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PHOTOPERIOD AND LONGEVITY IN *ANOPHELES CRUCIANS*

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ABSTRACT. The effect of photoperiod on longevity of nondiapausing members of *Anopheles crucians* was evaluated in laboratory experiments. First-generation adults reared from field collections illustrated the same trends. Individuals reared and maintained under short photoperiod (either 8 h light : 16 h dark or 11 h light : 13 h dark) lived longer than did those reared and maintained under long photoperiod (either 16 h light : 8 h dark or 15 h light : 9 h dark). Analysis of covariance showed that the greater longevity of short-photoperiod mosquitoes was not caused by their larger average body size. Also, a paired *t*-test confirmed the effect of photoperiod, independent of body size, on longevity; in mosquitoes paired according to thorax length, short-photoperiod individuals lived longer than did long-photoperiod individuals of the same sex and cohort. The reason short-photoperiod mosquitoes of this species live longer is not known.

INTRODUCTION

Photoperiod affects longevity in nondiapausing members of a mosquito species; individuals of a species in the *Anopheles quadrimaculatus* complex (probably species A) in peninsular Florida live longer under short-photoperiod than under long-photoperiod laboratory conditions (Lanciani and Anderson 1993). The cause of this longevity difference is not known. Although photoperiod triggers diapause in some anophelines (Washino 1970) and physiological changes in diapausing mosquitoes generally increase longevity (Washino et al. 1971, Washino 1977), *An. quadrimaculatus* from north Florida does not appear to enter diapause in either the field or the laboratory (Lanciani and Anderson 1993).

The greater longevity of these short-photoperiod mosquitoes may result from their greater body size. Larger insects usually live longer than smaller ones within the same species (King and Martin 1975, Akey et al. 1978), and in *An. quadrimaculatus,* short-photoperiod adults are larger than long-photoperiod adults of the same sex (Lanciani 1992). However, a correlation analysis between longevity and body size within photoperiod groups of *An. quadrimaculatus* was not attempted (Lanciani and Anderson 1993).

The objectives of the present study were to determine: 1) whether the relationship between photoperiod and longevity noted in *An. quadrimaculatus* also exists in another anopheline species, *Anopheles crucians* Wiedemann, and 2) whether longevity is correlated with body size in this species. Accordingly, mosquitoes from an Alachua County population of *An. crucians* were reared under short and long photoperiods, and their longevity and thorax lengths were measured.

MATERIALS AND METHODS

Rearing of immatures: Rearing followed the procedure described in Lanciani (1992). Gravid *An. crucians* were collected on October 25 and December 5, 1992, from daytime resting sites near Lake Alice, Gainesville, FL. The mosquitoes were kept in separate vials in a constant-temperature chamber set at 28°C and a 12 h light–dark cycle. The first batch of eggs laid from each of the collections was used. The first batch from the October 25 collection was oviposited on October 26 (the October cohort), and the first batch from the December 5 collection was oviposited on December 6 (the December cohort). Half of the eggs of a cohort were put in a short-photoperiod constant-temperature chamber set at 28°C and the other half in a long-photoperiod constant-temperature chamber also set at 28°C. The October cohort was exposed to photoperiods of 8 h light : 16 h dark (short) and 16 h light : 8 h dark (long). These photoperiods were selected to facilitate comparisons with other studies that use these common experimental photoperiods. However, at a latitude of 30° (close to that of Gainesville), the longest day length (including 56 min of twilight) is 15 h and 1 min, and the shortest day length (including 52 min of twilight) is 11 h and 4 min (List 1971). Accordingly, the December cohort was exposed to photoperiods of 11 h light : 13 h dark (short) and 15 h light : 9 h dark (long) to determine the effect of these more natural photoperiods on longevity.

A constant temperature of 28°C was maintained in both short- and long-photoperiod chambers by timed temperature controls set lower during light periods to compensate for heat given off by the lights (a pair of 20-W fluorescent lights in each chamber). Before experiments began, temperature controls were adjusted while temperature was checked with a thermometer placed in a water-filled 250-ml Erlenmeyer flask located next to the rearing pans. These thermometer readings agreed with average thermocouple readings recorded from the top 5 mm of water in different areas of the rearing pans, suggesting that the temperature experienced by the larvae, which occupy the upper few millimeters of water, was adequately controlled. Thermometers were monitored throughout the experiments to verify that temperature remained at 28°C.

