# CONSEQUENCES OF DELAYED MATING FOR LIFE-HISTORY TRAITS IN THE TROPICAL BUTTERFLY *BICYCLUS ANYNANA*

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## Summary

We examined the effects of a 12-day delay in mating on maternal and offspring traits for *Bicyclus anynana* females. The mating-delayed females did not differ in body or fat weight immediately after oviposition from females that had directly mated and oviposited. Both groups, however, had significantly lower fat weight (absolute and relative) than females that were not allowed to oviposit. Only fertility in the first laid eggs after mating was lower for the mating-delayed females. Egg number, weight, and fertility of a subsequent sample of eggs did not differ significantly.

Most of the variation in offspring pupal weight was explained by sex (59%, F>M), mean egg weight, which relates positively to pupal weight, significantly accounted for an additional 6% of the variation. Pupal weight itself explained most (59%) of the variance in starvation resistance.

## INTRODUCTION

Most studies on delayed mating are concerned with implications for pest control (e.g. van der Kraan & van der Straten, 1988). The rationale for this study is different: we sought to investigate possible confounding effects delayed mating may have in selection experiments on life-history. If, for example, maternal age partly determines development time, then selection on this trait can be hampered.

In a previous experiment we examined the effects of delayed female mating on reproductive traits such as fecundity and fertility (Zijlstra *et al.*, 1999). We found both maternal age and development time to be negatively correlated with the number of eggs laid. In this study we examined the effects of delayed mating on traits such as offspring development time and pupal weight.

Furthermore, several studies have reported a decline in egg weight with increasing maternal age for continuously laying females (e.g. Brakefield *et al.*, 1994 for *Bicyclus anynana*). Karlsson and Wiklund (1984) did not find any fitness-disadvantages for smaller eggs in *Lasiommata megera*, leading them to propose the mechanistic "resource depletion hypothesis" (Wiklund and Karlsson, 1984). However, Richards and Myers (1980) did observe a decline in hatching success for smaller eggs in *Tyria jacobaeae*. Here, we report on the effects of delayed mating on total egg weight and number. This also allows us to study the fitness consequences of variation in egg size.

## MATERIAL AND METHODS

The stock of *Bicyclus anynana* (Lepidoptera: Satyrinae) was founded in 1988 by >80 gravid females caught in Malawi. Subsequent laboratory populations have been kept at large population sizes. Larvae feed on maize, adults on banana. Butterflies were reared at 22.5°C (± 0.5°C), humidity of 75% (±15%), and 12:12 light:dark cycle.

Three experimental groups of females were established: females that did mate but were frozen before egg-laying, females that mated and oviposited shortly after emerging,

and a group of females that was delayed 12 days in their mating and egg laying. The latter two groups will be referred to as the direct and (12d-)delayed group, respectively. After egg laying, fresh, dry, and fat-extracted weights of these females were measured (using dichloromethane/methanol, see van Oosterhout *et al.* (1993) for full method). Fat weight and relative fat content can be calculated with these data.

Emerged females were individually marked and randomly assigned to an experimental group. Males were not marked. Sexes were kept separately with *ad libitum* banana; females at a low temperature (18°C) during their mating-delay.

Young males (<1 week old) and females were allowed to mate for three days in a 2:1 ratio in cylindrical hanging cages (diameter=0.3m), so multiple matings could occur. After this period, females were provided with an *Oplismenus* cutting for three concurrent one-day periods (here onwards referred to as period I-III) of oviposition. Egg laying occurred at 22.5°C in gauze-covered pots (0.15m diameter, 0.1m high). Egg samples from periods I and III were weighed, and (early) fertility and (early) fecundity assessed. Unhatched eggs could be classified as either sterile (unfertilized), or fertilized yet not emerged. Period II eggs were reared to adults. Each period II egg sample was placed in a separate sleeve cage containing maize plants. Pupae were weighed 24 hours after pupation. Egg to pupa, and egg to adult development times were also recorded. After all the offspring had emerged, they were tested for starvation resistance (only water provided).

MINITAB, release 12.2, was used for statistical analysis. All analyses performed on offspring traits, using egg weight and/or maternal body weight and composition as predictors, used family means. Trait values are followed by ± standard error.

## RESULTS

*Maternal body weight and composition*
Fresh weight did not differ between the three groups of females (ANOVA, $F_{2,68}$=2.25; NS, table 1). Females that were not allowed to lay eggs after mating did, however, contain more fat than their ovipositing counterparts both in absolute and relative terms (ANOVA, $F_{2,68}$=7.86, p<0.01, and $F_{2,68}$=5.63, p<0.01, respectively). The direct females did not differ significantly in fat weight from the delayed mated females (table 1). Water content did not differ ($F_{2,68}$=0.52, NS) between the groups.

