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## Effect of Genotype, Life-Stage and Temperature on Oxidative Stress

### Discussion

#### *Overview of Findings*

This study explored the effects of life stage, genotype, and temperature on catalase activity in *Drosophila melanogaster* to understand oxidative stress responses. Our results supported the hypothesis that catalase activity varies with life stage, with significantly higher activity observed in pupae compared to adults ( $p = 0.020$ , Figure 1, Table 1). However, neither genotype nor heat shock treatment significantly influenced catalase activity, leading us to reject the hypothesis for these factors under the conditions tested (Figure 1, Table 2).

#### *Biological Interpretation of Results*

The elevated catalase activity in pupae (Figure 1) aligns with their increased metabolic demands during metamorphosis, a stage requiring robust oxidative defenses due to extensive tissue remodeling and energy expenditure (Weber et al., 2012). Higher antioxidant activity in pupae highlights the importance of catalase in maintaining redox homeostasis, consistent with findings that ROS detoxification mechanisms are essential during physiologically intense developmental stages (Habib et al., 2021).

The absence of significant effects of heat shock or genotype on catalase activity (Figure 1, Table 2) contrasts with previous research showing that thermal stress often induces upregulation of stress-response enzymes (Zhang et al., 2016). A potential explanation is that the one-hour heat shock at 36°C was insufficient to generate substantial ROS accumulation or elicit

a significant enzymatic response. Additionally, the lack of genotype effects suggests limited genetic variation between the tested strains in alleles related to oxidative stress resistance, aligning with Weber et al. (2012), who found that not all genetic backgrounds show measurable differences in stress responses.

#### *Contextualization with Literature and Experimental Limitations*

The observed variation in catalase activity with life stage (Figure 1, Table 1) aligns with prior research, emphasizing developmental-stage-specific metabolic demands as key drivers of antioxidant activity (Habib et al., 2021; Hadwan, 2018). However, the lack of heat shock effects differs from studies such as Zhang et al. (2016), which reported temperature-dependent activation of stress-response pathways. These inconsistencies may arise from variations in the experimental protocols, stress exposure durations, or species-specific responses to thermal stress.

Experimental limitations include the relatively mild heat shock treatment, which may not have been sufficient to induce detectable oxidative stress. Furthermore, the study focused solely on catalase activity, which may not fully represent the complexity of oxidative stress responses, as other antioxidant enzymes and pathways likely contribute. The use of only two genotypes may have also restricted the ability to detect genotype-specific effects on catalase activity.

#### *Future Directions and Broader Implications*

Future research should employ a broader range of temperatures and exposure durations to better capture the effects of thermal stress on oxidative defenses. Including additional genotypes and analyzing other antioxidant enzymes, such as superoxide dismutase or glutathione peroxidase, could provide a more comprehensive understanding of oxidative stress resilience. Longitudinal studies examining changes in antioxidant activity throughout development could also yield valuable insights.

This study underscores the critical role of catalase in oxidative stress management during development (Figure 1) and provides a foundation for further investigations into the genetic and physiological mechanisms of stress tolerance. These findings contribute to a broader understanding of how organisms adapt to environmental challenges, with implications for stress resilience in both natural and applied contexts.

