SEXUAL RECEPTIVITY IN FEMALE *AEDES AEGYPTI*[1]

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INTRODUCTION

Various schemes involving the release of males have been suggested for the control of *Aedes aegypti* (L.). Control might be achieved through males sterilized with radiation (Morlan *et al.*, 1962), by males carrying a sex-ratio distorter (Hickey and Craig, 1966) or by a heritable chromosome translocation (Asman and Rai, 1966). In laboratory tests these methods have proved effective in controlling caged populations. To date, only the sterile male technique has been tested in the field. Morlan *et al.* (1962) released nearly 5 million radiation-sterilized males of *A. aegypti* in Pensacola, Florida; however, no significant depression of the field population was observed.

Several alternative explanations for this lack of success in the field have been suggested (Craig, 1967a). As one possibility, females may be inseminated at the breeding site soon after emergence. It has recently been established that females of *A. aegypti* are monogamous (Craig, 1967b; Spielman *et al.*, 1967). Hence, early insemination would reduce the opportunity for insemination by introduced males. Therefore, a knowledge of the age of the female at time of insemination becomes important in order to evaluate the prospects for released-male programs.

The age of the female *A. aegypti* at first copulation has been considered by a number of workers. MacGregor (1915) states that copulation takes place as soon as the mosquitoes are able to fly. Roth (1948) notes that most females are mated within 105 to 145 minutes after emergence. It has generally been assumed that copulation by newly emerged females results in insemination. Indeed, many mosquito workers have assumed that the terms "mating," "copulation" and "insemination" are synonymous.

Gwadz (1967) and simultaneously Lea (1967) reported that very young females are refractory to insemination. The purpose of this study is to further amplify this observation and to describe some behavioral responses of the female to mating attempts by the male.

MATERIALS AND METHODS

Data on the laboratory strains of *Aedes* species used in the study are as follows:

ROCK strain, from D. W. Jenkins, Fort Detrick, Md.; received 1959. (Laboratory rearing at least 20 years at Rockefeller Virus Foundation, N. Y.). KUALA strain, from John Reid, Institute for Medical Research, Kuala Lumpur, Malaysia; received 1956. (Field collected from Penang Island, Malaysia. Laboratory rearing since 1956). VIETNAM strain, from D. Do-Van-Quy, Pasteur Institute, Saigon, VietNam; received 1966. (Field collected from homes near the Pasteur Institute. Colony from field eggs). BANGKOK strain, from J. Mouchet, Office of Scientific and Technical Research, Bondy, France; received 1966. (Field collected from Huay-Kwank, Bangkok, Thailand, 1965). CURAÇAO strain, from H. Nijhout, University of Notre Dame; received 1967. (Field collected from Curaçao, Netherlands Antilles. Colony from field eggs). MERIDIAN strain, from H. Bond, U. S. Public Health Service, Meridian, Mississippi; received 1967. (Field collected from Meridian, Mississippi. Colony from field eggs). *A. aegypti formosus* Walk., from J. Mouchet, Office of Scientific and Technical Research, Bondy, France; received 1966. (Field collected from Kumba, Cameroun, 1965. Colony from F_1 of field eggs). *A.*

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mascarensis MacGregor, from R. Mamet, Medical and Health Department, Mauritius; received 1965. (Field collected from Mauritius. Colony from field eggs). A *albopictus* (Skuse), from M. Bat-Miriam, Israeli Institute for Biological Research, Ness Ziona, Israel; received 1959. (Colony from material collected in India, site unknown, prior to 1957).

Unless 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*. Larval rearing, pupal separation, and adult handling methods were in accordance with those of Craig and VandeHey (1962). Unless otherwise indicated, all rearing and testing were performed at 27±1° C. and 80±5 percent relative humidity. Day length was 16 hours.

To determine precise ages, emerging females were collected at hourly intervals. All females used in this study were of known age, within ±30 minutes. Males were of the same strain as the females being tested and were of mixed ages from 2 to 10 days old.

