ON THE BIONOMICS OF BROMELIAD-INHABITATING MOSQUITOES. III. THE PROBABLE STRATEGY OF LARVAL FEEDING IN *WYEOMYIA VANDUZEEI* AND *WY. MEDIOALBIPES*[1]

J. H. FRANK AND G. A. CURTIS

Florida Medical Entomology Laboratory, P. O. Box 520
Vero Beach, Florida 32960

ABSTRACT. Larvae of *Wyeomyia vanduzeei* and of *Wy. medioalbipes* demonstrate an ability to develop to the pupal stage when their supply of food is limited and intermittent. They develop slowly, are able to prolong their development for weeks or months under nutritional stress and are able to complete their development after additional potential food is added to their environment.

Analogies for this ability are suggested in larvae of other mosquito species, and the ecological significance is discussed.

INTRODUCTION

It has been suggested and demonstrated repeatedly in the literature (Nielsen and Haeger 1954; Wada 1965; Nayar 1967) that shortage of food prolongs the development period of mosquito larvae, just as in other holometabolous insects. Clements (1963) has suggested that, in nature, food is often insufficient for rapid growth of mosquito larvae.

It occurred to us that we might consider mosquito larvae to belong to 2 ecological groups based on their habitat. The first group would consist of those larvae which are found in ponds, ditches and marshes; the second, those larvae which are found in containers of various types such as water-filled leaf axils of plants, tree holes and man-made containers. The existence of the latter group has been recognized previously and those mosquito species which belong to it have been called "container breeders," presumably with reference to the adults rather than to the larvae to judge from the origin of the expression.

HYPOTHESIS

If we suppose the existence of the abovementioned 2 groups of mosquito larvae, then we might investigate the strategies available to the larvae of the 2 groups to overcome the disadvantages of their separate environments and, in particular, how they respond to shortage of food. We shall consider larvae of *Aedes taeniorhynchus* (Wiedemann) and *Culex nigripalpus* Theobald to belong to the first group, *Wyeomyia vanduzeei* Dyar and Knab, *Wy. medioalbipes* Lutz and *Toxorhynchites r. rutilus* (Coquillett) to the second group. All of these species occur in southern Florida; all but the last are prevalent, and we have first or second-hand observations on the natural history of all 5 species.

Females of *Ae. taeniorhynchus* lay their eggs on mud in salt marshes. Suitable areas for oviposition are provided by fluctuation of the water level. The larvae, which hatch after inundation of the eggs by flood water produced by high tides or heavy rains or both, may be stranded by the drying out of their habitat. Under these circumstances, the inhabitation of temporary pools, it may be seen to be to the advantage of the population for the larvae to develop rapidly. We find that the total larval development period, from eclosion of eggs to pupation, at 27°C and on an adequate diet in the laboratory, is about 5 to 6 days in duration (Nayar 1967). A total aquatic period (eclosion to emergence of adults) of 5 to 7 days, reported as "unpublished data given us by experienced observers," may occur in nature (Nielsen and Nielsen 1953), but other observations (loc. cit.) indicate that sometimes the total aquatic period may stretch to 9 to 12 days.

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Nielsen and Nielsen (1953) also reported that in one brood, observed in nature, most of the larvae died in a few days "because of drying breeding waters," but that a few larvae, which had survived in a pool, remained in instar IV for 12 days, then died when the pool dried out completely. W. L. Bidlingmayer (pers. comm.) has stated that after the emergence from a marsh near Vero Beach of a huge brood of adults, some larvae remained in the marsh and did not pupate. Some of these larvae were collected and given food, following which they pupated normally. This appears to be evidence that larvae of *Ae. taeniorhynchus* may sometimes face a food shortage in nature, but that larvae which fail to develop rapidly are exposed to an increased probability of death through desiccation. We therefore suggest that there may be a selective advantage to those larvae which succeeded in developing as rapidly as possible (5 to 6 days), that this is linked with an inability to delay larval development and that *Ae. taeniorhynchus* larvae have no effective method of dealing with food shortage. Any attempt to increase the probability of survival by prolonging the development period with the possibility of additional food being washed into the marsh by heavy rains in neighboring inland areas would also increase the probability of death through desiccation.

