Effects of Female Age, Female Mass, and Nutrients from Males on Monarch Egg Mass

Karen S. Oberhauser

INTRODUCTION

Reproductive success is determined not only by the quantity of offspring that individuals produce, but also by the quality of these offspring. It is often assumed that there is a trade-off between the size and fitness of offspring, and several studies have shown that their growth and survival are influenced by the resources allocated to eggs by their mothers (reviews in Fleming and Gross 1990 and Reznick 1991 [fish]; Kaplan 1991 [amphibians]; Fox 1994 [insects]). There is likely to be a negative correlation between the number and size of offspring, and thus egg size is likely to result from a balance between selection for large eggs and selection for large numbers of eggs. In many cases, the fitness consequences of egg size vary with environmental conditions, and large eggs are likely to be most advantageous in adverse environments (Janzen 1977; Braby 1994; Fox and Mousseau 1996; Fox et al. 1997; Fox 2000). However, several studies of offspring fitness and egg size in butterflies have shown no correlation between the two factors (Wiklund and Persson 1983; Karlsson and Wiklund 1984, 1985). It is possible that butterfly reproduction makes a correlation between egg size and offspring fitness unlikely. Newly hatched larvae are exposed to many kinds of mortality that may not be affected by variation in size, at least within the ranges of egg sizes that females can produce. Thus, female butterflies may maximize their reproductive success by laying as many eggs as possible, as long as these eggs are above a certain minimum size.

Research on lepidopteran egg mass has addressed the effects of interspecific and intraspecific differences in adult size, as well as female age. Wiklund and his collaborators found that egg mass increases with body size across several satyrid species (Wiklund and Karlsson 1984; Wiklund et al. 1987). However, there is not a consistent relationship between female size and egg mass across taxa; Jones and colleagues (1982) found a negative correlation in the cabbage white (Pieris rapae); Boggs (1986), a positive correlation in Mormon fritillaries (Speyeria mormonia); and Wiklund and Karlsson (1984), no correlation in 10 satyrid species. Egg mass decreases with female age in many species (Jones et al. 1982; Murphy et al. 1983; Wiklund and Persson 1983; Karlsson and Wiklund 1984, 1985; Wiklund and Karlsson 1984; Boggs 1986; Svärd and Wiklund 1988).

Across many groups of animals, egg size correlates with female body mass (Peters 1986). This relationship does not have to be a result of natural selection but could simply result from allometry, the scaling of body part size to overall size. Wiklund and colleagues (1987) argued that when fecundity is limited by the number of eggs females actually lay, owing to time constraints or the ability to find host plants, and not by the number they can physically produce, large females will lay larger eggs. This view does not imply that larger eggs are better, simply that egg size scales to female body size, often referred to as “nonadaptive scaling”. It implies a neutral relationship between egg size and offspring fitness. When females can produce more eggs than they can...
lay, there may not be a strong trade-off between egg size and egg number. However, when fecundity is limited by egg production, there will be a trade-off, resulting in selection on females to produce the smallest eggs possible, within limits posed by viability constraints. This could result in a weaker association between egg size and body size that is driven by natural selection. Since female monarchs do not seem to live long enough to lay all of the eggs they produce (Oberhauser 1997), a relationship between egg size and female body size in monarchs could support Wiklund and colleagues’ (1987) argument of nonadaptive scaling (table 3.1).

Information on egg mass can also be used to assess the total investment that females make in their offspring, and the source of the nutrients they use to produce eggs. In monarchs, as in most other butterflies, females utilize materials transferred by males during mating in egg production. Sperm are transferred to females within a protein-rich spermatophore, and the spermatophore is digested by the female and used in both somatic and reproductive tissue (Boggs and Gilbert 1979). I previously showed that the number of eggs that females produce is affected by the amount of spermatophore nutrients received (Oberhauser 1989, 1997); here I report how female investment in offspring is affected by the amount of nutrients acquired from spermatophores.

**METHODS**

All monarchs used in this study were the offspring of wild-captured adults and were reared on fresh cuttings of *Asclepia syriaca* (common milkweed) under ambient Minnesota summer photoperiod and light conditions in a room with open windows. On the day after eclosion, I weighed adults to the nearest 0.01 mg and measured their forewings to the nearest 0.1 mm. Female mass and mean forewing length ranged from 288 to 624 mg and 43 to 55 mm, respectively, reflecting a wide range of sizes. Butterflies were kept in glassine envelopes and fed a 20% honey solution ad libitum every other day until they were ready to mate.

