THE EFFECT OF COBALT 60 GAMMA RAYS ON THE BIOLOGY OF THE EYE GNAT *HIPPELATES PUSIO* LOEW

By

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INTRODUCTION

The eye gnat, Hippelates pusio Loew, belongs to the family Chloropidae of the order Diptera. The taxonomy of the genus Hippelates has been discussed by Aldrich (1929) and Sabrosky (1941). Hippelates gnats have been linked with the mechanical transmission of yaws (Kumm and Turner, 1936), conjunctivitis (Herms, 1926) and bovine mastitis (Sanders, 1940). Early reference to the pestiferous habits of the eye gnat was made by Schwarz (1895) who encountered large populations in Alabama, Texas, and Florida. Members of the genera are found abundantly throughout the southeastern and southwestern United States. They have also been recorded in Central and South America. Several extensive control programs have been initiated in southern California where eye gnats have become particularly troublesome. Recent work on chemical control has been performed by Dow and Willis (1959) and Mulla (1963). An effective and practical means of control has not been developed.

With the success of the screw-worm eradication project in Florida (Knipling, 1960), interest has been directed toward the use of the sterilized male technique for other insect pests. The use of sterilized insects is a form of control in which the males are sterilized by either radiation or chemicals and released in large numbers to compete with normal males for females in the natural population. Females which mate with sterile males lay infertile eggs. Continued release of sterilized male insects brings about a rapid reduction of the population.

Recent developments with compounds called chemical sterilants have overshadowed the use of radiation for inducing sterility in insects. But the use of radiation to sterilize insects may have advantages over the use of chemicals (Von Borstel, 1960) in that radiation doses can be controlled very accurately, affects only the desired insect species, and does not cause dangerous contamination. Radiation sterilization programs need not be concerned with insect resistance, residue hazards, and detrimental effects on fish and wildlife.

Response to radiation in insects may be immediate as with cessation of growth (Grosch, 1956) or apparent after longer periods such as shortening of the life span, reduction of fertility and fecundity, and failure to react normally to the environment. Subtle changes occur in the physiology and genetic transfer mechanism in the irradiated insect.

Research employing radiation effects have followed two main trends. These include application of radiation to insect control and fundamental studies in genetics and cytology. The numbers of species used in all radiation studies have been relatively few.

The author has been unable to find any literature on the effects of radiation on H. pusio. The objective of this study is to provide basic biological information concerning the effects of radiation on the eye gnat. The studies reported here deal specifically with: (1) the sterilizing dose for each sex treated in both the pupal and the adult stage, (2) the effects of the sterilizing dose on male competitiveness when treated in the pupal and the adult stage, (3) the lethal effects of radiation on the stages of the life cycle, and (4) the determination of the morphological and cytological effects of gamma radiation on the reproductive organs.

LITERATURE REVIEW

Radiosterilization of Insects

The first worker to observe that insects could be sterilized by radiation was Runner (1916) who was working with the cigarette beetle, Lasioderma serricorne Fabricius. He observed that although mortality was induced at higher dosages, fertility was lost with lower dosages and suggested this approach would be a useful tool for the control of the beetle. Subsequently, Muller (1927) demonstrated that heavy doses of X-rays also induced gene mutations in Drosophila melanogaster Meigen. He stated, "It has been found quite conclusively that treatment of the sperm with relatively heavy doses of X-rays induces the occurrence of true 'gene mutations' in a high proportion of the treated germ cells. In addition to gene mutations, it was found that X-ray treatment caused a high proportion of rearrangements in the linear order of the genes." Recently, Bushland and Hopkins (1953) have shown that gamma rays produce the same results when used at equal roentgen dosages.

Radiations which are deleterious to genetical material are of 2 kinds: the ionizing alpha, beta, and gamma rays, and non-ionizing ultraviolet radiation. Despite the fact that X-rays and gamma rays have a different origin they are precisely the same in their characteristics and can be measured in the same units. The biological action of radiation is most plausibly attributed to chemical changes resulting from ionization (Catchside, 1948). Some general effects of induced

ionization on the cell are reduction of DNA synthesis, depolymerization of DNA, inhibition of mitosis, induction of mutations and chromosomal breakage. The latter results in loss of pieces and translocations (Wilson and Morrison, 1960).

Radiation may cause sterility in either of 2 ways (LaChance and Bruns, 1963). The treatment may inhibit the formation and development of mature sperm (fecundity) or may not deter the production of these cells per se but induce dominant lethal changes in the hereditary material. The latter effect renders the gametes incapable of sustaining embryonic growth or causes death in the post-embryonic stages (fertility). The dominant lethal mutation is of primary importance in sterility studies with insects. In adults or in late stage pupae where adult structure is nearly complete, cell division proceeds slowly in the somatic tissues. Only in the gonads are mitosis and meiosis proceeding rapidly. A dose of radiation that is tolerated by the somatic cells selectively produces mutations in the germ cells. A dominant lethal mutation produced in 1 parent overcomes the effect of the corresponding gene from the other parent and the resulting zygote dies. The irradiated chromosomes may also stick together so that daughter cells do not receive the normal hereditary complement and these gross structural changes are also classified as dominant lethal mutations (Bushland, 1960). The dominant lethal mutations have been classified into several types by Von Borstel and Rekemeyer (1959) and Von Borstel (1959). The importance of dominant lethals is summarized by Bartlett and Bell (1962) as follows:

> An important aspect in the evaluation of radiation damage in any species is the ability of that species to perpetuate itself after irradiation. If the irradiated adults are sterilized by a particular dose, then the genetic consequences of irradiation are not of practical importance. On the other hand, if the irradiation only partially or insignificantly alters reproduction, then genetic damage becomes a matter of concern.

Dosages required to produce more than 95 per cent dominant lethal genes in gametes cause little impairment of the other biological functions of insects. Thus, although sperm and ova remain alive and the sperm retain full motility, the zygotes do not complete development. With the sterile male technique it is not necessary that the female be monogamous (Von Borstel, 1960) although the radiosterilized males must compete with normal males in mating with females (Knipling, 1955).

In addition to studies on the effect of radiation on the reproductive capabilities of insects, a great deal of research has been conducted on the concentrations needed to produce lethality in adults. The idea that an enormous dose must be used to irreversibly damage adult insects applies only if immediate mortality is sought. Lesser concentrations will produce the same effect over a longer period of time. Less than 10,000 r of X-rays or gamma rays can decrease the life span significantly. The radio-sensitivity of a certain species is not constant but varies with age, sex, and nutrition (Baxter and Tuttle, 1957).

Morphological and Cytological Effects of Radiation

The greatest amount of damage from radiation occurs in the reproductive tissues. The functions of these tissues are partially or completely destroyed at doses which are considerably lower than those needed to produce gross functional changes in other body tissues. LaChance and Bruns (1963) in their studies on oögenesis in Cochliomyia

hominivorax (Coquerel) showed that the effect of irradiation on the reproductive capacity of the female is largely dependent on the stage of development of the ovarioles at the time of treatment. The apparent failure of females to produce mature ova reflected the inability of irradiated nurse cells to support normal vitellogenesis. King and Sang (1959) suggested that an abnormal chromosomal complement in irradiated nurse cells of Drosophila inhibited normal vitellogenesis. Retardation of growth in the ovary is not due to a reduction in the number of ovarioles which are retained in normal numbers but to morphological changes in the individual ovarioles (Erdman, 1960a; LaChance and Bruns, 1963).

Ross and Cochran (1963) have made studies on irradiated ovaries and testes of the cockroach Blattella germanica Linnaeus. Their results indicated that continued growth of the gonads was inhibited when large nymphs were treated with gamma radiation. Annan (1955) showed that abnormally small ovaries in irradiated adult Drosophila were due to a process of atrophication. The exact cause of inhibition of growth or atrophy of treated gonadal tissue is not known.

Histological techniques have been used to reveal destruction of sensitive cell types in testes. Welshons and Russell (1957) demonstrated that a period of temporary sterility in Drosophila after treatment with 4,000 r was due to destruction of both secondary spermatogonia and young spermatocytes. A subsequent return in fertility was attributed to development of cells irradiated at a later spermatogenic stage. Recovery of fertility was also observed by Grosch and Sullivan (1954) in Habrobracon males irradiated with 3,300 r of X-rays.

Such recovery phenomena must be considered in control programs where insects are released into the natural population.

Cytological studies have shown that visible changes in irradiated chromosomes can be detected (Catchside, 1948; Lea, 1955). Koller and Ahmed (1942) made cytological studies on the larval salivary gland chromosomes of Drosophila pseudo-obscura Frolowa treated with 4,500 r of X-rays and found structural changes in 40 per cent of the chromosomes.

Several useful techniques for the study of insect chromosomes have been recently published (Breland, 1961; Oster and Balaban, 1963; French et al., 1962). Chromosome numbers are known for about 400 species of Diptera, many of which are species of Drosophila (Boyes, 1958).

