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EFFECT OF MATING SEQUENCE ON EGG-HATCH FROM FEMALE *AEDES AEGYPTI* (L.) MATED WITH IRRADIATED AND NORMAL MALES¹

J. A. GEORGE

Canada Department of Agriculture, Vineland Station, Ontario, Canada.

INTRODUCTION. It has been suggested that the release of sterile males would be an effective means of controlling insect populations even when the target species is polygamous (Knipling, 1955, von Borstel, 1960) or, more accurately, polyandrous, for if the spermatozoa from treated males are equally competitive with those from normal males, the proportion of sterile to fertile eggs obtained should depend exactly on the proportion of sterile to fertile males competing for the females.

Both sexes of the yellow fever mosquito, *Aedes aegypti* (L.), mate repeatedly. A single female placed with 11 males copulated 50 times in one hour, and a single male caged with 16 virgin females copulated 30 times in 30 minutes (Roth, 1948). Radiosterilized males, caged with normal males and females in the ratio of 20:1:1, reduced the egg-hatch of the females from the normal 76 percent to 1.5 percent (McCray *et al.*, 1961). But field releases

of irradiated males failed to effect any reduction in the population (Morlan *et al.*, 1962). Males sterilized by chemosterilants have since been found to be more effective than radiosterilized males in reducing the egg-hatch of virgin females placed with treated and normal males (Weidhaas and Schmidt, 1963).

Reported here are the results of an investigation of the relative competitiveness of spermatozoa from normal males and those from males sterilized by gamma-irradiation in the pupal stage.

MATERIALS AND METHOD. The Penang strain of *A. aegypti* was employed; it was obtained from Penang, Malaya and had been cultured for 7 years at the University of Western Ontario, London, Ontario. Larvae were fed on a 5:2:1 by weight mixture of brewer's yeast powder, blood albumin and ribonucleic acid. Unmated adults were obtained by separating the sexes by pupal size and taking the emergents within 12 hours of eclosion.

A group of 300 male pupae due to emerge within approximately 8 hours was divided into two equal lots, one of which was exposed to a dose of 8400 R. of

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gamma radiation and the other remained untreated as control males. Each lot of pupae was placed in an 18 x 12 x 12-inch screened cage for emergence. The adult males were ready for the experiments 1 day after all had emerged.

In Experiment 1, 50 one-day-old virgin females were placed in each of the 2 cages, irradiated and control, of 150 one-day-old unmated males (Fig. 1, upper). After 24 hours the two groups of females were interchanged from one cage to the other

