Behav Ecol Sociobiol (1987) 20:319–322

Behavioral Ecology and Sociobiology
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Female butterflies mated with recently mated males show reduced reproductive output

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Received July 14, 1986 / Accepted December 2, 1986

Summary. During copulation, males of the alfalfa butterfly, *Colias eurytheme*, transfer nutrients and sperm to the female. Recently mated males produce only about 40% of the quantity of material produced by males that have not recently mated (Rutowski and Gilchrist 1986). In this study, females that mated with recently mated males died sooner, had a lower lifetime egg output, and laid fewer eggs per day overall, but especially during the first 2 days after mating. We conclude that the effect of the materials (nutrients and sperm) passed by males at copulation on female oviposition and receptivity is such that the male's genetic return from nutrients passed is maximized.

Introduction

In many insects males provide females with nutrients as well as sperm during copulation (Gwynne 1984; Thornhill 1976). In the butterflies the nutrients are accessory gland secretions (Leopold 1976) that are placed with the sperm in a spermatophore that is formed in the female's reproductive tract during copulation. The nutrients are absorbed and used for oogenesis and body maintenance (Boggs 1981; Boggs and Gilbert 1976; Boggs and Watt 1981).

Male butterflies that remate within an hour of mating produce a mass of nutrients and sperm during copulation that is only about half of that produced by males that have not recently mated (Boggs 1981; Rutowski 1979, 1984; Rutowski and Gilchrist 1986; Sims 1979; Svard and Wiklund 1986). In this study we examined the reproductive output of females of the alfalfa butterfly (*C. eurytheme*) that were mated with males that differed in mating history. We tested the hypothesis that those females mated with recently mated males suffer a reduced reproductive output predicted because of the smaller supplies of nutrients, sperm or both that they receive. Recently mated *C. eurytheme* males pass only 40% the mass of nutrients and sperm passed by their unmated counterparts.

Methods

Virgin females were reared on alfalfa from eggs laid by field-caught *Colias eurytheme* females in cages. After emergence the virgin females were released in the field near free-flying males to elicit matings. Successfully mated pairs were held in vials until they separated.

After completion of his first mating a male was given a mark on his wings with a felt-tipped pen and released into the field. At this point we only used males whose first mating lasted 70 min or less. A longer copulation duration indicates that the male has recently mated (Rutowski and Gilchrist 1986). After release each male was permitted several minutes of recovery and then he was presented with a second virgin female from the same brood as his first female. Each male was mated with sisters to reduce the variance in fecundity due to differences in the genetic backgrounds of the females.

All females were placed in individual cages that were 10–15 cm below two 40 watt fluorescent bulbs (one soft white and one Grolux, Sylvania, Inc.). These lights were on from 6:00 to 18:00 hours every day. The temperature was about 30° C and the humidity unregulated. Each cage contained a piece of sponge saturated with a 10% sucrose solution and a sprig of alfalfa in a water-filled vial.

Every day at about 8:00 hours AM the alfalfa sprig was removed, inspected for eggs, and replaced with a fresh sprig. We also replaced the sponge with a newly saturated one and encouraged the female to feed by placing her on the sponge and extending her proboscis with an insect pin. Eggs were counted and kept for 24 h to see if they changed color from pale yellow to red-orange, a change which occurs only in fertilized eggs (Stern and Smith 1960).

As females died, they were frozen and later dissected by removing the abdomen from the female and making an incision along the right or left lateral midline. After the abdomen was

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pinned open and immersed in insect Ringer's solution, we noted the number of eggs and fat bodies it contained. We then removed the bursa copulatrix and noted the condition of material in the corpus bursae (the spermatophore) and the appendix bursae. Finally, we removed the spermatheca, prepared a squash mount of it, and examined it for sperm using phase contrast microscopy.

Statistical analysis of the survival and egg output of females involved pair-wise comparisons of sisters that had mated with the same male. Because our prior expectation was that female reproductive output would be positively correlated with the quantity of material received we used one-tailed tests at the 0.05 level in the evaluation of the significance of observed differences. All parametric summary statistics are given as mean ± standard error.

