EFFECTS OF MATING OR ASSOCIATION OF THE SEXES ON LONGEVITY IN *AEDES AEGYPTI* (L.)

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INTRODUCTION. In a previous report Liles and DeLong (1960) found that presumably mated (males and females together in the same cage) female *Aedes aegypti* (L.) outlived virgin females by approximately 17 percent when fed a non-egg-producing sucrose diet. Conversely, virgin males outlived mated males (males present in the same cage with females) by about 30 percent. In that work an equal number of males and females were present in each cage, if mated. The sexes were placed together two days following emergence as adults, then left together until death.

Similar observations on male life span have been reported with *Drosphila melanogaster,* but just the reverse with *Bombyx mori* (in Comfort, 1964). The shorter life span of mated males in *A. aegypti* may be, in part, related to the rate and extent of mating which Roth (1948) has shown to be quite high. The physical activity associated with the process, such as increased flying, as well as the drain of nutrient reserves for sperm production may account for the earlier death.

The effect on the female was unexpected. As a rule, mated females of an insect species, particularly where egg productivity is high, show a shortened life span. The extent of the decreased life span usually depends upon the rate and extent of egg production, and is apparently related rather directly to the nutritional drain caused by the process. For example, Griffiths and Tauber (1942) found that unmated female American cockroaches produced fewer egg capsules, and lived longer, than did mated females. The same has been found true in some species of moths (in Comfort, 1964). In these examples the effects on female life span are as would be expected since the females consumed the same diet regardless of whether eggs were produced or not. With respect to the anautogenous *A. aegypti,* however, egg production occurs only if the adult diet contains a protein source. Good longevity is obtained using diets of sucrose solutions alone, but no eggs are deposited. Therefore, effects of mating or association of the sexes may be assessed without having the picture distorted by productivity.

It seemed desirable to try to obtain answers to the following questions: (1) would additional groups of young males, added after the initial groups died or became old, further increase female longevity? (2) How long need the females be present with males to be benefited? (3) Do females, regardless of age, benefit from mating or association with males? (4) Can males regardless of age or condition transmit or induce the benefit? (5) Is male life-span shortened in proportion to the time the males spend with females?

MATERIALS AND METHODS. The mosquitoes used resulted from eggs laid on the same day by stock mosquitoes less than 3 weeks old (stock diet: caged mice every 4 days and 20 percent sucrose solution *ad lib.*). The eggs used were less than one month old, and were stored at 26–27° C and 77–81 percent r.h. The larvae were reared by the method of Burcham *et al.* (1956), and both larvae and adults were reared and maintained at 26–27° C and 77–81 percent r.h.

The method of separating the sexes was that described by Liles and Delong (1960).

Two sizes of cages were used to minimize any effects of cage size or population density on the results obtained. One type of cage was constructed of wood, covered with organdy. This measured 12 x 8 x 9 in. The other type was constructed from circular plastic cups, 3-½ inches in diameter, and 2-¾ inches high. A square 2 x 2

inches was cut from the lids and replaced with organdy, and a circular hole cut in the side to admit a ½ inch diameter plastic tube which contained cotton saturated with 20 percent sucrose solution. In some experiments, tests with one type of cage were repeated using the other type. In other cases convenience and space dictated the choice. Throughout the experiments it was found that total overall lifespan was approximately one-third less when the insects were kept in the smaller cages.

All insects were chilled in a cold room at 4–8° C before being added to or removed from cages. All cages in any given test were chilled if mosquitoes were added or removed from any cages in the test group.

The food for the adult insects was always 20 percent sucrose solution supplied on a cotton pad. The pads were changed every two days at which time mortalities were recorded. A cotton pad saturated with water was also supplied.

All experiments were run using a strain of mosquitoes obtained originally from Orlando, Florida which had been cultured continuously in the laboratory for about 15 years.

The standard one-way analysis of variance (Snedecor, 1946) was used to interpret the results.

RESULTS AND DISCUSSION. The effect of extent of mating or association was tested by comparing four groups of females. The first group contained young virgin females; the second contained females with an equal number of virgin males (added on the third day) which were left until they all died. In the third group an equal number of 6-day old virgin males were present for only one day (sixth day following emergence) and then removed; and in the fourth group a number of 3-day old virgin males were added every 2 weeks, equal to the number of remaining females, and the existing old males removed. The results are indicated in Table 1.

