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Fecundity, egg weight and longevity in relation to multiple matings in females of the monarch butterfly

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Summary. In many insects nutrients transferred by the male at mating are later incorporated into both the eggs and soma of the mated females. Accordingly, it has been suggested that insect females can use these male-derived nutrients for somatic maintenance and enhancement of their fecundity and fitness of their offspring. In this paper we tested the validity of these predictions by studying the longevity and reproductive output of 1, 2, 3, 4 or 5 times mated female monarchs *Danaus plexippus*, a nectar-feeding butterfly that is long-lived and strongly polyandrous and emerges as an adult without mature eggs. Females mated five times received ejaculates that corresponded to an average of 38% of their body weight at eclosion. However, we found that the number of times females had mated had no effect on their longevity, life-time fecundity, or egg weight. Although negative evidence should always be interpreted with caution, our study suggests that male-derived nutrients are less important for female longevity and reproductive output than are larval and adult food.

Introduction

In many insects ejaculate nutrients transferred by the male at mating are later incorporated in both the eggs and soma of the mated females (Friedel and Gillott 1977; Boggs and Gilbert 1979; Boggs 1981a; Boggs and Watt 1981; Bowen et al. 1984). Accordingly, it has been suggested that insect females can use male-derived nutrients for somatic maintenance and enhancement of their fecundity and fitness of their offspring (Thornhill and Alcock 1983; Gwynne 1984a; Rutowski 1984; Simmons 1988). However, although females have the physiological opportunity to use these nutrients, it has proven exceedingly difficult to demonstrate any positive effect of them on female longevity or fecundity (Walker 1980; Greenfield 1982; Gwynne et al. 1984; Jones et al. 1986). Resources allocated to reproduction by females can also be derived from larval and adult feeding (Labine 1968; Boggs 1981b). The difficulty in demonstrating an effect of male-derived nutrients is thus understandable if they contribute less to female longevity and fecundity than do larval and adult feeding. This line of reasoning is supported by the observation that a positive effect of male-derived nutrients on female egglaying rate in two orthopterans was visible only when females were maintained on a restricted or low-quality diet (Gwynne 1984b; Gwynne et al. 1984; Butlin et al. 1987; Simmons, 1988).

Male-derived nutrients could be especially important for female survivorship and fecundity in long-lived species in which eggs mature relatively late (after mating) and in which adult feeding contributes little to reproductive output of females, as in nectar-feeding insects (Boggs and Gilbert 1979; Boggs 1981a, b; Gwynne 1984b). Moreover, the potential importance of male-derived nutrients should correlate positively with the size of the male ejaculate and the degree of female polyandry.

The monarch butterfly *Danaus plexippus* has been suggested as a suitable candidate for investigating the possible role of male-derived nutrients in female reproductive output and survivorship (Gwynne 1984a). In the monarch females corporate male-derived nutrients in their soma and eggs (Boggs and Gilbert 1979); females eclose without mature eggs in their abdomen (Urquhart 1960; Barker and Herman 1976; Zalucki 1981); it is a long-lived species by butterfly standards (Urquhart 1960; Munger and Harriss 1969); and adults feed exclusively on nectar (Boggs and Gilbert 1979). Svärd and Wiklund (in press) report that ejaculate weights transferred by previously unmated males make up an average of 7.5% of female body weight, and Pliske (1973) reported that females mate up to eight times, old females having an average of between four and five spermatophores in their bursa.

In this laboratory study we investigated the possible influence of male ejaculates on female life-time fecundity, egg weight, and survivorship by comparing the values of these parameters for females allowed to mate between one and five times.

Methods

The experiments were performed in April and May and in October and November 1986. Pupae of *Danaus plexippus* were flown in from Florida and kept at approximately 25° C and a 16 h light : 8 h dark cycle. After eclosion, the butterflies were individually marked and weighed on a Sauter AR 1014 automatic electrobalance and put in a 0.8 × 0.8 × 0.5 m flight cage. The butterflies were given a 25% sucrose solution every day and were kept in the flight cage until mating took place between four and ten days after eclosion from the pupa.