The habitats of larvae of *Cx. nigripalpus* in Florida were listed by Provost (1969) and those most favorable were indicated to be ditches, grassy swales, puddles and edges of ponds, marshes and swamps. The larvae appear to have much in common with those of *Ae. taeniorhynchus*. We have performed laboratory experiments at 27.5°C with 12 hrs of daylight in which we have varied (a) number of larvae, (b) container size and (c) food supply. The details of these experiments will be reported elsewhere. Under optimal conditions the majority of larvae pupated by day 7 or 8, while under sub-optimal conditions of food supply most larvae which did not succeed in pupating had died by day 21, and the absolute maximum period for which a larva survived (of several thousand observations) was 44 days. We therefore suggest that *Cx. nigripalpus* larvae have adopted a strategy similar to that of *Ae. taeniorhynchus*, i.e. to develop as rapidly as possible, perhaps because their preferred habitat may be semipermanent waters or perhaps because the advantages of prolonging their development period may be outweighed by other disadvantages such as increased risk of predation.

We have collected eggs of *Tx. r. rutilus* from the field and placed them in individual containers at 27.5°C with 12 hrs. of daylight. The resultant predatory larvae (several hundred observations) were fed on different diets of *Wyeomyia* larvae. The details of the observations will be reported elsewhere. Under high diet conditions a *Toxorhynchites* larva completed development to the pupal stage in 17 days (instar I: 3 days, instar II: 4 days, instar III: 2 days, instar IV: 8 days), while under identical conditions except for low diet another *Toxorhynchites* larva took 153 days to develop to the pupal stage (instar I: 3 days, instar II: 3 days, instar III: 13 days, instar IV: 134 days). Although these are extremes, we have a complete range of intermediate data.

Other *Toxorhynchites* individuals, collected from the field as larvae rather than as eggs, so that our data are incomplete for all larval instars, have taken even longer to develop through instars III and IV. Wigglesworth (1929) reported that a young larva of *Tx. brevipalpis* Theobald was maintained on a minimal diet for 5 months at 24°C. Paine (1934) noted that the mean development time of *Tx. splendens* (Wiedemann) larvae with abundant food was 16.5 days, but with reduced food 105 days. We point out that the *Toxorhynchites* larvae with prolonged development periods seemed not to be in a state of diapause, but remained capable of completing development when presented with an adequate food supply. It might be suggested that the minimum recorded development period (17 days) is longer than that of *Ae. taeniorhynchus* (5 to 6 days) or *Cx. nigripalpus* (7 to 8 days) because larvae of *Toxorhynchites* are larger than those of

Aedes or *Culex*, but by reference to the data on *Wyeomyia* (below) we argue that this is not the case because *Wyeomyia* larvae are considerably smaller than those of *Ae. taeniorhynchus* or *Cx. nigripalpus* but take 13 to 14 days to develop. We suggest that any selection pressure to evolve rapid larval development has operated minimally on *Toxorhynchites* and that instead the larvae have become adapted to developing slowly on a minimal or intermittent diet. The reason for selection pressure of this nature is that *Toxorhynchites* larvae are found in nature confined in small containers and may be exposed frequently to temporary food shortages, these temporary shortages being relieved by females of other mosquitoes (and other insects) ovipositing in the containers, thus initiating a new food supply. The containers inhabited by *Toxorhynchites* larvae probably contain water permanently or very nearly so and the *Toxorhynchites* larvae, predators themselves, are probably less exposed to predation by organisms of other species than are larvae of *Aedes* and *Culex*. Intraspecific competition between *Toxorhynchites* larvae ultimately takes the form of cannibalism instead of competition for other food sources, and self-protection from cannibalism may explain the observation (Corbet and Griffiths 1963) that *Toxorhynchites* larvae attack and kill other mosquito larvae in their vicinity just before pupation.

