

# Establishing the Window of Selection (WoS) and level of resistance of knock down resistant (*kdr*) mutations for *Aedes aegypti* topically treated with deltamethrin



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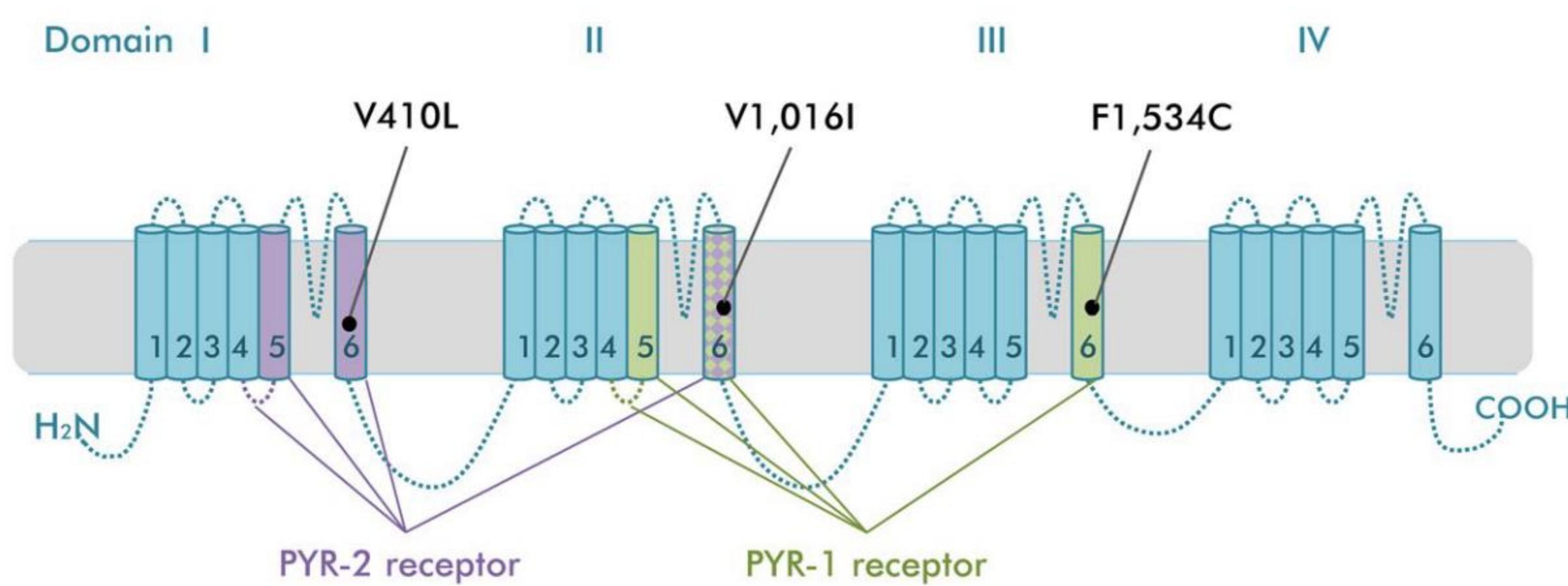


## General Background

- Insecticides** are one of the main **preventative tools** to reduce vector-borne disease transmission and burden.
- Insecticide resistance** has resulted from persistent use of insecticides by public health, agriculture, and the private sector.
- Repeated use of the limited insecticides available creates greater **selective pressure** for resistance.
- Insecticide resistance such as **knockdown resistance (*kdr*)** is especially prevalent against **pyrethroids** – one of the main insecticide classes used.
- There is a lack of evidence of the resistance levels *kdr* mutations confer for **males and heterozygous mosquitoes** [1].
- Genotype-specific and sex-specific resistance measurements** of *kdr*-containing mosquitoes are needed to better establish the selective pressures on *kdr* mutations in *Aedes aegypti* and predict the **evolutionary trajectory of insecticide resistant mosquitoes**.
- Aim 1:** Establish Window of Selection (WoS) – range of insecticide concentrations that exert positive selection for resistance – for *Ae. aegypti* and deltamethrin.
- Aim 2:** Determine level of resistance and dominance of *kdr* mutations present.

