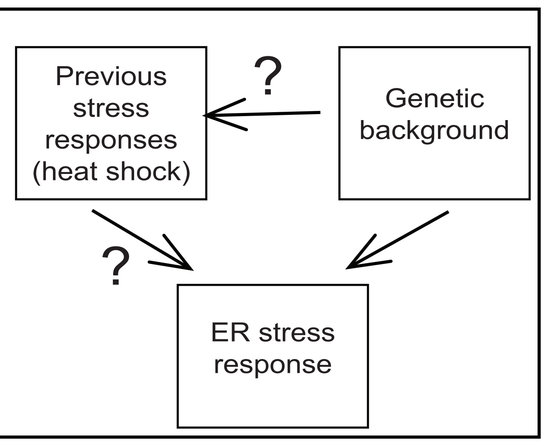
Using natural genetic variation in *Drosophila* to characterize the underlying mechanisms of hormesis

Katie G. Owings*, Clement Y. Chow

Department of Human Genetics, University of Utah, Salt Lake City, UT

1. Introduction

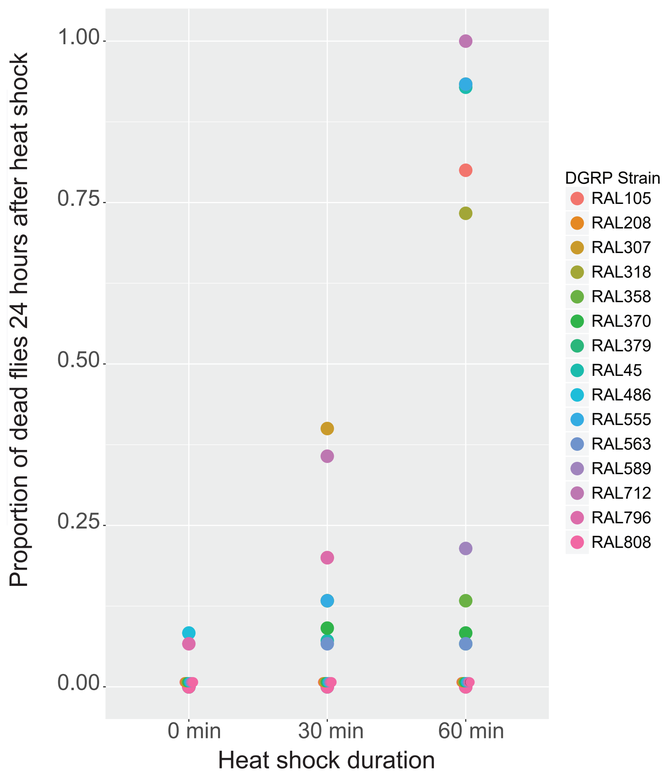
Many ER stress studies examine the ER stress response in isolation. In reality, however, ER stress occurs in a complex milieu of previous and ongoing stresses that likely impact how the cell responds. One example of this impact is the phenomenon of hormesis, whereby conditioning organisms with low levels of stress improves their ability to withstand subsequent stress and results in beneficial health outcomes. For example, it has been shown that pre-exposing C. elegans to mitochondrial stress improves their ability to deal with heat shock later in life (Morimoto et. al. 2017). Preconditioning can also result in a detrimental ability to deal with later stresses or, in some cases, no effect. The mechanisms behind stress preconditioning and what causes its various outcomes are still not understood. This study aims to utilize the *Drosophila* Genetic Reference Panel (DGRP) to characterize the impact of transient heat stress on subsequent ER stress resistance and investigate how genetic background influences this effect. Characterizing the impact of genetic diversity on stress preconditioning outcomes will allow us to identify modifiers of stress preconditioning that were overlooked when evaluating the phenomenon in a single strain. Here, we present data from preliminary experiments characterizing the phenotypic heterogeneity of heat shock recovery in a subset of DGRP strains and preliminary results of the DGRP stress preconditioning screen. Understanding how previous stress events and genetic background influence the ER stress response will have important implications for health and therapeutic development.



