REPRODUCTION AND CHEMICAL
STERILIZATION OF THE EYE GNAT,
HIPPELATES PUSIO LOEW

By
PAUL HENRY SCHWARTZ, JR.

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
August, 1964

ACKNOWLEDGMENTS

I would like to thank Dr. John T. Creighton, Head, Department of Entomology, who served as my chairman of the Supervisory Committee. I sincerely appreciate his encouragement and advice during the various aspects of the research.

Much appreciation also goes to Drs. F. S. Blanton, W. W. Smith, C. N. Smith, and A. Carr who served as members of the Supervisory Committee.

Without the kindness of Dr. Carroll N. Smith, Investigations Leader, and other members of the Laboratory of Insects Affecting Man and Animals, Entomology Research Division, United States Department of Agriculture; this study could not have been carried out. Much appreciation also goes to Dr. G. C. LaBrecque, Dr. D. E. Weidhaas, Dr. D. A. Dame, Dr. P. B. Morgan, Mr. J. B. Gahan, Mr. I. H. Gilbert, and Mr. C. Lofgren for their interest and encouragement throughout my course of study.

I am indebted to my wife Mary for her patience and understanding throughout the scope of this project.

This research was sponsored in part by the Insects Affecting Man and Animals, Entomology Research Division, United States Department of Agriculture, as a cooperative project with the Department of Entomology, University of Florida.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	ii
LIST OF TABLES.....	iv
LIST OF ILLUSTRATIONS.....	v
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	3
Organs of Reproduction in Insects.....	3
Chemosterilization of Insects.....	6
MATERIALS AND METHODS.....	8
RESULTS AND DISCUSSION.....	13
Anatomy of the Internal Reproductive System.....	13
Male.....	13
Female.....	18
Reproductive Behavior.....	28
Age at Time of Copulation.....	28
Multiple Mating.....	29
Testicular Maturation.....	31
Oviposition.....	34
Primary Follicular Development.....	36
Chemosterilization.....	37
Preliminary Tests.....	37
Sterility and Toxicity.....	38
Slope.....	40
Effect of Chemosterilants on the Two Sexes.....	42
Effects of Chemosterilants on the Reproductive System.....	43
Histopathology of Male Gonads.....	43
Histopathology of Female Gonads.....	47
SUMMARY.....	51
LITERATURE CITED.....	53
BIOGRAPHICAL SKETCH.....	60

LIST OF TABLES

Table	Page
1. The length, width, and proportional size of the oöcyte of primary follicles of the eye gnat.....	23
2. The frequency of copulation of male eye gnats.....	29
3. The length and width of the testes of eye gnats at different sex ratios.....	31
4. Oviposition of mated and unmated female eye gnats.....	34
5. Preoviposition period of mated and unmated female eye gnats.	35
6. The per cent mortality and fertility of eye gnats exposed to residual deposits of tepa on glass for 4 hours then held for 10 days.....	37
7. The per cent mortality and fertility of eye gnats when exposed to honey containing tepa for 3 days then held for 10 days.....	38
8. The per cent mortality and fertility of eye gnats when exposed for 3 days to a sugar solution treated with tepa, metepa, and apholate, then held for 10 days on honey.....	39
9. The per cent fertility of eye gnats when sexes were treated separately or simultaneously with tepa, metepa, and apholate in the food (sugar solution) for 3 days, then held for 10 days.....	42
10. The effect of tepa, metepa, and apholate on the length and width of the testes of eye gnats, and on the proportionate length of the zone of transformation.....	44
11. The stage of development of the primary follicles in ovaries of eye gnats treated with tepa, metepa, and apholate in the food.....	49

LIST OF ILLUSTRATIONS

Figure	Page
1. Mechanical aspirator used to manipulate eye gnats while anesthetized with carbon dioxide.....	10
2. The internal reproductive system of the male eye gnat. 100X	13
3. Cross-section of eye gnat testis. 226X.....	15
4. Mature sperm cells from eye gnat testis.....	16
5. The internal reproductive system of the female eye gnat. 90X.....	19
6. Five stages in the follicular development of the egg of the eye gnat.....	21-22
7. Distal end of eye gnat accessory gland shown in close association with lateral edge of ovary.....	27
8. Plot of the length of the zone of transformation expressed as the per cent of the length of the testes of eye gnats...	32
9. Testes from chemosterilized male and untreated male eye gnats. 133X.....	45
10. Sagittal section of testes from chemosterilized male (260X) and untreated male (360X) eye gnats.....	46
11. Ovaries from chemosterilized female and untreated female eye gnats. 110X.....	48

INTRODUCTION

Although a large number of papers on the biology and ecology of Hippelates species are available little information has been accumulated on reproduction in this group of insects. Similarly, a great deal of work has been done with insecticides for the control of Hippelates but the value of insecticidal applications for the reduction of Hippelates populations is questionable. Control of Hippelates is vital in some heavily infested areas because of their pestiferous habits (Schwarz, 1895) and medical (Bengston, 1933) and veterinary (Sanders, 1940) importance. Although Hippelates have never been shown to be vectors of any disease, they are mechanical carriers of the causal agent of yaws (Kumm and Turner, 1936) and other infectious diseases.

With the advent of sterilization techniques and the successful eradication of the screw-worm fly from Florida (Baumhover et al., 1959), a new approach to insect control and/or eradication seemed feasible. The success of the screw-worm program suggested the possible use of chemicals in adaptations or extensions of the sterility approach to Hippelates control. The induction of sterility in insects by exposure to several chemicals had already been demonstrated but little interest was generated in the practical usefulness of such compounds until the screw-worm program demonstrated the effectiveness of sterile males in reducing the reproductive potential of natural populations. Knippling discussed the possibilities of insect control through the use of sterile

males in 1955 and later, in 1959, he suggested the theoretical advantages of the use of chemicals to induce sterility in a large segment of the natural population. A year later, LaBrecque et al., demonstrated that several compounds induced sterility in house flies, Musca domestica Linnaeus. In 1961, LaBrecque showed that sterility of a type similar to that caused by radiation was induced in both sexes of house flies by aphamide, tepa, and apholate and he designated the compounds as chemosterilants.

The work of this dissertation was initiated in February, 1961, with the hope of showing the histological damage induced by chemosterilants on the reproductive system of Hippelates pusio Loew. At the start of this project no investigation of this type had been conducted on any insect nor had the reproductive system of any Hippelates species ever been described.

The major aims of this study were: (1) to anatomically define the male and female internal reproductive systems, (2) to chemically sterilize both sexes, (3) to determine the histopathological effects of the chemosterilants on the reproductive system, and (4) to make observations on the reproductive behavior of both sexes. It was hoped that these findings would provide a basis for further study and evaluation of chemosterilants against H. pusio leading to an ultimate program for the control or eradication of the species.

During this course of study many new and interesting observations were made in the various aspects of the work. Some of these findings have created new problems and questions which undoubtedly are a field for study in themselves. A further analysis of some of the various aspects of reproduction and chemical sterilization could lead to fruitful results.

REVIEW OF LITERATURE

Organs of Reproduction in Insects

A generalized description of the male reproductive organs is given by Snodgrass (1935). He stated that the internal organs of reproduction in male insects consist of a pair of testes, a pair of lateral ducts and an ejaculatory duct. Frequently accessory glands are present which may be in the form of pouches or blind tubes branching from the upper end of the ejaculatory duct. In Diptera, the single sac-like testis has many shapes and forms depending on the species studied. In Drosophila the testis may be ovoid, curved, conical or spiral (Stern, 1941). It is pear-shaped in Musca (West, 1951) and a tubular gland in Glossina (Buxton, 1955).

According to Wigglesworth (1953) the testis of insects is divided into four main zones: (1) zone of spermatogonia (undifferentiated sperm cells), (2) zone of spermatocytes (dividing spermatogonia inside spermatocysts), (3) zone of maturation and reduction (pre-spermatids and spermatids), and (4) zone of transformation (immature and mature sperm cells with a partially or fully differentiated tail). In adult Drosophila, Clayton (1957) noted that during the 13 days of adult life the average number of spermatogonia decreased only slightly but the zone of transformation gradually increased in size. However, in some insects (Blatta, Wassilieff, 1907; and Bombyx, Omura, 1936) spermatogenesis does not occur in the adult stage and the testes atrophy.

The testes connect at their base to vasa deferentia which are usually simple tubes with a thick cellular epithelium limited by a basement membrane and a strong muscular coat of circular fibers (Snodgrass, 1935). The vasa deferentia are direct derivatives of the posterior unspecialized parts of the genital ridge which are continuous with the ventral strands of the gonads. They connect to an ejaculatory duct which is formed as a median ventral invagination of the ectoderm at the posterior end of the ninth abdominal segment. The epithelium of the ejaculatory duct is surrounded by a strong muscular sheath usually consisting of circular and sometimes longitudinal fibers (Snodgrass, 1935). The male accessory glands generally arise from the anterior end of the ejaculatory duct. They usually function as secretory glands with a mucous or viscid discharge.

The internal organs of reproduction in female insects are in many respects similar to those of the male. The essential parts of the female reproductive system are: (1) a pair of ovaries, (2) two lateral oviducts converging posteriorly from the ovaries, and (3) a median oviduct receiving the lateral ducts anteriorly and opening posteriorly to the exterior at the gonophore (Snodgrass, 1935).

In most insects the ovary consists of a group of cylindrical or tapering units, the ovarioles. Wigglesworth (1953) described four zones of the ovariole: (1) the terminal filament, which serves to attach the ovary to the body wall, (2) the germarium, which consists of primordial germ cells which differentiate into oöcytes and nutritive or nurse cells), (3) the vitellarium, a series of oöcytes, each enclosed in an epithelial sac or follicle, which become progressively larger toward the lower end, and (4) an ovariole stalk, a thin-walled tube leading to the

oviduct. The ovariole may be of several types depending on the means by which the oöcyte is nourished. The polytrophic type of ovarioles each have an oöcyte and a number of nurse cells enclosed with it in its follicle (Wigglesworth, 1953).