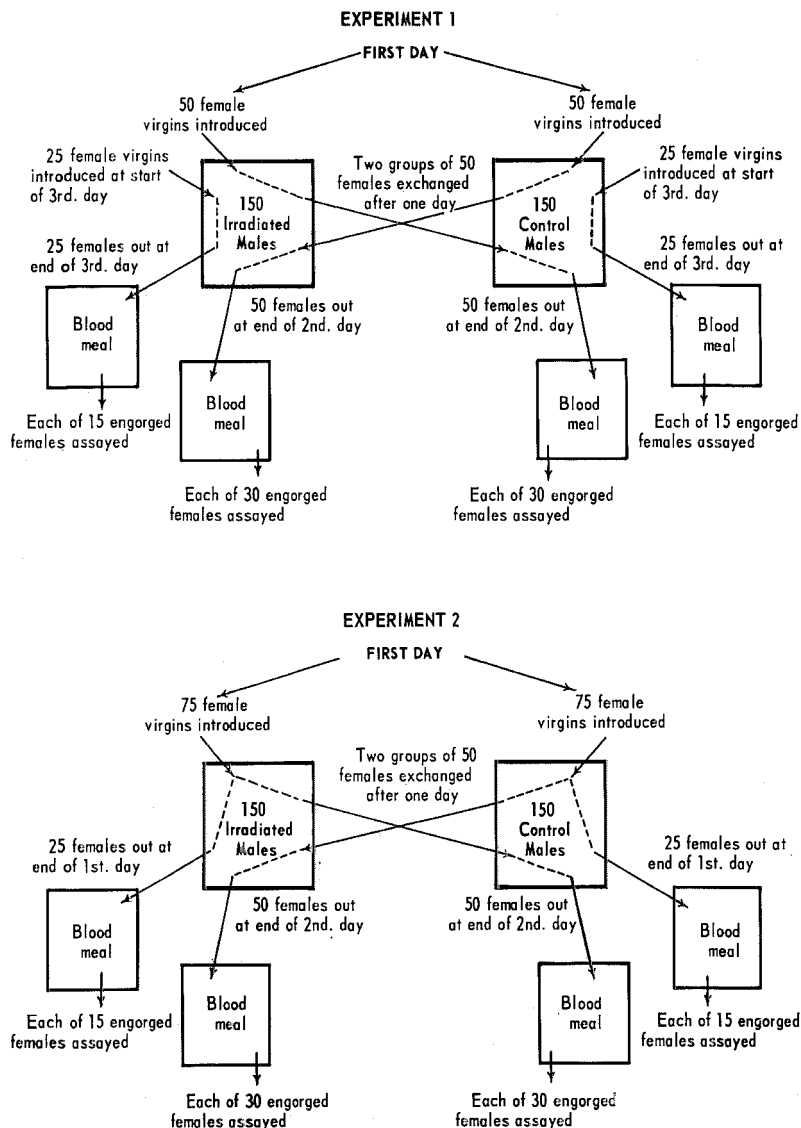


FIG. 1.—Experimental procedure employed to test the effect of mating sequence on egg-hatch from females mated with irradiated and normal males.

for a second 24-hour exposure period. Each group of females was then removed and placed in its own holding cage and fed on a guinea pig. After engorgement, 30 females from each group were placed in individual jars for egg laying and assessment of the number and percentage hatch of their eggs. Meanwhile, the 2 cages of males (irradiated and control) were now each supplied with a new set of 25 virgin females; the latter females were left with males for 24 hours only and then removed to their own holding cages and given a blood meal. Then 15 of each of the two new sets of females were placed in individual jars for assay of their oviposition and egg-hatch.

In Experiment 2 the procedure was the same except that sets of 25 females, comparable to those introduced on the third day in Experiment 1, were instead introduced along with the groups of 50 females on the first day, making 75 females per cage (Fig. 1, lower).

RESULTS. Females placed with only irradiated males produced as many eggs as those placed with only untreated males but very few of their eggs hatched (Table 1); only seven of the total of 30 such females produced larvae, and the maxi-

mum number of larvae per female was seven.

Females exposed first to untreated males and then to irradiated males laid normal numbers of eggs and most of them hatched normally; females with the converse exposure sequence laid normal numbers of eggs but most of these did not hatch. In both situations the eggs from a few females differed from the majority. Of those 60 females exposed first to untreated males, seven produced eggs of which less than 8 percent hatched, and of those 60 exposed first to irradiated males the eggs of ten had a hatch rate of at least 84 percent and of another 50 percent. Also, a small number of females in each of the four exposure groups did not oviposit.

With all four groups of females, however, the hatch of individual broods of eggs was either normally high (above 83 percent) or abnormally low (below 8 percent). There were two exceptions. One female that produced eggs of which 41 percent hatched was only exposed to untreated males while the second female produced eggs of which 50 percent hatched, as already mentioned, was exposed first to irradiated and second to

TABLE 1.—Fecundity of females exposed to irradiated or control males only, for 24 hours or in either sequence as indicated.

| | Expt. | Females mated with: | | | |
|---|-------|---------------------|-----------------------|--------------------------------|--------------------------------|
| | | Control males only | Irradiated males only | Control, then irradiated males | Irradiated, then control males |
| No. of females | 1 | 15 | 15 | 30 | 30 |
| | 2 | 15 | 15 | 30 | 30 |
| Percent of females that oviposited | 1 | 100 | 100 | 90 | 93 |
| | 2 | 80 | 60 | 47 | 53 |
| Mean eggs per female | 1 | 77 | 87 | 76 | 83 |
| | 2 | 49 | 47 | 39 | 48 |
| Percent egg-batches with over 83% hatch | 1 | 80 | 0 | 78 | 21 |
| | 2 | 83 | 0 | 93 | 25 |
| Percent egg-batches with under 8% hatch | 1 | 13 | 100 | 22 | 75 |
| | 2 | 17 | 100 | 7 | 75 |
| Mean percent of eggs that hatched | 1 | 79 | 1 | 75 | 26 |
| | 2 | 93 | 1 | 91 | 21 |

untreated males. Since in both instances of intermediate hatch untreated males were involved, it is probable that their sperm had been depleted by previous mating.

