MALE REPRODUCTIVE SUCCESS IN THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

by

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A THESIS

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In male Wyeomyia smithii mosquitoes in the laboratory, lifetime reproductive success does not depend upon the rate at which males encounter females but does increase with male longevity. Male longevity is also independent of the rate at which males encounter females. Males provided with a saturating number of females do not deplete themselves in a 24 hour period, instead; they pace their reproductive effort.

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TABLE OF CONTENTS

LIST OF GRAPHS

CHAPTER I

INTRODUCTION

An individual's fitness is defined as its contribution to the next generation, the number of descendants an individual leaves behind relative to the number of descendants left by other members of the population. Obviously, 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, animals that interact in groups, where males defend a harem, territory or lek (Downhower & Armitage, 1971; Howard, 1978, 1979; Payne, 1979; McGregor, Krebs & Perrins, 1981; Moore, 1989). A lek is an assembly of males in an area where there are no resources attractive to females, such as food or egg-laying sites; females visit the lek solely to copulate.

In more recent years, evaluations have been made of male reproductive success in non-social, non-territorial

insects. In these studies, 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; Simmons, 1986a; Hafernik & Garrison, 1986) or offspring sired over a brief period (McLain & Marsh, 1990).

Lifetime number of offspring sired is a more accurate representation of reproductive success than is lifetime mating activity, mating success or even offspring sired over a brief period. However, lifetime offspring sired has been measured for only one species in the field (Fincke, 1986) and two in the laboratory (Simmons, 1988; Rowe & Scudder, 1990). The first experiment in the present paper considers lifetime reproductive success (lifetime offspring sired) in the pitcher-plant mosquito, Wyeomyia smithii, in the laboratory.

Although sperm may be energetically cheaper to produce than eggs, the ejaculate transferred during mating contains millions of sperm, suggesting that males may incur non-trivial costs associated with mating (Dewsbury, 1982). Observations among a variety of insects support this contention. Experiments with butterflies have shown that the weight of both the spermatophore, a package of sperm, and the accessory substances delivered by males is highest

in the first mating compared with later matings (Svard & Wiklund, 1986) and that there is a significant dependence of the weight of the spermatophore on the number of days since the last mating (Svard, 1985). Fincke (1982) observed that 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. This observation suggests that males that have mated have incurred larger energetic costs than males who were unsuccessful in obtaining a mate. Mated males require a recovery period, and do not return to the breeding site until they are again able to mate, while unmated males return much sooner due to the fact that they are not constrained by the necessity of a recovery period. In the fruitfly, Drosophila melanogaster, a male's supply of mature sperm is reduced after a few consecutive matings, and continued mating results in the failure of the male to inseminate future females. Although the males replenished their sperm supply when allowed a recovery period, the amount of sperm was lower than it had been initially (Demerec & Kaufmann, 1941). Other experiments on D. melanogaster have shown that multiple copulations result in temporary depletion of the accessory glands, causing transient sterility (Markow, Quaid & Kerr, 1978).

Mahmood and Reisen (1982) showed that male Anopheles stephensi mosquitoes do not mate on every night of life despite the continual presence of sexually mature virgin females. Mating reocccured sporadically, indicating that males require a recovery period between matings during which they will not mate. Jones (1967) has shown that after multiple matings, male Aedes aegypti mosquitoes use up most or all of the sperm in their seminal vesicles, and two or three days are required to replenish their sperm supply. These observations suggest that males need time to recover after mating before they can successfully inseminate additional females. The second experiment in the present paper considers the effect of a recovery time between two mating events on male fitness.

CHAPTER II

MATERIALS & METHODS

Collection and Maintenance

Experiments used laboratory populations of Wyeomyia smithii collected from the 1987-1988 overwintering generation near Forge Village, Massachusetts (42.5°N; 71.5°W; 60 m elev.). A continuously reproducing population was initiated in 1988, and has been maintained since then in a 27 L plastic cage with rectangular screen holes 250 x 360 mm on two sides and a round screen hole 55 mm in diameter on the top upon which moist raisins were provided as a carbohydrate source. Pupae were placed in dessert dishes (80 mm diameter at the water's surface) filled with 60 ml of distilled water. Dishes were kept on the bottom of the cage. After eclosion, emergence of an adult from the pupa, adults had free access to mates and oviposited, deposited eggs, on the moist floor of the cage. Eggs were collected three times each week. Newly hatched larvae were reared in 150 x 25 mm petri dishes at a density of approximately 30 per dish and fed a mixture of ground brine shrimp and guinea pig pellets dissolved in distilled water. Pupae were

collected three times each week. At least 60% of the pupae were reserved for experiments, and the remainder were put into the stock population cage to insure that the population would continue to produce enough pupating individuals to maintain the experiments.

