

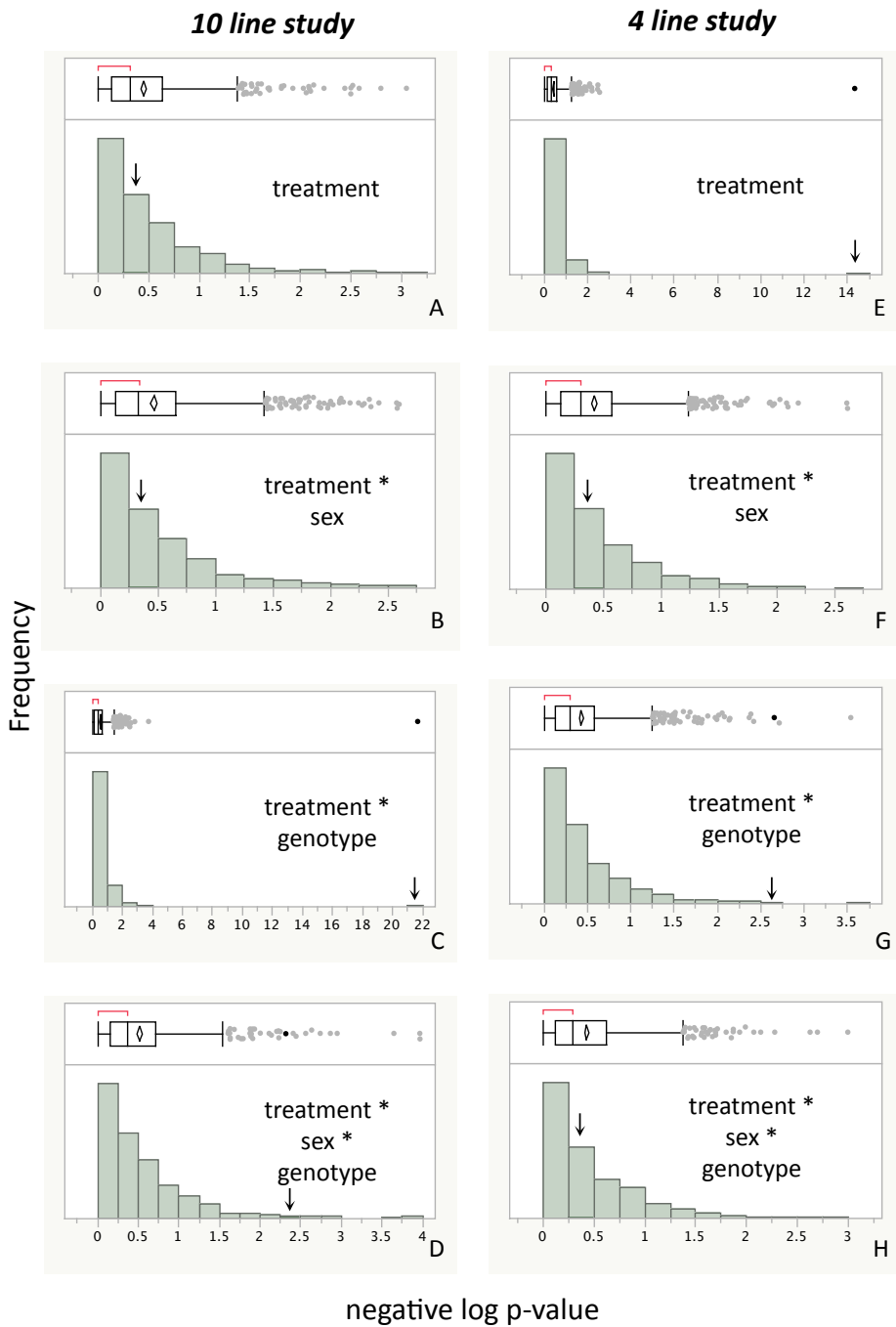
Supplemental Information: Genetic and Sex-specific Transgenerational Effects of a High Fat Diet  
in *Drosophila melanogaster*

