Male monarch butterfly spermatophore mass and mating strategies

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Abstract. Male monarch butterflies, Danaus plexippus, produce a spermatophore which can represent approximately 10% of their body mass. Spermatophore mass increased with age in virgin males, and with the time since last mating in non-virgin males. Male monarchs did not delay re-mating until they were able to produce a large spermatophore. Recently mated males were as likely as virgins to copulate with both virgin and non-virgin females. Monarchs provide an example of Bateman's principle, mating whenever possible, despite the non-trivial cost involved.

Bateman's (1948) study of reproductive success in Drosophila melanogaster showed that male reproductive success was correlated positively with the number of copulations they obtained. His study provided a quantitative example of Darwin's (1871) suggestion that males maximize their fitness by copulating as often as possible. However, there are exceptions to Bateman's principle. In species whose females mate synchronously, it may be impossible for males to show a high degree of polygyny (Knowlton 1979). In other species, it may be in the male's best interest to be monogamous if he can provide parental care that substantially increases offspring survival, or if guarding a mate prevents her from copulating with another male (Emlen & Oring 1977). A third category of exceptions may occur in polygynous species in which males delay re-mating to increase the expected number of offspring they gain from each mating. Bateman's principle is based on the assumption that males incur little or no cost in ejaculate production, but mating success in some species is affected by the transfer of substances that must be replenished after mating. Therefore, selection might favour males that wait between matings until the amount of material available for transfer to the female ensures paternity of more of her offspring (Dewsbury 1982).

In this paper I report a study of re-mating strategies in captive monarch butterflies, Danaus plexippus. Male monarchs, like all Lepidoptera, produce spermatophores consisting of a sperm sac embedded in a gelatinous body formed from accessory gland secretions. Several aspects of monarch biology suggest that males may increase their reproductive success by delaying re-mating: spermatophore size increases with the time elapsed since the last mating by a male (see below), monarchs are highly polyandrous (Pliske 1973: personal observation), and large spermatophores delay female re-mating (Oberhauser, unpublished data). Thus by waiting to re-mate, males might increase the number of ova from each mate that are fertilized by their sperm, thereby violating Bateman's principle.

METHODS

The study animals were offspring of five to seven wild females captured in Wisconsin and Minnesota in late May and June 1985–1987. These females were kept in oviposition cages (65 x 65 x 65 cm) with host plants, Asclepias syriaca, and their eggs were collected daily. Larvae were reared in outdoor cages and fed on host plants daily until pupation. On the morning following adult eclosion (when they had dried and were able to fly), the butterflies were individually numbered, weighed, fed and stored in glassine envelopes measuring 10 x 12 cm. Butterflies kept in envelopes were fed to satiation on a 30% honey solution every other morning, or daily during experiments when they flew in outdoor cages. In 1985, butterflies were kept at 25°C on an LD 16:8 photoperiod. In 1986 and 1987 they were kept at 20–25°C near a window, where they experienced natural daylength. There was no evidence that these two regimes affected the physiological or behavioural characteristics under study.

Mating experiments were carried out in large outdoor screen cages, measuring 2 × 2 × 2 m and 2 × 4 × 2 m (width × length × height). From 30 to 45 individuals at a time were introduced into these
cages, in sex ratios appropriate to particular experiments. The butterflies were released into the cages between 0900 and 1100 hours and were removed at dawn on the following morning. The cages were monitored for mating pairs every half hour until dark. Since monarchs remain in copula for several hours, and do not initiate matings at night (personal observation), it is unlikely that matings were missed.

To determine spermatophore mass, mated females were dissected in insect saline soon after removal from the mating cage. Spermatophores were dissected from the bursa copulatrix, blotted to uniform dryness, and weighed to the nearest 0.01 mg on a Mettler semi-micro analytical balance. Most copulations end early in the morning (usually between 0200 and 0600 hours, personal observation), so females were dissected from 3 to 8 h after copulation ended.

Two experiments were performed to determine whether males delay re-mating until they can produce larger spermatopores. First, mating propensity was compared in males with different mating histories. Virgin males and males that had mated from 1 to 8 days previously were exposed to virgin females, and all matings were recorded. In each cage 18–20 females and 22–25 males were used. Since weather affects mating probability (personal observation), I used males with many different mating histories simultaneously to even out weather effects. These experiments were carried out in 1985 (on 36 different days) and 1986 (23 days).