EXPERIMENTS WITH *WYEOMYIA*

Frank et al. (1976) have explained how the aquatic stages of *Wyeomyia* mosquitoes have been sampled from the leaf axils of the bromeliad *Tillandsia utriculata* L. in a study area near Vero Beach, Florida. The estimated density of *Wyeomyia* larvae has frequently exceeded 1 larva per ml of plant capacity. Food available to the larvae is provided by the breakdown of organic debris (leaves, seeds, twigs, etc.) falling into the leaf axils of the bromeliads and probably by the organisms (Protozoa, bacteria) assisting in the breakdown. If food shortage should occur, it would be expected to occur under conditions of high density of mosquito larvae. Under these circumstances rain or wind might provide additional food by causing debris from the tree canopies to fall into the leaf axils. Rain should also wash the leaves of the tree canopy and thus wash plant exudates into the leaf axils. If we were to hold *Wyeomyia* larvae at various densities under controlled conditions in the laboratory and simulate the effect of falling debris by adding leaves, we should be able to observe the response of the larvae.

We performed 4 experiments with larvae of *Wy. vanduzeei* in which we put newly-hatched larvae into water in petri dishes of diameter 9 cm and depth 2 cm which hold approximately 100 ml of water. We used filtered, purified well-water to which we added infusion from bromeliads containing Protozoa, filtered through glass wool. To each petri dish we added 1 g of dried oak leaves collected from the ground outside the laboratory. The petri dishes were held in an incubator with temperature controlled at 27.5°C and with a light regime of 12 hrs of light/12 hrs of darkness. The contents of the petri dishes were examined daily and *Wyeomyia* pupae were picked out by dropper and recorded. Petri dishes were topped up with water ca. every alternate day.

EXPERIMENT A. We put 5 *Wy. vanduzeei* larvae into the 1st petri dish, 10 into the 2nd and 50 into the 3rd. The results are recorded in Fig. 1. On the horizontal axis are recorded number of days from the beginning of the experiment, on the vertical bars the cumulative percentage of the initial number of larvae pupating in each petri dish. Thus in the 1st petri dish 3 pupae had appeared (and were removed) on day 11, a 4th pupa on day 12 and a 5th on day 13; the success of the larvae in pupating was 100% (Fig. 1: A 5 (100%)). In the 2nd petri dish the 1st 3 pupae had also appeared by day 11 and the last 3 by day 17; the success rate was also 100% (Fig. 1: A 10 (100%)). In the 3rd petri dish the 1st pupa appeared by day 15, a 2nd by day 33 and a 3rd by day 55. On day 60 most larvae were still alive and most were in

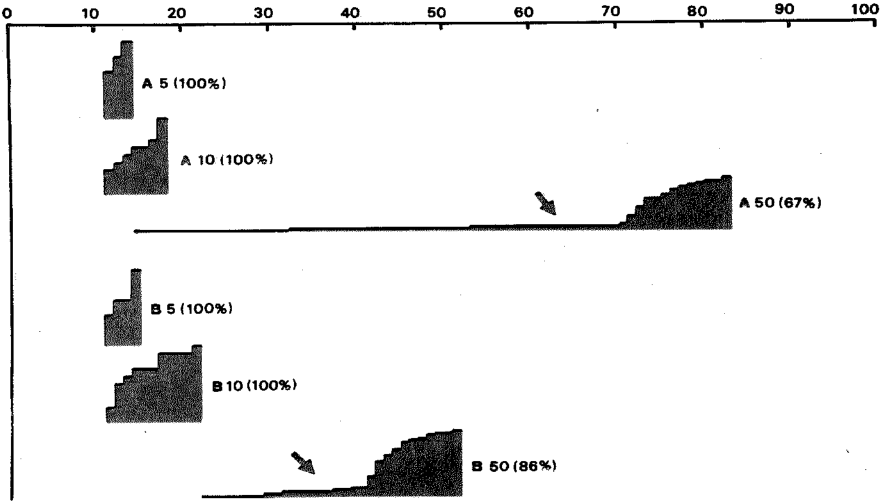

Fig. 1. Longevity and pupation success of *Wyeomyia vanduzeei* larvae when various numbers of larvae were crowded on the same quantity of food. The horizontal axis represents days from the beginning of experiments A and B. The vertical bars of each of the 6 treatments represent cumulative percentage of larvae pupated. The right vertical edge of each of the 6 blocks indicates the last day on which living *Wyeomyia* remained in each treatment, either because all larvae had pupated (and were removed) or because all larvae were dead. The arrows indicate addition of 1 g of oak leaves.