Christophers (1911) described five stages of the development of the primary follicle of the mosquito ovariole: (1) no oöcyte formation, (2) oöcyte occupying up to one-half of the follicle, (3) oöcyte occupying up to three-fourths of the first follicle and the second follicle in stage one, (4) follicle taking the shape of a mature mosquito egg, and (5) fully developed egg with the second follicle in stage two.

When the mature egg is released from the ovary it reaches the outside via the oviduct. The two lateral oviducts are very short in Musca and unite almost at once to form the median or common oviduct (West, 1951). The common oviduct of Aedes widens into a short muscular vagina and is attached to the body wall of the eighth abdominal segment by somatic muscles (Curtin and Jones, 1961). The vagina of insects is a direct derivative of the genital chamber and not a continuation of the oviduct. The point of union between the true oviduct and the vagina is marked approximately by the opening of the spermatheca into the anterior end of the vagina.

The spermathecae in most insects are sac-like receptacles for the storage of spermatozoa after insemination. There may be from one to several spermathecae depending on the species studied. There is usually a spermathecal duct and a gland associated with the spermatheca. The spermatheca is primarily an invagination of the body wall at the posterior end of the eighth abdominal segment and it lies in the dorsal wall of the vagina if the genital chamber has the form of a vaginal tube (Snodgrass, 1935).

Posterior to the location of the spermatheca on the vagina are the accessory glands which are thought to secrete a cement for attaching the eggs to a support or a material for covering the egg (Snodgrass, 1935). In insects having a vaginal pouch, the accessory glands open either at the end of the vagina or in its dorsal wall.

Chemosterilization of Insects

An insect's ability to reproduce may be totally or partially destroyed by chemical treatments. Two types of chemicals known to have this effect are antimetabolites and alkylating agents. The antimetabolites, according to Woolley (1952), are compounds essential to cell development which have been altered slightly and elicit signs associated with a specific lack of the metabolite which they resemble when introduced into an animal. The mode of action of antimetabolites has been recently discussed in Timmis (1961) and the reader is referred to this paper and Woolley's (1952) text for more detailed accounts. Antimetabolites such as the well-known folic acid antagonists have been found to induce sterility only in female insects (see Goldsmith and Frank, 1952; LaBrecque et al., 1960; and Mitlin et al., 1957) and are not considered potentially useful for insect control as chemosterilants.

The alkylating agents replace hydrogen with an alkyl group in fundamental genetic material with an effect similar to that of irradiation and are called radiomimetic compounds (Alexander, 1960), but the mode of action is not well understood. Information on this subject may be found in the papers of Bieseke (1958), Borkovec (1962) and Stacey et al. (1958). The chemical nature of alkylating agents in general is discussed by Ross (1962) and those which show greatest promise as insect chemosterilants (aziridine derivatives) are discussed by Borkovec and Woods (1963).

Of the 41 chemicals which have shown promise as insect chemosterilants, 14 are aziridine derivatives, including tepa, metepa, and apholate (Smith et al., 1964). Some of the insects which have been sterilized with aziridines are:

- Citrus red mite - aphamide, apholate, and tepa (Cressman, in press).
- Drosophila - apholate (Cantwell and Henneberry, 1963).
- German cockroaches - aphamide, apholate, methiotepa, and tepa (Burden and Smittle, 1963).
- House flies - apholate, metepa, and tepa (LaBrecque, 1961 and LaBrecque et al., 1963).
- Mexican bean beetle - apholate (Henneberry et al., in press).
- Mexican fruit fly - aphamide and tepa (Shaw and Sanchez Riviello, 1962).
- Mosquitoes - apholate (Weidhaas, 1962), and tepa (Weidhaas et al., 1961).
- Screw-worm fly - apholate (Chamberlain, 1962), metepa and tepa (Gouck et al., 1963), methiotepa, thiotepa, and tretamine (Crystal, 1963).
- Stable fly - apholate, metepa, and tepa (Harris, 1962).

Chemosterilants may act on the reproductive system of insects in three ways: (1) cause failure to produce ova or sperm, (2) cause death of sperm and ova after they have already been produced, and (3) produce multiple dominant lethal mutations, or injure the genetic material in sex cells. The third type of action is the most desirable because males sterilized in this manner compete readily with normal males for available females, and the transfer of motile sperm to the spermatheca of females satisfies the mating requirements as if the female had mated with a normal male (Smith et al., 1964).

Very little work has accumulated on the histological damage to insect reproductive systems resulting from exposure to chemosterilants. The observations which have been made indicate that the chemicals cause slightly smaller gonads and chromosomal damage in treated insects as compared to untreated (Drosophila, Cantwell and Henneberry, 1963; boll weevil, Lindquist et al., in ms.; house fly, Morgan and LaBrecque, 1962; and mosquito, Murray, 1963).

MATERIALS AND METHODS

The Orlando strain of H. pusio was used in all tests. The gnats were sexed while anesthetized with carbon dioxide. The transfer of gnats to test cages and the separation of the sexes was performed with a mechanical aspirator developed specifically for this purpose.

The mechanical aspirator consisted of four principal parts (fig. 1); a base (3) which contained the vacuum source and accessories, an anesthetization chamber (4), a collection chamber mounted on a wooden support (1), and a suction gun (2). In operation a collection cage was snapped into place and the insects were placed on the screen of the anesthetization chamber which was positioned under the low objective of a stereoscopic microscope. The gnats were picked up with the suction gun. The vacuum produced by the power source was just enough to pick up the gnats and hold them in the distal 1 inch of the pipette. Transfer from the suction gun was accomplished by attaching a tube from the second carbon dioxide valve to the tip of the glass pipette and blowing the insects into the collection chamber.

The holding cages used in the tests were of two types. One type, used for holding 25 or more pairs of gnats, consisted of a plastic refrigerator carton with the top modified to accomodate two 1/2-inch cloth-covered air holes and a center hole containing a cotton roll of the type used by dentists. The second type of cage was a 10-dram glass shell vial with a snap cap, used to contain 10 gnats or less. The

EXPLANATION OF FIGURE 1

1- Support Arm

VS - vertical support
HS - horizontal support
O - air outlet
I - air inlet
SV - screen
T - tops for collecting vials
CC - collection chamber

2- Suction Gun

SW - 3-position switch
I - air inlet
V - 10-dram plastic vial
PT - 3/8 in. plastic hose shim
F - funnel
SC - snap cap
G - glass pipette
1-3 - wires to power supply

3- Base

C - carbon dioxide inlet
VS - connection of vertical support arm
AO - air outlet
S - screw to fasten motor support
M - motor
MS - motor support and fan
VC - vacuum chamber
VCT - vacuum chamber removable top
W - wall of base frame
P - 1/8 and 1/4 in. partitions in base
B - batteries (4)
SP - spring tension on batteries
CV - carbon dioxide outlet valves
1-4 - wires to batteries, motor, and switch

4- Anesthetizing Chamber

Figure 1.--Mechanical aspirator used to manipulate eye gnats while anesthetized with carbon dioxide.

plastic top was modified so that it contained a hole 1-inch in diameter which was fitted with a 30 x 30 mesh plastic screen. A 35 mm. x 1 mm. capillary tube containing honey was inserted part-way through the screen. The breeding colony was maintained according to the method of Jay (1961).

All dissecting was done in E.B. saline (Ephrussi and Beadle, 1936). Observations on the reproductive system were made using a Spencer-Phase microscope in phase-contrast.

The chemical sterilants were obtained from the USDA and kept in concentrated form at -20° C until used. For the feeding tests, dilutions of the chemicals were made in solutions of sugar or honey in deionized water. For the residue tests, all dilutions were made in absolute methanol or absolute ethanol. At least 25 pairs of gnats, collected less than 36 hours after adult emergence, were exposed to each concentration tested. In the feeding tests a 3-day exposure period was used after which the gnats were held for 10 additional days. In the residue tests, 10-dram vials were treated with 1/2 ml. of the solution per vial. After the vials were treated they were allowed to dry overnight. Gnats were exposed to the residue for 4 hours. After the exposure the gnats were transferred to 1-pint containers, supplied with honey, and held for 10 days.

In both food and residue tests, gnats were allowed access to food prior to exposure. Mortality counts were made after the exposure period and after the 10-day holding period. Sterility was assayed by placing the eggs from each cage in water, holding 3 days and then determining the hatching rate by observation in a black, gridded petri dish. If more than 150 eggs were obtained from a cage, a 50-egg sample was separated

from the rest of the lot and used to determine the hatching rate. However, if the hatching rate in the sample was zero, the complete lot of eggs was examined and, if any had hatched, an estimate of the hatching rate in the complete lot was used.

Abbott's (1925) correction formula was applied to both mortality and sterility if they exceeded 10 per cent in the untreated gnats. The corrected data were analyzed by probit analysis according to the method of Litchfield and Wilcoxon (1949). The standard errors, "t" tests, "F" tests, and Chi squares were calculated according to the methods of Snedecor (1961). Tests for significant differences between means were conducted according to the method of Duncan (1955). In the statistical tests a level of 5 per cent probability was used for significant differences.

RESULTS AND DISCUSSION

Anatomy of the Internal Reproductive System

Male

The male reproductive system of H. pusio (fig. 2) was found to consist of a pair of testes, a pair of short vasa deferentia, paired accessory glands, an unpaired ejaculatory duct and the external genitalia.

Figure 2.--The internal reproductive system of the male eye gnat. 100X.

Testes.--The testes of H. pusio lay dorso-laterally in the region of the third abdominal segment with their extremes extending into the second and fourth segments. The testis was well tracheated but no

innervation was observed with phase-contrast microscopy. The double-layered testicular sheath contained orange-pigmented granules between the layers like that of Musca (West, 1951) and Drosophila (Stern, 1941). Stern (1941) believed the pigmentation of Drosophila testes was secreted by the external testicular layer (tunica externa). The testicular sheath of mosquitoes is neither pigmented nor double-layered (Hodapp and Jones, 1961).

