TESTS FOR MULTIPLE INSEMINATION OF FEMALES INVOLVING IRRADIATED AND UNIRRADIATED MALE *CULEX TARSALIS* (DIPTERA: CULICIDAE)[1]

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ABSTRACT. Multiple inseminations occurred among *Culex tarsalis* females simultaneously exposed to irradiated males and to unirradiated males that carried the black-eye genetic marker. Two of 38 egg rafts deposited by females simultaneously exposed to the irradiated males and unirradiated males produced individuals with normal-eyes and black-eyes indicating multiple insemination. No mixed hatch egg rafts were obtained when females were exposed 1st to irradiated males and after 3 days to unirradiated males.

Repeated copulations are observed in laboratory colonies of mosquitoes, but insemination usually occurs only once and females subsequently are refractory to insemination (Craig 1967). Multiple inseminations occur with limited frequency in *Aedes aegypti* (Spielman et al. 1967), *Anopheles gambiae* (Goma 1963) and irradiated *Culex pipiens fatigans* (de Alwis and Munasinghe 1974). Gwadz et al. (1971) found multiple inseminations in *Ae. aegypti* when several males copulated with a female in a short time. Multiple inseminations have not been reported for *Cx. tarsalis* (Asman 1975a), unirradiated *Cx. p. fatigans* or *Cx. pipiens molestus* (Kitzmiller and Laven 1958). The sterile-male-technique has possible application in the control of *Cx. tarsalis* (Darrow 1968, Ainsley et al. 1980). Zalom et al. (1981) demonstrated in small cage tests that irradiated male *Cx. tarsalis* fully inseminated fewer females than did unirradiated males. George (1967) observed 1 instance when a female *Ae. aegypti* exposed 1st to irradiated males and then to unirradiated males produced eggs of which 50% hatched as opposed to 100% sterility in eggs from females exposed to irradiated males alone. Multiple mating first by a male whose sperm had been depleted by previous mating and then by a normal male could explain the latter result. The objective of the present study was to determine if multiple insemination

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would occur if females were exposed to both irradiated and unirradiated males.

METHODS

The *Cx. tarsalis* used in this study were Knight's Landing (KL), a laboratory colony originally from Yolo Co., CA, with normal-eyes, and the black-eye mutant (*ble*), a colony developed by Asman (1975b) with black-eye acting as a recessive to normal-eye.

KL males were separated from the females within 24 hr of emergence and exposed to 5.5 kiloroentgens (kR) of irradiation at 0.15 kR/min from a cobalt 60 source, a treatment which had been shown to induce at least 95% sterility in *Cx. tarsalis* males while maintaining optimum mating competitiveness (Ainsley et al. 1980). *Ble* males and females remained unirradiated, but were also separated within 24 hr of emergence.

Each mating study was performed in cylindrical cartons 36.0 cm high and 16.5 cm in diameter that held a water-filled oviposition cup and a sucrose cube. Cage 1 contained 50 irradiated males (KL), 50 unirradiated males (*ble*) and 50 virgin females (*ble*). Cage 2 initially contained 50 irradiated males (KL) and 50 virgin females (*ble*). The males in cage 2 were removed after 3 days and replaced with 50 unirradiated males (*ble*). Cage 3 contained 25 irradiated males (KL) and 25 virgin females (*ble*). Cage 4 contained 25 unirradiated males (*ble*) and 25 virgin females (*ble*). All males and females were of the same age.

A mouse was provided as a blood source each day and females that had fed were isolated individually in 10 dram vials with water for oviposition. After 5 days the egg rafts were removed and scored for hatch rate and embryonation rate (McDonald et al. 1979). The larvae from each raft were reared separately. The eye color of each mosquito was determined within 48 hr of emergence as an adult. Normal eyes in the progeny should have indicated mating with an irradiated (KL) male. Black-eyes should have indicated mating with an unirradiated (*ble*) male.

RESULTS

CAGE 1. Female mosquitoes in cage 1 produced 45 egg rafts of which 2 were unembryonated, 25 had hatch rates less than 50% (low hatch), and 18 had hatch rates greater than 50% (high hatch). Some larvae from all high hatch and from 20 low hatch rafts were reared to adults. Of the low hatch egg rafts, 15 had normal eyes in all individuals reared, 3 had black eyes in all individuals reared and 2 had individuals with normal eyes and individuals with black-eyes potentially indicating multiple insemination. The 1st mixed hatch egg raft contained 200 eggs of which 8 hatched and an additional 19 were embryonated. Four adults were obtained, 2 with normal and 2 with black-eyes. The 2nd mixed hatch raft contained 160 eggs of which 3 hatched and an additional 8 were embryonated. All 3 larvae were reared to adults, 1 had normal and 2 had black-eyes.

All of the high hatch rafts contained only black-eyed individuals.

CAGE 2. Females in cage 2 produced 19 egg rafts of which 5 were unembryonated, 10 had hatch rates less than 50%, and 4 had hatch rates greater than 50%. Some larvae from all high hatch and 8 low hatch rafts were reared to adults. All 8 low hatch egg rafts contained only normal-eyed individuals. All 4 high hatch egg rafts contained only black-eyed individuals.

CAGE 3. Female mosquitoes in the irradiated male control cage produced 14 egg rafts of which 3 were unembryonated and 11 had hatch rates less than 50%. The mean (±SD) hatch rate of embryonated rafts from matings with irradiated fathers was 2.2±2.3%. The mean (±SD) embryonation rate was 4.7±2.8%. All egg rafts contained only normal-eyed individuals.

CAGE 4. Female mosquitoes in the unirradiated male control cage deposited 20 egg rafts all of which exceeded 90% hatch. The mean (±SD) hatch rate of egg rafts from matings with unirradiated fathers was 96.2±2.5%. The mean (±SD)

embryonation rate was 97.1±2.2%. All egg rafts contained only black-eyed individuals.

DISCUSSION

In 2 of 38 cases, *Cx. tarsalis* black-eyed females simultaneously exposed to irradiated KL males and unirradiated black-eyed males deposited egg rafts containing both normal-eyed and black-eyed individuals. Both examples were from low hatch egg rafts, with none occurring among the high hatch egg rafts. No mixed hatch rafts occurred when females were exposed to a group of unirradiated black-eyed males after 3 days of exposure to a group of irradiated KL males. This could be explained by the production of an ancessory gland substance preventing subsequent matings as described by Craig (1967). The complete effect of the substance is manifested only after several hours, during which multiple inseminations could occur. The mixed hatch egg rafts in our study might have been the result of a female mating 1st with an irradiated male and then by an unirradiated male within a limited period of time. Multiple insemination involving males of the same strain would be undetectable, and could have occurred in any of the cages.

The multiple inseminations found in this study occurred under laboratory cage conditions. Gwadz and Craig (1970) logically questioned the application of such results to field conditions where the chances of a female encountering an irradiated male whose sperm supply is depleted and then a normal male within a short time is probably small.

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