INFLUENCE OF TEMPERATURE AND LARVAL NUTRITION ON THE DIAPAUSE INDUCING PHOTOPERIOD OF *AEDES ALBOPICTUS*

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ABSTRACT. Photoperiod induced dormancy for 14 North American strains of *Aedes albopictus* were determined at 21, 26 and 29°C. Strains tested at 21°C and intermediate temperatures of 25–27°C demonstrated clear photoperiodic responses whereas temperatures of 29°C and above, greatly reduced or negated diapause incidence. A suboptimal larval diet increased the percentage diapause in eggs laid by resulting adults. This larval diet was also associated with a slight increase in critical photoperiod.

INTRODUCTION

In August of 1985, a large infestation of *Aedes albopictus* (Skuse) was reported in Harris County, Texas (Sprenger and Wuithiranyagool 1986). By 1989, *Ae. albopictus* was entrenched in the midwestern and eastern portions of North America (Moore et al. 1988). The successful colonization of North America by *Ae. albopictus* is due to its photoperiod-induced egg diapause and ability to tolerate low temperatures (Hawley et al. 1987).

When exposed to short days at 25°C, adult female *Ae. albopictus* from temperate latitudes lay diapause eggs (Wang 1966, Mori et al. 1981). The geographic distribution of the photoperiodic response is known (Hawley et al. 1987) and geographic variation in critical photoperiod documented for both North American and Asian populations. The present study investigates how 2 crucial environmental variables, temperature and larval nutrition, affect the photoperiodic response.

MATERIALS AND METHODS

Mosquito strains: Fourteen strains of *Ae. albopictus* (Skuse) from the United States were used in this study. Strain names and colonization history are listed in Table 1.

Standard rearing techniques: Eggs were hatched in deoxygenated nutrient broth solution (Novak and Shroyer 1978). One hundred fifty first instar larvae were placed in an enamel pan filled with approximately 2 liters of tap water and fed a suspension of liver powder in water (Munstermann and Wasmuth 1985). Larval rearing was conducted in an insectary maintained at 25–27°C, a relative humidity of 80–85% and a photoperiodic cycle of L:D 18:6. Pupae and adults were allowed to emerge into plastic 3.8 liter cages or 0.47 liter cardboard cages. Honey-soaked cellucotton was provided as a carbohydrate source for the adults. Female mosquitoes were blood-fed on white mice anesthetized with Nembutol.[2]

Photoperiod exposure: Two types of photoperiod exposure chambers were used. The first, a photocan system, was described by Beach and Craig (1977). Each photocan consisted of a light-tight metal can (25 cm diam. × 32 cm deep) equipped with 2 subminiature lamps (Type 2180, Lafayette Radio Electronics) and a Dayton 24-h time switch. Heat output of the subminiature lamps was negligible. The second were photoperiod cabinets constructed according to specifications provided by W. E. Bradshaw of the University of Oregon. Each box has 12 individual chambers measuring 35 × 35 × 45 cm each. Individual chambers were equipped with isolated, air-cooled (Dayton 2C781 shaded pole blower) 4 watt cool-white fluorescent lamps. Each chamber had its own timer switch that controlled day length. The boxes were located in constant temperature rooms at 21°C. Temperature in each chamber remained within 0.5°C of ambient.

Pupae and adults were exposed to different photoperiods. The INDY strain was used as a standard and run simultaneously with the other strains in each trial.

Egg handling procedures: A group of 30 to 40 females was held for 5 to 6 days following a bloodmeal and then provided with an oviposition substrate of water-soaked balsa wood strips. The number of eggs laid by each group averaged

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[2] Animal handling and treatment were according to the "Guide for the Care and Use of Laboratory Animals," as put forward by the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

Table 1. Strain histories of *Aedes albopictus* used.

