DEVELOPMENT AND SURVIVAL OF *ANOPHELES PHAROENSIS* AND *AN. MULTICOLOR* FROM FAIYUM, EGYPT

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ABSTRACT. Adults of *Anopheles pharoensis* and *An. multicolor* were held under cycling environmental conditions in the laboratory to examine the duration of the gonotrophic cycles, survival and life expectancy, and to examine the life table characteristics of F_1 larvae. The first gonotrophic cycle took 6.14 and 7.37 days for *An. pharoensis* and *An. multicolor*, respectively. Subsequent gonotrophic cycles for the 2 species were shorter. Daily survival rates of *An. pharoensis* and *An. multicolor* in the laboratory were 0.95 and 0.93, respectively. The parity rate of field-collected females and estimates of the duration of the gonotrophic cycle yielded daily survivorship estimates of 0.89 and 0.80 for *An. pharoensis* and *An. multicolor*, respectively. Mean life expectancy at emergence was 19.0 days for *An. pharoensis* compared with 17.9 days for *An. multicolor*. Survivorship from egg eclosion to adult emergence and development time were similar for both species. Both the duration of gonotrophic cycles and mean life expectancies indicated that *An. pharoensis* had a greater potential to serve as a malaria vector than *An. multicolor*.

INTRODUCTION

Anopheles pharoensis Theobald, *An. multicolor* Cambouliu and *An. sergentii* (Theobald) are common mosquito species in Faiyum Governorate, Egypt, an agricultural oasis where *Plasmodium vivax* and *P. falciparum* are endemic. *Anopheles pharoensis* and *An. sergentii* are proven malaria vectors, but *An. multicolor* has never been found infected in nature (Barber and Rice 1973, Halawani and Shawarby 1957, El Said et al. 1983, 1986). *Anopheles multicolor* has long been considered a suspected vector due to its relative abundance and susceptibility to infection under experimental conditions (El Said and Farid 1982). In Egypt, life table studies have only examined the survivorship and reproductive potential of *An. sergentii* under laboratory conditions (Beier et al. 1987a).

The present study compared the duration of the gonotrophic cycle, daily survivorship and life expectancy of field-collected *An. pharoensis* and *An. multicolor* adult females that were held under insectary conditions simulating field conditions. Both larval and adult life table parameters were determined for these 2 mosquito species to explain why *An. multicolor* apparently is not a malaria vector in Egypt.

MATERIALS AND METHODS

Adult collection and processing: Adult females of *An. pharoensis* and *An. multicolor* were collected during October and November 1990 in Tersa, a malaria-endemic village in Sinnuris District, Faiyum Governorate, Egypt. Mosquito were collected every 2 h throughout the night using a donkey-baited trap. A sample of the mosquitoes were dissected immediately to determine parity based on the coiling of ovarian tracheoles (Detinova 1962). In Cairo, observations were made on fed mosquitoes and the resultant F_1 progeny in a room with naturally cycling conditions of temperature (mean = 25.7°, range = 17.6–33.8°C), relative humidity (mean = 77.6%, range = 60–95%) and illumination to simulate the field conditions. For comparison, development of immature stages from eclosion to adult emergence also was studied in an insectary maintained at constant conditions of 25 ± 2°C temperature, 70 ± 5% RH and illuminated by fluorescent lighting for ca. 8 h daily.

Gonotrophic cycle, female survivorship and life expectancy: Blood-fed females of *An. pharoensis* ($n = 42$) and *An. multicolor* ($n = 66$) were placed individually in 60 ml screened plastic vials lined with filter paper and containing 10 ml distilled water for oviposition. The length of the gonotrophic cycle (g) was determined as the time from blood feeding (in the field) to oviposition. After oviposition, females were provided with 10% sugar solution on cotton (changed daily) and observed daily for mortality. Dead females were dissected and ovarian dilatations were counted to determine the number of the gonotrophic cycles completed (Detinova 1962). Based on the number and duration of the respective gonotrophic cycles plus a 2 day maturity period prior to the first blood meal, the calendar age of each female at the time of collection was determined. Age-specific survivorship (S) and life expectancy at emergence (e_1) in days for each parous group and the mean for all females were calculated according to methods described by Walter and Hacker (1974) and Reisen and Mahmood (1980). For comparison, the survivorship under field conditions, as expressed by the daily

Table 1. Duration of gonotrophic cycles of field-collected blood-fed females of *Anopheles pharoensis* and *An. multicolor*.*

Gonotrophic cycle	*An. pharoensis*		*An. multicolor*	
	No. ovi-posited females	Mean duration days ± SE	No. ovi-posited females	Mean duration days ± SE
1st (1-parous)	21	6.14 ± 0.14	54	7.37 ± 0.62
2nd (2-parous)	18	5.50 ± 0.56	12	5.50 ± 0.50
3rd (3-parous)	3	4.00 ± 0.00	0	—
Total	42	5.71**	66	7.03**

* Kept at mean cycling temperature of 25.7°C and RH of 77.6%.
** Weighted mean for all females.