By means of a lottery procedure females were assigned to one of five groups in which females were allowed to mate 1, 2, 3, 4, or 5 times. The number of females in each group were 11, 5, 5, 4, and 5, respectively. Immediately after the first mating, females were transferred together with a potted *Asclepias curassavica*, one of their natural host plants, to egglaying cages measuring 0.5 × 0.5 × 0.5 m. As previously, the females were fed every day. Dixon et al. (1978) reported that in their experiments the majority of monarch egglaying occurred between 11 a.m. and 2 p.m.; therefore, we maintained a 6 h light: 18 h dark cycle for egglaying females in order to give females ample time for ovipositing. The temperature was kept at 25° C during the dark period and 28°–30° C during the photophase.

Remating of females always took place after the end of the 6 h egglaying period, and all copulations ended before the start of the next photophase. Thus females were never hindered in their egglaying because of copulation time overlapping egglaying time. Females were remated at 3–4 day intervals, and all rematings occurred 4–14 days after the first mating (cf. Fig. 1), in an attempt to imitate the timing of rematings in natural populations of the monarch as indicated by Pliske (1973).

Throughout the lives of all 31 females, eggs laid by each individual female were removed, counted, and weighed daily on a Cahn 28 Automatic electrobalance. After weighing, the eggs were kept in 5 ml plastic capsules for a week and egg fertility was assessed by counting the number of larvae hatched from the laid eggs.

Males used for rematings were always allowed a three-day period of rest between matings after which they produced ejaculates as large as those delivered by unmated males (Svärd and Wiklund (unpublished).

Since we wanted to study the possible influence of male-derived nutrients on female survivorship, the longevity of females was measured from the day of their first mating.

Results

The number of matings and the weight of the female had no significant effect on female lifetime fecundity, mean egg weight, or female longevity (Table 1). We also did not find any short-term effect of male secretion when daily egg production, daily egg mass production and mean daily egg weight were examined (Fig. 1; *t*-test $P > 0.05$ for all of the three parameters throughout all of the 16 days analyzed). These parameters were compared for females mated once and females mated more than once. One multiple-mated female that died before the 16th day was omitted from this test. Monarch females laid an average of 663 ± 42 eggs (mean ± SE) and lived 28 ± 1 days after mating for the first time. The mean egg weight was 0.399 ± 0.005 mg and egg fertility was close to 100% for all females throughout the egglaying period.

Table 1 a–c. Multiple regression of number of matings and female weight on: **a**) female life-time fecundity, **b**) mean egg weight, **c**) female longevity. Significance was tested by analysis of variance

Variable	Coefficient	SE	Significance level
a) female life-time fecundity			
Constant	547.029	343.841	0.123
Female weight	0.147	0.706	0.837
Number of matings	17.700	29.217	0.550
$n = 31$			
Overall $F = 0.227$	$P = 0.789$		
$R^2 = 0.016$			
b) mean egg weight			
Constant	0.376	0.045	0.000
Female weight	0.000	0.000	0.615
Number of matings	0.000	0.004	0.937
$n = 31$			
Overall $F = 0.140$	$P = 0.869$		
$R^2 = 0.010$			
c) female longevity			
Constant	38.201	10.176	0.001
Female weight	−0.021	0.021	0.317
Number of matings	0.162	0.865	0.853
$n = 31$			
Overall $F = 0.520$	$P = 0.600$		
$R^2 = 0.036$			

Discussion

The hypothesis that male-contributed nutrients can be used by females to increase their reproductive output has been tested previously in four lepidopterans by analyzing the number of eggs laid by females that had all mated once but had received ejaculates of different sizes. In these studies material passed to the females at mating varied

