form the paraprocts, which are considered to be in whole tenth tergite and cerci.

(*c*) *The genital appendages*

The female flies have no genital appendages around the vulvar opening. Nevertheless, the present study supports the view that the genital appendages in the female, when present, rise from imaginal disks on the eighth and ninth abdominal segments. This conclusion is drawn from the fact that the same imaginal disks develop into evaginations which are the rudiments of the genital appendages, as well as invaginations which are the origin of the genital ectodermal ducts. This is exactly what happens in the case of the dipterous males which possess copulatory apparatus. But as the females do not possess such apparatus the imaginal disks give rise only to the genital ducts. In *Chironomus* and *Mycetophila* female larva two separate buds are present on the eighth and ninth abdominal segments.

The true appendicular processes of the male develop from two pairs of lobes, the coxal lobes and the paramere lobes of Singh-Pruthi. In all the cases the coxal lobes appear first, giving rise to the gonocoxites which are constricted off distally to form the styles. In *Chironomus*, where the gonocoxites are of huge size, the paramere lobes arise as detached parts from the base of the coxal lobes. In *Anisopus* and *Mycetophila* the paramere lobes arise at the entrance of a thecal pouch, quite independently from the coxal lobes. The paramere lobes in *Chironomus* do not divide secondarily but grow directly into the club-shaped parameres of the fly, for which I have in the section on definitions proposed the name paramerophores. In *Anisopus* and *Mycetophila* the paramere lobes divide into a median pair of penis valves (aedeagus) and a lateral pair of parameres. In both cases the penis valves and the parameres carry very little of the original function, which has been taken over by the thecal structure, the penis sheath.

(*d*) *The male intromittent organ and related structures*

In the types studied this organ may be essentially a duct merely opening as such on the surface (*Chironomus*), but may secondarily be, with its surrounding tissues, excavated to form a projection carrying this duct. The inner duct, the penis tube, is not developmentally, as mostly considered, merely the distal part of the ejaculatory ducts, but originates from a separate invagination here termed the penis invagination, a structure derived from a single median invagination which appears a little later and quite independently from those of the ejaculatory ducts. The intromittent organ reaches its greatest development in *Anisopus*, where, as in the Tipulidae, it forms a long filamentous projection; nevertheless, not a growth outwards, but an organ essentially excavated by an intersegmental inflexion, the genital pouch, part of which forms the large piston apodeme and its walls much of the pompetta.

A large part of the structures surrounding the intromittent organ in the three species studied, and perhaps in most Nematocera (notably the Tipulidae where the parts are often very similar to those in *Anisopus*), are not derived from an appendicular origin but from intersegmental sclerotizations here termed theca, penis sheath, etc., a fact clearly established in following the development of these parts in the immature stages. In *Chironomus* there is a thecal structure, endotheca, which is tucked inside the penis-tube cavity and is eversible. In *Anisopus* a thecal sclerotization forms a guide (penis sheath) for the filamentous penis. In *Mycetophila* the most conspicuous of the median structures is the large sclerotized prominence of intersegmental origin, the penis sheath.

(*e*) *Development of the ovary*

In all the forms studied the young gonad appears as a mass of small undifferentiated cells covered by a thin non-cellular peritoneal membrane. When differentiation of the germ cells starts some of the cells, those which are nearest to the periphery of the gonad, become larger than those more centrally situated. These larger cells aggregate in oogonial groups and become surrounded by the smaller follicular cells. Each oogonium is composed of several cells which look exactly the same until one of them, the nearest to the centre of the ovary, or the nearest to the oviduct, starts yolk formation within its cytoplasm. This oocyte receives nourishment mostly from the other cells of the oogonium (the nurse cells) and increases continually in size, while the bodies of the nurse cells diminish, until eventually they are completely used up.

The peritoneal coat of the gonad appears in the young larva as a non-cellular membrane or as a thin cellular structure with degenerate scattered cells. This becomes thinner as development proceeds, but meanwhile it may gain an additional cellular provision in that some of the surrounding fat cells may approach closely to the peritoneal membrane and eventually become adherent to it. In other cases some of the germ cells arrange themselves into an even layer, using the peritoneal coat as a basement membrane, so that eventually the two structures form a peritoneal epithelium.

(*f*) *Development of the testis*

The development of the testis starts, as in the case of the ovary, with small undifferentiated germ cells which remain undifferentiated for a comparatively longer period than those of the ovary. In *Chironomus*, spermatogenesis is completed during the larval stage, while in *Mycetophila* growth and nuclear division does not take place until the pupal stage. The nature of the peritoneal testicular coat is exactly the same as that of the ovary. In *Chironomus* the testis is divided internally into several loculi by septa from the outer peritoneal membrane. In *Anisopus* the peritoneal epithelium of the testis is composed of the united peritoneal membrane and fat cells. These latter contain in their cytoplasm brown particles which give the testes a brown appearance. This brown fatty tissue extends over the vasa deferentia and vesiculae seminales. In the female the primordial germ cells in the germarium have the potentiality to differentiate into follicular cells or oogonial cells; in the male all the primordial cells, except very few, are transformed into spermatozoa.