## Knockdown resistance (*kdr*)

- Pyrethroid resistance** can be conferred via knockdown resistance (*kdr*) mutations that cause confirmation changes or reduced binding sensitivity to voltage gated sodium channels (VGSC).
- V1016I** and **F1534C** are two *kdr* mutations associated with high levels of resistance.
- A recently discovered *kdr* mutation in *Ae. aegypti* is **V410L** [2] which has been found in both susceptible and resistant strains [3].
- V410L, V1016I and/or F1534C are frequently found together in North and South America [4].

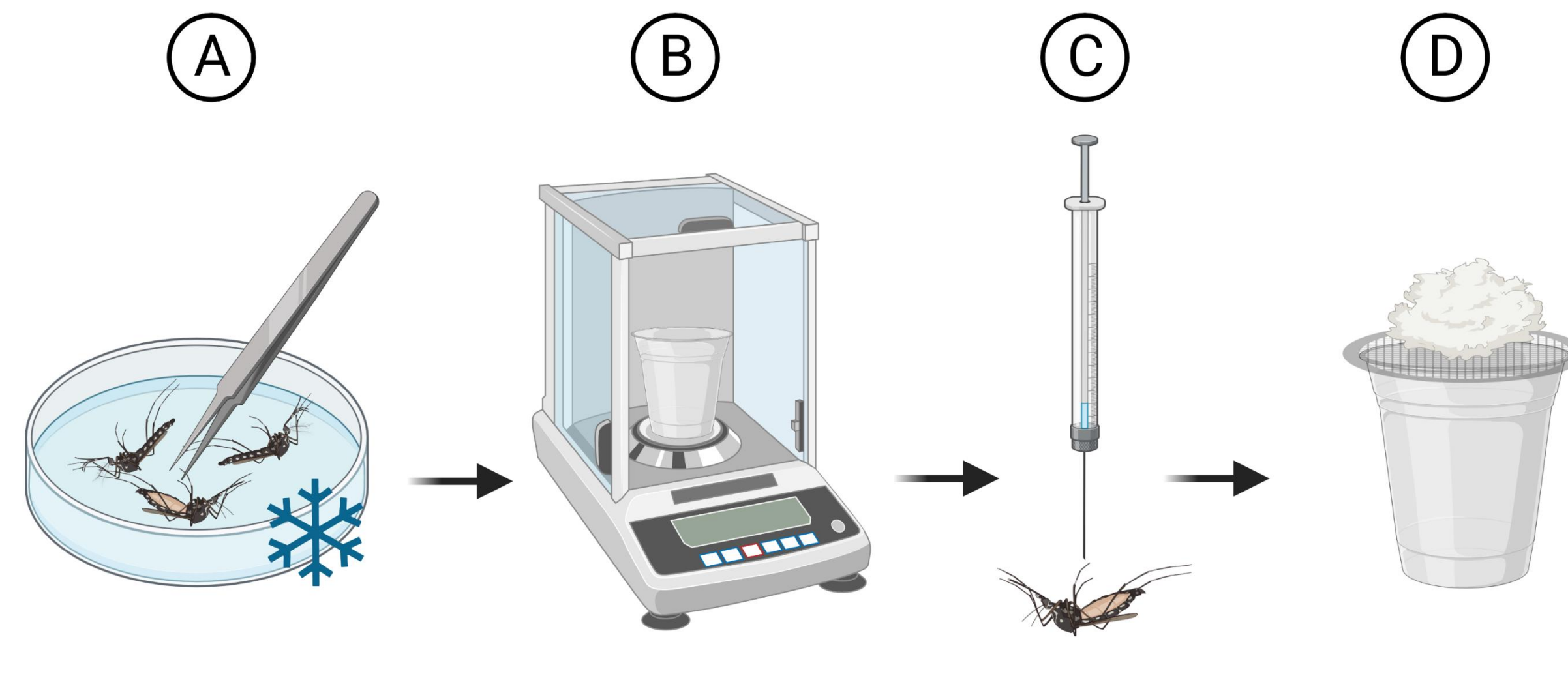


**Figure 1:** *Ae. aegypti* VGSC with three *kdr* mutations identified. Figure from [5].

**Table 1:** *Ae. aegypti* strains included in this study and their genotype at three loci (V410L, V1016I and F1534C respectively); S = susceptible allele, R = resistant allele. All strains, except for Rockefeller (ROCK), were isolated from St. Augustine (St.A.), Florida (2016).

