Body Size and Flight Activity Effects on Male Reproductive Success in the Pitcherplant Mosquito (Diptera: Culicidae)

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ABSTRACT   We investigated effects of body size and flight activity (cage size) on male reproductive success in the pitcherplant mosquito, *Wyeomyia smithii* (Coquillett). Small males were more likely to experience reproductive failure than large males; but, among reproducing males, there was no significant difference in longevity, lifetime offspring sired, or cost of reproduction between small and large males. Regardless of size, individual males in large cages experienced decreased longevity, decreased lifetime offspring sired, and increased cost of reproduction compared with males in small cages, suggesting an energetic trade-off between flight and reproductive success. Size is a less important determinant of fitness in males than in females, and this difference may underlie size dimorphism.

REPRODUCTIVE SUCCESS is a fundamental component of Darwinian fitness. Successful reproduction and the production of abundant offspring require the allocation of resources from the soma to the gonads and to behavioral and physiological activities adherent to reproduction. Although this allocation of resources may be energetically more costly in females than in males, costs of reproduction in males are not trivial (Markow et al. 1978, Dewsbury 1982, Svard 1985). In many insects, male reproductive success depends upon male size. Most observations show that larger males achieve greater reproductive success than smaller males (Alcock et al. 1977, Borgia 1981, Hieber & Cohen 1982, Partridge & Farquhar 1983, Gomulski 1985, Simmons 1988, Schatral 1990, Berrigan & Locke 1991, Markow & Ricker 1992). Still, a minority of studies has found reproductive advantage accruing to males of small (Steele & Partridge 1988, Neems et al. 1990) or intermediate (Fincke 1982, Boake 1989) size.

Size-dependent reproductive success in many insects involves flight activity. Egg production in dipterans is correlated negatively with tethered flight duration, and flies in large cages produce significantly fewer eggs than flies in small cages (Roff 1977). Flight distance in syrphids has a greater effect on small than on large females (Scott & Barlow 1984). Large females kept in large cages do not suffer decreases in fecundity or longevity relative to flies kept in small cages, but small flies in large cages experience reduced longevity and fecundity. Flight activity affects fecundity more severely than longevity. Tethered soybean looper moths, *Pseudoplusia includens* (Walker), encouraged to fly for 2 h every

night laid significantly fewer eggs than nonflown individuals (Mason et al. 1989). A carbohydrate source greatly replenished energy stores but did not entirely compensate for the decreased reproduction resulting from flight. Nonflown, fed moths had the highest fecundity, followed by flown, fed and nonflown, starved moths; flown, starved individuals had the lowest fecundity. Flight and starvation had the same effect on survivorship as on fecundity. In the African armyworm moth, *Spodoptera exempta* (Walker), there was a negative correlation between fecundity and flight duration (Gunn et al. 1989). Individuals with access to sucrose after flight did not suffer as great a reduction in fecundity as moths given only distilled water, and the latter had significantly reduced longevity compared with unflown moths. This result was eliminated when individuals had access to sucrose. The above results in female insects indicate that reproductive success and flight trade off physiologically with one another through reliance on a common energy reserve.

Trade-offs between flight and reproductive activity have not been studied as frequently in males as in females. Roff (1977) found that probability of dispersal increased with body size in male as well as female dipterans. Neems et al. (1990) found that, when given access to food, small and large male midges had equal flight duration and longevity. Only when starved did small males realize reduced flight duration and longevity. As with female insects, available observations indicate that male flight activity and reproduction draw upon common energy reserves.

Here we examine the effects of body size and cage size on male lifetime reproductive success

(number of offspring sired) in the pitcherplant mosquito, *Wyeomyia smithii* (Coquillett), in the laboratory. In *W. smithii*, Benjamin & Bradshaw (1993) concluded that mating appeared to be a low-cost activity because there was no decrease in longevity by large males that encountered and mated with the most females and sired the greatest number of offspring in their lifetimes. Donovan (1991) found that in *W. smithii* large males lived significantly longer than small males, but large and small males did not differ significantly in lifetime reproductive success. Both studies exposed males to abundant or a saturating number of females in small cages that may have reduced or eliminated costs to reproduction incurred by flight. Although typical flying distances in nature are not known for *W. smithii*, the study reported here reflects more natural conditions by using a lower frequency of female encounter and larger flight cages than did previous studies.