The testes of H. pusio (fig. 2) were single, sac-like organs as in most Diptera. They were pear-shaped in form, resembling those of mosquitoes (Hodapp and Jones, 1961). Internally they were not divided into distinct spermacysts such as those observed by Hodapp and Jones (1961) in the mosquito, nor was there an indication of spermacyst formation in the pupal testis, but a distinct regionalization of the testis did exist. At the small knob-like apical end (germarium), spermatogonia were predominant (fig. 2). This zone was followed posteriorly by the zones of spermatocytes, maturation and reduction, and transformation. The zone of transformation was the largest zone in the adult testis. This area contained sperm bundles in which the long, wavy spermatozoa were grouped together with cytoplasmic connections (fig. 3). The finding of sperm bundles in the testis is not an uncommon event. They have been observed in several insects such as Drosophila (Clayton, 1957), Chortophaga (Payne, 1933), and Bombyx (Omura, 1938b).

In H. pusio the sperm bundles persisted as very coherent fascicle-like structures with no trace of the individual spermatozoa remaining (fig. 4-b). These tube-like structures whipped about in E.B. saline but did not undulate like spermatozoa (fig. 4-a). The fascicles were observed to persist after transference to the female reproductive

system. This is not in agreement with the findings of other workers in other species. Omura (1938a) noted that in Bombyx the sperm bundles rapidly separated into individual cells after deposition in the female. The exact role which the bundles of sperm in H. pusio females play in fertilization is not known; however, a freshly laid egg was found to have a sperm bundle attached to its micropyle. Their persistence in the female reproductive system is a curious phenomenon and their function is yet to be fully explained.

Figure 3.--Cross-section of eye gnat testis (arrow points to sperm bundle). 226X.

The spermatozoa in the testes frequently contained cytoplasmic droplets along the flagellum. The sperm were rod-shaped (fig. 4-a) with no apparent head structure and appeared to be coiled within the testes (fig. 3). They remained motionless within the testis except when the contraction of the base of the testis moved them about.

The testes in very young males were light orange in color. However, as the male aged the density of sperm increased in the posterior portion of the testis, giving it a dark brown to black color.

The apical tip of the testis was sometimes in line with the remainder of the testis and sometimes recurved, occasionally even doubling back to touch the main body of the testis. Usually the recurvature did not exceed 90° .

Figure 4.--Mature sperm cells from eye gnat testis. (a) spermatozoa, (b) sperm bundle.

Vas deferens.--The vas deferens (fig. 2) was connected to the base of the testis on its inner surface. It was very short as in Musca (West, 1951). The wall of the vas deferens was whitish and somewhat translucent as seen with phase microscopy. The cells of the wall contained very small spherical nuclei. The vasa deferentia appeared to serve only as a means of communication between the testis and ejaculatory duct. In other insects the vasa deferentia may also serve as a sperm storage organ through a modification in the form of a seminal vesicle such as that found in mosquitoes (Hodapp and Jones, 1961) and the corn ear worm (Callahan and Cascio, 1963).

The vas deferens of H. pusio was contractile, as in Drosophila (Nonidez, 1920) and the corn ear worm (Callahan and Cascio, 1963). The vasa deferentia joined the ejaculatory duct on the same plane as the accessory glands.

Accessory gland.--The paired innervated accessory glands (fig. 2) were contractile in E.B. saline. Their ducts contained large granules that were moved by peristaltic action toward the ejaculatory duct. The house fly has no accessory glands (West, 1951). However, in the mosquitoes that have been studied, the accessory glands are very prominent and are attached to the vasa deferentia by a thin filament. In H. pusio the accessory glands were about 2,550 microns long by 40 microns wide with little or no taper. They were two to three times as wide as the vas deferens. Large secretory cells were observed in their walls.

In some instances spermatozoa were found clustered in areas of the accessory gland, increasing its diameter slightly. Areas invaded by sperm appeared to contain a finer granular substance than areas without sperm. This is an uncommon finding in insects, although Lum (1961a) found a few sperm occasionally in the accessory glands of male mosquitoes and attributed this to the pressure of the coverslip. However, the preparations of H. pusio were not coverslipped and the condition occurred more frequently in older males than in young males, which suggests that the accessory gland may serve as a sperm storage organ. The vasa deferentia of H. pusio probably could not serve as sperm reservoirs because of their extreme shortness. Therefore, the production of sperm by the testis may overload the ejaculatory duct and cause sperm migration or transfer into the accessory gland.

Ejaculatory duct.--The ejaculatory duct (fig. 2) was very muscular and contained many spherical nuclei in its walls. It was bulb-shaped at the anterior end and tapered into a narrow tube. The ejaculatory duct of H. pusio did not follow an irregular course to the

penis as in the house fly (West, 1951), nor did it enlarge into an ejaculatory sac as in Drosophila (Nonidez, 1920) and the house fly. It more nearly resembled the ejaculatory duct of Culicine mosquitoes (Hodapp and Jones, 1961) in general shape.

In Drosophila the ejaculatory sac acts as a pump to drive the sperm through the lumen of the duct. In H. pusio the bulbar portion of the ejaculatory duct may possibly act as a pump which forcibly ejects the spermatozoa from the lumen of the duct. However, the exact mechanism of sperm transfer in H. pusio remains unknown.

Within the lumen of the ejaculatory duct of H. pusio there were granules, possibly from the accessory glands, after the third day of adult life. The density of the spermatozoa in the lumen appeared to increase as the male aged. Lum (1961b) considered the fluid from the male mosquito accessory gland to act only as a medium for the spermatozoa during ejaculation and did not regard the glandular secretion as having a stimulatory effect on the spermatozoa as in the case of Bombyx (Omura, 1938b).

Female

The female reproductive system, occupying the posteriormost portion of the abdomen of H. pusio was found to consist of two ovaries, an efferent duct system, two sperm-storing organs, two accessory glands, and the external genitalia (fig. 5.) The ovaries were joined by two very short lateral oviducts to a common median oviduct. The median oviduct entered the tube-like genital chamber or vagina. The vagina opened to the exterior on the eighth abdominal segment. A bursa (located ventrally), a pair of spermathecae and a pair of accessory glands (located dorsally) opened into the vagina.

Figure 5.--The internal reproductive system of the female eye gnat. 90X.

Ovary.--The immature ovary resembled a bunch of grapes in shape, was well tracheated and contracted slightly in E.B. saline. As observed by Kumm (1935) for H. pallipes the ovaries were whitish and varied greatly in size according to the stage of development. The mature ovaries were ovate-shaped and occupied the greater portion of the abdomen, distending it by their growth. Their distal ends were unattached, with no terminal ligament such as that in Drosophila (Miller, 1950). The ovaries were supported in the abdomen by the numerous tracheal branches with which they were supplied. The ovarian sheath was very thin and appeared like a web of loose cross-striations over the ovarioles.

Each ovary consisted of a group of parallel egg tubes or ovarioles. The total number of ovarioles found in both ovaries of H. pusio averaged 40.2 ± 5.7 , as compared to 70 in the house fly (West, 1951) and 20 to 40 in Drosophila (Miller, 1950). The ovarioles were directed toward the internal calyx at an oblique angle and held together by the ovarian sheath. Each ovariole consisted of a terminal filament at the distal end, followed by the germarium, a tapered moniliform series of two to four follicles (fig. 6) and, at the basal end, a pedicel.

The terminal filament was short and attached to the germarium, which was oblong in shape and showed a granular cell structure (fig. 6). The germarium was not compartmented as in Drosophila (Miller, 1950) but more closely resembled the undifferentiated nucleated tissue of the mosquito germarium (Bertram, 1959). The basal portion of the germarium became enlarged as a follicle was being developed and at the completion of follicular development was separated from the distal portion by a short stalk. Each follicle became increasingly larger toward the basal portion of the ovariole. The basal-most follicle was the primary follicle which differentiated into a mature egg.

Primary follicular development was divided into five stages (after Christophers, 1911). In the first stage, the spherical primary follicle was the larger of the two follicles present (fig. 6-a) and contained granular cytoplasm with no oöcyte differentiation. In this stage the primary follicle was about 79 microns in length by 72 microns in width (table 1) and nurse cells became clearly defined as development progressed. The wall of the primary follicle was much thicker than that of the other follicle and the germarium.

Stage a. 234X.

Stage b. 215X.

Secondary follicle

Nurse cell
Oocyte

Stage c. 270X.

Follicular epithelium

Figure 6.--Five stages in the follicular development of the egg of the eye gnat.

Tertiary follicle

Stage d. 138X.

Micropyle

Stage e. 129X.

Figure 6.--Five stages in the follicular development of the egg of the eye gnat.

Table 1.--The length, width, and proportional size of the oöcyte of primary follicles of the eye gnat.

Follicle stage	Number measured	Length (μ)		Width (μ)		% of length occupied by oöcyte
		Average	SE	Average	SE	
1	6	79	0.94	72	1.9	0.0
2	5	102	1.5	79	1.2	25.9
2 (late)	4	104	0.81	91	1.2	36.4
3	7	272	5.9	151	0.75	72.6

In the second stage of development (fig. 6-b) oöcyte formation had begun and yolk granules were deposited. In this stage the oöcyte occupied up to 1/2 the length of the follicle. The follicle increased 10 to 25 per cent in size (table 1). The wall of the follicle became increasingly thicker, with the follicular epithelium constituting the greater portion. The nurse cells had approximately doubled in size by the end of this stage. The second follicle of the ovariole was in stage one and the germarium had not yet begun to develop a third follicle.

By the third stage (fig. 6-c), the primary follicle had become more ovate and was about 272 microns in length by 151 microns in width (table 1). The oöcyte occupied approximately one-half to three-fourths of the follicle. During this stage the nurse cells were quite large and the follicular epithelium did not surround them as in the earlier stages. The follicular epithelium contained two to three rows of columnar cells with large oval nuclei. The germarium had begun to differentiate a third follicle.

In the primary follicle the nurse cells had begun degeneration during stage four (fig. 6-d) and subsequently formed a polar cap

around the anterior of the oöcyte at the future location of the micropyle. The follicular epithelium surrounding the oöcyte had begun degeneration also. The third follicle was completed in this stage and the beginning of a fourth follicle appeared as a slight enlargement of the germarium. Both the second and third follicle were still in stage "a".

