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Life History Characteristics of *Wyeomyia smithii* from New Jersey

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ABSTRACT: We colonized *Wyeomyia smithii* (Coquillett) from southern New Jersey and studied life history characteristics in the laboratory. Males and females showed no significant difference in time spent from first to third instar, but female larvae remained in fourth instar 2.1 days longer than males. At 22±2°C females emerged 22.6±3 days after egg hatch; males emerged approximately two days earlier. Male emergence peaked five hours after dawn; females showed a trend to emerge late in the day. Rotation of male terminalia was completed 9 to 11 hours after emergence. Females were capable of mating immediately after emergence. *Wyeomyia smithii* females laid their first egg batch four to six days after emergence. Females were capable of laying up to seven batches of eggs, however the mean number of eggs per oviposition decreased significantly as the number of oviposition cycles increased.

Keyword Index: *Wyeomyia smithii,* larval development, emergence rhythms, sexual maturation, fecundity.

INTRODUCTION

Wyeomyia smithii (Coquillett) is distributed in eastern North America from the Gulf of Mexico to Labrador and northeastern Saskatchewan (Bradshaw and Holzapel 1996). The mosquito completes its immature development in aquatic habitat offered by the leaves of the purple pitcher plant, *Sarracenia purpurea* L. Dodge (1947) felt that *Wy. smithii* was limited to the northern subspecies of the plant, *S. p. gibbosa* (Rafinesque) and that *Wyeomyia haynei* followed the range of the southern subspecies, *S. p. venosa* (Rafinesque). The two species are now believed to represent a single species, *Wy. smithii*, that displays a north-south geographic cline of morphological and life history characteristics (Bradshaw and Lounibos 1977, Bradshaw and Holzapfel 1983).

Northern populations of *Wy. smithii* overwinter as larvae, frozen within the leaves of the host plant (Smith 1902), and are obligatorily autogenous throughout their life span (O'Meara and Lounibos 1981). Southern populations are autogenous for their first egg batch but generally require blood for subsequent batches (Bradshaw 1980).

We colonized *Wy. smithii* from southern New Jersey and used the F_3 generation to study various aspects of its biology, such as differences in larval developmental rates of females and males, diel emergence rhythms, sexual maturation of both sexes, ovarian development, and fecundity of females.

MATERIALS AND METHODS

Colony Maintenance

A colony of *Wy. smithii* was established from third-instar larvae that were collected from a stand of *S. purpurea* growing in the New Jersey pine barrens in Ocean County, NJ (latitude 39° 50'; longitude 74° 15'). Larvae were collected from the leaf liquor of plants in late March 1997 and reared in enamel pans containing deionized water and fed a mixture of 20 parts ground hamster food, 12 parts liver powder, and 1 part brewer's yeast. We kept the adults in a 0.3 m^3 cage at a 16L:8D photoperiod that included 1.5 hours of dusk and 1 hour of dawn provided by a 15 watt incandescent bulb. Adults were given constant access to 10% sucrose solution on cotton wicks as a source of carbohydrate and were never offered blood.

Larval Development.

To quantify immature life history characteristics, we reared 96 individual larvae at 22±2°C in separate wells of 24 well (2.0 ml each) Falcon® culture plates. We placed a single first-instar larva in each well with 1.5

ml of deionized water and placed covers on each unit throughout the rearing process. The food mixture used to rear the colony was mixed in slurry, and 100 ml was added to each well. The wells of the rearing plates were skimmed daily with a paper towel to remove scum formed by bacterial growth. Deionized water was added after the skimming process to replace water lost to evaporation. Food was added to individual wells every other day. We daily recorded the number of dead larvae and kept a record of exuviae to determine day of molting. No food was added after pupation and adults emerged directly in the wells of the covered culture plates. The date and sex of each individual was recorded at emergence, labeled by well number, and frozen in individual Wasserman tubes. Wing length of both sexes was measured with an ocular micrometer (Mahmood et al. 1997) and compared with a two tailed t-test ($P < 0.05$).