Costs of reproduction have been demonstrated for many species, and evidence for such costs is provided when there is "a negative association between . . . some measure of the investment of resources in reproduction and adult survival" (Reznick 1985). As an index of reproductive effort, we used lifetime offspring sired per lifetime female encountered. A physiological cost of reproduction in *W. smithii* then would be evidenced by a negative correlation between adult longevity and reproductive effort.

Based on the previous studies with insects cited above, we expected that males would suffer reduced longevity and reproductive success in larger cages that involved greater flying distances and that small males would realize lower reproductive success and a greater cost of reproduction than large males.

Materials and Methods

Collection and Maintenance. The population of *Wyeomyia smithii* used in this study was collected in eastern Massachusetts (42.5° N latitude; FV of Bradshaw & Holzapfel 1989) in May 1988. The laboratory population completed ≈20 generations before the start of the experiment. A continuously breeding population of 500–1,000 adults was maintained during the course of the experiment. Larvae and adults were kept in a controlled-environment room at constant 80% RH with a smooth sine-wave thermoperiod of 28:12°C (max:min.) that lagged the photoperiod by 3 h. Photoperiod was 18:6 (L:D) h with 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). This population of *W. smithii* is obligately autogenous and produces repeated egg clutches without blood meals (O'Meara et al. 1981).

Experimental Procedures. To determine the effects of male size on lifetime reproductive success, individual small (1.00–1.19 mg wet pupal weight) and large (1.80–1.99 mg) males were provided with one large–intermediate-size (2.40–2.69 mg) female every 4 d until male death. To determine the effects of cage size on male reproductive success, experimental males were maintained in small (1.1-liter), medium (4.2-liter), or large (16.0-liter) cages.

To generate experimental adults, larvae were raised on standard laboratory food (ground guinea-pig pellets and freeze-dried brine shrimp in a 3:1 mix by volume) at low or high food levels to produce small and large pupae. Pupae were separated by sex, blotted, and weighed to the nearest 0.01 mg on a microbalance. The pupae were maintained separately by sex and weight in distilled water in covered plastic dishes. The adults were collected daily by aspiration; adults that eclosed on a given day were pooled and maintained in screen cages until they were used in a trial. This procedure assured the virginity of both sexes before the start of an experiment and allowed control of adult age of each individual at the start of each experiment.

Experimental cages were constructed from high-density linear polyethylene food containers with screen covering holes on the side and top. A piece of dry filter paper was placed on the bottom of each cage. A scintillation vial with the top cut off and containing a strip of rolled filter paper was filled with distilled water and placed in the cage to allow adults access to moisture. A small glass jar containing a small leaf of the host plant, *Sarracenia purpurea* L., also was placed in the cage. Adults were provided a pesticide-free raisin as a carbohydrate source.

To begin a trial, an individual small or large male between 1 and 5 d after eclosion was placed in a cage of one of the three sizes with a virgin female between 0 and 7 d after eclosion for 24 h, after which the female was removed and placed in a small cage. We previously showed (Benjamin & Bradshaw 1993) that, within the same limits of variation as in this study, variation in mean female survivorship had no significant effect on male longevity or lifetime offspring sired. Seventy-two hours after the initial female was removed, a new virgin female was introduced into the male's cage for 24 h. This regimen was continued until the male's death. Females were left in cages with males for only 24 h to simulate a more natural rate of female encounter than continuous access to females. Cages with males were checked three times a week (Monday, Wednesday, Friday [MWF]) for male death.

