**Supplementary Material**

**Age-dependent male mating investment in *Drosophila pseudoobscura***

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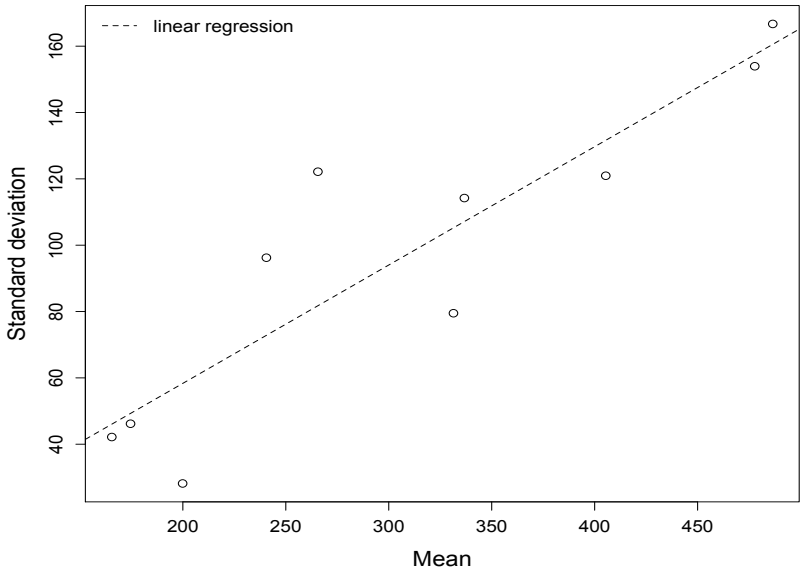
## I. Copulation Duration

**Table S1:** The copulation duration data are overdispersed relative to a Poisson distribution (variance to mean ratio > 1).

| Female age | Male age | Mean | Variance | Variance to mean ratio |
|---|---|---|---|---|
| 4 | 4 | 165.5 | 1778.0 | 10.7 |
| 4 | 8 | 336.7 | 13044.6 | 38.7 |
| 4 | 11 | 477.8 | 23697.6 | 49.6 |
| 4 | 15 | 486.5 | 27790.3 | 57.1 |
| 4 | 19 | 405.4 | 14626.7 | 36.1 |
| 11 | 4 | 174.6 | 2132.3 | 12.2 |
| 11 | 8 | 199.9 | 793.2 | 4.0 |
| 11 | 11 | 265.6 | 14922.5 | 56.2 |
| 11 | 15 | 240.6 | 9260.3 | 38.5 |
| 11 | 19 | 331.4 | 6318.3 | 19.1 |

**Figure S1:** Linear relationship between the mean and the standard deviation (quadratic relationship with variance) in copulation duration measured in the different male-female age combination groups.



Output for the generalized linear model:

Call:
glm(formula = Copulation.duration.seconds ~ factor(Male.age) * factor(Female.age), family = Gamma(link = log), data = copudata)

Deviance Residuals:

| Min | 1Q | Median | 3Q | Max |
|---|---|---|---|---|
| -0.65722 | -0.21524 | -0.04602 | 0.17905 | 0.79967 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| (Intercept) | 5.10897 | 0.07828 | 65.266 | < 2e-16 *** |
| factor(Male.age)8 | 0.71030 | 0.12264 | 5.792 | 1.03e-07 *** |
| factor(Male.age)11 | 1.06012 | 0.13558 | 7.819 | 1.02e-11 *** |
| factor(Male.age)15 | 1.07827 | 0.12622 | 8.543 | 3.32e-13 *** |
| factor(Male.age)19 | 0.89590 | 0.12622 | 7.098 | 2.95e-10 *** |
| factor(Female.age)11 | 0.05353 | 0.12622 | 0.424 | 0.672541 |
| factor(Male.age)8:factor(Female.age)11 | -0.57498 | 0.18614 | -3.089 | 0.002680 ** |
| factor(Male.age)11:factor(Female.age)11 | -0.64062 | 0.19491 | -3.287 | 0.001452 ** |
| factor(Male.age)15:factor(Female.age)11 | -0.75775 | 0.19935 | -3.801 | 0.000263 *** |
| factor(Male.age)19:factor(Female.age)11 | -0.25499 | 0.19935 | -1.279 | 0.204198 |

