# FEMALE POLYGAMY DUE TO INADEQUATE SEMEN TRANSFER IN *AEDES AEGYPTI* [1, 2]

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ABSTRACT. 1. Females of *A. aegypti* are essentially monogamous. However, under certain special conditions, they may accept and utilize sperm from more than one male.

2. Monogamy initially results from the filling of the bursa copulatrix of the female with sperm and seminal fluid. After a few hours this monogamy is made permanent through the action of male accessory gland substance on the female system.

3. Incomplete insemination can result from copulation with a partially depleted male, or from interruption of copulation. The end result would be a female having sperm from one male yet remaining receptive to a second insemination.

## INTRODUCTION

Females of the yellow fever mosquito, *Aedes aegypti* (L.), are monogamous. Craig (1967) and Spielman *et al.*, (1967) demonstrated that although copulation may take place many times, insemination occurs only once. Moreover, an inseminated female is refractory to subsequent insemination for life. In addition, these authors showed that monogamy results from the transfer of male accessory gland material as a component of the seminal fluid during the act of copulation.

The active material from male glands has been extracted (Craig, 1967), designated "matrone" (Fuchs *et al.*, 1968), and has been shown to be protein in nature (Fuchs *et al.*, 1969).

Although the monogamous nature of females of *A. aegypti* is well established, a number of authors have demonstrated, with genetic evidence, that multiple inseminations can occur (VandeHey and Craig, 1958; Adhami, 1964; Hickey, 1965; Spielman *et al.*, 1967).

This study was undertaken to demonstrate that females of *A. aegypti* are essentially monogamous and to describe some conditions under which multiple inseminations might occur.

## MATERIALS AND METHODS

Unless otherwise specified, all experiments were conducted with the ROCK strain of *A. aegypti*. ROCK is a relatively large, uniform, vigorous strain used in a number of laboratories and may be considered as characteristic of the type form of *A. aegypti aegypti*. This strain was originally obtained from D. W. Jenkins in 1959 and is the most commonly used strain for genetic and physiological research at our laboratory.

For experiments requiring genetic markers, the following mutant strains were utilized: MISS-MARK, homozygous for the dominant character, Silver mesonotum; COLORLESS, homozygous for three recessive genes, *re, ru,* and *ol,* which result in pupal eyes without color; BULBOUS, homozygous for the recessive character expressed as an inflated or bulbous apical antennal segment; and COLORLESS-BULBOUS, homozygous for both characters.

All rearing was conducted in accordance with the methods of Craig and VandeHey (1962). Sexes were separated by size as pupae, and sex separation was rechecked after emergence. Adults were fed on apple slices. All rearing and experimen-

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tation was performed at 27±1° C and 80±5 percent relative humidity. Day length was 16 hours.

Two methods were used to determine insemination. In one group of experiments, virgin females, 4 to 6 days old, were exposed to males, dissected in saline and examined for evidence of insemination. The degree of distention of the bursa copulatrix and the contents of the spermathecae were determined with a phase-contrast microscope at 100x. In the second group of experiments, virgin females were exposed to males of various marker strains either simultaneously or in sequence, and the progeny of these pairings were reared and examined for evidence of multiple insemination.

## RESULTS

TEST FOR MONOGAMY. Single virgin females were exposed to 5 males of 1 genotype for 24 hours and then placed with males of a second genotype for the remainder of life. All progeny from these experiments were fathered only by males of the first genotype. Table 1 presents data for the four possible exposure sequences using wild-type males and males with the genetic marker, Silver mesonotum (*Si*). Of 84 females tested, all were inseminated only by the first males to which they were exposed; progeny were either all wild-type or all Silver heterozygotes. Even though fresh males of the second type were provided weekly for the life of the female, there was no evidence of a successful second insemination.

A second experiment involved use of a different marker system. Females with colorless eyes were exposed to wild-type males for 24 hours and then to colorless males for several weeks. The reciprocal test, colorless females with colorless males followed by wild-type males, was also performed. There were 124 females tested; they produced 18,438 offspring. All offspring were fathered by the first male to which the female was exposed.

TEST FOR MULTIPLE INSEMINATION. Single virgin females were exposed to groups of six males, two each of three different marker strains (Table 2). There were 106 females tested; in each case, progeny

TABLE 2.—Results of crosses when one virgin female was simultaneously exposed to six males, two each of three different marker strains. Male success in insemination is indicated by incidence of markers in progeny.

| Inseminating male as determined by progeny | Females inseminated * | |
|---|---|---|
| | No. | Percent |
| Mutant I (COLORLESS) | 43 | 40.6 |
| Mutant II (SILVER) | 33 | 31.1 |
| Mutant III (BULBOUS) | 22 | 20.8 |
| Mixture (I & II–2 II & III–3 I & III–3) | 8 | 7.5 |

* 106 females tested, 11,380 progeny scored. All females tested were from the COLORLESS-BULBOUS strain.

