# LABORATORY REARING TECHNIQUES AND ADULT LIFE TABLE PARAMETERS FOR *ANOPHELES SERGENTII* FROM EGYPT[1]

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ABSTRACT. Techniques are presented for maintaining colonies of *Anopheles sergentii*, an important malaria vector in Egypt. Larval development time and survival rates were determined for 3 rearing solutions and 4 temperatures. Under optimal conditions larval survival rates averaged 85%. Mean life expectancy at emergence for mated *An. sergentii* was 23.3 days under insectary conditions of 27 ± 2°C, 70–80% R.H. The net reproductive rate, mean generation time and instantaneous rate of increase were respectively, 45.8 females per female per generation, 29.7 days and 0.127. In the context of vector potential for malaria transmission, *An. sergentii* has a daily survivorship rate of 0.95.

## INTRODUCTION

*Anopheles sergentii* (Theobald) is an important malaria vector throughout North Africa and the Middle East (Farid 1956). Recent malaria studies in Egypt have reexamined the ecology and host feeding behavior of this species in the Western Desert oases and in Faiyum Governorate (El Said et al. 1986; Kenawy et al. 1986a, 1986b; Beier et al. 1987). These studies raised questions concerning the vectorial capacity of *An. sergentii* for malaria transmission and indicated a need for corresponding laboratory studies.

Difficulty in colonization of *An. sergentii* has precluded detailed life history studies, although limited observations have been made on field-collected specimens (Saliternik 1955). Repeated colonization attempts have been unsuccessful, since this species is eurygamous and does not mate readily under laboratory conditions. Recently, Beier et al. (1986) reported the successful maintenance of 3 colonies for over 1 year employing force-mating techniques. This report describes experiments to improve colonization procedures and to determine life table parameters under insectary conditions.

## MATERIALS AND METHODS

Anopheles sergentii strain: A colony of *An. sergentii* was started with 400 larvae collected in Faiyum Governorate (Sinnuris District) in October 1983 and was maintained by force-mating techniques and rearing procedures described by Beier et al. 1986. Experiments were done with generations $F_2$–$F_8$. The insectary was maintained at 27 ± 2°C, 70–80% R.H., with illumination by fluorescent lighting for ca. 8 h daily.

*Effects of water types on larval development:* Three types of water were evaluated to determine the effects on larval survival and pupation time: tap water, distilled water and a mud slurry (2 kg clay soil stirred in 50 liters tap water; water was used for larvae after aging for 2 days). Replicates of thirty 1st-instar larvae were reared at 27°C in 30 cm diam round enamel pans containing 1 liter of water. Larvae were fed ground dog food pellets (fat-free) daily as a powder on the water surface. Water in pans was stirred daily to control scum formation and evaporated water was replaced as needed to maintain the 1 liter volume. Pupae were counted and removed daily from pans.

*Immature development at four temperatures:* The development and survivorship of immature *An. sergentii* was observed at 4 temperatures: 17, 22, 27 and 34°C. For each temperature, 8 replicates of fifty 1st-instar larvae were reared in 30 cm diam pans filled with 1.8 liter mud slurry solution. Larvae were fed as described above. Pupae were removed daily from rearing pans and placed in 400 ml screened emergence cups, separated by pan and date. Adults were counted by sex, and median pupation ($P_{50}$) and emergence ($E_{50}$) times were calculated.

*Gonotrophic cycles:* The length of successive gonotrophic cycles (gc) was determined as the time from blood feeding to oviposition. Force-mated, blood fed females (3–4 days old) were placed individually in 400 ml screened cartons lined with filter paper and containing 100 ml distilled water. Groups of mated females at 4 temperatures (17, 22, 27 and 34°C) were observed for 30 days. Eggs were collected and counted daily. Females were provided 5% sugar solution on cotton (changed daily) and after the first oviposition, females were allowed to refeed on human blood at 2-day intervals.

*Oviposition substrates:* Three replicates of 25 newly mated blood fed females held in 30 $cm^3$

[1] This study was supported by research contract No. NO1 AI 22667/NIH-NIAID, entitled: "Epidemiology and Control of Arthropod-Borne Diseases in Egypt" between Ain Shams University, Abbassia, Cairo, Egypt and the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA.

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screened cages, were tested to determine oviposition preference for either a shallow plastic bowl (12 cm diam) lined with filter paper and containing distilled water or a petri dish (10 cm diam) with moist cotton and covered by a filter paper. Females were maintained on sugar solution and were provided human blood at 3 day intervals. Eggs were collected daily over a 13 day period.