We calculated the mean duration for each larval instar by sex and compared differences in instar duration for each sex using a two way ANOVA and Tukey's HSD test. Survivorship to the adult stage was calculated as total number of adults divided by the total number of first-instar larvae at the onset of the experiment.

Diel Emergence Rhythms

We allowed females to oviposit on the water surface of 26.5 x 15.5 cm white enamel pans placed in the colony cage for 24 hours. Larvae that hatched from these eggs during a 24-hour period were transferred to 36.0 x 23.0 cm enamel pans and reared to pupation. A total of 600 pupae that were collected on six different days and placed in 500 ml beakers containing deionized water and covered with nylon netting was observed for adult emergence. Time of emergence was recorded at one-hour intervals for 24 hours for each group. Cohorts of adults that emerged during each hourly interval were placed in separate 0.5 liter cartons and later recorded by number and sex. Diel emergence rhythms were calculated as percent of total adults of each sex emerging during a one-hour interval divided by the total number of adults of that sex that emerged over a 24-hour period.

Sexual Maturation of Males

Groups of 10 to 24 males were isolated within one hour of emergence in separate 0.5 liter paper cartons. All of the insects were anesthetized with chloroform in individual cartons at intervals from 0 to 12 hours after emergence to record progression in rotation of their terminalia. We followed the classification system of Khan and Reisen (1977) and recognized four stages of rotation (Fig. 1): 1) 0° to <45° rotation, 2) 45° to 90° rotation, 3) 90° to 135° rotation, and 4) 135° to 180° rotation. Terminalia rotation was checked until all of the males in remaining cohorts had terminalia rotated to 180°. We also examined similar groups of males to determine whether the terminalia rotated clockwise or counter clockwise. Direction was determined visually by noting the position of the heavily scaled light pleuron of segment 8 in relation to the black tergum and white sternum of segment 7 (Fig 1). We calculated the percentage of males in each class of terminalia rotation by dividing the number of males in a rotation class by total number of males checked for that time interval.

Sexual Maturation of Females

We placed 22 female pupae in a 250 ml beaker and allowed them to emerge in a 3.8 liter carton with 20, five-day-old virgin males to determine if newly emerged females mate with sexually mature males. Similarly, we allowed 46 female and 39 male pupae to emerge together to determine if newly emerged adults were able to mate. Both groups were provided with 10% sucrose solution. After 30 hours, we dissected the spermathecae of each female in saline (Hayes 1953) for the presence of sperm. To determine the effect of age on female mating ability, we allowed groups of female and male pupae to emerge in a series of separate 3.8 liter paper cartons. Individual groups of virgin females were isolated from males for 1, 3, 4, and 5 days. Virgin males used for mating experiments were four days of age. Four to six replicates of 10 females in each of the above age classes were kept with 10 virgin males for 24 hours. The following day, we dissected the spermathecae of all females and recorded the number of inseminated females. The percentage of inseminated females (number inseminated in the sample/ total number dissected) in different age classes were compared by one way ANOVA after arcsine transformation and significant differences in means were compared by Tukey's HSD test.

Ovarian Development

We kept 100 newly emerged females and 100 newly emerged males together in a 3.8 liter carton for 72 hours and provided 10% sucrose solution as a source of energy. From this group we examined follicular development in the ovaries of five mated females at 24, 48, and 72 hours from the time of emergence. In addition, we examined the ovaries of five newly emerged females that had not been exposed to males. We recorded length and width of five individual ovarioles in each female and the length of the area occupied by yolk using the methods of Mahmood et. al. (1991). Significant changes in ovarian development over time were determined by comparing changes in the length of

