

Male Reproductive Success in *Wyeomyia smithii* (Diptera: Culicidae)

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ABSTRACT Lifetime offspring sired by males increases with both male longevity and the rate of female availability. High rates of female availability saturate male ability to sire increasing offspring but do not affect male survivorship. When presented with a saturating number of females in two sequential, 24-h bouts, offspring sired in the second bout is independent of offspring sired in the first bout and is independent of recovery time between bouts. Our results suggest that males will pass up the opportunity to mate and, in so doing, avoid a physiological cost to future reproductive effort or to residual longevity.

KEY WORDS *Wyeomyia smithii*, longevity, mating

REPRODUCTIVE SUCCESS makes a major contribution to an individual's fitness. Because eggs are easier to count and follow than are sperm, reproductive success has been evaluated more frequently among females than males (Sakaluk & Cade 1980, Banks & Thompson 1987, Wedell & Arak 1989, Thompson 1990). Many studies have been made of male reproductive success in social animals, where males defend a harem, territory or lek (e.g. Downhower & Armitage 1971; Wells 1977; Howard 1978, 1979; Payne 1979; McGregor et al. 1981; Moore 1989). In nonsocial, nonterritorial insects, male reproductive success has usually been measured by mating activity (courtship and copulation) (Fincke 1982, Banks & Thompson 1985, Hoffmann & Cacoyianni 1989, Oberhauser 1989), mating success (copulation and oviposition) (Partridge & Farquhar 1983, Hafernik & Garrison 1986, Simmons 1986) or offspring sired over a brief period (McLain & Marsh 1990). Few investigators have measured lifetime offspring sired in the field (Fincke 1986) or in the laboratory (Simmons 1988, Rowe & Scudder 1990). The first experiment in our article considers lifetime reproductive success (lifetime offspring sired) of the pitcherplant mosquito, *Wyeomyia smithii* (Coquillett), in the laboratory.

Although sperm may be energetically cheaper to produce than eggs, observations among a variety of insects indicate that mating incurs more than a trivial energetic investment in males. In butterflies, the weight of both the spermatophore and the accessory substances delivered by males is highest in the first mating compared with later matings (Svard & Wiklund 1986) and there is a significant dependence of the weight of the spermatophore on the number of days since the last

mating (Svard 1985). Male damselflies will spend more time away from the breeding site following a mating than they would if they had been at the breeding site but failed to mate (Fincke 1982). This observation suggests that mated males require a recovery period and do not return to the breeding site until they are again able to mate. In fruit flies (*Drosophilidae*) (Demerec & Kaufmann 1941), a male's supply of mature sperm can be reduced after a few consecutive matings, and continued mating can result in a male's failure to inseminate future females. Although the males replenish their sperm supply when allowed a recovery period, the amount of sperm is lower than it had been initially. Other experiments on fruit flies have shown that multiple copulations can result in temporary depletion of the accessory glands, causing sterility (Markow et al. 1978). These observations suggest that males need time to recover after mating before they can inseminate additional females successfully. The second experiment in our article considers the effect of a recovery time between two mating events on male reproductive success in *W. smithii*.

Materials and Methods

Collection and Maintenance. The population of *W. smithii* used in this study was collected during May 1988 in Eastern Massachusetts (42.5°N; FV of Bradshaw & Holzapfel 1989, Bradshaw & Lounibos 1977). Environmental effects were minimized by raising the population through five generations in the laboratory before the establishment of a continuously breeding population. This population consisted of ≈750 adults at any one time and completed ≈12 addi-

tional generations during the experiments below.

All stages of all the mosquitoes, stock and experimental, were reared under a long-day photoperiod of 18:6 (L:D) h at a constant 80% RH with a smooth sine-wave thermoperiod of 28:12°C (max:min) that lagged the photoperiod by 3 h. The 18-h photophase included two 0.5-h dim-light periods to simulate twilight. These conditions provided unambiguous long days that promoted continuous development without diapause (Bradshaw & Lounibos 1977). All larvae were fed standard laboratory food (ground guinea pig pellets and freeze-dried brine shrimp 3:1 [vol/vol]). All adults had access to pesticide-free raisins as a carbohydrate source. This population of *W. smithii* has obligate autogeny and produces repeated egg batches without blood meals (O'Meara et al. 1981).