Several papers concerning the effects of radiation on insect tissues have been reviewed by Grosch (1962).

MATERIALS AND METHODS

The strain of H. pusio utilized in these studies was from the USDA Orlando laboratory colony. This colony was originally established by Turner (1960) in 1958 from gnats collected in the vicinity of Orlando, Florida. It was then transferred to the University of Florida, Department of Entomology, where it was maintained for 3 years and is now in the F_{85} generation.

A rearing procedure similar to that described by Jay (1961) was used. When 5 to 10 thousand eggs had accumulated in a 1/2-gallon Mason jar containing several thousand adult gnats they were collected by adding approximately 1/2-pint of cool tap water directly through the screen lid and swirling to loosen the eggs. The water was poured back through the screen lid which retained the adult insects but allowed the eggs to pass. The egg suspension was poured into a 1-gallon bain-marie containing 3 quarts of larval rearing medium. The larval rearing medium consisted of 5 parts of number 4 vermiculite (Zonolite Company), 1 part CSMA (Chemical Specialties Manufacturing Association) house fly larval rearing medium and 2 parts of tap water. Tedion (Niagara Chemical Company) was added at 10 grams of a mixture containing 0.1 per cent by weight to prevent infestation of the colony with mites (Mulla, 1958). The bain-marie was covered with a piece of heavy cotton sailcloth held in place with a large rubber band and maintained at 82° ± 2° F. and about 70 per cent relative humidity. Under these conditions, the life

cycle was completed in about 12 days. The insects were allowed to emerge in a cage covered with 30 x 30 mesh plastic screen. The cage was fitted with a 1-pint Mason jar at one end into which the positively phototactic gnats moved when a black cloth was placed over the screen of the cage. Food was provided on cotton dental rolls saturated with honey and impaled on the sharp points of nails inserted through the wooden frame of the emergence cage. Insects were collected from the emergence cage and placed in a 1/2-gallon Mason jar which contained 1 or 2 cotton rolls saturated with honey.

Experimental Procedure

Insects were reared and tests conducted at 82° ± 2° F. and 70 per cent relative humidity except where noted. Carbon dioxide was used as an anesthetic when handling the insects. Transfer and separation of sexes for testing purposes was done using a battery operated device described by Schwartz (1964).

Containers for adult and pupal irradiation were 10-dram clear glass shell vials fitted with plastic snap lids modified to accomodate 3/4-inch 30 x 30 mesh screen lids. Insects were fed with pure honey in these containers with a 1-inch section of a 1-mm glass capillary tube partially inserted through the screen lid. All test insects were provided with food before and after irradiation.

Adult insects were held in 1-pint plastic food containers or in 1-pint Mason jars unless otherwise noted. The lids of the plastic cages contained a central 1/2-inch hole to accept a 1/2-inch cotton dental roll and 2 marginal holes approximately 3/4-inch in diameter which were covered by a coarse mesh linen cloth. The Mason jars were fitted with

30 x 30 mesh screen lids with a dental roll taped to the inner side of the lid. Mason jars were used in all mating agressiveness tests and the female insects were introduced into the jars 24 hours before the male insects were added.

Pupae for irradiation were collected from larval rearing medium by placing approximately 2 cups of medium in a gallon metal container and adding 1/2-gallon of water. After a short interval only the pupae and a small amount of media remained on the surface. The water was then decanted through a 30 x 30 mesh screen which retained the pupae. The pupae were then placed on blotting paper to dry. The dried material was placed in a narrow mouth 1/2-gallon jar and subjected to an air blast which removed the lighter media material and left the pupae.

The larvae to be irradiated were hand picked from the rearing medium using a fine brush. They were then returned to the original medium but not before it had been autoclaved at sufficient heat to kill uncounted larvae and pupae. The medium was re-used because it was found that survival of the test larvae was optimal when they were returned to the original medium. The larvae were irradiated in the medium contained in the 10-dram glass vials. The vials were then left uncapped in an upright position in 1-pint Mason jars fitted with screen lids. Adult food was provided on dental rolls when emergence began.

Eggs for irradiation were collected for testing by placing gravid adults in 1/2-gallon jars for periods of 1 to 3 hours to allow oviposition to take place. One-half cup of water was then added to the container and the accumulated eggs were loosened. The water and egg suspension was then poured into a petri dish and 50 eggs, selected with an eye dropper, were placed on dark colored blotting paper discs (1 x 1 inch) which had previously been boiled to remove excess dye. The eggs

were easily counted against the dark background. Each disc was placed in a 10-dram glass vial for irradiation. After treatment, the discs were retained in the capped vials for a period of 3 days from the known oviposition date and then removed. The number of hatched eggs were counted under a steroscopic microscope at a magnification of 15 diameters.

The Cobalt-60 Source

The University of Florida Cobalt-60 irradiator (Teas, 1959) used in the tests reported here contains 12 tubes loaded with Cobalt-60 wafers, and is submerged in a 13-foot water tank when not in the operating position. In the operating position in air, the irradiator is raised and dose rates determined by placing the objects to be treated varying distances from the source. In the operating position, the irradiator surrounds a galvanized iron can 8 inches in diameter and 21 inches high. Insects were treated in this central can at dose rates varying from 3155 r per minute in December, 1961, to 2185 r per minute in February, 1964. At doses of less than 1,000 r, specimens were placed on the table surrounding the irradiator and irradiated with a dose rate of about 70 r per minute to reduce error in exposure times. The dose rate used in testing was from 2,600 to 2,135 r per minute unless noted otherwise.

Histological Technique

All dissections were made in Ringer's saline. Whole ovaries and ovarioles were stained with aceto-carmine, squashed, and immediately examined under a phase microscope at 150 diameters. Ovarioles were

separated by either gently mincing the ovary with the tips of jeweler's forceps or by adding a cover slip and applying gentle pressure.

Larval brain and testicular tissue were prepared for chromosome studies by the technique of Morgan and LaBrecque (in press). The procedure is briefly outlined below. The dissected tissue was transferred to a drop of 1 per cent sodium citrate on a clean microscope slide for 10 minutes and then to 45 per cent glacial acetic acid on another slide. After 5 minutes a siliconized cover slip was added, a piece of blotting paper was placed over the slide to absorb excess fluid and heavy thumb pressure was applied in a straight line to avoid movement of the cover slip and squash the preparation. The cover slip was then tapped several times with a blunt instrument and the whole slide placed on a cake of dry ice for 30 minutes. The slide was then placed in 95 per cent ethyl alcohol for 5 minutes. The slide was allowed to drip excess alcohol and a drop of Gurr's natural orcein and fast green stain applied while the slide was still wet. A cover slip was placed on the slide and excess stain removed from the edges of the cover slip by careful blotting. The slide was placed in the refrigerator overnight and then examined under a phase contrast microscope using oil immersion at a magnification of 970 diameters.

Chromosome number determinations were made using mounts prepared by the Feulgen stain technique of Whiting (1950).

Statistical Methods

Abbott's (1925) formula was used to adjust sterility to that obtained in controls where probit analysis was used to calculate the sterility dose (SD-99). Analysis of variance and "F" tests were

conducted by the methods of Snedecor (1961). The Chi square tests were run following the procedures outlined by Freund and Williams (1961). Calculated probit values and LD-50 figures were obtained from the formula of Litchfield and Wilcoxon (1949). The term "significant difference" is used in this paper to denote a probability level of 95 per cent and a highly significant difference indicates a probability level of 99 per cent.

Replication as used in this paper indicates repetition of the test with another generation of insects at another time. Thus one replication is repeated independently of the other replications.

RESULTS AND DISCUSSION

The Effects of Radiation on the Biology of the Eye Gnat

Eye gnats were subjected to radiation treatment as pupae and adults to determine the approximate sterilizing dose, mating competitiveness of males, male recovery, mating effects, and egg production. The lethal effects on egg, larval, pupal, and adult stages of the life cycle were also tested. Preliminary tests indicated that pupae which were to emerge within 2 days and adults at least 24 to 36 hours after emergence were the most suitable age of treatment because of the low mortality and the insensitivity of the reproductive organs. In the literature, pupal age is generally given in units of days after pupation. It was found by the author to be more accurate to irradiate 2 days prior to the expected date of emergence. Only insects emerging on the expected date were then used in evaluation of results. In these studies, gnats were considered to be sterile when egg hatch was reduced to 1 per cent or less.

The Sterilizing Dose

In tests conducted to determine the minimum level of radiation needed to reduce the egg hatch to less than 1 per cent, 20 males were exposed to each of 6 doses ranging from 2,500 to 5,000 r and were crossed with 20 untreated females of the same age. The reciprocal crosses were made using equal numbers of insects. The results are shown in table 1.

