DEVELOPMENT OF SEVERAL SPECIES OF MOSQUITO LARVAE IN FULLY DEFINED DIETARY MEDIA: PRELIMINARY EVALUATION.

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ABSTRACT. A completely chemically defined dietary medium previously shown to support good development of aseptic, newly-hatched larvae of *Culex pipiens* and *Aedes aegypti* to the adult stage is also able to support growth and development of 7 other mosquito species. *Cx. quinquefasciatus, Cx. peus, Ae. taeniorrhynchus,* and *Ae. sierrensis* performed well (60% or more of the starters became adult). *Cx. coronator* and *Cx. tarsalis* developed well to the 4th instar, but increased mortality then resulted in adult percentages of generally less than 40. *Ae. dorsalis* developed well to the 3rd instar, when most died. Most *Anopheles freeborni* died without development in the 1st instar; the few that moulted once died shortly thereafter.

INTRODUCTION

Recently we described a chemically defined dietary medium in which newly hatched larvae of *Culex pipiens* L. may be reared aseptically with good growth rates and high survival to the adult stage (Dadd and Kleinjan 1976). Although the resulting adults were feeble and short-lived, indicating some as yet unrecognized deficiency, we have been able to determine most of the specific dietary requirements of *Cx. pipiens,* notably for nucleotides (Dadd and Kleinjan 1977), water-soluble vitamins (Kleinjan and Dadd 1977), amino acids, sterols and inorganic components (unpublished data).

As the *Culex* medium was improved over the past 3 years, we tried it out from time to time with the important Californian disease vector, *Cx. tarsalis* Coquillett. With this species results have been only partially successful. Many newly-hatched larvae failed to grow, although surviving as 1st instars for long periods, and those that did develop grew substantially more slowly than in crude stock medium; hence overall development to the adult stage was generally no more than 20%. On the other hand, virtually the same medium was highly successful as a basis for studying the effect of dietary sugar on development rate of *Aedes aegypti* L.; for this species the medium was apparently essentially complete; after excellent larval growth and survival, resultant adults were long-lived, vigorous fliers, and the females were avid biters which produced viable eggs after a blood meal (Sneller and Dadd, 1977, in press).

We have now tested several other species, with results that encourage us to believe that this synthetic medium could be adapted for many mosquito species with only slight modification. Here we summarize the growth characteristics of the 10 species whose rearing in synthetic dietary media was attempted.

MATERIALS AND METHODS

THE MOSQUITOES. The species studied and the sources of their eggs are listed below. Because of difficulties in obtaining eggs of species not locally held in culture, in most cases only 1 or 2 trials were carried out.

Cx. pipiens L. Our main nutritional studies use an autogenous strain which has been continously maintained in this laboratory since its earlier use in studies of feeding behavior (Dadd 1970). It originated from a sewage farm population at Dixon, California.

Cx. quinquefasciatus Say. Eggs were provided by C. Schaefer from stock cul-

tures at the University of California Mosquito Laboratory at Fresno.

Cx. tarsalis Coquillett. Several wild-type strains collected from various locations in California (Knights Landing, West Poso Creek, Dewarts) are maintained at Berkeley by M. Asman and colleagues of our *Cx. tarsalis* genetics group. Various strains used for the several trials carried out with this species are noted in the table.

Cx. peus Speiser. Egg rafts were supplied by J. Hardy of the School of Public Health, Berkeley, from stocks maintained at the Naval Biological Research Laboratory, Alameda, California.

Cx. coronator Dyar and Knab. This species was maintained by H. Terwedow of this department from eggs collected in Brazil.

Ae. aegypti L. Stocks of "Beirut strain," now maintained in our laboratory, were obtained from M. M. Wong of the Primate Center, University of California, Davis.

Ae. sierrensis Ludlow. Egg papers were provided by S. Bennett from cultures maintained in the Department of Entomology, University of California, Davis.

Ae. taeniorhynchus (Wiedemann). Field-collected eggs were provided by C. Schaefer of the University of California Mosquito Laboratory, Fresno.

Ae. dorsalis (Meigen). J. Hardy provided eggs from the stocks of the Naval Biological Research Laboratory, Alameda, California.

An. freeborni Aitken. Eggs were deposited by field-collected gravid females supplied by R. Washino, Department of Entomology, University of California, Davis.

EGG STERILIZATION, DIET PREPARATION AND GROWTH TRIALS. Eggs of *Cx.* spp. and *An. freeborni* were surface-sterilized before hatch with 0.2% Hyamine X10 as described by Dadd et al. (1973). Surface sterilization of *Ae. spp.* eggs with a mixture of Hypochlorite, Roccal and water in proportions of 2:1:7 and their subsequent hatching using reduced pressure were accomplished as described by Sneller and Dadd (1977).