Eggs were held in 500 ml of tap water in a white enamel pan, and on the day after oviposition, 0.05 g of a 2:1 mixture of baby-fish food and brewer's yeast was added to each pan. Two days later, groups of approximately 40 larvae of similar size were selected from each photoperiod group and were placed in separate pans containing 500 ml of tap water and 0.06 g of food. On subsequent days, larvae were transferred to clean pans with 500 ml of fresh tap water and were fed successively 0.06, 0.07, and then 0.09 g of food per pan until pupation. Because no more than one larva died in any pan, the number of larvae per pan remained close to 40 throughout larval development. The pans were covered with clear plastic sheets to reduce evaporation. As pupae appeared, they were held individually in screen-covered vials (2.5 cm in diameter and 8 cm in height) half-filled with tap water. Pupae were placed in the same constant-temperature chamber (with the same photoperiod) in which they developed. In the October cohort, pupation occurred 8.91 ± 0.04 days after oviposition in short-photoperiod individuals and 9.38 ± 0.07 days after oviposition in long-photoperiod individuals ($P = 0.0001$, F-test). In the December cohort, pupation occurred 8.37 ± 0.06 days after oviposition in short-photoperiod individuals and 8.53 ± 0.06 days in long-photoperiod individuals ($P = 0.046$, F-test). Although the short-photoperiod group developed significantly faster in these cohorts, 3 other rearings of *An. crucians* in my laboratory have shown either no difference in time to pupation between photoperiod groups ($P = 0.3$ and 0.8, F-test) or a significantly faster development in the long-photoperiod group ($P = 0.0001$, F-test).

Adult longevity measurement: Adults were held individually in the same vials they occupied as pupae and in the same constant-temperature chamber (with the same photoperiod) in which they developed as larvae. Thus, short- or long-photoperiod conditions were maintained throughout the duration of the experiments for each mosquito. Vials holding adults were kept ½ to ¼ filled with tap water. A drop of corn syrup was placed on the screen cover of each vial so that food would be available to emerging adults, and more syrup was added as the initial provision was eaten. Vials were briefly checked during light periods once each day (between 8:00 and 10:00 a.m.) for emergences and deaths until all adults died. In this way, survival times were obtained for each adult. Adult life spans were observed on 157 adults developing from 161 1st-instar larvae that made up the October cohort and 157 adults developing from 159 1st-instar larvae that made up the December cohort. Average life span remaining for an emerging adult, e_0, was calculated from

$$e_0 = \frac{\sum_{x=0}^{n} S_x}{S_0} - \frac{1}{2},$$

in which x is the number of days after emergence, S_0 is the number of adult mosquitoes alive at emergence ($x = 0$ days), S_x is the number of adult mosquitoes alive at the start of day x, and n is the maximum number of days survived after emergence (Lanciani 1987).

Thorax-length measurement: Adults were removed from vials within 1 day after death. Thorax length was measured parallel to the longitudinal axis of the mosquito's body with a dissecting microscope equipped with an ocular micrometer. Thorax length is an accurate index of body size in adult mosquitoes (van den Heuvel 1963) and is useful in longevity studies because it can be reliably measured on mosquitoes after they have died, thus avoiding measurement-related handling and injury of live, experimental mosquitoes. Wing length is not as suitable an index of body size in longevity studies because wing tips often eventually become eroded in container-held mosquitoes.

Statistical tests: Differences in longevity were evaluated with the logrank test (Peto et al. 1977) and differences in thorax length (and time from oviposition to pupation) with an analysis of variance. The effect of photoperiod on longevity, independent of any effect of thorax length on longevity, was tested with an analysis of covariance. Another test of photoperiod's independent effect was also run. Within a cohort and sex, short- and long-photoperiod individuals having the same thorax length were randomly paired (87 pairs with the same thorax length were found among the 314 mosquitoes). Then longevity differences between members of each pair were evaluated with a *t*-test for paired observations.