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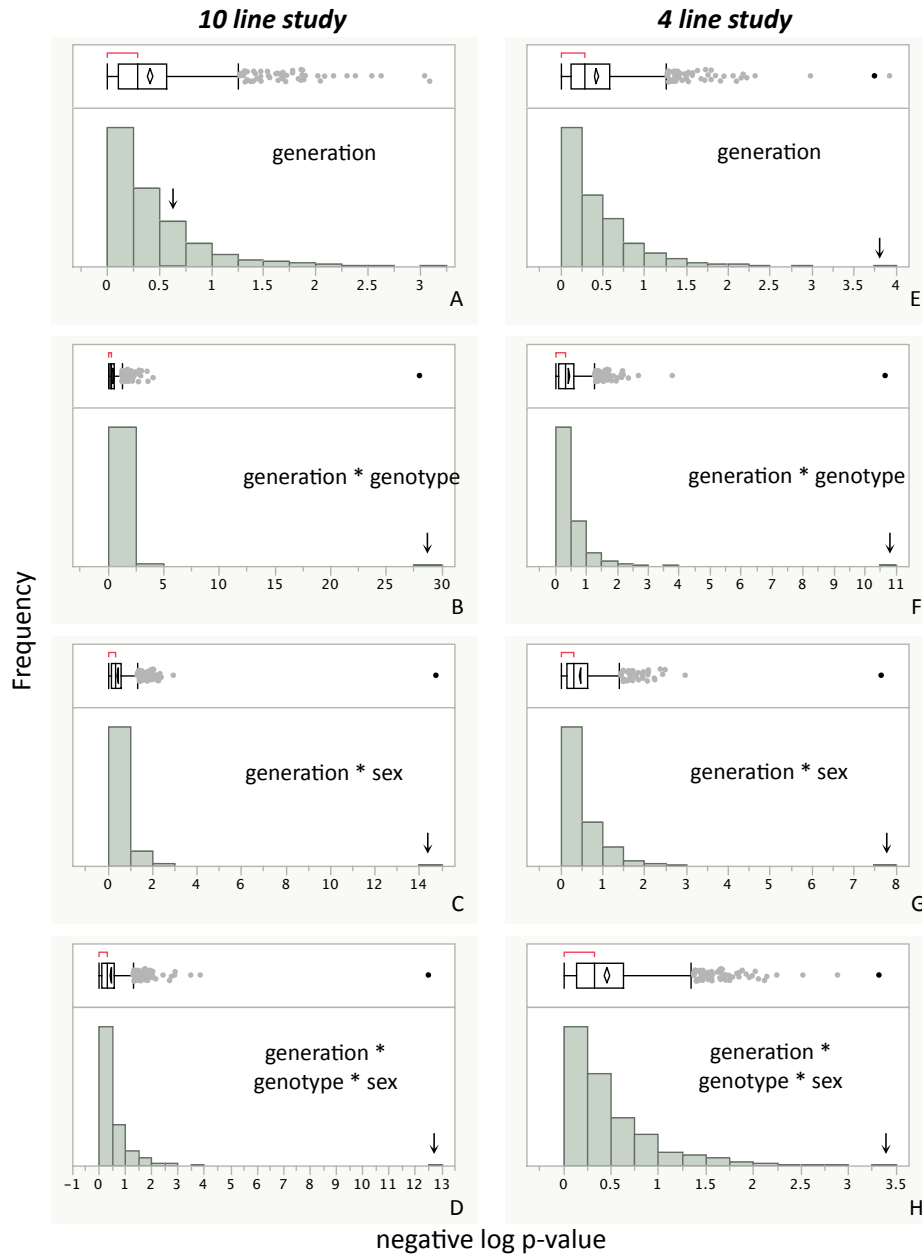
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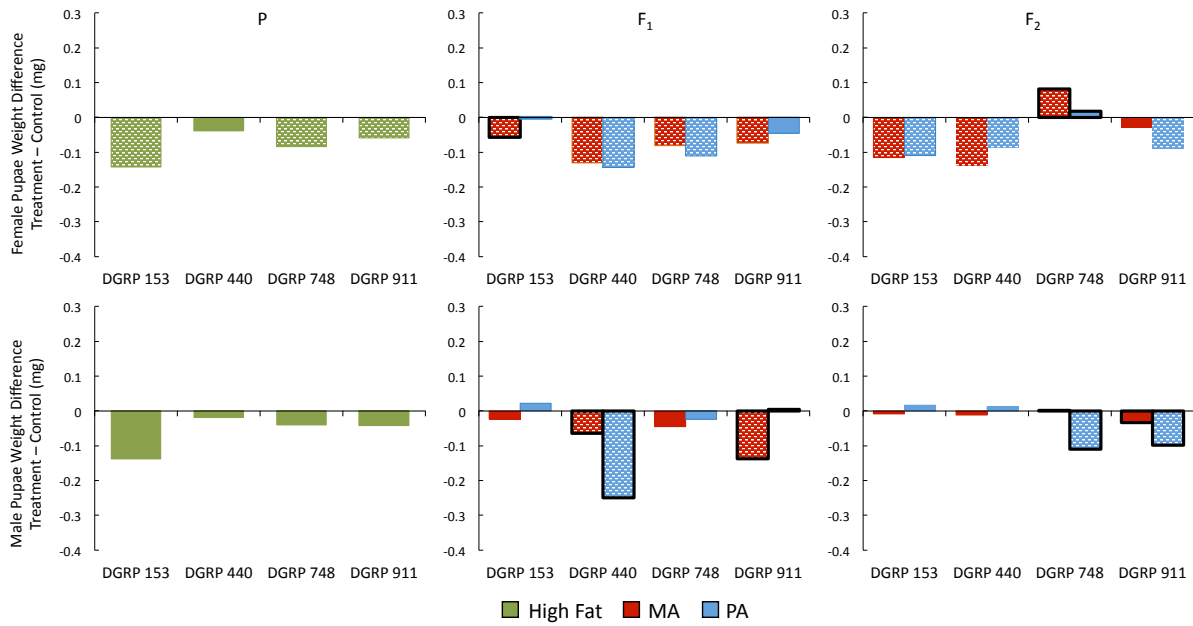
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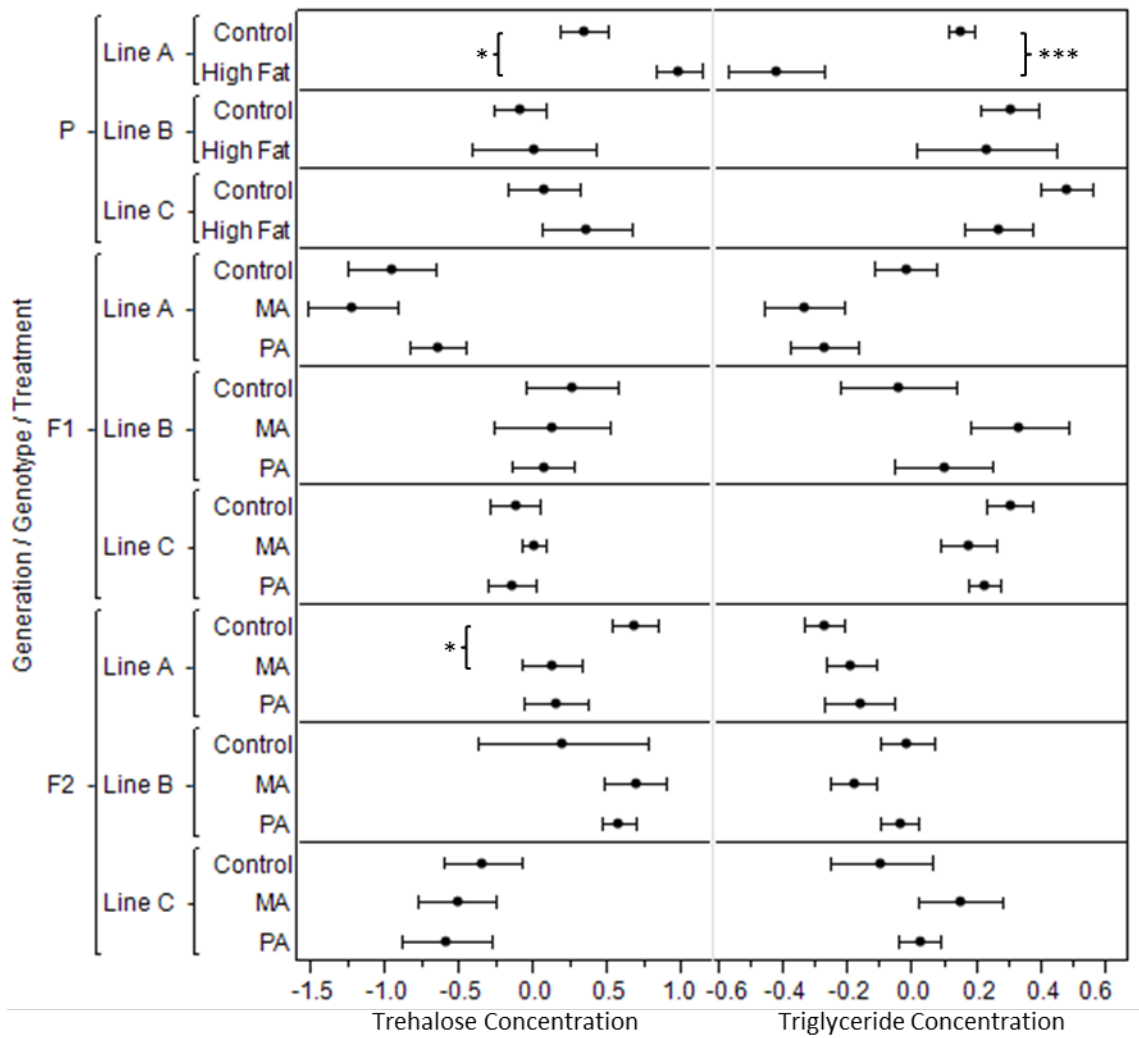
**Figure A. Parental generation distribution of p-values for ANOVA effects with permutation support significance of treatment effects.** Arrows indicate actual p-values. Distributions based on 1024 permutations within genetic line and sex to randomize treatment effects. Genetic line and sex distributions not shown since they remain highly significant under the permutation model. A-D are derived from the **10 line study** while E-H are derived from the fall and spring replicates of the **4 line study**. A and E are treatment main effects, B and F are the treatment interaction with sex, C and G are the treatment interaction with genetic line, and D and H are the three way interaction between treatment, genetic line, and sex.



