

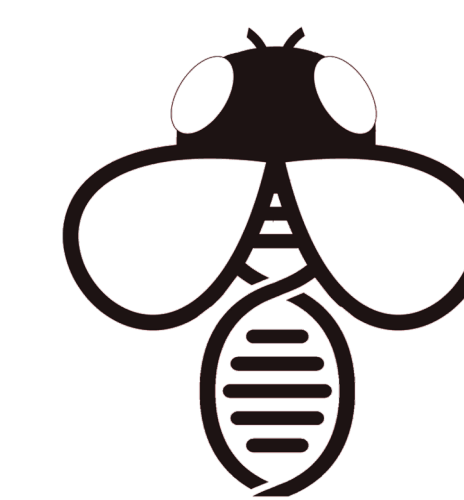


Determining the Effect of *Wolbachia* on Octanoic Acid Resistance in *D. sechellia*

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Drosophila sechellia

The fruit fly *Drosophila sechellia* has specialized on *Morinda citrifolia*, commonly known as noni fruit. This interaction is peculiar as noni fruit contains the toxic, volatile octanoic acid (OA). Previous research suggests that there is a notable difference among *D. sechellia* lab strain OA resistance (López et al., 2017). Previous work has also shown that when *D. sechellia* are exposed to OA, they have a down regulated immune response (Drum et al. 2021). This suggests that there may be other factors contributing to resistance. Gut microbes within these species may provide insight into the increased resistance to OA because of current evidence that suggests microbial assistance in degrading toxins, leading to dietary specialization in various organisms (Ceja-Navarro et al., 2015, Kohl et al., 2014, Hammerbacher et al., 2013). Microbes also play a crucial role in overall fitness and fecundity in multiple *Drosophila* species, depending on the diet composition (Bing et al., 2018). Although the microbiome may provide insight into OA resistance, a specific microbe that may be directly responsible for OA resistance is *Wolbachia*. One notable intraspecies difference in *D. sechellia* is the presence of a microbe known as *Wolbachia*. This intracellular, gram-negative microbe is passed from mother to offspring and has been implicated in increased fitness of some species of *Drosophila* (Sarwar et al., 2018). One way *Wolbachia* can increase the fitness of its host is by altering its dietary needs upon infection, which has been shown to increase lifespan (Ponton 2015). This direct effect on dietary needs, to increase survival, illustrates the ability for *Wolbachia* to have a direct impact on dietary consumption of its host, resulting in better fitness. In order to assess if microbes have an impact on *D. sechellia* survival, fly lines without *Wolbachia* and lines with decreased gut bacteria were created and survival assays were initiated.

