

# AUTOGENY IN *CULEX SALINARIUS* FROM TEXAS, FLORIDA AND NEW JERSEY

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**ABSTRACT.** Autogeny was studied in *Culex salinarius* from College Station, Texas; Vero Beach, Florida; and Edison and Dennisville, New Jersey. Autogenous egg development varied from 14% in the Florida colony to 23 and 27%, respectively, in colonies from Texas and New Jersey. The mean number of eggs per autogenous female ranged from 13.5 to 23.7. As in other autogenous mosquitoes, the expression of autogeny was dependent on larval and adult diets. Mating did not influence autogeny as it does in some mosquitoes. Autogeny in *Cx. salinarius* was obligate at warm temperatures, but females denied a bloodmeal appeared to undergo facultative autogeny at colder temperatures.

## INTRODUCTION

*Culex salinarius* Coquillett occurs throughout much of the United States, but is most abundant in the Atlantic and Gulf Coast regions (Carpenter and LaCasse 1955). Despite the extensive distribution of this mosquito, little is known about its biology and medical importance. *Culex salinarius* has been implicated as a possible vector of eastern equine encephalitis (Burbulis and Jobbins 1957) and St. Louis encephalitis viruses (Clark et al. 1977). Seeley and Bickley (1974) suggest that it is also a potential vector of *Dirofilaria immitis*, the causative agent of dog heartworm. Its importance as a nuisance mosquito is increasing along the coastal areas of the Atlantic and Gulf states. Several studies (cited by Slaff and Crans 1982) have shown that water impoundments in coastal areas, designed to control saltmarsh mosquito species and enhance waterfowl usage, produce large populations of *Cx. salinarius*.

*Culex salinarius* is predominately a fall and winter pest along the upper Texas Gulf Coast, usually ovipositing in tires, dredge spoils areas, and ditches (D. Sprenger, personal communication). To study this mosquito, we collected larvae from rain filled ditches on the Texas A&M University farm. Some of the adults emerging from this collection were autogenous; i.e., capable of producing an initial batch of eggs without a bloodmeal. Since autogeny has been reported in only seven *Culex* species (Chapman 1962, Vinogradova 1965, Rioux et al. 1975, Kay et al. 1986), the present study was conducted to investigate factors influencing autogeny in *Cx. salinarius*.

## MATERIALS AND METHODS

Laboratory colonies of *Cx. salinarius* from Texas, Florida and New Jersey were established for experimentation. The Texas colony originated from larvae collected from the Texas A&M University Swine Center in College Station, Brazos County. The colony was started in

February 1985, but died out by December 1985. A new colony was started in February 1986 with wild stock from the same location.

The Florida colony originated from a colony established in February 1986 at the Florida Medical Entomology Laboratory in Vero Beach, Indian River County. Our Florida colony was established in June 1986.

The New Jersey colony originated from wild females trapped July 1985 near Edison, Middlesex County but died out by early Spring 1986. A new colony was established in July 1986 from females trapped near Dennisville, Cape May County. Straight-line distance between Edison and Dennisville is approximately 95 miles.

Mosquitoes were reared according to the procedures of Readio and Meola (1985). Larvae were fed daily rations of a diet made from either 3 parts ground Purina laboratory rat chow and 1 part Brewer's yeast or a diet made from a 1:1:1 mixture of lab rat chow, Brewer's yeast, and lactalbumin. Daily rations were measured with a plastic scoop which delivered 45 mg of the 3:1 diet or 49 mg of the 1:1:1 diet. Feeding regimens varied with the diet used from a schedule of 1, 3, 3, 3, 5, -, 5, for the "low diet" to 1, 3, 5, 5, 3, 5, 3 for the "high diet". To test variations in larval diet, the 3:1 diet was used in two quantities: the low diet of 900 mg and the high diet of 1350 mg. The 1:1:1 diet fed at the low diet rate contained a total of 980 mg. In this experiment larval density was also varied from either 100 or 200 larvae per pan.

The rearing room was maintained at 27°C, 70% RH, and 14:10 (L:D) hours, including a simulated 1 hour sunrise and sunset. Refrigerated incubators were used for rearing larvae and maintaining adults at lower temperatures and shorter photoperiods. Fluorescent lights controlled by interval timers were used to maintain photoperiod in each incubator.