TABLE 1.—Results of crosses with the marker strain, Silver mesonotum (*Si*), and wild-type (+) to determine duration of female monogamy. The *Si* marker can be differentiated in homozygous and heterozygous conditions.

| Parental phenotype | | | | | |
|---|---|---|---|---|---|
| Female | Male | | No. females tested * | No. progeny | |
| | #1 | #2 | | Total | Fathered by second male |
| + | + | Si | 20 | 3199 | 0 |
| + | Si | + | 19 | 2200 | 0 |
| Si | + | Si | 24 | 2058 | 0 |
| Si | Si | + | 21 | 2791 | 0 |
| | | | 84 | 10248 | 0 |

* Single virgin female placed with 5 males of the first type for 24 hours, then with 5 males of the second type for the remainder of her life (fresh males of the second type added every week).

were reared to determine which male (or males) had inseminated the female. Over 92 percent of the females were monogamous, accepting sperm from only one male. However, 8 of 106 females (7.5 percent) produced offspring of mixed phenotype. This mixture indicated that these females were inseminated by more than one male.

QUANTITY OF SEMEN TRANSFERRED. If a freshly inseminated female is dissected and her bursa and spermathecae are examined in saline, the presence of sperm in these organs and the extent of their filling with sperm and seminal material is readily observable. Table 3 illustrates the quantity of semen transferred to virgin females exposed to males in different mating schedules. When 10 virgin females, 4 to 6 days old were individually exposed to males and allowed five copulations, all were inseminated; bursae were fully distended with seminal material and two of the three spermathecae were filled with sperm (the third spermatheca is rarely filled under any conditions). Similar results were obtained when a single female was allowed one copulation with a virgin male. Ten females were tested; all were fully inseminated.

The third line of Table 3 reports an experiment in which a single virgin male was exposed to 10 virgin females in sequence and was permitted one copulation with each female. In this case, not all of the females were inseminated. Furthermore, not all of those females received a full complement of sperm. Of the 10 males tested, 8 were able to fully inseminate 4 females. By copulation number 5 of each sequence, the male seemed to be running out of seminal material. All of the females from copulation number 5 showed only partial insemination. Indeed, 4 of 10 females showed only a few sperm in their spermathecae and a collapsed bursa. In copulations number 6 through 10, the females showed either a trace of sperm or no evidence of insemination.

In the fourth line of Table 3, a single virgin male was again allowed to copulate once with 10 virgin females in sequence. However, each female from these exposures was then allowed one copulation with a fresh male. In this case, all females were found to be fully inseminated or nearly so. Thus, it would appear that females receiving a reduced quantity of semen from the first copulation were subsequently filled by the second copulation.

INCOMPLETE FILLING LEADING TO MULTIPLE INSEMINATION. In order to prove that a second insemination sometimes supplements a reduced initial insemination, experiments with genetic markers were employed. Table 4 illustrates the results obtained from exposure of 10 females in sequence to a single wild-type male followed by exposure to a genetically marked male (Silver mesonotum). In general, the first three females of each series produced progeny fathered only by the first male. These females undoubtedly received a full complement of semen with the first copulation. However, the remainder of the females in each series showed progeny of various types. Some families were fathered by the first male, some by the second male and some were fathered by both males.

From the data in Table 4, one may calculate the insemination capabilities of males of the ROCK strain. On the average, a single male was capable of fully inseminating 3.7 females and partially inseminating an additional 2.6 females. These data are supported by results from line 3 of Table 3; here, single males fully inseminated an average of 3.7 females and partially inseminated an additional 2.8 females.

## DISCUSSION

As demonstrated by Craig (1967) and Spielman *et al.*, (1967), females of *A. aegypti* are refractory to a second insemination. Females may copulate repeatedly, but they are inseminated only once. This feature of the reproductive biology of *A. aegypti* is in agreement with the findings

TABLE 3.—Quantity of semen transferred to 10 virgin females exposed to males in different mating schedules.

| Treatment for each ♀ | No. ♂♂ tested | Quantity of semen transferred to each ♀ * | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Female no. | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Exposed to 10 ♂♂, allowed 5 copulations | .. | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Exposed to 1 ♂, allowed 1 copulation | 10 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Exposed to same ♂ in sequence, 1 copulation per ♀ | 10 | ++(10) | ++(10) | ++(9)<br>+(1) | ++(8)<br>+(2) | +(6)<br>±(4) | o(4)<br>±(6) | o(5)<br>±(5) | o(8)<br>±(2) | o(8)<br>±(2) | o(10) |
| Exposed to same ♂ in sequence, 1 copulation per ♀, followed by exposure to a second ♂, 1 copulation per ♀ | 10 | ++ | ++ | ++ | ++(7)<br>+(3) | +(1)<br>++(9) | ++ | ++ | ++ | ++ | ++ |

* ++ = full bursa and spermathecae.
+ = incomplete filling of bursa, full spermathecae.
± = empty bursa, few sperm in spermathecae.
o = no trace of sperm in bursa or spermathecae.