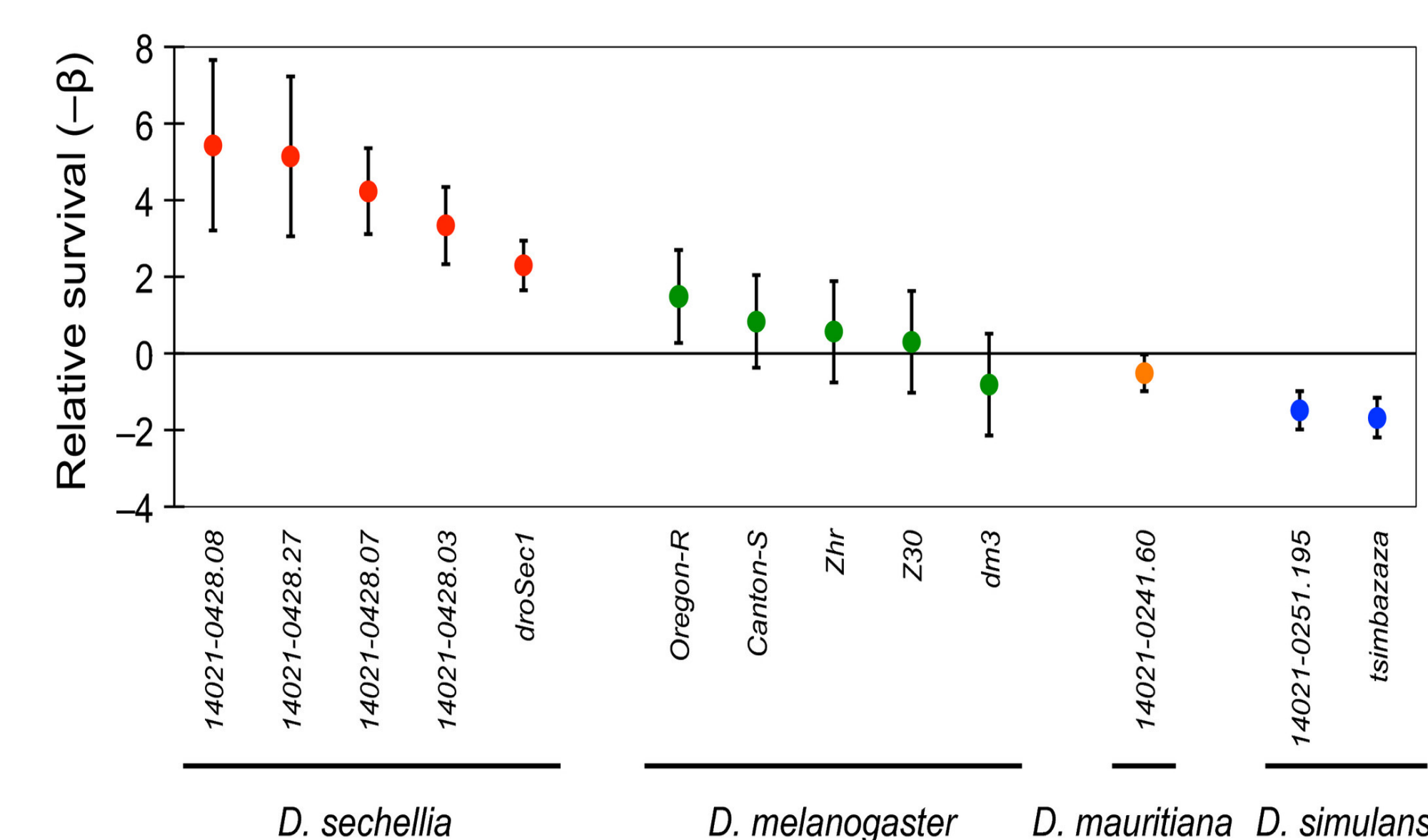