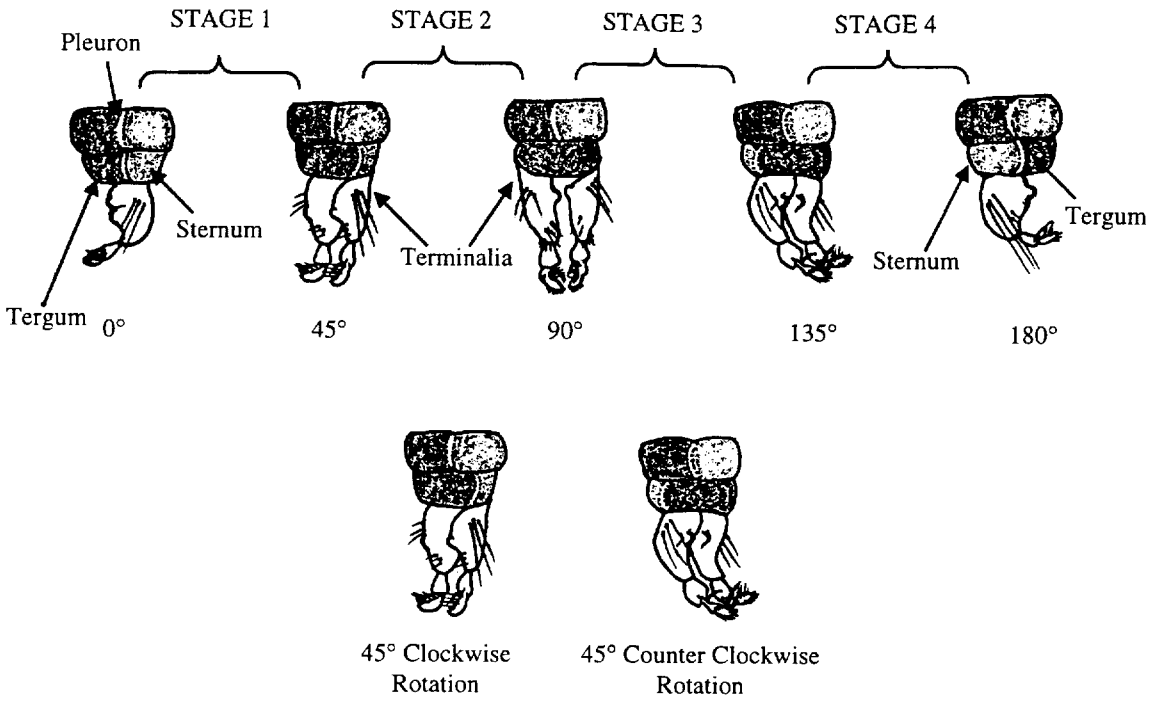

Figure 1. Stages of rotation in the male terminalia of *Wyeomyia smithii.*

ovarian follicles, width of ovarian follicles, and length of the follicles occupied by yolk using a one way ANOVA and Tukey's HSD test (Sokal and Rholf 1981).

Oviposition Cycles

We kept 50 newly emerged mosquitoes of each sex together in a 3.8 liter carton and isolated 30 females on the fourth day in individual glass vials half filled with deionized water. We plugged the vials with cotton soaked in 10% sucrose solution and checked the vials daily for evidence of oviposition. After ovi-position, females were transferred to fresh oviposition vials until all females laid their eggs and died. We recorded dates of oviposition, the number of eggs in each egg batch, the number of eggs that hatched in each egg batch, the number of days that elapsed between egg batches, and the total number of oviposition cycles completed by each female. We compared the mean number of eggs laid per female in each oviposition cycle using a one way ANOVA and Tukey's HSD test. Similarly, effect of increased oviposition cycles on mean percentage of hatched eggs per female was compared by the above tests after arcsine transformation of the data.