2. Establishing the parameters for a DGRP hormesis screen

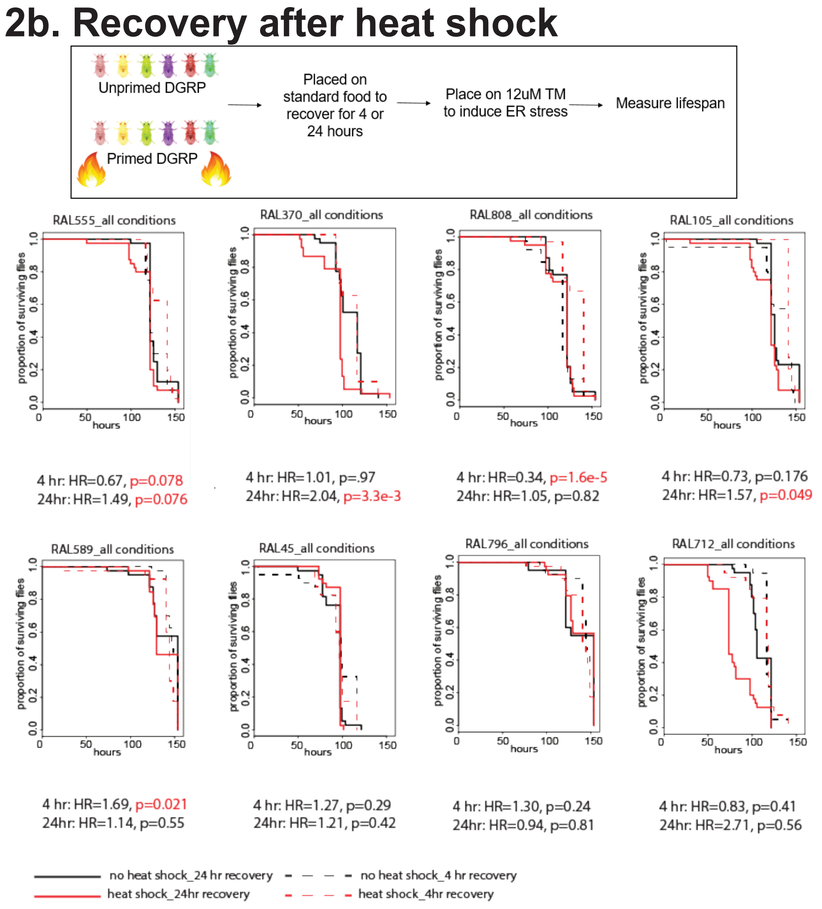
Prior to performing the DGRP hormesis screen, a sublethal and effective heat shock protocol needed to be established for use on the DGRP. The DGRP is a collection of 200 fully-sequenced, inbred lines derived from a natural population. We performed heat shock experiments on a subset of DGRP strains. Heat shock was performed at 35 ± 1 °C on adult males. There is a high level of phenotypic variability between different strains exposed to heat shock.

2a. Length of heat shock



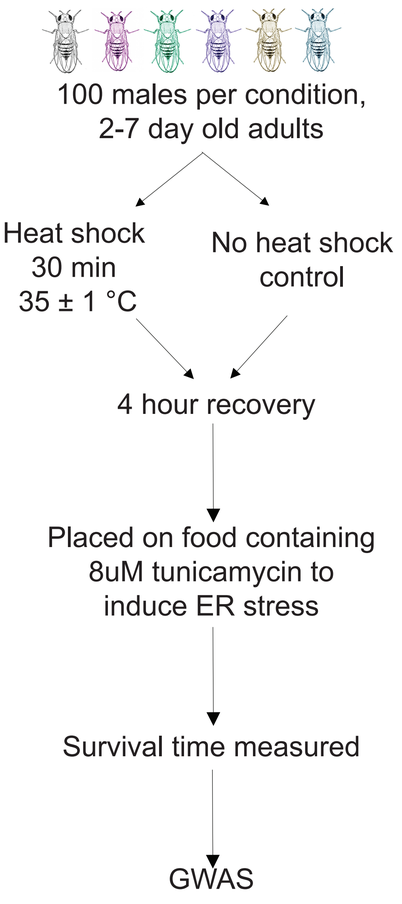
After 60 minutes of heat shock, lethality ranged from 0-100%. The striking variablilty in lethality can be attributed to differences in genetic background. Moving forward, heat shock will be performed for 30 min.

2b. Recovery after heat shock



A larger range of effects is observed with a 4 hour recovery vs a 24 hour recovery period.

