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Tricks Not Treats: *Wolbachia*'s Manipulation of Sex in Infected *D. subquinaria* Offspring

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Objectives

- Determine the phenotype of the old *D. recens* *Wolbachia* (wRec) strain in infected *D. subquinaria* crosses
- Determine the presence of a genetic suppressor of Male Killing in *D. subquinaria*

Cytoplasmic incompatibility:

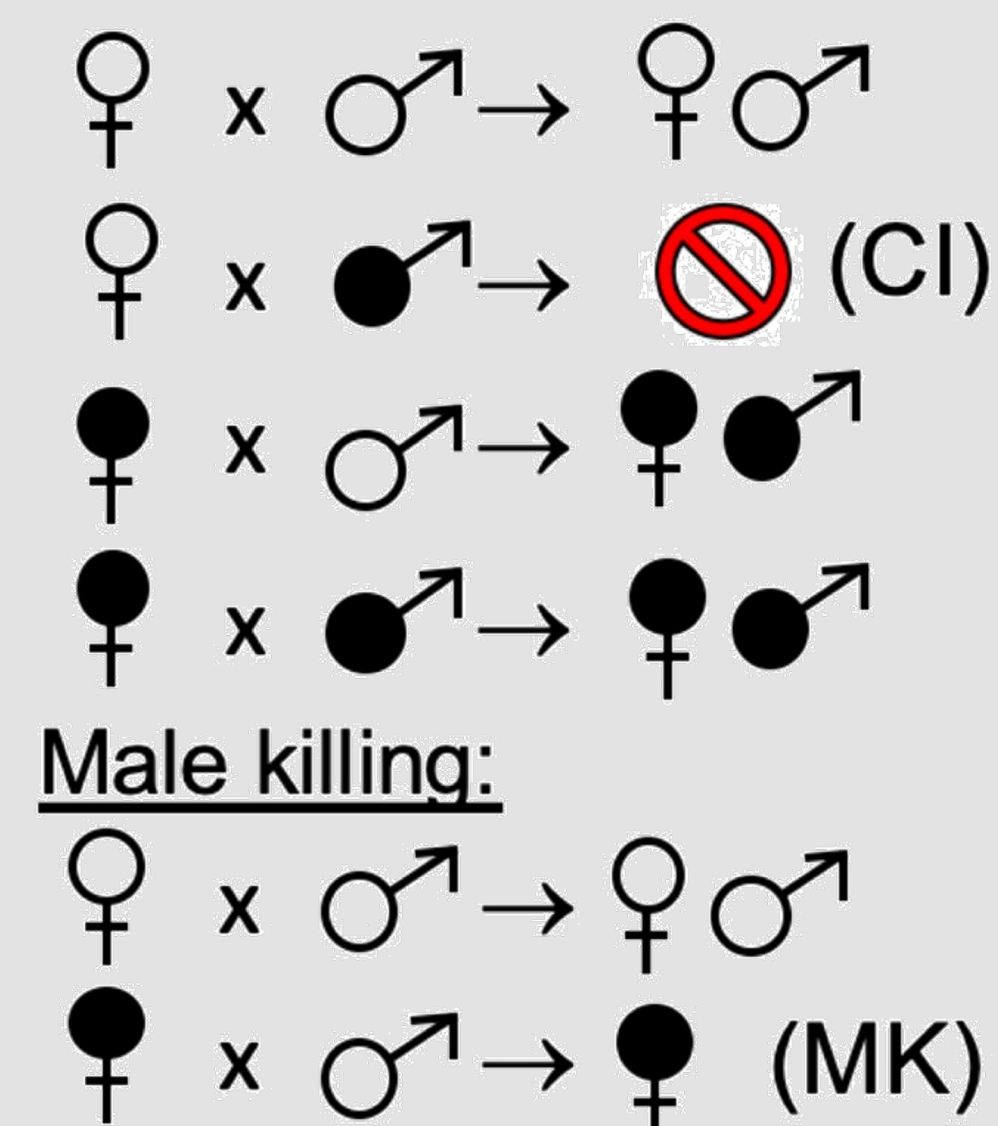


Figure 1

Cytoplasmic incompatibility causes the death of most infected offspring. Offspring have equal chances of being male/female

Male Killing:
The death of the sons of infected mothers

Methodology

- *Wolbachia* was introgressed from *D. recens* into *D. subquinaria* using backcrossing. This generates *D. subquinaria* females with *Wolbachia* infection
- Crosses were set with 2-3 infected *D. subquinaria* females, and 1 uninfected *D. subquinaria* male
- We tested 15 total lines of *D. subquinaria*,
- F1 offspring (~14 days) were collected and transferred into agar vials, where they are stored at room temperature
- We sexed each offspring by anesthetizing them with CO₂ and then looking at them under a microscope
- To verify that the offspring were infected with *Wolbachia*, we used PCR

References

- Hornett, Emily A, Daisuke Kageyama, and GDD Hurst. 2022. "Sex Determination Systems as the Interface Between Male-Killing Bacteria and Their Hosts." *Proceedings of the Royal Society B* 289 (1972).
- Kaur, Rupinder, J. Dylan Shropshire, Karissa L. Cross, Stewart Victoria, and Seth Bordenstein. 2021. "Living in the Endosymbiotic World of *Wolbachia*: A Centennial Review." *Cell Host & Microbe* 29 (6): 879–93.
- Figure 1 – Kelly Dyer

Results

- Four crosses failed to reproduce, and 1 cross failed to reproduce a significant number of flies (n>10)
- Of the 10 lines that produced offspring 4 showed no evidence of a suppressor and 6 indicate a suppressor is present