Table 1.--Fecundity and fertility of H. pusio treated as 24- to 36-hour adults.*

Dose	Sex treated in each cross**	Total eggs	Hatch (%)
0	Both untreated	234	70.3
2,500	Male	252	16.3
	Female	154	32.3
3,000	Male	269	6.0
	Female	134	11.6
3,500	Male	219	4.6
	Female	216	7.3
4,000	Male	228	2.3
	Female	187	6.6
4,500	Male	209	1.0
	Female	110	2.3
5,000	Male	216	0.6
	Female	138	0.5

*3 replicates.
**20 pairs of insects per cross.

At 5,000 r, or twice the starting dose, the gnat fertility was reduced to less than 1 per cent or a 26- to 65-fold decrease for males and females respectively over the initial reduction at 2,500 r. There was a significant difference between the fertility of males at each level of radiation. Moreover, it was found that male fertility was reduced about 5-fold at the 2,500 r level and female fertility was reduced by only 1/2 that of untreated gnats. A significant difference between the sterility induced in each sex was noted at the 2,500 r dosage. The number of eggs produced by females treated with 2,500 r was only about 1/2 that of untreated females.

Although there was no significant difference between the numbers of eggs laid by females treated at the different dosages, there was a significant difference in egg hatch. Fertility was decreased as the dosage increased but not proportionately.

The dose needed to reduce egg hatch to 1/2 that of controls was calculated to be approximately 2,200 r for males and 2,450 r for females. The female gnat was about 1.09 times more resistant to irradiation than male gnats at this level of fertility.

These results indicate a lower dose is required to sterilize the eye gnat than some other Diptera. Henneberry (1963) found that between 8,000 and 16,000 r was required to sterilize males of D. melanogaster and over 4,000 r for the females. Lea (1955) and Hassett and Jenkins (1952) have placed the sterilizing dose for Drosophila at approximately 8,000 r. Davis et al. (1959) showed that Anopheles quadrimaculatus Say required between 8,865 and 12,900 r to sterilize both sexes. Some other sterilizing doses for adult insects are: 5,000 r for the beetle, Onthophagus texanus Schaeffer (Howden, 1957), 5,000 r for Trogoderma sternale Jayne (Howden and Aurbach, 1958), 10,900 r for drone honey bees (Lee, 1958), 30,000 r for the cattle grub, Ostrinia nubilialis (Hubner) (Walker and Brindley, 1963) and 4,800 r for Habrobracon juglandis Ashmead (Grosch and Sullivan, 1954).

Tests showed that eye gnats could be sterilized with lower dosages when treated in the pupal stage than when treated as adults. Several hundred pupae were treated with doses ranging from 500 to 4,500 r. Twenty adults of each sex were collected from each dosage level as they became available. Adult insects were crossed when 24 to 36 hours old

with untreated insects of the same age. The females were virgin at this age since mating does not occur before approximately 36 hours after emergence (Schwartz, 1964).

At the lowest dose of 500 r there was a 30 per cent decrease in fertility with both sexes. At each subsequent level female fertility was 2 to 5 times greater than male fertility. The reduction in fertility was not proportional to dose. As shown in table 2, no viable eggs were produced from matings with either treated sex at 4,500 r as compared to the results obtained in the preceding test with adult irradiation where 99 per cent sterility was found at 5,000 r. There was no significant difference between egg production from untreated females at each dosage level. Treated females crossed with untreated males laid approximately normal numbers of eggs at 500 and 1,500 r. At higher doses there was a 4- to 100-fold decrease in egg production from treated females.

The dose needed to reduce egg hatch to 1/2 that of control was calculated to be approximately 850 r and 1,600 r for males and females, respectively. The females were about 1.8 times more resistant at this level of fertility.

These results compare closely to those obtained by Bushland and Hopkins (1951) for C. hominivorax who found that males were sterilized by 2,500 r when treated with X-rays as late pupae. Females required 5,000 r to induce sterility and produced greatly reduced numbers of eggs at this dose.

Table 2.--Fertility and fecundity of H. pusio treated as pupae 2 days prior to emergence.*

Dose	Sex treated in each cross**	Total eggs	Hatch (%)
0	Both untreated	226	75.0
500	Male	171	55.3
	Female	176	54.6
1,500	Male	211	17.1
	Female	163	48.0
2,500	Male	181	4.3
	Female	55	23.3
3,500	Male	153	3.7
	Female	12	14.0
4,500	Male	168	0.0
	Female	2	0.0

*3 replicates.
**20 pairs of insects per cross.

A comparison of the results of pupal and adult irradiation obtained in this study showed that a level of less than 1 per cent fertility was attained with both sexes when adults were treated with 5,000 r and pupae were treated with 4,500 r. At these doses, insects treated in the pupal stage produced very few eggs while treatment of adults reduced egg production to about 2/3 that of controls. As expected, treatment of the male at either stage had little or no effect on egg production in subsequent matings with untreated females. The SD-99 for gnats irradiated as adults was found to be 4,550 r for males and 4,900 r for females. The SD-99 for insects treated as pupae was 3,750 r for males and 4,700 r for females. At this level of fertility, females were 1.07 times more resistant to irradiation when the gnats were treated as adults and 1.24 times more resistant when gnats were treated in the pupal stage.

Mating Competitiveness

The effect of the sterilizing doses on male mating competitiveness was found to be dependent on whether the pupal or adult stage was treated. Treated males were added in various ratios with untreated males to virgin females of the same age. The expected per cent egg hatch was calculated from the egg hatch obtained in the control by using the ratio of sterilized males to untreated males.

The results of tests with the gnats irradiated as adults, as shown in table 3, indicate that the expected egg hatch was very close to the observed egg hatch at all cross ratios. There was no significant difference between the calculated egg hatch and the observed egg hatch. The expected value was most closely reached with the 5:1:1 ratio.

Table 3.--Competitiveness of H. pusio males treated with 5,000 r as 24- to 36-hour adults.*

Cross ratio**	Number of eggs	Hatch (%)	Expected hatch (%)
0:1:1	247	77.3	--
1:1:1	322	34.0	38.3
2:1:1	327	27.6	25.8
3:1:1	310	22.6	19.3
5:1:1	410	7.5	7.2
1:0:1	257	0.0	0.0

*20 females per ratio, 3 replicates.
**Treated male:untreated male:untreated female.

The test was repeated except that in this instance the males were treated in the pupal stage instead of the adult stage. Other conditions remained unchanged. The results given in table 4 show that the observed per cent egg hatch was not close to the expected per cent hatch at any of the test ratios. The obtained hatches were 1.4 to 2.3 times greater than expected although a significant reduction in fertility was obtained in all ratios using treated males. The expected reduction in fertility was most closely approached with the 5:1:1 ratio. Reduction in fertility was not proportionate to the numbers of treated males used.

Table 4.--Competitiveness of H. pusio males after treatment with 4,500 r as pupae 2 days prior to emergence.*

Cross ratio**	Number of eggs	Hatch (%)	Expected hatch (%)
0:1:1	167	78.3	--
1:1:1	189	62.5	39.1
2:1:1	225	59.0	26.1
3:1:1	164	38.3	19.5
5:1:1	234	19.5	13.5
1:0:1	201	0.5	0.0

*20 females per ratio, 3 replicates.
**Treated male:untreated male:untreated female.

The results of the two competitiveness tests are generally in accord with those obtained for other insects. Insects treated as adults are usually found to be fully competitive. Henneberry and McGovern (1963a) tested the effects of a sterilizing dose of 16,000 r on

competitiveness of D. melanogaster males and found them fully competitive except at 1 ratio. Males of O. nubilialis were shown to be competitive after treatment with a sterilizing dose of 30,000 r (Walker and Brindley, 1963).

Weidhaas and Schmidt (1963) reported males of Aedes aegypti L. were not able to compete with untreated males after a dose of 10,000 r. The same results were obtained by Davis et al. (1959) with males of A. quadrimaculatus when treated with 11,820 r as 1-day-old pupae. They noted that relatively large numbers of treated males had to be introduced into a population to bring about reduction in fertility. A field study using radiosterilized A. quadrimaculatus failed to reduce natural populations, probably due to a lack of male competitiveness based on irradiation effects and the use of a laboratory strain which was highly selected (Dame and Schmidt, 1962; Weidhaas et al., 1962). Steiner and Christenson (1956) and Christenson (1958) have reported that gamma radiation adversely affected the mating competitiveness of the oriental fruit fly, Dacus dorsalis Hendel. Treated males of Anastrepha ludens Loew were also noncompetitive when treated as pupae (Rhode et al., 1961). However, Bushland and Hopkins (1951) noted that screw-worm flies were fully competitive when treated as late pupae.

Undoubtedly the effect of the sterilizing dose on male competitiveness is closely determined by the stage of maturity of the insect at the time of irradiation. Late pupae have partially developed adult structure while freshly pupated insects retain large amounts of undifferentiated tissues.