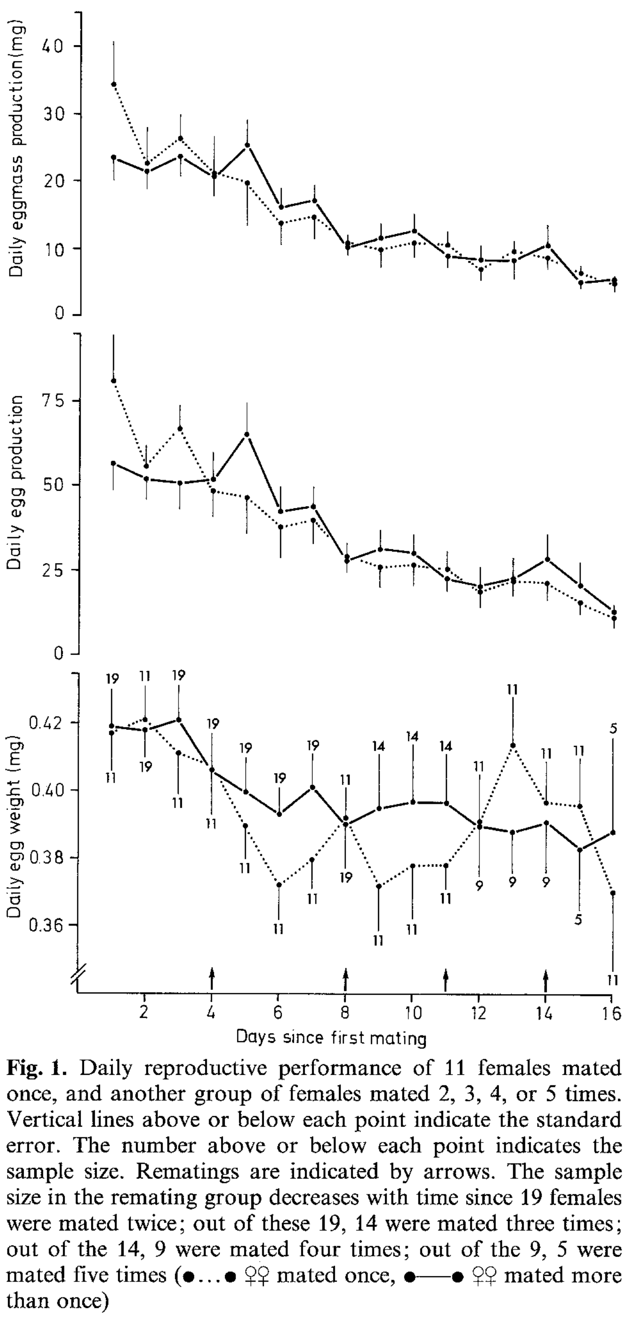

Fig. 1. Daily reproductive performance of 11 females mated once, and another group of females mated 2, 3, 4, or 5 times. Vertical lines above or below each point indicate the standard error. The number above or below each point indicates the sample size. Rematings are indicated by arrows. The sample size in the remating group decreases with time since 19 females were mated twice; out of these 19, 14 were mated three times; out of the 14, 9 were mated four times; out of the 9, 5 were mated five times (●...● ♀♀ mated once, ●——● ♀♀ mated more than once)

by a factor of not more than two. In *Colias eyrytheme* female fecundity correlated positively with the size of the ejaculate they had received (Rutowski et al. 1987), but in *Plodia interpunctella, Euphydryas editha,* and *E. chalcedona* male-contributed nutrients had no discernible effect on female fecundity or on egg fertility (Greenfield 1982; Jones et al. 1986). In the present study we increased the variance in the amount of materials transferred to the females at mating by allowing one group of females to mate repeatedly while another group of females was allowed to mate only once. Since the female monarchs in our study weighed an average of 486 mg and male ejaculates weighed an average of 37 mg (Svärd and Wiklund, in press), females that mated five times received a male contribution corresponding to 38% of their body weight at eclosion, whereas females that mated only once received an ejaculate corresponding to 7.5% of their body weight. (By comparison, in the seven butterfly species for which data were given by Rutowski et al. 1983, females received on an average ejaculates corresponding to only 4.9% of their body weight.)