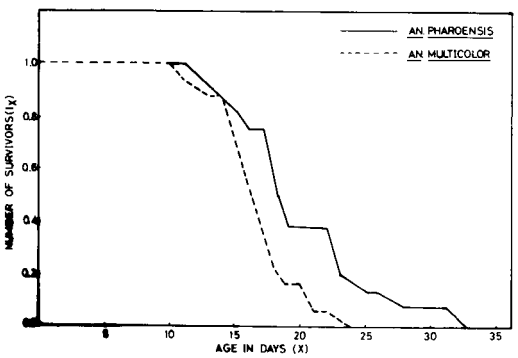

Fig. 1. Age-specific survivorship of females per day (l_X) plotted as a function of age (X) in days for *Anopheles pharoensis* (n = 42) and *An. multicolor* (n = 66).

probability of survival (P), was calculated for the field-collected females (Davidson 1954) by the expression $P = \sqrt[g]{p}$ where p = proportion parous and g = the mean duration of the gonotrophic cycle.

Egg hatch rates and duration: Eggs from individual females 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. Egg hatch rates and duration in days were determined under cycling and constant temperatures. Median time until hatching (H_{50}) was calculated by fitting a regression of the form P = a + b ln X (where P = cumulative proportion hatching on each day (X) transformed to probits) and solving the equation for P = 50%.

Immature development: Ten and 15 cohorts of 100 first instar larvae of *An. pharoensis* and *An. multicolor*, respectively, were reared under cycling temperatures (mean = 25.7°C) in 30 cm diam round enamel pans containing 2 liters mud slurry solution (Beier et al. 1987a). Five cohorts (100 larvae each) of each species were reared at constant temperature (25 ± 2°C). Larvae were fed powdered Tetramin® fish food sprinkled daily on the water surface. Pupae were removed daily from rearing pans and placed in 400 ml screened emergence cups, kept separated by pan and date. Emerged adults were counted and sexed. Immature developmental attributes were calculated according to Reisen et al. (1982). Survivorship (S) from egg eclosion to pupation, pupation to adult emergence and total S from eclosion to emergence were estimated by the expressions P/I, A/P and A/I, respectively, where P = no. of pupae, A = no. of emerged adults and I = no. of 1st instar larvae originally counted in the rearing pans. Median times to pupation (P_{50}) and adult emergence for each sex (E_{50}) were calculated by regression as for H_{50}.

RESULTS

Gonotrophic cycle, female survivorship and life expectancy: The mean duration of the 1st gonotrophic cycle was 6.14 days for *An. pharoensis* and 7.37 days for *An. multicolor* (Table 1). Subsequent cycles for both species were shorter. Dissections immediately after death indicated that 7.1% (3/42) of *An. pharoensis* females had 3 dilations (3rd gonotrophic cycle), but none of the 66 *An. multicolor* females had more than 2 dilations.

The mean survivorship rates (S) were 0.95 ± 0.01 per day for *An. pharoensis* (0.95 for 1-p and 0.96 for 2-p females) and 0.93 ± 0.002 per day for *An. multicolor* (0.93 for 1-p and 2-p females). The age-specific survivorship curves for the 2 species are shown in Fig. 1. The mean life expectancy (e_1) at emergence was 19.04 ± 3.26 days for *An. pharoensis* (15.78 and 22.30 days for 1-p and 2-p females, respectively) and 17.90 ± 2.10 days for *An. multicolor* (15.80 and 20.0 days for 1-p and 2-p females, respectively). The proportion parous (P) determined by dissection of field collected females was 0.50 for *An. pharoensis* (n = 106) and 0.20 for *An. multicolor* (n = 150). Based on these and gonotrophic cycle of 5.7 days for *An. pharoensis* and 7.03 days for

An. multicolor (weighted means for all females), the daily probability of survival (P) was 0.89 and 0.80 for the 2 species, respectively.

