INTERNAL GENITALIA OF THE FEMALE HOUSE FLY, 
*MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE): ANALYSIS 
OF COPULATION AND OVIPOSITION*

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Abstract—The morphology of the internal genitalia of the adult female *Musca domestica* L. was examined to determine the role of these structures during copulation and oviposition. Three major structures, the dorsal, ventral, and posterior valves, project into the lumina of the posterior common oviduct and anterior vagina in the region of the vaginal pouches. The dorsal valve is composed of 2 folds of epithelial tissue suspended from the dorsal vaginal wall, and houses the posterior openings of the spermathecal and accessory gland ducts. During copulation, the dorsal valve receives the aedeagus and is the site of sperm deposition. The ventral valve lies directly anterior to the dorsal valve and consists of a large, transverse muscle band and a posteriorly directed epithelial projection. Immediately beneath the epithelial projection, the vagina forms a blind, conical pocket, the anterior chamber. Fertilization is accomplished within this structure during oviposition. The posterior valve consists of 2 widely separated folds (or arms) of epithelial tissue arising from the ventral floor of the vagina. These arms, along with those of the dorsal valve, hold the egg in position while sperm penetration occurs. 

Index descriptors (in addition to those in the title): Insemination, sperm penetration, coitus.

INTRODUCTION

SUCCESSFUL mating among house flies, *Musca domestica* L., includes transfer of both the sperm and the male accessory secretion. Recent studies have demonstrated that male accessory secretion is synthesized by secretory cells that line the thickened anterior one-third of the ejaculatory duct, and is transferred to the vaginal pouches of the female during copulation where it is absorbed into the haemocoele (Leopold, 1970; Leopold et al., 1971). Once in the female, the accessory secretion causes loss of mating receptivity, and acts as a stimulus for oviposition (Riemann et al., 1967; Riemann and Thorson, 1969).

As a part of our investigation into the mechanisms of transfer of sperm and accessory secretion, and their subsequent utilization by the female house fly, we made a detailed study of the posterior internal female reproductive system. Previous investigations have dealt mostly with the external features of the female tract (Hewitt, 1914; West, 1951). For example, Berlese’s (1902) account of copulation in house flies includes illustrations taken from sections of the genital organs of both sexes, but he did not sufficiently label or describe the internal structure of the female reproductive tract. Perhaps the most extensive study to

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date of the female house fly reproductive structures was included in Graham-Smith's (1938) comparison of the histology and morphology of the generative organs of the blow fly, Calliphora vicina (≡ erythrocephala Meig.) with those of the house fly. Although the musculature of the reproductive tract of both insects was described in detail, certain features of the female house fly reproductive tract were not discussed. In the present paper, we describe the morphology of the female internal reproductive tract and present an interpretation of its role during copulation and oviposition.

MATERIALS AND METHODS

House flies used in this study were of the Fargo-W_2 strain, and larvae were reared on CSMA media (Soap Chem. Specialties, 1963). Adult flies were maintained on water and a diet of dried milk, sugar, and dried egg yolk (6 : 6 : 1 by weight).

Tissues to be examined were prepared by the freeze substitution method of Feder and Sidman (1958). Isopentane chilled with liquid nitrogen was the quenching solution, and absolute methanol or ethanol was the substituting medium. Initially, mercuric chloride (1-0%) and picric acid (5-0%) were added to the alcohols to enhance fixation, but substitution in alcohol alone for 7–10 days at −70°C subsequently proved to be sufficient.

Morphological observations on the ovipositors were made from 3 groups of females: virgin females, females in copu], and mated females with their ovipositors extended during the process of oviposition.

Ovipositors of virgin females were collected by pinning the female to a wax block, evertting the ovipositor, and severing it with a microscissors near its junction with the abdomen. Ovipositors of females in copu] were obtained by capturing mating pairs immediately after they coupled, allowing them to copulate for 5, 10, 30, 40, or 60 min, and then either dropping them intact into the quenching solution or severing the male and female abdomens prior to quenching. Twenty-five ovipositors were collected in this manner from each group.

