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EFFECT OF FLUCTUATING AND CONSTANT TEMPERATURES ON DEVELOPMENT, ADULT LONGEVITY AND FECUNDITY IN THE MOSQUITO *AEDES KROMBEINI*

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Abstract—1. The effects of fluctuating (16–12°C, 22–14°C, 26–18°C, 30–22°C, 33–27°C) and their mean constant temperatures (14, 18, 22, 26, 30°C, and also 33.5 and 35°C) on development, adult longevity and fecundity in *Aedes krombeini* were studied.

2. The developmental times at constant and fluctuating temperatures were statistically different in males ($P < 0.01$), whereas in females, they were not statistically different ($P > 0.05$).

3. Longevity times at constant and fluctuating temperatures were statistically different in both, males and females ($P < 0.01$).

4. The fecundity values at constant and fluctuating temperatures were also statistically different ($P < 0.01$). Copyright © 1996. Elsevier Science Ltd.

Key Word Index: Aedes krombeini; development; fecundity; longevity; temperature

INTRODUCTION

Several physiological processes in ectothermic organisms such as insects are influenced by environmental temperature. In nature, the insects are exposed to daily temperature cycles, the warm phase (thermophase) of which coincides with the photophase of the photoperiod, and the cool phase (cryophase) coincides with the dark phase (scotophase). There is voluminous literature describing the effects of temperature on insect growth, behaviour, fecundity, general physiology and ecology (reviews by Hagstrum and Hagstrum, 1970; Bursell, 1974; Beck, 1983).

The influence of constant temperature on larval development has been studied in several species of mosquitoes. For example, in *Aedes quadrimaculatus* (Huffaker, 1944); in *A. aegypti* (Bar-Zeev, 1958, Gilpin and McClelland, 1979); in *A. taeniorhynchus* (Nayar, 1967); in *Toxorhynchites brevipaplis* (Trpis, 1972) and in *Wyeomiya smithii* (Bradshaw, 1980). When the mosquito larvae were maintained at constant temperatures, their rate of development was usually directly proportional to the temperature imposed, within a certain range of temperature for that species (Clements, 1992).

Differences between the effects of fluctuating and constant temperatures on larval stages of various mosquito species have also been studied (Headlee, 1942; Huffaker, 1944; Brust and Kalpage, 1967; Bradshaw, 1980; Milby and Meyer, 1986). The rate of development may be faster or slower at a given thermoperiod than at an intermediate constant temperature, depending on the duration of the thermophase or cryophase (Clements, 1992).

Thus, there is wealth of experimental data on the relationship between temperature and larval development in mosquitoes; however, studies pertaining to the effects of temperature on longevity and fecundity in mosquitoes are few. In the adults of *A. taeniorhynchus*, for example, Nayar (1972) reported the effects of temperature on longevity. Similarly, in the pitcher-plant mosquito *Wyeomiya smithii*, the effects of fluctuating and constant temperatures on development and fecundity were investigated (Bradshaw, 1980).

The present report aims to study the effects of fluctuating and constant temperatures on the duration of development from hatching to eclosion, adult longevity in males and females, and the fecundity in the tropical mosquito *A. krombeini*. This investigation is an extension of earlier studies on longevity and period of light–dark cycles in *A. krombeini* (Joshi, 1994).

MATERIALS AND METHODS

The colony of *A. krombeini* for the present study started from one virgin female and one male collected as pupae in a remote hamlet of the coastal area of Maharashtra State, India, so that the genetic and physiological variabilities were minimized in the experimental insects. The method for rearing the larvae and adults of this mosquito was described by Joshi (1994).

Programmable environmental test chambers were used for maintaining the desired experimental temperatures. The following five fluctuating and seven constant temperatures were employed: (a) 16–12°C and mean 14°C; (b) 22–14°C and mean 18°C; (c) 26–18°C and mean 22°C; (d) 30–22°C and mean 26°C; (e) 33–27°C and mean 30°C; and (f) constant temperatures 33.5°C and 35°C.

During thermoperiods, the temperature transitions were essentially complete within 18 min after switching on, and the new changed temperature was established in less than 30 min. In all experiments the light–dark 12:12 h cycles were imposed with 12 h of fluorescent light (Philips tube light, 6500 K, 20 W, 240 V) of *ca* 150 lux, and 12 h of absolute darkness. The photoperiod was from 06:00 h to 18:00 h. In all thermoperiods, the thermophase coincided with photophase, and the cryophase coincided with the scotophase.