Egg hatch rates and duration: Under cycling temperature with a mean of 25.7°C, egg hatch rates of 79.8% and 85.0% were obtained over a mean period of 4.02 and 3.32 days for *An. pharoensis* and *An. multicolor*, respectively (Table 2). The median time for 50% hatching (H_{50}) was similar (t = 1.47, d.f. = 35, $P > 0.05$) for *An. multicolor* (2.7 days) and *An. pharoensis* (3.1 days). At 25°C constant temperature, eggs of *An. multicolor* hatched in shorter periods than at the mean cycling temperature.

Immature development: Seven developmental attributes were examined for immatures of *An. pharoensis* and *An. multicolor*, reared under the cycling room temperature (Table 3). Survivorship rates from eclosion to adult emergence (A/I) were similar (t = 1.27, d.f. = 23, $P > 0.05$) for *An. pharoensis* (0.13) and *An. multicolor* (0.22). Developmental time to pupation (P_{50}) was significantly faster (t = 5.56, d.f. = 23, $P < 0.01$) for *An. multicolor* (16.5 days) than for *An. pharoensis* (20.0 days), but survivorship to the pupal stage (P/I = 0.14 and 0.24 for the 2 species, respectively) was similar (t = 0.96, d.f. = 23, $P > 0.05$). Median emergence time (E_{50}) for males was significantly shorter for *An. multicolor* (18.5 days) than for *An. pharoensis* (21.0 days) (t = 2.82, d.f. = 23, $P < 0.01$). Likewise, E_{50} for females was shorter for *An. multicolor* (19.5 days) than for *An. pharoensis* (22.0 days) (t = 2.69, d.f. = 23, $P < 0.05$). Sex ratios of both species did not differ significantly (χ^2 = 0.32 for *An. pharoensis* and χ^2 = 0.00 for *An. multicolor*, d.f. = 1, $P > 0.05$) from the expected 1:1 ratio.

Under constant temperature the 2 species had similar survivorship A/I rates (t = 0.25, d.f. = 8, $P > 0.05$) and E_{50} (♀-♂) periods (t = 0.11, d.f. = 8, $P > 0.05$ for females and t = 0.01, d.f. = 8, $P > 0.05$ for males). The 2 species also had similar E_{50} (♀-♂) times and survivorship A/I rates at constant and cycling temperatures.

DISCUSSION

Daily survivorship rates for field-collected females of *An. pharoensis* and *An. multicolor* based on parity rates averaged 0.89 and 0.80 for the 2 species, respectively. The age-specific survivorship rates of 0.95 and 0.93 determined for *An.*

Table 2. Egg hatching rates and duration for *Anopheles pharoensis* and *An. multicolor* at cycling (mean = 25.7°C) and constant (25 ± 2°C) temperatures.

Species/temperature	n	No. eggs (Mean ± SE)	% Hatch (Mean ± SE)	Hatch days (Mean ± SE)	H_{50} (days)* (Mean ± SE)
An. pharoensis					
Cycling	15	209.2 ± 36.8	74.8 ± 5.49 A	4.07 ± 0.50 A	3.10 ± 0.31 A
Constant	10	73.3 ± 16.9	86.0 ± 2.55 A	3.50 ± 0.08 A	2.73 ± 0.05 A
An. multicolor					
Cycling	22	164.8 ± 61.5	85.0 ± 3.6 A	3.32 ± 0.22 A	2.70 ± 0.10 A
Constant	10	75.0 ± 15.8	88.6 ± 2.4 A	2.33 ± 0.27 B	1.41 ± 0.28 A

* H_{50} = median time to 50% hatch.

** Means with same letters in each column are not significantly different (t = test), $P > 0.05$.

Table 3. Immature development attributes of *Anopheles pharoensis* and *An. multicolor* reared at cycling (mean = 25.7°C) and constant (25 ± 2°C) temperatures.