TABLE 4.—Results of crosses involving a single wild-type (+) male exposed to 10 females in sequence, allowing one copulation per female. Each female was then exposed to a fresh male of the marker strain; the second copulation took place within 30 seconds of the first.

| No. of male tested | Type of insemination in single females sequentially exposed to test male, followed by a marker male * | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Female no. | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | + | + | + | + | + | S | S | S | +/S | +/S |
| 2 | + | + | + | + | +/S | +/S | +/S | S | S | S |
| 3 | + | + | + | + | +/S | +/S | S | S | S | S |
| 4 | + | + | + | + | S | S | S | S | +/S | +/S |
| 5 | + | + | + | +/S | +/S | + | +/S | +/S | S | S |
| 6 | + | + | + | +/S | + | +/S | +/S | +/S | S | S |
| 7 | + | + | + | +/S | +/S | S | S | S | S | S |
| 8 | + | + | + | +/S | +/S | +/S | S | S | S | S |
| 9 | + | + | + | S | +/S | +/S | S | S | S | S |
| 10 | + | + | S | + | +/S | +/S | S | S | S | S |

* + = insemination by wild-type male, ROCK strain.
S = insemination by Silver mesonotum male, MISS MARK strain.
+/S = insemination by males of both types.

of Kitzmiller and Laven (1958) with *Culex pipiens* and Goma (1963) with *Anopheles gambiae*. They used genetic markers to show that these species are inseminated only once; however, these authors did not rule out the possibility of multiple inseminations under special circumstances.

The earlier reports of multiple inseminations in *A. aegypti* (VandeHey and Craig, 1958; Adhami, 1964; Hickey, 1965; Spielman *et al.*, 1967), were based on experiments utilizing marker strains. All of these reports noted mixed progeny, indicative of multiple insemination, in fewer than 20 percent of their crossing experiments. VandeHey and Craig, Adhami, and Hickey all conducted their experiments as tests of mate choice or competition, and exposed individual virgin females to males of various marker strains simultaneously. On the other hand, Spielman and his co-workers allowed a 5-hour delay between exposures to males of the first and second types.

As indicated in Table 2, multiple inseminations occurred at a low frequency when females were exposed simultaneously to males of several genotypes. However, when females were held for 24 hours with males of the first type before the second exposure, the progeny were all fathered by males of the first type. There was no evidence of multiple insemination (Table 1).

Craig (1967), on the basis of experiments involving the implantation of male accessory glands into female *A. aegypti*, suggested that several hours might be required before the monogamizing effect of the gland material was accomplished. Some females remained receptive to insemination from 4 to 6 hours after gland implant. However, if these females were held for 24 hours after gland implant, they were completely refractory to insemination. Utilizing genetic marker strains, Spielman *et al.* (1967) similarly concluded that multiple insemination was occasionally effective when less than 5 hours separated the first and second matings.

As indicated in Table 3, a male is generally capable of fully inseminating only three or four females. After these initial inseminations the amount of sperm and seminal fluid transferred during copulation is markedly reduced. Spielman *et al.*, (1967) showed that the mechanical interruption of the act of copulation can also result in a reduction in the amount of seminal fluid transferred. Females inter-

rupted in the act of copulation showed a reduction in the quantity of semen in the bursa.

Observations of cages containing several males and a single female indicate that copulations are often interrupted by superfluous males drawn to the copulating pair in flight. In cages containing a number of males of different marker strains, interrupted copulations certainly could occur. This would result in a female being only partially filled with the sperm of one male and still receptive to a second insemination.

A single uninterrupted copulation of a virgin female with a virgin male normally results in a full insemination as indicated by a fully distended bursa and full spermathecae (Table 3). In addition, a full insemination renders a female refractory to a second insemination within 30 seconds of the completion of the first (Table 4). However, incomplete inseminations do not render females refractory. In Table 4, most of those females that were expected to receive reduced amounts of seminal fluid from the first male did indeed show multiple insemination.

As indicated by Craig (1967), male accessory gland material may not act on the female for some time after introduction, yet females are refractory almost immediately after a full insemination. It is possible that this rapid onset of refractory behavior results from the fully distended bursa acting via stretch receptors on the nervous control of sexual receptivity. The initiation of refractory behavior is apparently not a result of the act of copulation itself. Partially inseminated females remain receptive even though the act of copulation was normal and some seminal material was transferred.

Although multiple inseminations may take place under laboratory cage conditions, it is questionable whether they have any significance in the field. The frequency with which a single female may copulate in the field is unknown, but the chances of encountering depleted males or of being interrupted during copulation are probably small. This point might be of some concern to those involved in the use of genetic methods for mosquito control. Experiments to determine the frequency of multiple insemination in field populations are needed. However, when discussing the field biology of *A. aegypti* it is probably safe to consider the species as monogamous.

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