The method for determining female age at insemination was similar for all strains tested. Groups of 10 to 15 females were placed in gallon cardboard ice cream containers covered with bolting cloth, each container with 30 to 40 males. Exposure time was 24 hours. Scoring for insemination was on the basis of presence or absence of sperm in the spermathecae.

In order to characterize the behavioral responses of female mosquitoes to mating attempts by males, observations were made on individual free-flying females of various ages in the presence of males. In addition, observations were made with a 20X stereoscope on females in tethered flight in the presence of free-flying males. Tethering consisted of gluing the mesonotum of a female to the head of a pin in the manner of Jones and Wheeler (1965). Flight was stimulated by directing a stream of air over the female.

A modification of the forced-mating technique of McDaniel and Horsfall (1957) was used to determine the responses of the female terminalia to stimuli from the terminalia of decapitated males. This technique was also used to determine the capacity of teneral females for insemination.

RESULTS

AGE AT INSEMINATION. Initial tests on the relation of age to receptivity showed that insemination does not take place as early as had been indicated in the literature. Figure 1 illustrates the results obtained when females of the ROCK strain were tested at 6-hour intervals from 24 to 72 hours post-emergence. At least 500 females were tested at each time interval. Table 1 lists the rate of insemination for

TABLE 1.—Effect of age on receptivity to insemination in six strains of *Aedes aegypti*, and in *Aedes aegypti formosus*, *Aedes mascarensis* and *Aedes albopictus*.

Strain	No. ♀♀ tested per age group	Percent of ♀♀ inseminated at hrs. post-emergence							
		24	30	36	42	48	54	60	72
A. aegypti									
ROCK	500	0	1	12	20	63	73	89	96
KUALA	120	0	..	..	..	55	..	..	89
BANGKOK	170	0	..	..	..	69	..	..	92
VIETNAM	145	0	..	..	..	64	..	..	95
CURAÇAO	140	0	..	..	..	17	..	..	95
MERIDIAN	115	0	..	..	..	19	..	..	94
A. aegypti formosus	140	0	..	..	..	5	..	..	78
A. mascarensis	105	0	..	..	..	76	..	..	94
A. albopictus	100	0	..	..	..	11	..	..	93

seven strains of *A. aegypti* as well as *A. mascarensis* and *A. albopictus*. With the exception of the ROCK strain, all were tested only at 24, 48 and 72 hours after emergence.

In every case, no insemination occurred in females at 24 hours. Even at 48 hours a significant portion of each group remained uninseminated. Forced-copulation was applied to females less than 24 hours old in order to determine whether they were structurally capable of being inseminated. Teneral females, less than 8 hours after emergence, were soft and pliable; however, 2 of 8 were inseminated following repeated attempts. At 10 hours, 9 of 20 females were inseminated. At 20 hours, 17 of 20 were inseminated using this technique. In all cases of forced-insemination, repeated and persistent attempts were required; however, the filling of the bursa and spermathecae appeared normal.

The effects of rearing at high and low temperatures are compared in Table 2. In order to enhance conditions for mating, females which had been reared at 20° C. were exposed to 27° C. for a 2-hour pe-