In 1987, I did a second mating experiment to determine whether recently mated males were less likely than virgins to mate with recently mated and presumably reluctant females, as suggested by Rutowski (1979) in his study of *Pieris protodice*. On day 1, 40 virgin males and females were released into mating cages. Mated individuals from day 1 (30 pairs) and 30 virgin males and 30 virgin females were assigned to four pairwise treatments 2 days later (day 3): 15 non-virgin males confined with 15 non-virgin females, 15 non-virgin males with 15 virgin females, 15 virgin males with 15 non-virgin females, and 15 virgin males with 15 virgin females. All matings were recorded. Butterflies in each treatment were confined in separate 2 x 2 m cages in an open field, such that none was shaded at any time during the experiment. Butterflies were assigned to treatments randomly, and all were 6 ± 1 days old on day 1. Those that had been in cages on day 1 and had not mated were not used again on day 3. Data were examined using a loglinear analysis (Fienberg 1985), in which the dimensions of a three-dimensional contingency table were male (virgin or non-virgin), female (virgin or non-virgin) and mated (yes or no).

**RESULTS**

**Spermatophore Mass: Virgin Males**

Male age at mating (in days since eclosion) and mass at emergence both had significant positive effects on spermatophore mass of virgin males (multiple regression coefficients (± 1 se): age = 1.855 ± 0.149, P < 0.001; mass = 0.048 ± 0.013, P < 0.001; $R^2 = 0.722$; overall $F = 86.57$, $P < 0.001; N = 67$). The effect of male age, the most important predictor, is illustrated in Fig. 1a. Adult males weighed 539 ± 48 mg (mean ± 1 se; N = 276) 1 day after eclosion. Spermatophore mass ranged from about 25 mg in 4-day-old males (younger
Table I. Copulations by males with different mating histories to virgin females

<table>
<thead>
<tr>
<th>Time since last mating (days)</th>
<th>1985</th>
<th>1986</th>
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<tr>
<td></td>
<td>Number tested</td>
<td>% Mated</td>
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<tr>
<td>0*</td>
<td>396</td>
<td>33</td>
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<tr>
<td>1</td>
<td>49</td>
<td>57</td>
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<td>2</td>
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<td>21</td>
<td>62</td>
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<td>8</td>
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<td>38</td>
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</tbody>
</table>

* Virgins.

males did not mate) to about 50 mg in males over 2 weeks old (Fig. 1a). Thus, spermatophores from virgin males represent investments of roughly 5–10% of body mass.

Spermatophore Mass: Previously Mated Males

Three factors had significant effects on spermatophore mass of previously mated males: the amount of time that had elapsed since the last mating (in days, log-transformed to linearize data), the number of previous matings, and mass at emergence (multiple regression coefficients (±1 se): log time since last mating = 26.788 ± 1.558, \( P < 0.001 \); number of matings = -1.479 ± 0.325, \( P < 0.001 \); mass = 0.032 ± 0.010, \( P < 0.001 \); \( R^2 = 0.858 \); overall \( F = 123.7, P < 0.001, N = 62 \)). The effect of time since last mating, the most important predictor, is illustrated in Fig. 1b. The very small spermatophore mass of males that mated on 2 consecutive days consisted mainly of sperm and sperm sac, with little or no accessory gland material.

Male Re-mating

Table I summarizes the results of the first experiment designed to determine whether males delay re-mating. A lower percentage of males mated in 1986 than in 1985 (paired \( t = 3.3, df = 8, P = 0.011 \)) because it was an unusually cool summer and fewer matings occur on cold days (personal observation). In neither year did recently mated males mate less than males with other mating histories. A chi-squared analysis of the 1985 data revealed a significant deviation from the null hypothesis that mating history has no effect on likelihood to mate (\( \chi^2 = 51.84, df = 8, P < 0.001 \)), but observed matings by males that had mated 1 and 2 days previously were higher than expected. A chi-squared test of the 1986 data was not significant at the 0.05 level of confidence (\( \chi^2 = 14.43, df = 8, P = 0.071 \)).

Male Re-mating and Female Mating History

Thirteen of 15 possible pairs mated in the two treatments in which virgin and non-virgin males were combined with virgin females. Two previously mated females re-mated to virgin males, and four to non-virgin males. The best loglinear model for these data is one that includes an interaction between female type and mating, and none between male type and mating (\( G^2 = 0.85, df = 2, P = 0.659 \)). The loglinear test compares models to find the one that best fits a data set. Therefore, a low \( G^2 \) and high \( P \) correspond to the best model. Non-virgin females were more reluctant to mate than were virgins, but the two groups of males showed no differences in propensity to mate.