Male Recovery

In tests with H. pusio males, significant recovery was found at several dosage levels when gnats were treated in both the pupal and adult stage. Gnats were irradiated as 24- to 36-hour adults with doses ranging from 2,500 r to 5,000 r. The males were isolated from each treatment level and further divided into 2 subgroups. One subgroup at each dosage level was crossed immediately with untreated virgin females. The second subgroup from each dosage level was held in a 10-dram vial for 18 days. After 18 days, these males were crossed with virgin females which were 24 to 36 hours old. Ten pairs of insects were used in all crosses. The tests were terminated 7 days after the crosses had been made and egg hatch was determined. These same procedures were repeated using males which had been treated in the pupal stage 2 days prior to emergence.

The results shown in table 5 indicate that when males were treated as pupae, there was significant recovery except at the 500 r level. Egg hatch from the virgin females crossed with virgin males which had been held 18 days after irradiation ranged from 3- to 7-fold above that obtained from the virgin females which had been mated to the freshly emerged irradiated males. The greatest recovery was shown at the 2,500 r dosage where egg hatch went from approximately 7 per cent to 50 per cent. Recovery was found at the highest dosage of 4,500 r although the insects were 93.5 per cent sterile.

Insects treated as adults had a significant recovery of fertility at the 2,500 and 3,000 r levels. Thereafter, there was no statistical

difference between egg hatches from initial and delayed crosses. At the 2,500 r dosage, fertility of the treated males increased approximately 2-fold during the second cross.

Table 5.--Fertility recovery in males of H. pusio treated with gamma radiation as adults and pupae.*

Dose	Egg hatch (%) when mated at indicated interval: 1-8 days	18-26 days	Chi square**
	Pupal stage		
0	77.5	72.0	0.84
500	48.0	56.0	2.73
1,500	12.5	44.0	157.29
2,500	5.3	35.5	372.27
3,500	3.5	14.0	171.56
4,500	0.0	6.5	109.00
	Adult stage		
0	72.5	73.0	0.44
2,500	18.5	35.5	258.00
3,000	3.5	12.5	46.31
3,500	4.5	3.5	0.45
4,000	1.0	1.5	1.00
4,500	0.5	2.0	5.00
5,000	0.5	1.5	4.00

*10 pairs of insects per cross, 2 replicates.
**Chi square value at .05 equals 5.991.

Results similar to those obtained by the author with gnats irradiated as adults were observed by Grosch and Sullivan (1954) who demonstrated permanent sterility in H. juglandis adults after treatment with 4,800 r but at 3,300 r temporary sterility was obtained. Welshons and Russell (1957) state that temporary sterility is due to depletion of spermatogonia. The spermatogonial stage is especially sensitive to irradiation. They treated Drosophila adults with 4,000 r and dissected the testes at various intervals after treatment. At 48 hours after

treatment, there was a great reduction in gonial cells which subsequently were found to repopulate as age increased.

Controlled Mating

A controlled mating test showed that multiple mating occurred and that the reversal of treated and untreated males affected viability of eggs from untreated females. Isolated females were mated with single males which had been treated in the adult stage with 2,500 r or 5,000 r or were untreated. At the end of 9 days the order of treated males and untreated males were reversed at each level of radiation and the accumulated eggs were removed and held for hatch. At the end of 16 days or 7 days after the second cross had been made, the test was terminated and the second batch of eggs held for hatch. A pair of insects in each of 5 cages were used in each test cross. The test was replicated twice.

Table 6.-- The effect of alternate crosses with untreated males and males treated with 2,500 and 5,000 r on untreated females.*

Dose	Order of crosses	Cross**	Hatch (%)
2,500	First	TM x UF	12.5
	Second	UM x UF	25.5
	First	UM x UF	72.5
	Second	TM x UF	32.5
5,000	First	TM x UF	11.5
	Second	UM x UF	32.5
	First	UM x UF	82.5
	Second	TM x UF	51.5

*5 isolated pairs per cross, 2 replicates.
**UM = untreated male, TM = treated male, UF = untreated female.

The sperm from the initial mating were apparently retained by the female and subsequently diluted by sperm obtained in a second mating. The resultant fertility of the gnat would then be due to the ratio of untreated sperm to sperm bearing dominant lethal mutations. The ratio of sperm after the second crossing appeared to be close to 1:1 in these tests. At both 2,500 r and 5,000 r there was an approximate doubling of fertility or reduction of fertility by 1/2, depending on the order of crossing. These results are in accord with those obtained by Henneberry and McGovern (1963b) with Drosophila and by Steiner and Christenson (1956) with Dacus.

Egg Production

Fertility of female gnats was greatly reduced by treatment with 5,000 r. In a test designed to determine whether egg deposition from treated insects was also reduced, adult insects were irradiated with 5,000 r and crosses were made using treated males with treated females, untreated males with treated females, treated males with untreated females, and untreated males with untreated females. Twenty insects of each sex were used in each cross. Eggs were collected daily and counted. Three replicates were made of the test.

There was no difference between egg production when females were mated with treated or untreated males. The data were pooled across the 3 replicates. Egg production with treated and untreated females is presented graphically in figure 1. Both treated and untreated females began oviposition on the 5th day after emergence. Oviposition with treated females was 2.3 and 1.6 times greater than from untreated females on the 5th and 6th day, respectively. After the 6th day,

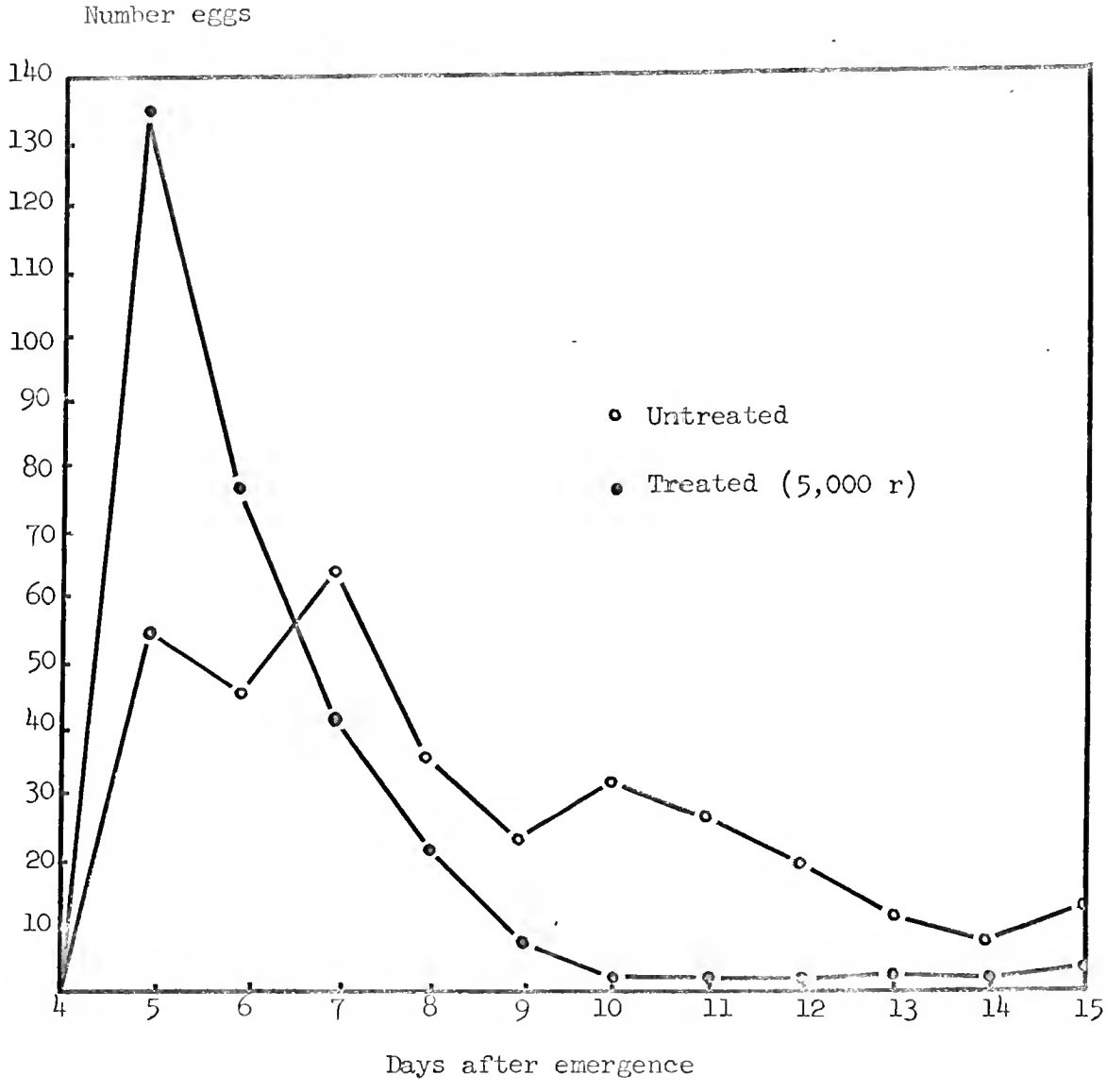


Figure 1.--Egg production from untreated females of H. pusio and females treated with 5,000 r as 24-to 36-hour adults in 3 replications (20 insects per test).

untreated females produced greater numbers of eggs daily until the end of the test. The average total egg production by untreated females was 17.1 eggs as compared to 14.8 eggs for the treated females, indicating an approximate reduction of 12 per cent in egg production from treated insects.

