DNA Repair in the Functional Consequences and Cell Biological Regulation of Reproductive Arrest in *Drosophila melanogaster*

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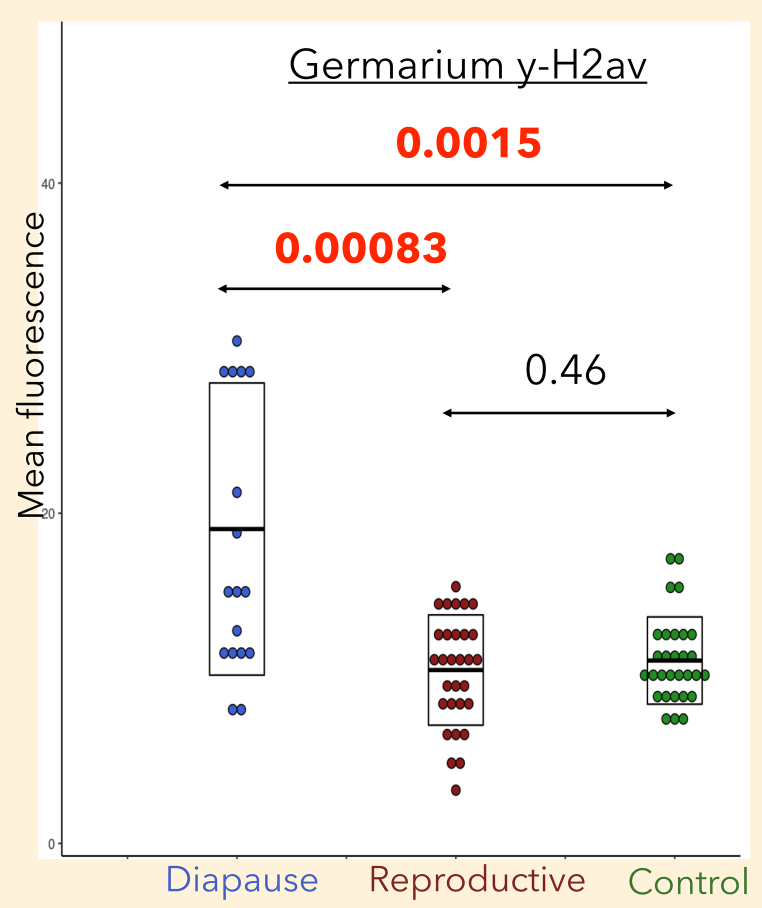
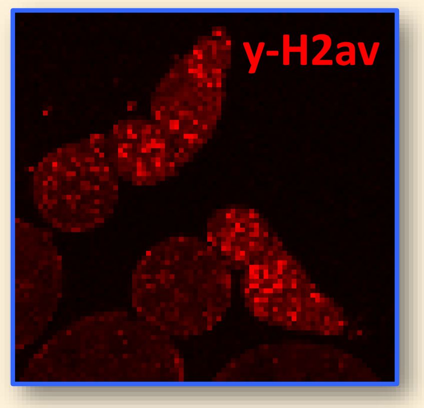
DNA Damage Signaling

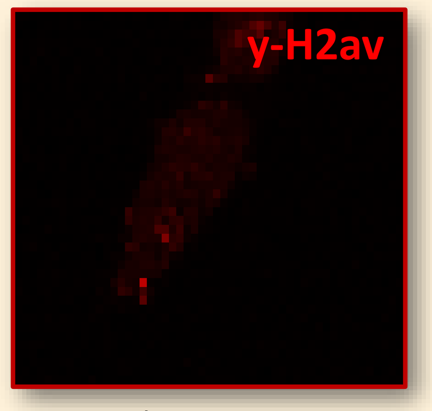
- Subject genetically identical flies to winter-like conditions for 28-days to induce diapause in some, but not all, flies
- Immunofluorescence staining of ovaries for γH2AV, which marks double stranded breaks in DNA



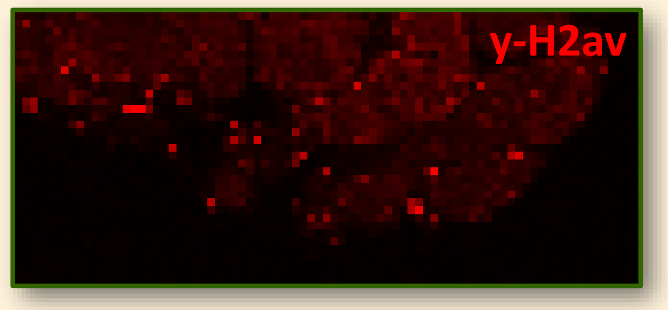
Confocal microscopy of *Drosophila melanogaster* ovaries



Diapause



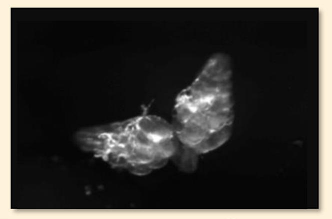
Reproductive

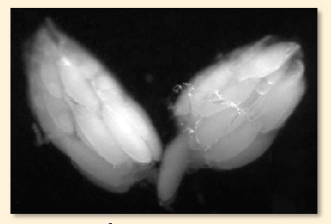


Room Temperature Control

DIAPAUSE – an alternative developmental fate

- Programmed state of arrest in many animal taxa[1]
- Diapause improves survival in adverse environmental conditions e.g. drought, cold temperatures
- Female ***Drosophila melanogaster*** in diapause arrest egg development to survive the winter
- Diapause substantially extends lifespan