*Egg hatching rates:* Eggs from individual females (<10 days of age) used in gonotrophic cycle experiments were counted and placed in 200 ml paper cups lined with filter paper and containing distilled water. Hatched 1st-instar larvae were removed and counted daily up to 6 days. Egg hatching duration and rates were determined for replicates held at 17, 27 and 34°C.

*Adult survivorship and life table:* For life table studies (at 27 ± 2°C), 7 replicates of 20 blood fed, force-mated females (3–4 days old) were placed in 30 $cm^3$ screened cages. They were provided with a plastic oviposition bowl (12 cm diam) containing distilled water, and 5% sugar solution on a 5 x 10 x 3 cm sponge suspended inside the cage. Oviposition bowls and sugar were changed daily and females were offered a human blood source daily. Each morning all dead adults were removed and counted. Life table parameters were calculated according to Reisen and Mahmood (1980) and included: mean life expectancy ($e_x$), net reproductive rate ($R_o$), mean time for cohort reproduction ($T_o$), intrinsic rate of increase ($r_m$), mean generation time (G) and age-specific survivorship (s).

Age-specific survivorship and life expectancies were also determined at 27°C for 3 replicates of 25 males, 25 unfed females, and 25 fed, unmated females which did not receive additional blood meals after cohorts were established. Two replicates of the same groups were observed at 17°C.

## RESULTS

*Effects of water types on larval development:* The mud slurry solution provided better larval rearing conditions than tap or distilled water (Table 1). Larvae reared in the mud slurry pupated significantly faster (3 and 5 days earlier) than in tap and distilled water, respectively (Model I ANOVA, df = 1, P < 0.05). The survival rate of 0.82 for larvae reared in the mud slurry was also significantly higher than the other 2 groups (Model I ANOVA, df = 1, P < 0.05).

Table 1. Comparison of 3 water types for rearing *Anopheles sergentii* larvae.

| Water type | No. of replicates | Mean pupation time (days ± S.D.) | Larval survival (rate ± S.D.) |
|---|---|---|---|
| Tap | 10 | 13.2 ± 2.3 A* | 0.56 ± 0.21 A |
| Distilled | 8 | 15.2 ± 2.2 A | 0.37 ± 0.25 A |
| Mud slurry | 13 | 10.4 ± 0.4 B | 0.82 ± 0.15 B |

* Values with the same letter, vertically, are not significantly different, Model I ANOVA, df = 1, p < 0.05.

*Immature development at four temperatures:* Seven developmental attributes were examined for 8 replicates of *An. sergentii* reared at 4 temperatures (Table 2). Survivorship from 1st-instar to adult emergence was over 80% at 22 and 27°C, 63% at 17°C and less than 50% at 34°C. Development to pupation ($P_{50}$) was fastest at 34°C (9.15 days) but survivorship in the pupal stage at this high temperature was only 37%. Survivorship from the pupal stage to adult emergence was over 95% for the 3 lowest temperatures. Median adult emergence times ranged from 11 days at 34°C to 28 days at 17°C. Similar emergence times were observed for both sexes at each temperature. Sex ratios were similar for all temperatures.

*Gonotrophic cycle:* The length of time from blood feeding to oviposition was determined for individual females at 4 temperatures (Table 3). The duration of the 1st gonotrophic cycle ranged from 4.2 days at 27°C to 11.8 days at 17°C; 2nd and 3rd gonotrophic cycles were shorter at each temperature. During this experiment only about half of the force-mated females oviposited and difficulties were encountered in refeeding.

*Oviposition substrates:* Newly mated blood fed females, tested in 3 groups of 25 individuals, preferred to oviposit in plastic bowls with distilled water. Over 10 days an average of 651 ± 219 eggs were collected from the plastic bowls but no eggs were collected from petri dishes with moist filter paper. On days 11–13, the plastic bowls were removed to determine if the females would oviposit in the petri dishes. On days 12 and 13, 51 eggs were collected from the petri dishes.

*Egg hatch rates:* Egg hatch rates were over 85% at 17 and 27°C, but no eggs hatched at 34°C (Table 4). Eclosion was synchronous at 27°C, with 95% hatching on day 2. At 17°C, most eggs hatched on days 4 and 5.