Adults were maintained in 50 × 50 × 50 cm cages, and fed *ad libitum* with 10% sucrose solution. When sucrose was withheld for experimentation, deionized water was provided. Mosquitoes were blood-fed on a young chicken re-

strained in a nylon stocking and taped to the bottom of the cage.

To assess autogeny in 8–10 day-old females, ovarian follicles were measured at 80× magnification with an ocular micrometer. Follicles 500  $\mu\text{m}$  or greater in length were classified as eggs. Resting stage follicles ranged in length from 62 to 100  $\mu\text{m}$ .

Wing length was used as an indicator of overall size of a mosquito. Wing measurements were made from the point of attachment to the wing tip, excluding the fringe. To confirm insemination of mated females, spermathecae were removed from the abdomen, crushed, and examined for the presence of sperm.

Statistical significance of percentages was determined by a test for comparing two binomial proportions (Ott 1984). The significance of other measurements was assessed using analysis of variance under the General Linear Models procedure in SAS. In all instances, differences were considered significant if  $P \leq 0.05$ .

## RESULTS

*Expression of autogeny in different geographical populations of Cx. salinarius:* Differences in the expression of autogeny in the three *Cx. salinarius* colonies are shown in Table 1. Data presented are from ovarian dissections of the F1 generation of each colony. Autogeny ranged from 14% in the Florida colony to 27% in the

New Jersey (1986) colony. Autogenous females developed from 1 to 50 eggs ( $\bar{x} = 13.5\text{--}23.7$ ).

Wing length is also shown in Table 1. Differences in mean wing length between concurrently reared autogenous and anautogenous females were compared in a variety of experiments subjecting test groups to variations in temperature, photoperiod, and larval diet. Size differences between autogenous and anautogenous mosquitoes were not significant in any of the comparisons. Likewise, there was no correlation between wing length and the number of eggs in autogenous females reared under the same regimen.

*Influence of mating on autogeny:* Autogenous females of the Texas (1985) and New Jersey (1985) colony were examined to determine whether mating influenced autogeny. Less than 20% of the autogenous females in the Texas colony and none in the New Jersey colony had mated. Since both colonies had similar percentages of autogenous females, mating did not appear to affect egg production. On the other hand, mating did appear to affect raft formation in both autogenous and anautogenous females. Well formed, normal appearing rafts were generally fertile, whereas broken or oddly shaped rafts were often infertile.

*Effects of larval nutrition and overcrowding:* Results comparing the effects of various larval diets and densities are shown in Table 2. The highest expression of autogeny was found in the groups fed the 3:1 high diet and the 1:1:1 diet.

Table 1. Autogeny and wing length of females in newly established colonies of *Culex salinarius* reared at 27°C 14:10 (L:D).

Colony	No. females examined	Percent autogeny	Eggs/autogenous female ( $\bar{x} \pm \text{SE}$ )	Female wing length in mm ( $\bar{x} \pm \text{SE}$ )
TX (1985)	300	16	23.2 $\pm$ 1.5	3.60 $\pm$ 0.01
TX (1986)	196	23	18.5 $\pm$ 1.3	3.74 $\pm$ 0.01
NJ (1985)	177	20	17.9 $\pm$ 1.9	3.76 $\pm$ 0.02
NJ (1986)	212	27	23.7 $\pm$ 1.4	3.84 $\pm$ 0.01
FL (1986)	170	14	13.5 $\pm$ 1.6	3.75 $\pm$ 0.01

Table 2. Effect of larval nutrition and density on size and autogeny in the Texas (1986) colony of *Culex salinarius* reared at 27°C 14:10 (L:D).