**Table 1.** *Maternal body weight and composition traits (all weights in mg). Same letters indicate no significant differences (Tukey, p>0.05).*

| Group | N | Fresh weight | Water weight | Fat weight | Fat% |
|---|---|---|---|---|---|
| Not oviposited | 25 | 71.6 ± 1.86[a] | 45.8 ± 0.68[a] | 6.6 ± 0.51[a] | 9.2 ± 0.33[a] |
| Direct | 25 | 67.9 ± 1.86[a] | 45.3 ± 0.67[a] | 5.3 ± 0.47[b] | 7.8 ± 0.39[b] |
| 12d-delay | 21 | 64.4 ± 3.36[a] | 43.2 ± 0.78[a] | 4.9 ± 0.50[b] | 7.6 ± 0.38[b] |

*Fertility and Fecundity*
Mating-delayed females laid the same number of eggs as females that had mated straight away (data not shown). Total number of eggs laid also did not differ significantly between the laying periods (table 2). Fertilities (arcsine transformed) were significantly different between direct and delayed mated females (table 2), because extremely low proportions of eggs from delayed females hatched in period I (35.7% versus ±75% for the other groups). These unhatched eggs were in 89% of the cases unfertilized. Excluding completely sterile females did not qualitatively alter the results.

*Table 2. GLM on fecundity (total number of eggs) and arcsine fertility (% hatched).*

| | | Fecundity | | | | Fertility | | |
|---|---|---|---|---|---|---|---|---|
| Source | df | Adj MS | F | p | df | Adj MS | F | p |
| Mating delay | 1 | 4.4 | 0.05 | .821 | 1 | 1.69 | 7.88 | .006 |
| Period | 2 | 176.0 | 2.06 | .131 | 1 | 1.06 | 4.94 | .029 |
| Interaction | 2 | 33.0 | 0.39 | .680 | 1 | 1.66 | 7.74 | .007 |
| Error | 132 | 85.4 | | | 77 | 0.21 | | |

The egg to adult survival, measured on the period II sample, was also similar for the two groups: 61.4% for the direct group, 53.4% for the delayed mating group (t= 1.01, df=32; NS).

*Egg weight*
Neither mean egg weight, nor total egg weight of a sample laid by directly mated females differed from delayed mated females. There were also no significant differences in this respect between the laying periods (table 3). No correlations between female body weight or composition, and weight of eggs laid, were found. Furthermore, mean egg weight did not correlate with total number of eggs laid.

*Table 3. Results of GLM on total and mean egg weight.*

| | | Total egg weight | | | Mean egg weight | | |
|---|---|---|---|---|---|---|---|
| Source | df | Adj MS | F | p | Adj MS | F | p |
| Mating delay | 1 | 4.81 | 0.26 | .614 | 0.0014 | 0.66 | .420 |
| Period | 1 | 51.51 | 2.75 | .101 | 0.0000 | 0.00 | .981 |
| Interaction | 1 | 8.28 | 0.44 | .508 | 0.0004 | 0.18 | .668 |
| Error | 77 | 18.71 | | | 0.0021 | | |

*Offspring traits*
The progeny of the delayed females had significant higher pupal weights, and shorter pupal and development times than those from the directly mated group (tables 4,5).

*Table 4. Summary of various offspring traits.*

| Group | Sex | N | Pupal time (days) | Pupal weight (mg) | Development time (days) |
|---|---|---|---|---|---|
| Direct | ♂ | 101 | 9.9 ± 0.06 | 171.5 ± 1.6 | 42.1 ± 0.22 |
| | ♀ | 108 | 9.1 ± 0.06 | 215.7 ± 2.0 | 44.4 ± 0.30 |
| 12d-delay | ♂ | 63 | 9.1 ± 0.08 | 177.9 ± 2.1 | 38.9 ± 0.29 |
| | ♀ | 58 | 8.5 ± 0.08 | 227.3 ± 3.1 | 41.5 ± 0.40 |

To test which variables predicted these offspring traits best, we performed stepwise regressions. All predictors listed in table 5 could have been incorporated.

The model for pupal weight contained five of these predictors, with sex accounting for most (59.1%) of the variation. Mating delay, egg weight, and both maternal and offspring development time explained small but significant portions of the variation.

Sex, mating delay, and pupal weight explained almost half of the variance in pupal time. Variance in offspring development time could only be explained by mating (20.3%) and an additional 13.2% by sex (data not shown). Males have a faster overall development, but a longer pupal phase than females (see table 4). Mating delay correlated negatively with both measures of development time.