*Adult survivorship and life table:* Life table parameters were determined for 7 replicates of 20 females each at 27°C. The mean life expectancy ($e_x$) was 23.30 ± 3.90 days. The age-specific survivorship (s) was 0.95 ± 0.20; s was calculated by the regression of the ln of $l_x$ on x and the mean coefficient of determination ($r^2$) was 0.74. The net reproductive rate ($R_o$) was 45.81 ± 13.56 females per female per generation (a, the mean proportion of females that survived from egg to adult emergence averaged 0.85 for *An. sergentii*, and p, the proportion of offspring that were

Table 2. Immature developmental attributes of *Anopheles sergentii* reared in a mud slurry solution at four temperatures (N = 8 replicates of 50 larvae per temperature).

| Attribute | Mean ± S.D. by temperature (°C) | | | |
|---|---|---|---|---|
| | 17 | 22 | 27 | 34 |
| Survivorship, L1 to P* | 0.63 ± 0.08 A** | 0.86 ± 0.12 B | 0.85 ± 0.14 B | 0.46 ± 0.15 C |
| $P_{50}$ | 24.29 ± 0.77 A | 14.75 ± 0.85 B | 10.24 ± 0.34 C | 9.15 ± 0.56 D |
| Survivorship, P to A | 0.99 ± 0.21 A | 0.95 ± 0.04 A | 0.99 ± 0.01 A | 0.37 ± 0.15 B |
| $E_{50}$ | | | | |
| ♀♀ | 28.72 ± 1.09 A | 17.19 ± 0.80 B | 12.09 ± 0.65 C | 11.33 ± 0.82 C |
| ♂♂ | 28.29 ± 0.96 A | 17.07 ± 0.92 B | 12.23 ± 0.48 C | 11.26 ± 0.92 D |
| Survivorship, L1 to A | 0.63 ± 0.08 A | 0.83 ± 0.12 B | 0.85 ± 0.14 B | 0.37 ± 0.15 C |
| Sex ratio (No. males/total) | 0.50 ± 0.14 A | 0.53 ± 0.13 A | 0.53 ± 0.07 A | 0.43 ± 0.16 A |

* L1 = first-instar larvae, P = pupae, A = adult, $P_{50}$ = median time (days) to 50% pupation, $E_{50}$ = median time to 50% adult emergence.

** Means with the same letter, horizontally, are not significantly different, Model I ANOVA, df = 1, $p < 0.05$.

Table 3. Duration of *Anopheles sergentii* gonotrophic cycles (time from blood-feeding to oviposition) at 4 temperatures.

| Temperature (°C) | 1st gonotrophic cycle | | 2nd gonotrophic cycle | | 3rd gonotrophic cycle | |
|---|---|---|---|---|---|---|
| | ♀ | Mean days ± S.D. | ♀ | Mean days ± S.D. | ♀ | Mean days ± S.D. |
| 17 | 35 | 11.8 ± 2.9 | 18 | 5.9 ± 0.9 | 4 | 5.5 ± 0.6 |
| 22 | 8 | 7.0 ± 2.4 | 2 | 4.0 ± 0.0 | 0 | |
| 27 | 71 | 4.2 ± 0.9 | 18 | 3.3 ± 0.8 | 6 | 2.3 ± 0.5 |
| 34 | 9 | 5.8 ± 2.2 | 0 | | 0 | |

Table 4. *Anopheles sergentii* egg hatch rates at 3 temperatures.

| Temperature (°C) | No. replicates | No. eggs/replicate | Mean % hatch ± S.D. | % hatch/day postoviposition | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 2 | 3 | 4 | 5 | 6 |
| 17 | 25 | 25–108 | 88.5 ± 12.9 | | | 51.1 | 45.0 | 3.9 |
| 27 | 56 | 25–141 | 85.2 ± 18.3 | 94.6 | 5.4 | | | |
| 34 | 9 | 11–104 | 0 | | | | | |

females was 0.5 assuming a 1:1 sex ratio). The age at mean cohort reproduction ($T_o$) was 13.10 ± 1.60 days. The intrinsic rate of increase ($r_m$) was estimated to be 0.127 ± 0.005 and the mean generation time (G) was 29.70 ± 1.56 days.