Ovipositors of mated females were obtained during the process of oviposition as follows: specially constructed egging tubes were fashioned from 8-dram glass shell vials after the bottom half of the vials had been cut away and discarded. Then a single layer of clear plastic film (commercial food wrap) was tightly stretched across one end of the cylinder and held in place by an elastic band. From 15 to 20 females that had been immobilized by chilling on ice for 5–10 min were crowded into the vials with their venters against the plastic film. Foam plastic plugs, inserted into the open end of the vial, held the females in place, but allowed the insertion of an eyedropper that was used to apply 4–5 drops of 2.5% aqueous ammonium carbonate, the latter acting as an ovipositional stimulant. Once oviposition had begun, the egging vials were placed under a dissecting microscope, and a sharply pointed microscissors was inserted through the plastic film so its tips straddled the ovipositor. When an egg had passed down the common oviduct and come to rest briefly within the anterior vagina, the ovipositor was quickly severed from the abdomen and immediately transferred to the quenching solution. Thus, the position of the egg and sperm within the female genital chamber during the process of sperm penetration could be determined.

The tissues that were to be examined were then dehydrated, cleared, embedded in Paraplast(R), sectioned at 8 μ, stained by the Feulgen reaction, and counterstained with 0.05% alcoholic Fast Green.
The anatomy of the various internal structures of the female house fly reproductive tract was found to be similar to that of the mosquito, *Aedes aegypti*, as described by Spielman (1964) and Jones and Wheeler (1965). Therefore, we adopted their terminology for the description of these structures in the female house fly.

**Gross morphology of the internal female reproductive tract**

The internal genitalia of the female house fly utilized during copulation and oviposition are located entirely within the eversible ‘ovipositor’ (= ovitubus, DeWilde, 1964). This ovipositor consists of the terminal abdominal segments VI through IX, each connected by a highly flexible intersegmental membrane. When the ovipositor is fully extended and viewed ventrally, the 3 dark-brown spermathecae are visible through the membrane that connects segments VI and VII. If the body wall of the ovipositor is opened along the dorsal side and the hindgut removed, the remaining surface features of the reproductive tract are visible within segments VI and VII (Fig. 1). They include the spermathecal ducts, the paired accessory glands and their ducts, the paired vaginal pouches, and 2 lateral muscle bands.

![Fig. 1. Dorsal view of structures associated with posterior common oviduct and anterior vagina. AG = accessory gland; AGD = accessory gland duct; ATM = anterior transverse muscle; CO = common oviduct; S = spermatheca; Sa = sacculus; SD = spermathecal duct; TM = transverse muscle of the ventral valve; VP = vaginal pouch. Scale = 0.4 mm.](image-url)
The region of the dorsal vagina from which the spermathecal and accessory gland ducts emerge (Fig. 1) has been termed the "sacculus" (Hewitt, 1914) or "camera dell'ovidutto" (Berlese, 1902) and was depicted by Berlese as a spherical swelling immediately dorsal to the lateral openings of the vaginal pouches. Our external observations did not indicate that the sacculus was a readily distinguishable feature of the reproductive tract, but internally it was found to contain structures related to copulation and oviposition and also the posterior opening of the common oviduct or gonopore. The genital chamber (= vagina) is distinguished from the common oviduct in this region in that it receives the spermathecal ducts into its anterior end (Snodgrass, 1935).

The walls of the common oviduct and vagina are similarly constructed and consist of 3 distinct layers: an outer muscular sheath, an underlying single layer of epithelial cells, and an intima. The vaginal musculature consists of an external layer of transverse muscle surrounding an inner layer of longitudinal muscle (Fig. 2), the latter greatly reduced in size and in number in the area of the valve complex. The musculature of the common oviduct consists of 2 semi-circular bands of muscle that arise laterally from either side of the oviduct, and nearly encircle it. Thus, this arrangement presents a latticelike pattern on the dorsal and ventral surfaces. When observed in transverse section, these 2 muscle layers appeared similar to the circular and longitudinal muscle layers of the vagina.