DISCUSSION

Two interesting features of monarch mating biology are illustrated by this study. (1) Males incur a non-trivial cost in mating, producing spermatophores that can represent up to 10% of their body mass. It took males in this experiment approximately 5 days to replenish the accessory gland material used to make spermatophores. This pattern is consistent with that reported in several other Lepidoptera (Rutowski 1979, 1984; Sims 1979; Boggs 1981; Svärd 1985; Rutowski & Gilchrist 1986; Svärd & Wiklund 1986). (2) Males do not delay re-mating until they can produce large spermatophores. This was true whether they were exposed to virgin or to non-virgin females.

Two aspects of my study make the second conclusion slightly tentative, and must be considered. It is possible that high butterfly densities or some other feature of the experiments made males more likely to mate. Since sample size and space considerations made lower densities impractical and the experiments could not be carried out in the wild, I have assumed the propensity to mate is similar in the wild and captivity. Second, I com-
pared males that had mated 2 days earlier to virgins when testing whether female mating history affects males' propensity to re-mate. At this time, accessory gland material is partially replenished (Fig. 1b). It is possible that males that had mated 1 day earlier would have been less likely than virgins to mate with reluctant females. The most conservative conclusions that can be drawn from both sets of remating data are: (1) males that produce the smallest spermatophores (those that mated 1 day previously, Fig. 1) are as likely to mate with virgin females as males that can produce much larger spermatophores (Table I), and (2) males that mated 2 days previously are as likely to mate with reluctant females as are virgin males, and very likely to mate with virgin females (Table I), despite the fact that these males produce smaller spermatophores than all but males mated 1 day previously (Fig. 1a, b).

Boggs (1981) suggested two ways in which butterfly spermatophores may benefit males. They may represent a parental investment, since spermatophore constituents are incorporated in ovaries, eggs and female somatic tissue (Boggs & Gilbert 1979; Marshall 1980, cited in Marshall 1982; Boggs 1981; Boggs & Watt 1981; Greenfield 1982). However, in polyandrous species such as the monarch it is not clear that males always benefit by providing these nutrients, since they may increase the fitness of other males' progeny (but see Rutowski et al. 1987). The second benefit males may gain by providing large spermatophores is an increase in the time before female re-mating. Studies of sperm precedence in Lepidoptera indicate that the last male to mate fertilizes most subsequent eggs (Drummond 1984). Thus, males of polyandrous species increase their fitness by delaying female re-mating. Studies of several Lepidoptera (Labine 1964; Boggs 1979; Sugawara 1979; Rutowski 1980; Rutowski et al. 1981), including monarchs (Oberhauser, unpublished data), have indicated that larger spermatophores delay female re-mating. Svärd (1985) found lower investment in the monandrous Pararge aegeria than in polyandrous butterflies, arguing that males do not invest as heavily when there is no advantage to delayed re-mating.

An explanation of the male monarch strategy of copulating whenever possible can be sought using a cost–benefit analysis. If a recently mated male encounters a female, he could attempt to copulate with her, or wait until he is able to contribute a larger spermatophore to a future mate. Copulating with the first female would deplete the small amount of accessory gland material available, and may not provide him with many offspring if the female re-mates after a short time. Waiting could benefit a male by increasing the number of inseminations gained in a future mating, since a larger spermatophore should increase the time before this female re-mates. However, giving up an opportunity to mate in favour of allowing accessory gland material to be replenished would be a costly strategy if the male is unlikely to find another mate. While it is difficult to quantify benefits and costs of delaying re-mating, their magnitude depends on several factors, including the effectiveness of large spermatophores in delaying female re-mating, the chances of encountering potential mates, the condition of the female encountered by the recently mated male, and the male's age and condition. If the conclusions suggested by this study are representative of wild monarch behaviour, it may be that the chances of obtaining copulations are low enough that it is always best for a male to mate whenever he has the opportunity. Low summer population densities of monarchs may make this true, especially since Asclepias sp. are characteristically in disturbed habitats, suggesting that summer densities in which monarchs evolved may have been even lower.

Dewsbury (1982, page 603) suggested that selection pressures associated with costly ejaculates and sperm competition should lead males 'not to inseminate as many females as possible but to ensure that the number of ejaculates with each female ensures effective paternity'. While monarchs deliver only a single spermatophore during mating, this argument might be extended to include ejaculate size as well as number, since large ejaculates should correlate with paternity of more offspring from each female. The monarch strategy of mating whenever possible appears to be more consistent with the viewpoints of Darwin (1871) and Bateman (1948).

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REFERENCES


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