A further test on egg production was made using insects treated as pupae with 2,500 and 5,000 r to compare the effects with adult irradiation. Crosses were made using 10 pairs of gnats per vial. Eggs were collected every 2 days and the test was terminated after 15 days. Two replicates were made.

From the results of the pupal irradiation test, no difference was found between egg production of females mated to treated and untreated males as expected from the results of the sterility test. The data were pooled and only the differences between treated and untreated females were calculated. The untreated females produced an average of 282 eggs per test. Females treated with 2,500 r produced an average of 69 eggs per test and those treated with 5,000 r produced an average of 7.5 eggs per test, or a 4-fold and 37-fold decrease from untreated insects. Females treated with 2,500 r produced low numbers of eggs continuously throughout the test; initial deposition began on the 5th day with both treated and untreated females.

Egg production from irradiated insects has been studied with several species. A linear decline in egg production has been found with Tribolium castaneum Herbst (Bartlett and Bell, 1962) and H. juglandis (Grosch and Sullivan, 1954) when females were treated with increasing doses of radiation as adults. Egg production may be eliminated with high doses such as 11,000 r with Habrobracon (Grosch, 1958) and 10,000 r

with Ae. aegypti (Terzian and Stahler, 1958). The lack of egg production in Drosophila after treatment with 10,000 r has been attributed to destruction of germ calle (Ives et al., 1955).

Reduced egg production in insects is common with low doses although the exact cause is not clear. Increased initial egg production as found by the author in this study has not been commonly observed with females treated as adults. However, Davis et al. (1959) found the numbers of eggs deposited by A. quadrimaculatus females which had received 1,500 and 2,500 r were almost twice the number deposited by checks.

King et al. (1956) found that egg deposition in Drosophila was unchanged over a 35-day period after treatment with 2,000 r. King (1957) noted that radiation treatment generally causes a slowing of oögenesis. He observed that a reduction in total egg production of treated females was due to inhibition of cell division and reduced numbers of ovarioles and oöcytes per ovary in Drosophila.

The Effects of Radiation on Stages of the Life Cycle

Adults.--One of the first observations made by the author was that treatment of adults with levels of radiation below about 10,000 r caused no apparent increased mortality over controls. It was apparent that doses of about 5,000 r caused no increased mortality when administered to either 24- to 36-hour adults or to pupae 2 days prior to emergence.

The test periods, 7 to 15 days, were not long enough to show the effect of low doses on life span. Since these low doses produced sterility, little effort was made to determine the effect of higher doses on the life span. In a test made to compare the survival for

several weeks of both sexes after a dose of 5,000 r, 50 adult insects of each sex were irradiated and 50 insects of each sex were held untreated. Food was supplied as needed, clean 10-dram vials were supplied once each week and mortality was recorded each week. Temperature during this test was 76° ± 5° F. and relative humidity about 50 per cent. The results are given in table 7.

Table 7.--Mortality of 50 H. pusio treated with 5,000 r as 24- to 36-hour adults.

Sex	Treatment	Mortality at indicated week			Total
		1-5	6	7	
Male	5,000 r	0	1	4	5
	None	0	2	3	5
Female	5,000 r	0	0	1	1
	None	0	0	0	0

The results indicate no increased mortality due to the radiation treatment after 7 weeks.

The apparent low lethality of doses of approximately 5,000 r is commonly observed among various insect species when treated as adults. Cork (1957), working with T. confusum, found that 20,000 r killed all the test beetles in 20 days whereas a chronic daily dose of 100 r or a single dose of 3,000 r extended the life span by several per cent. He suggested that these results might be due to a general retardation of the physiological processes, thus slowing the aging process, or to stimulation of some repair mechanism with a resultant increase in the ability to withstand normal damage to the system. Bushland and Hopkins (1953) found that there was no apparent increase in mortality of females of C. hominivorax irradiated with 5,000 r although fewer males

died in the controls when males were treated with the same dose. Ross and Cochran (1963) showed no increase in mortality of B. germanica when treated as adults with 3,200 r. However, Baxter and Tuttle (1957) concluded that the life span of Drosophila was reduced proportionally to dose when corrected for survival of the controls and the same effect was demonstrated in Habrobracon by Clark (1961).

A test was conducted to determine what doses were necessary to cause a reduction in life span and the level needed to cause death within a few days. Forty insects of each sex were exposed to radiation levels proceeding from 0 to 135,000 r in 15,000 r increments. Exposure time at 135,000 r was 58 minutes and 4 seconds. Unmated insects were held in 10-dram glass vials and food was provided as needed. Mortality was recorded daily. Temperature and humidity were as in the preceding test. The LT-50 and LT-100 for each sex at each dose level are given in table 8.

Table 8.--The LT-50 and LT-100 of H. pusio males and females irradiated as 24- to 36-hour adults.*

Dose	Days after irradiation			
	Males		Females	
	LT-50	LT-100	LT-50	LT-100
0	61	66	64	94
15,000	29	41	24	54
30,000	15	21	17	31
45,000	11	14	11	17
60,000	7	9	8	12
75,000	5	7	6	8
90,000	5	6	5	7
105,000	4	4	4	5
120,000	4	4	4	4
135,000	3	4	3	4

*40 insects per treatment.

With the lowest dose, 15,000 r, the LT-50 for males and females was reduced to approximately 1/2 that of untreated insects. No difference occurs between the LT-50 and LT-100 of males and females at doses of 120,000 r and 135,000 r. Below 105,000 r the LT-100 of females was always higher than that of males although the difference decreased with increasing dose. The same statement was true of the LT-50 except at 15,000 r where the LT-50 for males was higher. The control gnats lived longer than the treated gnats. Doses greater than 45,000 r produced an immediate comatose or moribund state which lasted less than an hour except at 105,000 r and up where no recovery of activity was observed. No insects were ever observed to feed at doses of 105,000 r or higher. At these dosages the gnats were immobile on the walls and floor of the cages and made no attempt to reach food.

At very high doses of radiation, the treated insect may become moribund, as observed by the author at doses over 45,000 r. This reaction was observed by Heidenthal (1945) who termed it "sluggishness," and by Hassett and Jenkins (1952) and Sullivan and Grosch (1953). Grosch applied the term "radiation induced lethargy." Insects treated with doses sufficient to induce permenent lethargy will usually survive longer than controls when both are kept under starvation conditions. This is due to the inactivity of the treated insect (Grosch, 1956).

The use of very high doses has been suggested as a control measure by Hassett and Jenkins (1952) who observed that 65,000 r could serve as a quick knockdown dose for control of most insects. Bletchly and Fisher (1957) found Lyctus and Anobium beetles could be controlled in situ by a dose of 48,000 r.

Eggs.--Preliminary tests on the sensitivity of eggs at different ages showed a wide variation in the sensitivity in that the more mature eggs were insensitive while the freshly laid eggs were extremely sensitive. Eggs 1 to 3, 24 to 36, and 47 to 49 hours from oviposition were tested. The 1 to 3 and 24 to 26 hour eggs were irradiated with 100, 500, and 1,000 r at a dose rate of 68 r per minute plus a control. The 47 to 49 hour eggs were irradiated with 4,000, 8,000, and 12,000 r at a dose rate of 2,310 r per minute. Fifty freshly oviposited eggs were placed on moist blotting paper at each level so that development could proceed normally until treatment. The per cent hatch was recorded and the LD-50 was calculated using probit analysis. The LD-50 for 1- to 3-hour-old eggs was 126 r, for 24- to 36-hour-old eggs 1,350 r, and for 47- to 49-hour-old eggs the LD-50 was 20,000 r.

Cole et al. (1959) gave the LD-50 as 136 r at 2 days for 1/2-day-old house fly eggs, that of pupae as 15,000 r. Erdman (1960b) found that treatment of 24-hour-old eggs of Habrobracon with 2,400 r prevented the eclosion of any adult insects. These results are essentially the same results obtained for H. pusio by the author.