Strain	Collection data (generation tested)
BAL	Baltimore, Baltimore Co., MD, January 1988, from eggs sent by K. Sweeny. (F_3).
CARBEACH	Caroline Beach, New Hanover Co., NC, July 1987, collected from a barrel-drum by B. Engber. (F_3).
DOMINICK	Chicago, Cook Co., IL, August 1987, from a tire yard by S. Nawrocki and B. Farmer. (F_2).
DUNN	Evansville, Vanderburg Co., IN, September 1986, biting females collected from a tire yard, by M. Sinsko. (F_2).
ESL	East St. Louis, East St. Louis Co., IL, June 1988, from a tire dump, by S. Hanson and D. Wesson. (F_1).
INDY	Indianapolis, Marion Co., IN, September 1986, at a tire recycling company, by M. Sinsko. (F_3).
HOUSTON	Houston, Harris Co., TX, June 1986, from a tire dump by D. Sprenger. (F_4).
JAX-2	Jacksonville, Duval Co., FL, August 1987, this second collection by W. Hawley. (F_3).
LEX	Lexington, Fayette Co., KY, August 1987, from a tire yard by J. Cilek. (F_3).
MEMPHIS (MEM)	Memphis, Shelby Co., TN, June 1988, a second collection by C. Pumpuni. (F_1).
MIL	Milford, Sussex Co., DE, September 1987, from a tire recapping company by C. Stachecki. (F_3).
NEW ORLEANS	New Orleans, Jefferson Parish, LA, June 1986, collected M. Andis and E. Bordes of the New Orleans Mosquito Abatement District (F_3).
OAK	Oak Hill, Jackson Co., OH, October 1987, eggs from field-collected adults by R. Berry. (F_3).
SAVANNAH	Savannah, Chatham Co., GA, July 1988, eggs sent by O. Fultz of the Chatham Co. Mosquito Control Commission (F_2).

between 900 and 3,000. Eggs were held for 6 to 8 days to ensure embryonation, a process completed in about 72 hours. Under normal conditions (all rearing stages under a light cycle of 16:8 L:D), fresh eggs flooded with hatching solution give 99% hatch within 12 hours. Unhatched eggs were bleached with a weak solution of sodium hypochlorite to determine viability (Trpis 1970). Bleached eggs became transparent and changed color from black to yellowish-brown. The bleach was then rinsed from the eggs with tap water. The criteria for determining whether embryos were viable (Shroyer 1979)[3] were: 1) plate of hatching spine darkly sclerotized, 2) eyes large and darkly pigmented, and 3) absence of abnormal pigmentation, discoloration, decomposition or obvious deformity. To determine percent egg hatch, the number of hatched larvae was divided by the total number of viable eggs (hatched larvae plus remaining embryonated eggs).

Effect of temperature on photoperiod response: Photoperiod responses were determined at 15 and 30 min intervals, for 14 strains at temperatures ranging from 21 to 29°C. All 14 strains were run at 21°C; 10 strains were tested at 25–27°C and 5 strains at 29°C. Photoperiod experiments at 25 and 29°C were carried out in photocans (Beach 1977).[4]

Effect of larval nutrition on photoperiod response: The INDY strain was raised according to the "½×" (underfed) or "2×" (well-fed) diet protocols of McCombs (1980).[5] Since nutritionally deprived larvae take a longer period to pupate, the larvae were hatched a week earlier than larvae on "2X" diet, and were also fed an extra day to increase the rate of pupation (Pumpuni and Walker 1989). Wing measurements (from the base of the costa to the tip of the wing) and egg size (from the micropyle to the pointed end

[3] Shroyer, D. A. 1979. Seasonal aspects of egg hatching in *Aedes triseriatus* (Say): sex ratio distortion and diapause. Ph.D dissertation. University of Notre Dame, IN.

[4] Beach, R. F. 1977. The clock mechanism for photoperiodic induction of egg diapause in *Aedes atropalpus* (Diptera: Culicidae) Ph.D dissertation. University of Notre Dame, IN.

[5] McCombs, S. D. 1980. Effect of differential nutrition of larvae on adult fitness of *Aedes triseriatus*. M.S. thesis, Univ. of Notre Dame, IN.

of the egg) of both small- and large-sized adults were determined. Photoperiodic response curves for both well-fed and poorly fed groups were determined at 22°C.

RESULTS

Effect of temperature on photoperiodic response: All 14 strains of *Ae. albopictus* demonstrated clear photoperiodism at 21°C (Fig. 1). At photoperiods at or below 13 h of light and 11 h of darkness, egg hatch in each strain was below 50% and at photoperiods at or above 14 h of light and 10 h of darkness, egg hatch was above 50%. The critical photoperiod for all 14 strains in this study was therefore between 13 and 14 h of light (Fig. 1). A 5°C rise in temperature from 21°C to 26°C did not affect the photoperiodic response of the groups examined. In contrast, temperatures at or above 29°C greatly reduced (NEW ORLEANS) or completely negated the photoperiodic response (INDY, LEX, MEM, SAVANNAH, BAL and DOMINICK) (Fig. 1).