Here the mating or association proved of benefit even if the time was as short as one day, but the addition of new males every 2 weeks proved detrimental. This detrimental effect may have resulted from the fact that the females were "bothered" excessively or they simply may have acquired too much of something which affected their physiology under the particular set of circumstances at hand. The latter is probably correct in that the same experiment was repeated except that the females were given the opportunity of a blood meal (hemolyzed bovine blood plus 10% sucrose) every fourth day. This resulted in high egg productivity and no decrease in female longevity when new males were added every 2 weeks. In the blood diet test all groups containing males outlived virgins, to a small degree, and deposited considerably more eggs.

TABLE 1.—Effect of extent of mating or association on female lifespan (large cages, 50 females per cage if virgins; 30 females per cage where mated)

	Av. days lived		
	Rep. No.		
Group No.	1	2	Av.
1. Virgins	56.20	57.00	56.60
2. Females with males until males died	68.10	56.50	62.30
3. Females with males one day	69.10	70.10	69.60
4. Females with multiple groups of males	44.40	45.70	45.05

By analysis of variance there was a significant difference between the various groups at the five percent level. In separate analyses there was a significant difference at the one percent level between the presumed virgins and those mated or associated with males for one day, but no significant difference between the virgins and those females present with the initial group of males.

Next a test was designed to determine whether young males might transmit the "effect" to young females in less than one day's time. Again, four groups of females were used as follows: (1) virgins, (2) virgin females confined with males for 1 hour at 3 days of age, (3) 3-day-old virgin females confined with virgin males of

TABLE 2.—Effect of mating or association of less than one day on female lifespan (small cages, 15 females per cage)

Group No.	Av. Days Lived				
	Rep. No.				
	1	2	3	4	Av.
1. Virgins	36.5	45.0	32.6	35.5	37.4
2. Females with males one hour	44.0	41.8	38.1	47.2	42.8
3. Females with males one day	52.0	37.2	36.8	45.0	42.9
4. Females with males until males died	37.2	46.8	47.6	37.2	42.2

the same age for 1 day, and (4) females with an initial group of males which were left until dead. The results are shown in Table 2.

Here an increase of from 14–15 percent more than the virgins' lifespan occurred in all of the mated groups. There was a statistically significant difference at the one percent level when all groups were considered.

The next test was devised to study the effect of mating or association on females of different ages. As before, four groups of females were used as follows: (1) virgins, (2) 4-day-old virgin females confined for one hour with 4-day-old virgin males, (3) 2-week old virgin females confined for one hour with 4-day-old virgin males, and (4) 3-week-old virgin females confined for one hour with 4-day-old virgin males. The results are shown in Table 3.

From several separate analyses of variance it appeared that one hour's association was sufficient to bring about an increase in female lifespan only in the 4-day-old age group of females. It was decided, therefore, that the age of the female might be critical, and that further investigation was justified.

Since previous tests showed one hour's mating or association was sufficient to insure greater female lifespan, using young males and females, but not with older females and young males, the following test was run. Four groups of females 13–14 days of age were placed with males of different ages and conditions as follows: (1) virgin females, (2) virgin females placed with an equal number of young males (3 days old) until all males were dead, (3) virgin females placed with 14-day-old virgin males and left until the males died, and (4) virgin females placed with young males for one day. The results are shown in Table 4.

In this experiment only one group indicated a life-span increase. It was the group in which the 13–14-day-old females were mated or associated with the 13–14-day-old males. These females lived significantly longer (one percent level) than the virgins by approximately 17 percent. Thus it appeared that the ages of the older females and males must be similar in order to result in an increase in female life-span.

Since longevity was increased in only the one group in the previous experiment it was decided to try even older females. A group of females was kept as virgins for 17 days and divided into three groups as follows: (1) virgins; (2) females plus young males (3 days old) and (3) females plus 17-day-old males. The results are shown in Table 5.

TABLE 3.—Effect of mating or association of the sexes for one hour on female lifespan; females of different ages (small cages, 15 females per cage.)