Since adult size of female Wyeomyia smithii and male size of other Diptera species (Partridge, Ewing & Chandler, 1987) can affect reproductive success, the size of both sexes used in experiments was restricted. Large intermediate sizes of both sexes were used in order to maximize survivorship and fecundity. Up to 25 male pupae weighing 1.60-1.79 mg or female pupae weighing 2.40-2.69 mg were placed in 60 ml of distilled water in dessert dishes labeled with the sex of the pupae. The dishes were checked daily for adults; adults were transferred to a holding cage consisting of a plastic cylinder 50 mm in diameter and 110 mm in height with a screen top and a removable dish containing 30 ml of distilled water as the base. The cages were labelled according to sex and eclosion date.

Experimental cages consisted of inverted 0.95 L plastic containers with a screen hole 55 mm in diameter on the top and a screen hole 60 x 80 mm on one side. To permit the addition of females into a male's cage, a hole 12 mm in diameter was drilled in one corner of the top of the cage and a rubber stopper (size 00) inserted. A piece of filter

paper was placed on the bottom of the cage and kept moist with distilled water. A 60 x 15 mm petri dish filled with 10 ml of distilled water was placed in the cage. Since this population of Wyeomyia smithii produces repeated clutches of eggs without blood feeding, vertebrate hosts were not necessary. A raisin at the top of the cage provided carbohydrates for adults. All experimental animals and the stock population were kept in a controlled environment room, which simulated natural temperature and photoperiod, the proportion of light to dark in a 24 hour period, (Bradshaw & Holzapfel, 1989) and which was held at 80 + 5% relative humidity.

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 day until death. Five replicates, exact duplications of experimental procedures to increase accuracy of results, were obtained for each rate of female availability. For each replicate, a single male between 0 & 7 days after eclosion was placed in a cage with 1, 2 or 4 virgin females between 0 & 7 days after eclosion for 24 hours, 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, 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 (a) new female(s) every 24 hours. As soon as the female(s) from the previous day was/were removed from the male's cage, the appropriate number of sexually mature virgin females were 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 hours, but after the female was removed, the male remained alone for 24 hours (0.5 female/day) or 72 hours (0.25 female/day) 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 collected by washing them from the filter paper or small petri dish into a 150 x 25 mm petri dish so that the eggs floated on the water's surface. A few drops of food were added to the water. After ten days, the eggs were checked for hatching. If larvae were present, they were counted and the actual number of offspring sired and oviposition date were recorded. If none of the eggs hatched after ten days, it was assumed that the eggs were infertile, and they were

discarded. 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, more females than a male could inseminate, for approximately 24 hours, then isolated for a 1, 2, 4 or 8 day recovery period before receiving another saturating number of females for 24 hours. 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. To begin a trial, a single male was placed in a cage with ten sexually mature virgin females for 24 hours. After 24 hours, the male was removed and placed in a separate cage. After the male was isolated for the appropriate recovery time, a new group of ten sexually mature virgin females was introduced to the male for 24 hours, after which the male was again isolated.

Male longevity was recorded by checking the cages three times each week for dead adults. The female cages were 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

To diminish the effect of the tendency of the variances to be proportional to the mean, log/log regressions were used to analyze the data.

CHAPTER III

RESULTS

Experiment 1

The rate of female availability does not significantly affect male longevity (Fig. 1a) or lifetime number of offspring sired (Fig. 1b). Lifetime number of females mated increases with rate of female availability (Fig. 1c) and with the number of females encountered during a male's lifetime (Fig. 2). Lifetime number of offspring sired increases with male longevity (Fig. 3). Lifetime offspring sired per female encountered declines with rate of female availability (Fig. 1d).

Experiment 2

To determine if offspring sired in the second bout of mating depends upon either offspring sired in the first bout or upon recovery time (Fig. 4), we used the general linear models procedure in SAS. We set offspring sired in the second bout as the dependent variable, 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 ($F_{1,12}=4.55$, $p>0.05$), recovery time ($F_{3,12}=1.14$, $p>0.05$) or their interaction ($F_{3,12}=2.37$, $p>0.05$).

CHAPTER IV

DISCUSSION

Male Wyeomyia smithii that live longer encounter and mate with more females than shorter lived males, and consequently, sire more total offspring, regardless of the rate at which they encounter females (Fig. 3). Similarly, in damselflies it has been shown that reproductive success depends mostly on the duration of a male's life (Fincke, 1982; Banks & Thompson, 1985). The number of females a male mates with is dependent upon the rate of female availability, although lifetime number of offspring sired is not significantly correlated with rate of female availability (Fig. 1b). These results illustrate the potential pitfalls of measuring reproductive success in terms of mating activity or mating success. Measuring male reproductive success by the number of females mated instead of lifetime number of offspring sired would have lead to the erroneous conclusion that male fitness increases with rate of female availability, when in fact it does not. The number of offspring a male sired by any given female varied greatly, revealing that using data from a few matings to

generalize about an insect's lifetime reproductive success is highly misleading.