RESULTS

Immature Development

Wyeomyia smithii eggs hatched on the fifth day after oviposition at 22±2°C. Times spent in different immature instars differed significantly ($F = 432.263$; $df = 4, 420$; $P < 0.001$) and the mean duration of instars differed significantly between sexes ($F = 432.263$, $df = 1, 4$; $P < 0.001$). Tukey's HSD test revealed that males and females showed no significant differences in mean times spent in the first to third instars or as pupae. The second instar was of significantly shortest duration in both sexes. Female larvae remained in the fourth instar significantly longer, and as a result, emerged two days later than males ($P<0.01$) (TABLE 1). Of the 96 first-instar larvae used in these tests, 90% survived to adult emergence. Females were significantly larger (wing length = 2.35±0.01 mm, n=38) than males (wing length = 2.08±0.01 mm, n=41) ($t = -11.688$; $df = 77$; $P<0.001$, 2-tailed) and the sex ratio (males/total adults) was 0.53.

Adult Emergence

Emergence occurred primarily during the photophase for both sexes (Fig. 2). A total of 554 pupae emerged successfully during this study. Out of a total of

246 females that emerged successfully, only nine females emerged at night. There was a trend for increased emergence of females late in the day with maximum emergence one to three hours before dusk. Similarly, out of 290 males emerging successfully, only eight males emerged at night. Male emergence peaked five hours after dawn, with a second peak one hour before dusk.

Rotation of Male Terminalia

In the 236 specimens we examined, only slightly more than half (54%) rotated in a clockwise direction. Rotation to 180° was completed by 9 to 11 hours after emergence (Fig. 3).

TABLE 1. Duration of larval development in *Wyeomyia smithii*.

	Duration in Days	
	Female (n=40)	Male (n=46)
Instar	Mean ± SD [1,2]	Mean ± SD [1,2]
1st	3.0 ± 0.0^{cx}	3.1 ± 0.3^{cx}
2nd	2.1 ± 0.2^{dx}	2.1 ± 0.4^{dx}
3rd	3.0 ± 0.9^{cx}	2.8 ± 1.1^{cx}
4th	9.6 ± 2.7^{ax}	7.5 ± 1.6^{ay}
Pupa	5.1 ± 0.8^{bx}	5.0 ± 0.8^{bx}
Total	22.6 ± 3.0^{x}	20.4 ± 1.4^{y}

[1]Means followed by same letters (a, b, c, or d) in columns were not significantly different when tested by Tukey's HSD test ($P > 0.05$).
[2]Means followed by same letters (x, y) in rows were not significantly different when tested by Student's t test. ($P > 0.05$).