To minimize effects of cage size on female survivorship, all females were maintained in small (1.1-liter) cages after their 24-h encounter with a male. All females exposed to the same

male were pooled into a single cage after being separated from the male. A piece of moistened filter paper and a small dish of distilled water were placed on the bottom of each cage with females to maintain high humidity. The cages with females were inspected three times a week (MWF), at which time eggs were collected and dead adults were recorded. Eggs were washed with distilled water into a 150-by-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 10 d, the eggs were checked for hatching. If larvae were present, they were counted and the number recorded. Embryonic diapause is unknown in W. *smithii*, and first instars hatch at the completion of embryogenesis within 5–7 d of oviposition; consequently, eggs that did not hatch after 10 d were scored as infertile. Lifetime number of offspring sired by a male was calculated as the total number of larvae produced by all females during their lifetimes. Male longevity was interpolated between the last day a male was observed alive in his cage and the day he was found dead.

Statistical Procedures. Loglinear models (G tests) according to Sokal & Rohlf (1969) were used to analyze reproductive failure. The GLM procedure of SAS (SAS Institute 1985) was used to analyze variable reproductive success and cost of reproduction. In the latter case, cage size and male size were entered as class variables (treatments) and longevity, lifetime offspring sired, offspring per female encountered, male age at the start of the experiment, and mean age of females at male encounter were entered as continuous variables (covariates). Initially, longevity or reproductive effort, cage size, and their interaction were entered into the analysis. Next, male size and its two- and three-way interactions with the previous variables were entered into the analysis. Finally, male and female age, and all of their two-, three-, and four-way interactions were entered into the analysis. At each step, the coefficient of multiple determination (R^2) and the significance of each newly entered variable was determined. In each case, because of the unbalanced data, Type III sums of squares were used. To correct for positive skewness, longevity, lifetime offspring sired, and offspring sired per female encountered were $\log_{10}$ transformed before being entered into analysis.

Results

Reproductive success was discontinuous between those males failing to sire any offspring (zero offspring sired) and those that sired at least 20 offspring (20–700 offspring sired). Consequently, we first examined the bases of reproductive failure (zero offspring sired) and then considered the bases of variable reproductive success among males that sired any offspring.
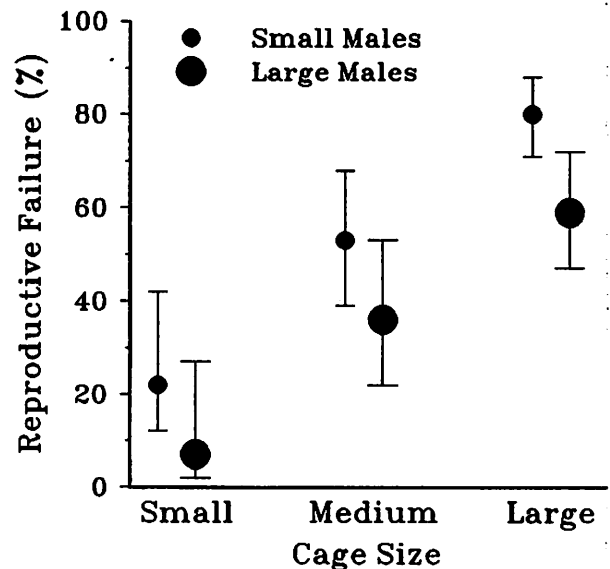

Fig. 1. Influence of cage size and male size on reproductive failure (percentage of males failing to sire any offspring). Bars indicate 95% confidence limits.

Percentage of males failing to reproduce was related to both cage size and male size (Fig. 1). Small males were more likely to experience reproductive failure than large males ($G = 11.90$, df = 1, $P < 0.001$), and reproductive failure increased with cage size, regardless of male size ($G = 59.94$, df = 2, $P < 0.001$). There was no cage-size-by-male-size interaction ($G = 1.28$, df = 2, $P > 0.05$) (i.e., small males did not experience disproportionate reproductive failure in large cages relative to large males).