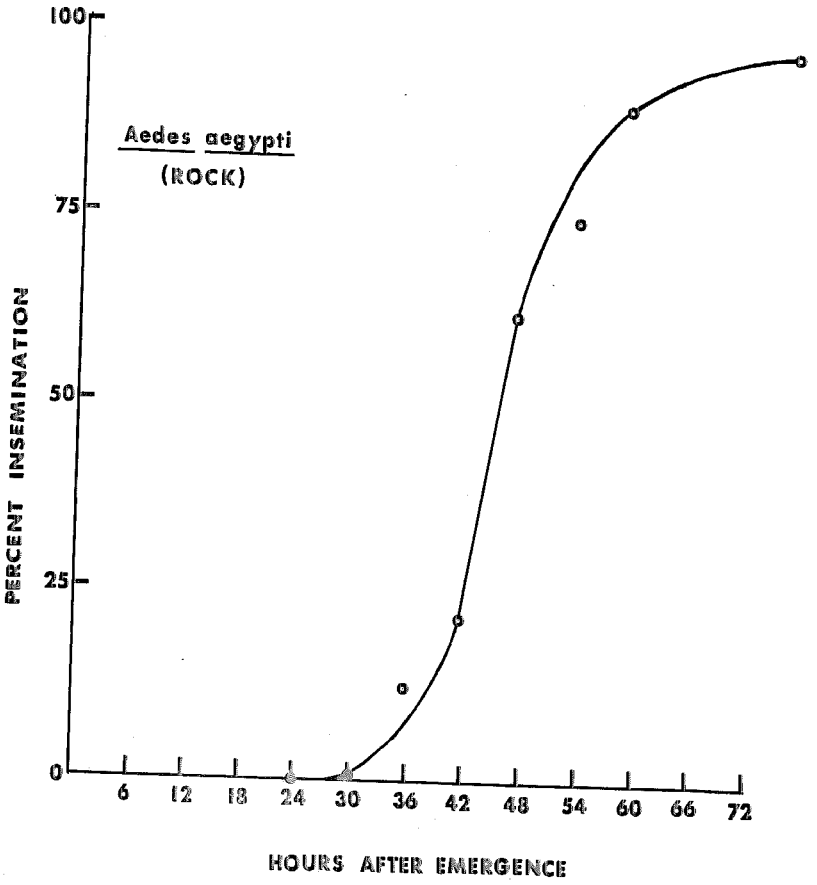

FIG. 1.—Onset of receptivity to insemination measured at 6 hour intervals. Each point represents a minimum of 500 females.

TABLE 2.—Effect of rearing temperature and larval crowding on the age of onset of receptivity to insemination in the ROCK strain of *Aedes aegypti*.

Larval rearing conditions		♀♀				
			Percent inseminated at hours post-emergence			
Temperature (° C.)	Crowded	No. tested per age group	24	48	72	96	144
30	no	100	11	90	..	..	..
27	no	500	0	63	96	..	..
20	no	100	..	0	0	24	41
27	yes	100	0	41	96	..	..

riod; females were then exposed for 3 hours to males which had been reared at 27° C. The onset of insemination was definitely delayed by rearing at 20° C. and was accelerated by rearing at 30° C.

Table 2 also gives data for females reared as larvae under crowded conditions. Crowding was achieved by rearing approximately 2000 larvae in a container having 500 cm^2 of water surface. Normal rearing conditions consisted of 200 larvae per container. Crowding produced significantly smaller adults but appeared to have little effect on the rate of insemination.

The results of tests to determine the duration of the receptive period are presented in Table 3. Virgin females were isolated and fed on apple slices until the desired time had elapsed. Exposure to males was for 24 hours. It would appear that a virgin female remains receptive for life.

FEMALE MATING RESPONSES. *Forced-copulation.* Experience with forced-copulation led to the observation that females of different ages showed a differential response to contact with male genitalia. Stimulation of a lightly etherized female with the terminalia of a decapitated male elicited either extrusion or retraction of the female eighth abdominal segment and cerci. The type of reaction depended on the receptive state of the female. Very young females showed an avoidance response to contact with male genitalia; older virgins were fully receptive. The avoidance response was also observed in inseminated females and virgin females injected with male accessory gland substance.

Receptive females responded to stimuli from the male genitalia by extruding the eighth segment and cerci. These receptive females were virgins over 72 hours old. This active extrusion of the female terminalia facilitated clasping, union and sperm transfer. Young virgin females (less than 24 hours post-emergence) responded to the male stimuli by retracting the eighth segment and cerci. This retraction prevented union of the terminalia and insemination. As previously noted,

TABLE 3.—Effect of aging on receptivity to insemination in females of the ROCK strain of *Aedes aegypti*.