Larvae.--In a test using last instar larvae to observe the effects of irradiation on this stage of the life cycle, larvae which had not reached the prepupal stage were hand picked and placed on larval rearing media contained in 1-pint jars. One hundred larvae were used per jar at each level of radiation. An initial test had shown no emergence after a dose of 5,000 r so the highest dose given was 3,500 r. A dose rate of 68 r per minute was used. The jars were connected by 2-way lids to another pint jar and the emerging insects were collected

daily. Crosses were made using 10 insects of each sex when sufficient insects were available. The results are compiled in table 9.

Table 9.--The emergence and fertility of H. pusio adults treated as last instar larvae.*

Dose	Emergence (%)	Cross**	Number eggs in 10 days	Hatch (%)
0	57	UM x UF	49	71
500	52	UM x TF	129	66
		TM x UF	106	79
1,500	47	UM x TF	144	39
		TM x UF	99	65
2,500	42	--	--	--
3,500	7	--	--	--

*100 larvae per treatment.
**UM = untreated male, UF = untreated female, TM = treated male, TF = treated female.

Emergence in the control was 57 per cent while 7 per cent emerged at 3,500 r. Fertility was reduced in females but not in males at 1,500 r. Fecundity appeared to be low only in the control. Emergence of gnats at 2,500 and 3,500 r was delayed 2 to 3 days beyond the untreated gnats and emergence was spread over a longer period of time.

These results compare favorably with those of Henneberry (1963) who found 56 per cent survival in controls of larval irradiation tests with Drosophila, and only 17 per cent survival to adult stage when larvae were treated with 4,000 r. He also found no difference in the life span of males and females irradiated as pupae or adults with doses up to 16,000 r.

Pupae.--In a test to determine the effect of dose on pupal emergence, 50 pupae were treated at each of 5 levels of radiation. Dose rate was 3,130 r per minute, the temperature during rearing and up till the time of the last eclosion was 70° ± 5° F. The test was replicated twice. The results are shown in table 10.

Table 10.--The mortality of 50 H. pusio treated as pupae 2 days prior to emergence.*

Dose	Number emerged		Average emergence (%)
	Test 1	Test 2	
0	45	47	92
1,000	45	49	94
5,000	40	49	89
10,000	29	36	65
15,000	15	27	42
20,000	4	2	6

*2 replicates.

There was no significant difference in gnat emergence between the control and after treatment with 1,000 r and 5,000 r. At the 10,000 r level there was a 30 per cent decrease in adult emergence as compared to the untreated gnat emergence. The LD-50 dose was calculated by probit analysis to be 12,000 r. Insects treated with 10,000 r and higher doses were frequently found with the ptilinum extended and were partially clear of the pupal case but were unable to complete emergence. These were counted as not emerged.

Morphological and Cytological Effects

Studies of the cytological effect of gamma radiation on the screw-worm fly (LaChance and Leverich, 1962; LaChance and Bruns, 1963), the German cockroach (Ross and Cochran, 1963), Drosophila (King, 1957;

Cantwell and Henneberry, 1963) showed that morphological damage is induced in insect gonads when they are treated with sterilizing doses of radiation. This part of the study was undertaken to correlate the observed effects of radiation on fecundity and fertility with morphological and cytological damage to the gonads. The project was divided into a study of the morphology of irradiated ovaries, testes, and chromosomal aberrations.

The Ovaries

The ovaries of treated and untreated insects were observed to determine the effects of radiation on the whole ovary and the ovarioles. Insects were treated as pupae with doses ranging up to 4,500 r. Approximately 10 insects were dissected at each treatment level. Dissections were made 2, 5, and 8 days after emergence.

Each ovariole consisted of a terminal filament at the distal end, followed by the germarium, a tapered moniliform series of 2 to 4 follicles and, at the basal end, a pedicel. The development of the primary follicles of the eye gnat was divided into 5 stages by Schwartz (1964) and consisted briefly of the following: stage "a", no oöcyte visible in the primary follicle; stage "b", oöcyte visible but occupying less than 1/2 the volume of the follicle; stage "c", oöcyte occupying 51 to 75 per cent of the follicle; stage "d", 76 to 100 per cent oöcyte formation; and stage "e", egg fully developed with chorionic pattern and/or micropyle visible. This classification scheme was used to evaluate the effects of irradiation on oöcyte development in this investigation. In the author's study, the oöcyte was observed to enlarge as the nurse cells atrophied. The second follicle of the ovariole did

not undergo oöcyte development until the primary follicle had discharged its mature egg. The second follicle then proceeded to develop as did the first follicle. A third and fourth follicle are usually present but did not develop until the preceding follicle had discharged its egg. The germarium contained the gonial cells which develop into oöcytes and nurse cells within the follicles.

A comparison of treated and untreated ovarioles was made by examining the primary follicle and recording its stage of development. The second follicular cell was scored as being normal, abnormal, or absent. Normal indicates the follicle was at stage "a" and appeared the same as the controls at that age. The term abnormal was applied to second follicles which were markedly smaller, irregular in shape and/or showed loss of normal cell structure. When the second follicle could not be differentiated from other tissue it was scored as absent.

The germarium was scored as normal if it resembled the control of the same age. A germarium was noted as abnormal if it had lost its characteristic shape or cell structure. At the higher dosages used, the germarium was sometimes not distinguishable from other tissues and in these cases it was scored as absent. Provision was also made in the table for recording additional follicles, properly termed third, fourth, or fifth follicles. If any more than the first and second follicles were present, the ovary was scored for additional follicles. The results are given in table 11.

Table 11.--Effects of gamma radiation on the ovarioles of H. pusio treated as pupae 2 days prior to emergence. (See text for definition of column headings.)

Dose	Age in days	Number of insects	First follicle stage						Second follicle*			Third follicle:	Germarium*		
			A	B	C	D	E	mal	nor.	abn.	abs.	present	nor.	abn.	abs.
0	2	10	10	0	0	0	0	0	10	0	0	9	10	0	0
	5	9	1	0	0	2	6	0	9	0	0	4	9	0	0
	8	9	4	1	0	0	4	0	9	0	0	6	9	0	0
500	2	10	1	1	5	1	2	0	10	0	0	7	10	0	0
	5	10	0	0	0	2	8	0	10	0	0	7	10	0	0
	8	10	2	3	0	0	5	0	10	0	0	5	10	0	0
1,500	2	10	0	5	3	2	0	0	10	0	0	4	10	0	0
	5	10	2	1	1	2	4	0	10	0	0	0	9	0	0
	8	10	0	0	0	3	7	0	8	2	0	1	9	0	0
2,500	2	8	0	1	0	1	0	6	1	8	0	1	1	7	0
	5	5	0	0	0	0	3	2	3	2	0	0	3	0	2
	8	1	0	0	0	0	0	1	0	1	0	0	0	1	0
3,500	2	10	0	0	0	0	0	10	0	0	10	0	0	0	10
	5	10	0	0	0	3	1	6	0	0	10	0	0	0	10
	8	9	0	0	0	1	0	8	0	0	9	0	0	0	10
4,500	2	10	0	0	0	0	0	10	0	0	10	0	0	0	10
	5	10	0	0	0	0	0	10	0	0	10	0	0	0	10
	8	10	0	0	0	0	0	10	0	0	10	0	0	0	10

*Normal = nor.; Abnormal = abn.; Absent = abs.

The ovaries of untreated gnats 2 days after emergence were all found to have stage "a" follicular development and in 90 per cent of the ovarioles a third follicle could be distinguished besides the second follicle and germarium. All the ovarioles in each ovary appeared in the same stage of development. After 5 days, over 1/2 the ovaries contained primary follicles with fully mature eggs and only 1 adult was found with stage "a" oöcytes. This was probably due to oviposition and subsequent development of the second follicles. After 8 days, approximately 1/2 the ovaries had apparently discharged their mature eggs and the second follicles were in the early stages of oöcyte development. At least 50 per cent of all untreated ovaries were found at 5 and 8 days to have ovarioles with 3 or more follicles.

Gnat pupae treated with 500 r showed oöcyte development very similar to untreated insects. The only difference observed between treated and untreated ovaries was a wider variation in the developing stages of the follicles. With 2-day-old adults, some ovaries contained nearly mature eggs. The remaining follicles and germaria showed no variation from normal. At 1,500 r there was an apparent reduction in the number of third follicles and 2 of the second follicles were found to be abnormal. First follicle development appeared normal at 2 days. Three 8-day-old adults showed loss of nurse cell differentiation in their developing follicles. Some ovaries contained follicles in several stages of development or with a reduced number of ovarioles, a condition not observed previously by the author.

At 2,500 r a large proportion of the ovaries were classified as malformed. These ovaries had ovarioles which showed loss of cellular differentiation in all follicles. That proportion of the ovaries which

were not malformed contained oöcytes in an advanced stage of development but these oöcytes contained no distinct nurse cells. These were classified by size alone. Some ovaries which showed development contained as few as 2 distinguishable ovarioles. With this exposure the germarium was usually present but in many cases was abnormal in shape, being only a terminal clump of tissue.