Group No.	Av. days lived				
	Rep. No.				
	1	2	3	4	Av.
1. Virgins	38.40	36.50	37.60	32.60	36.27
2. 4-day-old females with males one hour	44.40	46.75	41.63	38.10	42.70
3. 2-week-old females with males one hour	35.46	31.30	36.20	31.40	33.60
4. 3-week-old females with males one hour	27.80	34.40	33.30	34.00	32.40

TABLE 4.—Effect of mating or association of the sexes on female lifespan; females 13–14 days old and males of different ages (large cages, 50 females per cage)

Group No.	Av. Days Lived		
	Rep. No. 1	Rep. No. 2	Av.
1. Virgins	41.10	40.00	40.05
2. Two-week-old virgin females with young males until males died	38.10	42.20	40.10
3. Two-week-old virgin females with 13–14-day-old males until males died	48.90	48.71	48.80
4. Two-week-old virgin females with young males for one day	38.80	41.90	40.30

There were no statistical differences among the groups, which indicated that at least under the conditions of these experiments females beyond 15 or so days of age receive no longevity benefit from mating or association with males. The possibility exists that little or no mating occurs in older females. At least there is little flying in females beyond 2 weeks of age.

Previous tests indicated that when an equal number of young males and females are placed together, male lifespan is shortened by about one-third (Liles and Delong, 1960).

The effect of the age of the male at time of mating or association, the length of time with females, and the presence of more than an equal number of females on male lifespan was tested by these experiments. The first involved the following groups: (1) 6-day-old virgin males; (2) 6-day-old virgin males mated with an equal number of 6-day-old virgin females and left together until all the males were dead. The results are shown in Table 6.

Here those males in constant association with females had a 45 percent shorter lifespan than did the virgin males. This was higher than usually recorded when the insects are placed together earlier in adult life. The males which were confined with females for one day (group 2) had an 18 percent shorter life span than the virgins.

TABLE 5.—Effect of mating or association of the sexes on female lifespan using 17-day-old females (large cages, 50 females per cage)

Group No.	Av. days lived		
	Rep. No. 1	Rep. No. 2	Av.
1. Virgins	41.2	35.6	38.4
2. 17-day-old virgin females with young males until males died	39.3	34.0	36.5
3. 17-day-old virgin females with 17-day-old males until males died	37.5	40.5	39.0

In another test four more groups were used. They included (1) virgin males; (2) 9-day-old virgin males confined for one day with 9-day-old virgin females; (3) 9-day-old virgin males confined for two days with 9-day-old virgin females and (4) a group of 9-day-old virgin males confined with virgin females of the same age until death. The results are shown in Table 7.

All of the males in the fourth group were dead within 4 days of being placed with the females. In other tests not listed, males up to 30 days of age (virgins) have been used, and in such cases all were dead within 2 days, although several of the virgins in the check groups were still alive up to 10 days beyond. In spite of the fact that the males died quickly, the

TABLE 6.—Lifespan of males confined with females various lengths of time in large cages. (Equal Nos. of males and females, 50 of each per cage)

Group No.	Av. days lived		
	Rep. No. 1	Rep. No. 2	Av.
1. 6-day-old virgin males	38.70	36.10	37.40
2. 6-day-old virgin males with 6-day-old females one day	32.50	28.36	30.43
3. 6-day-old virgin males with 6-day-old females until death	20.10	21.26	20.63

TABLE 7.—Lifespan of males confined with females various lengths of time in large cages. (Equal Nos. of males and females, 50 of each per cage)

Group No.	Av. days lived		
	Rep. No.		
	1	2	Av.
1. Virgins	31.00	28.70	29.85
2. 9-day-old virgin males with females one day	27.00	26.70	26.85
3. 9-day-old virgin males with females two days	22.30	20.70	21.50
4. 9-day-old virgin males with females until death	12.73	12.80	12.76

progeny which they produced were quite healthy and appeared normal in every way. Although there appeared to be a reduction in life span between the virgins and those males mated just one day, it was not statistically significant. The lifespan of the other groups was significantly less then the virgins.