(*g*) *The efferent system*

The present work has shown that in both sexes the accessory organs and the efferent system, except probably a small portion of the lateral genital ducts near the gonads, originate from the hypoderm.

The mesodermal strands which, when present, extend posteriorly from the peritoneal coat of the gonads form fine syncytial cords, and in both sexes are present initially as two branches, one extending from the gonad to the seventh and the other to the ninth sternite. These strands establish the linkage between the gonads and the ectodermal efferent ducts. After junction of these two parts has been established an ectodermal encroachment takes place from the proximal part of the hypodermal efferent ducts to invade and largely replace the original tissue of the mesodermal ducts. This encroachment has been shown in both sexes of the three species studied. The condition in the male *Mycetophila*, where the mesodermal strands are very short or absent, is evidence that anterior extension from the ectodermal ducts could reach the gonads even without the intervention of a mesodermal tissue tract.

At the point where a mesodermal strand ends distally it is usually fixed to the hypoderm through some myoblastic tissue, which is clearly distinct from that of the rest of the mesodermal strand. This fact has not been realized by many authors, who have described the mesodermal strands as extending posteriorly further than they really do go. This has led to the description of terminal ampullae, which are, in fact, hypodermal rudiments of the secondary reproductive system. The myoblastic tissue does not merely run between the distal ends of the mesodermal strands and the hypoderm, but it also covers the growing rudiments of the ectodermal organs. It is transformed later into the muscular tissue of the hypopygium.

(*h*) *The efferent system in the female*

The efferent system, excluding the two mesodermal oviducts, and the accessory organs originate in all the forms studied from hypodermal rudiments, one on the eighth and one on the ninth sternite. Each of these rudiments starts in the young larva as two separate podial buds which soon coalesce at the mid-ventral line into a single structure.

The hypodermal rudiment on the eighth sternite gives rise to the spermatheca and the common oviduct, the degree to which these organs arise from a double origin varying in the case of the three species studied. In *Mycetophila* both the spermatheca and the common oviduct arise as double invaginations, the first remaining double up to the adult stage, the second coalescing distally into one duct. In *Chironomus* the spermatheca arises from a double origin and more or less remains so up to the adult stage, whilst the common oviduct develops from the beginning as a single invagination which lies slightly in front of the spermathecal invagination. In *Anisopus* both the spermatheca and the common oviduct develop from the very early stage as a single invagination.

The spermathecal and vulvar openings are enclosed in a short vestibule. This is the vagina which is originally the peripodial cavity of the imaginal disk in the larva. It should not be considered as an extended part of the common oviduct, a view that has sometimes led to the spermatheca being considered as a secondary structure springing from the dorsal wall of the common oviduct. Actually the spermathecal invagination appears before that of the common oviduct.

The hypodermal common oviduct invagination extends anteriorly until it joins the distal ends of the mesodermal oviducts. In *Mycetophila* this invagination is branched before it joins the mesodermal structures, and in *Anisopus* there is also a slight division at the proximal end of the common oviduct rudiment. In these two cases therefore the oviducts are from the beginning ectodermal in their distal portions.

In the three species studied there is present over some portion of the spermathecal duct an outer layer of huge glandular cells. In *Anisopus* and *Mycetophila* the sac of the spermatheca proper is also coated with a layer of glandular cells.

The hypodermal rudiment on the ninth abdominal sternite gives rise to the caecus (the accessory glands of other insects). This rudiment also starts from a definite double origin, which it soon loses, though revealing its original double origin by a slight notch at its anterior end or by being divided anteriorly into two lobes.

(*i*) *The efferent system in the male*

The whole of the rudiments of the male efferent system appear within the ninth abdominal segment. The first structures to appear are the double invaginations of the ejaculatory ducts. These keep their double nature throughout in *Chironomus*. In *Anisopus* and *Mycetophila* their distal ends coalesce into a single duct. Each duct is divided into a distal non-glandular part and a proximal glandular part. This latter becomes swollen by reason of the thickness of its wall and develops into the vesiculae seminales, structures which play the part of accessory glands as well as sperm stores. In *Chironomus* the glandular part is separated from that part which acts as a store, the former consisting of the proximal glandular part of the ejaculatory duct, while the vesiculae are dilated parts of the vasa deferentia. This is due to the glandular part of the ejaculatory duct producing its secretion before receiving the sperms, the secretion then blocking the passage of the sperms which have of necessity to accumulate behind the ejaculatory duct, i.e. in the region of the vasa deferentia.

The lateral gonoducts vary in structure in the three species. In *Mycetophila* the vasa deferentia are completely ectodermal as the gonads have no mesodermal strands, and so the proximal portions of the ejaculatory ducts have to take their place and extend to join the peritoneal epithelium of the testes. In *Chironomus* the lateral gonoducts are the result of a linkage between the mesodermal vasa efferentia and the ectodermal vasa deferentia. In *Anisopus* the lateral gonoducts are for the greater part of their length derived from the mesodermal vasa efferentia. In the latter two cases ectodermal encroachment takes place from the proximal part of the secondary efferent system as in the case of the female.

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