RESULTS

The longevity of reared adults is illustrated in the survivorship curves of Fig. 1. In both cohorts, short-photoperiod individuals lived longer ($P = 0.0001$–0.008). Longevity differences are also reflected in the higher e_0 values (average life span remaining for emerging adults) of short-photoperiod members of both cohorts (Table 1). Although each cohort experienced different short and long photoperiods, both cohorts responded

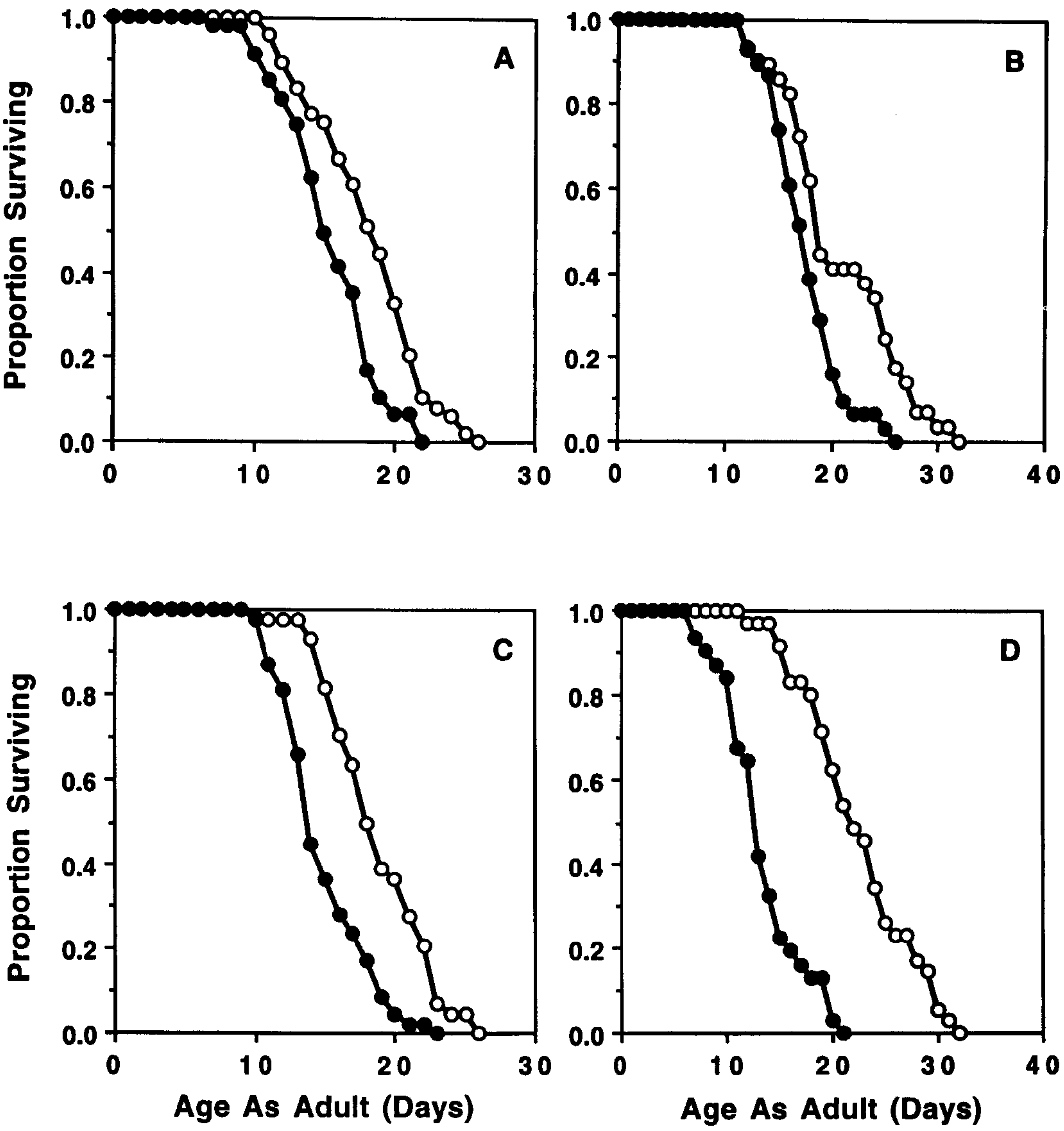

Fig. 1. Survivorship curves of adult males and females from cohorts of *Anopheles crucians* reared and maintained under short and long photoperiods. Open circles represent short-photoperiod rearings and closed circles represent long-photoperiod rearings; A. Survivorship of 49 short-photoperiod and 48 long-photoperiod males from the October cohort. B. Survivorship of 29 short-photoperiod and 31 long-photoperiod females from the October cohort; C. Survivorship of 44 short-photoperiod and 47 long-photoperiod males from the December cohort; D. Survivorship of 35 short-photoperiod and 31 long-photoperiod females from the December cohort.