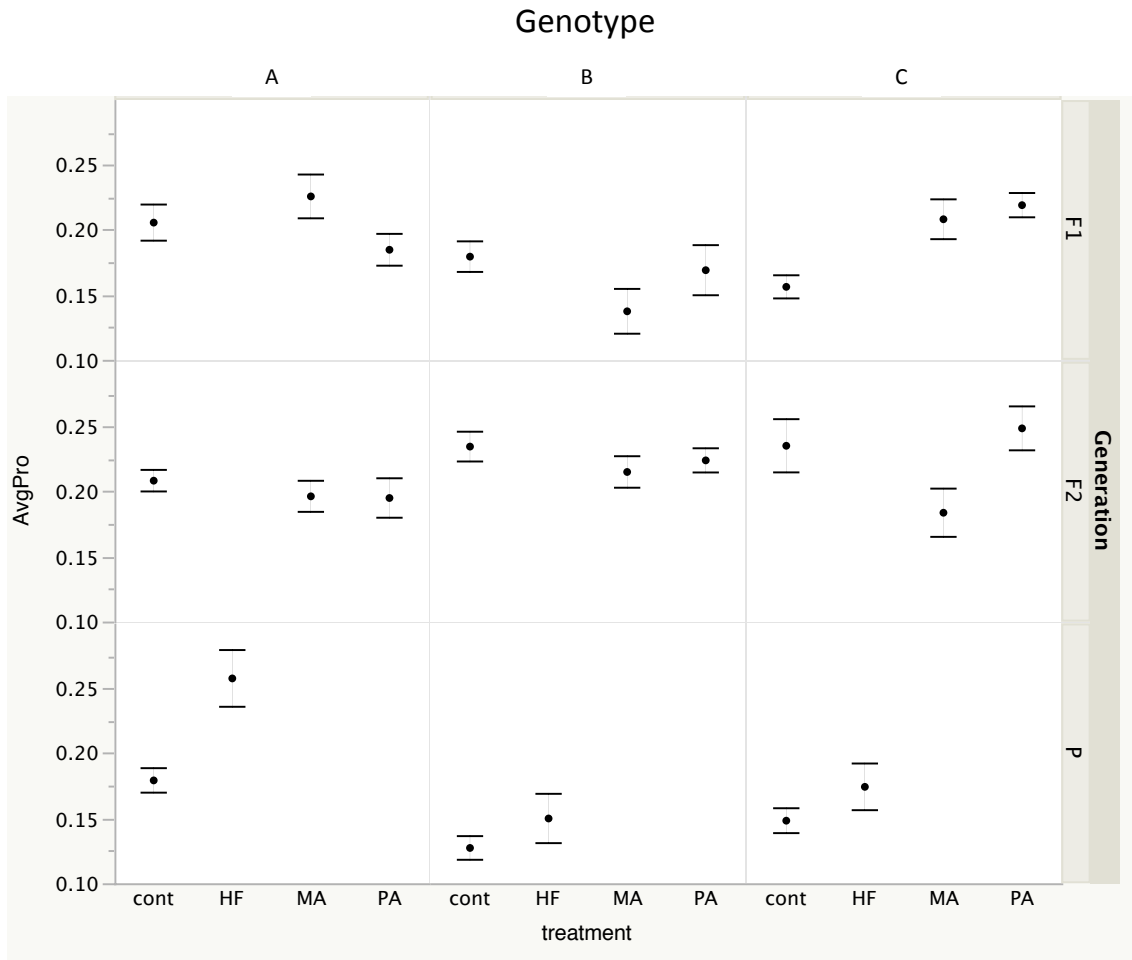
**Figure B. F1 and F2 generation distribution of p-values for ANOVA effects with permutation support significance of effects other than treatment.** Arrows indicate actual p-values. Distributions based on 1024 permutations within genetic line and sex to randomize treatment effects. Genetic line and sex distributions not shown since they remain highly significant under the permutation model. A-D are derived from the **10 line study** while E-H are derived from the fall and spring replicates of the **4 line study**. A and E are generation main effects, B and F are the generation interaction with genetic line, C and G are the generation interaction with sex, and D and H are the three way interaction between generation, genetic line, and sex.