	Diet <sup>1</sup>			
	3:1 900 mg	3:1 900 mg	3:1 1350 mg	1:1:1 980 mg
No. larvae per pan	100	200	100	100
Total larvae	300	400	300	300
% survival to adult	80	78	80	81
No. females examined	76	60	60	60
% autogeny	20a	5c	28b	22ab
Eggs/autogenous female ( $\bar{x} \pm \text{SE}$ )	15.9 $\pm$ 1.0a	7.0 $\pm$ 2.0b	17.0 $\pm$ 1.9a	23.2 $\pm$ 2.0b
Female wing length (mm) ( $\bar{x} \pm \text{SE}$ )	3.71 $\pm$ 0.01a	3.49 $\pm$ 0.02c	3.77 $\pm$ 0.02b	3.75 $\pm$ 0.02ab

<sup>1</sup> Diet used is either 3 parts lab rat chow and 1 part Brewer's yeast, or a 1:1:1 mixture of lab rat chow, Brewer's yeast and lactalbumin.

Note: Values within a row not followed by the same letter differ significantly ( $P < 0.05$ ).

Differences in rates of autogeny were not significant between these two groups, but autogenous females on the 1:1:1 diet produced significantly more eggs. Conversely autogeny was significantly higher in the 3:1 high diet group than in the 3:1 low diet group, but the two did not produce significantly different numbers of eggs. The low density (100 larvae/pan) group also had a significantly higher rate of autogeny than the high density (200 larvae/pan) group with the same diet.

In addition to their effects on rate of autogeny, larval diet and population density likewise affected the size of the adult female. The longest wing lengths were recorded for the 3:1 high diet and the 1:1:1 diet groups, and the shortest wing lengths were found in the crowded 3:1 low diet group.

**Effects of adult nutrition:** In another experiment, the effect of sucrose feeding on autogeny was tested. The control group was fed 10% sucrose *ad libitum* from the time of emergence. The experimental group was provided with deionized water until the mosquitoes were 48 hours old, then given 10% sucrose. Ovaries in both groups were dissected 10 days after emergence. The control groups exhibited 15–20% autogeny, while females that were denied access to sucrose for 2 days after emergence failed to develop autogenous eggs.

**Effects of temperature and photoperiod:** Simulated summer and winter conditions had variable effects on the expression of autogeny. In the Texas (1985) colony, there was no significant difference in percentage of autogeny or number of eggs per female between groups reared at 27°C 14:10 (L:D) and 21°C 10:14 (L:D). However, tests with the Texas (1986) and Florida colonies showed that when the rearing temperature was decreased to 18°C or 15°C and photoperiod was modified to 8:16 (L:D), percentage of autogeny decreased significantly (Table 3). Likewise, significantly fewer eggs per autogenous female were produced at 15°C 8:16 (L:D) than at 27°C 14:10 (L:D).

**Follicular development and egg maturation:** In both the Texas (1986) and Florida colonies, the rate of follicular growth after emergence was influenced by temperature and photoperiod. Preresting stage follicles at 27°C, 14:10 (L:D) took approximately 3–4 days to reach the resting stage, while most females at 18°C or 15°C and 8:16 (L:D) did not develop resting stage follicles until day 5 or 6 after emergence (Figs. 1, 2).

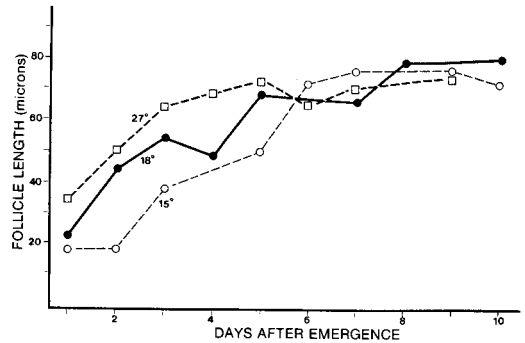


Fig. 1. Rate of development of preresting stage follicles in the Texas (1986) *Culex salinarius* colony reared at 27°C 14:10 (L:D), 18°C 8:16 (L:D), and 15°C 8:16 (L:D).

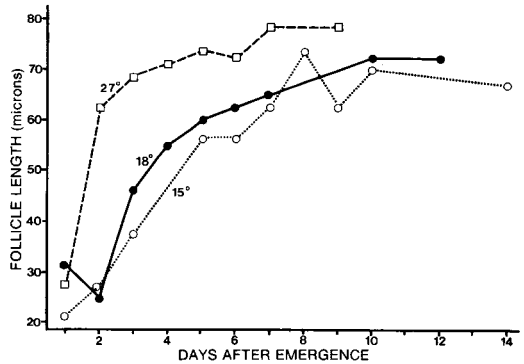


Fig. 2. Rate of development of preresting stage follicles in the Florida *Culex salinarius* colony reared at 27°C 14:10 (L:D), 18°C 8:16 (L:D), and 15°C 8:16 (L:D).

