EFFECT OF PHOTOPERIOD ON LONGEVITY AND METABOLIC RATE IN *ANOPHELES QUADRIMACULATUS*

CARMINE A. LANCIANI AND JOHN F. ANDERSON

Department of Zoology, University of Florida, Gainesville, FL 32611

ABSTRACT. The effect of photoperiod on longevity of nondiapausing members of a species in the mosquito species complex *Anopheles quadrimaculatus* was tested in laboratory experiments. First-generation adults reared from 2 field collections showed the same trends: those reared under short photoperiod (8 h light: 16 h dark) lived longer than did those reared under long photoperiod (16 h light: 8 h dark). Adult metabolic rates were not consistently related to longevity. In adults reared from the first collection, metabolic rates were higher in long-photoperiod individuals but in adults reared from the second collection, metabolic rates were higher in short-photoperiod individuals. Longevity appears to be another of many insect features that are affected similarly by temperature and photoperiod.

INTRODUCTION

Temperature and photoperiod can have similar effects on insects (Hoffmann 1985). For example, insects reared under short photoperiods often are larger (Ratte 1979, Lanciani 1992), have higher metabolic rates at the same test temperature (Giesel et al. 1989, Lanciani et al. 1990), and recover faster or survive better after chilling (van der Woude and Verhoef 1988, Lanciani et al. 1990) than those reared under long photoperiods. These differences are also commonly observed between insects reared at low and high temperatures. Temperature and photoperiod have similar effects perhaps because they are normally associated in particular ways in nature: low temperature is usually coupled with short photoperiod and high temperature with long photoperiod. As a result, both temperature and photoperiod may signal the same near-future environmental conditions and thus may trigger similar adaptive changes in insects.

Another insect characteristic influenced by temperature is longevity. Typically, insects survive longer when reared under low temperatures (King and Martin 1975, Butler and Foster 1979, Moscardi et al. 1981). In particular, the mosquito *Anopheles sergentii* (Theobald) was observed to live significantly longer at 17° than at 27°C in laboratory cultures (Beier et al. 1987). To test whether photoperiod affects longevity similarly, we reared mosquitoes from an Alachua County, Florida population of a species in the complex *Anopheles quadrimaculatus* (probably species A on the basis of locale and habitat; Kaiser et al. 1988) under short and long photoperiods and observed their adult life spans. In addition, because of the possible effect of metabolic rate on longevity, we also measured metabolic rates of individuals from the same cohorts.

Short photoperiod is known to trigger diapause in some anophelines (Washino 1970), including northern U.S. populations of *An. quadrimaculatus* (Hitchcock 1968), and physiological changes in diapausing mosquitoes do increase longevity (Washino et al. 1971, Washino 1977). However, mosquitoes in peninsular Florida generally do not enter diapause (Van Handel 1984, Mitchell 1988), and the population in the present investigation shows no signs of diapause. For example, many males, gravid females and larvae have been observed in the field throughout the fall and winter, as well as the rest of the year. In addition, female mosquitoes reared in this study did not show fat body enlargement typical of mosquitoes in diapause (Depner and Harwood 1966, Washino and Bailey 1970). Thus, in the present study, we tested the effects of photoperiod on longevity of nondiapausing adult mosquitoes.

MATERIALS AND METHODS

Rearing of immatures: Rearing followed the procedure described in Lanciani (1992). Gravid *An. quadrimaculatus* were collected on 2 dates (August 3 and September 22, 1991) from daytime resting sites near Lake Alice, Gainesville, Florida. The mosquitoes were held in separate vials in a constant-temperature chamber set at 28°C and a 12 h light-dark cycle. Only a single clutch of eggs, that laid by the first ovipositing female, from each of the 2 collections was reared. The first clutch from the August 3 collection was oviposited on August 5 (the August 5 cohort), and the first clutch from the September 22 collection was oviposited on September 23 (the September 23 cohort). Half of the eggs from a clutch were placed into a short-photoperiod constant-temperature chamber (8 h light: 16 h dark) set at 28°C and the other half into a long-photoperiod constant-temperature chamber (16 h light: 8 h dark) also set at 28°C. Eggs at each photoperiod were held in 500 ml of tap water in a white enamel pan. On the day after oviposi-

tion, 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 sizes 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 each succeeding day until pupation, larvae were transferred to clean pans with 500 ml of fresh tap water and were fed. The remaining feeding schedule was 0.06, 0.07 and then 0.09 g of food per pan on successive days continuing until pupation of all larvae. The pans were covered with clear plastic sheets to reduce evaporation. As pupae appeared, they were held individually in screen-covered vials in the same constant temperature chamber where they developed. Thermocouple readings taken from the top 5 mm of water in the rearing pans verified that the temperature experienced by larvae, which frequent the top few mm of the water column, was 28°C. Perhaps because of the low density of larvae in the pans (0.05 larvae per square cm of water surface), this method of rearing larvae produced high survival rates to adult emergence; usually no more than 1 or 2 deaths were observed among the 4 lots of 40 larvae initially placed in pans.

