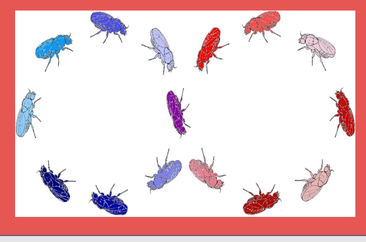
# Identifying genetic background effects for cancer susceptibility

Andrew Jones, Chiswili Yves Chabu, Elizabeth King

Division of Biological Sciences University of Missouri Columbia



## Introduction

The study of cancer susceptibility is incredibly important due to the harmful effects of cancer and the difficulty in predicting and preventing it. Individuals can vary in their degree of cancer susceptibility due to different genetic backgrounds (4). A major goal in biomedicine is to identify genomic regions that influence cancer severity. Here, we used the *Drosophila melanogaster* model organism to identify genetic background effects on the severity of a cJUN NH2-terminal kinase (JNK) mutation. Under normal circumstances, JNK serves as a tumor-suppressor and regulator of cell proliferation (1). When its upstream regulator, Egr is mutated, the JNK gene loses control of its regulatory and developmental capabilities. This causes cells to enter premature death cycles. The loss of regulation can also lead to increased prevalence of oncogenes. One of the noticeable effects of the JNK mutation is that yields a phenotypic variation of decreased eye pigment due to increased cell death (2). Eye expression was the emphasis of this project.

## Objectives

- Cross DSPR flies with Egr mutated flies
- Observe heritability of mutated EGR gene across generations
- Analyze differences between eye expression among lines

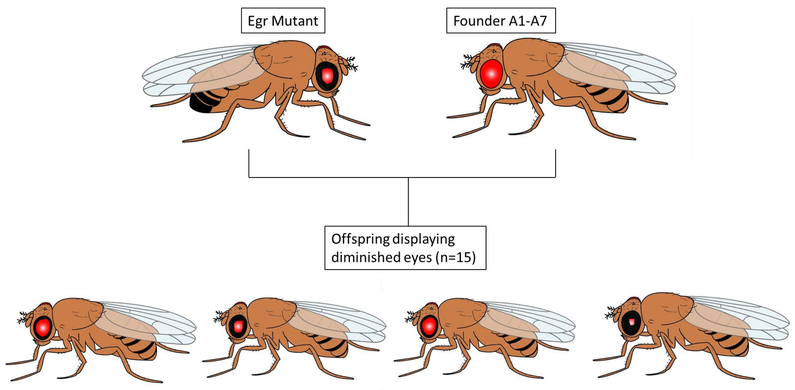



Fig. 1

## Crossing Design

To create a new line with the mutation in question, Egr mutant flies were bred with 7 lines of DSPR founders who acted as balancers to observe the mutation. Egr serves as an upstream regulator of JNK and is affected by the driver GMR GAL4. Lines of mutant and DSPR flies were bred separately over several generations before being crossed. Male Egr flies with straight wings and short bristles were crossed with females of each founder line with long bristles and straight wings. Adults were removed from vials prior to larvae eclosion. Flies were phenotyped before imaging.

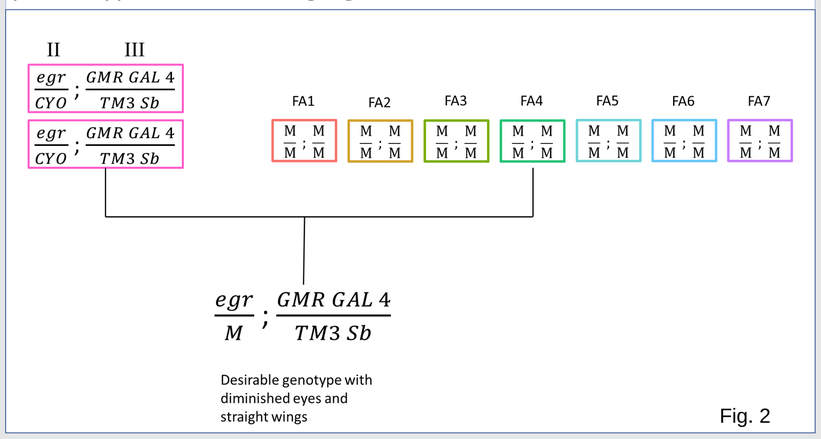
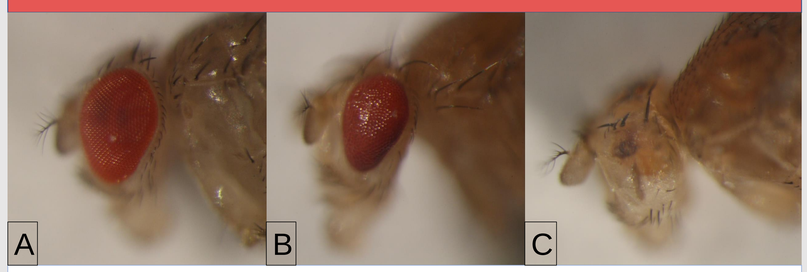