Diapause Ovaries


Reproductive Ovaries

Genome Integrity during Diapause

Insights from Diapause Literature

- Germline of diapausing insects and related arthropods tend to exhibit resistance against gamma radiation
- Diapausing insects and related arthropods do not tend to exhibit general resistance against gamma radiation

	Effects	
Species	***Germline***	***Soma***
Plodia interpunctella[2,,4] (Indian meal moth)	**Resistant**	**Mixed**
Ostrinia nubilalis[3,10] (European corn borer)	**Resistant**	**Resistant**
Pectinophora gossypiella[4] (pink bollworm)	**Resistant**	**not tested**
Cydia pomonella[5] (codling moth)	**Resistant**	**Mixed**
Rhagoletis mendax[6] (blueberry maggot)	**not tested**	**Sensitive**
Trogoderma granarium[7] (khapra beetle)	**not tested**	**No difference**
Heliothis virescens[8] (tobacco budworm)	**Resistant**	**not tested**
Tetranychus urticae[9] (two-spotted spider mite)	**Resistant**	**Resistant**

Insights from Data Generated by the Levine Lab

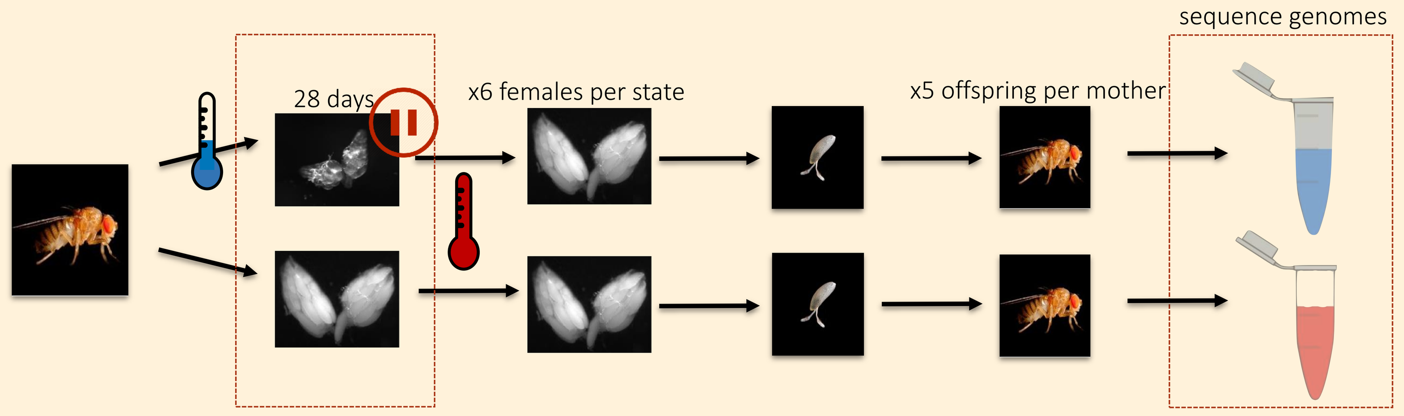
- DNA damage signaling and repair genes are upregulated in diapausing female fruit flies
- Flies successfully reproduce after diapause

Hypothesis: Flies co-opt the DNA damage signaling pathway to maintain germline genome integrity during diapause.

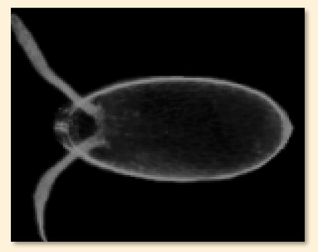
Predictions: Despite the increase in DNA damage signaling, there will be 1) no increase in mutations and 2) no increase in egg defects characteristic of DNA damage.

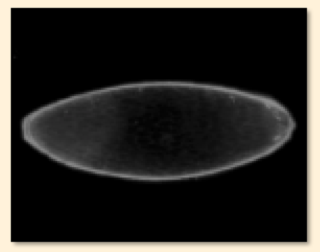
Mutation Accumulation

- Sequence offspring of recovered post-diapause and post-winter reproductive females to assess for mutations acquired during winter incubation period



Analysis of genomes revealed **~27 mutations** acquired per individual for both diapause and winter-reproductive flies.


Wild type (normal)[11]


Defective

	Diapause	***Reproductive***
Wild type	232	205
Defective	2	1

Conclusion: Upregulation of DNA damage signaling without an accompanying increase in DNA damage suggests flies may poise ovaries for DNA repair to maintain genome integrity and reproductive youth throughout the prolonged lifespan experienced during diapause

Future Directions

- Analyze DNA damage repair mutants
- Challenge diapause and winter reproductive flies with DNA damaging agents

Acknowledgements

We are grateful for funding from CURF in the form of the Benjamin Franklin Scholars Summer Grant and the Ruth Marcus Kanter College Alumni Society Undergraduate Research Grant.

Author Contributions

A.D. designed and performed immunofluorescence experiments with input from R.A.F. R.A.F. and A.D. designed mutation accumulation experiment. A.D. conducted immunofluorescence and mutation accumulation experiments. R.A.F quantified immunofluorescence images. A.D. and R.A.F. prepared sequences for analysis and R.A.F. conducted the analysis. M.T.L. provided input into research design.

References

1) Renfree & Shaw, 2000 2) Pentz & Krause, 1968 3) Cloutier & Beck, 1963. 4) Brower, 1980 5) Mansour & Mohamed, 2004 8) Proshold & North, 1978 9) Suzuki et al. 2009 10) Raun et al.,1967 11) *Egg morphology images from Hawkins, 1997)*