

PHOTOPERIOD AND THE RELATIONSHIP BETWEEN WING LENGTH AND BODY WEIGHT IN *ANOPHELES QUADRIMACULATUS*

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ABSTRACT. The effect of photoperiod on wing length, body weight, and relationship between wing length and body weight was investigated in the mosquito species *Anopheles quadrimaculatus*. Individuals reared under a short photoperiod (8 h light: 16 h dark) had longer wings and larger weights than did those reared under a long photoperiod (16 h light: 8 h dark). Covariance analysis showed that photoperiod and wing length interacted so that photoperiod did not have a uniform effect on body weight at all wing lengths. At small mosquito sizes, body weight was higher in short than in long-photoperiod individuals of the same wing length, but at large mosquito sizes, body weight was higher in long than in short-photoperiod individuals of the same wing length. Thus, among smaller mosquitoes of this species, wings were disproportionately longer in long-photoperiod individuals, but among larger mosquitoes, wings were disproportionately longer in short-photoperiod individuals. These results, together with previous studies, suggest that photoperiod and temperature have similar effects on the developing insect.

INTRODUCTION

Photoperiod and temperature often have similar effects on the developing insect (Hoffmann 1985). For example, *Drosophila melanogaster* Meigen reared under short photoperiods have higher metabolic rates at a given temperature than do those reared under long photoperiods, just as expected if low and high rearing temperatures had been substituted for short and long photoperiods (Giesel et al. 1989, Lanciani et al. 1990). Similarly, *Chaoborus crystallinus* de Geer develop into larger adults if reared under either short photoperiods or low temperatures (Ratte 1979). This phenomenon may exist because photoperiod and temperature are normally associated in particular ways in nature. Short photoperiods are usually coupled with low temperatures and long photoperiods with high temperatures. As a result, similar adaptive changes in insects may be triggered by changes in photoperiod and temperature.

The effect of developmental temperature on insect morphology has been well established, e.g., in mosquitoes (van den Heuvel 1963, Nayar 1968), biting midges (Linley et al. 1970, Akey et al. 1978) and blackflies (Colbo and Porter 1981). These studies exemplify a practically axiomatic trend that low temperature leads to the development of larger insects. Temperature also affects the relationship between wing length and body weight; in *Culex pipiens pallens* Coq. (Hosoi 1954), *Aedes aegypti* (Linn.) (van den Heuvel 1963), and *Culex nigripalpus* Theobald (Nayar 1968), individuals reared at low temperatures develop disproportionately longer wings than do those reared at high temperatures. Thus, although both wing length and body weight generally increase as temperature experienced during development decreases, wing length in-

creases more. Considering the similar influences of temperature and photoperiod already mentioned, one might expect that photoperiod, too, affects the relationship between wing length and body weight in insects. To test this expectation, I reared mosquitoes of the species *Anopheles quadrimaculatus* Say under short and long photoperiods and analyzed the relationship between their wing lengths and body weights.

MATERIALS AND METHODS

Cultures: Gravid *An. quadrimaculatus* were collected on 2 dates (January 14 and June 23, 1991) from daytime resting sites near the shore of Lake Alice, Gainesville, Florida. The mosquitoes were held separately in plastic vials (3 cm diam \times 7 cm height) with a few ml of water, the vials were placed in covered plastic boxes containing moistened paper towels, and the boxes were kept in a constant-temperature chamber set at 28°C and a 12 h light-dark cycle. The first clutch of eggs laid by a female from each of the 2 collections was used in rearing experiments. Half of the eggs from a clutch were placed into a short-photoperiod (8 h light: 16 h dark) constant-temperature chamber set at 28°C and the other half into a long-photoperiod (16 h light: 8 h dark) constant-temperature chamber also set at 28°C. A constant temperature was assured in each chamber by timed temperature controls set lower during light periods to compensate for heat given off by the lights. Before experiments began, these controls were adjusted while checking temperature with a thermometer placed in a 250 ml water-filled Erlenmeyer flask located next to the rearing pans. These thermometer readings agreed with average thermocouple readings taken from the top 5 mm of water in different areas of the rearing pans, suggesting

Table 1. Wing lengths (expressed in mm) and dry weights (expressed in mg) of *Anopheles quadrimaculatus* reared under short and long photoperiods. Symbols used are WL (wing length), Wt (dry weight), SE (standard error), and *n* (sample size).

