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Taste of Attraction: Observing the effects of starvation resistance on mating success in D. melanogaster

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TASTE OF ATTRACTION

Observing the effects of starvation resistance on mating success of *D. melanogaster*

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INTRODUCTION

- Experimental evolution was utilized to generate starvation resistant flies (*Drosophila melanogaster*, Meigen 1830) with enhanced response to starvation. Selection for survival of **starvation resistant flies** (S flies) on a non-caloric agar diet was compared to a population of **fed control flies** (F flies).
- Increased survival of the S flies is partly due to the exaggerated elevation in lipid levels. The **excess lipid storage** is due to extended larva feeding.
- Selected flies display several adaptive traits, such as **increased body size** and **pheromone production**, but they also have **reduced fecundity**, suggesting a trade-off between starvation resistance and reproduction.
- To distinguish between the effect of genetic changes due to the evolutionary selection and the acquired obesity, we set up a **'rescue' experiment** to reduce flies' weight without changing their genetics. This results in **smaller but viable adults**.
- Here we removed larvae from food prematurely to restore their 'normal' weight and measured their mating success compared to genetic and handling controls.
- We set up a **'pheromone' exchange experiment** to alter pheromone composition
- Here we vortexed F flies with dead starvation resistant flies to observe pheromone transfer and its effect on mating

HYPOTHESIS

1. Starvation resistant flies that with **lower weight** will **increase mating success.**
2. Transfer of pheromones from starvation resistant flies onto fed control flies will **decrease** mating success of the fed control flies

METHODS

EXPERIMENT 1: Flies on a Diet

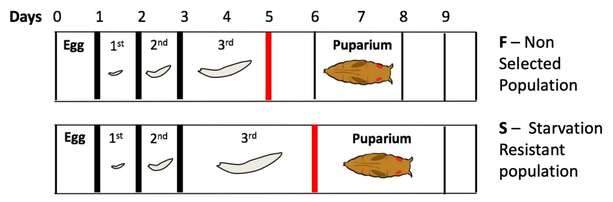

Fig 1. Larval Development. Collection begins once Non-selected larvae would begin to Wander which is typically around day 5. Since Starvation resistant flies are 1-2 day behind developmentally, they were scooped at the time non-selected flies would wander. This is the "rescue."

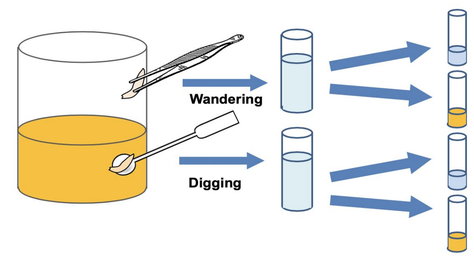

Fig 2. Washing, Scooping and Plucking Larvae. Larvae were scooped with a small spatula or plucked with a tweezer and placed into density vials. These vials consists of 10 ml PBS and 20 ml of 20% sucrose. After being washed the plucked wandering larva or scooped larva will be put into either food or agar

EXPERIMENT 2: Pheromone Transfer

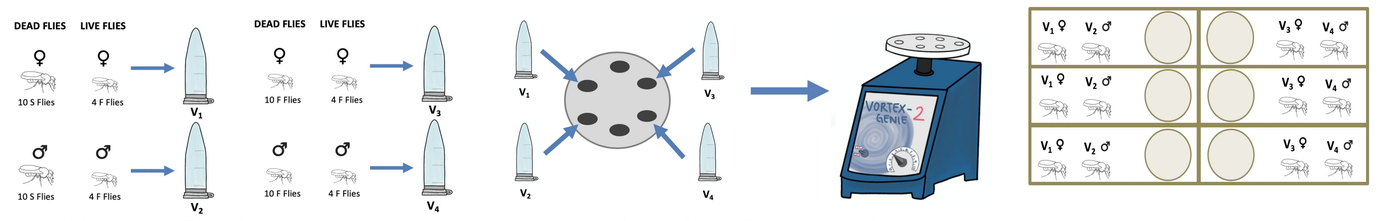

Fig 3. Pheromone Transfer. In order to commence pheromone transfer, 10 **S** or **F** flies that were frozen were placed in an eppendorf tube with 4 living **F** flies. The dead flies were frozen 1-2 days after hatching. $\mathbf{V_1}$ = Live female F flies with dead female S flies. $\mathbf{V_2}$ = Live male F flies with dead male S flies. $\mathbf{V_3}$ = Live female F flies with dead female F flies. $\mathbf{V_4}$ = Live male F flies with dead male F flies. Flies were vortexed at a **speed of 4** for **20 seconds** three times with a **20 second rest** in between pulses (Grillet 2012). Once they are all vortexed, flies were allowed to recover for 30-45 minutes before placing them in mating chambers. After the recovery period, flies were pipetted into mating chambers were copulation was recorded under conditions of 22°C and 80% Humidity for 10 minutes.