Experimental animals were taken haphazardly over several oviposition dates from the stock colony and maintained separately to minimize selection imposed on the stock. The pupae were sexed, blotted, and weighed to the nearest 0.01 mg on a microbalance. Because size of adult female *W. smithii* (Bradshaw & Holzapfel 1992) and male size in other Diptera (Partridge et al. 1987) can affect reproductive success, both sexes used in both experiments were restricted to a large intermediate size of 1.60–1.79 mg male pupal weight and 2.40–2.69 mg female pupal weight. The sexes were maintained separately in covered plastic cages. The adults were collected daily and transferred to fresh screen cages until they were used in a trial. This procedure assured the virginity of both sexes before the start of any experiment and allowed the adult age of each individual to be tracked throughout the experiment. During a trial the mosquitoes were maintained in a 0.95-liter plastic container which had a screened side and top. Moist filter paper and a dish of water were placed on the bottom of the cage to facilitate egg collection. Adults were provided a pesticide-free raisin.

Experiment 1. To determine the effect of female availability on male lifetime reproductive success, individual males were provided with females at rates of 0.25, 0.5, 1, 2, or 4 per d until death. Five replicates were obtained for each rate of female availability. For each replicate, a single male between 0 and 7 d after eclosion was placed in a cage with one, two, or four virgin females between 0 and 7 d after eclosion for 24 h, after which the females were removed. Females from experimental regimens of two females or fewer per day were maintained in separate cages to determine if one or both females had been inseminated. In trials with four females per day, because of logistical limitations, the females were kept together in a single cage; therefore, it was not possible to determine how many females had been inseminated. In trials with at least one

female per day, the male was given new females every 24 h. As soon as the females from the previous day were removed from the male's cage, the appropriate number of sexually mature virgin females was introduced into the male's cage. In trials where the male received fewer than one female per day, the female remained in the male's cage for 24 h, but after the female was removed, the male remained alone for 24 h (0.5 female per d) or 72 h (0.25 female per d) before the next female was introduced into the male's cage.

After the females were separated from the males, the female cages were inspected three times each week for eggs until all females had died. If eggs were present, they were washed with distilled water into a petri dish (150 × 25 mm) so that the eggs floated on the water's surface. A few drops of food were added to the water. After 10 d, the eggs were checked for hatching. If larvae were present, they were counted and the number recorded. If eggs did not hatch after 10 d, they were scored as infertile. Lifetime number of offspring sired was then calculated as the total number of larvae produced by all females during their entire lifetimes. Males that failed to sire at least one offspring were not included in the data.

Experiment 2. Individual males were provided with a saturating number of females for 24 h, then isolated for a recovery period of zero, one, two, four, or eight d before receiving another saturating number of females for 24 h. Five replicates were obtained for each recovery time. For a male to qualify as a replicate, he had to sire at least one offspring in the first round of mating. In a separate study using this same population of *W. smithii* (S. Donovan & W.E.B, unpublished data), the maximum number of females mated in 1 d by one male was six. Consequently, the availability of 10 females per day was considered saturating. To begin a trial, a single male was placed in a cage with 10 sexually mature virgin females for 24 h. After 24 h, the male was removed and placed in a separate cage. After the male was isolated for the appropriate recovery time, a new group of 10 sexually mature virgin females was introduced to the male for 24 h, after which the male was again isolated.

Male longevity was recorded by checking the cages three times each week (MWF) for dead adults. The female cages were also inspected three times each week for eggs until all females had died. If eggs were present, they were collected and checked for hatching as described above.

Statistical Procedures. Because variances of both the dependent and independent variables tended to be proportional to the mean, \log_{10} - \log_{10} regressions were used to analyze the data with the GLM procedure in SAS (SAS Institute 1985).