Females were allowed to mate with one or two males. Females were 5 to 10 days old at their first mating, and their mates were 5 to 11 days old and either unmated or recently mated. Recently mated males transfer much smaller spermatophores (about 7 mg) than virgin males (over 25 mg) (Oberhauser 1988). I used five different mating treatments in which the amount of spermatophore nutrients females received varied (Oberhauser 1988). Females (total n = 47) mated with either (a) two unmated males, receiving two large spermatophores (large-large treatment); (b) an unmated male followed by a mated male, receiving a large followed by small spermatophore (large-small treatment); (c) a mated male followed by an unmated male, receiving a small followed by large spermatophore (small-large treatment); (d) two mated males, receiving two small spermatophores (small-small treatment); or (e) one mated male, receiving one small spermatophore (small treatment). Thus, the total amount of spermatophore material received by females ranged from about 7 mg (delivered by one recently mated male) to over 50 mg (delivered by two unmated males). Intermating intervals ranged from 3 to 7 days, and females were kept in 0.7-m³ cages with potted *A. curassavica* plants and fed daily until they died or stopped laying eggs. I counted the number of eggs laid by each female every day and weighed 10

<table>
<thead>
<tr>
<th>Relationship between egg size and offspring survival?</th>
<th>Is a trade-off expected between egg size and number of eggs that can be laid?</th>
<th>Degree of scaling of egg size to body size (allometry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak (expected in butterflies)</td>
<td>Yes, if fecundity is limited by the number of eggs produced.</td>
<td>Less: selection produces small eggs to maximize fecundity.</td>
</tr>
<tr>
<td></td>
<td>No, if fecundity is limited by the number of eggs females can lay.</td>
<td>More: egg size doesn’t affect fitness, so the only factor that affects egg size is female size (allometric scaling).</td>
</tr>
</tbody>
</table>
eggs/female/day to the nearest 0.01 mg on a Mettler analytical balance. Since the balance was not sensitive enough to weigh individual eggs, I weighed batches of 5 eggs.

I used multiple regression analyses to test the effects of treatment, time, and female size on egg mass, and one-way analyses of variance to compare treatment means of total fecundity and total egg mass.

RESULTS

Egg mass

The mass of a single monarch egg ranged from 0.242 to 0.588 mg, with a mean mass of 0.460 mg. This mean equals approximately 1/1000th the mass of a female (about 480 mg).

Several factors affected egg mass. The most important factor was time; females tended to lay lighter eggs as they aged (figure 3.1). I used time since mating rather than absolute age as a predictor in this analysis, since eggs are formed within the female before mating. Thus, a female that doesn’t mate until she is 10 days old will actually lay eggs that were manufactured when she was younger. The shape of the plot of egg mass on day of egg laying is concave, and using the log of time as a predictor significantly improved the regression over using untransformed data. When time was controlled, there was an effect of female mass on egg mass; larger females laid larger eggs (figure 3.2). In addition, females in the large-small and small spermatophore treatments laid smaller eggs than did other females (table 3.2).

Total reproductive effort

I measured female reproductive output in two ways; the number of eggs produced and the total egg mass produced. Egg mass was calculated by multiplying the average mass of the 10 eggs by the number of eggs laid by each female on each day of egg laying, and summing this over the female’s egg-laying life span. Table 3.3 summarizes means for both of these measures in each mating treatment, and table 3.4 shows the results of a multiple regression of several predictors on total egg mass.

The total number of eggs produced by females ranged from 290 to 1179 (mean = 715). There was a treatment effect on total fecundity, with females that received a large first spermatophore tending to lay more eggs (see table 3.3), although only two treatments were significantly different from each other. The total egg mass laid by females ranged from 129 to 510 mg (mean = 323 mg). Again, females that received a larger first spermatophore tended to lay a greater total mass of eggs (see tables 3.3 and 3.4),

Figure 3.1. Egg mass over the period of egg laying. Bars represent one standard error. From Oberhauser 1997, reprinted with permission of Blackwell Publishing.

Figure 3.2. The mean mass of individual eggs laid by each female on the day after her first mating, as a function of female mass 24 h after eclosion.
although only the large-small, small-large, and small spermatophore treatments differed significantly. Female mass at eclosion and the total amount of time over which females laid eggs also affected total egg mass. Females that laid eggs over a longer period of time and those that weighed more at eclosion tended to lay a higher total mass of eggs (see table 3.4).

### DISCUSSION

Even though individual eggs are small (weighing about 1/1000th as much as the female herself), female monarchs invest a large proportion of their mass in egg production. On average, females produce eggs that equal 70% of their initial body mass, with a range of 30% to 114%.

Female monarch butterflies lay smaller eggs as they age (see figure 3.1, table 3.2). Larger females tend to lay larger eggs (see table 3.2) and a greater total mass of eggs (see figure 3.2, table 3.4). While the amount of nutrients that females receive from male spermatophores affects their total egg mass, it does not appear to affect the mass of individual eggs. These results augment our understanding of how females utilize available nutrients for egg production. Since larger females tend to lay larger eggs, it appears that the nutrients that females consume as larvae affect egg mass. Nutrients that females receive from males do not affect egg mass in a predictable way; there were statistically significant negative effects of two mating treatments, but these treatments varied a great deal in the amount of spermatophore material received. In addition, as egg mass dropped most rapidly (days 3 to 7 of egg laying, see figure 3.1), most females are still obtaining nutrients from spermatophores (Oberhauser 1992).