Among reproducing males, lifetime offspring sired (Fig. 2A) increased with male longevity ($F = 55.91$; df = 1, 136; $P < 0.001$) and was not significantly affected by cage size ($F = 1.98$; df = 2, 136; $P > 0.05$) but was affected by the interaction between cage size and male longevity ($F = 3.89$; df = 2, 136; $P < 0.05$) ($R^2 = 57.3\%$). Fig. 2A shows that the regression coefficient (slope) declined with increasing cage size, indicating that longevity had a decreasing effect on lifetime offspring sired in larger cages. Addition of male size (Fig. 2B) to the analysis increased R^2 by 2.2%, and neither male size nor any of its interactions with the other variables was significant ($P > 0.05$). Addition of male and female age to the analysis increased R^2 by an additional 5.0%, but neither female age, male age, nor any of their interactions with other variables was significant ($P > 0.05$). Consequently, male longevity and its interaction with cage size as shown in Fig. 2A were the main determinants of lifetime offspring sired.

Among reproducing males, adult longevity (Fig. 3A) declined with increasing reproductive effort (offspring sired per female encountered) ($F = 28.44$; df = 1, 136; $P < 0.001$) but was not
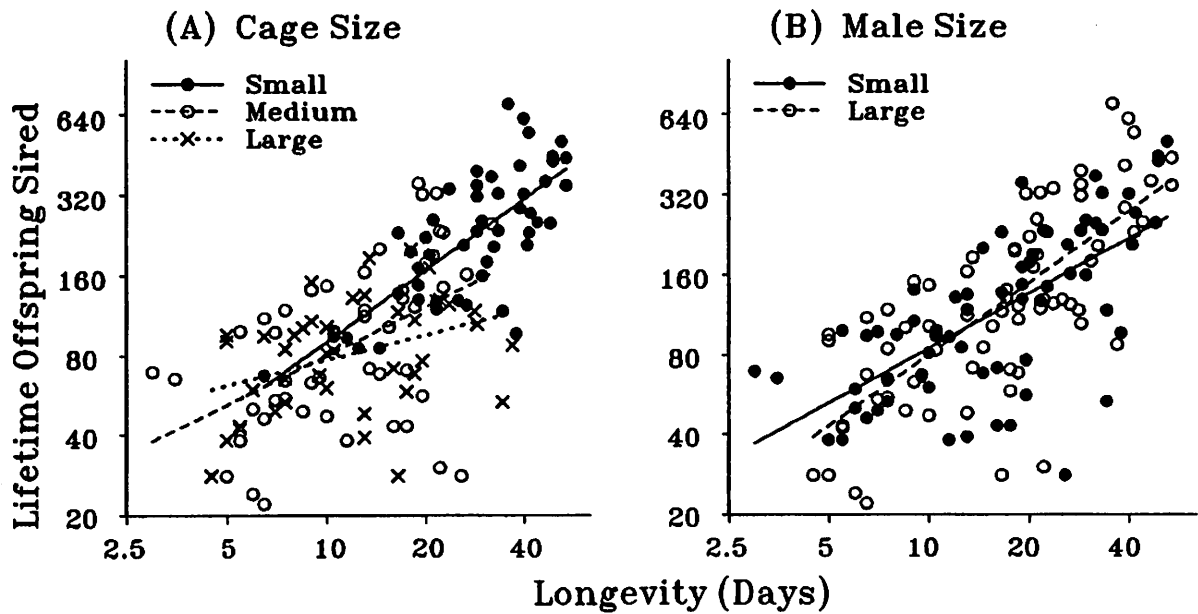
Fig. 2. Effect of (A) cage size and (B) male size on lifetime offspring sired, taking male longevity into account.

significantly affected by cage size ($F = 1.63$; df = 2, 136; $P > 0.05$) or the interaction between cage size and reproductive effort ($F = 2.81$; df = 2, 136; $P > 0.05$) ($R^2 = 53.2\%$). Addition of male size (Fig. 3B) to the analysis increased R^2 by 1.2%, and neither male size nor any of its interactions with the other variables was significant ($P > 0.05$). Further addition of male and female age to the analysis increased R^2 by an additional 8.9% and revealed significant effects of male-age-by-female-age-by-male-size interaction ($F = 3.99$; df = 1, 124; $P < 0.05$), retained the significant

