THE DURATION OF LARVAL LIFE OF *AEDES AEGYPTI* AS AFFECTED BY TIME OF HATCH

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ABSTRACT. When *Aedes aegypti* eggs (1-10 wk old) were submerged in water, larvae that hatched in the 24 hr period following exposure to reduced oxygen developed significantly faster than larvae from the initial hatch. There were significant differences in the sex ratios between first- and second-hatch groups; however, adult size and fecundity did not differ significantly.

INTRODUCTION

In the field, eggs of the mosquito *Aedes aegypti* (Linn.) are deposited singly in an environment that is potentially susceptible to flooding. Extensive field (Fielding 1919, Gjullin et al. 1950) and laboratory (Shannon and Putnam 1934, Putnam and Shannon 1934, Thomas 1943, Travis 1953; Gillett 1955a, 1955b) observations document that eggs of *Ae. aegypti* and other flood-water mosquitoes hatch over a period of several days. Atkin and Bacot (1917) proposed that asynchrony in egg hatch was due to genetically-based variation in response to seasonal changes and Gillett (1955b) selected for variable response to a hatch stimulus, and demonstrated that it was a genetically acquired trait. However little work has appeared on the relationship of variability in hatch time and the rate of larval development.

During the course of routine maintenance of an *Ae. aegypti* colony, what appeared to be significant differences in the rate of development of larvae hatching at various times, were noted. In this paper the duration of larval development, sex ratios, and adult size and fecundity as a function of hatch time are described.

MATERIALS AND METHODS

Aedes aegypti, NIH-Rockefeller strain, were obtained from Dr. D. J. Sutherland, Mosquito Research and Control, New Jersey Agricultural Experiment Station, Rutgers, the State University of New Jersey. Females in the first gonotrophic cycle and less than 2 wk old were allowed to oviposit on moist filter papers, which were removed at 24 hr intervals. Eggs were collected over a period of several weeks and maintained at 25±2°C and > 50% relative humidity for up to 10 wk before being hatched. Eggs that appeared normal were counted and placed in tap water under vacuum for 1 hr. Most of the eggs hatched within 10-20 min. Larvae were briefly chilled on ice, counted and maintained at a density of 0.5-0.75 larvae/cm^2 surface area in water ca. 1-2 cm deep. Finely pulverized rat chow was provided according to the schedule of Gerberg (1970).

After removal of the larvae resulting from this initial hatch, the eggs were returned to the water to which was added ca. 5 mg of pulverized rat chow. At 24 hr intervals, newly emerged larvae were removed, counted and established as described above. Hatch times are designated as follows: first-hatch (reduced oxygen hatch), second-hatch (larvae emerging in the first 24 hr period), third-hatch (larvae emerging in the second 24 hr period) and fourth-hatch (larvae emerging in the third 24 hr period).

Larvae were maintained at 27±0.5°C. Pupae were counted at 24 hr intervals and pooled in separate containers according to hatch time. Adults were separated according to sex, chilled and weighed in groups of 25, 0-2 days after emergence. Adults were maintained at ca. 25°C and provided with water and raisins for 4-5 days before a blood-meal on guinea pigs was provided. Eggs were counted to evaluate fecundity.

To control for the possibility that larvae which emerged from the first hatch could have been at some disadvantage relative to larvae that hatched subsequently (the latter being in the presence of a more nutrient-rich environment), variations in the feeding regime were tested. A semiliquid diet containing mouse chow, yeast hydrolysate and lactalbumin hydrolystat, autoclaved in water (Baker et al. 1983), was substituted for the pulverized rat chow. In a second variation, first-hatch larvae were placed in water which had been "preconditioned" with rat chow, simulating the environment of second-hatch larvae. With both dietary variations, the unhatched eggs were returned to water to which was added 2 ml of semiliquid diet or ca. 5 mg dry rat chow, respectively.

Data were analyzed with SAS (Statistical Analysis System) using 2 X 2 contingency tables, ANOVA, Duncan's Multiple Range tests and *t*-tests.

RESULTS

In these experiments, ca. 94% of *Ae. aegypti* eggs, which had been maintained for as long as 10 weeks, hatched during a 72 hr period. During a 1 hr exposure to reduced oxygen tension 67.3-84.1% of the eggs hatched. During the succeeding 24 hr period, an additional 8.6-18.5% of the eggs hatched, and between 24 and 48 hr, 0.6-3.7% of the eggs hatched. Only 2-wk old eggs had a fourth hatch. Although 100% hatch was not attained in any group, presumably because some eggs were dead, 4 days after the first-hatch was designated as a termination point. Since the number of third- and fourth-hatch larvae was insufficient for significant data analysis, attention was focused on differences in developmental rates between first- and second-hatch larvae.