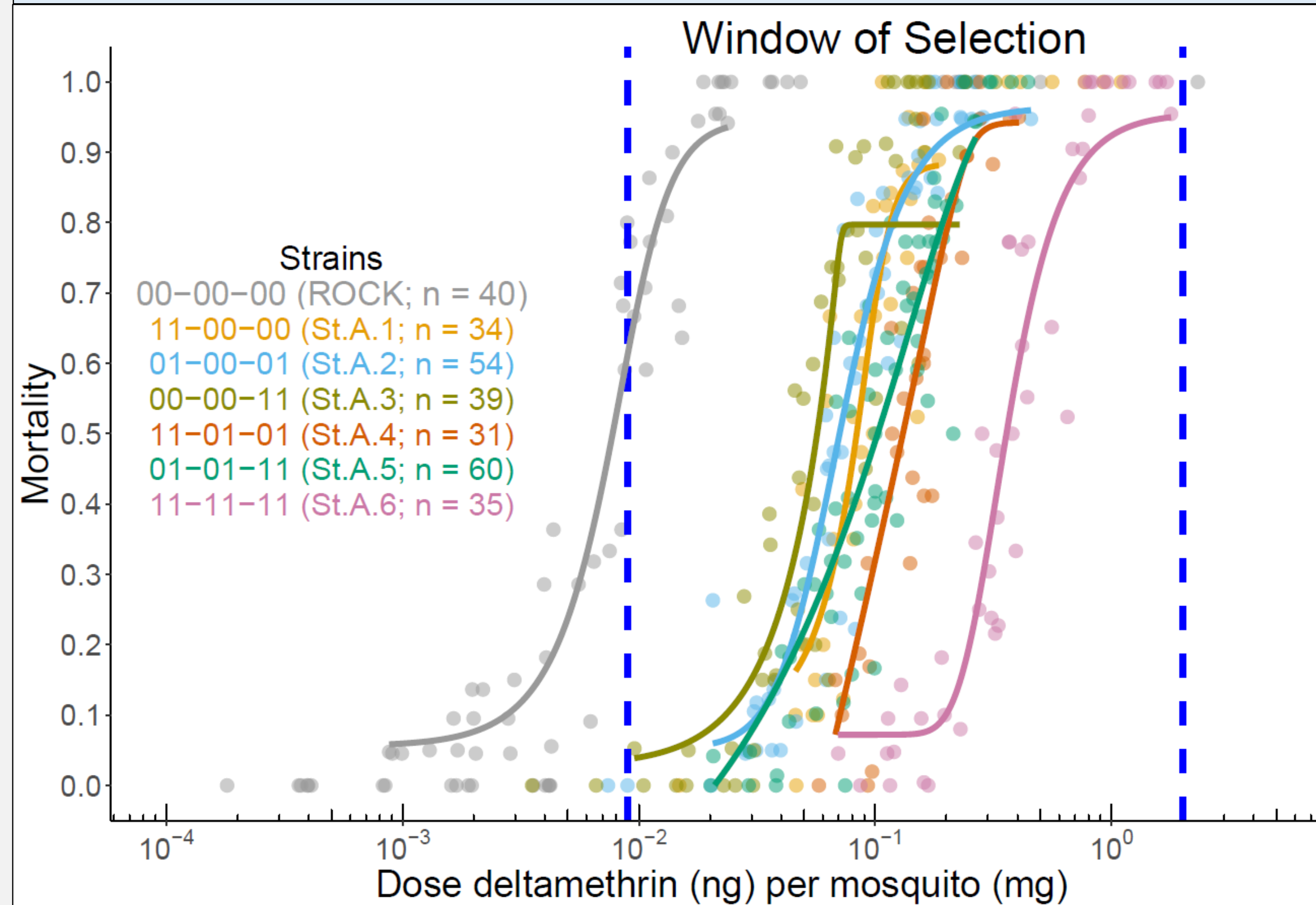
Strain	Locus 1 (410)	Locus 2 (1016)	Locus 3 (1534)	Total <i>kdr</i> mutations	Genotype CODE
ROCK	SS	SS	SS	0	00-00-00
St.A.1	RR	SS	SS	2	11-00-00
St.A.2	SR	SS	SR	2	01-00-01
St.A.3	SS	SS	RR	2	00-00-11
St.A.4	RR	SR	SR	4	11-01-01
St.A.5	SR	SR	RR	4	01-01-11
St.A.6	RR	RR	RR	6	11-11-11