Collection	Sex	Photoperiod	Mean WL (mm) ± SE	Mean Wt (mg) ± SE	<i>n</i>
January 14, 1991	Female	Short	4.227 ± 0.033	0.945 ± 0.014	10
		Long	3.863 ± 0.026	0.838 ± 0.021	16
	Male	Short	3.687 ± 0.019	0.757 ± 0.012	18
		Long	3.454 ± 0.016	0.644 ± 0.010	19
June 23, 1991	Female	Short	4.131 ± 0.038	0.875 ± 0.023	33
		Long	3.929 ± 0.031	0.762 ± 0.021	40
	Male	Short	3.613 ± 0.015	0.648 ± 0.012	42
		Long	3.388 ± 0.017	0.528 ± 0.012	36

that the temperature experienced by the larvae, which frequent the top few mm of the water column, was adequately controlled. The thermometers were monitored throughout the experiments to verify that temperature was held at 28°C.

Eggs at each photoperiod were held within a floating paper ring in 500 ml of tap water in a white enamel pan. 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 sizes were selected from each pan and were placed in separate pans after 500 ml of tap water and 0.06 g of food had been added. On each succeeding day until pupation, larvae were transferred to clean pans containing 500 ml of fresh tap water and were fed. On these succeeding days, feeding followed this schedule: 0.06, 0.07 and then 0.09 g of food per pan continuing until pupation of all larvae in a pan. The pans were covered with clear plastic sheets throughout the experiments to retard evaporation. As pupae appeared, they were held individually in screen-covered vials in the same constant-temperature chamber in which they developed.

Measurements: Adults were removed from the constant-temperature chamber within 8 h of emergence and were placed in a freezer. Later, they were dried for 2 days at 60°C and were weighed individually with a Cahn electrobalance. Then one wing was removed from each specimen, placed between 2 glass slides, and measured from the axillary incision to the apex, excluding scales. Drying apparently did not change wing length but sometimes warped the wing. However, the wing could still be accurately measured by placement between 2 glass slides. Sample sizes of analyzed adults were 63 (collection of January 14) and 151 (collection of June 23).

Statistical analysis: Analysis of variance was used to test body weight (dry) and wing length differences between short and long-photoperiod

mosquitoes. Separate analyses of variance were done for each sex and collection. Covariance analysis was used to determine the relationship between wing length and body weight (dry) of short and long-photoperiod mosquitoes of each sex. Separate covariance analyses were done for each collection date. The variables wing length (the independent variable) and body weight (the dependent variable) were logarithmically transformed to improve the fit to the linear covariance model. The linear relationship between body weight and cube of wing length suggested by scatterplots in van den Heuvel (1963) implies a linear relationship between the logarithms of body weight and wing length.

RESULTS

Analysis of variance showed that average wing lengths of short-photoperiod mosquitoes were significantly longer than those of long-photoperiod mosquitoes in both sexes and collections ($P = 0.0001$; Table 1). Likewise, average body weights of short-photoperiod mosquitoes were significantly higher than those of long-photoperiod mosquitoes in both sexes and collections ($P = 0.0001-0.0012$; Table 1).

Covariance analysis of the wing length-body weight relationship in the January 14 collection showed that body weight was significantly affected by photoperiod ($P = 0.0001$), wing length ($P = 0.001$), and a photoperiod-wing length interaction ($P = 0.001$). This model accounted for 92.4% of the variation in body weight. In the collection of June 23, body weight was significantly affected by photoperiod ($P = 0.0434$), sex ($P = 0.0047$), wing length ($P = 0.001$), and a photoperiod-wing length interaction ($P = 0.0463$). This model accounted for 86.8% of the variation in body weight.

The significant interactions in both collections mean that the regression lines predicting body weight from wing length have different slopes for each photoperiod. The parameters of

the regression lines are listed in Table 2. Because the line with the smaller slope (short photoperiod) also has the larger (less negative) intercept within the same sex grouping in each collection, the short and long-photoperiod lines must intersect. Through algebraic means, the lines were found to intersect at a wing length of 3.72 mm in the collection of January 14 (both sexes) and 3.85 mm in the collection of June 23 (both sexes). Of the mosquitoes included in the study, most females (83/99) are above and most males (111/115) below these wing length boundaries. At wing lengths below the boundaries, short-photoperiod mosquitoes are heavier (because of the larger intercepts) than long-photoperiod individuals of the same wing length, but at wing lengths above the boundaries, long-photoperiod mosquitoes are heavier (because of the larger slopes) than short-photoperiod individuals of the same wing length. These results can be expressed differently to facilitate comparisons with related studies, e.g., van den Heuvel (1963) and Nayar (1968): in small mosquitoes, wings are disproportionately longer in long-photoperiod individuals, but in large mosquitoes, wings are disproportionately longer in short-photoperiod individuals.