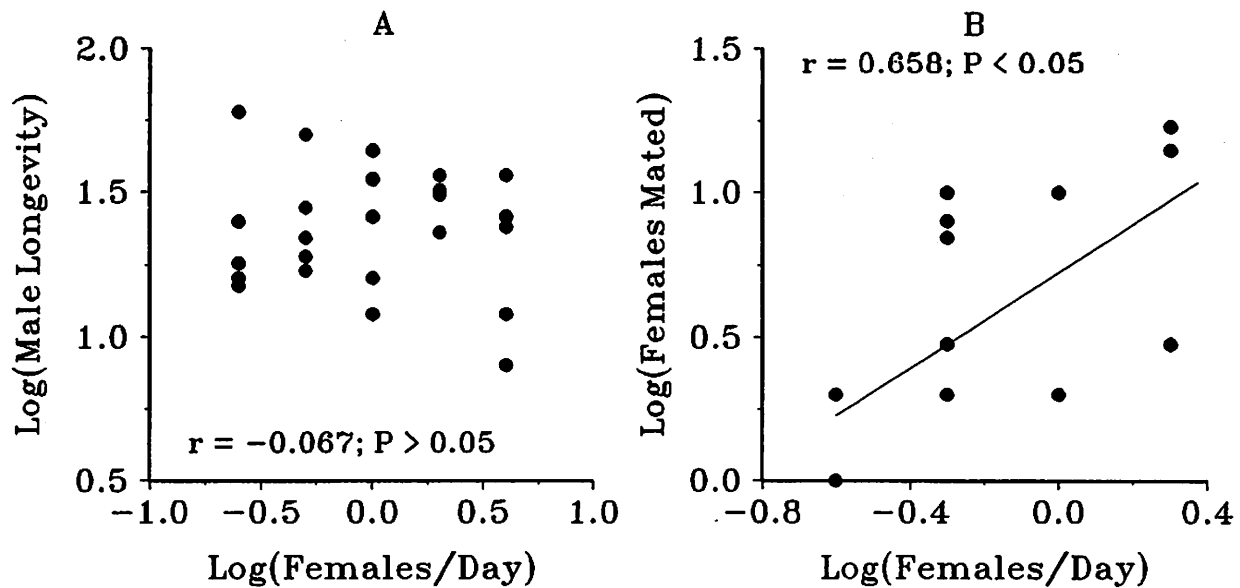


Fig. 1. Influence of the rate of female availability on individual male performance. (A) Measured as adult longevity. (B) Measured as lifetime number of females mated. Note that in all treatments five males are represented but fewer than five points show on the graphs because of overlapping values.

Voucher Specimens. Preserved specimens (adults) have been deposited in the collections of the Department of Entomology, Oregon State University, Corvallis, OR 97331, and identified as "*Wyeomyia smithii* (Coq.) (Diptera: Culicidae) collected May, 1988, at Forge Village (Middlesex Co.), MA, by W. E. Bradshaw & J. Hard, and reared for 24 generations in the laboratory."

Results

Experiment 1. The rate of female availability did not significantly affect male longevity (Fig. 1A) but did affect the lifetime number of females mated (Fig. 1B). Lifetime number of offspring sired (Fig. 2) increased with both male longevity

($b \pm 2SE = 1.17 \pm 0.73$; $P < 0.01$) and with female availability ($b = 0.63 \pm 0.32$; $P < 0.001$). The latter regression coefficient is significantly less than 1.0 ($t = 2.31$; $P < 0.05$), indicating lifetime offspring sired approached a plateau at higher rates of female availability. When mean female longevity and male and mean female age at the start of the experiment were added to the regressions, the coefficient of determination (R^2) increased by 0.095, 0.066, and 0.019 in Fig. 1 A and B and Fig. 2, respectively. Thus, variation in mean female survivorship or male or mean female age at the start of the experiment had no significant effect on male longevity, on lifetime number of females mated, or on lifetime offspring sired.

Experiment 2. To determine if the number of offspring sired in the second bout of mating depended upon either the number of offspring sired in the first bout or upon recovery time, we set the number of offspring sired in the second bout as the dependent variable, the number of offspring sired in the first bout as a covariate and recovery time as a fixed effect. The number of offspring sired in the second bout was not significantly affected by either the number of offspring sired in the first bout ($F_{1,15} = 0.72$, $P > 0.05$), by recovery time ($F_{4,15} = 2.10$, $P > 0.05$), or by their interaction ($F_{4,15} = 1.50$, $P > 0.05$).

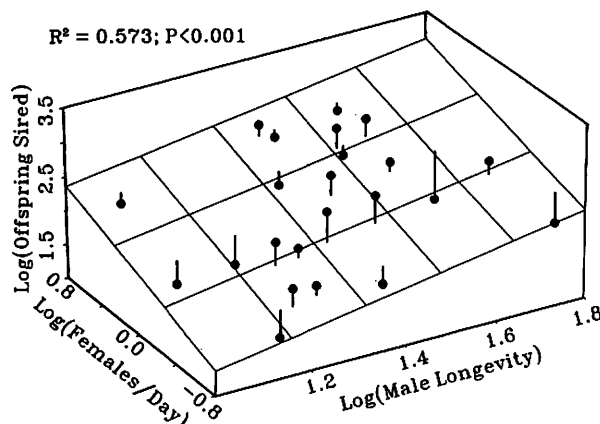


Fig. 2. Multiple regression of lifetime offspring sired on rate of female availability and individual male longevity. Grid shows the regression plane; short vertical lines show deviation of individual males from the regression plane.