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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Gamma family taken to be 0.0980428)

Null deviance: 23.975 on 98 degrees of freedom
Residual deviance: 8.892 on 89 degrees of freedom
AIC: 1169.9

Number of Fisher Scoring iterations: 4

Confidence intervals:

| | Estimate | 2.5 % | 97.5 % |
|---|---|---|---|
| (Intercept) | 5.10897 | 4.9593716 | 5.2664281 |
| factor(Male.age)8 | 0.71030 | 0.4714257 | 0.9527546 |
| factor(Male.age)11 | 1.06012 | 0.7979036 | 1.3301991 |
| factor(Male.age)15 | 1.07827 | 0.8329134 | 1.3283389 |
| factor(Male.age)19 | 0.89590 | 0.6505508 | 1.1459762 |
| factor(Female.age)11 | 0.05353 | -0.1918258 | 0.3035996 |
| factor(Male.age)8:factor(Female.age)11 | -0.57498 | -0.9408627 | -0.2106510 |
| factor(Male.age)11:factor(Female.age)11 | -0.64062 | -1.0248667 | -0.2601819 |
| factor(Male.age)15:factor(Female.age)11 | -0.75775 | -1.1481843 | -0.3659588 |
| factor(Male.age)19:factor(Female.age)11 | -0.75775 | -0.6454264 | 0.1367991 |

Type III anova of the above glm model:

Analysis of Deviance Table (Type III tests)

Response: Copulation.duration.seconds

| | LR Chisq | Df | Pr(>Chisq) |
|---|---|---|---|
| factor(Male.age) | 96.010 | 4 | < 2.2e-16 *** |
| factor(Female.age) | 0.181 | 1 | 0.6708971 |
| factor(Male.age):factor(Female.age) | 20.663 | 4 | 0.0003693 *** |

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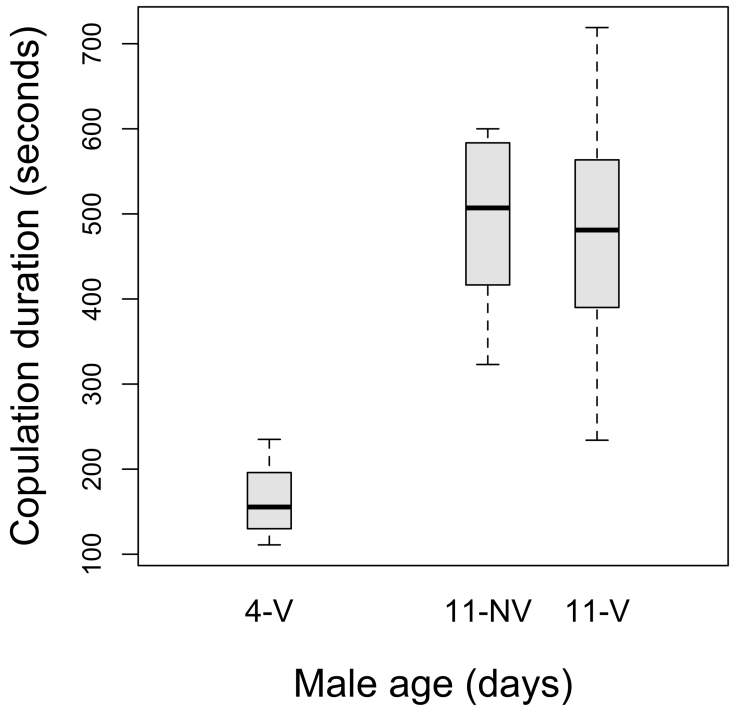
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Figure S2:** Copulation duration of virgin (V) and non-virgin (NV) males of two ages. Eleven-day old males copulate for longer than young 4-day old males regardless of mating status.



