**Supplementary Material**

Evolution of reproductive isolation in a long-term evolution experiment with *Drosophila melanogaster*: 30 years of divergent life history selection

**Figure S1.** The proportion of homotypic compared to heterotypic matings recorded during the female (a), male (b), and group (c) mate choice assay for each replicate population. An asterisk represents a significant deviation from a random mating ratio as indicated by a repeated G-test for goodness of fit (p<0.05).

**Table S1.** The results of the repeated G-test for goodness of fit for the female mate choice assay.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Population** | **Heterogeneity G** | **df** | **P-value** | **Pooled G** | **df** | **P-value** | **Total G** | **df** | **P-value** |
| ***A1*** | *3.7* | *3* | *0.30* | *1.55* | *1* | *0.21* | *5.25* | *4* | *0.26* |
| ***A3*** | *3.87* | *3* | *0.28* | *5.40* | *1* | *0.02* | *9.27* | *4* | *0.05* |
| ***A5*** | *4.54* | *3* | *0.21* | *5.47* | *1* | *0.02* | *10.02* | *4* | *0.04* |
| ***Overall A*** | *0.82* | *2* | *0.66* | *11.59* | *1* | *<0.001* | *12.42* | *3* | *0.01* |
|  |  |  |  |  |  |  |  |  |  |
| ***C1*** | *5.68* | *3* | *0.13* | *11.13* | *1* | *<0.001* | *16.81* | *4* | *< 0.01* |
| ***C3*** | *5.4* | *3* | *0.15* | *9.16* | *1* | *<0.01* | *14.56* | *4* | *0.01* |
| ***C5*** | *3.87* | *3* | *0.28* | *4.42* | *1* | *0.04* | *8.29* | *4* | *0.08* |
| ***Overall C*** | *0.85* | *2* | *0.65* | *23.86* | *1* | *<0.00001* | *24.71* | *3* | *< 0.0001* |

1 A repeated G-test was conducted on the level of each replicate population, then on the level of the overall selection regime. A total p-value of less than 0.05 indicates significance.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *Assay* | *Response* | *Factor Type* | | *MS* | *df* | *F* | *P* |
| Female Choice | Latency | Fixed | **Male** | ***11.36*** | ***1, 1107.2*** | ***25.71*** | ***<0.0001*** |
|  |  |  | Female | *0.006* | *1, 1098.7* | *0.01* | *0.92* |
|  |  |  | Male x Female | *0.16* | *1, 1103.9* | *0.36* | *0.55* |
|  |  |  |  | *% var* | *Df* | *X2* | *P* |
|  |  | Random | **Trial/Replicate** | ***4.85*** | ***2*** | ***28.77*** | ***<0.0001*** |
|  |  |  |  | *MS* | *df* | *F* | *P* |
| Female Choice | Duration | Fixed | Male | *0.05* | *1, 1105.1* | *0.003* | *0.96* |
|  |  |  | **Female** | ***788.89*** | ***1, 1098.3*** | ***52.19*** | ***<0.0001*** |
|  |  |  | **Male x Female** | ***268.75*** | ***1, 1101.5*** | ***17.78*** | ***<0.0001*** |
|  |  |  |  | *% var* | *Df* | *X2* | *P* |
|  |  | Random | **Trial/Replicate** | ***4.48*** | ***2*** | ***24.91*** | ***<0.0001*** |
|  |  |  |  | *MS* | *df* | *F* | *P* |
| Male Choice | Latency | Fixed | Male | *0.16* | *1, 1086.9* | *0.36* | *0.55* |
|  |  |  | Female | *0.23* | *1, 1087.4* | *0.52* | *0.47* |
|  |  |  | Male x Female | *0.01* | *1, 1087.9* | *0.02* | *0.90* |
|  |  |  |  | *% var* | *Df* | *X2* | *P* |
|  |  | Random | **Trial/Replicate** | ***2.11*** | ***2*** | ***8.37*** | ***0.01*** |
|  |  |  |  | *MS* | *df* | *F* | *P* |
| Male Choice | Duration | Fixed | Male | *79.74* | *1, 1084.2* | *3.12* | *0.08* |
|  |  |  | **Female** | ***982.57*** | ***1, 1084.4*** | ***38.42*** | ***<0.0001*** |
|  |  |  | Male x Female | *46.32* | *1, 1085.3* | *1.81* | *0.18* |
|  |  |  |  | *% var* | *Df* | *X2* | *P* |
|  |  | Random | **Trial/Replicate** | ***3.86*** | ***2*** | ***21.25*** | ***<0.0001*** |

**Table S2.** Full LMM results for the latency and duration of matings in the female and male mate choice assays.

1Bold font indicates statistical significance (P < 0.05). LMMs followed the general format: log(Latency +1) ~ Male Population\*Female Population + (1|Trial/Replicate Population) for latency models, and Duration ~ Male Population\*Female Population + (1|Trial/Replicate Population).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Population** | **Heterogeneity G** | **df** | **P-value** | **Pooled G** | **df** | **P-value** | **Total G** | **df** | **P-value** |
| ***A1*** | *11.62* | *3* | *0.01* | *13.61* | *1* | *<0.001* | *25.22* | *4* | *<0.0001* |
| ***A3*** | *1.42* | *3* | *0.70* | *11.37* | *1* | *<0.001* | *12.79* | *4* | *0.01* |
| ***A5*** | *3.11* | *3* | *0.38* | *17.85* | *1* | *<0.0001* | *21.11* | *4* | *<0.001* |
| ***Overall A*** | *0.41* | *2* | *0.81* | *46.85* | *1* | *<0.00001* | *47.27* | *3* | *<0.00001* |
|  |  |  |  |  |  |  |  |  |  |
| ***C1*** | *0.23* | *3* | *0.97* | *0* | *1* | *1* | *0.23* | *4* | *0.99* |
| ***C3*** | *2.18* | *3* | *0.54* | *0.29* | *1* | *0.59* | *2.47* | *4* | *0.65* |
| ***C5*** | *0.55* | *3* | *0.91* | *0.05* | *1* | *0.83* | *0.6* | *4* | *0.96* |
| ***Overall C*** | *0.15* | *2* | *0.93* | *0.19* | *1* | *0.67* | *0.34* | *3* | *0.95* |