## Methods



**Figure 2:** Topical application bioassay methodology. (A) mosquitoes are knocked down with cold, counted, and sorted. (B) cups of 15-25 three-to-six-day old non-blood fed females (or males) are weighed. (C) insecticide droplet (0.5 uL) is applied to ventral side of mosquito at the thorax-abdomen joint. (D) sucrose is provided to each cup and mortality is assessed 24 hours later. Insecticide used was deltamethrin. Strains of *Ae. aegypti* used seen in Table 1.

## Results



**Figure 3:** Dose-response curves of seven *Ae. aegypti* strains (see legend and Table 1) exposed to deltamethrin. Insecticide dose was calculated by ng deltamethrin per mg of mosquito mass. Data show males and females combined for each strain. Trendline shows the 5-parameter logistic regression model fit for each strain. N-values include only data points where mortality was greater than 0% and less than 100%. Window of Selection bounds are indicated with blue dotted lines and range from LD99 of St.A.6 to LD01 of St.A.3.

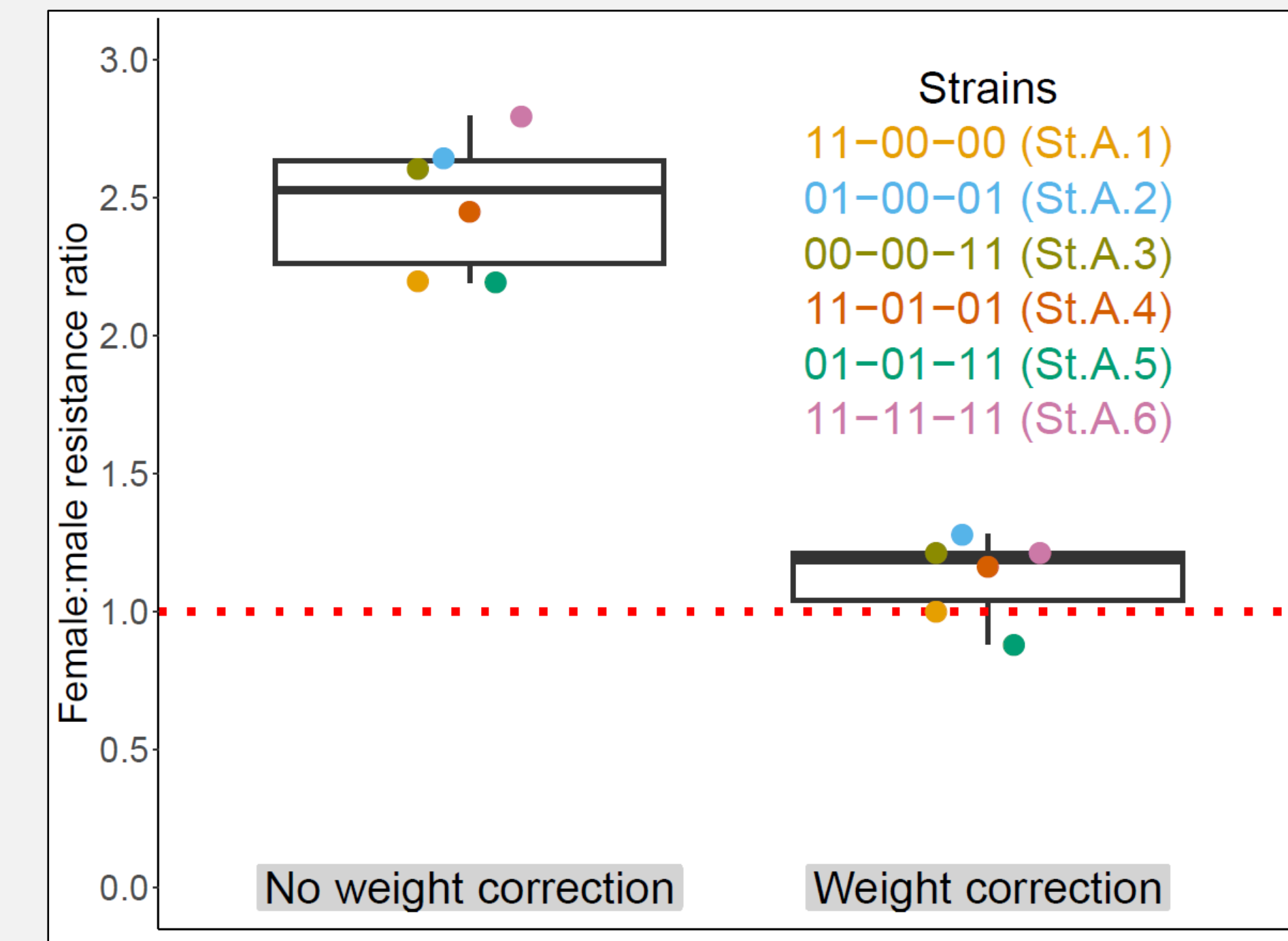
## Acknowledgments

This research was supported by a CAREER award by the National Science Foundation (NSF) to SH under award number 2047572, as well as an NSF Collaboration grant, award number 2052363. We thank the Centers for Disease Control and Prevention for sharing the Rockefeller strain and the United States Department of Agriculture Center for Medical Agricultural and Veterinary Entomology for sharing the six *kdr* isolate strains. Figure 2 was created with BioRender.com.

