

EXPANSION OF EGGS OF *CULEX TARSALIS* COQUILLET AND *AEDES NIGROMACULIS* (LUDLOW) (DIPTERA: CULICIDAE)

BETTINA ROSAY

California State Department of Public Health, Bureau of Vector Control, Fresno

Water absorption in arthropod eggs usually takes place during embryonic development (Edney, 1957; Wigglesworth, 1950). The process of water absorption can be studied indirectly by measuring the change in size of eggs after they are laid. This type of investigation has previously been done for mosquito eggs by Downs (1951) and Gander (1951). The present study is an extension of their works and is intended to provide a foundation for the study of the permeability of eggs to insecticides.

MATERIALS AND METHODS. *Culex tarsalis* eggs were obtained from a laboratory colony (Bakersfield strain) maintained at the State Department of Public Health Field Station in Fresno. Groups of eggs were separated from newly-laid rafts and placed individually in a horizontal position in rows on a strip of filter paper. The paper strip, wet with distilled water, was supported by a glass slide. Measurements of length and width of eggs were made at intervals until hatching occurred. Measurements were made with a compound microscope fitted with an ocular micrometer. To test the effect of an environment of limited moisture, newly-laid eggs were drained briefly on absorbent paper, then submerged in mineral oil (Nujol) for subsequent measurements. Eggs from the same rafts were kept on wet filter paper for controls. In all tests the normal hatching time was determined by the unused portion of each raft which was left in the usual upright position on a water surface. In all tests the developmental times of control and test eggs were the same. Infertile *C. tarsalis* eggs were easily differentiated from embryonated eggs by observing the state of development through the transparent shells.

Aedes nigromaculis eggs were obtained in the laboratory from field-caught females captured in the Fresno area. These eggs were kept in the same manner as described above; only lengths of eggs were measured. After 80 hours of incubation the shells of these eggs were bleached with sodium hypochlorite so that embryonated eggs could be distinguished from infertile ones.

Initial measurements were made when the eggs were less than one hour old; at this age the eggs were white. Final measurements of *C. tarsalis* were made immediately before hatching of fertile eggs which took place 30 to 33 hours after oviposition. For *A. nigromaculis*, final measurements were made 76 hours after oviposition.

Except where otherwise specified, the eggs were incubated at 90° F.

All data were analyzed statistically, and statements of significance are based on a chance probability of less than one in one hundred.

RESULTS. Measurements of *C. tarsalis* eggs are shown in Table 1. If the egg is considered to be a prolate spheroid, the initial and final volumes can be calculated to be 0.0065 mm³ and 0.0124 mm³ respectively, which is a change in volume of 47.5 percent. Weighings were made to estimate the weight changes and densities of eggs, but the results were inconclusive due to technical difficulties.

C. tarsalis eggs from the same raft exhibited similar magnitudes of expansion when incubated at different temperatures. The rates of expansion, however, were influenced by temperature as shown in Figure 1. There was a direct relationship between temperature and rate of expansion. In a refrigerator at 40° F. lengthening of the eggs took place very slowly;

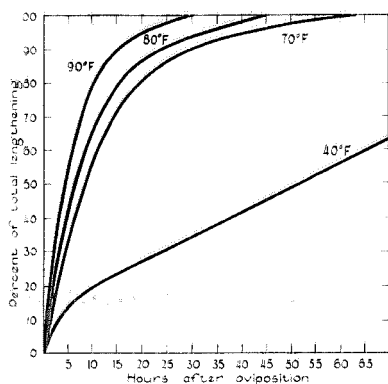


FIG. 1.—Change in length of *Culex tarsalis* eggs at different temperatures.

six to ten days were required for these eggs to reach a size comparable to eggs incubated at warmer temperatures.

There was no significant difference in initial lengths of fertile and infertile eggs (Table 1). Since lengthening occurred in infertile as well as fertile eggs embryonic development was not necessary for expansion. A comparison of lengths of fertile and infertile eggs at 30 hours after oviposition showed that in the same period of time infertile eggs had become significantly longer. The expansion of infertile eggs was 4.8 percent greater than fertile eggs (Figure 2).

Some newly-laid *C. tarsalis* eggs were placed in mineral oil to restrict water uptake. Lengths of eggs in oil were compared with eggs that had developed on wet filter paper. In the absence of excess water eggs lengthened 6.1 percent less than the control eggs (Table 1). Very few of the eggs submerged in oil embryonated in a normal manner; many were abnormal and many did not undergo any embryonic development. Control eggs from the same rafts which were incubated on wet filter paper developed normal embryos. The factors that limited embryogenesis of eggs submerged in oil were not determined. A comparison of eggs from this control group

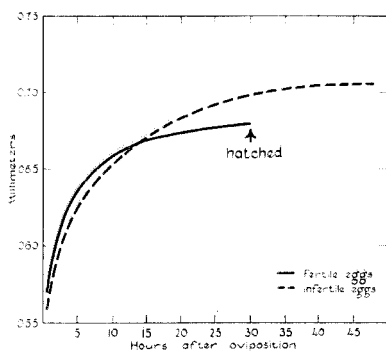


FIG. 2.—Change in length of *Culex tarsalis* eggs after oviposition (90° F.).