Adult longevity measurement: A drop of corn syrup was placed on the screen cover of each vial holding a pupa so that food would be available to emerging adults, and more syrup was added as the initial provision was eaten. Vials were checked each morning for emergences and deaths until all adults died. In this way, survival times in days were obtained for each adult in both photoperiod groups. Adult life spans were observed in 102 individuals from the August 5 cohort and 117 individuals from the September 23 cohort. Average life span remaining for an emerging adult, e_0, was calculated from the equation:

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

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).

Metabolic rate measurement: Rates of metabolism were determined on individual mosquitoes sampled from the longevity experiments. Each mosquito was placed in a separate closed-system metabolic chamber (Vleck 1987), which consisted of a disposable 30 cm syringe (adjusted to a volume of 20 cm). At the end of an approximately 6-h run, which extended from about 0800 h to about 1400 h, the fractional O_2 concentration of syringe gas was determined with an Ametek Applied Electrochemistry S-3A Oxygen Analyzer (Pittsburgh, PA) supplied with a model N-22M sensor. A 6-h run was chosen because the decrease in oxygen fraction over this time is sufficient to allow accurate measurement. Three or 4 syringes without mosquitoes were treated exactly as were the experimental syringes and served as controls for each series of measurements. The small changes in oxygen fraction observed in the controls were used to adjust experimental syringe readings. A more complete description of our method is found in Giesel et al. (1989), and a justification of the method's use in animals with potential diel cycles in rates of metabolism is found in Anderson et al. (1989).

Metabolic rate, as measured by oxygen consumption ($\dot{V}_{O_2}$) in μl per hour, was calculated using the following equation (Vleck 1987):

$$\dot{V}_{O_2} = \frac{V\,(FI_{O_2} - FE_{O_2})}{(1 - FE_{O_2})t},$$

in which V is the initial volume of dry CO_2-free room air in the syringe at STP, FI_{O_2} and FE_{O_2} are the O_2 fractions within the syringe at the beginning and end of the run, and t is the duration of the run in hours.

We next dried the mosquitoes for 2 days in a 60°C oven and weighed them to the nearest 0.002 mg using a Cahn electrobalance. The mosquitoes were not weighed before metabolic-rate runs because any method of immobilization to permit weighing might have affected metabolic rates.

Metabolic rates were measured usually on samples of 5 adults of each sex, photoperiod and age (1 or 4 days after emergence in the August 5 cohort and 0 or 4 days after emergence in the September 23 cohort). In the August 5 cohort, members of the younger age class (i.e., those tested one day after emergence) were held either in the light or dark during the 6-h runs. Because no statistically significant differences existed between metabolic rates of groups held in the light or dark, the results were pooled and members of the older age class (i.e., those tested 4 days after emergence) as well as mosquitoes from the September 23 cohort were held only in the light during the 6-h runs.

Statistical tests: Differences in longevity between short and long-photoperiod groups were evaluated with the logrank test (Peto et al. 1977). Differences in rates of metabolism between short and long-photoperiod groups were

evaluated with an analysis of covariance. Analysis of covariance was used because it shows the effect of photoperiod on metabolic rate after the effects of other variables, such as body size, have been removed (Packard and Boardman 1987). Metabolic-rate and body-weight values were transformed logarithmically before the analysis to improve the linear relationship between these 2 variables.

RESULTS

Adult longevity: The longevity of reared adult mosquitoes is illustrated in the survivorship curves of Fig. 1. In both cohorts, short-photoperiod individuals lived longer than did long-photoperiod individuals (P = 0.001 to 0.008). Longevity differences are also reflected in e_0 values, i.e., average life span remaining for