Age in weeks post-emergence when ♀♀ first exposed to ♂♂	♀♀ surviving (initial no.=50)		♀♀ inseminated	
	No.	%	No.	%
2	47	94	44	94
4	40	80	38	95
6	32	64	28	88
8	24	48	16	67

insemination of young unreceptive females is possible, albeit difficult, generally requiring repeated attempts with a number of males. Inseminated females gave a response similar to that of unreceptive young virgin females. When subjected to the male stimuli, these females also withdrew the terminalia. Females implanted with male accessory glands or injected with male accessory gland substance responded much like inseminated females. As with the unreceptive virgins, repeated efforts at forced-copulation resulted in a few inseminations. When 16 gland-implanted females were subjected to forced-copulation, 3 were inseminated, but all required repeated attempts. Unsuccessful attempts often resulted in deposition of semen on the vaginal lips of the female, producing a sticky white thread when the male was withdrawn.

On the basis of the differential response to males, an attempt was made to determine the receptive state based on behavioral observations of females. Table 4 lists the results of three sets of experiments where the observer was presented with a mixed blind array of receptive and refractory females. The observer made the correct determination for 72, 77 and 78 percent of females for the three experiments. In all observations the probability that the number of correct determinations was a chance guess was less than .001.

Tethered flight. Females in tethered flight showed definite patterns of acceptance or rejection of mating attempts by free-flying males. Table 5 gives data for three types of females tested in tethered flight. Receptive virgin females did not attempt to push males away and were usually inseminated soon after being placed with males. Of 25 females tested, 23 were inseminated within 2 minutes. Young virgin females respond to mating attempts by kicking males away and by bending their terminalia dorsally and away from the male genitalia. When 25 females 20–24 hours post-emergence, were individually exposed to males, none were inseminated. In all cases at least 3 males were allowed to attempt copulation and in all cases the copulations appeared successful. Similar tests were conducted with females injected with male accessory gland substance and in all cases these females were refractory to insemination. These injected females responded like inseminated or young virgin females by rejecting most attempts. Even those at-

TABLE 4.—Determination of receptivity based on behavioral reaction of females of the ROCK strain of *Aedes aegypti* during forced copulation.

Females				Estimate of mating state by observer *		
Mating state	Age (hrs.)	Receptive to insemination	No. tested	No. correct determinations	Percent correct	χ^2
Virgin	20	No	31	21	72	11.26 ***
Virgin	72–120	Yes	29	22		
Inseminated	72–120	No	24	17	78	13.52 ***
Virgin	72–120	Yes	26	21		
Virgin injected **	72–120	No	22	15	77	13.30 ***
Virgin	72–120	Yes	25	21		

* Observer presented receptive and unreceptive females in a mixed blind array; observer estimated receptivity based on response of female terminalia to contact with male genitalia.

** Extract of male accessory glands injected into virgin female.

*** P less than .001.

tempts which appeared successful did not result in insemination.

Free-flight. Free-flying receptive virgin females are soon inseminated when exposed to males. A 15-minute exposure of 50 females (96 hours post-emergence) resulted in the insemination of 39 (78 percent). Table 5 lists the results of large numbers of three types of females exposed to males for 24 hours. Mature virgin females were inseminated. Young virgin females were not inseminated under cage conditions. Although over 2,000 females were exposed to males for the first 24 hours after emergence, none were inseminated. During this time, copulation attempts were frequent and both males and females were active. Mature females rendered refractory by accessory gland substance injection were not inseminated after a 24-hour exposure to males.

DISCUSSION

Males will attempt copulation frequently and persistently with females of all ages. However, only one copulation results in insemination. Insemination does not take place immediately after emergence, but is delayed for several days. This delay should allow time for female dispersal from the emergence site. Craig and Hickey (1966) have shown that field populations of *A. aegypti* are heterozygous for a large number of mutations. Sibling mating could have deleterious consequences. Dispersal, however, allows for outcrossing and maintains the heterozygous nature of the mutational load. The delay in insemination may have evolved as a mechanism to avoid inbreeding.