In stage five (fig. 6-e) the oöcyte occupied the whole primary follicle, the chorion pattern was easily discernible, and the micropyle was apparent. The follicular wall degenerated until it resembled a thin tunica and was closely associated with the ovarian sheath. The second and third follicles had remained unchanged and the basal enlargement in the germarium had undergone very little additional differentiation. It took approximately 72 hours to complete follicular development at about 24° C. This was about the same rate of development King et al. (1956) observed for Drosophila follicles.

Oviducts and vagina.--The lateral oviducts were short tubes without an expansion such as that observed in Drosophila (Miller, 1950). They united medially to form the common oviduct which was about 232 microns long and 78 microns wide when empty. The common oviduct entered the vagina dorsally between the openings of the ventral bursa and the dorsal spermathecae and accessory glands (fig. 5).

The vagina was a highly muscular organ about 600 microns in length and about 133 microns wide when empty. It was capable of considerable distention during egg deposition. The vagina was attached to the walls of the abdominal segment by somatic muscles. The antero-ventral portion of the vagina was modified into a bursa. This organ

is homologous with the seminal receptacle of Drosophila (Miller, 1950) but bears little resemblance to it. In H. pusio the bursa was a nodule which protruded from the point of fusion of the vagina and common oviduct. It appeared to be a non-contractile sperm receptacle. When empty its cellular structure was much like that of the common oviducts and vagina but when full of spermatozoa its cell structure appeared hyaline probably due to the extension by the sperm. Spermatozoa were present in this structure when the eggs were ready to be deposited and were subsequently transferred back to the spermathecae after egg deposition. The sperm were quite motile in the bursa and revolved within it at a great speed. Sperm dissected from it were of two types. The first type was typical of spermatozoa in general, being long and containing numerous undulations along the flagellum. The second type was the fascicle-like structure discussed previously under the male. This type was unchanged from that which were found as a compact bundle of mature sperm located in the basal portion of the male testis. Freshly inseminated females contained seminal fluid in the vagina. It is not known whether the sperm were subsequently transferred first to the bursa or to the spermatheca.

The transfer of sperm from the spermatheca to the bursa when eggs are about to be laid is an unexplained phenomenon. It may be that the enlargement of the ripened ovaries causes a pressure on the spermathecae located between them, thus creating a stimulus which causes the migration of the spermatozoa. The forces which bring about this transfer are not known. The movement of sperm in female insects may be passive or active. The sperm are transferred to the spermathecae by movement

of the reproductive system (Ephestia and Flodia (Norris, 1932; Rhodnius, Davey, 1958; Aphis queen, Snodgrass, 1956; and Zygaena, Hewer, 1934) or by their own movement (Bombyx, Omura, 1938a; Cinex, Abraham, 1934; and Drosophila, Nonidez, 1920).

Spermathecae and accessory glands.--Attached immediately posterior to the bursa and dorsal to it were the ducts of the two spermathecae (fig. 5). The spermathecae were translucent tubes attenuated at the distal end and terminating in a gland. The basal portion of the tube was about 48 microns wide with a wall about 6.8 microns thick and was very lightly sclerotic. The spermathecae were quite constricted where they empty into the vagina. This region of the duct is very contractile and may aid in the transfer of sperm into and out of the duct. The attenuated portions of the ducts were narrow tubes that were coiled like watch springs as in H. pallipes (Kumm, 1935). At the distal end of the attenuated portion, each duct was attached to a spherical gland, located at the center of the coil, which contained large secretory cells. The basic structure of the spermatheca of H. pusio was essentially similar to that of other insects but appeared to be a radical departure from the heavily sclerotized organ found in most Diptera, e.g. usually spherical in mosquitoes (Snodgrass, 1959), and house fly (West, 1951), but mushroom-shaped in Drosophila (Nonidez, 1920).

Within the spermathecal ducts of H. pusio, the sperm were not actively motile as in the bursa but undulations of the flagellae occurred. No spermatozoa were observed in the gland attached to the end of the spermathecal duct.

Immediately posterior to the attachment of the spermatheca on the vagina were a pair of contractile nodular filaments about 525

microns long. The distal end of each was in a close association with the base of the ovary near its lateral margin (fig. 7). The duct of this organ was nebular and its wall contained elliptic protruding nodules (fig. 5). The nodules were nebular like the duct but contained several dark granules clustered in the center. In very young females the nodules were larger and more conspicuous than those of older females which have finished egg deposition. This organ appeared glandular but no secretory products were observed in its duct. It is thought to be an accessory gland because of its position on the vagina, although its true nature has not been revealed in this study. In other insects, the female accessory gland has been designated by various names such as mucus gland (Christophers, 1923), cement gland (Howard et al., 1913), and spermathecal gland (Detinova, 1962) in the mosquito and colleterial gland or parovaria in Drosophila (Nonidez, 1920). In these instances the authors designated the gland according to the function they felt it performed; however, no such attempt was made in this study.

Figure 7.--Distal end of eye gnat accessory gland shown in close association with lateral edge of ovary (arrow points to blind end of gland).

Oviposition.--Gravid females were observed in the process of oviposition. First, the modified portion of the abdomen or ovipositor (Hall, 1932) was extended to its full length. Then an egg was released from the ovary and passed into the common oviduct via the lateral oviduct. It paused in the common oviduct close to the bursa, which was filled with motile sperm, for 7 to 20 seconds. The egg was forced through the constriction of the common oviduct where it empties into the vagina by contraction of the former. It paused in the vagina for 20 to 25 seconds and constrictions of the vagina forced the egg out of the atrium located ventrally on the eighth abdominal segment. As the egg came through the atrium, the ninth and tenth segments lifted to about a 75° angle and returned to their original position after the egg was released. The stimulus for egg deposition in H. pusio is not known. In A. aegypti, Curtin and Jones (1961) indicated that ovulation and oviposition are regulated by the nervous system.

Reproductive Behavior

Age at Time of Copulation

Dissections of females confined with males of similar age showed no spermatozoa in the reproductive tract up to 36 hours after emergence, but sperm were found between the 36th and 48th hours after emergence. When newly emerged females were confined with males that were at least 3 days old, no spermatozoa were observed in the females until a period of 36 hours had elapsed. Likewise females confined with older males failed to produce hatchable eggs during the first 36 hours after emergence. When males were less than 36 hours old they did not inseminate females which were more than 3 days old.

These data were similar to those obtained by Murvosh et al. (in press) who showed that newly emerged house fly males and females did not mate for 16 and 24 hours respectively. In the mosquito, Wheeler and Jones (1960) showed that an actively mating male does not copulate freely with a newly emerged female partly because her terminalia are so greatly retracted and partly because she uses her metathoracic legs to prevent his clasping. In the case of the male mosquito, mating is prevented because of the unrotated terminalia (Hodapp, 1960). In males of H. pusio, there is an anatomical indication of sexual immaturity in that there are very few or no sperm present in the ejaculatory duct for 1 or 2 days after emergence. However, in females no such lack of sexual development is apparent during the first 36 hours of life, except for the apparent immaturity of the ovaries.

Multiple Mating

When a sexually mature male was offered five sexually mature females for a 24-hour period he inseminated as many as four (table 2).

Table 2.--The frequency of copulation of male eye gnats.

Male No.	Number of females fertilized during indicated day									
	1	2	3	4	5	6	7	8	9	10
1	0	4	2	2	0	3	1	0	0	1
2	0	1	2	3	1	1	0	2	0	1
3	2	2	1	1	1	0	0	0	0	0
4	0	0	2	2	0	1	1	-	-	-
5	0	2	3	3	1	1	1	-	-	-

Individual males were capable of inseminating from 6 to 14 females each over periods ranging up to 10 days. Copulation occurred most frequently from the second to the sixth day, at which time the males were

5 to 9 days old. Whether or not a male copulates more than once with the same female could not be determined.

Aedes males were force-copulated by Jones (1961), who observed that they would copulate with about five females. However, he stated that copulation may occur without insemination. Virgin male Aedes were found to inseminate about five females in an hour when allowed to copulate freely. He found that the condition of sexual depletion in males was maintained for about 24 hours after the males were removed from the females.

Tests were conducted to determine whether or not multiple mating had an observable gross effect on H. pusio testes. Males confined with females at a 1:10 ratio had slightly larger testes than those confined at a 1:1 ratio (table 3). This difference was significant at the 5 per cent level. However, those males mated at a 1:1 ratio had significantly smaller testes than unmated males (table 3). It appeared that mating at a 1:1 ratio caused a slight decrease in testicular length over unmated males whereas mating at a 10:1 ratio appeared to increase the size of the testes. There was no apparent difference in the proportionate length of the zone of transformation between males at the various mating ratios. In Aedes, Schwartz (1961) found no difference between the size of the testis of mated and unmated males but observed that sexual activity definitely hastens the full maturity of the spermatozoa.

Table 3.--The length and width of the testes of eye gnats at different sex ratios.

Sex ratio (male:female)	Number of testes measured	Size of testes (μ)			
		Length		Width	
		Average	SE	Average	SE
<u>0 to 12 day period (daily observations)</u>					
1:0	86	466.7	5.2	187.4	1.8
1:1	90	442.9	6.6	186.1	1.8
<u>10 to 24 day period (4 observations*)</u>					
1:1	50	405.4	4.2	152.0	1.4
1:10	50	434.2	4.4	161.2	1.8

* Observations made on the 10th, 14th, 20th, and 24th days of adult life.

Testicular Maturation

Measurements of the zone of transformation converted to a percentage of the length of the testis of H. pusio indicated the rate of spermatogenesis in the adult. This area increased in length at a decreasing rate with age (fig. 8). By the fifth day one-half the length of the testis was occupied by the zone of transformation, an increase of 40 per cent in the first five days of adult life. Beyond the fifth day, the zone of transformation occupied approximately 70 per cent of the length of the testis as a maximum for 24 days of life. In rare instances with males greater than 12 days of age, the zone of transformation occupied up to 100 per cent of the length of the testis. Clayton (1957) noted that the zone of transformation of Drosophila testes also increased in size during adult life.

Spermatogenesis appeared to be initiated in the pupal stage of H. pusio and apparently continued throughout the life of the male. This finding is in agreement with that observed for Drosophila

Figure 8.--Plot of the length of the zone of transformation expressed as the per cent length of the testes of eye gnats.