Eggs used for the present experiments were obtained from the laboratory colony maintained for 1 year in light–dark 12:12 at 25°C and 80% relative humidity (r.h.). The freshly laid eggs on wet pieces of coconut shell were incubated at 25°C and 80% r.h. for 3 days and then stored in an air-tight desiccator in the same room until needed. Such fully embryonated eggs, when immersed in water, hatched simultaneously within 10 min, at water temperatures ranging from 25°C to 35°C. The eggs were about 1 month old, and they were immersed in the water for hatching at the onset of lights on, i.e. at 06:00 h in all experiments. Dependent on the water temperature, they hatched immediately or within 60 h from immersion; nevertheless, all eggs for one particular experimental temperature hatched simultaneously within 10 min.

Using small pipettes, 100 larvae were transferred to 500 ml of water in an enamelled trough, and four such troughs, i.e. 400 larvae, were used for each experiment. The developmental time from hatching to eclosion was determined for each group as follows.

As soon as pupae were formed, 50 of them were removed with a pipette and transferred to a beaker of water. Twelve of these beakers were kept in a Nylon netting cage (60 × 60 × 60 cm) for eclosion of adults. The eclosion events were recorded at 2 h intervals till 50% of the adults were eclosed.

The longevity in days for adults was determined as follows. After eclosion, males and females were separated and maintained as 40 adults per plastic container (1.5 l vol.) at 80% r.h. in the environmental test chambers. Six of these containers for each gender were maintained at the desired temperature for each group, and the containers were perforated for ventilation. The dead mosquitoes were counted daily. Longevity was determined for each gender by counting the number of days after which only 50% adults were still alive. Females used for the determination of longevity were not given blood-meals. The detailed procedure for the maintenance of adults and the determination of longevity was described by Joshi (1994).

The fecundity for each group was determined as follows. Ten freshly eclosed virgin females and 30 males were confined in a Nylon netting cage. Six of these cages for each group were maintained in light–dark 12:12 h cycles at the desired temperatures and 80% r.h. The females were offered a pigeon for blood meals three times in a week. It was always made certain that there were 30 males in the cage by replacing the dead ones, since the males always died earlier than the females. The eggs laid on wet pieces of coconut shell were counted every day. Thus, the number of eggs laid by all females during their entire lifespan was totalled, and the means with SE were calculated for a group of 10 females.

RESULTS AND DISCUSSION

The duration of development, adult longevity and fecundity of *A. krombeini* reared at constant and fluctuating temperatures are summarized in Table 1. The data were subjected to statistical analysis using the method developed by Connolly (1981). Third-degree polynomial was fitted using the Lotus 1-2-3 software package. The null hypothesis, that the response pattern remains the same regardless of whether temperature was constant or fluctuating, was tested using a standard procedure in regression analysis. Here the polynomial was fitted to data of constant and fluctuating temperatures separately, and then to the combined data. The difference between the two indicates rejection of null hypothesis. It was checked using an F-test. For brevity, only the results of the combined data are given here. The following regression equation was used for analysing the data:

$$1/D = B_0 + B_1T + B_2T^2 + B_3T^3.$$

When the combined data of developmental time of males at constant and fluctuating temperatures were fitted, the values of constants were as follows:

$B_0 = 0.329087$
$B_1 = -0.05563$
$B_2 = 0.003105$
$B_3 = -0.00004.$

This shows that the developmental times of males at constant and fluctuating temperatures were statistically different ($P < 0.01$).

When the combined data of developmental time of females at constant and fluctuating temperatures were fitted, the values of constants were as follows:

$B_0 = 0.138794$
$B_1 = -0.02420$
$B_2 = 0.001455$
$B_3 = -0.00001.$

This shows that the developmental times of females at constant and fluctuating temperatures were not statistically different ($P > 0.05$).

When the combined data for adult longevity in males at constant and fluctuating temperatures were fitted, the values of constants were as follows:

$B_0 = -280.482$
$B_1 = 28.91950$
$B_2 = -0.66553$
$B_3 = 0.001735.$

This shows that the adult longevity in males at constant and fluctuating temperatures was statistically different ($P < 0.01$).

Similarly, when the combined data for the female longevity at constant and fluctuating temperatures were fitted, the values of constants were as follows:

$B_0 = -236.844$
$B_1 = 20.90048$
$B_2 = -0.19654$
$B_3 = -0.00620.$

This also shows that the adult longevity in females at constant and fluctuating temperatures was statistically different ($P < 0.01$).

