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SEPARATION OF FIRST-INSTAR LARVAE OF FOUR FLORIDA *CULEX (CULEX)*[1]

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While conducting field studies on ovipositional activities of wastewater mosquitoes, we collected several thousand egg rafts from sites in central and southern Florida. The vast majority of these field-collected egg rafts had been deposited by females of the following species: *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, *Cx, salinarius* Coq. and *Cx. restuans* Theobald. These are the four most common *Culex (Culex)* that will be encountered in Florida where they occur throughout the state. Rather than identify these rafts from the morphological characteristics of the eggs, we have relied on an indirect approach based on the examination of first-instar larvae following their eclosion. Most mosquito identification keys for first-instar larvae use only morphological traits (Bohart 1954, Price 1960, Dodge 1966). However, we have found that the process of identifying first-instar larvae can be greatly facilitated if consideration is given to both morphological and behavioral traits. This report gives a brief description of our procedure for distinguishing live first-instar larvae of the four above-mentioned mosquito species. *Culex bahamensis* Dyar and Knab and *Cx. tarsalis* Coq., the two other members of the subgenus, *Culex* in Florida, have very restricted distributions within the state. Therefore, we have not included them in this study.

Field-collected egg rafts were isolated individually into short, flat-bottom vials (25 mm diam, 35 mm height, 10 ml capacity) containing ca. 5 ml of tap water. Approximately 0.5 mg of liver powder was added to each vial shortly after the larvae had hatched. Without removing the larvae from the vial, species identifications were made 12–24 hours later using a dissecting microscope at low magnification (15X to 25X).

In addition to the taxonomic characters described by Dodge (1966), we found several others which were useful for distinguishing the first-instar larvae of *Cx. nigripalpus*, *Cx. quinquefasciatus*, *Cx. salinarius* and *Cx. restuans*. For example, in *Cx. nigripalpus* larvae the abdomen appears to have a clear band in the middle because the fourth abdominal segment is much less pigmented than the adjacent segments. This pigmentation pattern is much more difficult to discern in preserved specimens than it is in live material. As larval development in *Cx. nigripalpus* proceeds to later stages similar levels of pigmentation are attained by the third, fourth and fifth abdominal segments. In contrast, first-instar larvae of the three other species of *Culex (Culex)* mosquitoes found in our study areas did not possess a readily distinguishable difference in the amount of pigmentation among abdominal segments.

Another diagnostic character for separating these species of *Culex* mosquitoes involves the dorsal brush (setae $\underline{2}$, 3–$\overline{X}$) of the anal segment (abdominal segment $\overline{X}$). Actually, many components of the dorsal brush are structurally very similar among the four species. For the first-instar larvae of each species, the dorsal brush consists of two long caudal setae (a single upper seta, Z–$\overline{X}$, and a single lower seta, 3–$\overline{X}$) on each side of the anal segment. The upper and lower caudal setae, which are about the same size, are often more than twice the length of the anal segment. There is, however, a major interspecific difference which can be used to distinguish *Cx. salinarius* from the three other species. When *Cx. salinarius* larvae come to the surface for air, they hold their upper and lower caudal setae separated by an angle of 30° to 45° in a plane parallel to the water's surface. This angle is usually more acute in *Cx. nigripalpus* and *Cx. restuans* larvae. Practically no lateral separation occurs between the upper and the lower caudal setae of *Cx. quinquefasciatus* larvae. Thus, when observed from above the upper caudal seta often obscures the view of the lower caudal seta.

Vibrations induced by handling the shell vials invariably caused the larvae to dive toward the bottom of the vial. Following this alarm reaction *Cx. quinquefasciatus* larvae normally spent at least one minute and often longer away from the surface, whereas larvae of the three other species usually returned to the surface in less than 20 seconds. Even when un-

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disturbed *Cx. quinquefasciatus* larvae tended to forage away from the surface for longer periods of time between air breathing bouts than did the larvae of *Cx. nigripalpus*, *Cx. salinarius* and *Cx. restuans*.