Fig. 2

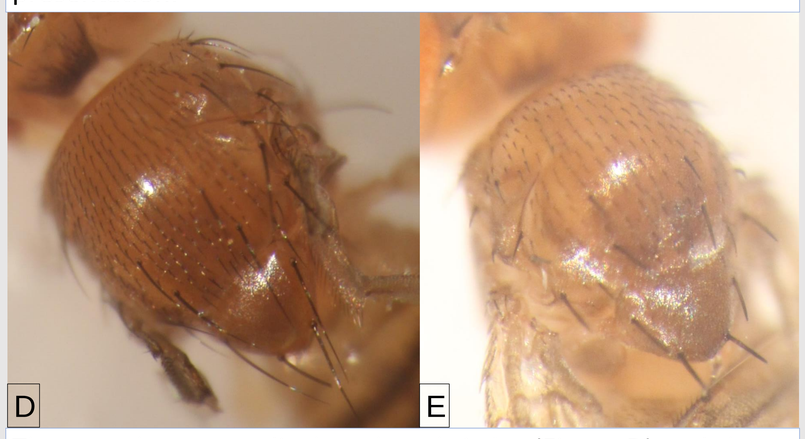
## Imaging Technique

To image, flies were anesthetized and laid on a CO2 pad. Flies with the desired phenotype were selected while others were returned to the vial. Selected flies had their legs and wings removed with forceps to set them in position. A small amount of glue was used to minimize movement and allow for a high replicability of position. Magnification was 10X for eye images. Images were then uploaded to image software ImageJ where total eye area was measured using a tracing tool. A micrometer was used to establish the scale for each image.

## Eye and Body Phenotypes

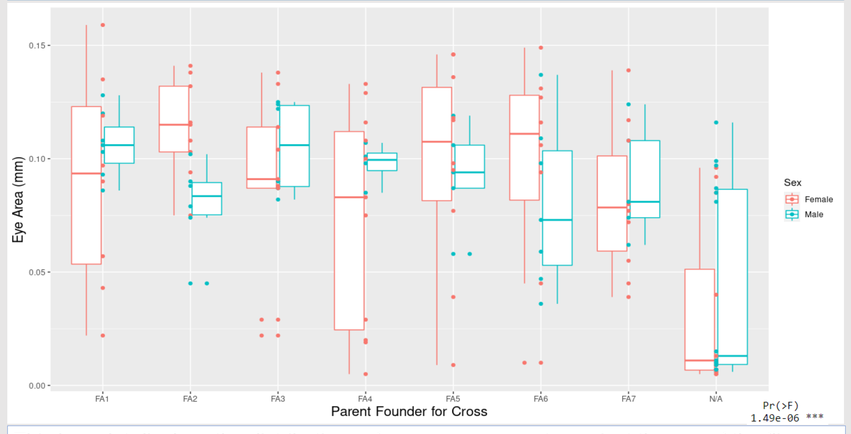



Varying degrees of loss-of-function displayed in eyes. Figures A and B are the offspring of a Founder-Mutant cross while Figure C is an original Egr mutant fly with the mutant EGR gene presentation.




The two phenotype displays: long bristles (Figure D) and short bristles (Figure E). Short bristles indicated the presence of the TM3 Sb gene.

## Eye Area by Sex




This box plot displays the distributions of eye measurements against the sex of the flies. The top line represents Q3, the middle is the median, and the low line is Q1. Vertical lines represent maximums and minimums. Longer lines signify higher variance in values for that line. This plot shows that there was a large degree of variation between the size of eyes for both sexes . There was not a discrete pattern, but sex was found to have a background effect on the eye expression.