TABLE 1.—Size increases of eggs of *Culex tarsalis* and *Aedes nigromaculis*

	Number of eggs	Initial measurement in millimeters			Final measurement in millimeters			Per- cent increase
		Mean \pm	S.E. _m ^a	S.D. ^b	Mean \pm	S.E. _m	S.D.	
<i>Culex tarsalis</i>								
Width, fertile	13	0.153	0.000	0.000	0.191	0.003	0.010	19.9
Length, fertile	28	0.565	0.005	0.026	0.680	0.006	0.032	16.9
Length, infertile	28	0.556	0.005	0.025	0.713	0.008	0.039	21.7
Length in oil	17	0.527	0.003	0.015	0.585	0.009	0.039	9.9
Control	14	0.526	0.004	0.014	0.626	0.007	0.026	16.0
<i>Aedes nigromaculis</i>								
Length, fertile	8,18 ^c	0.586	0.008	0.022	0.703	0.004	0.016	16.4
Length, infertile	19,28 ^c	0.578	0.004	0.019	0.719	0.002	0.013	19.3

^a Standard error of the mean.

^b Standard deviation of the frequency distribution.

^c All figures were not available for analyses of initial measurements.

with normal eggs from the previously described test showed that they differed significantly in length even though their proportionate increases in length were the same. This demonstrates the desirability of using the same raft for test and control eggs.

Fertile *A. nigromaculis* eggs incubated at 90° F. lengthened very rapidly at first, reaching their maximum length between 15 and 21 hours after oviposition (Figure 3). After this time there was no detectable change in size. At this temperature *A. nigromaculis* eggs were capable of

this assumption. Downs (1951) demonstrated that hypertonic solutions interfered with lengthening of *Anopheles* eggs. Gander (1951) found that *Aedes aegypti* (Linnaeus) eggs lengthened less when kept on a dry surface in a saturated atmosphere than when they were on a damp surface. Gander's work with *A. aegypti*, as well as the present work with *C. tarsalis*, showed that eggs placed in mineral oil did not lengthen as much as eggs exposed to water. The increase in length that did occur was probably due to water trapped in the exochorion.

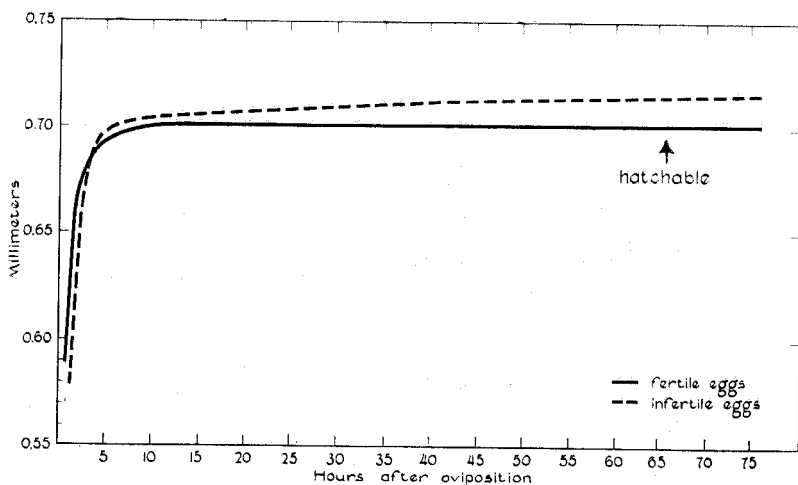


FIG. 3.—Change in length of *Aedes nigromaculis* eggs after oviposition (90° F.).

hatching 66 hours after they were laid. Infertile eggs also lengthened rapidly at first, but they continued to lengthen after the time when fertile eggs had reached maximum expansion. Initial lengths of fertile and infertile eggs were the same, but the final lengths of infertile eggs were significantly greater. The difference was 2.9 percent (Table 1).

DISCUSSION. Studies on increases in size, weight, and volume of arthropod eggs laid in moist environments have been reviewed by Wigglesworth (1950) and Edney (1957). The evidence implies that the changes were due to water absorption. Studies with mosquito eggs have supported

Since infertile eggs of *A. nigromaculis* and *C. tarsalis* also increased in size, water absorption is not dependent on embryonic development. Infertile eggs lengthened more than fertile ones in the same period of time and continued to lengthen thereafter.

At 90° F. fertile *A. nigromaculis* eggs reached their maximum size within 21 hours after oviposition; actually the lengthening was practically complete at 6 hours. *C. tarsalis* eggs continued to lengthen until they hatched. These patterns of expansion can be related to the oviposition sites of each species. *C. tarsalis* eggs are laid on a water surface where water is continually available to developing eggs. *A.*

nigromaculis eggs are laid on drying soil where free water is available for only a short time. Beckel (1955 a, b, 1958), Telford (1957), and Judson and Rosay (unpublished) have shown that fertile *Aedes* eggs become impermeable to water within a day after being laid. This is coincident with the appearance of an embryonic membrane which appears to be the most important water-proofing layer. After completing embryonic development, *Aedes* eggs are dormant until they are flooded and stimulated to hatch.

Downs' measurements of *Anopheles* eggs were similar to those reported in the present work, but the three species studied did not show a common pattern of lengthening.

SUMMARY. Eggs of *Culex tarsalis* and *Aedes nigromaculis* increased in size after oviposition. The rate of expansion varied directly with temperature, but the final size was independent of temperature. The expansion was not dependent on embryonic development since infertile eggs as well as fertile ones increased in size. Infertile eggs, however, expanded more than fertile eggs. *C. tarsalis* eggs exposed to free water lengthened more than eggs placed in mineral oil. This supported the assumption that expansion was due to water absorption. The eggs of the two species studied had different expansion rates which were related to their respective habitats. *C. tarsalis* eggs are laid on water, and they increase in size until they hatch. *A. nigromaculis* eggs are laid on drying soil where free water is available for only a short time. These eggs increased in size

rapidly at first, then no further change was apparent. The termination of expansion was correlated with the appearance of an embryonic membrane.

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