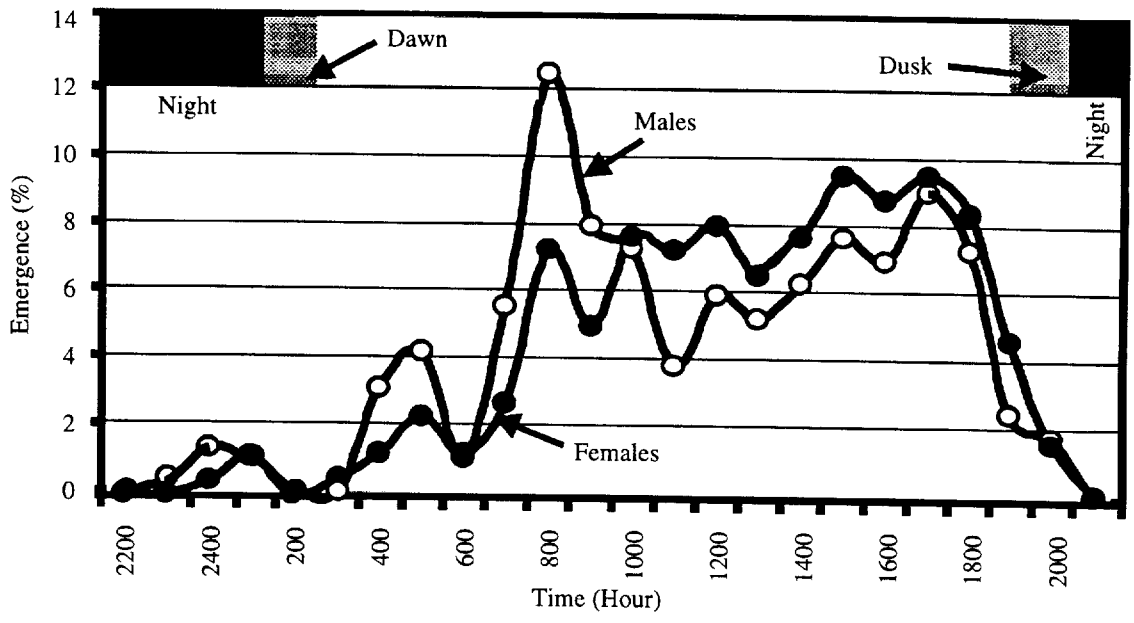

Figure 2. Emergence rhythms of male and female *Wyeomyia smithii* over a 24 hr period.

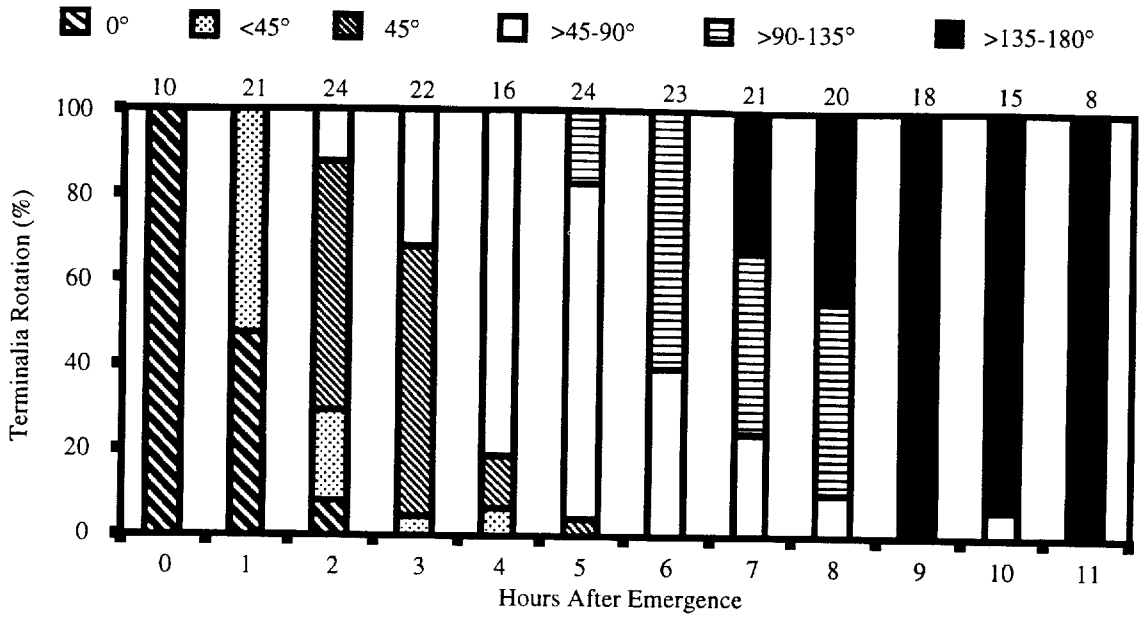

Figure 3. Progression of male terminalia rotation following emergence. Numbers on top of the bars indicate total males examined at each hourly interval.

Female Sexual Maturation

Five-day-old males inseminated 45.5% of newly emerged females, indicating that females are capable of mating immediately after emergence. We observed males approaching freshly emerged females that were resting on the side of the emergence container and still pumping air to expand the wings, similar to the observations of O'Meara and Lounibos (1981). Males mated during daylight with no noticeable swarm. Males hovered near resting females, landed nearby, and immediately attempted to clasp. When males and females of the same age were allowed to emerge together, 28% of the females were inseminated in the first 24 hours. No significant effect of age was observed on the mean percentage of inseminated females when compared by one way ANOVA after arcsine transformation of the data ($F = 2.721$; $df = 3, 13$; $P > 0.05$) (TABLE 2).