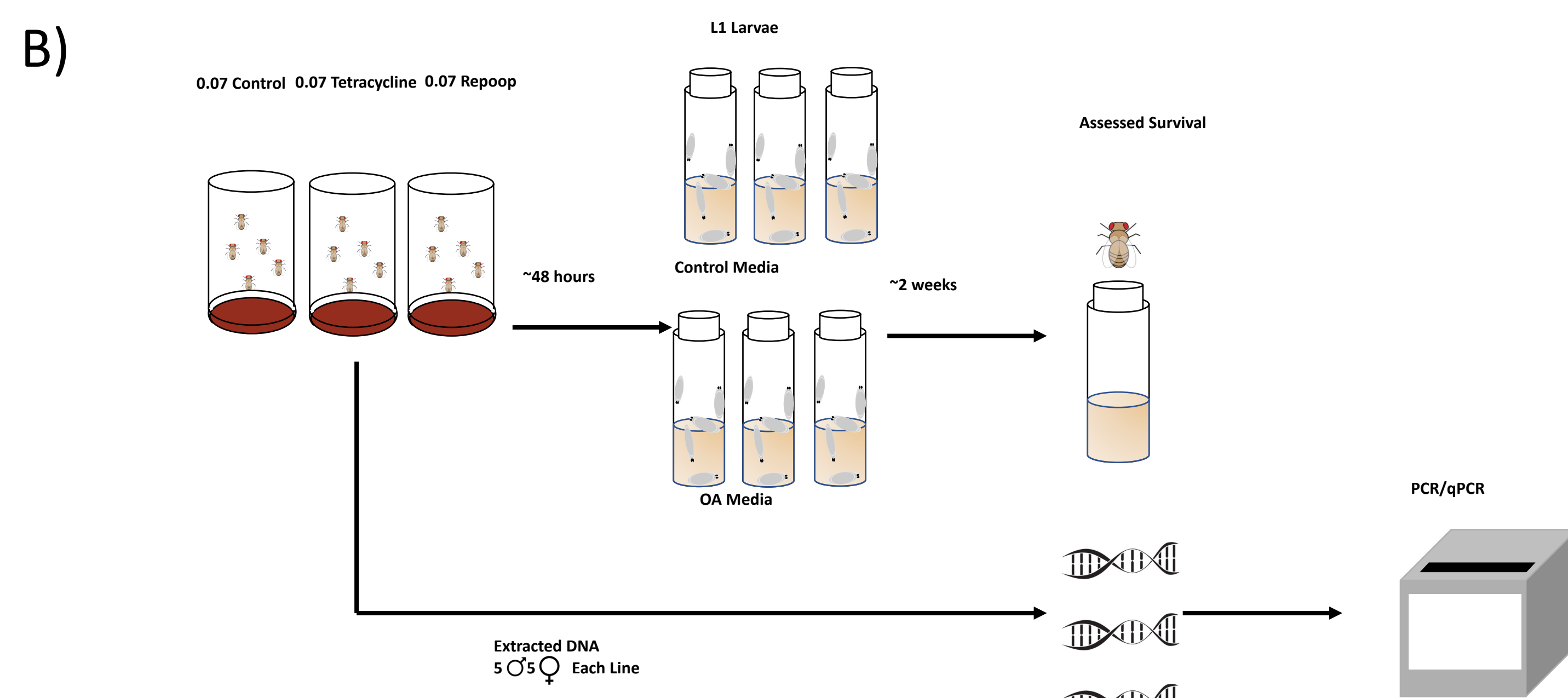
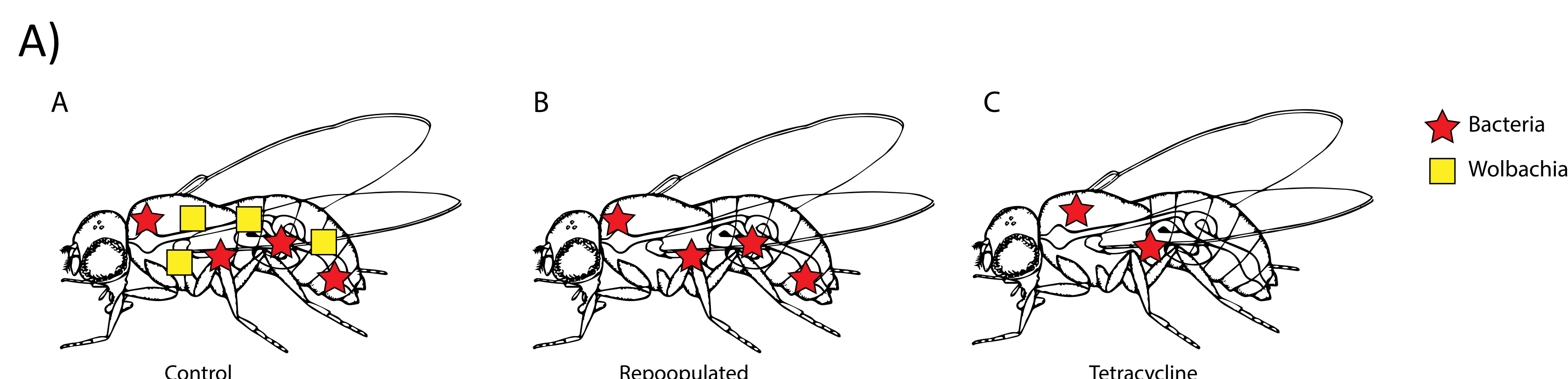