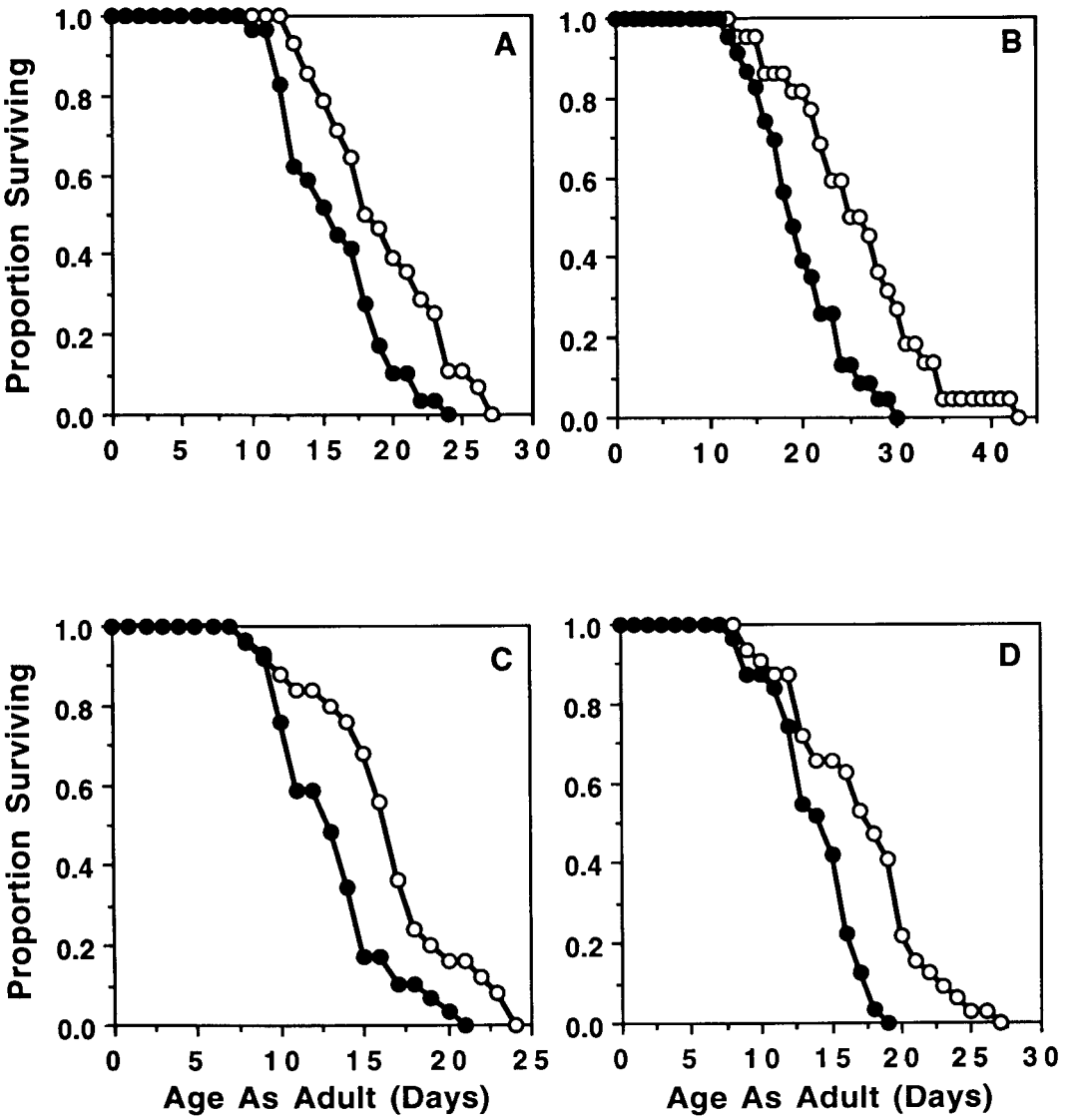

Fig. 1. Survivorship of adult males and females from 2 cohorts of *Anopheles quadrimaculatus* reared under short and long photoperiods. Open circles represent short-photoperiod rearings and black circles represent long-photoperiod rearings. A. Survivorship of 28 short-photoperiod and 29 long-photoperiod males from the August 5 cohort. B. Survivorship of 22 short-photoperiod and 23 long-photoperiod females from the August 5 cohort. C. Survivorship of 25 short-photoperiod and 29 long-photoperiod males from the September 23 cohort. D. Survivorship of 32 short-photoperiod and 31 long-photoperiod females from the September 23 cohort.

Table 1. Mean metabolic rates ($\bar{x}$ MR) expressed in μl O_2/h and mean dry weights ($\bar{x}$ DW) expressed in mg of different groups of *Anopheles quadrimaculatus*. Other symbols are DAE (number of days after emergence), SE (standard error) and *n* (sample size)