**Figure C. Weight 4 Line Study Data.** Polka dots indicate treatment is significantly different from Control. Black border indicates treatments are significantly different from each other. Multiple testing was corrected using false discovery rate of 0.05.



**Figure D. Trehalose and triglyceride concentrations (mean  $\pm$  SE) of Control, High Fat, Maternal Ancestor (MA), and Paternal Ancestor (PA) for three generations.** Raw data was log transformed and the residuals for batch effects are graphed. Triglycerides were corrected for protein concentration of the sample. Significance values indicated ( $p < 0.05$  \*,  $p < 0.01$  \*\*,  $p < 0.001$  \*\*\*).



**Figure E. Protein concentrations (mean ± SE) of Control, High Fat, Maternal Ancestor (MA), and Paternal Ancestor (PA) for three generations.**

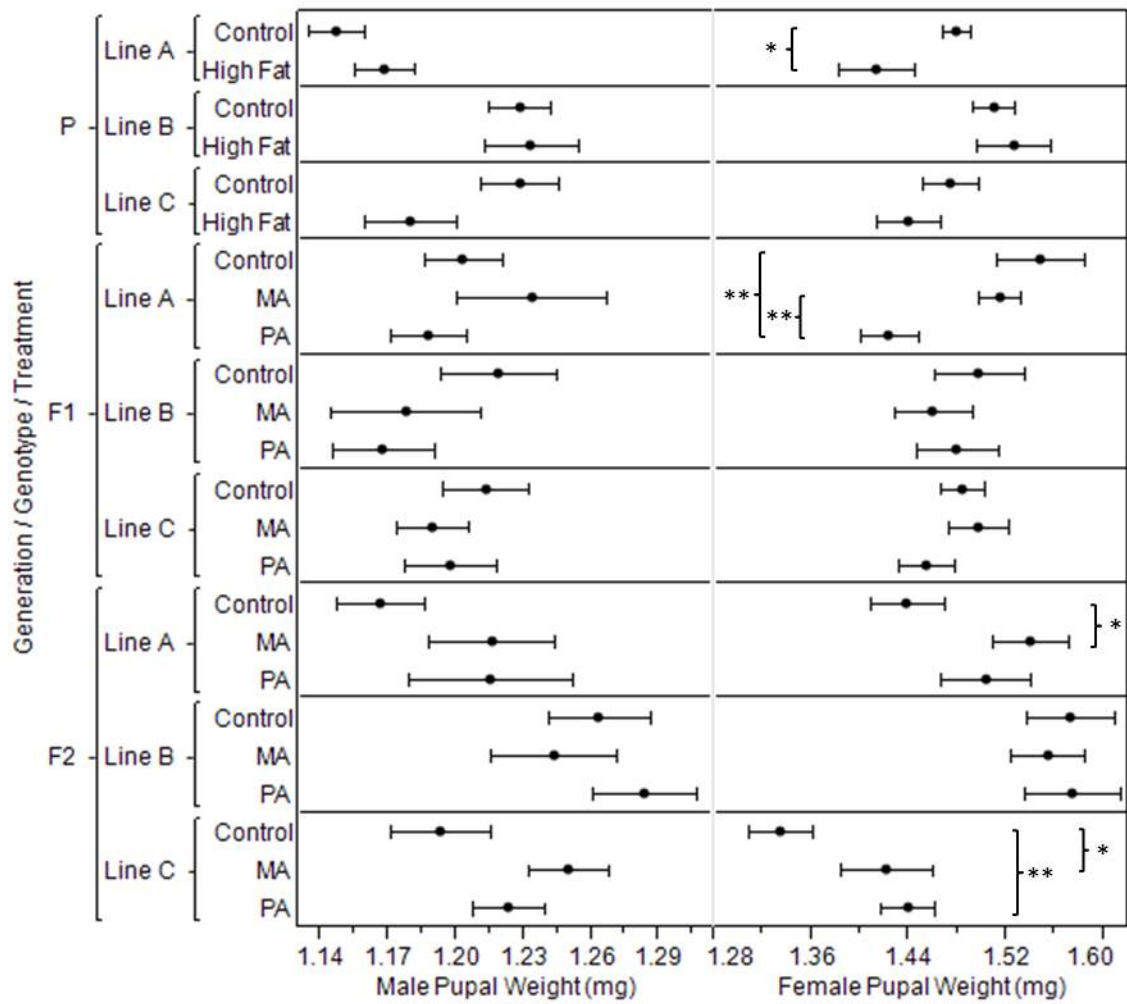


Figure F. Wet male and female pupal weight (mean  $\pm$  SE) of Control, High Fat, Maternal Ancestor (MA), and Paternal Ancestor (PA) for three generations. Significance values indicated ( $p < 0.05$  \*,  $p < 0.01$  \*\*).