(Clayton, 1957), Blatta (Wassilieff, 1907), Passalus (Krause, 1947), Bombyx (Omura, 1936), Camponotus (Whelden and Haskins, 1953), Mycetophila (Abul-Nasr, 1950), and Ctenicera (Zacharuk, 1958). These workers also observed that spermatogenesis began in the prepupal or pupal stage.

The continuation of spermatogenesis in the adult of H. pusio appeared to have a slight effect on the size of the testes. The average length of the testes was 436 microns with a range of 310 to 580 microns and the average width of the testes was 168 microns with a range of 120 to 280 microns. In a twelve-day study (table 3) there was a slight increase in the size of the testes as determined by an "F" test. The testes appeared to increase in length by about 60 microns by the fourth day. By the seventh day the testes decreased in length by about 60 microns but appeared to regain the 60 microns during the remainder of the twelve-day period. Correlating these observations with those on the rate of spermiogenesis it can be seen that the increased size of the testis by the fourth day occurs at about the same time at which the testis is making its greatest increase in the zone of transformation (fig. 8). After about the seventh day the length of the zone of transformation appeared to increase at a much slower rate (fig. 8), and this would account for the gradual increase in the length of the testes. There is little indication from figure 8 as to why the testis should decrease in size during the sixth and seventh day. However, the testis may not actually be growing during adult life but rather the testicular sheath is stretched to accomodate the increased production of spermatozoa. As the sperm become tightly packed within the testes by the sixth day it probably

returns to normal size and again becomes gradually stretched as the sperm are produced throughout life. The increased size of the testis is about 13 per cent beyond that of a 1-day-old male. The above theory was tested to see if the testicular sheath was elastic enough to accomodate a 13 per cent increase. Testes were placed in a hypotonic solution and the maximum distension was measured. It was found that the testicular sheath would stretch up to about 25 per cent of its original dimension before breaking. Thus it seems possible that the increased size of the testes of H. pusio could be due to expansion of its testicular sheath rather than to its growth. In some insects (Bombyx, Omura 1938b; Blatta, Wassilieff, 1907), spermatogenesis does not continue in the adult stage and the testes atrophy.

A paired "t" test indicated no significant difference between the pairs of testes of each male. However, in the mosquito Hodapp and Jones (1961) observed that one of the testes is usually smaller than the other.

Oviposition

A study was conducted to determine the number of eggs laid by mated and unmated females and to determine whether or not mating had an influence on the time of oviposition.

Table 4.--Oviposition of mated and unmated female eye gnats*

Number of females	Number of females laying indicated number of eggs									
	0	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41 up
<u>Mated</u>										
50	15	5	0	1	1	9	4	5	4	6
25	2	3	1	1	4	3	6	2	0	3
25	5	2	1	2	0	3	3	3	3	3
<u>Unmated</u>										
25	9	2	1	1	4	3	3	0	1	1
25	10	2	0	0	2	2	7	1	1	0

*18-day confinement period.

Table 5.--Preoviposition period of mated and unmated female eye gnats.*

Number of females	Number of females with indicated preoviposition period (days)				
	5-8	9-12	13-15	16-18	(Average)
			<u>Mated</u>		
50	13	15	6	1	9.6
25	9	10	3	1	9.5
25	6	8	5	1	11.0
			<u>Unmated</u>		
25	1	3	12	0	12.8
25	0	1	8	6	15.4

*18-day confinement period.

There was no significant difference between those females that oviposited in each test in the average number of eggs laid per female (25.8 eggs) as determined by an "F" test (table 4). However, the preoviposition period was somewhat longer for unmated gnats than for mated gnats (table 5). In 5 to 8 days a maximum of 39 per cent of the mated females had begun to oviposit whereas a maximum of only 6 per cent of the unmated females had begun in the same period. By the 12th day, maximum oviposition had reached 83 per cent for mated females as compared to 25 per cent for unmated.

The over-all average of about 26 eggs per female is in general agreement with that of 24 eggs observed for *Hippelates* by Hall (1932) and of 47.8 eggs by Mulla (1962) (10-day longer average oviposition period). The slight delay in oviposition by unmated females is in agreement with the results of Glaser (1923) for the house fly and Curtin and Jones (1961) for the mosquito.

Primary Follicular Development

Because of the difference in the length of the preoviposition period observed in the preceding section, a study was conducted to compare primary follicular development for 12 days in mated and unmated gnats. Actual follicular counts were made on those primary follicles which had initiated oöcyte development which corresponded to stages "b" through "e" (fig. 6).

The data indicated no significant differences in the number of advanced primary follicles per day between mated and unmated gnats as determined by a paired "t" test. The average number of advanced primary follicles per day per female was 23.5. This is in very close agreement with the average number of eggs laid per female in an 18-day period, 25.8.

It appeared quite evident in this study that slightly more than one-half of the ovarioles per ovary had primary follicles in an advanced stage of development. There was no alternation of ovarian development since both ovaries appeared to contain an equal number of developing primary follicles. Advanced primary follicular development had not begun until the third day.

By ranking the mean number of advanced primary follicles per day per female and using Duncan's test for significant differences it was found that no significant change occurred until the tenth day. (See results below.)

Days	3	4	6	5	7	8	9	12	10	11
Ranked	28	27	27	27	22	22	18	16*	14	10
mean										

*Means for days three through nine are significantly different from days ten through twelve.

The results of this test indicate the development of a second set of primary follicles by about the tenth day which was significantly smaller than the first. The sum of these two ovipositions (28 and 16) correspond very closely with the average number of ovarioles per female (40).

Chemosterilization

Preliminary Tests

Preliminary tests were conducted to determine an effective means for exposing both sexes of the eye gnat to chemosterilants. Residual deposits of apholate on glass were not effective at 10 mg./sq. ft. This finding was in agreement with that obtained with house flies (Meifert et al., 1963) and mosquitoes (Weidhaas and McDuffie, 1963) exposed to residual deposits of 250 mg./sq. ft. However, tepa was effective at both 5 and 10 mg./sq. ft. (table 6).

Table 6.--The per cent mortality and fertility of eye gnats exposed to residual deposits of tepa on glass for 4 hours then held for 10 days.*

Mg. of tepa per sq. ft.	Treated sex	% Mortality and Fertility (10 days)	
		Mortality	Fertility
10	Both	77	0.5
10	Male	0	0.0
10	Female	50	8.0
5	Both	10	0.0
5	Male	0	0.0
5	Female	0	0.0

*Abbott's correction formula applied to mortality and fertility.
(Two replicates, 25 to 30 pairs of gnats per test.)

Food tests indicated that tepa sterilized both sexes at concentrations of 1/2 to 1 per cent when honey was used as a carrier. However, the results appeared somewhat inconsistent at different concentrations (table 7).

Table 7.--The per cent mortality and fertility of eye gnats when exposed to honey containing tepa for 3 days then held for 10 days.*

Concentration of tepa (% wt/vol)	Treated sex	% Mortality and fertility after 13 days	
		Mortality	Fertility
1.0	Both	1.8	1.0
1.0	Male	0.0	20.0
1.0	Female	0.0	5.0
0.5	Both	0.0	0.0
0.5	Male	21.0	10.0
0.5	Female	3.7	22.0
0.1	Both	11.0	49.0
0.1	Male	29.6	29.0
0.1	Female	0.0	57.0

*Abbott's correction formula applied to mortality and fertility.
(Two replicates, 25-30 pairs of gnats per test.)

Subsequently it was stated by Borkovec (1962) that the acidic nature of honey was capable of polymerizing the chemical. For this reason sugar solution was substituted for the honey. A 10 per cent concentration of a saturated solution was used. In subsequent tests sugar solution appeared to be an effective carrier and became the method of choice for administering the chemicals to determine a sterilizing concentration and for observing the effect of the sterilant on the reproductive system.

Sterility and Toxicity

A study was conducted to determine the sterility and toxicity induced by tepa, metepa, and apholate when administered in the adult food for the first 3 days of life. The average per cent mortality and fertility in three generations of treated gnats are presented in table 8.

Sterility.--The data indicated that all the chemicals at the concentrations used (0.5 to 0.001 per cent) completely or substantially

reduced the fertility of surviving gnats. No eggs were deposited by gnats exposed to 0.5 per cent tepa and metepa. No egg hatch was obtained with tepa at concentrations of 0.1 to 0.01 per cent and fertility was less than 50 per cent at concentrations of 0.005 and 0.001 per cent (table 8).

Table 8.--The per cent mortality and fertility of eye gnats when exposed for 3 days to a sugar solution treated with tepa, metepa, and apholate, then held for 10 days on honey.*

Sterilant concentration (%)	Mortality of adult gnats in 13 days	% of eggs hatched
	<u>Tepa</u>	
0.5	84	0
.1	26	0
.05	8	0
.01	19	0
.005	3	42
.001	1	49
	<u>Metepa</u>	
0.5	99	0
.1	20	1
.05	3	14
.01	3	36
.005	1	33
.001	1	49
	<u>Apholate</u>	
0.5	27	0
.1	3	0
.05	7	4
.01	2	30
.005	15	67
.001	10	54

*Abbott's correction formula was applied where mortality exceeded 5 per cent and fertility was less than 90 per cent in the untreated gnats. (25 pairs of gnats per concentration; 3 generations).

Metepa was less effective than tepa or apholate in reducing the fertility of gnats at concentrations of 0.5 to 0.01 per cent. Similar

results with tepa and apholate were obtained with mosquitoes (Weidhaas et al., 1961; Weidhaas, 1962) and with house flies (LaBrecque, 1961).

Toxicity.--Tepa and metepa at 0.5 per cent had a harmful effect, resulting in high mortality (84 and 99 per cent, respectively). Mortality at concentrations of 0.1 to 0.001 per cent of tepa and metepa ranged from 1 to 26 per cent. However, apholate at a concentration of 0.5 per cent produced only 27 per cent mortality.