instar III with a few in instar IV. At this stage the larvae, which were evidently undergoing intraspecific competition, had greatly exceeded the survival time of *Cx. nigripalpus* under similar conditions (*Cx. nigripalpus* mostly dead by day 21, *Wy. vanduzeei* mostly still alive by day 60). By day 63 there still had been no more pupation and we added 1 g of oak leaves. By day 67 another larva had pupated and between days 71 and 83 a further 29 larvae pupated. The last surviving larva had pupated by day 83 (Fig. 1: A 50 (67%)). It seemed probable that the pupation response by day 67 and subsequently had been a result of the oak leaves we added on day 63.

EXPERIMENT B. The same numbers of *Wy. vanduzeei* larvae were added to 3 petri dishes as in Experiment A. The results in the first 2 petri dishes (Fig. 1: B 5 (100%) and B 10 (100%)) were almost identical to those of Experiment A with perhaps slight indication of greater competition because of delay in appearance of the last pupa in the 2nd petri dish until day 22. In the 3rd petri dish 4 pupae had appeared by day 35, at which time most larvae were in instar III, and we added 1 g of oak leaves. Pupation of the surviving larvae began by day 38 and finished by day 52 at which time no living larvae remained. Fully 86% of the initial number of larvae succeeded in pupating (Fig. 1: B 50 (86%)). It was obvious that the onset of pupation could be controlled by the addition of potential food.

EXPERIMENT C. Numbers of larvae added to the 3 petri dishes were respectively 5, 25 and 125. Although all larvae in the 1st dish (Fig. 2: C 5 (100%)) pupated, there was some delay in appearance of the last pupa. In the 2nd dish (Fig. 2: C 25 (60%)) the 1st pupa appeared by day 16, and 12 more pupae by day 35. In the 3rd dish no pupation had taken place by day 35. To each of the 2nd and 3rd dishes 1 g of oak leaves was added on day 35; this

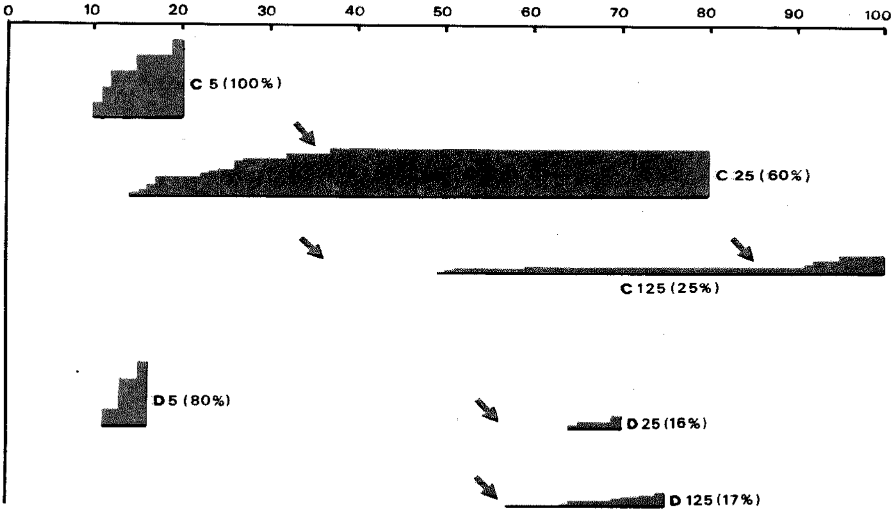

Fig. 2. Explanation is as for Fig. 1, but for experiments C and D.

