Rearing of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae) from Florida and Pennsylvania with Notes on Their Pre-diapause and Pupal Development[1]

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Larvae of the carnivorous tree-hole mosquito, *Toxorhynchites rutilus septentrionalis* (Dyar and Knab), overwinter as diapausing fourth instars from northern Florida to Pennsylvania. When reared in the laboratory at 21°C after induced mating, larvae from Florida require a longer period for prediapause and pupal development than those from Pennsylvania.

T. rutilus septentrionalis occurs in eastern North America from Florida to New Jersey and Pennsylvania and west to the Great Plains (Jenkins and Carpenter 1946). Larvae are usually found in the rot-holes of trees and are of particular interest because of their carnivorous habits, feeding on larvae of other mosquitoes sharing their habitat. Here we describe rearing and certain aspects of development of these mosquitoes from disjunct portions of their range.

MATERIALS AND METHODS

We collected 4th instars from tree-holes in Lahaska, Bucks County, Pennsylvania, and Iamonia, Leon County, Florida. We shipped the larvae to Eugene, Oregon, and reared them to adulthood on long-day photoperiod (L:D = 15:9) at 21°C. We achieved insemination of adults by induced mating. We employed methods similar to those of Trimble and Corbet (1975) with several exceptions. We have achieved a high rate of insemination using carbon dioxide as an anesthetic. We anesthetize males for 2–3 min until they fall to the bottom of the cage but are still struggling. We then remove them, excise the head, wings, and legs, and affix them to a female anesthetized for 3–6 minutes. Pairs remain joined for a few seconds to several minutes.

We placed mated females in a 60×60×45 cm screen cage and provided molasses containing mold inhibitor (paramethylhydroxybenzoate) for food. We maintained adult cages at high humidity at 25±1°C and a changing photoperiod. The females oviposited into an artificial tree-hole constructed by cutting a 5 cm semicircle into one of 2 Jetware® 8-ounce dessert dishes which had been glued together and painted black. We removed the eggs daily and reared the resulting larvae on short-day photoperiod (L:D = 10:14) at 21±1°C. For food, 1st instars received washed *Artemia* (Arthropoda: Crustacea); 2nd and 3rd instars received *Enchytraeus* (Annelida: Oligochaeta); 4th instars received *Tubifex* (Annelida: Oligochaeta). After 10–42 days as 4th instars, we transferred the larvae to long days (L:D = 15:9) at 21±1°C.

In a separate experiment, we inseminated 8 ♀ 5–8 days after adult emergence and provided them with food, but no oviposition dishes. Seven days after insemination, we dissected the females and counted the number of mature eggs.

To compare the durations of various stages of development, we considered only animals that survived from hatch to adult ecdysis. We omitted the 4th instar since individuals spent varying times on short-day photoperiod as 4th instars prior to transfer to diapause-terminating long days. To obtain equal sample sizes for the analysis of variance, excess larvae were eliminated by assigning numbers to each larva and reading the numbers to be eliminated from a table of random numbers.

RESULTS AND DISCUSSION

Females began ovipositing as early as 24 (Pennsylvania) or 36 (Florida) h after insemination; females from both localities may delay oviposition for up to 10 days. The 8 mated females from Pennsylvania which were denied an oviposition site matured an average of 37 eggs each (range = 24–48). With

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Table 1.—Durations in days (mean ± 2 SE) of 1st, 2nd and 3rd larval instars reared on short days at 21°C and then transferred to long days as 4th instars, and of the pupal stage reared on long days at 21°C; sample size is 62 for each locality.

Locality	Stage of development I	II	III	P
Florida	4.44±0.18	5.84±0.26	7.82±0.26	8.39±0.13
Pennsylvania	4.02±0.19	4.76±0.25	6.72±0.17	7.60±0.13

Analysis of variance

Sources of variation	Degrees of freedom	Mean square	F
Localities	1	88.91	140.42***
Stages	3	375.11	592.41***
Interaction	3	3.13	4.95**
Residual	488	0.63	

few exceptions, hatching occurred 24–48 h after oviposition among eggs from both localities.

For the 1st, 2nd, and 3rd instars and pupae, analysis of variance indicated very highly significant ($P < 0.001$) differences in developmental time between localities and stages (Table 1). Duncan's multiple range test showed a highly significant ($P < 0.01$) difference between each stage at each locality and between each locality for each stage. Thus, larvae spend a longer time at each successive instar, and larvae and pupae from Florida take longer to develop at each stage than those from Pennsylvania. Among larvae from Florida, only one of those spending the normal 6–10 days as a 3rd instar died later when the resulting 4th instar was transferred to long days (Fig. 1). By contrast, all of those larvae which required more than 10 days to complete the 3rd instar subsequently died. All of the larvae from Pennsylvania which successfully completed the 3rd instar did so within 5–9 days and thereafter survived to adulthood. Hence, under controlled conditions, healthy *T. rutilus* are highly synchronized in their prediapause and pupal development.

