# Comparative Analysis of Cross-Resistance to Insecticide in Four *Drosophila* Species

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## Introduction

The continuous growth of resistance to new insecticides and the further development of more aggressive chemicals. The constantly emerging aggressive insecticides have had major implications for the surrounding environments of agricultural land and non-target organisms (Aktar et al. 2009; Igbedioh 1991).

- **Deltamethrin (DM):** first introduced in 1974 as a commercial crop protection made to interfere with neural signaling in insects through target disruption in voltage-gated sodium channels, potentially leading to paralysis (Casida and Quistad 1998; Lu *et al.* 2019).
- **Dibrom:** an organophosphate, is made to target the enzyme acetylcholinesterase, leading to neural shutdown and eventually paralysis within the organism(Steppuhn *et al.* 2004).
- **Nicotine:** often found in tobacco, is used as an insecticide. Nicotine focuses on acetylcholine receptors, overstimulating the nervous system and leading to paralysis (Steppuhn *et al.* 2004; Whittaker 1990).

These three modern synthetic insecticides previously mentioned all target the central nervous system of insects, but many species of insects have developed ways to resist these odorant toxins through metabolic breakdown.

## Research Question

This project aims to see the relationship of how different treatments of insecticides interact with the resistance of different species of *Drosophila*.

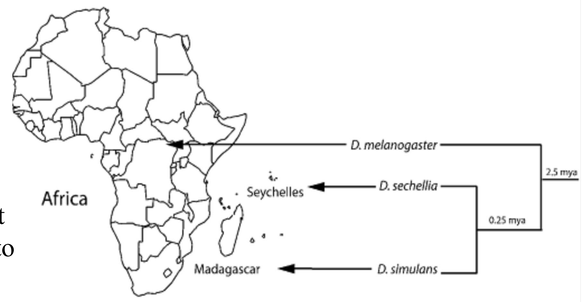
## Methods

**Sample**

The different *Drosophila* species utilized were *D. melanogaster*, *D. sechellia*, *D. simulans*, and *D. mauritiana*. The reason *D. melanogaster* is a subject of interest is largely due to the extensive insecticide work on the species, because it is a model organism. A point of interest is *D. sechellia,* known for its particular resistance to natural toxins such as those found in the noni fruit (Lanno and Coolon 2019). Potentially, there can be cross-resistance with *D. sechellia* and synthetic man-made insecticides. Few studies have examined the effect insecticides have on both *D. simulans* and *D. mauritiana*, being closely genomically related to *D. melanogaster, allowing* for a better understanding of these species and our data.

The compounds of insecticides tested were deltamethrin, dibrom, and nicotine as they are popular insecticides reguraly utilized in modern pesticides.

On the right four species of *Drosophila* that are closely related together genetically where *D. melanogaster* is a model organism and *D.sechellia* that has high resistance to naturally occurring toxins.

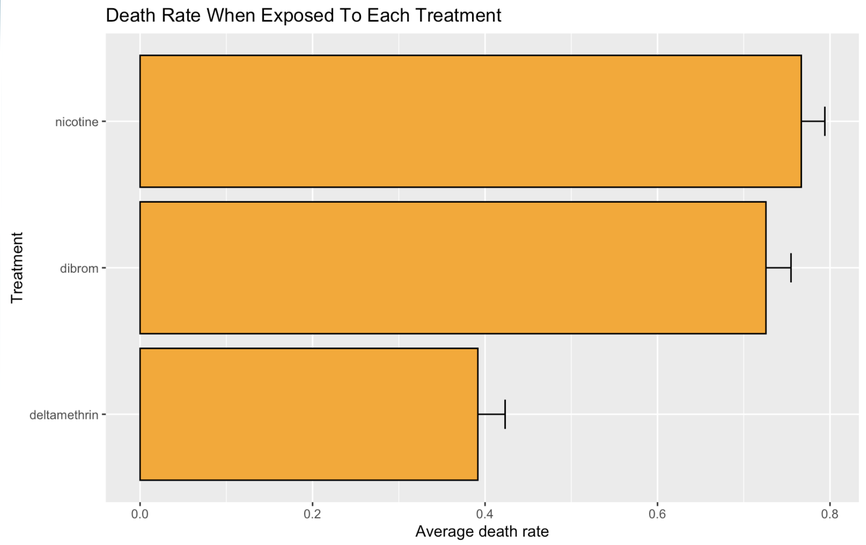



**Measures**


For survival assays, 10 female flies of each selected species are placed in vials containing sublethal concentrations of the chosen insecticide mixed with artificial food, exposing the flies to the insecticide. Female flies were chosen because they can live longer and undergo greater physiological stress than their male counterparts. Mortality (death is recorded by the fly's inability to move) was recorded every five minutes to measure the rate of death over an hour. In total, 2872 flies were assayed in 2018 in the Coolon Lab at Wesleyan University.

