

The following is a list of materials necessary to build the described apparatus:

- 24 Sealed ball bearing pillow blocks with 1.3-cm bores
 - 1 Pulley 20.3 cm
 - 1 Pulley 5.1 cm
- 10 Pulleys 10.2 cm
- 5 V-belts Truflex® 2330
- 1 V-belt Demco® 4L340
- 30 Shaft collars, 1.3-cm bore
 - 6 Lengths water pipe 1.9 cm ID x 25.4 cm
 - 12 Lengths cold rolled steel shafts 1.3 x 45.7 cm
 - 12 Lengths flexible tubing PVC, Nalgon® size (31.8 cm x 2.5 cm ID)
 - 1 Roll carpet tape
 - 1 AC motor 1/3 HP, 1725 rpm, 115-V
 - 1 Shut-off timer and buzzer ¼ HP, 120-V, 1600-W
 - 3 Lengths slotted angle iron 186.7 cm
 - 3 Lengths slotted angle iron 38.1 cm
 - 4 Mounting brackets of slotted angle iron 7.6 cm
 - 8 Shield mounting brackets of slotted angle iron 2.5 cm
 - 1 Box slotted angle serr. nuts & bolts 3-8 x 5-8
 - 2 Lengths slotted angle iron 72.4 cm
 - 2 Lengths slotted angle iron 49.5 cm
 - 1 Length wood slat 1.3 x 5.1 x 186.7 cm
 - 4 Rubber wheel castors 10.2 cm
 - 4 Rubber stoppers 3.8 cm diam.
 - 6 Pipe, "T" joints 2.5 cm
 - 8 Pipe, flanges 2.5 cm x 8.9 cm diam.
 - 1 Length pipe 2.5 x 170.2 cm
 - 4 Pipe, end pieces 2.5 x 11.4 cm
 - 4 Pipe, leg pieces 2.5 x 47.0 cm
 - 4 Pipe, leg pieces 2.5 x 26.7 cm
 - 1 Sheet aluminum (pulley & belt shield) 50.8 x 189.2 x 0.10cm
 - 2 Sheet aluminum end brackets 14.0 x 15.2 x 0.10cm
 - 12 Sheet aluminum fins 11.4 x 20.3 x 0.10 cm
 - 1 Box Pop-Rivets®, grip range 0.3 x 0.3 cm
 - 6 Carboys, wide mouth with handles, Nalgene®, 8.9 cm diam. mouth, 8 liter capacity

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References Cited

Bailey, D. L., D. A. Dame, W. L. Munroe and J. A. Thomas. 1978. Colony maintenance of *Anopheles albimanus* Wiedemann fed

preserved blood through natural membrane. Mosquito News 38(3): 403-408.

U.S. Department of Agriculture (USDA). 1967. Materials evaluated as insecticides, repellents, and chemosterilants at Orlando and Gainesville, Fla., 1952-1964. Agric. Handb. 340. 424 p.

THE MALE REPRODUCTIVE SYSTEM OF THE BLACK FLY, *SIMULIUM PICTIPES* HAGEN¹

L. N. RAMINANI AND E. W. CUPP

Department of Entomology,
Cornell University,
Ithaca, New York 14853

Thorough reports of genital tract development and spermatogenesis in the Simuliidae are relatively rare. Aside from scattered gross morphological descriptions of the male reproductive system (see Ramirez-Perez 1977), and several accounts describing chiasmatal aspects of meiosis in subarctic species (Rothfels and Mason 1975, Rothfels and Nambiar 1975, Procnier 1975), very little histological-cytological information is available for this important group of biting flies. Because a better understanding of the reproductive processes in black flies is necessary for behavioral and colonization research, we studied the microanatomy of the reproductive tract of *Simulium pictipes* during the pupal and early imaginal stages. Descriptions of basic developmental steps are given here and comparisons are made with related Diptera.

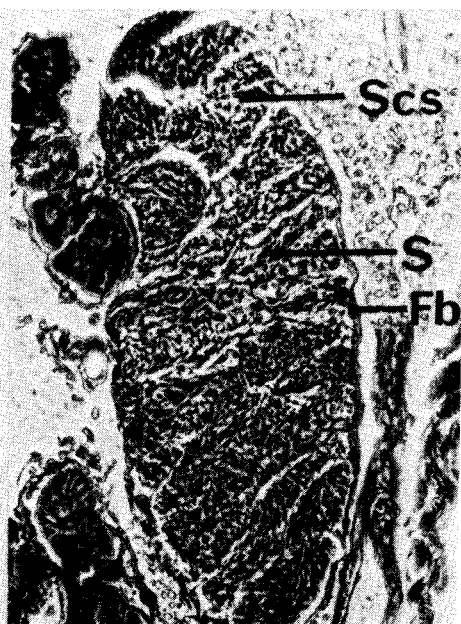
PREPARATION OF MATERIAL. Larvae were collected in Tompkins Co., N.Y. and reared in the laboratory (Muirhead-Thomson 1968). Specimens were fixed in Bouin's fluid and embedded in paraffin. Sections approximately 6 μ m thick were cut with a rotary microtome. Hematoxylin and eosin were used as stains.