### Literature Cited

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## Supplemental Figures

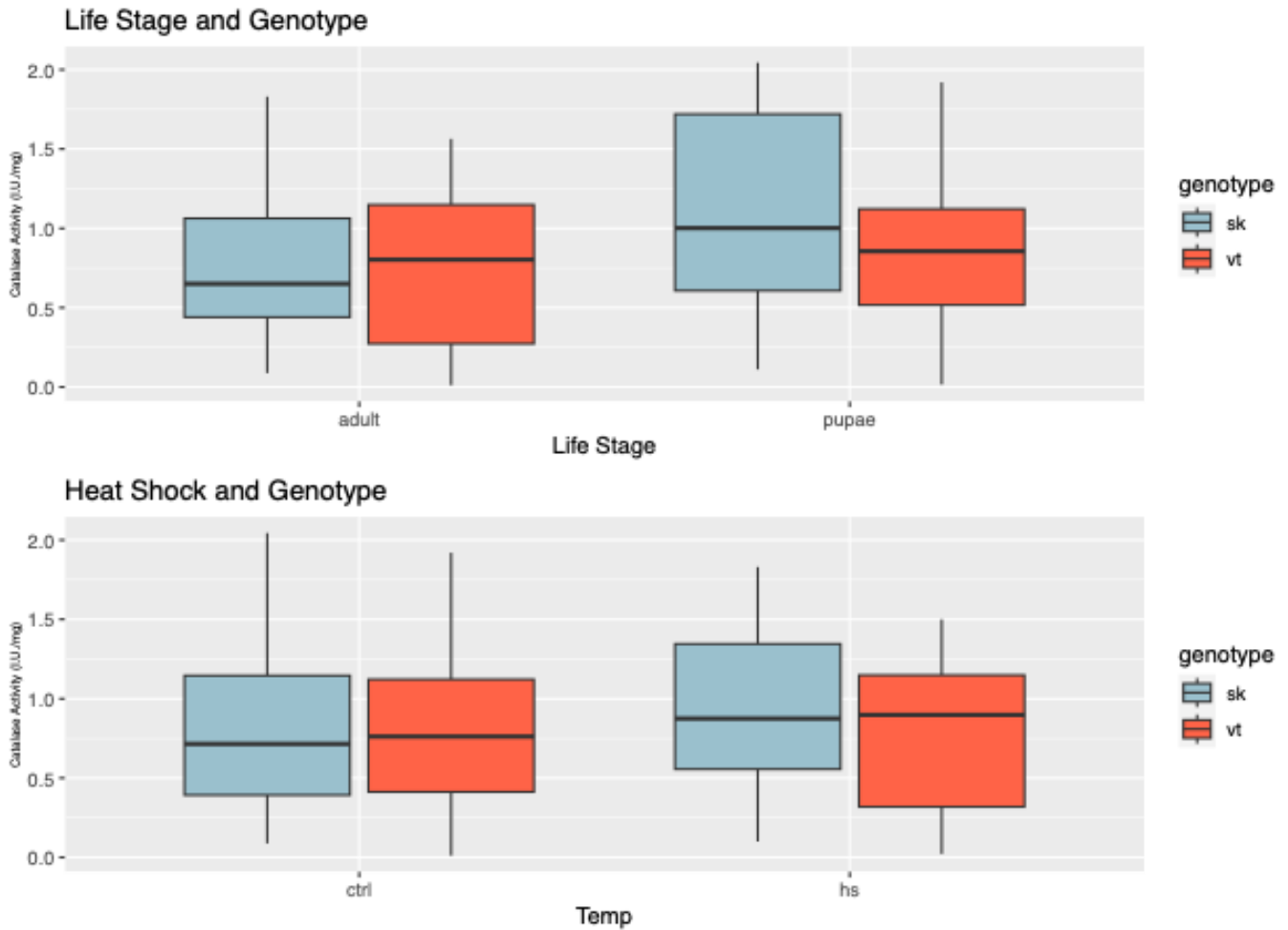


Figure 1: Box plots showing catalase activity (I.U./mg) in different experimental groups based on life stage, genotype, and temperature treatments. In the first plot, a significant effect of life stage on catalase activity was observed ( $p = 0.020$ ), with higher activity in one stage over the other, as indicated by the ANOVA. Genotype and the interaction between life stage and genotype were not significant ( $p > 0.05$ ). In the second plot, neither temperature treatment nor genotype showed significant effects on catalase activity.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Life S.	1	1.38	1.3752	5.54	0.02
Genotype	1	0.23	0.2274	0.916	0.34
Life S. ~ Genotype	1	0.42	0.4228	1.703	0.194
Residuals	140	34.75	0.2482		

*Table 1: Analysis of Variance (ANOVA) summary for the effects of life stage, genotype, and their interaction on the response variable. Degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F-values, and p-values (Pr(>F)) are shown for each factor and interaction term. A statistically significant effect of life stage was observed at the 0.05 significance level, with a p-value of 0.020. No significant effects were found for genotype or the life stage and genotype interaction.*

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
<b>Temp</b>	1	0.08	0.07977	0.307	0.581
<b>Genotype</b>	1	0.23	0.22745	0.874	0.351
<b>Temp ~ Genotype</b>	1	0.05	0.05366	0.206	0.65
<b>Residuals</b>	140	36.42	0.26013		

*Table 2: Analysis of Variance (ANOVA) summary for the effects of temperature (temp), genotype, and their interaction on the response variable. Degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F-values, and p-values (Pr(>F)) are presented for each factor and interaction term. No statistically significant effects were observed at a 0.05 significance level.*