

Tina Ngo, Gayani Nanayakkara, Aylin Rodan

Department of Internal Medicine, Division of Nephrology and Hypertension, Molecular Medicine Program and Human Genetics, University of Utah

HOSA (Future Health Professionals) University of Utah Chapter

## ABSTRACT

Our lab uses *Drosophila melanogaster* (fruit fly) to understand the effects of salt on the body. We previously showed that a heterozygous mutation (*EY-P283/+*) in the gene locomotion defect (*loco*) protects against death on a high salt study. To further characterize *loco* mutant flies, we performed the following assays: 1) Wing measurements: *EY-P283/+* flies have larger body weight relative to controls. To determine whether the larger body weight in *EY-P283/+* flies is due to larger body size, I dissected out the left wing of male and female flies and imaged them with a microscope. Male but not female *EY-P283/+* wings were larger than controls. 2) Climbing assay: Homozygous *loco* mutation impairs the climbing ability of flies. However, it was not known whether *EY-P283/+* flies also have impaired locomotion abilities. My data revealed that female *EY-P283/+* doesn't show significant differences in their climbing ability. However, relative to the controls, male *EY-P283/+* flies showed better capability at climbing. 3) SMurf assays: Gut barrier function deteriorates with age. As *EY-P283/+* flies have a longer life span, we tested whether they have a better gut barrier function using the SMURF assay. According to my data, there is no significant difference between the gut barrier function of aged *EY-P283/+* and control flies. This concludes that the longer life span observed in *EY-P283/+* flies is not due to a differential gut barrier function between the genotypes. Overall, the aim of this study was to understand the characteristics of this mutant to better understand its salt resistance.

## BACKGROUND

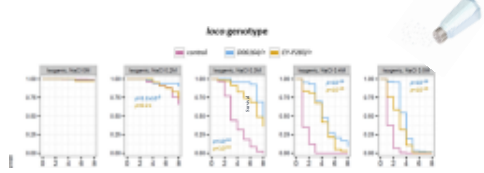


Figure 1. Heterozygous flies' survival observed under different salt concentrations. The mutant flies were better able to survive a high salt diet compared to the controls.

Mahajan et al. The American Journal of Human Genetics 99: 636-646

- Homozygous *loco* mutant flies were reported to have a deficiency in locomotion/climbing ability.
  - Does the heterozygous *EY-P283/+* flies also have impaired locomotion?
- The *loco* mutant flies have larger body weight.
  - Is the larger body weight in heterozygous *loco* mutant flies due to larger body size or larger metabolic reserves?
- The *loco* mutant flies have a longer life span. During aging, gut barrier function of flies deteriorates which accelerates their mortality.
  - Do the aged *loco* mutant flies have a better gut barrier function?

## MATERIALS & METHODS

All data has been analyzed using the software Prism 8. Genotypes: Control-*yw*, Mutant-*yw*; *loco*<sup>EY-P283/+</sup>

### Climbing assay

- Used 1-week-old male and female flies.
- Counted number of flies that could climb 5, 10, and 15 cm.
- Given 10 seconds to climb as far as they could.

### Wing size measurement assay

- Used 10-day-old male and female flies.
- Dissected left wing and observed under microscope.



Figure 3a. Female wing.

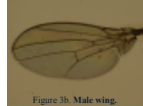


Figure 3b. Male wing.

### SMURF assay

- Used 7-week-old (aged) male and female flies.
- Prepared blue dyed high salt and normal salt food.
- Flies were fed blue food for 24 hours in vials.
- Visually scored for blue dye outside of gut.

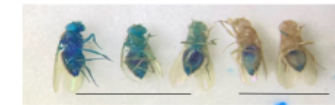


Figure 4. Image of SMURF flies.

## RESULTS

### Climbing Assay

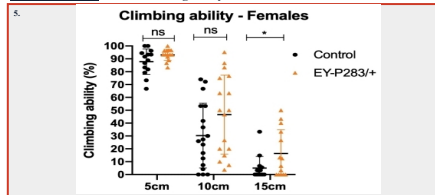


Figure 5. Percentage of male flies able to reach 5, 10, and 15 cm. For 5 and 10 cm, there were no changes in climbing ability. For 15 cm, the flies showed slightly improved climbing. *n*=150-200 flies for each genotype for each distance measured.