Synthetic dietary medium having the composition shown in Table 1 of Dadd and Kleinjan (1976) was used for all *Cx.* spp., *Ae. taeniorhynchus, Ae. dorsalis, Ae. sierrensis,* and *An. freeborni.* The medium used for *Ae. aegypti* differed slightly, having all nucleotides reduced to a half the concentrations given and glucose doubled. Medium was prepared, dispensed to culture tubes (5 ml per 25 mm diameter tube) and autoclaved as described elsewhere (Dadd et al. 1973; Dadd and Kleinjan 1976). Newly-hatched larvae from surface-sterile eggs were inoculated, 2 or 3 per tube, using a wire loop, and the tubes incubated at 27°C. On the 3rd day after inoculation and then every other day, all tubes were examined and the stage of development recorded; once pupation started, daily records were kept until all individuals had become adult or had died.

DEVELOPMENTAL PARAMETERS. The following were excluded from consideration in calculating developmental parameters: 1) All larvae in tubes which at any time showed contamination by bacteria or molds. 2) All larvae that died as 1st instars by day 5, it being assumed they were injured during egg sterilization or subsequent handling. 3) All larvae remaining alive in the 1st instar when developing larvae from the same run were, on average, 2 molts ahead; such larvae sometimes lived for 2–3 weeks without apparent growth, and were presumed to be in an anomolous, quiescent, physiological state. Quiescent larvae generally were infrequent, but as they occurred in rather substantial numbers for some species (e.g., *Cx. tarsalis*) their numbers are given in table 1. It should be emphasized that they were not included among starting larvae for the calculation of developmental criteria.

Four criteria of development are listed in table 1. Three of these, average number of molts per starting larva, percentage of starters to pupate, and percentage adults, give an indication of the amount of development achieved, regardless of rate of development. The fourth, average time (days) to pupation, provides a convenient index of the rate of development.

Table 1. Developmental criteria for several species of mosquitoes reared from aseptic, newly-hatched larvae in a chemically defined synthetic dietary medium.

Species (strain*)	Number of starters	Average # molts per starter	% pupation	% adults	Average days to pupation	% of quiescent 1st instar
Culex pipiens						
(a)	21	4.71	95	81	11.8	none
(b)	19	4.39	84	71	11.6	none
(c)	21	4.62	95	76	12.6	none
(d)	18	4.94	100	95	12.4	2
(e)	10	4.78	80	80	11.0	none
(f)	19	4.21	84	63	12.0	none
quinquefasciatus						
(a)	15	4.67	93	73	11.4	none
(c)	18	4.39	83	67	13.0	1
peus						
(b)	17	3.76	65	59	12.1	none
coronator						
(d)	19	2.61	37	29	13.4	3
tarsalis						
(cross) (b)	12	1.63	25	nil	18.3	4
(KL) (e)	16	2.82	36	4	14.4	2
(WPC) (f)	11	4.00	64	55	16.4	none
(KL)	14	3.79	71	43	13.4	none
(KL)	7	3.00	43	14	14.7	6
(DW)	9	3.61	78	28	18.1	12
(DW)	8	2.88	50	25	17.3	9
(WPC)	8	3.31	50	31	16.0	2
Aedes aegypti						
	20	4.85	95	95	10.3	none
	22	5.0	100	100	11.6	none
	17	4.76	94	94	12.3	1
taeniorhynchus						
(a)	14	4.57	93	64	15.3	1
sierrensis						
(a)	28	4.89	100	89	15.3	4
(b)	7	3.43	71	57	16.0	3
dorsalis						
(b)	18	1.78	6	nil	30 (1 pupa only)	none
Anopheles freeborni						
(b)	10	0.30	nil	nil	....	none

* KL: Knights Landing; WPC: West Poso Creek; DW: Dewarts.
(a) (b) etc. indicate tests using same batch of medium.

Tests of all species, excepting *Ae. aegypti* and some runs of *Cx. tarsalis*, were run in parallel with *Cx. pipiens* using the same lots of medium, and these correspondences are indicated in Table 1. To allow some comparison of performances, representative developmental data for *Ae. aegypti* in its slightly different medium are taken from separate experiments in progress over the same period.

RESULTS AND DISCUSSION

Developmental criteria for the 10 species studied are presented in Table 1. Nine species grew and developed well or to some extent. However, the medium was virtually a complete failure with *An. freeborni*; although inoculated 1st instars survived to the 5th day, most died trying to molt, and 3 which molted to 2nd instars died soon thereafter.