effect of reproductive effort ($F = 11.59$; df = 1, 124; $P < 0.001$), and indicated a significant cage size by offspring-sired-per-female-encountered interaction ($F = 5.33$; df = 2, 124; $P < 0.01$), but showed no other significant main effects or interactions ($P > 0.05$). These results indicate that male age at first female encounter and mean age of females encountered by a male may affect longevity when they interact with male size, but this effect is in addition to the main effect of reproductive effort and the interaction of reproductive effort with cage size.
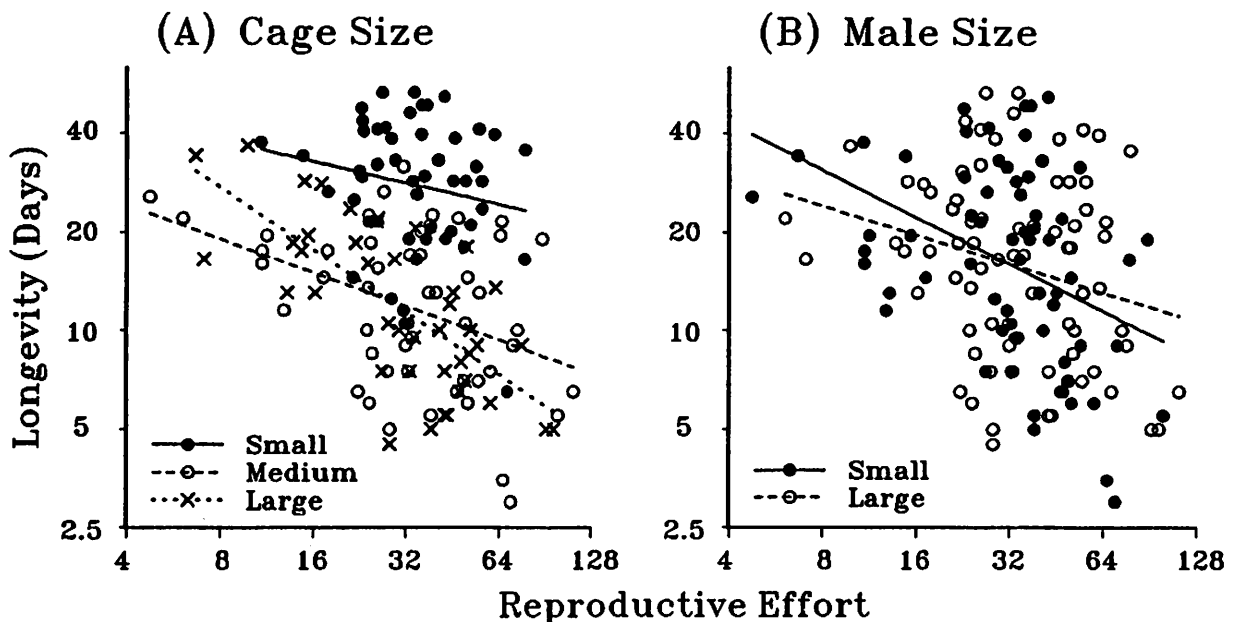

Fig. 3. Effect of (A) cage size and (B) male size on longevity, taking reproductive effort (offspring sired per female encountered) into account. A cost of reproduction is indicated by a negative slope.
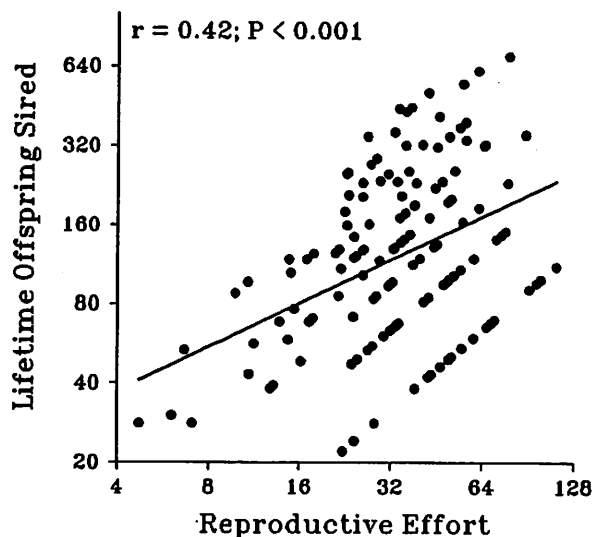
Fig. 4. Relationship between lifetime offspring sired and reproductive effort (offspring sired per female encountered).