Slope

The data from table 8 were used to calculate the concentration to kill 50 per cent of the gnats with tepa and metepa (LC-50) and the concentration to obtain 90 per cent sterility (SC-90) for all three chemicals. The calculated SC-90, LC-50, and the slope of the regression line for each chemical are given below with the standard errors of each:

<u>Chemical</u>	<u>LC-50</u>	<u>SE</u>	<u>Slope</u>	<u>SE</u>	<u>SC-90</u>	<u>SE</u>	<u>Slope</u>	<u>SE</u>
Tepa	0.191	.001	2.37	.79	.0077	.0016	1.58	.90
Metepa	.146	.001	4.28	.80	.037	.001	1.80	.79
Apholate	--	--	--	--	.042	.002	1.18	.36

In computing the regression lines for tepa, data from a test not shown in table 8 was used (3 per cent fertility at a concentration of 0.007 per cent).

The slopes of the sterility data were essentially the same and the SC-90's of apholate and metepa were similar, but that of tepa was five to six times lower than both of the other sterilants.

The SC-90 of tepa obtained in this test agrees closely with the SC-90 of tepa for house flies (Murvosh et al., 1964). The SC-90 of metepa and apholate obtained by Murvosh et al. (1964) for house flies

is about three times higher than that obtained in this study. However, this is not surprising since the wide variation obtained in the results of chemosterilant feeding studies indicates considerable differences in the response of an insect species to the chemical. This may be due to two causes: (1) there may be polymerization of the chemical causing differences attributable to the chemical itself and (2) there may be a deterrent effect of the chemical causing the insect to vary its uptake of the food containing the chemical. This latter factor may account for the apparent reversal effect at the lower concentrations whereby the insect is not deterred to the same degree and actually takes up more chemical. As suggested by Murvosh et al. (1964) less variable results might be obtained with a more precise method of administration. However, the feeding method serves to indicate whether or not the chemical will sterilize and the approximate concentrations at which sterility occurs.

The apparent wide range between toxicity and sterility for tepa obtained in this study indicated that tepa is capable of sterilizing at the lower concentrations without an apparent lethal effect such as that obtained with metepa, where the ratio was quite low. A high ratio between toxicity and sterility is a desirable characteristic in a chemosterilant, in that the dosage does not become a critical factor. This is especially apparent with alkylating agents in which polymerization occurs. The results of this study indicate the concentration of tepa can drop about 14-fold without causing a loss in the sterility effect.

Effect of Chemosterilants on the Two Sexes

Preliminary tests with tepa (table 7) indicated that both sexes were sterilized when given treated food. A subsequent test was conducted with all three chemicals to determine whether or not males and females were both being sterilized. The results of this test indicated that a slightly higher concentration was needed to sterilize either sex than if both sexes were treated and mated. Males treated with tepa or metepa required lower concentrations than females to induce sterility (table 9).

Table 9.--The per cent fertility of eye gnats when sexes were treated separately or simultaneously with tepa, metepa, and apholate in the food (sugar solution) for 3 days, then held for 10 days.*

Treated sex	% fertility at indicated % concentration of the chemosterilant					
	0.07	0.05	0.03	0.01	0.009	0.007
<u>Tepa</u>						
Both	--	0	0	0	0	3
Male	--	0	0	0	0	25
Female	--	0	1	45	50	50
<u>Metepa</u>						
Both	0	1	38	52	63	--
Male	0	38	100	100	100	--
Female	100	88	100	100	100	--
<u>Apholate</u>						
Both	--	0	0	0	0	14
Male	--	0	6	12	19	19
Female	--	0	0	5	12	50

*Abbott's correction formula applied where fertility in untreated gnats was less than 90 per cent. (25 pairs of gnats per concentration; 1 generation.)

That the concentration to sterilize either sex is somewhat higher than that needed to sterilize both sexes appears consistent with the work reported by LaBrecque (1961) for house flies.

Effects of Chemosterilants on the Reproductive System

Gnats were examined to determine the effect of the chemosterilants on the reproductive system between the 13th and 14th days after first exposure to the chemicals. The only histological damage apparently caused by the chemicals occurred in the gonads and their gamete-producing cells.

Histopathology of Male Gonads

Tepa at concentrations of 0.1 and 0.05 per cent and apholate at 0.5 and 0.1 per cent reduced the length of the testes by almost 100 microns, or 23 per cent of the length of the testes in the untreated gnats (table 10 and fig. 9). The length of the testes in gnats treated with 0.01 per cent of tepa or 0.05 per cent of apholate was approximately the same size as testes of untreated gnats. The highest concentrations of tepa (0.1 per cent) and apholate (0.5 per cent) also reduced the width of the testes by 17 to 23 per cent of the testes of untreated gnats (table 10). Metepa did not appear to have an effect on the length and width of the testes (table 10). A similar effect was observed in Drosophila (Cantwell and Henneberry, 1963), in the boll weevil (Lindquist et al., in ms.), and in Culex (Murray, 1963).

All three chemicals at the higher concentrations caused abnormalities of the testes in some instances. The germarial region showed the greatest abnormalities. In some testes it was slightly everted and in one testis it was quite distended. In several of the

males one testis appeared much smaller than the other. Another effect which was never observed in untreated gnats but occurred occasionally in treated gnats was a wave-like effect over the periphery of the testes, giving them a shriveled appearance (fig. 10).

Table 10.--The effect of tepa, metepa, and apholate on the length and width of the testes of eye gnats, and on the proportionate length of the zone of transformation.

Concentration (%)	Number of observations	Size of testis (μ)				Length of zone of transformation as % of length of testis	
		Length		Width		Average	SE*
		Avg.	SE*	Avg.	SE*		
<u>Tepa</u>							
0.1	28	349	19	134	7	89.2	5.7
.05	21	369	23	127	12	91.2	8.6
.01	16	465	20	146	12	64.9	8.0
<u>Metepa</u>							
0.1	19	412	23	152	11	54.2	18.5
.05	20	457	23	156	8	57.8	4.0
.01	27	447	20	155	8	69.4	5.0
<u>Apholate</u>							
0.5	32	377	21	126	6	97.2	2.3
.1	18	387	33	136	13	74.6	13.5
.05	15	486	27	151	9	80.4	10.4
<u>Untreated</u>							
--	54	447	12	164	12	61.6	3.4

*SE is the standard error times the value for "t" at the 5% probability level.

The zone of transformation appeared to increase in length at the higher concentrations of tepa and apholate. In most instances with tepa at 0.1 and 0.05 per cent and apholate at 0.5 and 0.1 per cent, and infrequently at 0.05 per cent, the testes had no well-defined separation of the zone of spermatogenesis as occurred in the testes of untreated gnats or those treated with metepa (fig. 10). When these testes were crushed at the germarial region to free the cells within,

only mature or nearly mature sperm were obtained. Observations with phase contrast microscopy showed no spermatogonial cells or spermatids from testes which had no well-defined separations of the zone of spermatogenesis (fig. 10).

Figure 9.--Testes from (a) chemosterilized male and (b) untreated male eye gnats. 133X.

As shown in table 10, the increase in length of the zone of transformation expressed as a per cent of the length of the testis ranged from 13 to 35.6 per cent for apholate at all three concentrations, and from 27.6 to 29.6 per cent for tepa at 0.1 and 0.05 per cent. Metepa did not affect the length of the zone of transformation. In all the testes crushed and examined for sperm, the sperm were found to be motile and did not appear different from those of untreated gnat testes. Other organs of the male reproductive system showed no gross abnormalities.

Figure 10.--Sagittal section of testes from (a) chemosterilized male (260X), and (b) untreated male (360X) eye gnats (arrows point to sperm bundle).

The significant reduction in size of the testes after chemosterilization of the adult is an indication of two possibilities; (1) the testes were retarded in growth by the effects of the chemosterilant and/or (2) the testes atrophied due to the degenerative effects of the chemical. The latter assumption appears to be more reasonable since the study of untreated adult testes indicated no further growth of the testes during the first twelve days of life. When insects are treated in the pupal stage a retardation of testicular development has definitely been shown to occur (Culex, Murray, 1963; and boll weevil, Lindquist et al., in ms.), and this may also happen in the adult stage (Drosophila, Cantwell and Henneberry, 1963). The substantial reduction in size of the testes of treated H. pusio observed in this study is probably correlated with the increase in the amount of mature sperm found in the treated testes over the untreated testes. It seems highly probable that the germarial region of the

testis was irreversibly affected preventing further meiotic and mitotic divisions (fig. 10). Those sperm cells which completed division subsequently elongated and differentiated into mature sperm while the other primary cells, grossly affected by the chemical, were possibly resorbed. This would result in a loss of cellular material within the testis and an expansion of the transformation zone throughout the length of the testis. The resulting sperm content would be somewhat less than the original resulting in a partial collapse of the testicular envelopes thus producing a wave-like effect over the periphery of the testis as was observed in this study (fig. 10). Due to the loss of primordial sperm cells, further increases in numbers of sperm would not be possible; therefore, each time the male mated with a subsequent loss of sperm the testicular content would be slightly reduced. This factor also could be a contributing cause to the shrinkage of the testis.

Since the gnats were treated during the first three days of adult life, the maximum size of the transformation zone would be about 40 per cent of the length of the testis. However, the germarial zone occupies about 20 per cent of the length of the testis and if destroyed, the remainder of the sperm cells after completion of development could occupy the whole testis. That the 20 per cent collapse of the testicular envelope does not result in a more severe effect on its perimeter is probably due to the elasticity of the tunicas.

Histopathology of Female Gonads

Of the 166 females observed, 131 had ovaries with the majority of the primary follicles in stage "a", 20 in stage "b", 3 in stage "c",

and 12 in stage "d" or "e" (table 11). Tepa at 0.1 and 0.05 per cent and apholate at 0.5 per cent caused at least 90 per cent of the females to have abnormalities in the ovarioles, particularly in the area where the nurse-cells were undifferentiated from the oöcytes. These cells were misshapen or degenerate and sometimes undistinguishable, in which case the area had a granular appearance (fig. 11). Smaller primary follicles were observed in many treated females, which resulted in ovaries reduced in size as compared to those from untreated females of the same stage (fig. 11). Primary follicles that had reached stage "b" and had more than 15 per cent oöcyte development did not appear to have degenerate or misshapen nurse cells. Those ovaries showing severe effects from the chemosterilant contained degenerated and atrophied germarial areas (fig. 11). Similar effects occurred in house flies as observed by Morgan and LaBrecque (1962).