Ovarian Development

TABLE 3 shows that the length of the follicles in the ovaries of *Wy. smithii* increased significantly as the mosquitoes aged ($F = 330.51$; $df = 3, 96$; $P < 0.001$). The increase in size was due largely to yolk accumulation, because the area of follicle occupied by yolk granules increased significantly with age ($F = 514.779$; $df = 3, 96$; $P < 0.001$). Although follicle width increased with time, the effect was only significant for the first two days after emergence ($F = 330.510$; $df = 3, 96$; $P < 0.001$). Thereafter, the length of individual follicles increased significantly, but width remained relatively static (TABLE 3).

Oviposition Cycles

Wyeomyia smithii females laid their first egg batch four to six days after emergence. Only 8% of females oviposited on the fourth day and most of them (67%)

TABLE 2. Effect of age on insemination success in *Wyeomyia smithii* when groups of 10 females of known age were kept with 10, four-day-old males.

Female (Days)	Age Replicate Groups Examined	% Inseminated per Group (Mean ± SD)[1]
0-1	3	10.0 ± 0.0[a]
3	4	15.0 ± 5.8[a]
4	4	18.6 ± 14.4[a]
5	6	37.5 ± 21.8[a]

[1]Mean ± SD in column followed by the same letters was not significantly different when arcsine transformed mean percent inseminated was tested by Tukey's HSD test.

laid eggs on the fifth day, while 25% laid on the sixth day after emergence. Females were capable of laying up to seven batches of eggs but only 37.5% survived to complete more than four oviposition cycles (TABLE 4). Although, the mean number of eggs laid per female decreased significantly with increasing oviposition cycles ($F = 4.837$; $df = 5, 86$; $P < 0.001$), the mean number of eggs laid/female in the first four oviposition cycles were not significantly different when tested by Tukey's HSD. No effect of increased oviposition cycles was observed on mean percentage of hatched eggs per female ($P > 0.05$) (TABLE 4). Females took progressively longer to lay later egg batches (TABLE 5). The mean total eggs laid per female was 43±19 and the total number of eggs laid per female ranged from 16 to 88. The mean total % hatch over the life span of females was 93±10 ranging from 57% to 100%.

DISCUSSION

Wyeomyia smithii exhibits protandry, with males emerging approximately two days earlier than females. The delay of female emergence appears to result from their increased developmental time during the fourth larval instar. Female fourth instars required 2.1 days longer than males. Females were able to mate immediately after emergence but males required eight to ten hours for terminalia rotation. Smith and Brust (1971) showed that females of northern *Wy. smithii* eclose with their ovaries in an advanced stage of development. Horsfall and Ronquillo (1970) showed that ovarian development accelerates in late fourth-instar larvae of *Aedes stimulans* (Walker). Moeur and Istock (1980) showed that nutrients accumulated in fourth-instar larvae of *Wy. smithii* are allocated to oogenisis. This indicates that the additional time spent

TABLE 3. Effect of age on ovarian follicle size in *Wyeomyia smithii.*

Age (Hrs)	Follicle Length mm (Mean ± SD)[1]	Follicle Width mm (Mean ± SD)[1]	Yolk Length mm (Mean ± SD)[1]
0-1	170.0 ± 15.0^{d}	117.6 ± 11.5^{b}	110.6 ± 21.4^{d}
24	252.4 ± 21.9^{c}	173.6 ± 10.9^{a}	214.2 ± 20.6^{c}
48	307.0 ± 38.5^{b}	170.4 ± 9.2^{a}	274.8 ± 36.5^{b}
72	397.0 ± 23.7^{a}	175.0 ± 7.2^{a}	397.0 ± 23.7^{a}

[1]Means ± SD in columns followed by different letters were significantly different when tested by Tukey's HSD test ($P < 0.001$; $df = 3, 96$) for each test.