For all 5 species of *Culex*, at least a few individuals were able to develop to adults, but only for *pipiens, quinquefasciatus* and *peus* could development be considered good—that is, with an average of about 4 molts or more per starter, and with a majority of starters becoming adult. The 2 trials of *quinquefasciatus* indicate performances equally as good as for *pipiens*, as might be expected for very close taxa considered by some to differ only subspecifically. The several trials of *tarsalis,* carried out over a period of 3 years, are notable for wide variability in outcome. This variability attaches mainly to mortality at pupation and adult emergence since the average molts achieved per starter indicate that if larval development commenced at all, it usually continued until the 4th instar with little mortality. Many trials of *tarsalis* were characterized by high proportions of quiescent 1st instars, contrasting markedly with our extensive data on *pipiens* showing that only an occasional larva enters a quiescent condition, perhaps 2% of all individuals in the several hundred runs using this basic medium. No evidence is at hand to indicate the cause of this developmental stasis in *tarsalis* or to a lesser extent in the other spp. In view of recent findings that mosquito development can be sensitive to day-length effects on larval stages (Sanburg and Larsen 1973), we wonder whether it may be related to the absence of light in our incubator, where the larvae were in complete darkness except for the brief period every few days when taken out for recording. The single test with *coronator* suggests that it resembles *tarsalis* in its response to our medium. Several 1st instars remained quiescent, and of those that developed, a majority became 4th instars before heavy mortality reduced survival to the adult stage to about a third of the starters.

Although the data for *Cx. pipiens* given in table 1 (selected because these particular tests were run at the same time and used the identical batches of medium prepared for various of the tests with other species) indicate average pupation times of 11–12 days, in the generality of our work with *pipiens*, pupation started on day 9 and averaged 10–11 days. In crude culture medium (based on rat pellet/liver extract as starter food), pupation begins on day 7 and averages 8–9 days, while the literature (e.g., Nayar and Sauerman 1970) indicates that under optimal, precisely-controlled conditions even faster development may be attained, with pupation commencing on day 5 and averaging 7–8 day. Thus, compared with normal crude rearings, pupation time for *pipiens* in synthetic medium is 10–40% delayed (depending on what is considered "normal"). A recent compendium of stock rearing methods for many mosquito species (Gerberg 1970) gives larval developmental periods of approximately 7 days for *pipiens* and the other *Culex species* here tested in synthetic diet; since our data indicate pupation times for *peus, quinquefasciatus* and *coronator* similar to those for *pipiens*, they apparently undergo a similarly moderate developmental retardation in synthetic medium. By contrast, *tarsalis* was markedly slower to pupate in synthetic medium than the 4 other species; if the normal pupation time for this species is also about a week at 28°C, long-delayed pupation provides another crite-

rion of the relatively poor performances of this species in synthetic medium.

Of the 4 *Aedes* species tested, 3 achieved good development but one, *dorsalis*, failed, virtually completely, after an initial 2 instars of good growth. *Ae. aegypti*, for which we have considerable data beyond the examples given in table 1, consistently outperforms *Cx. pipiens* in terms of all the developmental criteria considered, and its larval performance in synthetic medium is inferior to crude culture only in terms of a slightly increased pupation time as compared with the best examples to be found in the extensive literature for this species. Most notably, and in contrast to *Cx. pipiens*, synthetic diet-reared adults are long-lived and viably reproductive (Sneller and Dadd 1977). The data for *Ae. taeniorhynchus* and *Ae. sierrensis* indicate comparably good ultimate development, in terms of average molts achieved and high percentages of pupation and adult emergence. However, these 2 species took 50% longer than *Ae. aegypti* to pupate, so assuming their normal pupation time should be comparable to that for *Ae. aegypti*, as Gerberg (1970) indicates, they must be considered less successful in synthetic diet than *Ae. aegypti*. This conclusion is supported by the inability of emerging adults to free themselves from the slightly sticky surface of synthetic medium in these few tests, even though they were evidently more vigorous than any of the diet-reared *Culex* adults.

Of the several species of *Culex* and *Aedes* that developed well to adults, only adults of *Ae. aegypti* were vigorous and long-lived like adults reared in normal crude culture. Currently, we are obtaining vigorously flying adults of *Cx. pipiens* by augmenting our basic medium with minute amounts of various lipid materials of animal origin, such as mammalian serum lipoproteins or crude animal lecithins. This suggests that the diet for *Cx. pipiens* lacks an unknown growth factor which is not required by, or is adequately transmitted in the normal egg of *Ae. aegypti*. If this is the case, it seems likely that all other species studied in this work would, like *Cx. pipiens*, require the growth factor in their first generation on synthetic diet.

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