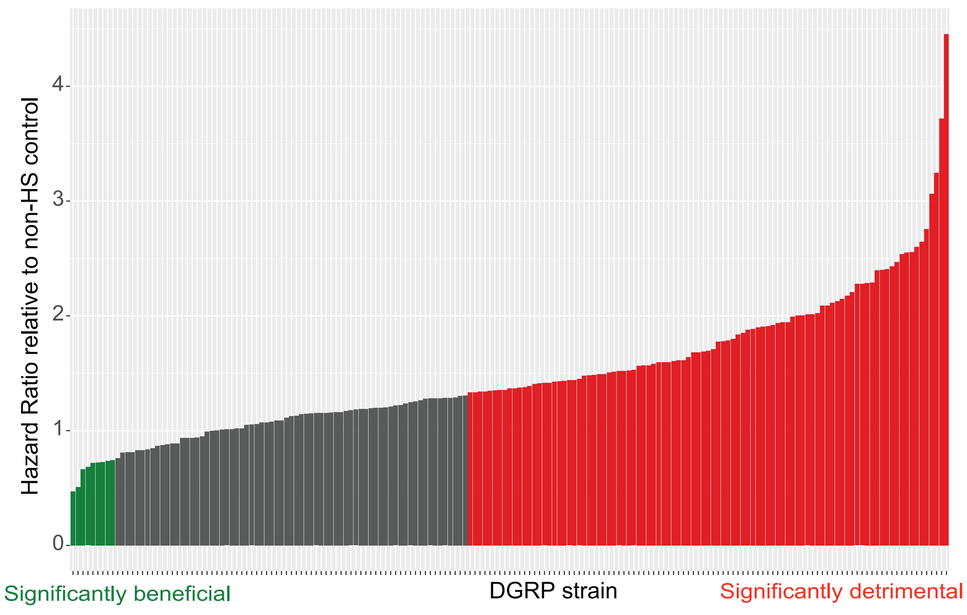
2c. Final experimental design



3. Preliminary results from the DGRP hormesis screen

The stress preconditioning screen was performed on 177 DGRP strains as shown in 2c. The impact of heat shock preconditioning on subsequent ER stress survival times was found to vary greatly with genetic background, ranging from beneficial to detrimental consequences of preconditioning.

3a. Hazard ratios of survival under ER stress of heat shock flies relative to unprimed



Impact of HS preconditioning on subsequent ER stress survival time varies with genetic background

3b. Top candidate modifiers

Gene	p_value	Function
Pdp1	4.97E-06	PAR domain bZip family of transcription factors
TwdlJ	5.13E-06	chitin-based cuticle development
CG13889	1.27E-05	compartmentalization of sillium, sperm movement
LpR2	1.57E-05	transmembrane receptor, cellular uptake of neutral lipids
ckn	1.73E-05	cytoplasmic adaptor protein, axon growth
hdc	2.83E-05	terminal branching in trachea
nmo	3.42E-05	serine/threonine kinase, roles in developmental processes
Tpl94D	4.36E-05	chromatin reorganization in spermatids
Wnt10	4.56E-05	member of the Wnt protein family
CG17197	4.70E-05	protein palmitoylation
fry	5.21E-05	mutations lead to multiplied hairs
side	6.03E-05	transmembrane protein of the immunoglobulin superfamily
TyrR	6.15E-05	Tyramine 2 class receptor, release of intercellular calcium
Hs6st	6.24E-05	heparan sulfate modifying enzyme
frac	6.92E-05	acts via an LIMK1- dependent BMP pathway to promote axon target recognition
fra	7.35E-05	DCC-like Netrin receptor that mediates axon guidance
TfIIA-L	7.56E-05	precursor protein for TFIIA, a transcription factor required for initiation by RNA polymerase II
sfl	8.20E-05	heparin- sulfotransferase activity, wing morphogenesis, trachea development
px	9.19E-05	nuclear matrix protein required to restrict excess wing vein development
Pino	9.29E-05	olfactory behavior
Drat	9.39E-05	member of the zinc-dependent ADH-like family, effector of ethanol-induced apoptosis.
lea	9.66E-05	member of the Robo receptor family, repulsive cue development

p-value < 1E-04, AF>0.05, gene has reported function

3c. Top 20 GSEA results

Term Name	p_value	#genes
pole plasm assembly	0.002	15
spindle microtubule	0.005	11
JAK-STAT cascade	0.008	8
regulation of heart contraction	0.009	6
procollagen-proline 4-dioxygenase activity	0.010	22
oxidoreductase activity	0.010	22
heterogeneous nuclear ribonucleoprotein complex	0.012	5
procollagen-proline 4-dioxygenase complex	0.015	15
negative regulation of growth	0.015	6
male meiosis I	0.016	8
histone-lysine N-methyltransferase activity	0.016	5
Set1C/COMPASS complex	0.017	7
acid phosphatase activity	0.020	9
negative regulation of Wnt receptor signaling pathway	0.021	15
histone H3-K4 methylation	0.022	6
protein-hormone receptor activity	0.025	5
spermatocyte division	0.026	8
meiosis	0.026	21
ATP binding	0.027	299
mesoderm development	0.027	56

p-value < 0.05, #genes>4

Indicates several major pathways that may play a crucial role in heat shock preconditioning

4. Conclusions

We found that different strains of the DGRP are affected by heat shock precoditioning prior to ER stress to varying degrees. This illustrates that genetic background is an important factor in the preconditioning effect in *Drosophila*. JAK-STAT, histone methylation, and Wnt singaling appear to play an important role in stress preconditioning. Moving forward, we aim to more fully understand the mechanism behind stress preconditioning and functionally validate candidate modifiers uncovered in our screen. This work has important implications for health.

NIGMS R35GM124780

Gentics Training Program T32GM007464

*Katie Owings: katie.owings@utah.edu

www.ChowLab.org twitter: @kgowings