However, in spite of the large amount of ejaculate received by multiple mated females and the fact that Boggs and Gilbert (1979) have shown that male-derived nutrients are rapidly incorporated into both the female eggs and soma of the monarch, we found no short- or long-term effect of male-derived nutrients on female fecundity, reproductive output, or egg fertility.

Male-derived nutrients have been shown to increase female reproductive output in three orthopterans: *Requena verticalis* (Gwynne 1984b), *Chorthippus brunneus* (Butlin et al. 1987), and *Gryllus bimaculatus* (Simmons 1988), but the effect is only discernible if females are maintained on a restricted or low quality diet.

Even though we agree with Jones et al. (1986) that more studies must be conducted before safe generalizations can be made, the studies performed to date on both orthopterans and lepidopterans suggest that the reproductive output by females in benign environments is not increased by male-derived nutrients, whereas that of females in harsh environments may be. In the Lepidoptera, females (and males) generally feed on a more protein-rich food in the larval stage than in the adult stage (Labine 1968; Dunlap-Pianka et al. 1977; Dunlap-Pianka 1979; Boggs 1981b), and therefore female reproductive output should be strongly dependent on the resources accumulated in the larval stage. In contrast, the food of adult orthopterans is as protein-rich as that of the larvae; (the spermatophylax that is delivered by the male at mating along with the sperm ampulla is consumed as any other morsel of food). Because adequate nutrients for egg production are probably limited in many lepidopterans, Gwynne (1984a) argued that "long-lived species of Lepidoptera with multiple-mating females are thus the most likely candidates for increased male nutrient investment". However, the fact that females of four out of five investigated species of Lepidoptera do not use male-derived nutrients to increase their reproductive output suggests that lepidopteran females should not be regarded as equivalent to undernourished orthopterans. Furthermore, the composition of lepidopteran spermatophores, about 85% water and only about 7% protein (Boggs 1981 a), make their nutritional contribution even less likely.

At the present time we feel that the possible role of male investment in female fecundity and longevity is uncertain; therefore, the question remains why males produce these copious secretions. Our explanation is that males of polyandrous species produce large ejaculates in order to cope with the high degree of male competition in those species (Svärd 1985; Svärd and Wiklund, unpublished). The rationale for this is as follows: Since there is a high degree of sperm displacement in butterflies, it would be to the male's advantage if he could prevent or delay further matings by the female, thereby increasing the number of eggs being fertilized by his sperm. A larger size of the ejaculate may mechanically prevent other males from being able to inseminate females. In addition, the size of the ejaculate has a positive influence on the time period females are unwilling to remate (Sugawara 1979).

Another question that arises, if male investment lacks importance for female fecundity or longevity, is why females mate more than once. Walker (1980) and Drummond (1984) give several alternative reasons why females may benefit from polyandry: (1) to achieve an adequate sperm supply, (2) to increase genetic diversity of offspring, or (3) to minimize loss of time and energy required to resist insistent males. In view of the high fertility of eggs in our study, there is little that indicates monarch females need to replenish sperm supply. The second hypothesis is more or less untestable. In reference to (3), evidence indicates that male butterflies transfer antiaphrodisiacs to females at mating (Gilbert 1976; Wiklund and Forsberg 1985), and since courtship by male monarchs appears brutal (Pliske 1975; Rothschild 1978), receiving antiaphrodisiacs through repeated matings might be beneficial to female monarchs. However, since male monarchs can enforce copulation on females (Pliske 1975; Rothschild 1978), this could be a satisfactory explanation for the high degree of polyandry observed in wild monarch butterflies.

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