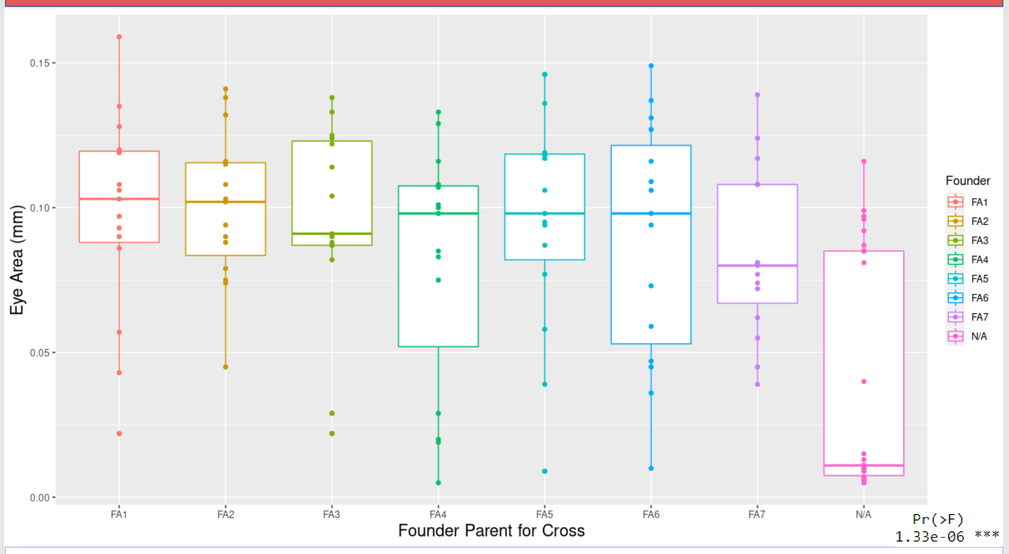
## Discussion

This project aimed to observe the effect of the EGR gene mutation and its degree of heritability. Across multiple lines there was a significant difference between eye measurements among founder lines. There was also a significant difference between the eye area of crossed offspring and original Egr flies. The presence of TM3 Sb gene (short bristles) was not found to be a significant background effector. The CYO (curly wing) gene did have significant differences but few flies with this gene were measured. Overall, it appears that the mutation and subsequent display in diminished eyes is passed down, but to a lesser extent in crossed flies. Further test could seek out the pathways for this.

## Significance and Future Directions

This project is an important first step in locating the background of cancer susceptibility and its ability to be inherited. Mapping the JNK gene and modulators of the EGR allele will allow us to further investigate tumor suppressors in flies. Over 60% of fly DNA is conserved in humans so discoveries made here could be indicators for human genes as well. By using natural variants with the DSPR founders, we can detect novel modifies in an unbiased way. Cancer is one of the leading diseases in the medical field, a breakthrough in understanding it could improve our ability to combat it.

## Eye Area by Parent Founder




This dot plot features eye area on the Y-Axis and the founder parent of the crossed fly on the X-Axis. It shows the distribution eye measurements for the different founder-mutant crosses. Note the column of pure mutant Egr flies labeled as (N/A) on the far right. There were significant differences between each founder line but more importantly between the mutant Egr flies and founders. This indicates that flies carrying the Egr mutation can regain some expression of eye pigmentation. Crossed flies displayed less degradation than their mutant parents.

## References

1. Tournier C. The 2 Faces of JNK Signaling in Cancer. *Genes Cancer*. 2013;4(9-10):397-400. doi:10.1177/1947601913486349
2. Igaki T, Kanda H, Yamamoto-Goto Y, et al. Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway. *EMBO J*. 2002;21(12):3009-3018. doi:10.1093/emboj/cdf306
3. Elizabeth G. King, Stuart J. Macdonald and Anthony D. Long *Genetics* July 1, 2012 vol. 191 no. 3 935-949; https://doi.org/10.1534/genetics.112.138537
4. Vidal M, Cagan RL. Drosophila models for cancer research. *Curr Opin Genet Dev*. 2006;16(1):10-16. doi:10.1016/j.gde.2005.12.004
5. Shimosako, Nana, Dafni Hadjieconomou, and Iris Salecker. “Flybow to Dissect Circuit Assembly in the Drosophila Brain.” In Brain Development: Methods and Protocols, edited by Simon G Sprecher, 57–69. Totowa, NJ: Humana Press, 2014. https://doi.org/10.1007/978-1-62703-655-9_4.

## Acknowledgements

- Funded by a grant from the National Institutes of Health
- I would like to thank all members of the Kinglab for their advice, assistance, and contributions to this project
- A special thanks also to the members of the Chabu Lab for their contributions to the project and its materials and methods