## II. Early post-mating fecundity

**Table S2:** Mean-variance relationship of egg count data for all male-female mating combinations

| Female age | Male age | Mean | Variance | Variance to mean ratio |
|---|---|---|---|---|
| 4 | 4 | 78.7 | 1916.0 | 24.4 |
| 4 | 11 | 125.1 | 2645.5 | 21.1 |
| 4 | 19 | 95.0 | 864.0 | 9.1 |
| 11 | 4 | 45.6 | 1344.5 | 29.5 |
| 11 | 11 | 71.0 | 2082.2 | 29.3 |
| 11 | 19 | 53.3 | 1405.9 | 26.4 |

The variance to mean ratios for all the groups fall within a small range, with the exception of 4-day old females mated with 19-day old males, suggesting a linear mean-variance relationship. Models with a binomial distribution with a linear mean-variance relationship ('NB2' distribution in the GAMLSS package) were found to fit the data better than corresponding models with a negative binomial distribution with a quadratic mean-variance relationship ('NB1' distribution) or a Poisson distribution. NB1 vs NB2 vs Poisson comparisons are shown for the three best fitting models in Table S3. Results for all NB2 models are shown in Table S4.

**Table S3:** AICc values for the best three models with NB1, NB2 and Poisson error distributions

| Distr. | Model | Predictors | K | AICc |
|---|---|---|---|---|
| **NB2** | Sans male age, sans interaction | ln(Copulation duration)+ Female age | 4 | 576.9 |
| **NB1** | Sans male age, sans interaction | ln(Copulation duration)+ Female age | 4 | 592.4 |
| **Poisson** | Sans male age, sans interaction | ln(Copulation duration)+ Female age | 4 | 1741.5 |
| **NB2** | One two-factor interactions | ln(Copulation duration)* Male age + Female age | 8 | 577.1 |
| **NB1** | One two-factor interactions | ln(Copulation duration)* Male age + Female age | 8 | 600.8 |
| **Poisson** | One two-factor interactions | ln(Copulation duration)* Male age + Female age | 8 | 1849.8 |
| **NB2** | Sans male age with interaction | ln(Copulation duration)* Female age | 5 | 579.1 |
| **NB1** | Sans male age with interaction | ln(Copulation duration)* Female age | 5 | 594.8 |
| **Poisson** | Sans male age with interaction | ln(Copulation duration)* Female age | 5 | 1848.2 |

The small difference between AICc values of the two best models (Table S4) and their low Akaike weights suggest uncertainty in exclusion of the effect of male age. However, the top four best fitting models consistently include a significant independent effect of copulation duration on early post-mating fecundity.

It should be noted that the AICc correction is designed for models with Gaussian error distribution, and the correction does not generalize in a straightforward way to models with non-Gaussian distributions. Correction for models with negative binomial distribution is not available. However, this correction is argued to be better than no correction for small sample sizes (Simonoff 2003).

**Table S4:** Akaike Information Criterion indices (AIC), Akaike Information Criterion indices corrected for small sample size (AICc), the number of parameters (K), Log Likelihood statistics (LL) and the Akaike weights (w) of different models are listed. The models are arranged by their AICc values.

| No. | Model | Predictors | K | LL | AIC | AICc | w |
|---|---|---|---|---|---|---|---|
| **1** | Sans male age, sans interaction | ln(Copulation duration)+ Female age | 4 | –284.1 | 576.1 | 576.9 | 32.8% |
| **2** | One two-factor interactions | ln(Copulation duration)* Male age + Female age | 8 | –279.0 | 574.0 | 577.1 | 29.8% |
| **3** | Sans male age with interaction | ln(Copulation duration)* Female age | 5 | –283.9 | 577.8 | 579.1 | 11.1% |
| **4** | Two two-factor interactions | ln(Copulation duration)* Male age + ln(Copulation duration)* Female age | 9 | –278.7 | 575.5 | 579.5 | 9.2% |
| **5** | Main effects only | ln(Copulation duration) + Male age + Female age | 6 | –283.1 | 578.1 | 579.9 | 7.4% |
| **6** | Sans copulation duration | Male age+ Female age | 5 | –284.5 | 579.0 | 580.2 | 6.4% |
| **7** | One two-factor interactions | ln(Copulation duration)* Female age + Male age | 7 | –282.8 | 579.6 | 582.0 | 2.6% |
| **8** | Three two-factor interactions | ln(Copulation duration)* Male age + ln(Copulation duration)* Female age + Male age*Female age | 11 | –278.3 | 578.5 | 584.7 | 0.7% |
| **9** | Female age only | Female age | 3 | –290.2 | 586.4 | 586.8 | 0.2% |
| **10** | 3-factor interaction | ln(Copulation duration)* Male age* Female age | 13 | –277.1 | 580.3 | 589.1 | 0.1% |

A summary of the two best models is shown below.
(Note: In gamlss models, “log” refers to the natural log.)