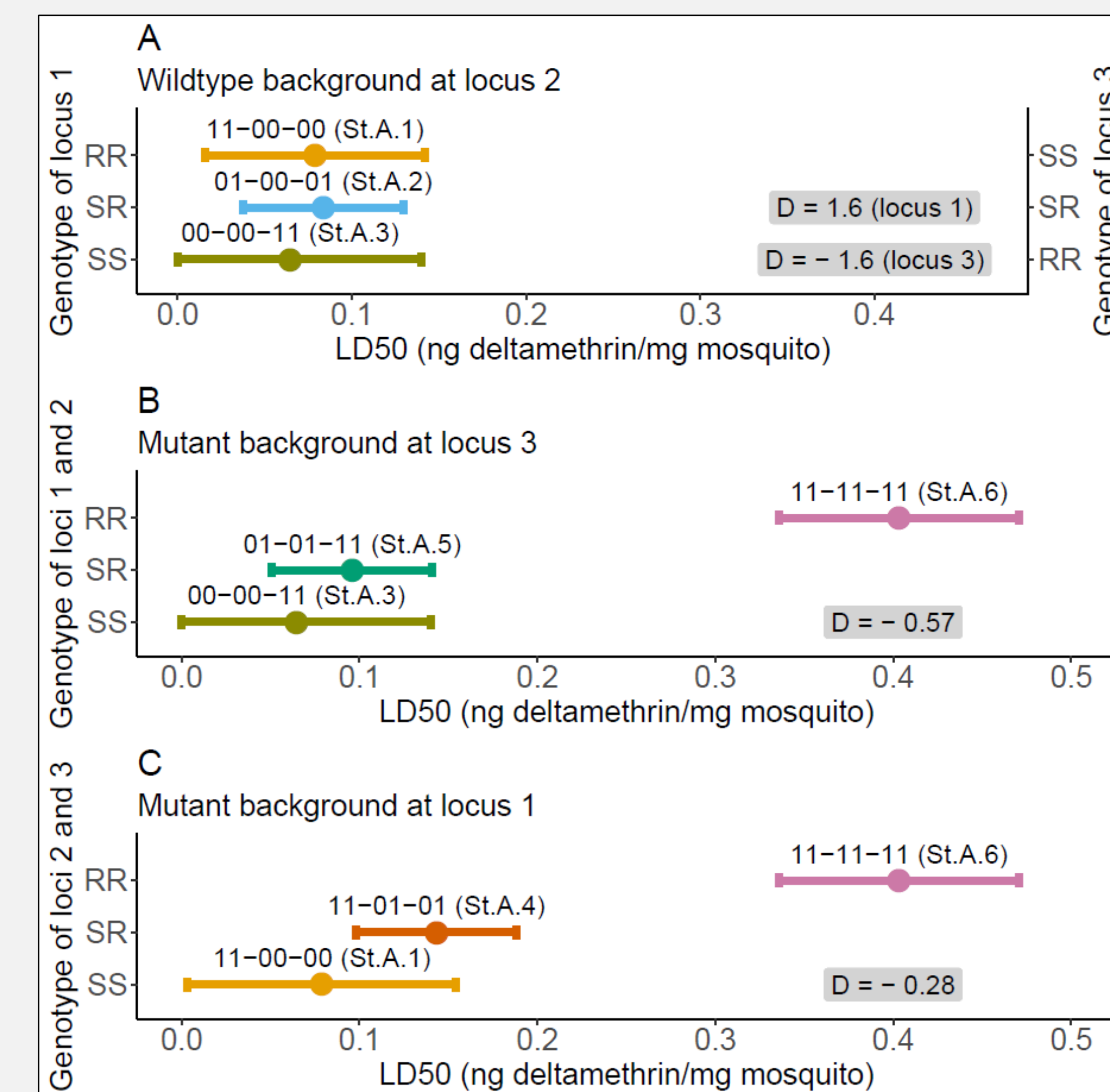
## Literature Cited

[1] South et al., 2020, *Evol. Appl.* [2] Haddi et al., 2017, *Sci. Rep.* [3] Mack et al., 2021, [4] Fan et al., 2020, *PLoS Negl. Trop. Dis.* [5] Saavedra-Rodriguez et al., 2018, *Sci. Rep.* [6] Stone, 1968, *Bull.*

## Results



**Figure 4:** Female:male resistance ratio (RR) comparisons with or without weight correction for dose of deltamethrin (ng) per mosquito (mg). ROCK used to calculate RR for six St.A. strains. Each point represents RR value of a strain.



**Figure 5:** Lethal dose values to kill 50% (LD50) and degree of dominance (D) values. Homozygous SS vs. heterozygous (SR) vs. homozygous RR compared for (A) locus 1 allele (primary y-axis) and locus 3 allele (secondary y-axis) with wildtype background at locus 2; (B) loci 1 and 2 haplotype with mutant background at locus 3; (C) loci 2 and 3 haplotype with mutant background at locus 1. D value calculations following [6].

## Conclusions

- The WoS covers a wide range of dosages of deltamethrin, meaning there is greater potential for selective pressure of resistance to occur in the field.
- An increase in resistance levels correlates with an increase in *kdr* mutations.
- When correcting for weight differences, male and female resistance level differences become almost negligible.
- The level of resistance is greatest for the triple resistant haplotype, as well as the level of dominance (see figure 5C).
- Kdr* mutations at **locus 2** are the **strongest predictor of resistance**.
- The **triple resistant haplotype** poses the greatest **threat to insecticide resistance management**.
- Future work will compare resistance allele frequency change over time for *Ae. aegypti* exposed to different deltamethrin resistance management strategies.

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