The epithelial layer underlying the musculature of the vagina is composed of a sheath of indistinct, flattened cells that extend throughout the length of the vagina. Although these epithelial cells are continuous with those underlying the cuticle of the body wall, they are separated from the intima of the vagina by a space (about 25 μ wide, Fig. 2) that is filled with a substance which is principally an acid mucopolysaccharide (Leopold, unpublished). The epithelial sheath of the common oviduct, a single layer of cells attached to the underlying cuticle, is continuous with that of the vagina. The squamous epithelial cells of the vagina are indistinctly separated; those of the common oviduct are cuboidal and have well-defined boundaries (Fig. 3).

The intima of the middle and posterior vagina is thin (< 0.5 μ) and contains many irregular folds, but in the anterior vaginal region, it is considerably thicker, often exceeding 5 μ, and surrounds the internal valvelike structures. The intima in the common oviduct is extremely thin, and was not detectable under brightfield illumination because of its close attachment to the underlying epithelial cells, except by staining with thiazine red and viewing under polarized light (Füller, 1965).

The 2 prominent muscle bands that project from the surface of the genital tract (Fig. 1—ATM, TM) extend laterally and attach to the body wall. The anterior band, which consists of 10–15 fibers, emerges from either side of the common oviduct at the level of the spermathecae, and extends to the posterior margin of abdominal segment VII, where the 2 ends attach midway between the rodlike tergal and sternal sclerites. The anterior transverse muscle band does not penetrate the epithelial layer of cells of the oviduct, but passes dorsal to it before emerging from either side of the oviduct (Fig. 3).

The transverse muscle band that emerges from the posterior end of the common oviduct (Fig. 1) appears similar to the anterior band, but it extends completely through the walls of the common oviduct into the lumen, and forms part of the internal valvelike complex that divides the posterior common oviduct from the anterior vagina. The morphology and histology of the vaginal pouches and their role in the absorption of the male accessory secretion have been previously described by Leopold et al. (1971). A description of the spermathecae and accessory glands is under preparation.
Fig. 2. Transverse section of vagina posterior to sacculus (phase contrast). C = cuticular intima; CM = circular muscle; E = epithelium; LM = longitudinal muscle. Scale = 50 μ.

Fig. 3. Transverse section of common oviduct showing anterior transverse muscle band (phase contrast). The cuticular intima is not visible because of its close attachment to the epithelial cells. ATM = anterior transverse muscle; E = epithelial cell; M = muscular sheath of the common oviduct. Scale = 50 μ.
Morphology of the valve complex

The dorsal valve (genital tubercle of Graham-Smith, 1938) is a large conspicuous structure formed by 2 folds of epithelial tissue that jut downward from the dorsal surface of the anterior vagina (Figs. 4–6). It varies from 0.06 to 0.08 mm in length, and extends nearly to the ventral floor of the vagina. Near its posterior end, the ventral margins of the valve fold inward to form 2 J-shaped arms (Figs. 4 and 6). The remainder of the valve is unmodified, with the walls of the 2 folds being roughly parallel. As shown in Fig. 6, the epithelial cells of the dorsal valve are densely packed along the periphery of the 2 arms, but they appear to be similar to the cells underlying the intima along the rest of the vagina.

Midway along the length of the dorsal valve in the cleft between the 2 folds of epithelial tissue, a single spermathecal duct arises within a small chamber or vestibule (Fig. 5, v). As the duct passes anteriorly and dorsally, it bifurcates to form 2 ducts. The third spermathecal duct arises by a secondary bifurcation of the left duct as it extends outward through the dorsal vaginal wall. Within the lateral walls of the vestibule are 2 minute orifices that gradually widen anteriorly to form the ducts of the accessory glands (Fig. 7, AGD). The accessory gland ducts continue to pass lateral to the spermathecal ducts and extend through the dorsal surface of the vagina slightly anterior to the dorsolateral openings of the vaginal pouches.
The ventral valve projects into the lumen of the genital tract immediately anterior to the dorsal valve. In sagittal section, the margins of the 2 valves form an interlocking S-shaped structure (Fig. 5). The ventral valve consists in part of a muscle band (Figs. 4, 5 and 7, TM) that extends transversely through the dorsolateral walls of the common oviduct near its junction with the vagina. This transverse muscle band is composed of 10–15 fibers that form a U-shaped structure when viewed in transverse section. After passing through the walls of the common oviduct, the transverse muscle band extends posteriorly and dorsally within segment VII where the 2 ends attach to the posterior part of the abdominal tergites of this segment.