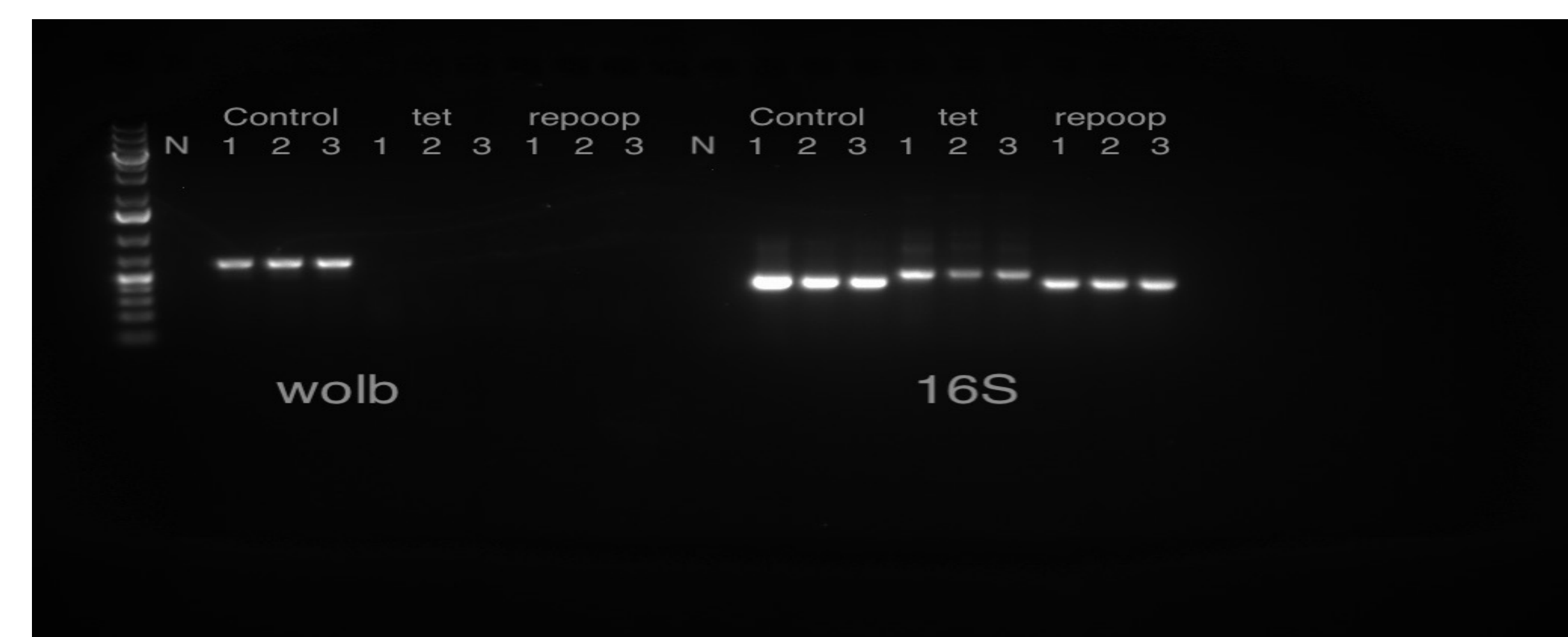