T. rutilus from North Carolina (Jenner and McCrary 1964, McCrary 1965) and Pennsylvania (Bradshaw and Holzapfel 1975) enter a photoperiodically mediated diapause in the 4th instar. We observed 68 *T. rutilus* for 20–40 days (mean ± 2 SE = 38.2 ± 1.7) on a short-day regimen at 21°C. Not one exhibited any sign of development but all, except those dying as discussed above, pupated within 10–16 days (mean ± 2 SE = 15.1 ± 0.5) upon transfer to long days. Under short-day conditions at 22°C, *T. rutilus* from North Carolina invariably enter a 4th instar diapause (McCrary 1965).[2] Under short-day conditions at 23°C, *T. rutilus* either develop within 9–17 days or enter a stable diapause (Bradshaw and Holzapfel 1975). It seems likely that photoperiod has also arrested development among the *T. rutilus* from Florida which had not developed after 20–42

[2] McCrary, A. B. 1965. The effect of photoperiod, temperature and food on diapausing and developing larvae of *Toxorhynchites rutilus* (Coq.). M. S. Thesis, Univ. of North Carolina, Chapel Hill.

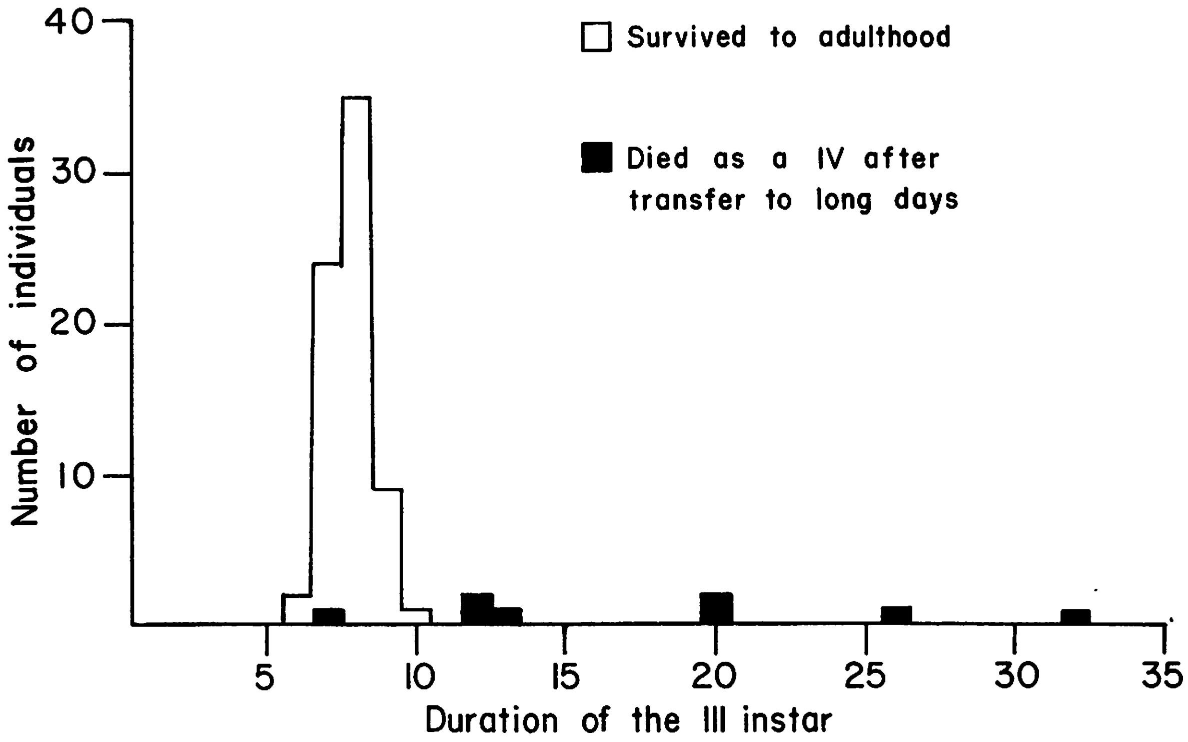

FIG. 1.—Relationship between the duration of the 3rd instar and subsequent survivorship.

short days at 21°C. We have collected overwintering *T. rutilus* near Baltimore, Baltimore County, Maryland; Durham, Durham County, North Carolina; and Clarkton, Bladen County, North Carolina; we have always found them only in the 4th instar. *Toxorhynchites rutilus septentrionalis* thus probably undergoes a photoperiodically induced 4th instar diapause throughout its range from north Florida to Pennsylvania, 30–40° N latitude.

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