Pupae treated with 3,500 and 4,500 r showed an almost complete lack of follicular or ovarian development as adults. At the 3,500 r level, 5-day-old adults had partially developed eggs which had no nurse cell differentiation. They had only 1 to 3 developed ovarioles per ovary. At 4,500 r no remnant of an ovariole was found. Figures 2 and 3 show ovarian damage obtained by irradiation of the pupal stage with these dosage levels.

The damage observed in ovaries of adults treated as pupae explains the egg production and fertility from gnats treated with the same doses in preceding tests. Females treated with 3,500 and 4,500 r laid an average of less than 1 egg per female in the egg production test and in the present test dissected ovaries showed complete lack of differentiation into normal structures at these doses.

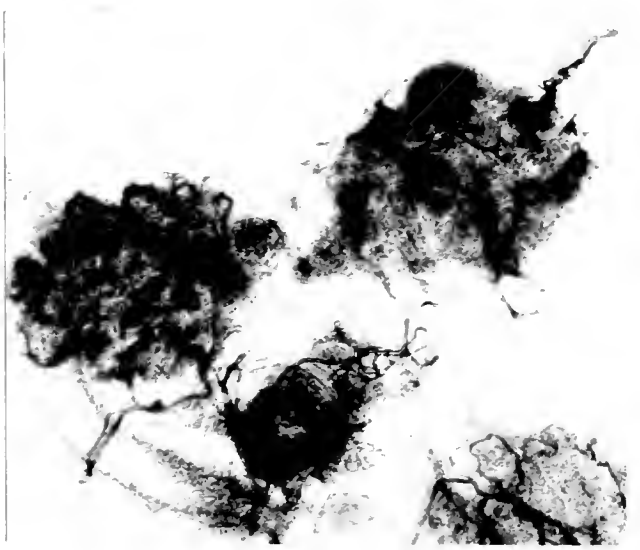
Similar results were obtained by LaChance and Bruns (1963) with C. hominivorax. These workers treated 5-day-old pupae with 4,000 r and found that primary follicles remained undeveloped and secondary follicles failed to develop or were atrophied. At 2,000 r they found retarded growth, but nurse cells, second follicles and germaria were generally present. They suggest that failure of treated females to produce mature ova reflects an inability of nurse cells to support

Figure 2.--Ovaries of H. pusio treated with gamma radiation and untreated.

a. Untreated ovaries with stage "b" oöcytes.

b. Treated ovaries from insect irradiated with 4,500 r as a pupae 2 days prior to emergence. Note complete lack of normal ovariole development.

a.

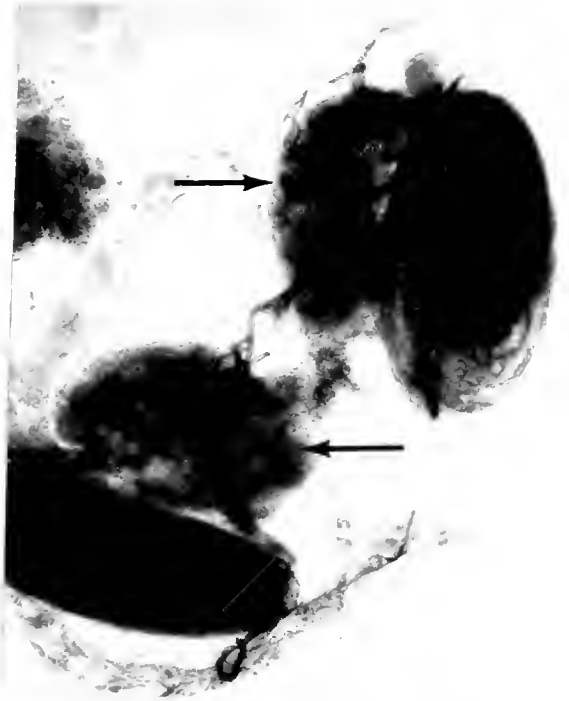


b.

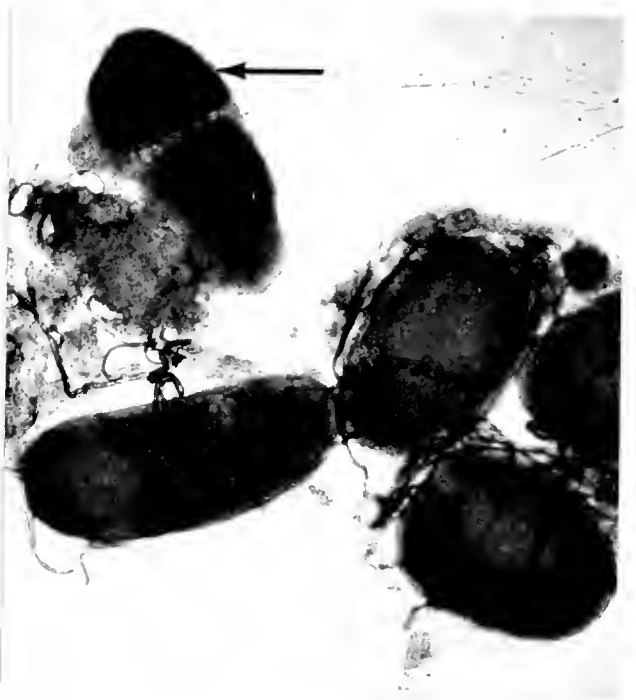
Figure 3.--Ovarian development of H. pusio after treatment with 3,500 r as pupae 2 days prior to emergence.

a. Arrows indicate ovaries, each with an abnormal oöcyte.

b. Abnormal oöcytes, arrow indicates area of undifferentiated nurse cells.



a.



b.

normal vitellogenesis. In the author's study of H. pusio ovarioles, nurse cells were found to be abnormal at doses as low as 1,500 r in that the nurse cells failed to differentiate into visible cells.

In studies with the German cockroach, Ross and Cochran (1963) found that insects treated as nymphs with doses from 3,200 to 6,400 r produced ovaries in the adult stage which appeared to have ceased growth at the time of irradiation. The dose of 3,200 r effectively sterilized female roaches. A dose of 1,600 r caused a high proportion of ovarioles to be abnormal in structure.

The Testes

The normal testis has been described by Schwartz (1964) as a pear-shaped body having a knob-like apical end and a broad base where it attaches to the vas deferens. The testis is divided into several distinct zones of spermatogenesis which are visible in untreated testes (see figure 4). Basally, there is a darkened zone of mature sperm or transformation zone. This zone of mature sperm fills approximately 1/2 the untreated testis by the seventh day.

The effect of irradiation was observed on the testes of males treated as 24- to 36-hour adults or treated in the pupal stage 2 days prior to emergence. The sterilizing dose of 4,500 r for pupal irradiation and 5,000 r for adult irradiation was used. Testicular measurements were made from males 7 days $\pm$ 1 day after emergence. The test gnats were from the same generation. Approximately 25 males were examined for each treatment. Measurements were made on both testes from each male using an ocular scale and the measurements converted to microns with an accuracy of $\pm$ 10 microns. Each observation in the table is from a single testis.

Figure 4.--Testes from untreated and treated H. pusio 7 days after emergence.

a. Testes from untreated male. Note distinct areas of spermatogenesis.

b. Testes from male treated with 5,000 r as an adult 24 to 36 hours after emergence.

a

b

The length and width of the testes from males treated in the pupal stage was significantly different from untreated testes. The smallest testis examined in this study was from a male treated in the pupal stage which had the dimensions of 250 x 112 microns in length and width respectively. The largest testis was from a male treated in the adult stage which had a length and width of 580 x 210 microns.

In males treated as adults or pupae, the darkened zone of mature sperm extended the full length of the testes and gave the entire testis an opaque appearance. Schwartz (1964) observed a similar effect from treatment of H. pusio with chemosterilants.

The shapes of testes from males treated as pupae were markedly different than those from either untreated males or males treated as adults. The whole testis had a shriveled and knobby appearance, the apical tip of the testis was often reflexed on the main axis of the testis at about 90°. The difference in size between 2 testes in an individual did not vary appreciably but occasionally a more reduced testes was found on one side. Several individual testes were seen with patchy areas of pigmentation. The areas without pigmentation appeared to be transparent. This effect was observed only in males treated in the pupal stage.

Testes from males treated as adults were somewhat longer than those from untreated males although widths were approximately the same. The shape of the treated testis was more oblong than the untreated testis due to slightly increased length. The testes of the gnats treated in the adult stage were perceptibly knobby. The apical tip was straight or slightly reflexed as observed in untreated testes. The

distortion of the apical tip of the testes from males treated as adults was less severe than that observed in testes from males treated in the pupal stage.

Testes from males treated in both stages of development with the sterilizing dose revealed active sperm and sperm bundles upon squashing of the whole testis.