**Table S3.** The results of the repeated G-test for goodness of fit for the male mate choice assay.

1A repeated G-test was conducted on the level of each replicate population, then on the level of the overall selection regime. A total p-value of less than 0.05 indicates significance.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Population** | **Heterogeneity G** | | **df** | **P-value** | **Pooled G** | **df** | **P-value** | **Total G** | **df** | **P-value** |
| ***A1*** | | *0.48* | *1* | *0.49* | *0.01* | *1* | *0.92* | *0.49* | *2* | *0.78* |
| ***A3*** | | *0.58* | *1* | *0.45* | *7.64* | *1* | *0.01* | *8.23* | *2* | *0.02* |
| ***A5*** | | *0.46* | *1* | *0.50* | *18.75* | *1* | *<0.0001* | *19.21* | *2* | *<0.0001* |
| ***Overall A*** | | *8.87* | *2* | *0.01* | *17.53* | *1* | *<0.0001* | *26.4* | *3* | *<0.00001* |
|  | |  |  |  |  |  |  |  |  |  |
| ***C1*** | | *2.75* | *1* | *0.10* | *1.8* | *1* | *0.18* | *4.55* | *2* | *0.10* |
| ***C3*** | | *0.3* | *1* | *0.58* | *5.13* | *1* | *0.02* | *5.43* | *2* | *0.07* |
| ***C5*** | | *0.002* | *1* | *0.96* | *16.21* | *1* | *<0.0001* | *16.21* | *2* | *<0.001* |
| ***Overall C*** | | *4.31* | *2* | *0.12* | *18.83* | *1* | *<0.0001* | *23.14* | *3* | *<0.0001* |

**Table S4.** The results of the repeated G-test for goodness of fit for the group mate choice assay.

1A repeated G-test was conducted on the level of each replicate population, then on the level of the overall selection regime. A total p-value of less than 0.05 indicates significance.

Table S5. Full GLMM results for the hatchability and larval to adult viability of flies from parental and hybrid crosses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Response* | *Factor Type* | | *χ2* | *df* | *P* |
| Hatchability | Fixed | **Cross Identity** | ***19.10*** | ***5, 154*** | ***<0.01*** |
|  |  |  | *χ2* | *df* | *P* |
|  | Random | Replicate Population | *0.02* | *1* | *0.89* |
|  |  |  | *χ2* | *df* | *P* |
| Larvae to Adult Viability | Fixed | **Cross Identity** | ***134.79*** | ***5, 154*** | ***<0.0001*** |
|  |  |  | *χ2* | *df* | *P* |
|  | Random | Replicate Population | *1.05* | *1* | *0.31* |

1Bold font indicates statistical significance (P < 0.05). GLMMs followed the general format: Response ~ Cross Identity + (1|Replicate Population), with family = binomial for hatchability and family = betabinomial for viability.

Table S6. Full LMM results for the development time and body size of flies from parental and hybrid crosses.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Response* | *Factor Type* | | *MS* | *df* | *F* | *P* |
| Development Time | Fixed | **Cross Identity** | ***4974.90*** | ***5, 154*** | ***987.18*** | ***<0.0001*** |
|  |  |  |  | *Df* | *LRT* | *P* |
|  | Random | Replicate Population |  | *1* | *0.43* | *0.51* |
|  |  |  | *MS* | *df* | *F* | *P* |
| Body Size | Fixed | **Cross Identity** | ***0.01*** | ***5, 64*** | ***41.36*** | ***<0.0001*** |
|  |  |  |  | *Df* | *LRT* | *P* |
|  | Random | Replicate Population |  | *1* | *1.41* | *0.23* |

1Bold font indicates statistical significance (P < 0.05). The LMMs followed the formula:

Response ~ Cross Identity + (1|Replicate Population).

Table S7. Full GLMM results for the fertility of female and male flies from parental and hybrid crosses.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Sex* | *Response* | *Factor Type* | | *χ2* | *df* | *P* |
| Female | Proportion Red Eyed | Fixed | **Cross Identity** | ***67.49*** | ***5, 515*** | ***<0.0001*** |
|  |  |  |  | *χ2* | *df* | *P* |
|  |  | Random | Replicate Population | *0* | *1* | *1* |
|  |  |  |  | *χ2* | *df* | *P* |
| Male | Proportion Red Eyed | Fixed | **Cross Identity** | ***19.47*** | ***5, 502*** | ***<0.01*** |
|  |  |  |  | *χ2* | *df* | *P* |
|  |  | Random | Replicate Population | *0* | *1* | *1* |

1Fertility was determined as the proportion of red eyed offspring produced in competition with brown eyed competitors. Bold font indicates statistical significance (P < 0.05). GLMMs followed the general format: Proportion Red Eyed ~ Cross Identity + (1|Replicate Population), family = betabinomial.