When the combined data for fecundity at constant and fluctuating temperatures were fitted, the values of constants were as follows:

$B_0 = -27638.7$
$B_1 = 1866.763$
$B_2 = 28.24893$
$B_3 = -1.73777.$

This shows that the fecundity at constant and fluctuating temperatures was statistically different ($P < 0.01$).

The critical low temperature for egg hatching is 16°C. Only *ca* 9% and *ca* 25% of the eggs hatched at 14°C and 15°C, respectively, after 50 h of immersion; but there was 100% mortality among newly hatched larvae. However, the larvae that had hatched at 26°C and were maintained at the same temperature for 30 h, and then transferred as early II instars to 14°C, developed very slowly (Table 1) and pupated; only *ca* 6% of the females and *ca* 80% of the males eclosed. Such adults did not move from the water

Table 1. Duration of development from hatching to eclosion (50%), adult longevity in days (50% alive) and fecundity (no. of eggs/10 females) in *Aedes krombeini* maintained at constant and fluctuating temperatures

Temperature (°C)	Developmental time (days) Males Mean	±SE	Females Mean	±SE	Adult longevity (days) Males Mean	±SE	Females Mean	±SE	Fecundity (eggs/10 females) Mean	±SE
Experiment I: Results of constant temperatures										
14[a]	29	2.71	39	6.27	2	0.29	4.0	0.15	0	0
18	21	2.28	24	2.83	18	1.9	23	1.8	1027	9.8
22	7.4	0.75	10.3	0.76	56	3.46	64	5.58	8430	159.6
26	5.1	0.36	6.3	0.71	56	2.48	70	7.23	10,090	31.3
30	4.6	0.42	5.0	0.36	22	2.14	29	2.1	3970	66.25
33.5	4.1	0.47	5.5	0.35	2	0.09	3	0.7	0	0
35[b]	4.0	0.68	4.0	0.49	1	0.03	0	0	0	0
Experiment II: Results of fluctuating temperatures										
16–12	21	1.7	26	1.81	4.0	0.61	5.06	0.39	0	0
22–14	16	2.05	18	2.62	26	2.11	32.8	4.0	4850	50.83
26–18	8.3	1.39	9.3	0.83	64	4.1	71	5.95	10,650	220.4
30–22	4.6	0.49	7.1	0.43	64	4.02	81	3.79	12,600	363.0
33–27	4.1	0.41	5.2	0.41	29	3.18	41	3.7	6140	24.12

[a] Larvae hatched and maintained at 26°C and then transferred to 14°C as early second instars.
[b] Duration up to pupal development, the females did not eclose.

surface, where they eclosed and died without taking food.

The critical higher temperature for survival and fecundity was between 30 and 33.5°C. At 30°C, the longevity and fecundity were drastically reduced compared to those at 26°C ($P < 0.01$). At 33.5°C, the longevity for females was reduced to *ca* 3 days and fecundity was 0. At 35°C, only males completed development, and they eclosed as weak adults and died in *ca* 1 day, whereas the females completed the development up to pupal stage and died without eclosing. In *Culiseta inornata* (Diptera: Culicidae), very high mortality also occurred among late instar larvae and pupae above 21°C (Hanec and Brust, 1967).

The development of mosquito larvae takes place within a fairly narrow range of temperature, and within this range, it is positively correlated with temperature (Clements, 1992). The development from hatching to adult eclosion in *A. krombeini* occurred only within a narrow range from 22–14°C to 33–22°C; and constant temperatures from 18 to 30°C. The development was protracted at lower temperatures, for example, *ca* 39 days at 14°C, whereas it was rapid at higher temperatures, for example, only *ca* 5 days at 30°C in females (Table 1). Similarly, *A. taeniorhynchus* completed larval development in *ca* 144 h at 22°C, and in just 72 h at 32°C (Nayar, 1967).

In the present study, the development and adult survival of *A. krombeini* were possible, although to a lesser extent, at fluctuating temperatures 16–12°C or at constant temperatures 14, 33.5 and 35°C, but reproduction did not take place at all. The females did not attempt to take blood-meals, nor did the males attempt to mate. Reproduction in this mosquito appears to take place in the narrow range of fluctuating temperatures from 22–14°C to 33–27°C, and constant temperatures from 18 to 30°C only (Table 1). Incidently, in the coastal area of western India, where these mosquitoes are found, the winter and summer temperatures are near the values of the experimental temperature mentioned above (Joshi, unpublished observation).

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