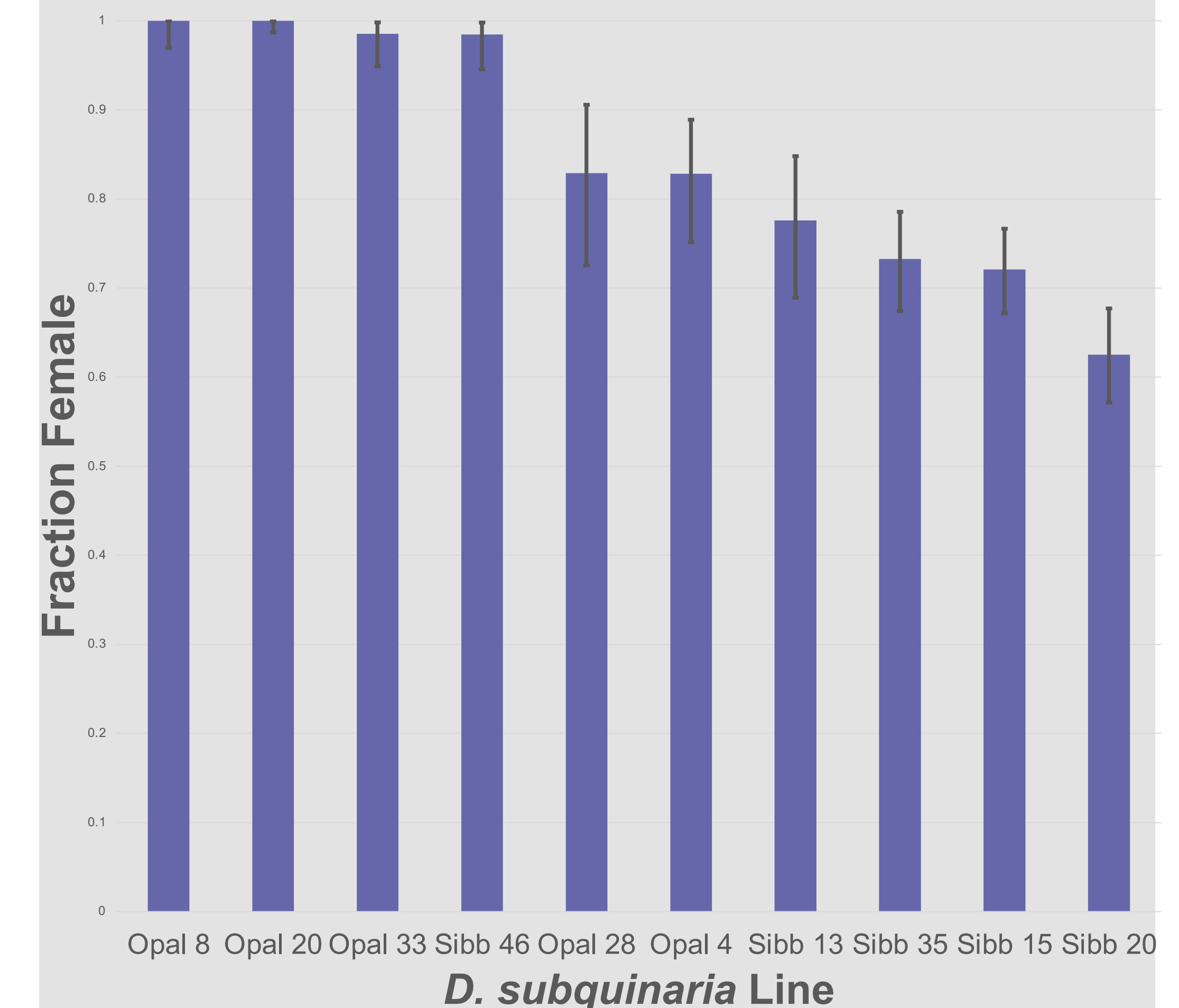
Male Population	Percent Female	Percent Male
Opal 8	100	0
Opal 20	100	0
Opal 33	98.56	1.44
Sibb 46	98.46	1.54
Opal 28	82.89	17.11
Opal 4	82.81	17.19
Sibb 13	77.59	22.41
Sibb 35	73.26	26.74
Sibb 15	72.1	27.9
Sibb 20	62.54	37.46

Fig. 2: table comparing the percentages of male and female offspring per line

Conclusions and Future Directions

- *D. recens* crosses were positive for *Wolbachia* as expected
- None of the tested lines expressed a complete MK suppressor
- Use PCR to determine whether the males present were infected with *Wolbachia* and survived or escaped infection and were not subject to MK
- Allow the F1 generation to reproduce to determine if males are sterile

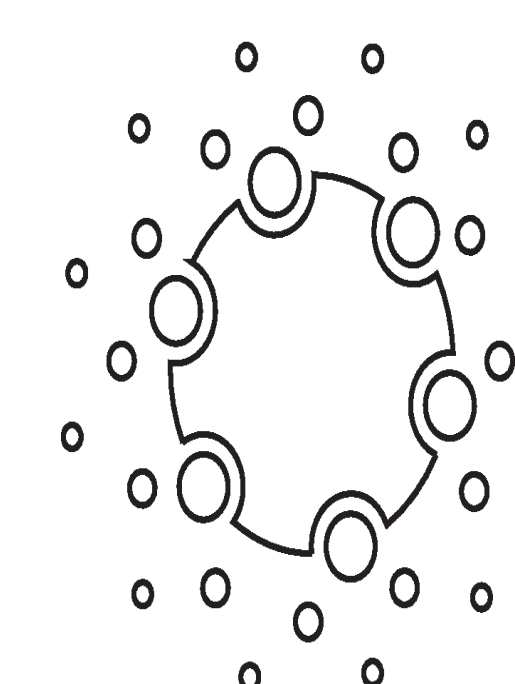
Distribution of infected Female offspring



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