Effect of nutrition on critical photoperiod: Groups of adults nutritionally deprived during the larval stage showed a lower egg hatch than groups on normal diet at most of the photophases tested (Fig. 2). Indeed, 30 and 40% of eggs laid by adults nutritionally deprived as larvae remained in diapause at photophases of 14-¼ and 15 h, respectively. Further, the critical photoperiod in the nutritionally deprived group was lengthened by 15 minutes. The lengthening of critical photoperiod, exhibited by adults nutritionally deprived in the larval stage, was significant ($\chi^2 = 182$, $P < 0.001$). Egg length measurements in both test groups, adults on a normal diet and adults nutritionally deprived as larvae, were not significantly different (0.48 ± 0.02 mm and 0.47 ± 0.03 mm, respectively, $P > 0.05$, paired t-test; $n = 30$). There was a significant difference in body size of adults from the poorly fed and well-fed groups (well-fed females, 3.2 ± 0.2 mm; poorly fed females, 2.7 ± 0.11 mm; $P < 0.05$, paired t-test; $n = 40$).

DISCUSSION

Effect of temperature on photoperiodic response: In general, insects that enter photoperiod-induced diapause in response to lengthened daylight have an intense diapause when raised at low temperatures, whereas high temperatures either override or greatly decrease incidence of such diapause (Saunders 1982). For *Aedes* mosquitoes, interactions between temperature and photoperiod have been examined for *Aedes atropalpus* (Coq). (Anderson 1968, Beach 1977),[4] *Ae. albopictus* (Imai and Maeda 1976) and *Aedes taeniorhynchus* (Wied.) (Parker 1986). The TALLULAH strain of *Ae. atropalpus* from Georgia (34°N) reverted from a 100% diapause response to 20% response when the rearing temperature was changed from 22 to 27°C at L:D 12:12, while the photoperiodic response of the FITZROY strain from Ontario (45°N) was unaffected (Beach 1977).[4] In *Ae. albopictus*, Imai and Maeda (1976) observed an egg hatch of 10 and 14% for adults raised in short photoperiod at 20 and 27°C, respectively. Egg hatch subsequently increased to 54% for adults kept at 27°C over 47 days. In the present study, strains of *Ae. albopictus* exhibited a clear photoperiodic response at 21°C and 25–27°C. This response was absent or greatly reduced at 29°C. Photoperiodic responses at 21°C were the most reliable and showed the least fluctuation with respect to egg hatch at the different photophases. It has further been observed that temperatures below 21°C further increase the incidence of photoperiod induced dormancy in *Ae. albopictus* (G. B. Craig, Jr.; unpublished data).

Effect of nutrition on photoperiodic response: Nutritionally deprived larvae gave rise to adults that laid eggs with a high incidence of diapause. At 21°C the critical photoperiod in nutritionally deprived adults of the INDY strain was lengthened by 15 min, and percent egg hatch laid by these adults in long photoperiod (L:D 15:9) was only 65%. The lengthening of the critical photoperiod associated with nutritional deprivation may have an adaptive significance. In the field, adult mosquitoes emerging from habitats with little nutrition may lay eggs programmed to go early into diapause, thus avoiding low larval food supplies. Further, females nutritionally stressed as larvae will begin laying diapause eggs earlier in fall than their well-fed conspecifics.

Food has been shown to modify the primary response to photoperiod in several species of insects thereby altering the incidence and duration of diapause (Tauber et al. 1986). In *Ae. triseriatus* (Say), larval diapause increases when short day larvae are provided with an inadequate diet or kept at low temperatures (Clay and Venard 1972). Similar observations have been reported in *Ae. atropalpus* (Beach 1977).[4] Beach (1977)[4] noted that starved larvae of *Ae. atropalpus* took longer to develop, thus extending the larval sensitive period even at high temperatures which led to a higher incidence of diapause.

The photosensitive stages in *Ae. albopictus* are the pupae and adults (Wang 1966). This then suggests a different mechanism may be associated with increased incidence of diapause and nutritional deprivation.