RESULTS

EXPERIMENT 1: Flies on a Diet

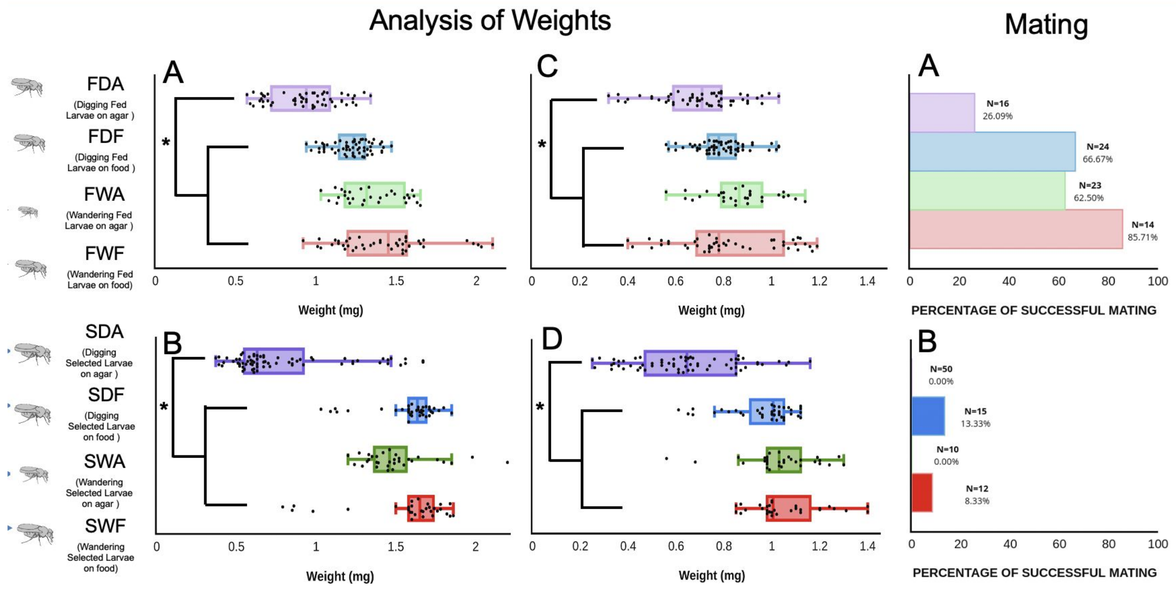

Fig 4. Differences in weight distribution vary A, B) Female weight of the four-treatment group. **(A)** The FDA group has significantly decreased weight from all other groups. (**B**) The SDA group has significantly decreased weight from all other groups.
C, D) Male weight of the four-treatment group. **(C)** The FDA group has significantly decreased weight from all other groups. **(D)** The SDA group has significantly decreased weight from all other groups. A one-way ANOVA and tukey's multiple comparison test was utilized (* - $p<0.05$)

Fig 5. Copulation Success. Mating success was observed under 10 minutes. **(A)** FWF, FWA, and FDF demonstrated mating success above 50%. **(B)** SWA and SWF both had low mating success which ranged from 0-8%. The S population that was rescued (scooped and placed on agar, SDA) showed no success in mating.

EXPERIMENT 2: Pheromone

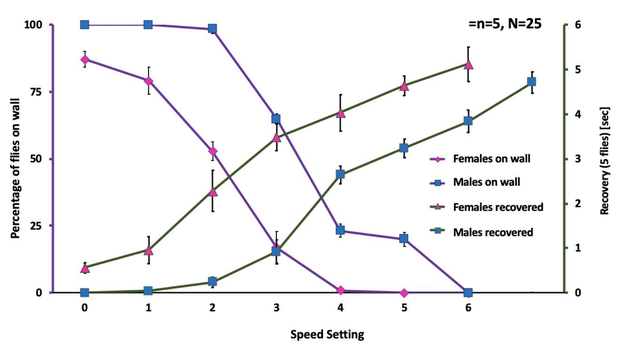

Fig 6. Vortex Method. In order to determine what speed would yield the best results for the pheromone transfer we vortexed males and females separately to see if they are have different tolerances to the change in speed. Females were quick to recover at a speed of 2-3 with less than 50% of flies on the wall. Males were quick to recover at a speed of about 4 with less than 50% of flies on the wall. Ultimately to maintain consistency, we decided to move forward using a speed of 4 for both males and females.

Male Vortex Speed	Female Vortex Speed	Recovery Time (min)	Mated Pairs
2	2	> 15	0/6
3	3	>15	0/6
4	4	>15	0/6
3	4	>15	0/6
2	4	>15	0/6
2	3	30 – 45	4/6
4	4	30 – 45	4/4

Fig 7. Determining Recovery Time. In order to further pinpoint the best speed and recovery time that will allow for the high copulation we vortexed males and females at varying speeds three times for 20 seconds times with 20 second rest between each pulse. We either allowed them to recover after being vortexed for less than 15 minutes or 30-45 minutes. Flies mated when given more than 30 minutes to recover after being vortexed.

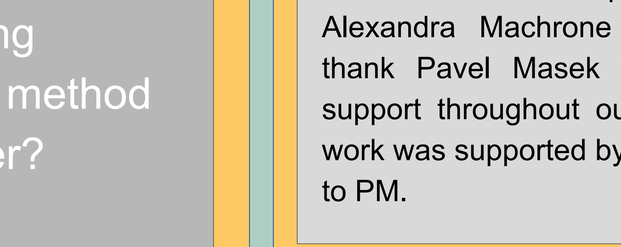

Fig 8. Pheromone Transfer and Copulation. Following the methods denoted in Figure 3, F flies were mated after being vortexed with either dead S or F flies. There was not variability in the percent of successful mating due to flies being vortexed with either dead S or F flies. Both groups resulted in 100% successful mating.

CONCLUSIONS AND FUTURE RESEARCH

Experiment 1

1. Removing flies early from food reduces their weight.
2. Reducing the size of the obese flies does not restore normal mating

Experiment 2

Vortexing live F flies with dead S flies in order to have pheromone transfer does not negatively impact copulation

1. Is mating affected by phenotypic differences between S and F flies
2. Do dead fruit flies lose their pheromone?
3. Would stripping S fruit flies pheromone and coating eppendorf be a better method for pheromone transfer?

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NSF