The remainder of the ventral valve consists of an infolding of the epithelial sheath along the ventral midline of the transverse muscle band. We have designated this structure as the epithelial projection of the ventral valve (Figs. 4, 5 and 7, EP). The posterior end of the epithelial projection in transverse section appears as a U-shaped fold about 0.45–0.65 mm wide that is loosely attached to the transverse muscle band (Fig. 4). As it passes anteriorly, the fold of epithelium gradually closes upon itself to form a solid plug of closely packed cells. The main body of the epithelial projection is situated directly mesad of the dorsolateral openings of the vaginal pouches.

Directly beneath the ventral valve, a blind, conical cavity of the vagina extends anteriorly to form the anterior vaginal chamber (Figs. 4, 5 and 7, AC). The epithelial projection of the ventral valve forms the roof of the anterior chamber, and the ventral surface of the vagina forms the floor. Laterally, the anterior chamber opens directly into the paired vaginal pouches to form 3 interconnected compartments. Numerous flexible spines (Fig. 10, CS) extend from the cuticle into the cavity of the anterior chamber and continue along the ventral floor of the vagina to a point nearly opposite the vestibule of the dorsal valve (Fig. 5).

A third infolding of the epithelial lining occurs along the ventral floor slightly posterior to the point where the dorsal valve projects from the opposite wall and forms the posterior valve (Figs. 5 and 6, PV). In transverse section, the posterior valve is bowl-shaped with widely separated margins. When the ovipositor is only partly everted, the posterior valve extends forward and partially envelopes the ventral margins of the dorsal valve (Fig. 6).
The valve complex during copulation

Copulation in house flies occurs by a partial extension of the female terminalia into the genital atrium of the male without an active intromission of the male organ. As a result, positioning of the ejaculate during insemination is determined by directional movements of the ovipositor.

In the present study, the term *aedeagus* is used to refer to the whole copulatory organ (after Van Emden and Hennig, 1956); it consists of a tubular basal portion, the phallobase, joined to the membranous tip, the endophallus (Fig. 8). Graham-Smith (1938) referred to the expanded portion of the phallosome immediately preceding the endophallus as the collar. The male gonopore is located in the furrow between the 2 membranous ridges along the dorsal surface of the endophallus (Figs. 8 and 9). The entire intromittant apparatus is housed within the genital atrium along the ventroposterior surface of the abdomen.

At the onset of copulation, the ovipositor is partially extended and positioned within the male genital atrium so that the female genital aperture (vulva) receives the aedeagus. As intromission occurs, the secondary forceps of the male (the ventral arch of segment VII of Hewitt, 1914) grasp the dorsal and lateral surfaces of the ovipositor and assist in positioning the ovipositor within the atrium. Once coupling is achieved, the forceps press against the sides of the ovipositor in the region of the dorsal valve (Fig. 9), apparently to aid in insuring firm union of the male and female genitalia during coitus. Within 5 min after coupling, the endophallus is situated between the 2 lobes of the dorsal valve, and sperm are present within the terminal portion of the ejaculatory duct. Although the endophallus was situated within the dorsal valve of all females that had mated for 5 min, the extent of intromission was variable. In 55% of the females examined at 5 min post-coupling (20 observations), the endophallus was located within the vestibule of the dorsal valve or slightly posterior to it; in the other 45% of the females, the endophallus was anterior and ventral to the vestibule and often extended as far anteriorly as the epithelial projection of the ventral valve. Although none of the spermathecae was filled with sperm within 5 min after initiation of copulation (64 observations), 12.5% had small-to-moderate amounts of sperm and many of the spermathecal ducts contained sperm.

