

Supplementary Material

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

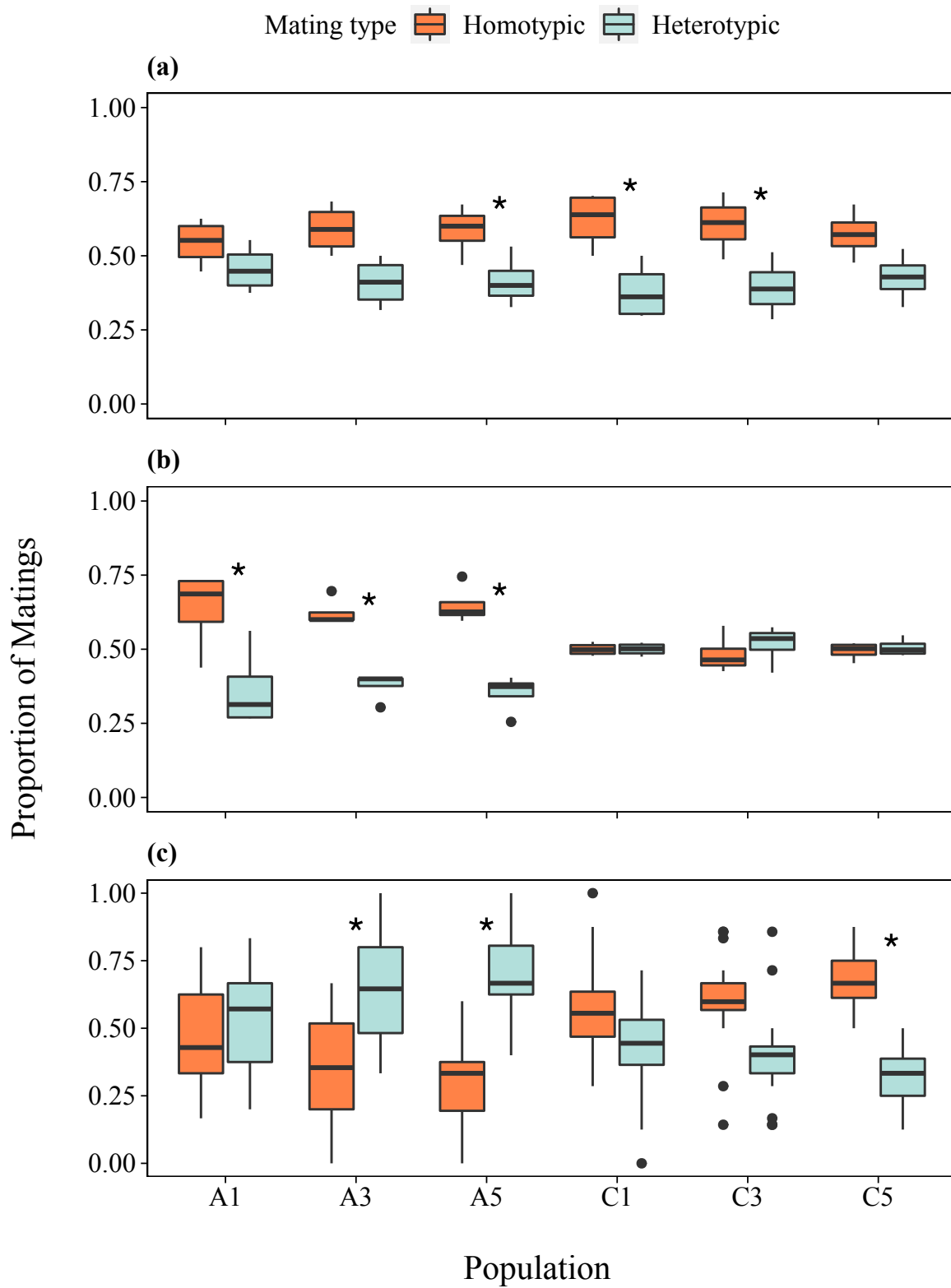


Figure S1. The proportion of homotypic compared to heterotypic matings recorded during the female (a), male (b), and group (c) mate choice assay for each replicate population. An asterisk

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
<i>A1</i>	3.7	3	0.30	1.55	1	0.21	5.25	4	0.26
<i>A3</i>	3.87	3	0.28	5.40	1	0.02	9.27	4	0.05
<i>A5</i>	4.54	3	0.21	5.47	1	0.02	10.02	4	0.04
<i>Overall A</i>	0.82	2	0.66	11.59	1	<0.001	12.42	3	0.01
<i>C1</i>	5.68	3	0.13	11.13	1	<0.001	16.81	4	< 0.01
<i>C3</i>	5.4	3	0.15	9.16	1	<0.01	14.56	4	0.01
<i>C5</i>	3.87	3	0.28	4.42	1	0.04	8.29	4	0.08
<i>Overall C</i>	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.

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

<i>Assay</i>	<i>Response</i>	<i>Factor Type</i>		<i>MS</i>	<i>df</i>	<i>F</i>	<i>P</i>
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	X ²	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	X ²	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	X ²	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	X ²	P
		Random	Trial/Replicate	3.86	2	21.25	<0.0001

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).

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

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

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

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

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.

<i>Response</i>	<i>Factor Type</i>		χ^2	<i>df</i>	<i>P</i>
Hatchability	Fixed	Cross Identity	19.10	5, 154	<0.01
	Random	Replicate Population	0.02	1	0.89
Larvae to Adult Viability	Fixed	Cross Identity	134.79	5, 154	<0.0001
	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.

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

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.

<i>Sex</i>	<i>Response</i>	<i>Factor Type</i>		χ^2	<i>df</i>	<i>P</i>
Female	Proportion Red Eyed	Fixed	Cross Identity	67.49	5, 515	<0.0001
		Random	Replicate Population	0	1	1
Male	Proportion Red Eyed	Fixed	Cross Identity	19.47	5, 502	<0.01
		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.