Results

General

Twenty-two fresh or slightly worn males were mated with a total of 44 females. The duration of first copulations averaged 46 ± 2.22 min (range, 31–66 min) while that for second copulations was 602 ± 19.1 min (range, 476–799 min). In 4 of the 22 pairs of females mated with the same male, one of the females laid no eggs. In one pair the female died the day after she was mated. In the other 3 pairs, one of the females laid no eggs although they survived 4, 23, and 31 days in the cages, respectively. These females and, where appropriate, the pairs to which they belonged were excluded from subsequent analyses.

Infertile eggs appeared in both groups of females. Two females produced eggs of which more than 80% were infertile. These females were both from second matings and upon dissection neither was found to have sperm present in the spermatheca. The results from these females and their sisters were excluded from subsequent analysis.

In 7 pairs one female died prematurely after catching an antenna in the hardware cloth from which the cages were constructed. Data from these pairs were used in analyses of egg production up to the point at which the female died, but were excluded from paired comparisons of lifetime egg output and survival.

Nineteen females (10 from first and 9 from second copulations) were dissected after they died for no apparent reason. All but one female (from a first mating) had sperm remaining in the spermatheca. Only one female of 14 that were checked had any fat bodies remaining in the abdomen. Mature eggs were found in the abdomen of 16 of the 19 females.

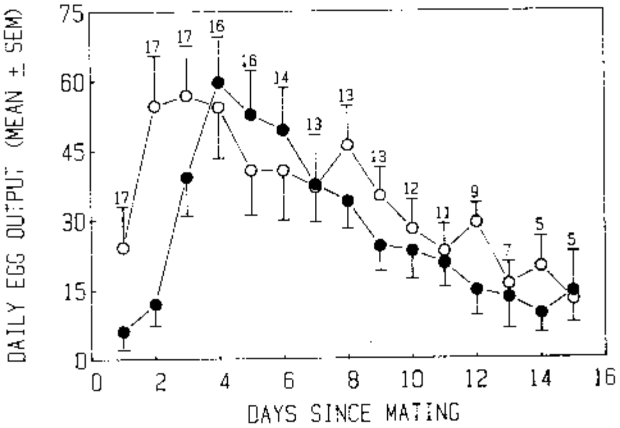

Fig. 1. The mean daily egg output for females from first (open circles) and second (closed circles) matings as a function of days since mating. Vertical lines above or below each point show one SEM. The number above each point is the number of pairs from which the means were calculated

Effect of secretions on egg output and survival

The 11 females that (1) did not die prematurely, (2) produced at least one fertile egg, and (3) mated with a male that was not recently mated (first mating) lived an average of 15.5 ± 1.3 days (range: 9–23 days) and laid an average total of 490 ± 37 eggs (range: 319–675 eggs). In contrast, under similar conditions, the 11 sisters of these females that were mated with males thad had recently mated (second mating) lived for significantly shorter periods of time (12.1 ± 2.2 days, range: 3 27; paired t-test, $t=2.02$, 10 *df*, $P<0.05$) and laid significantly fewer eggs (314 ± 61 eggs, range: 78–774; paired t-test, $t=3.44$, 10 *df*, $P<0.005$).

The daily rate of egg production for both groups of females rose sharply during the first 5 days after mating and then gradually declined (Fig. 1). Differences between the two groups on individual days were significantly different only on the first two days after mating (paired t-test, $P<0.05$). However, the mean difference observed over all 15 days (8.1 ± 2.8 eggs, $n=185$) was significantly different from zero (paired $t=2.88$, $P<0.05$).

In these 11 pairs few infertile eggs were produced. Of the females mated with recently mated males, three produced infertile eggs (0.5%, 2.8%, and 6.6% of all eggs, respectively). Of the sister group, two produced infertile eggs (0.6% and 1% of all eggs, respectively). The two groups did not differ significantly in the proportion of females laying infertile eggs ($\chi^2=0.25$, $P>0.05$) or the proportion of total egg output that was infertile (Wilcoxon rank sum test, $P>0.05$).