At 10 min after coupling, over 96% of the spermathecae contained sperm (69 observations), and globules of male accessory secretion and small amounts of sperm were frequently observed within the vaginal pouches. The endophallus was still within the dorsal valve at 10 min post-coupling, but it extended beyond the vestibule in only about 18% of the females. Throughout the remainder of the mating sequence, as the time spent *in copulo*
increased, the incidence of females with the endophallus anterior to the vestibule also increased. At 30, 40 and 60 min after coupling, there was deep intromission of the endophallus in ca. 32, 77 and 91 % of the females, respectively. The vaginal pouches of females that had mated at least 30 min were greatly distended with the male accessory secretion, and occasionally small masses of sperm were observed within the lumina of the pouches. Thus, the dorsal valve was utilized extensively during insemination, but the ventral and posterior valves appeared to have no active part in the copulatory act.

The female genital apparatus during oviposition

Oviposition begins by the female making a series of telescoping movements of the ovipositor, with the tip apparently probing the surrounding area for a suitable place to deposit the eggs. The ovipositor, which is only partially extended for copulation, is usually fully extended for oviposition. After an egg has been released from an ovariole into the lateral oviduct, it passes rapidly and continuously down the common oviduct, enters the genital chamber, and is held briefly before being deposited. A series of timed observations of 61 eggs from 11 females disclosed that eggs were held in the genital chamber for an average of 5.4 sec (range from 3.1 to 10.1 sec). The amount of time that eggs were held in the genital chamber was fairly uniform within individual females but varied among females. After deposition of an egg, the ovipositor was withdrawn slightly. The walls of the common oviduct and vagina underwent considerable muscular contraction before each succeeding egg entered the oviduct.

Observations from sectioned material revealed that as an egg passed from the common oviduct into the genital chamber, the orifice between the ventral and dorsal valves became greatly enlarged, and the 2 valves were pushed wide apart. The floor and roof of the anterior vaginal chamber were pressed together and the 2 arms of the dorsal valve were forced against the dorsal vagina. The egg descends the common oviduct with the slightly tapered end bearing the micropyle directed anteriorly. The direction of movement of the egg was reversed after it entered the genital chamber and its anterior end was thrust slightly forward and ventrally into the anterior vaginal chamber.

The examination of ovipositors removed from females during the short interval between deposition of successive eggs showed that sperm were directed toward the anterior vaginal chamber after they left the spermathecal ducts. Sperm reaching the chamber appear to become trapped among the cuticular spines that cover the walls. Subsequently, as the anterior end of the egg is forced into the conical anterior vaginal chamber, the spines are bent anteriorly, and sperm are directed forward toward the entrance of the micropyle (Fig. 10, S, M). During sperm penetration, the arms of the dorsal valve press the egg tightly against the venter of the genital chamber, while the arms of the posterior valve are forced apart and compressed by the egg. In Fig. 11, 2 sperm appear to be penetrating the micropyle simultaneously. Usually, from 2 to 4 sperm were observed within the anterior chamber prior to the entry of the egg.

DISCUSSION

The examination of the female house fly internal reproductive tract indicated that certain structures were utilized mainly for copulatory purposes; others appeared to be used chiefly for oviposition. Also, although the dorsal, ventral, and posterior valves of the female house fly are morphologically similar to structures in the reproductive tract of
female mosquitoes, they appear to have different primary functions. According to Jones and Wheeler (1965), the dorsal valve of *Aedes aegypti* has from 10 to 15 denticles along its dorsal surface that mesh with a similar dentate area along the distal half of the aedeagus, thus strengthening the connection between the 2 structures during copulation. They also indicated that pressure from the tip of the aedeagus forced the dorsal valve ventrally and posteriorly to form a coital cavity that matched the shape of the remaining central portion of the aedeagus where the male gonopore is located. The spermathecal ducts were said to open directly into the bursal orifice rather than into the vagina or the dorsal valve. Thus, in *Aedes*, the dorsal valve serves chiefly to insure a firm connection during coitus and to form a copulatory chamber by its displacement.