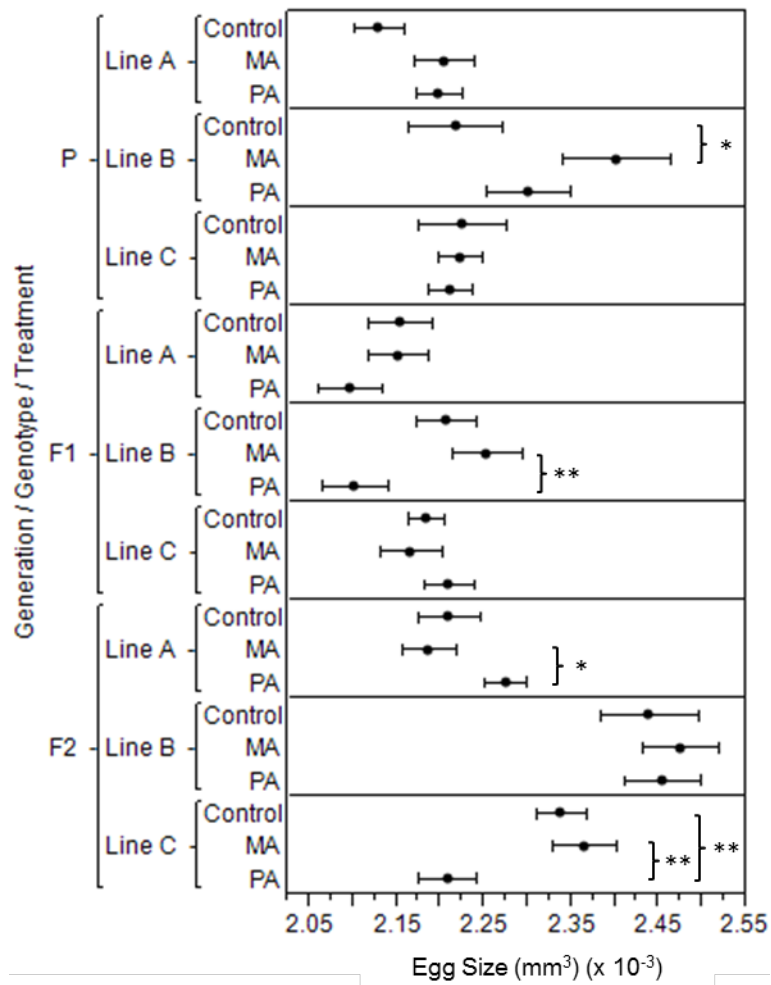


Figure G. Egg Size (mean  $\pm$  SE) of Control, High Fat, Maternal Ancestor (MA), and Paternal Ancestor (PA) for three generations. Significance values indicated ( $p < 0.05$  \*,  $p < 0.01$  \*\*).



**Table A. Parental generation variance partition for pupal weight.**

<u>Effect</u> <sup>1</sup>	<u>10 line study</u> variance explained <sup>2</sup>	<u>NLP</u> <sup>3</sup>	<u>4 line study</u> variance explained <sup>4</sup>	<u>NLP</u> <sup>3</sup>
Genotype	0.2179	114.4 5	0.2595	134.9 0
Treatment	0.0001	<i>ns</i>	0.0166	14.92
Sex	0.5326	203.9 6	0.5278	207.4 9
Treatment*Genotype	0.0308	20.72	0.0036	2.65
Sex*Genotype	0.0149	9.05	0.0094	7.33
Treatment*Sex	0.0000	<i>ns</i>	0.0004	<i>ns</i>
Treatment*Sex*Genotype	0.0047	1.75	0.0003	<i>ns</i>
time replicate	<i>na</i>	<i>na</i>	0.0101	9.50

<sup>1</sup> ANOVA model effects, <sup>2</sup> variance in **10 line study** <sup>3</sup> negative log p-value; *ns*, non-significant, <sup>4</sup> variance in the **4 line study**

**Table B. Egg size and pupal weight correlations.** Correlation reported as  $R^2$ -value with +/- sign representing the direction of significant correlations. Significant  $p$ -values reported parenthetically.

	<u>Male Pupae</u>				<u>Female Pupae</u>			<u>Overall</u>
	<u>Line A</u>	<u>Line B</u>	<u>Line C</u>	<u>Overall</u>	<u>Line A</u>	<u>Line B</u>	<u>Line C</u>	
Pupae → Egg Size <sup>a</sup>	0.078	+0.697 <b>(0.019)</b>	0.174	+0.498 <b>(0.0004)</b>	0.059	+0.754 <b>(0.011)</b>	-0.638 <b>(0.031)</b>	0.062
Egg size → Pupae <sup>b</sup>	0.001	-0.748 <b>(0.026)</b>	0.322	0.156	0.146	-0.668 <b>(0.047)</b>	0.518	0.276

<sup>a</sup> Tests if pupal weight correlates with the egg size of offspring

<sup>b</sup> Tests if egg size correlates with the future pupal weight