Differences in the time of onset of receptivity exist on both the strain and species level. It is interesting to note that ROCK, KUALA, VIETNAM, and BANGKOK are strains which have been in laboratory colonies for at least 2 years. During this time these strains have been subjected to the selective pressures inherent in laboratory colonization. On the other hand, CURAÇAO and MERIDIAN were both tested within one or two generations after field collection. These strains had not been subjected to laboratory selection and showed a significantly longer refractory period. The CURAÇAO and MERIDIAN strains were highly receptive once the refractory period was over, as indicated by the uniformly high rate of insemination at 72 hours.

Patterns of sexual receptivity have been described in the Diptera and a number of other insect groups. In the crab-hole mosquito, *Deinocerites cancer,* inseminated females are unreceptive to males and will kick away males attempting to copulate (Provost and Haeger, 1967). Females of *Drosophila melanogaster* are unreceptive to male courtship for at least 24 hours after emergence. During this refractory period females will ignore courting males

TABLE 5.—Receptivity to insemination in tethered and free-flying females of the ROCK strain of *Aedes aegypti.*

	Females			
Type of flight	Mating state	Age (hrs.)	No. tested	Percent inseminated
Tethered, held with ♂ ♂ for a minimum of 3 copulations	Virgin	20–24	25	0
	Virgin	72–120	25	92
	Virgin injected *	72–120	25	0
Free-flying, held with ♂ ♂ for 24 hours	Virgin	24	2,000	0
	Virgin	72	2,000	95
	Virgin injected *	72	2,000	0

* Extract of male accessory gland injected into virgin female.

and will actively struggle with males attempting to mount. Moreover, mated females are unreceptive for some time after insemination and respond to courtship by extruding their ovipositor. This extrusion normally discourages males from attempting to mount (Manning, 1967). Bateman (1948) has characterized the mating pair in *Drosophila* as the male being "indiscriminantly active" while the female is "discriminatingly passive." This description might well be applied to the mating behavior of *A. aegypti*. Jones and Wheeler (1965) state that the genital apparatus of the male "recognizes" the terminalia of an inseminated female. However, it would appear from the present work that the "recognition" is a function of the female.

Endocrine regulation of sexual receptivity has been described in the grasshopper, *Gomphocerus rufus* (Loher and Huber, 1966) and in *Drosophila melanogaster* (Manning, 1967). In both species, sexual receptivity appears to be regulated by the corpora allata. Lea (1968) has recently shown that sexual receptivity in *A. aegypti* is influenced by a hormone from the corpora allata. When the corpora allata were removed from newly-emerged females, these females remained refractory to insemination for at least 12 days. Implantation of allata into allatectomized females initiated receptivity within 72 hours.

The existence of a post-emergence refractory period has been demonstrated in the laboratory. These laboratory experiments have established minimum times for the onset of receptivity. In the field, the actual time of insemination will also depend on the time of encounter with males. It is this period between the onset of receptivity and encounter with field males that is critical for success in male release programs. Additional studies are needed on dispersal before and after insemination and on the place where insemination occurs in the field. Finally, the recognition of age-dependent receptivity may modify plans for genetic control programs involving male release.

SUMMARY

1. Females of *A. aegypti* begin copulating soon after emergence, but are refractory to insemination for about 48–72 hours; hence, copulation by very young females does not result in insemination.

2. Rearing temperature and genetic constitution can modify the time of onset of receptivity.

3. The state of receptivity is indicated by the female's behavioral responses to mating attempts. Receptive virgin females respond to males by extending their terminalia and in general allowing the male to complete insemination. Unreceptive young virgins and previously inseminated females respond to mating attempts by withdrawing their terminalia and actively repelling males.

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