seemed to have had little effect in the 2nd dish but appeared to initiate pupation (by day 50) in the 3rd dish (Fig. 2: C 125 (25%)). In the 2nd dish the last 2 pupae had appeared by day 38, although a few larvae survived longer, the last of these dying by day 81. In the 3rd dish only 5 pupae had appeared by day 85, at which time a further 1 g of oak leaves was added; subsequently 24 more larvae pupated between days 92 and 96, then no more although several larvae survived considerably longer, mostly in instar III, and the last larva survived until day 149. Prolongation of the larval period in the 3rd dish was remarkably long.

Experiment D. The same numbers of larvae were added to the petri dishes as in Experiment C. Four of the five larvae in the 1st dish (Fig. 2: D 5 (80%)) had pupated by day 16; the 5th larva died. No pupation had occurred in the 2nd and 3rd dishes by day 56, at which time 1 g of oak leaves was added to each dish. Larvae subsequently pupated and the last pupae were found in the 2nd dish by day 71 (Fig. 2: D 25 (16%)) and in the 3rd dish by day 76 (Fig. 2: D 125 (17%))); no living larvae were found in either dish subsequent to the appearance of these pupae.

A set of experiments identical to those with *Wy. vanduzeei* larvae was carried out with larvae of *Wy. medioalbipes*. The results were very similar with a slightly longer development period, e.g., with *Wy. vanduzeei* at 5 to a dish the average larva (19 observations) developed to the pupal stage in 13 days, while at 10 to a dish the average (20 observations) was 15 days; in the case of *Wy. medioalbipes* the equivalent figures were 17 days (16 observations) and 19 days (20 observations). Percentage survival was also slightly lower with *Wy. medioalbipes*, for while the survival figure for *Wy. vanduzeei* at 5 and 10 to a dish combined was 97.5% (40 observations) the equivalent figure for *Wy. medioalbipes* was 90% (40 observations). The difference in development periods between the 2 species were significant ($P < 0.01$ at both 5 per dish and 10 per dish) and probably reflects the greater size of *Wy. medioalbipes* larvae and the concomitantly greater nutritional requirements. Similar prolongation of the larval development period was obtained at higher densities of larvae, and it was possible to induce larvae to pupate following addition of potential food just as with *Wy. vanduzeei.*

In 1 replicate with an initial number of 50 larvae in a dish, additions of leaves had induced pupation on 3 occasions: some larvae were still alive after 159 days and addition of a further quantity of leaves induced the last remaining larva to pupate before day 173. Where prolongation of development occurred, at high larval densities, the majority of larvae was observed to be arrested in instar III. Conditions for the development of larvae of *Wy. medioalbipes,* as described above, were probably not optimal because measurable mortality occurred even with larvae at only 5 and 10 to a dish. Under better conditions the development period might be shortened slightly; J. K. Nayar (pers. comm.) has used an artificial diet for larvae of *Wy. medioalbipes* and informs us that under optimal conditions the average development period for the larvae at 27°C is about 14 days, i.e. very similar to that we obtained for *Wy. vanduzeei.*

CONCLUSIONS AND DISCUSSION

The average development periods for the larvae of *Ae. taeniorhynchus* and of *Cx. nigripalpus* at 27°C under optimal conditions are 5 to 8 days, while for *Wy. vanduzeei* and *Wy. medioalbipes* the development periods are 13 to 14 days at the same temperature, and for *Tx. r. rutilus* in excess of 17 days. Larvae of the first 2 species are not able to prolong their development period for much more than 21 days under sub-optimal nutritional regimes, while larvae of *Wyeomyia* and of *Toxorhynchites* may prolong the development period for many weeks or even months. Marcovitch (1960) reported that larvae of *Ae. aegypti* (L.), which occur in small, confined habitats, are also capable of prolonging their development for considerable time (in excess of 100 days) under a sub-optimal nutritional regime. We suggest that the physiological mechanisms regulating development of larvae of the first group (including *Ae. taeniorhynchus* and *Cx. nigripalpus*) may be different from those of larvae of the 2nd group (including the 2 *Wyeomyia* species, *Ae. aegypti* and *Toxorhynchites*) and that this difference may be maintained by selective pressures operating on the larvae in the environments they inhabit.