Howard (1979) states that "higher order estimates of reproductive success (ERS) 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 survive to hatching in oviparous species is defined as a higher order ERS than both the number of zygotes produced and the number of observed copulations. Therefore, the lifetime number of offspring sired (defined as the number of larvae sired in this paper) 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 Wyeomyia smithii, the rate of female availability affects the number of females a male mates with during his lifetime (Fig. 1c) 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 and Farquhar (1981) showed that increased sexual activity in fruitflies resulted in decreased male lifespan. Male Wyeomyia smithii apparently reach a saturation point in their ability to continue mating since offspring sired per female encountered declines with increasing rate of

encounter (Fig. 1d). This saturation does not, however, affect male longevity (Fig. 1a), suggesting that males pace their reproductive activities. This same conclusion follows from the results of the second experiment shown in Figure 4. Males sire less than their lifetime potential offspring when encountering a saturating number of females and reproductive effort in a subsequent mating bout is not significantly affected by either reproductive effort in the early bout or by recovery time between bouts. These observations suggest, but do not show, that males will pass up the opportunity to mate and, in so doing, avoid a cost to future reporductive effort or residual longevity.

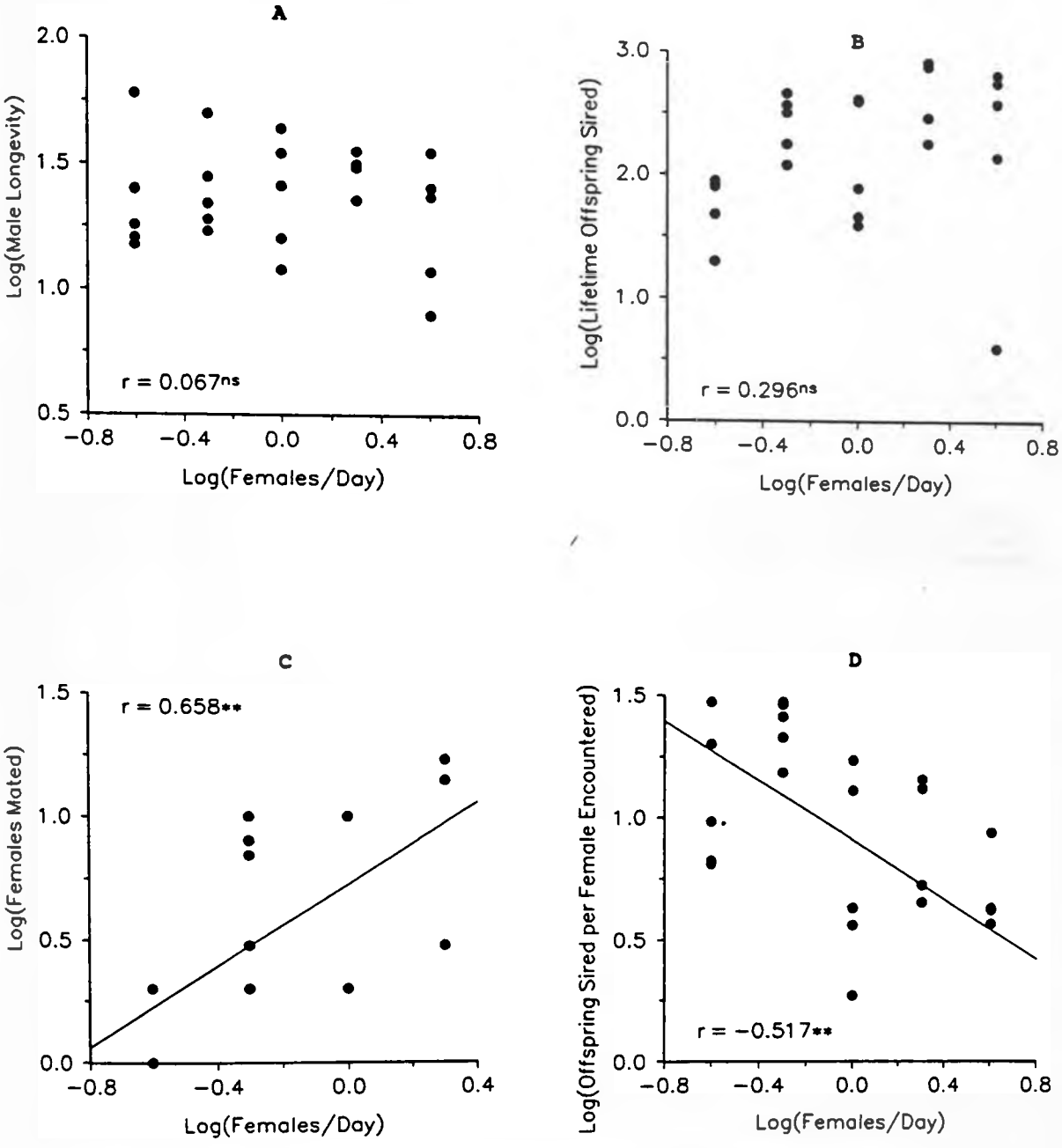

Fig. 1. Effect of female availability on (A) male longevity, (B) male lifetime offspring sired, (C) lifetime number of females mated and (D) offspring sired per female encountered throughout a male's lifetime.