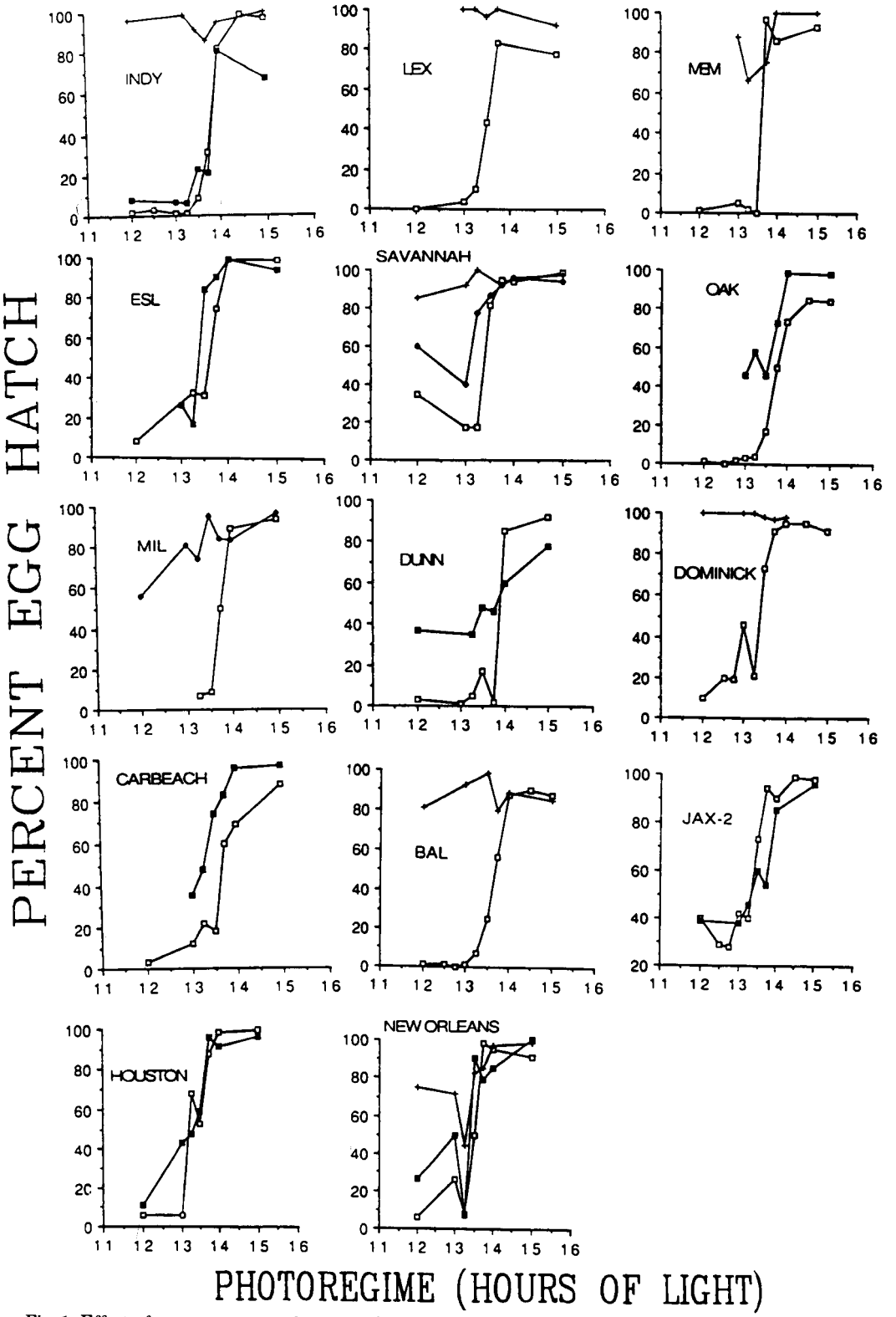

Fig. 1. Effect of temperature on photoperiodic response of *Aedes albopictus*. All 14 strains were run at 21°C; 10 strains were run at 26°C and 7 at an additional 28–29°C. Groups run at 21°C are represented by open squares (□), those run at 26°C, by closed squares (■) and those run at 29°C by pluses (+).

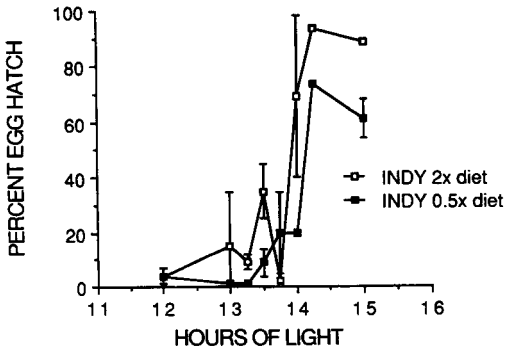

Fig. 2. Effect of nutritional deprivation on critical photoperiod of *Aedes albopictus*. Photoperiodic responses of adults starved when larvae are represented by closed squares (■) while responses by adults on normal diet are represented by open squares (□). The vertical bars represents the standard error around the mean for 2 replicates.

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