Discussion

The effect of materials produced by males: causes

The data support the prediction that quantity of material passed by males to females during mating influences female reproductive output. Compared to those that received small amounts of material, females that received large amounts laid more eggs overall, lived longer, and on average, were likely to produce more eggs per day. There are at least three possible explanations for these results. First, females in second matings may lay fewer eggs because of sperm depletion. The impression reported in Rutowski and Gilchrist (1986) is that recently-mated *C. eurytheme* males produce less sperm and this has been observed in other butterflies (Sims 1979; Svard and Wiklund 1986). However, even if less sperm is produced we do not know the relationship between sperm received and egg output. Several observations argue against the sperm depletion hypothesis: (1) the presence of sperm in the spermathecae of both groups of females after death, (2) the survivorship differences between the groups, and (3) the differences in daily rate of egg production in the first few days after mating.

Second, females in second matings may have received lesser amounts of an oviposition stimulant such as that documented in other insects (Leopold 1976). The fact that both groups of females ultimately attained similar oviposition rates and, again, the positive effects of the secretions on survivorship argue against this explanation.

The third alternative is that the difference in reproductive output between the sisters resulted from differences in the quantity of nutrients they received from males. As such these data represent a demonstration that the nutrients contributed do have a positive effect on the female's reproductive energy budget. Other attempts to document such effects for the secretions produced by males have been unsuccessful (Greenfield 1982; Jones et al. 1986). The absence of fat bodies in the abdomens of females at the time of death suggests that nutrients of the sort stored in these organs are the limiting factor in egg production.

The effect of materials produced by males: consequences

In the field, females will vary in the amount of material that they receive from males because their mates vary in size, because the males have recently mated, or for other reasons. In several butterflies and moths (Boggs 1981; Greenfield 1982; Jones et al. 1986) including *Colias eurytheme* (Rutowski and Gilchrist 1986) there is a significant positive correlation between male size and the quantity of material produced at copulation. In the study of *C. eurytheme* the largest males produced almost twice as much material as the smallest males, a difference paralleling that found between the males of different mating histories used in this study (Rutowski and Gilchrist 1986).

Will this variation produce differences between females in reproductive output in the field? Under the conditions of this experiment females were prevented from remating but in the field females will remate (Rutowski et al. 1981), typically about 4–8 days after first mating (Rutowski and Gilchrist 1986). Hence females may compensate for receiving only a small amount of material from a male by remating. In spite of this some of the most consistent differences in egg output between females from first and second matings were in the first 3 to 5 days after mating (this study) when a female is least likely to remate (Rutowski and Gilchrist 1986; Suzuki 1979).

What selective consequences has the enhancement of female reproductive output of male-imparted materials had on male behavior? The material passed by males to females has at least two functions. First, the material received by a female may supply sperm and nutrients available to females for reproduction and so enhance male reproductive success. Second, they may influence female receptivity to subsequent courtship attempts and so maximize the fertilizations a male gains from a copulation. In the cabbage butterfly (*Pieris rapae*) the larger the quantity of material passed by a male the more likely the female is to reject subsequent courtship attempts (Sugawara 1979). In *C. eurytheme*, females apparently remate only after secretions from previous matings have been depleted (Rutowski et al. 1981).

Remating by the female in *C. eurytheme* because of last male sperm precedence (Boggs and Watt 1981) means that a first mate's nutrients will be used to produce eggs in which he has no genetic investment. However, the incorporation of nutrients from a male's spermatophore into the eggs in *C. eurytheme* is highest in the first five days after mating (Boggs and Watt 1981) and the enhancement of egg production is greatest during this same time period. Moreover, it is only after this time that a female in the field is likely to remate (Rutowski and Gilchrist 1986). It seems clear, then, that the two functions of the materials discussed above (enhancement of egg output and induction of a refractory state) have coevolved so that the effect of the secretions on a female's behavior maximizes the male's genetic return on nutrients passed to the female.

Acknowledgements. We thank Cathleen Shelton for assistance in the laboratory and Dr. Darryl Gwynne, Dr. Carol Boggs and two anonymous reviewers for constructive criticism of early drafts of the manuscript. Financial support for this work was provided by the National Science Foundation Grant No. BNS 83-00317 (to R.L.R.).

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