Age-specific survivorship and mean life expectancies were similarly compared for cohorts of males, unfed females, and females blood fed once. Respective age-specific survivorship rates for 17 and 27°C were 0.93 and 0.91 for males, 0.94 and 0.92 for unfed females, and 0.94 and 0.92 for females blood fed once. Respective mean life expectancies at 17 and 27°C were 25.38 ± 3.14 and 17.70 ± 4.75 days for males; 30.68 ± 0.23 and 20.87 ± 5.94 days for unfed females; and 33.82 ± 4.50 and 23.43 ± 4.61 days for once fed females. Figure 1 illustrates age-specific survivorship curves for all groups and the reproductive effort in the number of female offspring per living female per day ($l_x m_x$) for mated females at 27°C.

## DISCUSSION

Laboratory rearing methods were examined to determine optimal conditions for colony maintenance. The mud slurry solution used for larval rearing provided better conditions than tap or distilled water, presumably because this medium contained higher levels of microorganisms for larval consumption. The optimal rearing temperature was 27°C based on total survivorship (0.85) and pupation time (10.2 days). Adult females routinely were fed human blood since difficulties were initially encountered in feeding *An. sergentii* on other hosts (M. S. Beier, unpublished data). Ovipositing females preferred plastic bowls with distilled water rather than petri dishes with moist filter paper. This proved convenient since eggs could be left in oviposition bowls for hatching and egg transfer was not necessary. Despite requirements for force-mating (Beier et al. 1986), the number of