TABLE 4. Fecundity in *Wyeomyia smithii.*

No. Oviposition Cycles	Females Alive[1]	Percent Surviving	Eggs laid per female (Mean ± SD)[1]	% Hatch (Mean ± SD)[1,2]
1	24	100	19.6 ± 13.4^{a}	97.1 ± 9.9^{a}
2	22	91.7	12.3 ± 10.4^{ab}	97.5 ± 7.0^{a}
3	19	79.7	7.7 ± 6.4^{ab}	85.6 ± 25.0^{a}
4	15	62.5	10.1 ± 7.7^{ab}	79.9 ± 30.4^{a}
5	7	29.2	6.9 ± 7.4^{b}	79.9 ± 36.5^{a}
6	5	20.8	3.6 ± 1.1^{b}	90.0 ± 22.4^{a}
7	1	4.2	6.0	50.0

[1]Means ± SD in columns followed by same letters were not significantly different when compared by Tukey's HSD test.

[2]Percentages were arcsine transformed.

TABLE 5. Time interval between lifetime ovipositions of *Wyeomyia smithii*.

Oviposition Cycle	Total Females	Time Interval between Ovipositions (Percentage of Females)					
		1 Day	2 Days	3 Days	4 Days	5 Days	8 Days
2	22	68	18	5	5	0	5
3	19	42	26	11	11	11	0
4	15	38	6	13	19	25	0
5	7	60	40	0	0	0	0
6	5	67	33	0	0	0	0
7	1	0	0	0	100	0	0

in the fourth instar by females in this northern strain of *Wy. smithii* is used primarily to accumulate proteins for ovarian maturation.

Emergence rhythms in insects may be based on environmental cues such as photoperiod, ambient temperature, and humidity. Clements (1992) recognized species with no apparent emergence periodicity, species in which the time of emergence is determined by larval-pupal ecdysis as well as ambient temperature, and species in which ecdysis is either fine tuned during the pupal stage or controlled by an endogenous rhythm. In our studies, *Wy. smithii* male emergence peaked five hours after dawn with increased emergence of females later in the day. The differential appears to allow time for complete rotation of male terminalia in time to mate with females that eclose that same day.

Southern strains of *Wy. smithii* emerge with undeveloped ovaries (O'Meara and Lounibos 1981), mature a single batch of eggs without blood (O'Meara et al. 1981), and blood-feed for development of subsequent egg clutches. Northern strains of *Wy. smithii* emerge with their ovaries in an advanced stage of development (Smith and Brust 1971) and produce multiple clutches of eggs without blood (O'Meara et al. 1981). The Massachusetts strain of *Wy. smithii* used by O'Meara et al. (1981) laid three to five discrete egg batches at an interval of one to three days between clutches. In their study, the Massachusetts strain laid a mean of 64 eggs over the first three days, whereas, our New Jersey strain laid a mean of only 32.2 eggs over the same time period. Similar differences between Massachusetts and New Jersey strains occur in total fecundity. New Jersey *Wy. smithii* averaged only 43 lifetime eggs compared to 82 lifetime eggs from Massachusetts females. Larval diet, rearing temperatures, and larval density can influence fecundity in *Wy. smithii* (Smith and Brust 1971, Bradshaw and Lounibos 1972, Lang 1978, Bradshaw and Holzapfel 1992). A progressive decrease in the mean number of total eggs laid per female with increasing oviposition was similar to the observation of O'Meara et. al. (1981) for the Massachusetts (BT) strain. A decrease in the number of eggs laid per female is expected because protein reserves acquired as larvae are drawn upon for egg development during repeated ovipositions. Bradshaw (1986) found no significant differences among mean fecundities of six geographic populations of *Wy. smithii* and concluded that variation in fecundity is largely attributed to nutritional qualities of larval diet. Lang (1978) found that the protein content of the larval diet significantly increased oocyte production in this species. Diet, however, may not be entirely responsible for the wide variation in fecundity among geographic strains of this species. Differences in fecundity between the Massachusetts strain of *Wy. smithii* used by O'Meara et al. (1981) and the New Jersey strain used in this investigation suggests that genetic variation may be broad over the geographic distribution of this species.

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