**Table 5**. *Results of stepwise regressions on offspring traits, F-to-Enter=3.84 (p=0.05), F-to-Remove= 2.71 (p=0.10). N.A.= not applicable. Bold numbers: step included. T-values are for the final model and have the same sign as the coefficient. Percentages: total $r^2$ [additional $r^2$ after inclusion of predictor] x100%.*

| Response:<br>Predictor | Pupal weight<br>(N=61) | Pupal development<br>time (N=60) | Starvation resistance<br>(N=59) |
|---|---|---|---|
| Sex<br>($\male$=1, $\female$=2) | **1**; t= 9.56<br>59.1 % | **1**; t= -5.67<br>22.9 % | **4**; t= 2.47<br>74.9 [2.8] % |
| Mating delay<br>(0 or 12 d) | **3**; t= 5.59<br>70.0 [5.1] % | **2**; t= -4.93<br>37.9 [15.0] % | - |
| Maternal<br>development time | **5**; t= -2.49<br>78.7 [2.4] % | - | - |
| Maternal fat<br>percentage | - | - | - |
| Mean egg weight | **2**; t= 3.24<br>64.9 [5.9] % | - | **3**; t= -2.56<br>72.1 [5.4] % |
| Pupal weight | N.A. | **3**; t= 3.37<br>48.4 [10.5] % | **1**; t= 3.81<br>58.6 % |
| Offspring<br>development time | **4**; t= 3.65<br>76.3 [6.4] % | N.A. | **2**; t= 4.06<br>66.7 [8.1] % |

The main predictor (58.6%) of starvation resistance was pupal weight. Sex, offspring development time and egg weight also contributed, the last being negatively correlated to starvation resistance. However, without correction for pupal weight (which occurs in the model when it is incorporated in step 1), egg weight did not correlate with starvation resistance. The effects of sex are also mediated through pupal weight. Regressing only sex on starvation resistance rendered an $r^2$ of 0.578. When we separate the sexes, only female pupal weight regressed significantly on starvation resistance (females: $r^2$=0.41, p<0.01; males: $r^2$=0.0, NS).

## DISCUSSION

We found two effects of delayed mating on life history traits in *Bicyclus anynana*: delayed females had a lower fertility for the first sample of eggs laid than females which had directly mated and oviposited. Furthermore, delayed mated females produced faster developing offspring with heavier pupae than directly mated females.

The decrease in development time of offspring from mating-delayed females was surprising. Most literature cites the opposite (e.g. Rossiter, 1991). We think, however, that these results are attributable to differential environmental circumstances, like bad food quality, and do not constitute a real effect of delayed mating. Because the cohorts of larvae (offspring) were separated in time, differences in environmental variables, such as food supply, could have occurred. Drought stress in food plants can add several days to development time (Kooi *et al.*, 1998).

The difference in fertility in period I is mainly due to mating-delayed females laying unfertilized eggs. These females probably already had begun to produce (sterile) eggs, which they have to oviposit before they can start laying fertilized eggs. This hypothesis is supported by the lack of difference in fertility in period III. In previous work (Zijlstra *et al.*, 1999) we did find similar results for one group (slow females, 14 days mating-

and 14 days delayed females. The initial low fertility probably only manifests itself after a prolonged period (12 days or more) of pairing delay. The absence of an effect of mating delay on fecundity (number of eggs) is in accordance with literature (Hiroki & Obara, 1997, Zijlstra et al., 1999).

We did not find differences in total sample weight or mean egg weights between the direct and mating-delayed group. Furthermore, no differences between directly mated and delayed mated females were found with regard to body weight and composition. However, females that did not oviposit had higher fat contents (absolute and relative). This probably reflects the physiological and genetical trade off between egg production and fat content (e.g. Zwaan et al., 1995).

With these data, the decline in egg size with age reported by Brakefield et al. (1994) could be explained by the "resource depletion hypothesis" (Wiklund & Karlsson, 1984): egg laying does deplete fat reserves whilst an extra 12 days of somatic maintenance does not. The energy income through fruit-feeding seems to be sufficient for body fat maintenance, but not for egg production. Furthermore, we did not find negative effects of maternal age (without oviposition history) on egg weight. However, a 12-day delay might be too short for this long-lived butterfly (Brakefield & Kesbeke, 1995) to be able to detect effects of senescence on egg weight.

The absence of a relation between maternal and offspring development time would suggest non-heritable variation for this trait. However, selection experiments (P.M. Brakefield & F. Kesbeke, unpublished results) have shown that development time has a substantial genetic component. Moreover, as noted above, the differential rearing conditions may confound results.

We did find a small, albeit significant positive relation between egg and pupal weight. However, egg weight did not correlate positively with development time and starvation resistance. It also did not trade off with egg number. Van Oosterhout et al. (1993) did not find a significant relation between egg weight and larval weight or other life history traits. The literature on this issue of maternal (age) effects mediated through egg size is inconclusive (see e.g. Fox, 1993 and references therein). Whether this is due to differential biological circumstances or the protocols used, remains to be seen. The polyphenic Bicyclus anynana experiences markedly different seasonal environments (Brakefield & Larsen, 1984). Differential selection pressures on the delays in mating are thus very likely.

## ACKNOWLEDGMENTS

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