	Cycling temperature**		Constant temperature**	
Attributes*	*An. pharoensis* (Mean ± SE)	*An. multicolor* (Mean ± SE)	*An. pharoensis* (Mean ± SE)	*An. multicolor* (Mean ± SE)
Survivorship (P/I)	0.14 ± 0.03 A	0.24 ± 0.06 A	—	—
P_{50} (days)	20.00 ± 0.60 A	16.50 ± 0.30 B	—	—
Survivorship (A/P)	0.83 ± 0.06 A	0.85 ± 0.04 A	—	—
E_{50}				
♂♂	21.00 ± 0.93 AC	18.50 ± 0.34 BC	18.40 ± 2.32 C	18.38 ± 2.18 C
♀♀	22.00 ± 0.85 AC	19.50 ± 0.47 BC	19.53 ± 2.32 C	19.01 ± 2.63 C
Total survivorship (A/I)	0.13 ± 0.04 A	0.22 ± 0.05 A	0.16 ± 0.07 A	0.18 ± 0.04 A
Sex ratio (♀♀/total)***	0.45 ± 0.04 NS	0.51 ± 0.04 NS	—	—

* Abbreviations: I = 1st instar larvae; P = pupae; A = adults; P_{50} = median time to 50% pupation; E_{50} = median time to 50% adult emergence.

** Means with the same letters in each row are not significantly different (t-test), $P > 0.05$).

*** Sex ratios tested for departure from 1:1 by χ^2; NS = not significant ($P > 0.05$).

pharoensis and *An. multicolor* females, respectively, under the experimental conditions were similar to rates ranging from 0.92 to 0.95 reported for *An. sergentii* females held at 27°C (Beier et al. 1987a). These rates for survivorship under field and cycling conditions indicate that more *An. pharoensis* than *An. multicolor* females would survive to become infective for malaria.

To transmit *Plasmodium vivax* and *P. falciparum*, the anopheline female must survive for ca. 9 and 11 days, respectively (at 25.7°C), after taking an infective blood meal (Macdonald 1957). Assuming that females take the first blood meal 2 days after emergence, then the potentially infective females will not be less than 11–13 days of age required for *P. vivax* and *P. falciparum* transmission, respectively. Under field conditions, the proportion of female populations surviving to infective age for *P. vivax* and *P. falciparum* transmission would be 31.4% and 25.4% for *An. pharoensis* and 8.6% and 5.5% for *An. multicolor*, respectively. Life expectancy at 11 and 13 days averaged 10.89 and 9.80 days for *An. pharoensis* and 6.02 and 5.21 days for *An. multicolor*, respectively. Based on gonotrophic cycles of 5.3 days for *An. pharoensis* and of 5.5 days for *An. multicolor* (weighted average for all females from the 2nd cycle), it appears that mosquito females will take only 2 and one additional blood meals, for the 2 species, respectively, after completing the sporogonic cycle if *Plasmodium* infection was acquired during the first blood meal. In comparison, *An. sergentii* has the potential to imbibe up to 4 blood meals after completing sporogony (Beier et al. 1987a).

The developmental rates of immature *An. multicolor* under cycling temperature based on P_{50} and E_{50} values were significantly higher than those of *An. pharoensis*, but survivorship of the different stages was similar for the 2 species. Adults of both species emerged at shorter periods (E_{50} ♀-♂) under constant than under cycling temperature, but their survivorship rates (A/I) were similar under both temperatures. Similar results were obtained by Reisen et al. (1982) for *An. culicifacies*. Under both the constant and cycling temperatures, *An. multicolor* immatures developed at higher rates and in shorter periods than *An. pharoensis*. Similarly, *An. multicolor* hatched more eggs and in less time than *An. pharoensis* eggs. Such higher egg hatching rates together with the observed faster development of *An. multicolor* may explain the abundance of its immature stages in the field.

In Egypt *An. pharoensis* and *An. multicolor* are zoophilic (Beier et al. 1987b, Kenawy et al. 1987). Although immature stages of *An. multicolor* develop faster than *An. pharoensis*, adult survival is a major factor which may limit the capability of *An. multicolor* to transmit malaria. These results likely explain why *An. multicolor* has never been found naturally infected (Kenawy 1988). Studies on the longevity of these 2 species in the field are needed to verify the above hypothesis.

ACKNOWLEDGMENTS

The author is grateful to M. Soualem (Ain Shams Center) for his assistance in the field collections and laboratory experiments, to S. El Said (Ain Shams Center), A. Merdan (Ain Shams Center) and R. Gwadz (NIH) for their support in facilitating this research, and to J. Beier (The Johns Hopkins University) for comments on the manuscript.

This study was supported by the Regional Project entitled: "Epidemiology and Control of Arthropod-Borne Diseases in Egypt-NO1 AI 22667" between the Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt and, the National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD, USA.

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