Figure Legend: These data from (López et al., 2017) illustrate that *D. sechellia* are more resistant than other species of *Drosophila*. Additionally, there is an intraspecies difference in OA resistance among *D. sechellia*.

Data Collection



C)



D)

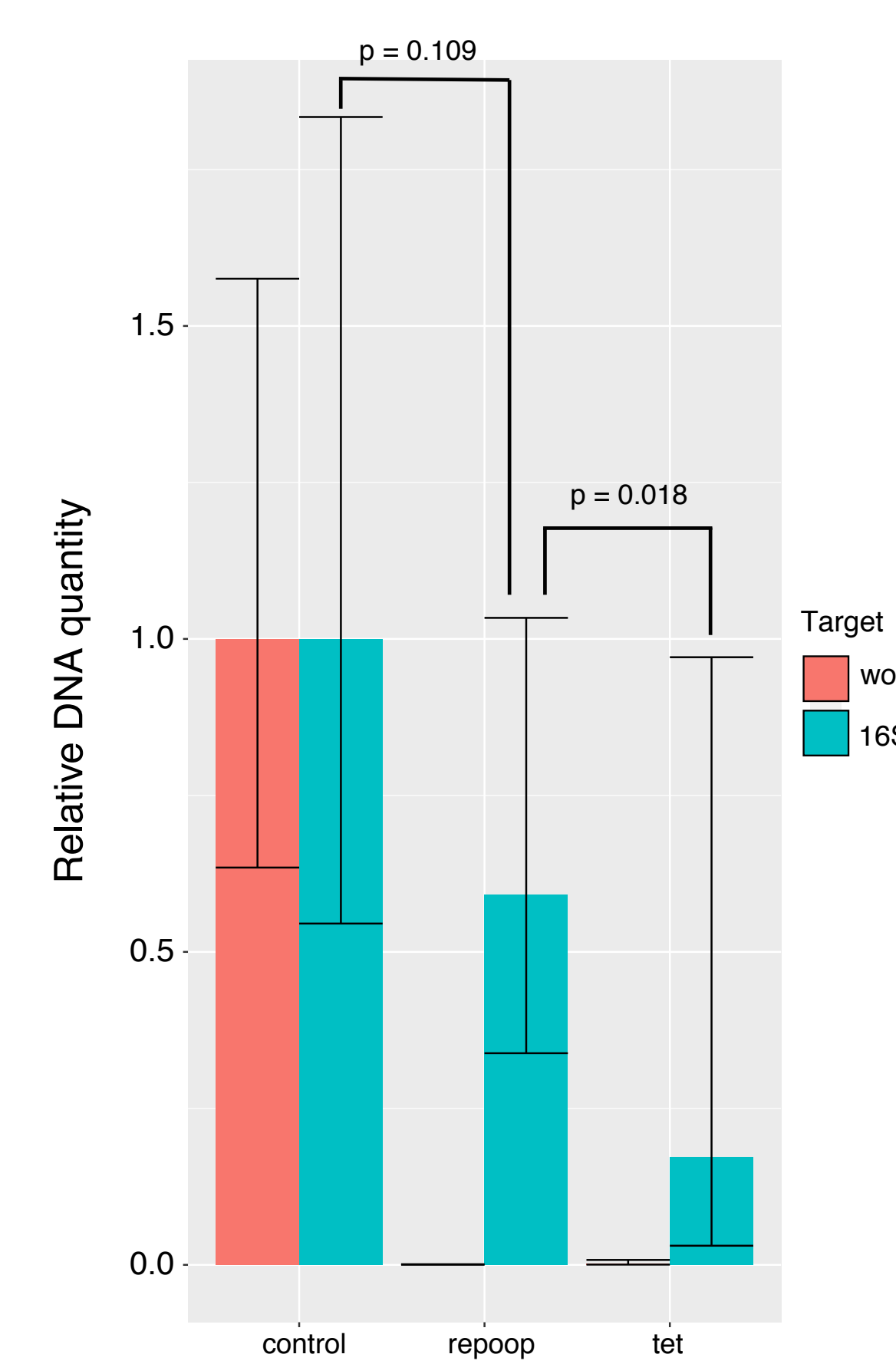


Figure Legend: A) Illustrates the three different fly lines with a depiction of the relative amount of gut bacteria and *wolbachia* in each line. B) Depicts the experimental pipeline that was utilized. C) PCR gel that confirms the presence of *Wolbachia* in the 0.07 control line and the absence of *Wolbachia* in the tetracycline and repopulated fly lines. Furthermore, it illustrates the presence of 16S in all three fly lines. D) qPCR data that illustrates similar levels of 16S found in the repopulated and control lines and a significantly decreased amount in the tetracycline treated lines. There is also a negligible amount of *Wolbachia* found in the repopulated and tetracycline treated flies.