Table 12.--Testicular measurements of H. pusio treated with 4,500 r as pupae 2 days prior to emergence, 5,000 r as 24- to 36-hour adults, and untreated.*

Treatment	Number observed	Dimensions in microns ± SE		Range	
		Length	Width	Length	Width
None	46	428.0±5.22	181.5±2.86	530-330	220-150
4,500 r on pupae	50	380.2±6.20	132.0±3.50	470-280	170-90
5,000 r on adults	48	459.3±7.32	174.5±3.28	580-350	220-110

*7 days after emergence.

Chromosome Aberrations

A study was undertaken to (1) define the somatic number of chromosomes in H. pusio, and (2) determine if there was morphological damage to the chromosomes with the sterilizing doses. It was the objective of this work to relate the chromosomal damage to the reproductive performance of the insect induced by the sterilizing dose.

The untreated larval brain was selected for chromosome number studies. Last instar larvae were taken from the laboratory colony and squash preparations were made of the brain tissue. The brain was found

to be dorsally located about 1/4 the body length from the anterior end. It was identified by the bilobed structure and milky opaque color which differentiated it from surrounding tissue.

Chromosome figures were readily obtained in the larval brain preparations using both the orcein-fast green stain technique of Morgan and LaBrecque (in press) and the Feulgen staining procedure of Whiting (1950). Slides prepared by the Feulgen method showed the chromosome number to be 2N = 8. Four distinct pairs of homologous chromosomes were observed.

The phenomenon of somatic pairing was consistently encountered. Somatic pairing is characterized by a close association between homologous chromosomes during mitotic divisions and is commonly observed in Diptera (LaChance, 1964). At least 25 preparations were stained by the orcein-fast green method but in no case was a full chromosome complement of 8 observed. Instead there were 3 pairs of chromosomes which were somatically paired plus an additional chromosome which stained identically to the others but which had no observable homolog. From comparisons with the Feulgen prepared slides it was determined that there were actually 2 homologous chromosomes, 1 of which could not be shown with the orcein-fast green stain. As shown in the Feulgen preparations, these chromosomes appear morphologically identical and it is suggested by the author that they are the sex chromosomes. Thus, no morphologically differentiated heterochromosomes (XY) were observed although differences in stain characteristics were found.

LaChance (1964) studied the chromosomes of the horn fly, Haematobia irritans (L.), and the stable fly, Stomoxys calcitrans (L.), and found

the diploid (2N) chromosome number to be 10 in both species. He found no morphologically differentiated heterochromosomes using aceto-orcein stain and concluded the sex chromosomes were not distinguishable from the autosomes.

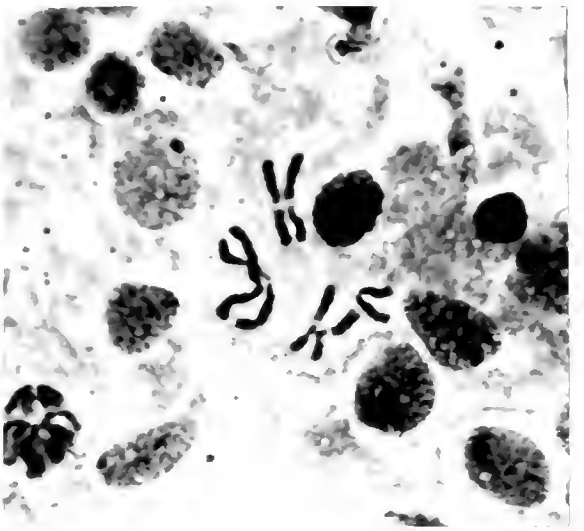
Preparations were made from the testes from treated pupae and adults. Pupae were treated 2 days prior to emergence and the testes were removed 0 to 12 hours after emergence. Adults were treated 24 to 36 hours after emergence and the testis removed immediately. The dissected testes were stained using the orcein-fast green method which produced darkly stained chromosomes.

Initial trials using doses of 4,500 r on pupae and 5,000 r on the adults produced slides with no or very few chromosome figures. The dose was reduced to 2,500 r for both the pupae and the adults, and the same dissection schedule was maintained. There was an increase in number of chromosome figures although the chromosome figures from treated testes were never found in numbers approaching those on slides from the larval brain. Damaged chromosomes were not frequently encountered but figures were found where chromosome breakage was evident. In such cases the chromosomes were fragmented and the pieces scattered so that individual chromosomes could not be recognized. Many more chromosomal patterns were observed from insects treated in the pupal stage, probably due to greater activity in cell division.

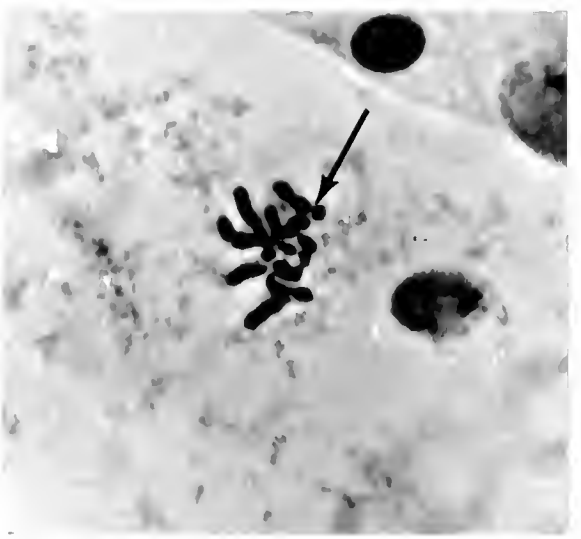
Figure 5.--Chromosomes of H. pusio.

a. Untreated larval brain tissue stained with orcein-fast green.

b. Chromosome figure from testis of insect treated with 3,500 *r* as a late pupae. Arrow indicates a broken chromosome.



a.



b.

SUMMARY

1. The dose required to sterilize insects treated as 24- to 36-hour adults was determined to be approximately 5,000 r for both males and females. When insects were treated as pupae 2 days prior to emergence, the sterilizing dose was found to be approximately 4,500 r for both sexes.

2. Males treated as adults with the sterilizing dose were found to be fully competitive with untreated males for untreated virgin females. Males treated in the pupal stage were partially competitive with untreated males for virgin females at the ratios and doses tested.

3. A partial recovery of fertility was observed in males after 18 days when treated as pupae at doses up to and including 4,500 r. Males treated as adults recovered partial fertility at doses of 2,500 and 3,000 r. Beyond 3,000 r a permanent sterility was induced.

4. In multiple mating tests, females initially crossed with males treated with 2,500 and 5,000 r produced a high percentage of infertile eggs. Upon subsequent substitution of untreated males, fertility was increased. Females crossed initially to untreated males produced a high percentage of fertile eggs but on subsequent substitution of treated males, the percentage of egg hatch was reduced. These results indicate the females to be polygamous and a carry-over of sperm from the initial cross.

5. Females treated with 4,500 r as pupae 2 days prior to adult emergence did not produce eggs. Females treated as 24- to 36-hour adults with 5,000 r produced initially greater numbers of eggs but total production during the 15-day test period was reduced as compared to untreated females. Untreated females appeared to exhibit 3 peaks of oviposition but did produce eggs continuously over the 15-day test period.

6. The lethal effects of radiation were determined for each sex treated as adults with doses up to 135,000 r which produced 100 per cent mortality in 4 days. A dose of 5,000 r caused no increased mortality in either sex during a 7-week test period.

7. The LD-50 dose for pupae treated 2 days prior to emergence was found to be 12,000 r. No significant difference was found in emergence of pupae treated with 5,000 r as compared to controls.

8. The lethal effect of radiation was tested on larvae and eggs. Last instar larvae were very susceptible to a dose of 3,500 r as measured by adult emergence but showed resistance to doses of 2,500 r and lower. Eggs which were 1 to 3 hours old had an LD-50 of 126 r. After 47 to 49 hours of development, the eggs had an LD-50 of 20,000 r.

9. A cytological study of ovarioles was conducted to determine the state of development in untreated insects at 2, 5, and 8 days when compared to those irradiated with various doses as pupae. The effects of radiation on ovarian growth were the loss of ovariole differentiation at 3,500 r and 4,500 r and a loss of nurse cell organization in the developing oöcytes at lower doses.

10. The chromosomes of treated and untreated insects were studied and abnormalities were observed in those treated with the sterilizing dose. The 2N chromosome number is 8.

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BIOGRAPHICAL SKETCH

Hollis Mitchell Flint was born May 28, 1938, at Miami, Florida. He was graduated from Mainland High School in Daytona, Florida, in 1956. In June, 1960, he received the degree of Bachelor of Science from Stetson University, DeLand, Florida. In 1960 he enrolled in the Graduate School of the University of Florida. He held a National Defense Education Act fellowship from 1961 to 1964 in the Department of Entomology. From 1960 until the present time he has pursued his work toward the degree of Doctor of Philosophy.

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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.

June 18, 1964.

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