OBSERVATIONS. The testis in a pharate pupa is a well-developed ovoid structure with an anuclear sheath and a closely adherent fat body (fig. 1). The anterior half of the organ consists of spermatogonia and spermatocytes; the posterior is filled with spermatids in various stages of differentiation. Occasionally

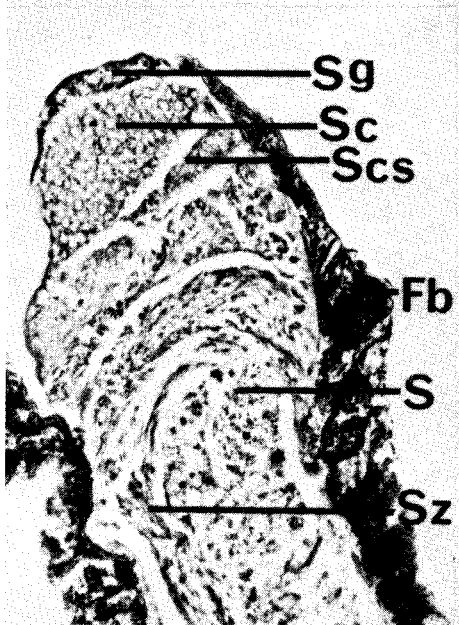
¹ Supported by USDA Specific Cooperative Agreement #12-14-1001-606.



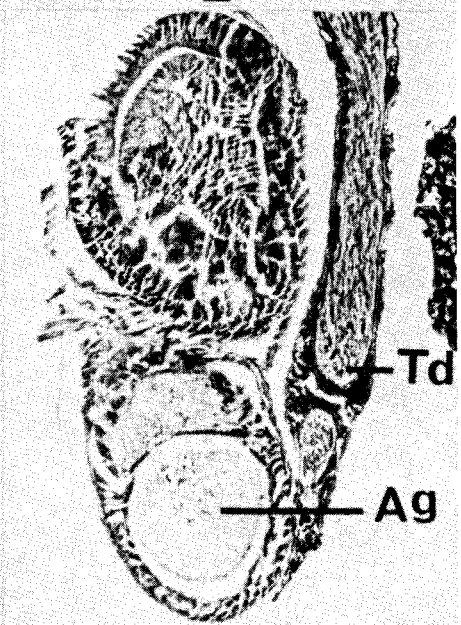
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clusters of mature spermatozoa are present, indicating that spermatogenesis may begin during the larval stage. In 1-day-old pupae the fat body becomes thickened and contains conspicuous dark brown granules (fig. 2). At this time, spermiogenesis is more obvious, with a large number of mature spermatozoa present, but a few spermatogonia and spermatocytes can still be seen in the anterior part of the testis.

In newly emerged adults, the testis is enveloped in a thickened fat body that extends posteriorly to the testicular ducts (fig. 3). The anterior tip of the testis is free of this covering and contains spermatogonia and spermatocytes. Spermatocysts are observed in the anterior half of the testis. Each compartment is separated by a thin membrane. These spaces are obliquely arranged and are leaf-like in appearance, extending half way across the testis. In the posterior portion of the gonad these units have histolysed, releasing gametes into the efferent ducts and forming characteristic lacunae within the testis.

Spermatogenesis in older males can occasionally be observed. The division of spermatogonia to form spermatocytes is preceded by the condensation of chromatin to form individual chromosomes. The resultant spermatocytes are spherical in shape, about $2.5 \mu\text{m}$ in size. First stage spermatids appear as dark dots, that differentiate into short, rod-like structures (fig. 3). Following gradual elongation of their posterior ends, mature spermatozoa are formed. However, the testes of the majority of adult males examined showed spermatids in various stages of differentiation except for the posterior tips which were filled with sperm.

The accessory glands are paired structures with a well-developed layer of circular muscle surrounding them. Each gland is filled with a

granular secretory material (fig. 4), presumably the product of the conspicuous epithelial cells that make up the cortex of each gland. A small amount of this secretion is also scattered along the periphery of each gland. The testicular ducts and the seminal vesicle are filled with sperm.