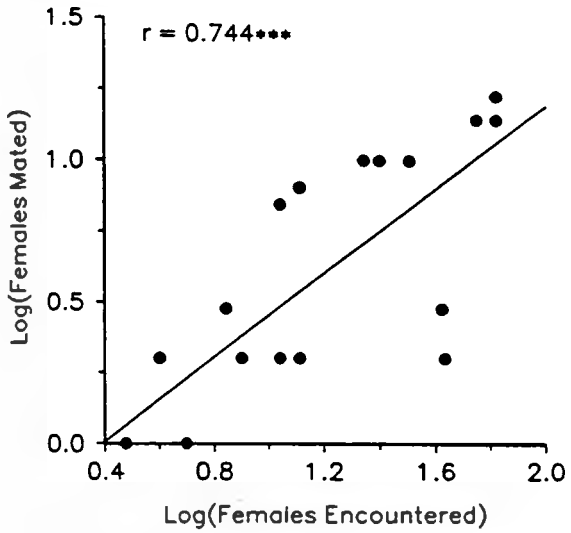

Fig. 2. The number of females mated increases with the number of females a male encounters in his lifetime.

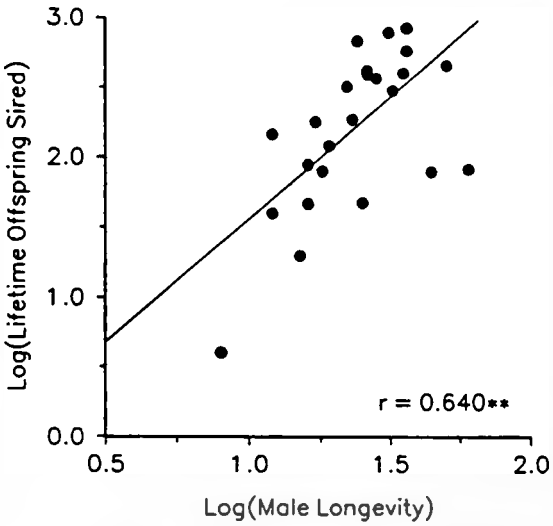

Fig. 3. The lifetime number of offspring sired by a male increases with male longevity

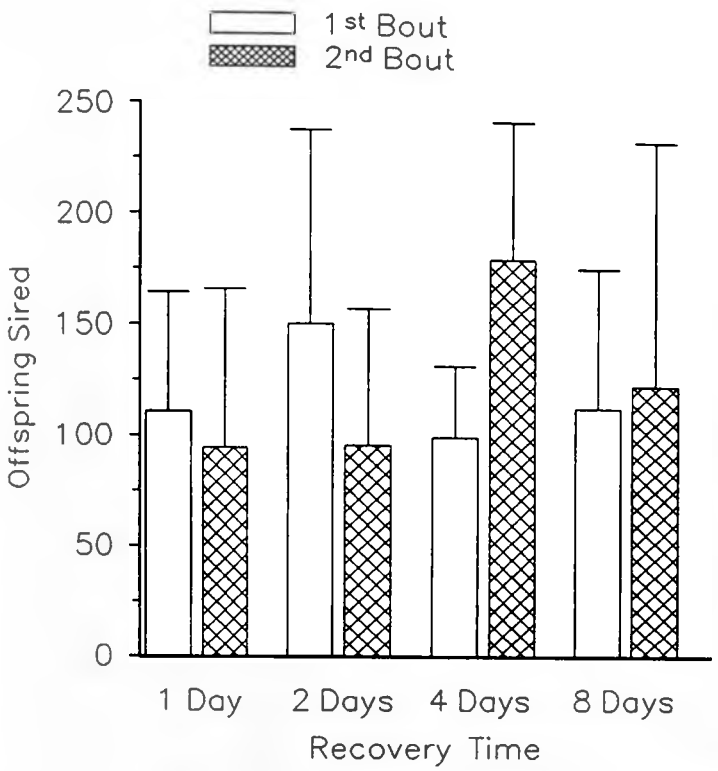

Fig. 4. The mean number of offspring sired by males that encountered 10 females for 24 hours (1st bout), were allowed a recovery time of varying duration, and then allowed to mate with 10 more females for 24 hours (2nd bout). The error bar shows 2 S.E.

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