Table 3. Effect of simulated summer and winter conditions on adult size and autogeny in the Texas (1986) and Florida colonies of *Culex salinarius*.

	Colony					
	Texas (1986)			Florida		
Temperature	27°C	18°C	15°C	27°C	18°C	15°C
Photoperiod (L:D)	14:10	8:16	8:16	14:10	8:16	8:16
No. females examined	80	109	79	62	60	70
% autogeny	39a	19b	20b	37a	18b	7c
Eggs/autogenous female ( $\bar{x} \pm SE$ )	20.9 $\pm$ 2.6a	16.5 $\pm$ 3.7ab	10.4 $\pm$ 1.6b	20.1 $\pm$ 1.9a	17.3 $\pm$ 1.7ab	13.2 $\pm$ 2.1b
Female wing length in mm ( $\bar{x} \pm SE$ )	3.65 $\pm$ 0.02a	4.30 $\pm$ 0.01b	4.27 $\pm$ 0.01b	3.80 $\pm$ 0.03a	4.01 $\pm$ 0.02b	4.00 $\pm$ 0.02b

Note: Within a colony, values within a row not followed by the same letter differ significantly ( $P < 0.05$ ).

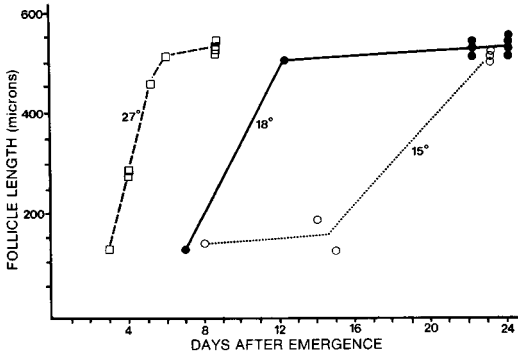


Fig. 3. Rate of egg development in autogenous females of the Florida *Culex salinarius* colony reared at 27°C 14:10 (L:D), 18°C 8:16 (L:D), and 15°C 8:16 (L:D).

Autogenous females typically needed 6 days to develop eggs at 27°C, 14:10 (L:D). However, at lower temperatures and shorter photoperiods fully developed eggs were not observed until day 12 at 18°C 8:16 (L:D) and day 22 at 15°C, 8:16 (L:D) (Fig. 3).

Rate of follicular growth was variable at all temperatures. Follicles under 20  $\mu\text{m}$  were observed as late as day 11 after emergence in females from the Texas (1986) colony reared at 18°C. However, most females developed resting stage follicles.

## DISCUSSION

Autogeny in newly established colonies of *Cx. salinarius* varied slightly with the geographic site of origin and year of collection, and ranged from 14 to 27%. The average number of eggs per autogenous female was less than 25, and individual autogenous females never developed more than 50 eggs. Autogeny in *Cx. salinarius* is therefore comparable to that seen in *Culex annulirostris* Skuse, *Culex peus* Speiser, and *Culex erythrothorax* Dyar, in which wild populations generally exhibit autogeny rates of less than 20% and produce less than 50 eggs per female (Chapman 1962, Washino and Shad-Del 1969, Kay et al. 1986). In contrast, some populations of *Culex tarsalis* Coq. may exhibit over 80% autogeny in the summer months, with individual females producing as many as 220 eggs (Moore 1963).

While much research has been done on the role of the male accessory gland substance that regulates autogeny in *Aedes* (O'Meara and Evans 1977), the effect of mating on autogeny in *Culex* is not well understood. Kay et al. (1986) suggested that autogeny in *Cx. annulirostris* is male-induced, but they did not examine ovarian development in non-mated females. Spielman (1957) suggested that in *Cx. pipiens* Linn., mating acts as a stimulus to oviposition, but does

not affect the autogenous development of ovarian follicles. *Culex salinarius* may be similar to *Cx. pipiens* in this respect. Ovarian dissections of *Cx. salinarius* showed that mating took place in less than 20% of the autogenous females.