Despite the positive correlation between lifetime offspring sired and longevity and the negative correlation between longevity and reproductive effort, lifetime offspring sired still increased with reproductive effort (Fig. 4), although with diminishing returns because the slope of the log–log regression was <1.0 ($b \pm 2$ SE = 0.552 $\pm$ 0.202).

Discussion

In *W. smithii*, male size and flight activity are both important determinants of an individual's fitness. Reproductive failure is more common among small males than large males and in larger cages, regardless of male size (Fig. 1). Although small males experience higher reproductive failure than large males, small males that reproduce successfully do not have a further fitness disadvantage in comparison with large males (Figs. 2B, 3B). Among both large and small males, reproductive success increases with individual longevity (Fig. 2A), but longevity confers decreasing advantage with increasing cage size and, by implication, with increasing flight activity.

Among successfully reproducing males, higher reproductive effort incurred a physiological cost of reduced life expectancy (Fig. 3A). The most inclusive analysis established a significant cage size by reproductive effort interaction. In Fig. 3A, the steeper slope in large than in small or medium cages indicates that the cost of reproduction is higher in the larger flight space.

The effects of cage size on reproductive failure (Fig. 1), on lifetime offspring sired (Fig. 2A), and on cost of reproduction (Fig. 3A) outweigh the effects of male size on these same contributors to male fitness (Figs. 1, 2B, 3B). This result suggests that flight imposes a cost to reproduction

that is the same in all reproductively successful males, regardless of size. Large male size is most advantageous in averting reproductive failure (Fig. 1) rather than in augmenting offspring sired (Fig. 2B) or ameliorating the costs of reproduction (Fig. 3B).

In female *W. smithii*, gross lifetime fecundity increases linearly (Bradshaw et al. 1993) or exponentially (Bradshaw & Holzapfel 1992a) with female size. A large reproductive effort (total egg volume/mg body weight) early in adult life does not affect subsequent survivorship, reproductive effort, or reproductive rate [(total egg volume)/(mg body weight × day)] (Bradshaw et al. 1993). In male *W. smithii*, a previous study (Benjamin & Bradshaw 1993) concluded that males pace their reproductive activities and thereby avoid a physiological cost to future reproduction or longevity. In the study reported here, a high reproductive effort was found to incur a cost of reduced longevity (Fig. 3). Nonetheless, males that make a high reproductive effort also sire more offspring during their lifetimes (Fig. 4). The cost to subsequent longevity of a high reproductive effort, therefore, is more than compensated for by the increased total lifetime offspring sired.

Female fitness is determined by the number of eggs produced over a female's lifetime. Because both fecundity and longevity of *W. smithii* females increase with size, females should be under selective pressure to maximize size at emergence. Male fitness is determined by the number of offspring sired over a male's lifetime. In a seasonal environment with synchronous generations like *W. smithii* in eastern Massachusetts (Lounibos & Bradshaw 1975), the availability of unmated females declines with time. Under these circumstances, higher fitness accrues to early emerging males that have access to the greatest number of unmated females (Kleckner 1993). This early emergence of males relative to females (protandry) may require a trade-off of smaller male body size. The experiments reported here found that small males incurred a cost of increased reproductive failure relative to large males. This cost of male size was small compared with the cost, regardless of size, of a larger flying space in which to feed and mate. With unlimited flight opportunity in nature, early emergence of males, even of small size, may outweigh, therefore, the cost of possible reproductive failure. *Wyeomyia smithii*, like most other mosquitoes (Hawley 1985, Bradshaw & Holzapfel 1992a,b), are size-dimorphic; females are larger than males. This sexual dimorphism, then, may reflect the differential contribution of size to fitness in the two sexes.

Acknowledgments

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