Figure 11.--Ovaries from (a) chemosterilized female and (b) untreated female eye gnats. (Arrow points to germarium of ovariole.) 110X.

Table 11.--The stage of development of the primary follicles in ovaries of eye gnats treated with tepa, metepa, and apholate in the food.

Concentration (%)	Number of females with ovaries at indicated stage of primary follicle development*			
	a	b	c	d and e
<u>Tepa</u>				
0.1	16**	0	0	2
.05	8**	1	0	2
.01	11	2	1	1
<u>Metepa</u>				
0.1	11	4	0	0
.05	9	2	0	0
.01	10	1	2	3
<u>Apholate</u>				
0.5	18**	0	0	0
.1	9	0	0	2
.05	12	3	0	0
<u>Untreated</u>				
--	27	7	0	2

*Stage a - oöcyte undifferentiated from nurse cells; stage b - oöcyte occupying up to 50 per cent of the follicle; stage c - oöcyte occupying more than 50 per cent of the follicle and nurse cells without atrophy; stage d - nurse cells beginning to atrophy but chorion pattern not distinguishable; stage e - mature with micropyle and chorion discernible.

**Ninety per cent or more of the ovaries observed contained primary follicles with abnormalities.

The reduction in the size of the adult ovary observed in this study is in agreement with that for house flies (Morgan and LaBrecque, 1962; and in press). The effects of chemosterilization on the egg chambers of house flies as observed by Morgan and LaBrecque showed a retardation in the development of the egg chambers and a reduction in the size of the ovary.

In the author's study the ovary was decreased in size but the most significant effects were seen in the nurse cells and the germarial regions. The germarium was partly or completely destroyed. This effect is consistent with that observed in the male. Evidently those cells within the follicle which have finished the meiotic divisions are capable of subsequent development at least to the extent of nurse cell formation. However, those cells which have not undergone meiotic divisions or are in the process are partially or completely destroyed. This meiotic destruction in treated females parallels the effects in apholate-treated boll weevils observed by Lindquist et al. (in ms.) who found extremely few meiotic divisions and a lack of general organization in the meiotic stages. Lindquist et al. (in ms.) postulated that apholate chemically interfered with the spermeogenic transformations and/or meiotic II divisions. The latter effect appears to account for some of the germarial effects observed in this study. It is not known exactly what stages of meiosis and mitosis are affected, if any, but there appears to be a definite atrophy of the germarial regions of the treated ovaries observed in this study.

SUMMARY

1. The orange-pigmented, pear-shaped testes of H. pusio adult males lie dorso-laterally in the second to fourth abdominal segments. They are approximately 436 microns long and 168 microns wide.
2. The zone of transformation appears to occupy about 10 per cent of the length of the testis during the first day, increasing at a descending rate of growth to approximately 72 per cent by the 12th day, and remains at approximately this percentage.
3. The vas deferens of the male is short and attaches to the ejaculatory duct next to the long, secretory accessory glands. The ejaculatory duct gradually narrows as it reaches its point of attachment to the ninth segment.
4. The ovaries of H. pusio contain approximately 40 ovarioles and about half of these appear to develop at approximately the same rate. The spermathecae are somewhat atypical of that observed in other insects studied, in that they are coiled ducts rather than hollow spheres and are without heavy sclerotization.
5. Sperm are transferred from the spermathecae to the bursa in the female at the approximate time of egg deposition and are subsequently transferred to the spermathecae after oviposition is completed. The mechanism by which this transfer occurs is not known.

6. The mature sperm cells of H. pusio males may be in the form of bundles or may occur singly and persist in both forms in the female.
7. Both sexes of H. pusio undergo a sexual maturation following adult emergence, after which they are capable of mating. This development apparently requires the same time for both sexes and was found to be about 36 to 48 hours in this study.
8. Males of H. pusio may mate with several females (up to four a day).
9. Females of H. pusio are capable of laying an average of 26 eggs in an 18-day period. There is no apparent difference in the number of eggs laid by mated and unmated females although a slight difference in the onset of oviposition indicating a delay in oviposition by unmated females was noted.
10. Both sexes of H. pusio are capable of being sterilized in the adult stage by feeding on food treated with tepa, metepa, and apholate. Generally the concentration required to sterilize either sex was slightly higher than that concentration required to sterilize both sexes simultaneously. The female usually required a slightly higher concentration for sterility than the males.
11. The most apparent histological damage caused by the chemosterilants occurred in the germarial region of the gonads of both sexes. This region appeared to be either completely or partially destroyed in a 13-day period. Once development and differentiation of the sex cells had begun, it did not appear to be stopped by the chemosterilants, but the sex cells were nevertheless rendered sterile.
12. The chemosterilants caused treated gnats to have smaller gonads than untreated gnats.

LITERATURE CITED

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
- Abraham, R. 1934. Das Verhalten der Spermien der weiblichen Bettwanze (*C. lectularius* L.) und der Verbleib der überschüssigen Spermasse. Z. Parasiten. 6: 559-591.
- Abul-Nasr, S. E. 1950. Structure and development of the reproductive system of some species of Nematocera. Phil. Trans. Roy. Soc. London (B) 234: 339-396.
- Alexander, P. 1960. Radiation-imitating chemicals. Sci. Am. 202: 98-108.
- Baumhover, A. H., Eisman, C. N., Skipper, C. C., and New, W. D. 1959. Field observations on the effects of releasing sterile screw-worms in Florida. J. Econ. Entomol. 52: 1202-1206.
- Bengston, Ida A. 1933. Seasonal acute conjunctivitis occurring in the southern states. Publ. Hlth. Repts. (U.S.) 48: 917-926.
- Bertram, D. S. 1959. The ovary and ovarioles of mosquitoes. "Course in advanced entomological techniques applied to malaria eradication", WHO mimeo pub. L. J. Bruce-Chwatt, Ed. 238: 170-180.
- Bieseke, J. J. 1958. Mitotic poisons and the cancer problem. Elsevier Publ. Co., New York. 214 p.
- Borkovec, A. B. 1962. Sexual sterilization of insects by chemicals. Science 137: 1034-1037.
- Borkovec, A. B., and Woods, C. W. 1963. Aziridine chemosterilants. Sulfur-containing Aziridines. Adv. in Chem. Series 41: 47-55.
- Burden, G. S., and Smittle, B. J. 1963. Chemosterilant studies with the German cockroach. Fla. Entomol. 46: 229-234.
- Buxton, P. A. 1955. The natural history of tsetse flies. An account of the biology of the genus *Glossina* (Diptera). London School Hyg. and Trop. Med. Memoir No. 10. H. K. Lewis & Co., Ltd. 816 p.

- Callahan, P. S., and Cascio, T. 1963. Histology of the reproductive tracts and transmission of sperm in the corn ear worm, Heliothis zea. Ann. Entomol. Soc. Am. 56: 535-556.
- Cantwell, G. E., and Henneberry, T. J. 1963. The effects of gamma radiation and apholate on the reproductive tissues of Drosophila melanogaster Meigen. J. Insect. Path. 5: 251-264.
- Chamberlain, W. F. 1962. Chemical sterilization of the screw-worm. J. Econ. Entomol. 55: 240-248.
- Christophers, S. R. 1911. The development of the egg follicle in Anophelines. Paludism. 2: 73-88.
- Christophers, S. R. 1923. The structure and development of the female genital organs and hypopygium of the mosquito. Ind. J. Med. Res. 10: 698-720.
- Clayton, F. E. 1957. Absolute and relative frequencies of spermatogenic stages at different pupal periods in Drosophila virilis. J. Morph. 101: 457-476.
- Cressman, A. W. (In press) Response of citrus red mite to chemical sterilants. J. Econ. Entomol.
- Crystal, M. M. 1963. The induction of sexual sterility in the screw-worm fly by antimetabolites and alkylating agents. J. Econ. Entomol. 56: 468-473.
- Curtin, T. J., and Jones, J. C. 1961. The mechanism of ovulation and oviposition in Aedes aegypti. Ann. Entomol. Soc. Am. 54: 298-313.
- Davey, K. G. 1958. The migration of spermatozoa in the female of Rhodnius prolixus, Stal. J. Exp. Biol. 35: 694-701.
- Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance. WHO monograph. 47. 216 p.
- Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1-42.
- Ephrussi, B., and Beadle, G. W. 1936. A transplantation technique for Drosophila. Am. Nat. 70: 218-225.
- Glaser, R. W. 1923. The effect of food on longevity and reproduction in flies. J. Exp. Zool. 38: 383-412.
- Goldsmith, E. D., and Frank, I. 1952. Sterility in the female fruit fly, Drosophila melanogaster, produced by the feeding of a folic acid antagonist. Am. J. Physiol. 171: 726-727.

- Gouck, H. K., Crystal, M. M., Borkovec, A. B., and Meifert, D. W. 1963. A comparison of techniques for screening chemosterilants of house flies and screw-worm flies. *J. Econ. Entomol.* 56: 506-509.
- Hall, D. G. 1932. Some studies on the breeding media, development, and stages of the eye gnat, Hippelates pusio Loew. *Am. J. Hyg.* 16: 854-864.
- Harris, R. L. 1962. Chemical induction of sterility in the stable fly. *J. Econ. Entomol.* 55: 882-885.
- Henneberry, T. J., Smith, F. F., and McGovern, W. L. (In press) Some effects of gamma radiation and a chemosterilant on Mexican bean beetle. *J. Econ. Entomol.*
- Hewer, H. R. 1934. Studies in Zygaena. II. The mechanism of copulation and the passage of sperm in the female. *Proc. Zool. Soc. London.* 2: 513-527.
- Hodapp, C. J. 1960. The mechanism of terminalia rotation in male Aedes aegypti (Linnaeus). M. S. Thesis. University of Maryland. 43 p.
- Hodapp, C. J., and Jones, J. C. 1961. The anatomy of the adult male reproductive system of Aedes aegypti (Linnaeus) (Diptera, Culicidae). *Ann. Entomol. Soc. Am.* 54: 832-844.
- Howard, L. O., Dyar, H. G., and Knab, F. 1913. The mosquitoes of North and Central America and the West Indies. Vol. 1. A general consideration of mosquitoes, their habits, and their relations to the human species. Carnegie Inst. Washington Publ. 159. 520 p. (Dated 1912).
- Jay, E. G. 1961. Laboratory and field studies on the ecology of Hippelates pusio Loew (Diptera: Chloropidae) and related species. M. S. Thesis. University of Florida. 84 p.
- Jones, J. C. 1961. Observations on sexually depleted male Aedes aegypti (L.). (Abstr.) *Am. Zool.* 1: 362.
- King, R. C., Rubinson, Ann C., and Smith, R. F. 1956. Oögenesis in adult Drosophila melanogaster. *Growth* 20: 121-57.
- Knipling, E. F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.* 48: 459-462.
- Knipling, E. F. 1959. Sterile male method of population control. *Science.* 139: 902-904.