**Best model:**

**********

Family: c("NBII", "Negative Binomial type II")

Call: gamlss(formula = Eggs ~ log(Copulation.duration.seconds) + factor(Female.age), family = NBII, data = eggdata)

Fitting method: RS()

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Mu link function: log
Mu Coefficients:

| | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| (Intercept) | 0.9157 | 1.0496 | 0.8725 | 0.3870502 |
| log(Copulation.duration.seconds) | 0.6459 | 0.1794 | 3.5993 | 0.0007213 |
| factor(Female.age)11 | -0.5451 | 0.2113 | -2.5801 | 0.0127990 |

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Sigma link function: log
Sigma Coefficients:

| Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|
| 3.908e+00 | 2.291e-01 | 1.706e+01 | 1.018e-22 |

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No. of observations in the fit: 55
Degrees of Freedom for the fit: 4
Residual Deg. of Freedom: 51
at cycle: 5

Global Deviance: 568.1132
AIC: 576.1132
SBC: 584.1425
**********

Confidence intervals:

| | Estimate | 2.5 % | 97.5 % |
|---|---|---|---|
| (Intercept) | 0.9157 | -1.1571737 | 2.9886597 |
| log(Copulation.duration.seconds) | 0.6459 | 0.2909656 | 1.0008074 |
| factor(Female.age)11 | -0.54511 | -0.9606749 | -0.1295458 |

**Second best model:**

**********
Family: c("NBII", "Negative Binomial type II")
Call: gamlss(formula = Eggs ~ log(Copulation.duration.seconds) * factor(Male.age) + factor(Female.age), family = NBII)

Fitting method: RS()
---
Mu link function: log
Mu Coefficients:

| | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| (Intercept) | -7.589 | 3.6375 | -2.086 | 0.042390 |
| log(Copulation.duration.seconds) | 2.298 | 0.6944 | 3.309 | 0.001804* |
| factor(Male.age)11 | 11.868 | 4.1556 | 2.856 | 0.006370* |
| factor(Male.age)19 | 11.660 | 5.9299 | 1.966 | 0.055176 |
| factor(Female.age)11 | -0.713 | 0.2289 | -3.114 | 0.003137* |
| log(Copulation.duration.seconds):factor(Male.age)11 | -2.182 | 0.7758 | -2.813 | 0.007146* |
| log(Copulation.duration.seconds):factor(Male.age)19 | -2.202 | 1.0505 | -2.097 | 0.041436* |

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Sigma link function: log
Sigma Coefficients:

| Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|
| 3.752e+00 | 2.283e-01 | 1.643e+01 | 4.128e-21 |

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No. of observations in the fit: 55
Degrees of Freedom for the fit: 8
Residual Deg. of Freedom: 47
at cycle: 4

Global Deviance: 557.9931
AIC: 573.9931
SBC: 590.0518

**********

Confidence intervals:

| | Estimate | 2.5 % | 97.5 % |
|---|---|---|---|
| (Intercept) | -7.589 | -14.70528089 | -0.4735016 |
| log(Copulation.duration.seconds) | 2.298 | 0.93725102 | 3.6582401 |
| factor(Male.age)11 | 11.868 | 3.78774969 | 19.9482951 |
| factor(Male.age)19 | 11.660 | -0.04658854 | 23.3673123 |
| factor(Female.age)11 | -0.713 | -1.16331291 | -0.2626987 |
| log(Copulation.duration.seconds):factor(Male.age)11 | -2.182 | -3.68557690 | -0.6786359 |
| log(Copulation.duration.seconds):factor(Male.age)19 | -2.202 | -4.26885266 | -0.1360741 |

**References:**

Simonoff JS (2003) Analyzing Categorical Data. New York: Springer-Verlag.