In the case of larvae of the 1st group the selective pressures may operate to promote rapid development because delay in development causes excessive exposure to unfavorable environmental factors such as desiccation and predation. In the case of *Wyeomyia* larvae, high densities of larvae may result in frequent temporary shortages of food which may be overcome by prolonging the development period to take advantage of additional food input. In the case of *Toxorhynchites* larvae, food shortages are probably seldom the result of high larval densities but instead are associated with the larvae being predatory and at the top of a food pyramid in a confining habitat; *Toxorhynchites* larvae also may prolong their development period to take advantage of additional food input.

It is interesting that we found delay in development of *Wyeomyia* larvae to occur principally in instar III under the experimental conditions. This observation may help explain relatively large numbers of instar III larvae we have found to occur in the natural population in our study area. It is also curious that the pitcher plant mosquito *Wy. smithii* Coquillett may diapause in southern Canada and the northern United States primarily in larval instar III (Smith and Brust 1971), secondarily in larval instar IV (Lounibos and Bradshaw 1975). However, we have no evidence that diapause of any type occurs in larvae of *Wy. vanduzeei* or of *Wy. medioalbipes.* The onset and termination of larval diapause in *Wy. smithii* are controlled by photoperiod (Smith and Brust, 1971; Lounibos and Bradshaw 1975); since day length in southern Canada is much more variable than at the latitude of our study area (27°) it is hardly surprising that such effects have not been observed in *Wy. vanduzeei* or *Wy. medioalbipes* which, along with the bromeliads they inhabit, are tropical and subtropical in distribution.

The experiments we described above were performed with small numbers of larvae since they were intended to demonstrate only that the larval development period may be prolonged under a suboptimal nutritional regime to support our hypothesis. They were not intended to form the basis for any precise logistic curve because we believe it to be more realistic to attempt to demonstrate such curves in the natural population in our study area. However, they do demonstrate that larvae under conditions of food shortage may be induced to pupate by the provision of additional potential food. Further, they suggest that the great majority of surviving larvae may be induced to pupate even if these larvae have been subject to a suboptimal nutritional regime for more than 80 days (173 days in the case of a *Wy. medioalbipes* larva). This is in marked contrast to the results obtained by Istock et al. (1975) working with *Wy. smithii;* these workers reported "the substantial survival of larvae out to the 53rd day" under suboptimal nutritional regimes, but that "the formation of pupae in all treatments stops well before the 53rd day" and "the 3rd instar larvae remaining at day 53 are definitely in a 'summer diapause' and unable to metamorphose," also, "nothing . . . suggests that [this] diapause can be broken as a response to increased food." The data obtained by Istock et al. (1975) suggest the evolution in *Wy. smithii* of a mechanism more complex than in *Wy. vanduzeei* and *Wy. medioalbipes* for survival through periods when food supply is inadequate: there is no evidence for a "summer diapause" in either *Wy. vanduzeei* or *Wy. medioalbipes*.

The ability of *Wyeomyia, Toxorhynchites* and *Ae. aegypti* larvae to survive periods of inadequate and intermittent food supply, either by nutritional diapause (*Wy. smithii*) or by nutritionally delayed development, suggests the action of a selective evolutionary pressure, especially when contrasted with the inability of larvae of *Cx. nigripalpus* and *Ae. taeniorhynchus* to do the same. This ability is probably analogous to that described by den Boer (1968) as spreading of risk in time.

It seems possible that larval nutrition is, in nature, a very important factor limiting population size in *Wyeomyia, Toxorhynchites* and *Ae. aegypti*, but that in *Cx. nigripalpus* and *taeniorhynchus* other factors (desiccation, predation) are of primary importance as natural regulatory agents.

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