In the female house fly, the dorsal valve serves to position the endophallus near the opening of the spermathecal ducts into the vagina. The dorsal valve also functions as a type of internal 'clasper' for the male intromittant organ. As the secondary forceps of the male press against the lateral surfaces of the ovipositor, the arms of the dorsal valve are forced against the aedeagus, thus insuring a firm connection during coitus. According to Hewitt (1914), the vaginal pouches (accessory copulatory vesicles) insure firm attachment of the male and female genitalia during mating by expanding as copulation progresses. However, a recent study by Leopold *et al.* (1971) indicated that the primary role of the vaginal pouches is probably for collection and assimilation of the male accessory secretion. We have frequently observed that in recently mated females, the vaginal pouches will remain greatly expanded after termination of copulation. This indicates that the pouches appear to play a minor role, if any, in insuring firm coitus.

Our observations agree with those of Murvosh *et al.* (1964) who found that complete sperm transfer occurred in 10 min or less and that the spermathecae were usually filled within 10 min after initiation of copulation, even though copulation often lasted for over an hour. The remaining time spent *in copulo* is apparently utilized for deposition of male accessory secretion (which reached the highest level about 40 min after the start of copulation) and for the assimilation of this material by the female (Leopold *et al.*, 1971). The increased incidence of females with the endophallus positioned anterior to the entrance of the spermathecal ducts after 10 min *in copulo* indicates that deep intromission occurs as accessory secretion is being deposited. We cannot account for the relatively high incidence of females (45%) exhibiting deep intromission of the endophallus 5 min into coitus. Variability in the extent of intromission of the aedeagus within the dorsal valve also indicates that the ovipositor undergoes a considerable amount of extension and retraction during copulation.

After deposition within the dorsal valve, sperm apparently are pushed anteriorly into the vestibule by the spatula-shaped endophallus by means of posteriorly directed movements of the ovipositor. How sperm reach the spermathecae once they are in the spermathecal ducts is unknown. In a previous investigation of the female reproductive apparatus, we found a longitudinal muscular sheath surrounding the distal half of the spermathecal ducts; this sheath was innervated by small branches arising from a main trunk of the median abdominal nerve (Degrugillier and Leopold, 1972). Since severance of the main abdominal nerve trunk resulted in a lack of sperm in the spermathecae in over 73% of the females tested, sperm migration to the spermathecae may be aided by muscular contractions of their ducts.

During oviposition, the arms of the dorsal valve retain sperm after they are released from the spermathecal ducts, and the sperm are subsequently directed forward to the
anterior chamber. Whether sperm reach the anterior chamber independently or whether they are transported by muscular contractions of the vagina was not resolved in this study. The intense contractions of the vagina prior to passage of the egg down the common oviduct suggest that sperm may be at least partially transported from the dorsal valve to the anterior chamber by contraction of vaginal muscles.

Attachment of the transverse muscles of the ventral valve posteriorly on the tergites of segment VII apparently provides a means for effectively closing the genital tube. The contraction of fibers within this band forces the interfacing margins of the dorsal and ventral valves together, thereby sealing off the vagina from the common oviduct. This response appears to be of primary importance to oviposition since it prevents the anterior end of the egg from retreating into the common oviduct and allows it to enter the anterior chamber. In addition, closure of the tract in this region may prevent sperm and accessory secretion from entering the common oviduct during insemination.

Graham-Smith (1938) reported a median muscle band connecting the inner walls of the vaginal pouches that apparently provides a means for opening and closing the entrance to these structures. Although we were unable to verify the presence of this muscle band, it seems probable that a mechanism would be required for retaining the male accessory secretion within the vaginal pouches after the aedeagus has been withdrawn from the dorsal valve.

Our observations concerning the number of sperm retained within the anterior vaginal chamber immediately preceding sperm penetration of the egg generally coincided with the observations of LaChance and Leopold (1969) concerning the incidence of polyspermy within the house fly egg after oviposition. They reported that usually from 1 to 4 sperm are found within the egg during or shortly after fertilization, and that over 50% of the eggs contained 2 sperm. In the present study, we again observed that from 2 to 4 sperm are normally released from the spermathecal ducts and enter the anterior chamber between the deposition of successive eggs; thus, essentially all the sperm released from the spermathecae during oviposition appear to enter the egg.

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