Focusing on *D. melanogaster*, *D. sechellia*, *D. simulans*, and *D. mauritiana* interactions with each insecticide taking the overall status of mortality at the end of the one-hour survival assay of each fly and calculated average mortality rate to compare between species of *Drosophila* and the insecticides they interacted with.

## Results





**Bivariate**

Bivariate bar graph looking at relationship between insecticides and rate of mortality.

Chi-square test: X-squared = 86.788, df = 2, p-value < 2.2e-16

Across species there is a resistance to deltamethrin compared dibrom and nicotine.

**Multivariate**

*D. melanogaster*, *D.sechellia*, *D. simulans*, and *D. mauritiana* adult females were placed in vials containing food with sublethal concentrations of the chosen insecticide mixed with artificial food, exposing the flies to the insecticide. Survival was recorded every 5 minutes for one hour to measure rate of death. Each fly was inputted into a logistic regression graph model above tested based off Insecticide, Species, and average mortality rate.

In reviewing the two multivariate graphs present in results it's found that *D.sechellia* and *D.simulans* have a strong resistance compared to *D. Mauritiana* and *D.Melanogaster.* looking at dibrom it is clear *D. simulans* has a very low resistance to the chemical compared to *D.sechellia*, *D. Mauritiana*, and *D. Melanogaster*. *D. Melanogaster, which* has a low death rate and higher resistance compared to the other species when exposed to nicotine.

## Discussion

Due to past studies of *D. sechellia* having a larger resistance to toxins (e.g., octanoic acid, hexanoic acid, methyl octanoate, ethyl octanoate, methyl hexanoate, ethyl hexanoate), there could be a good amount of cross-resistance to insecticides (Lopez et al. 2017). Potentially, along with the metabolizing detoxification with family genes like cytochrome P450, GST, and esterases that break down the insecticide. It's possible that there could be other factors of resistance, such as thicker cuticle production or nerve mutations that insecticides target. In past studies, *D. sechellia* has displayed strong resistance to specific toxins that otherwise species, *D. simulans*, *D. melanogaster*, and *D. mauritiana* are not able to be metabolized or detoxified, leading to lower survival rates of these species (Lanno and Coolon 2019).

The major implication of getting to know how particular species of insects interact with insecticides, is the possibility of understanding the genetic motivators of resistance within an organism. Learning the genetic pathways to resistance to insecticides within these species of *Drosophila* could allow us to produce highly targeted insecticides, RNAi-based insecticides, or microbial insecticides, potentially breaking the cycle of growing insecticide resistance.

## References

**Aktar, W., Sengupta, D., & Chowdhury, A.** (2009). Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology, 2*(1), 1–12. https://doi.org/10.2478/v10102-009-0001-7

**Andrade López, J. M., Lanno, S. M., Auerbach, J. M., Moskowitz, E. C., Sligar, 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*(5), 1148–1160. https://doi.org/10.1111/mec.14001

**Casida, J. E., & Quistad, G. B.** (1998). Golden age of insecticide research: Past, present, or future? *Annual Review of Entomology, 43*, 1–16. https://doi.org/10.1146/annurev.ento.43.1.1

**Igdebioh, S.** (1991). Effects of agricultural pesticides on humans, animals, and higher plants in developing countries. *Physical Therapy, 81*, 218–224. https://doi.org/10.1177/017084068800900203

**Lanno, S. M., & Coolon, J. D.** (2019). Derived esterase activity in *Drosophila sechellia* contributes to evolved octanoic acid resistance. *Insect Molecular Biology, 28*(6), 798–806. https://doi.org/10.1111/imb.12587

**Lu, Q., Sun, Y., Ares, I., Anadón, A., Martínez, M., Martínez-Larrañaga, M. R., ... Martínez, M. A.** (2019). Deltamethrin toxicity: A review of oxidative stress and metabolism. *Environmental Research, 170*, 260–281. https://doi.org/10.1016/j.envres.2018.12.045

**Whittaker, V. P.** (1990). The contribution of drugs and toxins to understanding of cholinergic function. *Trends in Pharmacological Sciences, 11*(1), 8–13. https://doi.org/10.1016/0165-6147(90)90034-6