Since, in all probability, it is the mating process or something connected with it which causes the decrease in male lifespan and increase in female lifespan, I decided to set up an experiment involving stimulation of the males in the absence of females. Roth (1948) found that the males are incited to fly and mate by the wing beat frequency of the females. Tuning forks at 480 and 512 vps. appeared to be the most effective. Therefore, an experiment was performed in which tuning forks of the mentioned frequencies were struck above the experimental cages for two periods of 15 minutes each per day. The experiment involved the following groups: (1) 3-day-old virgin males; (2) virgin males subjected to the tuning forks; (3) virgin males with 2 times the equivalent number of females, and (4) virgin males with an equal number of females. The results are shown in Table 8.

Here the insects subjected to the tuning forks had a shorter average lifespan than the virgins, but not significantly so. Such an experiment, using hand-struck equipment cannot be well controlled. Perhaps if electric forks had been used, operating for a longer period of time, significance might have resulted. The males when stimulated by the forks appeared to make every attempt to "mate" with the sides of the organdy covered cages.

The males which were with an equal number of females lived about 27 percent less time than the virgins, which was about as usual, and those mated with two times the number of females lived about 49 percent less time than the virgins. The entire experiment recorded in Table 8 was repeated, with similar results. In addition, male mosquitoes of a totally different origin (reared for at least 20 years at the Rockefeller Foundation) showed essentially the same results.

The explanation of the lifespan increase in mated females and decrease in mated males is unresolved, but more rapid utilization of nutrient reserves in the males is the likely reason for them. The males consumed and digested only carbohydrate materials yet reserves of many types are utilized in sperm production. Energy expenditure is also probably greater in mated than in virgin males, and preliminary studies indicate that mated males consume more sucrose per day than do virgins. Perhaps in the females hormone cycles are initiated or hormone levels increased by the mating (or association) process which are beneficial in some way. Also, it should be made clear that the effects may be due to association of the sexes alone rather than to the mating process. Mating was

TABLE 8.—Lifespan of males, confined with different numbers of females, and stimulated with tuning forks, (large cages, 75 males per cage)

Group No.	Av. days lived		
	Rep. No.		
	1	2	Av.
1. Virgin males	24.90	26.17	25.93
2. Virgin males, tuning forks	19.20	23.38	21.29
3. Males with 2 X No. of females	14.12	12.41	13.26
4. Males with equal No. of females	19.10	18.80	18.95

observed in all cases where the sexes were together, but at a much greater rate in the younger mosquitoes. Females two weeks of age and older mate at a much lower rate than do younger females, in all probability because they spend much less time in flight, where mating is initiated.

SUMMARY AND CONCLUSIONS. 1. Under the conditions of the experiments, young mated females (i.e. those in association with males) of *Aedes aegypti* lived 12–17 percent longer than did virgin females (females never in association with males).

2. The repeated addition of young males to a group of females shortened the lifespan of mated females or females associated with males, compared with virgins, if the insects were fed a non-egg-producing diet of sucrose. This was not true with an egg-producing blood and sucrose diet.

3. Up to about 2 weeks of age, association or mating with males of similar age increased the female lifespan over that of virgins even if the mating or association period was as brief as 1 hour.

4. Male lifespan was shortened apparently in proportion to length of time in association with females, as well as in proportion to the number of females with which they were present.

ACKNOWLEDGMENTS. The writer wishes to thank Dr. D. M. DeLong, Mr. Roger Meola, and Mr. R. E. Williams for their help in the various experiments.

This investigation was supported by research grant E 528 (C9), the National Institutes of Health, U. S. Public Health Service; and grant No. E 20.18-15 Army FD-GR-4, the U. S. Army Biological Laboratories.

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SOME EFFECTS OF RESIDUAL INSECTICIDES ON ADULT MOSQUITOES

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In the study of the habits of the anopheline vectors of malaria, in areas where residual insecticides are applied inside houses, consideration must be given to the influence exerted on the mosquitoes by the sprayed surfaces of all the houses of the locality and of all the localities of the area. The habits that receive most attention are resting, or looking for shelter, inside or outside of the houses, and the frequency of biting man or animals inside or outside of the houses. With the introduction of insecticides, of greatest importance is the study of susceptibility, irritability and longevity.

The purpose of this note is to call atten-