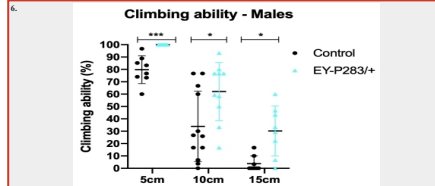


Figure 6. Percentage of male flies able to reach 5, 10, and 15cm. For 5cm, the flies had better climbing ability. For 10 and 15cm, the flies slightly improved climbing. *n*=150-200 flies.

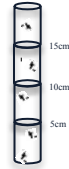


Figure 2. Climbing assay set-up.



Figure 7a, and 7b. Females. No significant difference in the wing size and relative wing size between mutant and control flies. *n*=40-50 flies.

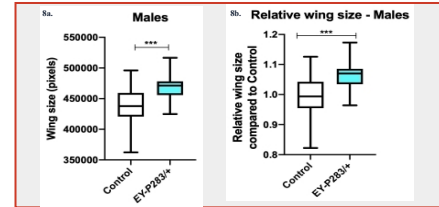


Figure 8a, and 8b. Males. Significant difference in wing size and relative wing size between mutant flies and controls. Larger wings in mutants. *n*=40-50 flies.

### SMURF Assay

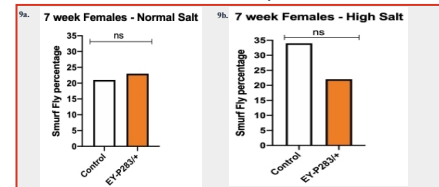


Figure 9a, and 9b. Percentage of female flies becoming a SMURF from normal and high salt diet. No significant between the control and *loco* mutant flies. *n*=60-70 flies.

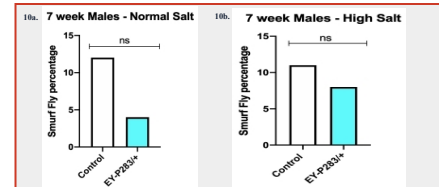


Figure 10a, and 10b. Percentage of male flies becoming a SMURF normal and high salt diet. No significant between the control and *loco* mutant flies. *n*=60-70 flies.

## CONCLUSIONS

### Climbing assay

- Heterozygous *loco* mutation in an isogenic background does not impair locomotion ability in *loco* mutant flies.
- Female *loco* mutant climbing ability was not significant compared to the controls.
- Male *loco* mutant climbing ability was significant compared to the controls and show that the mutants do have higher climbing abilities.

### Wing size measurement assay

- Female *loco* mutant wing size was not significant compared to the controls so there isn't a significant difference in their body sizes.
- Male *loco* mutant wing size was significantly larger compared to controls and show that mutants have a larger body size.

### SMURF assay

- The percentage of SMURF flies were not significantly different between the two genotypes so there is no significance difference in the gut barrier function between the aged *loco* mutant and control flies.

## FUTURE DIRECTIONS

Our lab has shown that *loco* mutant flies are better at tolerating high salt stress. Despite having a larger body weight, my wing measurement data shows that *loco* mutant female body size does not significantly differ to that of controls. This suggests that the larger body weight in female flies is maybe due to larger metabolic reserves in *loco* mutant flies. Therefore, future studies can be carried out to determine whether having larger energy depots help the *loco* mutant flies to survive better under high salt stress compared to controls. The future experiments can be carried out on how *loco* mutation affects aging, ion exchange and transport etc. Future studies on *loco* mutant flies will provide insights into aging and impacts of high salt stress, which may provide insights into how the modern high salt diet adversely affects human health.

## REFERENCES

1. Mahajan S, et al. The American Journal of Human Genetics 99: 636-646
2. ...
3. ...
4. ...
5. ...
6. ...
7. ...
8. ...
9. ...
10. ...

## ACKNOWLEDGEMENTS

- Many thanks to
- University of Utah for hosting the RUUTE Summer Undergraduate Research program
  - Aylin Rodan M.D. Ph.D. for giving me this opportunity to work in her lab as well as her support and mentorship
  - My mentors Gayani Nanayakkara Ph.D. and Daryl Morrison B.S. for their support and guidance during journey of this Summer 2021 internship
  - All participating community leaders, community professionals, and University of Utah faculty whose devoted time and patience has been greatly appreciated and has helped with the research.