The main characters for separating the first-instar larvae of the four species of *Culex (Culex)* are presented in the following key:

1. Fourth abdominal segment unpigmented .... *nigripalpus*
   Pigmented 2
2(1). Clear crescent anterior to egg breaker *restuans*
   no crescent 3
3(2). Upper and lower caudal setae of anal segment separated by 30°–45°; larva returns to surface in 20 sec. or less after disturbance . *salinarius*
   Upper and lower caudal setae not unseparated; larva returns to surface 1 min. or longer after disturbance .... *quinquefasciatus*

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INCREASED ADULT SIZE CORRELATED WITH PARITY IN *AEDES TRISERIATUS*[1]

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Parous rates frequently have been utilized to analyze the age structure of mosquito populations. A population with a large proportion of parous females is aging, and potentially contains more individuals that have had an opportunity to become infected with a transmittable pathogen. Detinova (1962, 1968) and Ungureanu (1974) have reviewed the significance of parous rates to the epidemiology of mosquito-borne disease.

The observed parity rate in a population is known to be influenced by the method of collecting, periodicity of blood-feeding, duration of the gonotrophic cycle, and weather (Service 1976), but the relationship between female size and parity has not been investigated. Container-breeding mosquitoes vary markedly in size; variation in larval diet can produce adults that vary 7× in dry weight[3]. Size variation is of epidemiological significance because longevity of adult female *Aedes triseriatus* (Say) in the laboratory increases with size[3]. Consequently, large females may be disproportionally dangerous as vectors. The object of this study was to determine if the percentage of parous females in *Ae. triseriatus* varied with the size of adult females.

MATERIALS AND METHODS

On September 4, 8, 11 and 24 of 1981, *Ae. triseriatus* females were collected at Western Iron and Metal Co., South Bend (St. Joseph Co.), Indiana. Thousands of tires, many of which contained *Ae. triseriatus* larvae, are stored in the salvage yard (Beier et al. 1983). The mosquitoes were collected with a mechanical aspirator (Nasci 1981) from low understory vegetation (primarily Virginia creeper, *Parthenocissus* sp.; stinging nettle, *Urtica* sp.; spicebush, *Lindera* sp.; and poison ivy, *Rhus radicans*) in a tree line along the western edge of the salvage yard. Total time expended collecting was approximately 6.5 hours.

In the laboratory, the mosquitoes were anesthetized with ether and females of *Ae. triseriatus* were frozen at −70°C. Wing length was used as a measure of body size because this character is significantly correlated with dry weight ($n=178$, $r=0.94$, $P<0.01$)[3]. Females were thawed and the length of one wing from the base of the costa to the tip of the wing margin excluding the apical scales was measured with an ocular micrometer. The observed range of the wing lengths (2.74–4.48 mm) was evenly divided among 3 size-classes: small (2.71–3.30 mm); medium (3.31–3.90 mm); and large (3.91–4.50 mm). Skewness and kurtosis of the entire wing-length distribution of the pooled samples were calculated by the method of Sokal and Rohlf (1969) and the distribution was tested for departure from normality by Chi square goodness of fit (Snedecor and Cochran 1967).

To determine the gonotrophic state of the mosquitoes, each female was washed with a 1% soap solution to wet the cuticle and the ovaries were dissected in several drops of deionized water. Females were classified as nulliparous or parous based on differences in the tracheal system of the ovaries (Detinova 1962). Nulliparous females with ovarioles at stage S-1 (Chrostophers' stages as in Detinova 1962) were designated as non-gonoactive. Gonoactive females were: 1) nulliparous females with ovarioles at stage S-2; 2) blooded or gravid females at stage S-3, 4 or 5; and 3) parous females. Gravid and parous females were pooled into a gravid or parous gonotrophic state because both had completed part of at least 1 gonotrophic cycle. The percentages of the gravid or parous females of the size classes were compared with a test for equality of percentages (Sokal and Rohlf 1969). Non-gonoactive females were assumed to be newly emerged, thus would have had less time to seek hosts than gonoactive nullipars. The pooling of non-gonoactive and gonoactive females could have altered the relative proportion of nullipars in each size class, which would have distorted the results of statistical analysis. However, the non-

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