Second-hatch larvae developed significantly faster than first-hatch larvae ($F = 1121.43$, $P = 0.0001$) regardless of the age of the eggs. Larval development, as a function of time after hatching, was represented by a positively skewed unimodal distribution (Fig. 1). The general shape of this distribution (for both first- and second-hatch larvae) is not unlike that previously reported for field (Keirans and Fay 1970) or laboratory (Shannon and Putnam 1934) observations. The duration of the larval stage was significantly affected by the age of the eggs and the time of hatch, (ANOVA, $F = 71.52$, $P = 0.0001$; Fig. 2). The age of the eggs had a significant effect on the duration of the larval period, but not in a linear manner. Larvae from eggs 1-2 and 6-9 wk old took slightly longer to pupate than larvae from eggs 3 wk old. When the average developmental time was plotted for each hatch, a statistically significant (as analyzed by Duncan's multiple range test, alpha level = 0.05) bimodal distribution was observed.

Variation in the diet affected the duration of the larval period (Table 1). With all three types of diet (semiliquid diet of Baker et al. (1983), presoaked rat chow or dry rat chow), organic material appeared to be in excess; therefore differences in developmental rates were probably not due to starvation (Shannon and Putnam 1934). Second-hatch larvae developed faster than first-hatch larvae under all feeding regimes. When larvae were fed on the semiliquid diet, first-hatch larvae took significantly longer ($t = 41.44$, $p = 0.0001$) to develop to the pupal stage relative to larvae fed on pulverized rat chow. These results were consistent with those of Baker et al. (1983) under whose feeding regime larvae started to pupate on the ninth day after eclosion. The difference between the rate of development of second-hatch larvae fed on semiliquid diet (6.65 days) and the dry food

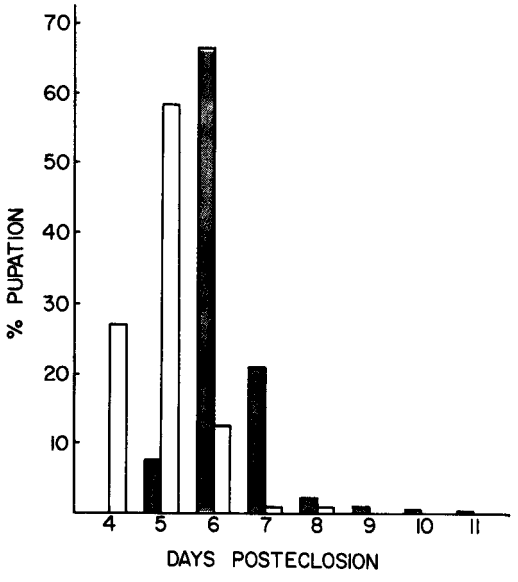

Fig. 1. Pupation of *Aedes aegypti* larvae as a function of time after hatching. Calculations were based on a total of 662 first-hatch larvae (shaded bars) and 103 second-hatch larvae (open bars).

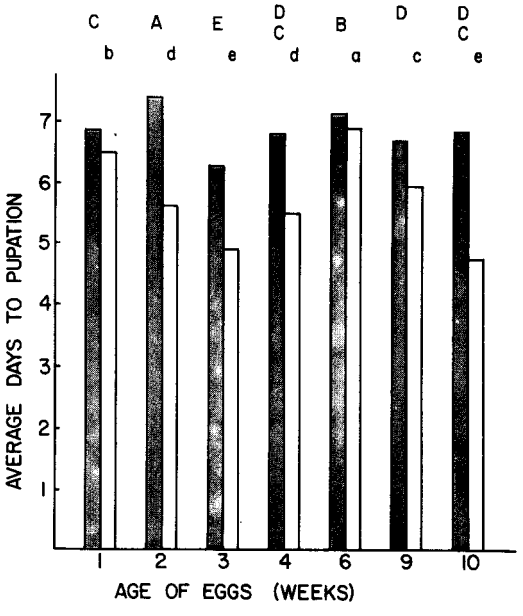

Fig. 2. Effect of age of *Aedes aegypti* eggs on the duration of larval development. Shaded bars indicate first-hatch larvae, open bars indicate second-hatch larvae. Capital and lower case letters indicate results of Duncan's Multiple Range test for first- and second-hatch larvae, respectively. Different letters indicate means that differ at the 0.05 level.