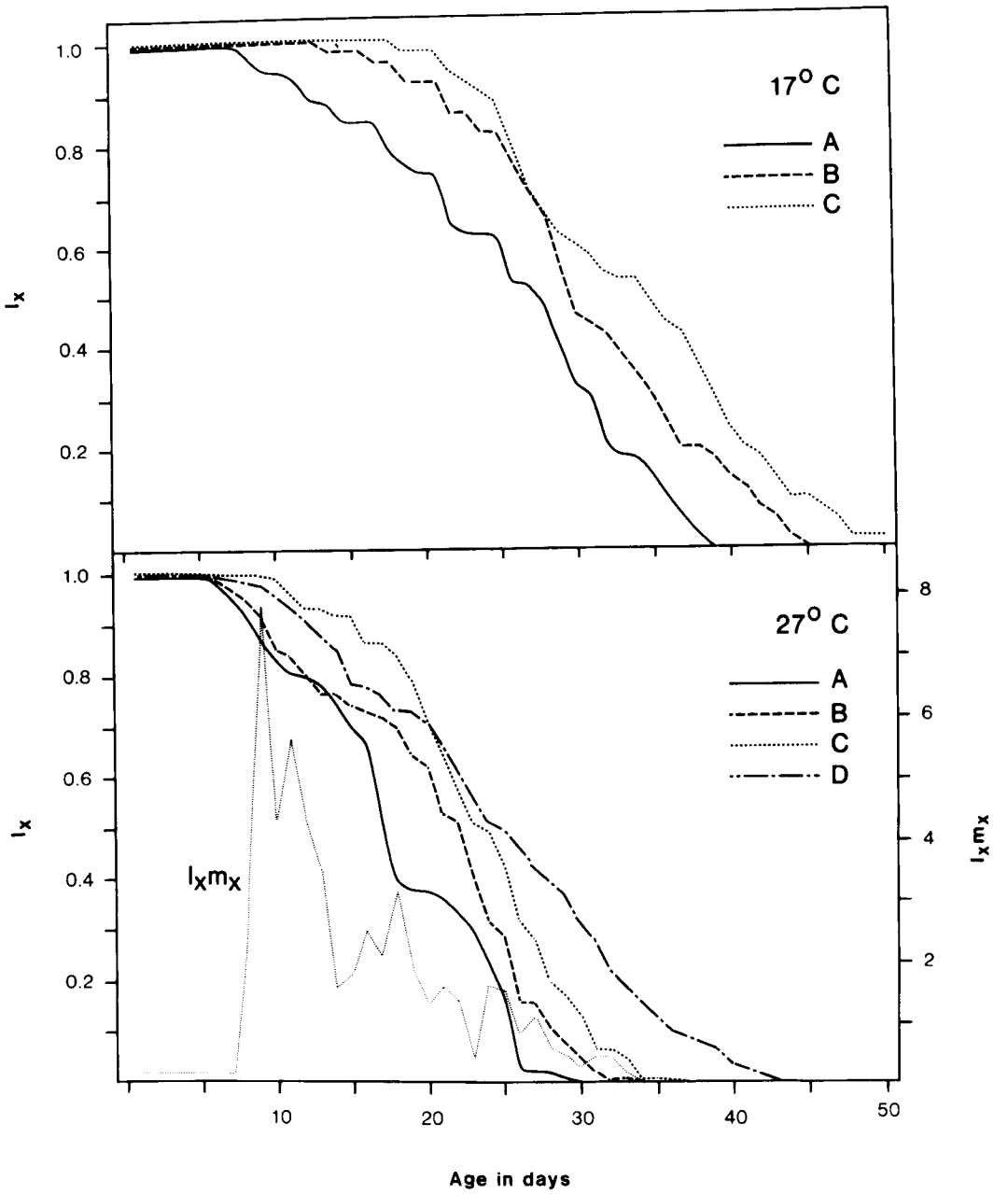


Fig. 1. Age specific survivorship in individuals per individual per day ($l_x$) for *An. sergentii* adults reared at 17 and 27°C (A, males; B, unfed females; C, unmated females blood fed once; and D, mated females offered a blood source daily). The number of female offspring produced per living female per day ($l_x m_x$) is shown for group D, at 27°C.

females mated per generation does not have to be great for routine colony maintenance, since this species has a high survivorship and reproductive potential.

The gonotrophic cycle of *An. sergentii* measured as the time from blood feeding to oviposition is directly related to feeding cycles since this species usually refeeds on the same night as oviposition under field conditions (El Said et al. 1986). At the 4 observation temperatures, the duration of the 1st gonotrophic cycle ranged from 4.2 to 11.8 days and 2nd and 3rd gonotrophic cycles were shorter. Further studies are needed to determine gonotrophic cycles of *An.*

*sergentii* under field conditions since the present data were attained under laboratory conditions where only about half of the tested females oviposited. Many females that failed to oviposit after the initial blood meal were refed, but this did not result in oviposition. Subsequent dissection of spermathecae showed that many females were uninseminated, perhaps explaining the low oviposition rate.

Life tables constructed under optimal insectary conditions provide information on maximum expression of species genetic potential since parameters are measured in the absence of environmental pressures experienced by field populations. Survivorship and reproductive strategies have extensively been studied for culicine mosquitoes (Crovello and Hacker 1972, Walker and Hacker 1974, Reisen et al. 1979); however complete adult life tables have been constructed for relatively few anophelines (Reisen and Mahmood 1980). Age specific survivorship of 0.95 for *An. sergentii* is considerably higher than values of 0.87 and 0.81 reported for laboratory reared *An. culicifacies* Giles and *An. stephensi* Liston (Reisen and Mahmood 1980).

To transmit *Plasmodium falciparum* or *P. vivax*, the anopheline host must survive for ca. 12 and 9 days, respectively, after taking an infective blood meal (with incubation at 27°C) (Macdonald 1952). Considering that initial host-feeding by *An. sergentii* occurs on the second or third night after emergence, the potentially infective portion of the population would consist of females not less than 11–14 days of age. Infective life expectancy for laboratory-reared *An. sergentii* averaged 12.3 and 9.3 days for *P. falciparum* and *P. vivax* transmission, respectively, based on the life expectancy of females 2 days of age. Based on the gonotrophic cycle length determinations at the same temperature (27°C) and a survival rate of 0.95, *An. sergentii* could imbibe up to 4 blood meals after completing sporogony, if a *Plasmodium* infection was acquired during the first blood meal.

The survivorship of *An. sergentii* under field conditions is considerably lower. In Faiyum Governorate, Egypt, *An. sergentii* survivorship determined by parity averaged 0.835 (El Said et al. 1986). Under such field conditions, the proportion of an *An. sergentii* population surviving to infective age would be 16.5% and 9.6% for *P. vivax* and *P. falciparum*, respectively. In Faiyum Governorate and in the Western Desert oases of Egypt malaria transmission by *An. sergentii* is limited further by the zoophilic feeding habits of this species (Beier et al. 1987, Kenawy et al. 1986b). Further studies will examine the vectorial capacity of *An. sergentii* in the Egyptian oases. Based on the present laboratory study, *An. sergentii* has the physiological potential for long term survival under suitable field conditions.

## ACKNOWLEDGMENTS

We are grateful to the Egyptian Ministry of Health for facilitating this study. We thank Ms. Ebtesam El-Kordy for her dedicated assistance. We thank Dr. Sherif El Said (Ain Shams University) and Dr. Robert Gwadz (NIH) for their excellent support and advice.

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