- Krause, J. B. 1947. The development of the gonads of the wood-eating beetle, Passalus cornutus Fabricius. Ann. Entomol. Soc. Am. 40: 172-202.
- Kumm, H. W. 1935. The digestive mechanism of one of the West Indian "eye gnats", Hippelates pallipes Loew. Ann. Trop. Med. Parasitol. 29: 283-302.
- Kumm, H. W., and Turner, T. B. 1936. The transmission of yaws from man to rabbits by an insect vector, Hippelates pallipes Loew. Am. J. Trop. Med. 16: 245-263.
- LaBrecque, G. C. 1961. Studies with three alkylating agents as house fly sterilants. J. Econ. Entomol. 54: 684-689.
- LaBrecque, G. C., Adcock, P. H., and Smith, C. N. 1960. Tests with compounds affecting house fly metabolism. J. Econ. Entomol. 53: 802-805.
- LaBrecque, G. C., Meifert, D. W., and Gouck, H. K. 1963. Effectiveness of three 2-methyl-aziridine derivatives as house fly chemosterilants. Fla. Entomol. 46: 7-10.
- Lindquist, D. A., Gorzyski, L. J., Meyer, M. S., Scales, A. L., and Davich, T. B. (In ms.) Laboratory studies on sterilization of the boll weevil with apholate.
- Litchfield, J. T., and Wilcoxon, F. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. and Exp. Therapy. 96: 94-113.
- Lum, P. T. M. 1961a. The reproductive system of some Florida mosquitoes. I. The male reproductive tract. Ann. Entomol. Soc. Am. 54: 397-401.
- Lum, P. T. M. 1961b. The reproductive system of some Florida mosquitoes. II. The male accessory glands and their role. Ann. Entomol. Soc. Am. 54: 430-433.
- Meifert, D. W., Fye, R. L., and LaBrecque, G. C. 1963. Effect on house flies of exposure to residual applications of chemosterilants. Fla. Entomol. 46(2): 161-168.
- Miller, A. 1950. The internal anatomy and histology of the imago of Drosophila melanogaster. p. 420-534. In "Biology of Drosophila", M. Demerec, Ed., John Wiley & Sons, Inc., New York.
- Mitlin, N., Butt, B. A., and Shortino, T. J. 1957. Effect of mitotic poisons on house fly oviposition. Physiol. Zool. 30: 133-136.

- Morgan, P. B., and LaBrecque, G. C. 1962. The effect of apholate on the ovarian development of house flies. *J. Econ. Entomol.* 55: 626-628.
- Morgan, P. B., and LaBrecque, G. C. (In press) Effect of tepa and metepa on ovarian development of house flies. *J. Econ. Entomol.*
- Mulla, M. S. 1962. Mass rearing of three species of Hippelates eye gnats (Diptera: Chloropidae). *Ann. Entomol. Soc. Am.* 55: 253-258.
- Murray, W. S. 1963. The effect of apholate on the mosquito, Culex pipiens quinquefasciatus Say. Ph.D. Thesis. University of Maryland. 75 p.
- Murvosh, C. M., Fye, R. L., and LaBrecque, G. C. (In press) Studies on the mating behavior of the house fly, Musca domestica L. *Ohio J. Sci.*
- Murvosh, C. M., LaBrecque, G. C., and Smith, C. N. 1964. Effect of three chemosterilants on house fly longevity and sterility. *J. Econ. Entomol.* 57: 89-93.
- Nonidez, José F. 1920. The internal phenomena of reproduction in Drosophila. *Biol. Bull.* 39: 207-230.
- Norris, Maud J. 1932. Contributions toward the study of insect fertility. I. The general structure and operation of the reproductive organs of the genera Ephestia and Plodia (Lepidoptera, Phycitidae). *Proc. Zool. Soc.* (no vol. no.) 595-611.
- Omura, S. 1936. Studies on the reproductive system of the male of Bombyx mori. I. Structure of the testis and the intratesticular behavior of the spermatozoa. *J. Fac. Agr. Hokkaido Imp. Univ. Sapporo.* 38: 151-181.
- Omura, S. 1938a. Structure and function of the female genital system of Bombyx mori with special reference to the mechanism of fertilization. *J. Fac. Agr. Hokkaido Imp. Univ. Sapporo.* 40: 111-128.
- Omura, S. 1938b. Studies on the reproductive system of the male of Bombyx mori. II. Post-testicular organs and post-testicular behavior of the spermatozoa. *J. Fac. Agr. Hokkaido Imp. Univ. Sapporo.* 40: 129-170.
- Payne, M. A. 1933. The structure of the testis and movements of sperms in Chortophaga viridifasciata as demonstrated by intravitem technique. *J. Morph.* 54: 321-346.
- Ross, W. C. J. 1962. Biological alkylating agents. Butterworth and Co., Ltd., London. 232 p.

- Sanders, D. A. 1940. Musca domestica and Hippelates flies; vectors of bovine mastitis. *Science*. 92: 286.
- Schwartz, P. H., Jr. 1961. Behavior of spermatozoa of Aedes aegypti (L.). M. S. Thesis. University of Maryland. 45 p.
- Schwarz, E. A. 1895. The Hippelates plague in Florida. *Insect Life*. 7: 374-379.
- Shaw, J. G., and Sanchez Riviello, M. 1962. Sterility in the Mexican fruit fly caused by chemicals. *Science*. 137: 754-755.
- ✓ Smith, C. N., LaBrecque, G. C., and Borkovec, A. B. 1964. Insect chemosterilants. *Ann. Rev. Entomol.* 9: 269-284.
- Snedecor, G. W. 1961. Statistical methods applied to experiments in agriculture and biology. 5th ed. Iowa State Univ. Press, Ames, Iowa. 534 p.
- Snodgrass, R. E. 1935. Principles of insect morphology. McGraw-Hill Book Co., Inc., New York. 667 p.
- Snodgrass, R. E. 1956. The anatomy of the honeybee. Comstock Publ. Assoc., Ithaca, New York. 334 p.
- Snodgrass, R. E. 1959. The anatomical life of the mosquito. *Smith. Miscell. Coll.* 139(8): 1-87.
- Stacey, K. A., Cobb, M., Cousens, S. F., and Alexander, P. 1958. The reactions of the "radiomimetic" alkylating agents with micro-molecules in vitro. *Ann. N. Y. Acad. Sci.* 68: 682-701.
- Stern, C. 1941. The growth of testes in Drosophila. I. The relation between vas deferens and testis within various species. *J. Exp. Zool.* 87: 113-158.
- Timmis, G. M. 1961. Antagonists of purine and pyrimidine metabolites and of folic acid. *Adv. in Cancer Res.* 6: 369-401.
- Wassilieff, A. 1907. Die Spermatogenese von Blatta germanica. *Arch. f. Mikros. Anat.* 70: 1-42.
- Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. *Nature*. 195: 786-787.
- Weidhaas, D. E., Ford, H. R., Gehan, J. B., and Smith, C. N. 1961. Preliminary observations on chemosterilization of mosquitoes. *Proc. 48th Ann. N. J. Mosq. Exterm. Assoc.* p. 106-109.
- ✓ Weidhaas, D. E., and McDuffie, W. C. 1963. Highlights of recent research on chemosterilants for the control of insects of medical and veterinary importance. *Bull. Ent. Soc. Am.* 9: 268-272.

- West, L. S. 1951. The house fly, its natural history, medical importance, and control. Comstock Publ. Assoc., Ithaca, New York.
- Wheeler, R. E., and Jones, J. C. 1960. The mechanics of copulation in Aedes aegypti (L.) mosquitoes. (Abstr.) Anat. Rec. 138: 388.
- Whelden, R. M., and Haskins, C. P. 1953. Cytological and histological studies in the Formicidae. I. Chromosome morphology and the problem of sex determination. Ann. Entomol. Soc. Am. 46: 579-595.
- Wigglesworth, V. B. 1953. The principles of insect physiology. E. R. Dutton and Co., Inc., New York. 546 p.
- Woolley, D. W. 1952. A study of antimetabolites. John Wiley and Sons, Inc., New York. 269 p. ✓
- Zacharuk, R. Y. 1958. Structures and functions of the reproductive systems of the prairie grain wireworm, Ctenicera aeripennis destructor (Brown) (Coleoptera: Elateridae). Canad. J. Zool. 36: 725-751.

BIOGRAPHICAL SKETCH

Paul Henry Schwartz, Jr., was born December 19, 1936, at Baltimore, Maryland. He attended Catonsville Elementary and Junior High School in Catonsville, Maryland, and was graduated from Howard County Senior High School in June, 1955. In June, 1959, he received the degree of Bachelor of Science from the University of Maryland and in February, 1961, the degree of Master of Science from that institution. While at the University of Maryland he received a research fellowship and subsequently worked for six months for the State Experiment Station as a Biological Aid. He enrolled in the Graduate School of the University of Florida in February, 1961, and worked on a cooperative project between the University of Florida and the U. S. Department of Agriculture while pursuing the degree of Doctor of Philosophy.

Paul H. Schwartz, Jr., is married to the former Mary Frances Cox and is the father of two children. He is a member of the Entomological Society of America and the Florida Entomological Society and a member and past officer of Alpha Gamma Rho.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 8, 1964.

W. D. Bricker
Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

John T. Breighton
Chairman

F. S. Hanton

Carroll N. Smith

W. W. Smith

A. E. Carr Jr.