Table 1. Effect of diet on average days to pupation of *Aedes aegypti* larvae.

Egg group	Diet			
	Semiliquid (N)	Dry (N)	Presoaked (N)	Dry (N)
First-hatch	9.83 (546) A[1]	6.86 (540) B	6.49 (195) a	6.82 (382) b
Second-hatch	6.65 (23) C	6.51 (386) C	4.38 (16) c	5.53 (253) d

[1] Capital and lower case letters indicate results of Duncan's Multiple Range test for semi-liquid and dry diet, and presoaked and dry diet, respectively.

(6.51 days) was not significant. The more rapid growth of second-hatch larvae relative to first-hatch larvae, when fed semiliquid diet, was highly significant (t = 11.36, p = 0.0001) and more pronounced than when larvae were fed dry food.

When pulverized rat chow was presoaked for 24 hr before being fed to first-hatch larvae, these larvae developed significantly faster (t = 4.14, p = 0.0001) than first-hatch larvae fed on the dry food. Similarly, second-hatch larvae that were fed the presoaked food also developed faster than second-hatch larvae fed on dry food. Presumably, the presoaked rat chow provided a milieu conducive to the growth of microorganisms, thus providing first instar larvae with a more abundant food supply. First-hatch larvae developed to pupation more slowly than second-hatch larvae when both were fed presoaked food (t = 32.873, p = 0.001).

There were significant differences (χ^2 = 13.82, p = 0.0002) in the sex ratios between first- and second-hatch mosquitoes. The ratio of males to females from first-hatch larvae was 50.2 : 49.8, and from second-hatch larvae 38.6 : 61.4.

Although the second-hatch larvae developed faster than those from the first hatch, the second-hatch females weighed slightly more (0.0567 g/25 and 0.0531 g/25; second- and first-hatch, respectively); these differences in the average weight of adult females were not significant (t = 1.68, p = 0.10). There were no significant differences in the weight of males from each hatch (0.0295 g/25 and 0.0298 g/25, first- and second-hatch respectively; t = 0.329, p = 0.745).

The fecundity (based on 60 females) was not significantly affected by the differences in weight (t = 0.578, p = 0.59), although as expected the larger second-hatch females laid more eggs. First-hatch females laid an average of 93 eggs/female and second-hatch females laid an average of 99 eggs/female.

DISCUSSION

The various conditions stimulating egg hatch in *Ae. aegypti* have been enumerated. Gillett (1955b) demonstrated that, while application of a strong hatch stimulus would induce hatching in all eggs, a moderate stimulus could be selected such that mature *Ae. aegypti* eggs would have variation in hatching response (i.e., eggs would hatch over several days). By regular application of this moderate stimulus, he was able to select for variable response to the hatching stimulus, and demonstrate that it was a genetically acquired trait. The data presented herein demonstrate that a strong hatch stimulus will also yield variable hatch responses and the ensuing larvae differ significantly in their developmental rate and sex ratios.

The reasons for the differential rate of development between first- and second-hatch larvae are unclear. Shannon and Putnam (1934) demonstrated that male *Ae. aegypti* larvae develop slightly faster than do females (8.09 ± 0.03 versus 8.72 ± 0.03 days, respectively, temperature was not controlled). If, in the present experiments, there had been a greater percentage of males in the second-hatch, one might suspect that the faster development rate was due to sexual differences; this however was not the case, for the sex ratio of second-hatch larvae was skewed in favor of females.

Little work has appeared on the possible contribution of innate genetic factors on rates of larval development. Differential rates of larval development and levels of adult fecundity have, however, been correlated with resistance or susceptibility of arthropod strains to various pesticides (Ferrari and Georghiou 1981, Roush and Hoy 1981, Roush and Plapp 1982). Any inherent differences, such as those due to the time of hatch, among populations of organisms may be potentiated or mitigated when challenged with pesticides.

One may speculate that in the case of floodwater mosquitoes in the field it is "advantageous" that all of the eggs do not hatch immediately, since the duration of appropriate environmental conditions may be transient. It may further be advantageous for those larvae which do hatch later, to develop more quickly. The environment in which the eggs were laid may become less than optimal; evaporation causes the water level to drop, the food supply

may decrease and waste products may accumulate.

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