similarly. The short photoperiod of 8 h light : 16 h dark and long photoperiod of 16 h light : 8 h dark produced results qualitatively similar to those produced by the more realistic short photoperiod of 11 h light : 13 h dark and long photoperiod of 15 h light : 9 h dark.

Thorax lengths of reared adults are listed in Table 1. In both cohorts, short-photoperiod individuals had greater thorax lengths than did long-photoperiod individuals of the same sex (P = 0.0001–0.02). Analysis of covariance, however, showed that greater thorax length did not contribute significantly to the greater longevity of short-photoperiod mosquitoes. In the October cohort, longevity was significantly affected by photoperiod ($P = 0.0005$ in males and 0.008 in females) but not by thorax length ($P = 0.5$ in males and 0.09 in females). Similarly, in the De-

Table 1. Mean thorax length (mm) and mean adult life span remaining (e_0) for emerging adults (days) in different groups of *An. crucians*.

Cohort	Sex	Photo-period	Mean thorax length ± SE	e_0 ± SE	*n*
October	♂	Short	1.435 ± 0.004	17.77 ± 0.56	49
	♂	Long	1.381 ± 0.005	15.06 ± 0.49	48
	♀	Short	1.513 ± 0.006	20.43 ± 1.01	29
	♀	Long	1.482 ± 0.004	17.24 ± 0.60	31
December	♂	Short	1.471 ± 0.004	18.39 ± 0.53	44
	♂	Long	1.460 ± 0.003	14.48 ± 0.44	47
	♀	Short	1.561 ± 0.004	22.07 ± 0.87	35
	♀	Long	1.540 ± 0.005	13.98 ± 0.67	31

cember cohort, longevity was significantly affected by photoperiod ($P = 0.0001$ in males and females) but not by thorax length ($P = 0.06$ in males and 0.2 in females). Thus, the greater longevity of short-photoperiod *An. crucians* is related to photoperiod and not to body-size difference. Further support for this observation arose from the paired *t*-test. Within the same cohort and sex, short-photoperiod mosquitoes lived significantly longer than did their long-photoperiod pair-members of the same thorax length ($P = 0.0001$).

DISCUSSION

Short photoperiod affected longevity in *An. crucians* just as in *An. quadrimaculatus* (Lanciani and Anderson 1993): mosquitoes reared and maintained under short photoperiod lived significantly longer than did those reared and maintained under long photoperiod. This longevity difference was not due to body-size difference even though short-photoperiod mosquitoes were larger on average. Within the same sex and cohort, short-photoperiod individuals lived longer than did their long-photoperiod counterparts of the same thorax length.

The reason short photoperiod mosquitoes live longer remains unclear. Short photoperiod is known to initiate diapause in mosquitoes, and diapausing mosquitoes live longer. But diapause cannot explain the present results. Most mosquito species in peninsular Florida are thought not to enter diapause (Van Handel 1984, Mitchell 1988), and *An. crucians* shows no signs of diapause in north Florida. Males, gravid females, and larvae from natural populations have been observed in Gainesville throughout the year, and reared females lack the extensive fat-body enlargement of females in diapause (Depner and Harwood 1966, Washino 1970, Washino and Bailey 1970). Photoperiod-induced change in metabolic rate is a possible cause of the observed longevity difference in *An. crucians*. This possibility is weakened, however, by observations on a related species; in *An. quadrimaculatus*, short-photoperiod individuals live longer also, even though their metabolic rate (i.e., their rate of oxygen consumption) is not consistently lower as measured in the laboratory (Lanciani and Anderson 1993). Additional research is needed to discover how photoperiod alters life span in these mosquitoes.

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