Nutritional factors that regulate autogeny in other species of mosquitoes (Corbet 1964, Laurence 1964, Lea 1964, O'Meara and Krasnick 1970, Kay et al. 1986) appear to function in a similar manner in *Cx. salinarius*. Decreasing the quality or quantity of larval diet or increasing larval density led to a decrease in the expression of autogeny. Adult *Cx. salinarius* also required carbohydrates for the expression of autogeny, like many other autogeneus species. Indeed, sucrose deprivation for 2–4 days after emergence completely eliminated autogenous egg development.

Autogenous mosquitoes are often larger than concurrently reared anaotogenous females (Spielman 1957, Reisen et al. 1984). However, comparisons of autogenous and anaotogenous *Cx. salinarius* reared under the same conditions did not show a significant size difference.

Another relationship commonly found in autogenous species is that larger individuals produce greater numbers of eggs (Spielman 1957, Laurence 1964, O'Meara and Krasnick 1970). In *Cx. salinarius*, however, there was no correlation between wing length and the number of autogenous eggs in females reared under the same regimen. In species where this relationship exists, autogenous females usually exhibit a much greater range in the number of eggs produced per female than the number found in *Cx. salinarius*.

The effects of temperature and photoperiod on autogeny in *Cx. salinarius* differed substantially from the effects seen in other autogenous *Culex* species. *Cx. tarsalis* and *Cx. peus* undergo ovarian diapause under low temperature and short photoperiod, which causes arrest of follicular growth before the resting stage is reached (Reisen et al. 1984). In contrast, winter-type rearing conditions did not prevent follicular growth in *Cx. salinarius*, even though the rate of growth was slowed considerably. The percentage of autogenous females was significantly lower when *Cx. salinarius* were reared at low temperature and short photoperiod. However, the decrease in autogeny was not as great as that seen in *Cx. tarsalis* (Moore 1963).

Before initiating this study, we suspected that some southern *Cx. salinarius* populations might aestivate during periods of high summer temperatures. However, the present results suggest that the Texas and Florida colonies do not aestivate under summer conditions or enter diapause under winter conditions. Likewise winter conditions do not appear to induce diapause in

northern populations of *Cx. salinarius*. Slaff and Crans (1981) showed that New Jersey populations remained relatively active during the winter, and attempted to bloodfeed on mild days. This finding was supported by the laboratory work of Eldridge et al. (1972). However, in northern areas, the fate of eggs that may be developed over the winter is not clear, and the overwintering site of this species remains uncertain.

The rate of autogenous follicular development in *Cx. salinarius* is much slower than the rate described for *Cx. pipiens* (Speilman 1957). At 27°C, follicles over 500 µm in length were first observed in *Cx. pipiens* 70 hours after emergence while female *Cx. salinarius* took over 140 hours to develop eggs to this size.

Most autogenous species with sufficient nutrient reserves for egg production begin maturing eggs soon after emergence. These females generally forego a bloodmeal until the second gonotrophic cycle, and are considered to undergo obligate autogeny (Spielman 1971). Some species, however, are capable of facultative autogeny. These facultative species develop their follicles to the resting stage and actively seek a bloodmeal. However, if they do not feed on blood within a few days, the follicles resume growth and autogenous eggs are produced (Spielman 1971, O'Meara 1985).

Autogeny in *Cx. salinarius* fits the description of obligate autogeny at warm temperatures. Although egg maturation was not as rapid as in *Cx. pipiens*, female *Cx. salinarius* began autogenous egg development soon after emergence, with follicles advanced beyond the resting stage by day 4. At low temperatures, however, fully developed eggs were not observed in *Cx. salinarius* until day 12 after emergence, closely resembling the situation of facultative autogeny.

Assuming that the results of this study are indicative of autogeny in wild populations of *Cx. salinarius*, then this species may reproduce autogenously throughout the year in the southern part of its range, and at least during the summer months farther north. This type of reproduction could allow a population to maintain itself at low levels during periods of unfavorable environmental conditions. In times of extreme heat, cold, or drought, autogenous mosquitoes could remain in protected habitats and produce eggs without the hazards involved in seeking a bloodmeal.

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