DISCUSSION. Testicular development and the basic structure of the secondary reproductive system in *Simulium pictipes* follow a pattern similar to that in other nematocerans. The general appearance and adhesion of the fat body to the gonads is much like that reported for *Anisopis* (Abul-Nasr 1950), and the occurrence of meiotic divisions and spermatogenesis during the late larval-early pupal stage is essentially the same as in *Aedes stimulans* (Horsfall and Ronquillo 1970). But, the timing of spermatogenesis in this black fly varies considerably from that in *Chironomus dorsalis* (Abul-Nasr 1950) and *Culiseta inornata* (Breland et al. 1964), in which most meiotic activity and sperm production occur in fully grown larvae, and from that of *Ae. aegypti*, in which sperm differentiation takes place mainly in older pupae (Jones 1967). However, the general period required for differentiation of spermatogenous cells is essentially the same as that reported for other black flies.

The tissue-cellular organization of the efferent ducts and seminal vesicle in *Simulium pictipes* is generally similar to that of various species of Culicidae (Hodapp and Jones 1961). The structure and secretory activity of the accessory glands are also worthy of mention, since it is the sequential activation of these organs with the seminal vesicle that is responsible for the discharge of sperm and the formation of spermatophores in the Simuliidae (Davies 1965). While no material was seen in the seminal vesicle of newly emerged *S. pictipes*, the cellular nature of the accessory glands and the

Figure 1. The testis of a pharate pupa of *Simulium pictipes*. Note the differentiating spermatids (S). Longitudinal section, X 66. (Sg = spermatogonia; Sc = spermatocytes; Ts = testicular sheath).

Figure 2. The testis of a one-day-old pupa. Spermatocysts (Scs) are present and the fat body (Fb) has begun to thicken. Longitudinal section, X 66. (S = spermatids).

Figure 3. The testis of a pharate adult. Note the presence of spermatogonia (Sg), spermatocytes (Sc), and spermatocysts (Scs). Spermatids (S) and spermatozoa (Sz) are evident at the posterior portion of the organ. Longitudinal section, X 132. (Fb) = fat body).

Figure 4. The accessory gland (Ag) and testicular duct (Td) of a pharate adult. Spermatozoa fill the testicular ducts and seminal vesicle. Note also the disc of secretory material in the accessory gland. Longitudinal section, X66.

accumulation of secretory discs suggests they play a primary role in sperm transfer.

Literature Cited

- Abul-Nasr, S. E. 1950. Structure and development of the reproductive system of some species of Nematocera (Order Diptera: Suborder Nematocera). Phil. Trans. R. Soc. (Ser. B) 234:339-396.
- Breland, O. P., G. Gassner and J. G. Riemann. 1964. Studies of meiosis in the male of the mosquito *Culiseta inornata*. Ann. Entomol. Soc. Amer. 57:472-279.
- Davies, L. 1965. On spermatophores in Simuliidae (Diptera). Proc. R. Entomol. Soc. Lond. (A) 40:30-34.
- Hodapp, C. J. and J. C. Jones. 1961. The anatomy of the adult male reproductive system of *Aedes aegypti* (Linnaeus) (Diptera, Culicidae). Ann. Entomol. Soc. Amer. 54:832-843.
- Horsfall, W. R. and M. C. Ronquillo. 1970. Genesis of the reproductive system of mosquitoes. II. Male of *Aedes stimulans* (Walker). J. Morph. 131:329-358.
- Jones, J. C. 1967. Spermatocysts in *Aedes aegypti* (Linnaeus). Biol. Bull. 132:23-33.
- Muirhead-Thomson, R. C. 1968. A laboratory technique for establishing *Simulium* larvae in an experimental channel. Bull. Entomol. Res. 59:533-536.
- Procunier, W. S. 1975. A cytological study of two closely related blackfly species: *Cnephia dacotensis* and *Cnephia ornithophilia* (Diptera: Simuliidae). Canad. J. of Zool. 53:1622-1637.
- Ramirez-Perez, J. 1977. Estudio sobre la morfología de *Simulium metallicum*, vector de la oncocercosis humana en Venezuela. Publicacion Cientifica No. 338. Organizacion Panamericana de la Salud, Washington. 140 pp.
- Rothfels, K. H. and G. F. Mason. 1975. Achiasmatic meiosis and centromere shift in *Eusimulium aureum* (Diptera-Simuliidae). Chromosoma 15:111-124.
- Rothfels, K. H. and R. Nambiar. 1975. The origin of meiotic bridges by chiasma formation in heterozygous inversions in *Prosimulium multidentatum* (Diptera: Simuliidae). Chromosoma 52:283-292.