Conclusion

- Control line had both *Wolbachia* and 16S present
- Repoop line had a similar amount of 16S present, compared to the control line. No *Wolbachia* was present.
- Tetracycline line had significantly less 16S present compared to the control and repoop line, in addition to an absence of *Wolbachia*.

Future directions and predictions

- Survival assays to determine if there is a statistically significant difference in the survival
- Attempt to make different foods to increase control fly survival such as “M Food” or food made from noni juice
- Determine the microbe diversity and quantity

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Citations

Bing, X., Gerlach, J., Loeb, G., & Buchon, N. (2018). Nutrient-Dependent Impact of Microbes on *Drosophila* Development. *MBio*, 9(2), e02199-17. <https://doi.org/10.1128/mBio.02199-17>

Ceja-Navarro, J. A., Vega, F. E., Karaoz, U., Hao, Z., Jenkins, S., Lim, H. C., Kosina, P., Infante, F., Northen, T. R., & Brodie, E. L. (2015). Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nature Communications*, 6, 7618. <https://doi.org/10.1038/ncomms8618>

Drum, Z. A., Lanno, S. M., Gregory, S. M., Shimshak, S. J., Ahamed, M., Barr, W., Bekele, B., Biester, A., Castro, C., Connolly, L., DelGaudio, N., Humphrey, W., Karimi, H., Karolczak, S., Lawrence, T., McCracken, A., Miller-Medzon, N., Murphy, L., Park, C., ... Coolon, J. D. (2021). Genomics analysis of hexanoic acid exposure in *Drosophila* species. *Biorxiv*, 2021.06.08.447576. <https://doi.org/10.1101/2021.06.08.447576>

Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L. P., Schneider, B., Bohlmann, J., Brand, W. A., Fenning, T. M., Gershenson, J., & Paetz, C. (2013). A Common Fungal Associate of the Spruce Bark Beetle Metabolizes the Stilbene Defenses of Norway Spruce. *Plant Physiology*, 162(3), 1324-1336. <https://doi.org/10.1104/pp.113.218610>

Kohl, K. D., Weiss, R. B., Cox, J., Dale, C., & Dearing, M. D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. *Ecology Letters*, 17(10), 1238-1246. <https://doi.org/10.1111/ele.12329>

López, J. M. A., Lanno, S. M., Auerbach, J. M., Moskowicz, E. C., Sliagar, L. A., Wittkopp, P. J., & Coolon, J. D. (2017). Genetic basis of octanoic acid resistance in *Drosophila sechellia*: Functional analysis of a fine-mapped region. *Molecular Ecology*, 26(4), 1148-1160. <https://doi.org/10.1111/mec.14001>

Ponton, F., Wilson, K., Holmes, A., Raubenheimer, D., Robinson, K. L., & Simpson, S. J. (2015). Macronutrients mediate the functional relationship between *Drosophila* and *Wolbachia*. *Proceedings of the Royal Society B: Biological Sciences*, 282(1800), 20142029. <https://doi.org/10.1098/rspb.2014.2029>

Sarwar, M. S., Jahan, N., & Shahbaz, F. (2018). Molecular Detection and Characterization of *Wolbachia* pipiens from *Culex quinquefasciatus* Collected from Lahore, Pakistan. *The American Journal of Tropical Medicine and Hygiene*, 98(1), 154-161. <https://doi.org/10.4269/ajtmh.17-0329>