Cohort	Photoperiod	Sex	DAE	$\bar{x}$ MR ± SE	$\bar{x}$ DW ± SE	*n*
Aug. 5, 1991	Short	Male	1	3.8 ± 0.1	0.653 ± 0.020	10
			4	2.8 ± 0.2	0.656 ± 0.021	5
		Female	1	4.3 ± 0.3	0.842 ± 0.039	10
			4	3.3 ± 0.3	0.990 ± 0.056	5
	Long	Male	1	4.7 ± 0.4	0.716 ± 0.033	10
			4	3.8 ± 0.7	0.661 ± 0.023	5
		Female	1	5.3 ± 0.4	0.985 ± 0.050	9
			4	4.3 ± 0.5	1.080 ± 0.078	5
Sept. 23, 1991	Short	Male	0	4.6 ± 0.2	0.624 ± 0.025	5
			4	2.9 ± 0.2	0.626 ± 0.048	5
		Female	0	5.1 ± 0.2	0.800 ± 0.055	5
			4	3.0 ± 0.1	0.861 ± 0.069	5
	Long	Male	0	3.9 ± 0.1	0.572 ± 0.017	5
			4	2.4 ± 0.2	0.566 ± 0.026	5
		Female	0	4.5 ± 0.1	0.652 ± 0.023	5
			4	2.4 ± 0.3	0.696 ± 0.056	5

emerging adults: 18.96 vs. 15.57 days in males and 25.64 vs. 19.33 days in females from the August 5 cohort; 16.06 vs. 12.81 days in males and 16.88 vs. 13.66 days in females from the September 23 cohort. Also, mosquitoes from the August 5 cohort lived longer than did those of the same photoperiod and gender from the September 23 cohort ($P = 0.0001$ to 0.047).

Adult metabolic rates: Adult metabolic rates are listed in Table 1. The metabolic rates of adult mosquitoes from the August 5 cohort were significantly affected by body weight ($P = 0.001$), age ($P = 0.0001$) and photoperiod ($P = 0.002$). Specifically, higher metabolic rates were observed in larger, younger, and long-photoperiod individuals. From the covariance model applied to the August 5 cohort, metabolic rates were 3.53 and 4.22 μl O_2/h in short and long-photoperiod mosquitoes with the average dry body weight of 0.79 mg. The metabolic rates of mosquitoes from the September 23 cohort were also significantly affected by body weight ($P = 0.0007$), age ($P = 0.0001$) and photoperiod ($P = 0.004$). Specifically, higher metabolic rates were observed in larger, younger and short-photoperiod individuals. From the covariance model applied to the September 23 cohort, metabolic rates were 3.65 and 3.24 μl O_2/h in short and long-photoperiod mosquitoes with the average dry body weight of 0.66 mg. Thus, despite the similar relationship between survivorship and photoperiod in both cohorts, metabolic rate was not consistently related to photoperiod. Short-photoperiod mosquitoes lived longer than did their long-photoperiod counterparts in both cohorts, but had lower metabolic rates only in the first cohort. Also, gender had no effect on metabolic rates after the effect of body size was removed by the covariance analysis.

DISCUSSION

Short photoperiod affects longevity of *An. quadrimaculatus*: individuals reared under short photoperiod lived longer than did those reared under long photoperiod. Reasons for the greater longevity of these short-photoperiod mosquitoes are unclear. Short photoperiod is known to initiate diapause in adult anophelines, and adult diapause is associated with extended life spans (Washino et al. 1971, Washino 1977). As mentioned earlier, however, diapause is not likely to occur in natural populations of this species in Alachua County, Florida and was not triggered in the laboratory by the short photoperiod used in this study.

Insects reared under high temperature have been hypothesized to die sooner because of high metabolic rates (Dingle and Baldwin 1983). This reasonable hypothesis suggests that short photoperiod decreases life spans because short photoperiod is apparently associated with high metabolic rates in some insects (Giesel et al. 1989, Lanciani et al. 1990). But short photoperiod was found to increase, not decrease life spans in the present study. Short-photoperiod mosquitoes lived longer even when their metabolic rates were higher than those of long-photoperiod mosquitoes. Thus, metabolic rate differences between short and long-photoperiod mosquitoes do not explain the longevity differences observed in the present study.

One variable that appears to be associated with longevity in insects is body weight; within a species, heavier individuals often live longer (King and Martin 1975, Akey et al. 1978). Weights could not be estimated on mosquitoes from the longevity experiments, but short-photoperiod mosquitoes from the metabolic-rate study were not consistently heavier (Table 1). However, other rearings from the Lake Alice population of *An. quadrimaculatus* have been analyzed, and short-photoperiod individuals were found to be consistently heavier (Lanciani 1992). Although weight and longevity may be positively correlated in some insects, a physiological mechanism that causally links these variables has not been identified.

The effect of short photoperiod on longevity as demonstrated in this study, thus, is similar to the effect of low temperature on longevity as demonstrated in previous studies (King and Martin 1975, Butler and Foster 1979, Moscardi et al. 1981, Beier et al. 1987). The effects of photoperiod on insect longevity, morphology and physiology often parallel the effects of temperature on these same features, illustrating the similarity between the action of photoperiod and temperature on insect biology.

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