In every case I have observed, female monarchs have eggs remaining in their ovaries at the end of their lives (Oberhauser 1997), suggesting that monarch fecundity is limited by female egg-laying life span, and not the total number of eggs produced. Thus, my finding that larger females lay larger eggs supports the hypothesis of Wiklund and colleagues (1987) that there will be nonadaptive scaling of egg size to body size when fecundity is limited by the number of eggs females can actually lay (see table 3.1). If there was strong correlation between egg mass and offspring fitness, all females should

#### Table 3.2. Significant predictors of egg mass

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient (SE)</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.620 (0.221)</td>
<td>2.80</td>
<td>0.005</td>
</tr>
<tr>
<td>Log (time since first mating)</td>
<td>-0.280 (0.024)</td>
<td>-11.67</td>
<td>0.000</td>
</tr>
<tr>
<td>Female mass</td>
<td>0.036 (0.004)</td>
<td>8.44</td>
<td>0.000</td>
</tr>
<tr>
<td>Small spermatophore treatment</td>
<td>-0.0176 (0.005)</td>
<td>-3.53</td>
<td>0.000</td>
</tr>
<tr>
<td>Large-small spermatophore treatment</td>
<td>-0.0161 (0.004)</td>
<td>-4.13</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: n = 614, adj. $R^2 = 0.231$. Time since first mating correlates with female age. There was no effect of interactions between treatments and time since first mating, or of first spermatophore size. SE, standard error.

#### Table 3.3. Lifetime fecundity means and total egg mass in the five mating treatments

<table>
<thead>
<tr>
<th>Mating treatment</th>
<th>n</th>
<th>Mean fecundity</th>
<th>Total egg mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Large-large</td>
<td>11</td>
<td>711ab</td>
<td>321ab</td>
</tr>
<tr>
<td>b. Large-small</td>
<td>9</td>
<td>936b</td>
<td>413a</td>
</tr>
<tr>
<td>c. Small-large</td>
<td>12</td>
<td>596b</td>
<td>275</td>
</tr>
<tr>
<td>d. Small-small</td>
<td>9</td>
<td>700ab</td>
<td>317ab</td>
</tr>
<tr>
<td>e. Small</td>
<td>6</td>
<td>664a</td>
<td>302b</td>
</tr>
<tr>
<td>Total or mean</td>
<td>47</td>
<td>715</td>
<td>323</td>
</tr>
</tbody>
</table>

Note: Mating treatments refer to the sizes of the two (or in the case of treatment e, one) spermatophores received from males. Means followed by the same letter are not significantly different at the 0.05 level of confidence (Tukey’s least significant difference comparisons).

#### Table 3.4. Significant predictors of total egg mass

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient (SE)</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-84.12 (107.3)</td>
<td>-0.78</td>
<td>0.438</td>
</tr>
<tr>
<td>Egg-laying life span</td>
<td>8.471 (1.834)</td>
<td>4.62</td>
<td>0.000</td>
</tr>
<tr>
<td>Female mass at eclosion</td>
<td>0.5313 (0.2145)</td>
<td>2.48</td>
<td>0.018</td>
</tr>
<tr>
<td>Large first spermatophore</td>
<td>50.24 (24.07)</td>
<td>2.09</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Note: n = 44, adj. $R^2 = 0.500$. SE, standard error.
produce eggs of similar size. Wiklund and Karlsson (1984) suggested that females use a proportion of available reserves to produce eggs and as these reserves are depleted, females lay smaller eggs. My finding that egg mass decreases with female age supports this suggestion.

The arguments presented here assume that egg mass and offspring fitness are not correlated, as long as eggs are above some minimum size required for viability. This has not been tested in monarchs, and an important test of my conclusions would be to look for effects of egg mass on offspring fitness.

These findings have important implications for the population dynamics of monarchs. The fact that offspring number appears to be limited by the number of eggs females are able to lay, rather than produce, means that the density and quality of milkweed available will be an important determinant of population growth. If females spend more time searching for suitable oviposition sites, their realized fecundity will be decreased. Factors that result in an early departure from overwintering sites, such as lipid depletion and early mating (Oberhauser and Frey 1999), may result in less time for egg laying if females leave before milkweed is ready. The fact that female size influences egg numbers means that factors that affect one generation may have cascading effects into the next generation. Since adult size is determined during the larval stage, the quality and quantity of milkweed available to larvae will impact the number of eggs in the subsequent generation.

Acknowledgments

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References