DISCUSSION

The body-weight and wing-length observations on *An. quadrimaculatus* support the contention that photoperiod and temperature have similar effects on insect morphology. The higher weights and longer wing lengths of individuals reared under a short photoperiod and the lower weights and smaller wing lengths of individuals reared under a long photoperiod indicate that short photoperiod and low temperature similarly influence mosquito morphology as do long photoperiod and high temperature.

Table 2. Parameters of regression equations predicting log (base 10) dry body weight (mg) from log (base 10) wing length (mm) in *Anopheles quadrimaculatus* reared under short and long photoperiods. The equations are of the form $Y = bX + a$, in which Y is log dry body weight, b is the slope, X is log wing length, and a is the Y intercept. For example, log dry body weight of short photoperiod adults of the January 14, 1991 collection is equal to 1.635 log wing length -1.045.

Collection	Photoperiod	Sex	Slope	Intercept
January 14, 1991	Short	Both	1.635	-1.045
	Long	Both	2.450	-1.510
June 23, 1991	Short	Female	2.715	-1.737
	Long	Female	3.065	-1.942
	Short	Male	2.715	-1.703
	Long	Male	3.065	-1.908

The photoperiod by wing-length interaction found to affect body weight was not noted previously in mosquito studies. Photoperiod, like temperature, appears to change the proportionality between wing length and body weight in a mosquito species. However, because of the interaction, the photoperiod associated with disproportionately longer wings is not the same at all mosquito sizes. Short-photoperiod individuals have disproportionately longer wings, as do low-temperature individuals, but only in large mosquitoes. In small mosquitoes, long-photoperiod individuals have disproportionately longer wings.

The effect of temperature on the relationship between wing length and body weight may also be nonuniform at different mosquito sizes, but previous analyses did not test this possibility. However, scatterplots of dry weight vs. cubed wing length of female *Ae. aegypti* presented in van den Heuvel (1963) contain the appropriate data. This information was extracted with a scanner, microcomputer and software and was then converted to a form comparable to data in the present study. Results from the 2 lower rearing temperatures (17 and 25°C), but not the higher (34°C), agreed with results of the present study: temperature and wing length interacted ($P = 0.0001$) in the same way as did photoperiod and wing length, i.e., in small mosquitoes, high-temperature individuals had disproportionately longer wings, but in large mosquitoes, low-temperature individuals had disproportionately longer wings. Thus, this comparison showed that even at a more complex level, photoperiod and temperature can similarly affect the relationship between wing length and body weight.

Mosquito workers, recognizing the linear relationship between body weight and cube of wing length, have suggested that the ratio of body weight to cube of wing length is constant in a species reared under constant conditions (Hosoi 1954, Christophers 1960, van den Heuvel 1963). However, Nayar (1968, 1969) and Nayar and Sauerman (1970) experimentally observed that this ratio was not constant in mosquitoes reared at any one temperature. Similarly, the ratio was not constant in *An. quadrimaculatus* reared at any one photoperiod in the present study. These experimental observations have a logical mathematical basis: a linear relationship between 2 variables does not necessarily establish a constant ratio between them. A linear relationship between a dependent variable Y, which in this case represents body weight, and an independent variable X, which in this case represents cube of wing length, implies the following equation: $Y = a + bX$, in which a is the Y intercept and b is the slope. The ratio of body weight to cube of

wing length is Y/X , which can be seen as the left side of the above equation after both sides are divided by X . The ratio Y/X then is equal to $a/X + bX/X$ or $a/X + b$. The expression $a/X + b$ cannot be constant in general, even though a and b are constant, because of the variable X ; as X changes in value, so does the entire expression. For example, as cube of wing length increases, the ratio of body weight to cube of wing length is generally not constant but converges on b from values above or below, depending on the sign of a . Only if a is zero will the ratio be constant (equal to the value of b). The ratio of body weight to cube of wing length does not reliably indicate how mosquitoes respond to different rearing conditions and should not be used for this purpose. The problem of working with ratios has long been recognized by statisticians (e.g., Snedecor 1956), and more recently Packard and Boardman (1987) recommended using regression or covariance instead of ratios to study features that vary allometrically with some index of body size.

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