During these experiments there was no indication that males from irradiated pupae suffered a reduction in mating vigor as compared to normal males; indeed, equally virgorous mating activity was observed in both cages containing either type of male.

DISCUSSION. In the two groups of females placed with irradiated and normal males, in either sequence, the hatch of eggs from a few females differed widely from the majority. Moreover, not all of the females exposed only to untreated males produced larvae, while some did not lay eggs. Therefore a small proportion of females was apparently not inseminated during the 24-hour period allowed for mating; such females would still be unmated when subsequently placed with irradiated males and, after mating with the irradiated males, their eggs would be expected to differ from the majority by showing a negligible egg-hatch of less than 8 percent. Conversely, a few of the females exposed first to irradiated males and then to normal males would have their first mating with the normal males and thus would produce eggs with a normal rate of hatch. Thus the average egg-hatch of the females exposed to irradiated males was raised from about 1 percent to around 24 percent by subsequent exposure to normal males. The converse effect, however, was only slight. That is, the average egg-hatch of females exposed to normal males was reduced only slightly by subsequent exposure to irradiated males. Some females placed with only normal males, laid only inviable eggs, so the hatch of eggs from similar females could not be reduced any further by insemination by irradiated males.

The suggestion of Knippling (1955) and von Borstel (1960) that the "sterile male technique" could also be effective against

polygamous insects involved the assumption that spermatozoa from different matings have an equal opportunity to fertilize ova. The experiments reported here, however, reveal that with *A. aegypti* the spermatozoa acquired by the female at the first mating have a great advantage over spermatozoa acquired subsequently. Thus when virgin females were exposed for 24 hours to gamma-irradiated males and then for 24 hours to normal males the egg-hatch was very low, whereas when the order of mating was reversed egg-hatch was entirely normal. This "all-or-none" result is the same as that obtained by Weidhaas and Schmidt (1963) from mating competition trials with *A. aegypti* in which females were exposed to a mixture of normal and irradiated males. They concluded that "successful multiple matings by sterile and normal males might be expected to result in many egg batches giving intermediate hatches (1-59 percent). However, such a result was not observed; the majority of egg batches hatched normally or not at all." Thus it appears that though copulation may occur repeatedly, the only effective one is the first. However, it is possible that some of the sperm from subsequent matings may be eventually utilized when the seminal fluid from the first mating has been absorbed and the mating-plug has dissipated.

In these experiments irradiated males appeared to mate as effectively as normal males. Thus the greater mating competitiveness of chemosterilized males as compared to radiosterilized males reported by Weidhaas and Schmidt (1963) may indicate a greater ability of the chemosterilized males to be the first to mate with virgin females, but does not necessarily imply that the radiosterilized males mate any less frequently over their reproductive period or that the competitiveness of their sperm is reduced.

The results reported here emphasize the importance of timing and placement when sterile males are released. The overall

ratio of sterile to fertile eggs produced will approximate the ratio of sterile to fertile males if both types have equal opportunity for being the first to contact virgin females. As the males in the field emerge in the proximity of wild females they are likely to have an advantage over released males. Though these results show that once females of *A. aegypti* have mated with normal males their egg-hatch cannot be reduced by subsequent mating with irradiated males, it is also fortunately true that the egg-hatch from females recently mated with irradiated males is not increased by subsequent mating with normal males.

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THE PESTICIDE PROGRAM OF THE U. S. PUBLIC HEALTH SERVICE, ATLANTA, GEORGIA announces a five day course entitled "Pesticides and Public Health" to take place May 15 through 19 at the National Communicable Disease Center, Atlanta, Georgia.

This course is specifically designed to present an overall view of the health aspects of pesticides. The course will consist of lectures and specialized topics that relate to problems encountered with the use of pesticides. The course is tailored for personnel employed by federal agencies, local and state health departments, arthropod control districts, pest control operators, conservation groups, biologists, ecologists, and other individuals whose employment includes the responsibility for performing or supervising pest control activities.

There is no tuition for this course. However, attendees will be expected to pay their own rooming and boarding expenses. For further information, please contact Chief, Pesticide Program, Public Health Service, Atlanta, Georgia 30333.