Discussion

In *W. smithii*, adult males that live longer encounter and mate with more females than shorter-lived males and consequently sire more total offspring (Fig. 2). Similarly, in damselflies it has been shown that reproductive success de-

pends mostly on the duration of a male's adult life (Fincke 1982, Banks & Thompson 1985). Howard (1979) states that "higher order estimates of reproductive success should be more biologically accurate indications of fitness because more information concerning the potential genetic contribution of an individual is known." The number of offspring that survives to hatching in oviparous species is defined as a higher order estimate of reproductive success than both the number of observed copulations and the number of zygotes produced. Therefore, the lifetime number of offspring actually sired should and can be measured without much difficulty in the laboratory, although this method may not be feasible for some species in the field.

In *W. smithii*, the rate of female availability affects the number of females a male mates with during his lifetime (Fig. 1B) but not his survivorship (Fig. 1A). This observation indicates that increased mating frequency does not result in decreased male survivorship. Fincke (1982) observed the same relationship in damselflies, but Partridge & Farquhar (1981) showed that increased sexual activity in fruit flies resulted in decreased male lifespan. The regression coefficient of log(offspring sired) on log(females per day) (Fig. 2) is less than 1.0, meaning that higher rates of female encounter yield diminishing returns in offspring sired. Thus, male *W. smithii* apparently reach a saturation point in their ability to continue inseminating females. This saturation does not, however, affect male longevity (Fig. 1A). We have never observed copulation durations longer than about 30 s or any ability of males to capture the attention of females when not in copulo. Mating, per se, appears to be a low-cost activity in *W. smithii*. Our results therefore suggest that males pace their reproductive activities, whether by modifying their behavior or as a consequence of sperm or accessory gland depletion. The same conclusion follows from the results of the second experiment (Fig. 3). Like *Anopheles stephensi* Liston (Mahmood & Reisen 1982), male *W. smithii* sire fewer than their lifetime potential offspring when encountering a saturating number of females in a single day; but, unlike *Aedes aegypti* (L.) (Jones 1967), reproductive success in a subsequent mating bout is not significantly affected either by reproductive success in the early bout or by recovery time between bouts. Female availability does not affect male longevity (Fig. 1A), but longer-lived males encounter more females and do sire more offspring over their lifetimes (Fig. 2). These observations indicate, but do not directly show, that males will pass up the opportunity to mate and, in so doing, avoid a physiological cost to future reproductive effort or to residual longevity.

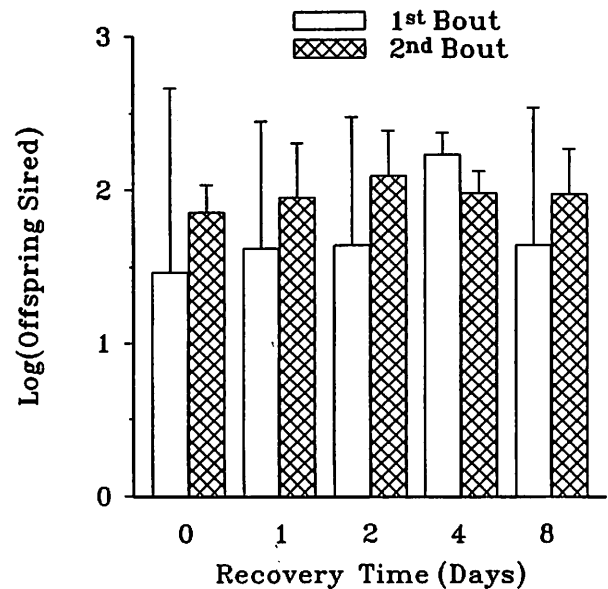


Fig. 3. Effect of recovery time on male performance. Open bars show mean (± 2 SE) number of offspring sired during an initial 24-h exposure (first bout) to a saturating number of females. Cross-hatched bars show mean (± 2 SE) number of offspring sired during a second 24-h exposure (second bout) to a saturating number of females after a recovery time (no females present) of varying duration.

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