The role of seminal fluid proteins in speciation

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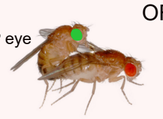
1 Does knock-down of seminal fluid genes in *D. melanogaster* affect sperm competitiveness?

Sperm Competition Assays

P_1
Virgin females first mated with knock down males.

After two days of isolation, mated females second mated with GFP eye males.



OR

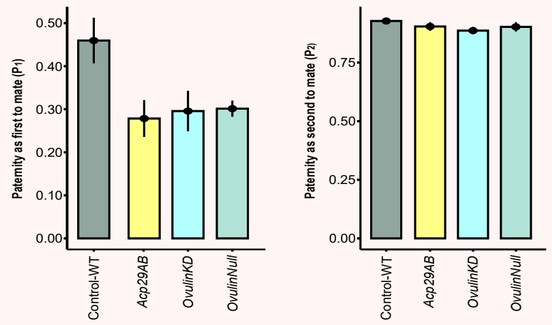
P_2
Virgin females first mated with GFP eye males.

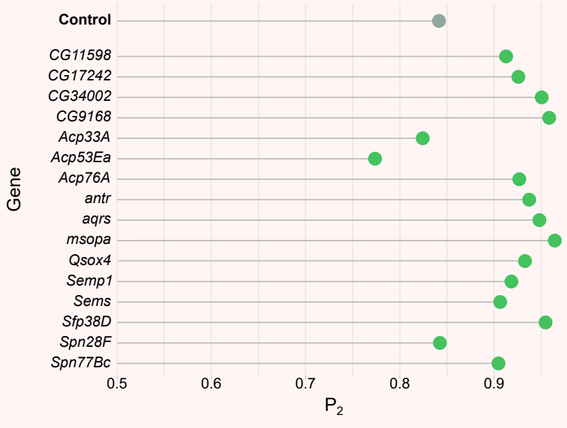
with knock-down males.

FOLLOWING, paternity assessment was done by counting the offspring from each female as GFP expressing or not.

Results

We first tested efficiency of our double mating assays using two genes, ***Ovulin*** and ***Acp29AB***, known to affect P_1 but not P_2 [1-2].





Knockdown of SFP genes show no significant effects on second male paternity success (P_2) when compared to control. Green plots show average P_2 value of knock-down males and grey control males.

P_1 assays are ongoing...

Introduction

Post-mating male-male competition known as **sperm competition** drives rapid diversification of male ejaculate that can be a driver for speciation. **Conspecific sperm precedence (CSP)** is a taxonomically widespread form of reproductive isolation mediated by sperm competition between sperm of males from different population/species. So far, the selective causes and ejaculate traits responsible for CSP are poorly understood. **Seminal fluid proteins (SFPs)** that are transferred during mating along with sperm have diverse function conferring male advantage in sperm competition and can be one major factor affecting CSP among species. Although a substantial number of studies have been conducted on SFPs of common model organism *Drosophila melanogaster*, only a handful of genes were identified having function in sperm competition and/or CSP in this species. In this project we expand assays of SFPs in sperm competition, with the ultimate goal of determining their role in CSP and speciation.

2 Do the genes involved in sperm competition have a role in reproductive isolation?

Gene selection: Genes involving sperm competition [2]:

Gene	Experimental evidence	Affecting CSP
Acp62F	*Gp, Ga*	?
Lectin-46Cb	*Gp, Ga, Gc*	?
Lectin-46Ca	*Gp, Ga, Gc*	?
Ovulin	*Gp, Ga, Gc*	?
CG17575	*Gp, Ga, Gc*	?
Acp29AB	*Gp, Ga, Gc*	?
Acp36DE	*Gp, Ga, Gc*	yes
CG9997	*Gp, Ga, Gc*	yes

Gp: Gene perturbation revealed role in sperm competition / fertilization
Ga: Gene association revealed relation with paternity success and/or fecundity
Gc: Gene correlation in expression with a known CSP gene CG9997

P_1 & P_2 (CSP) assays are ongoing...

References:

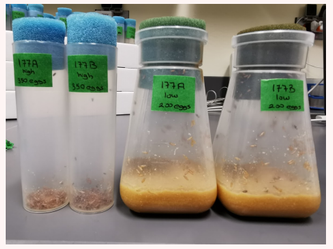
1 Wong, Alex, et al. "A role for Acp29AB, a predicted seminal fluid lectin, in female sperm storage in Drosophila melanogaster." Genetics 180.2 (2008): 921-931.
2 Civetta, Alberto, and Jose M. Ranz. "Genetic Factors Influencing Sperm Competition." Frontiers in genetics 10 (2019).
3 Castillo, Dean M., and Leonie C. Moyle. "Intraspecific sperm competition genes enforce post-mating species barriers in Drosophila." Proceedings of the Royal Society B: Biological Sciences 281.1797 (2014): 20142050.

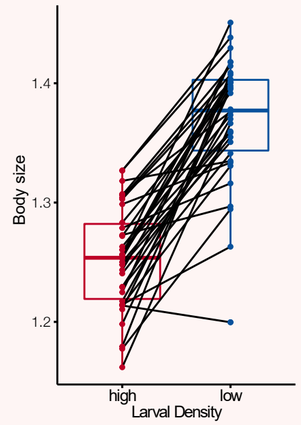
We thank M. Wolfner (Cornell, USA) for providing ovulin-driver flies, L. Fulham for helping to maintain DGRP lines.

3 Does genotype-by-environment interaction (GEI) influence seminal fluid gene expression and CSP outcomes?

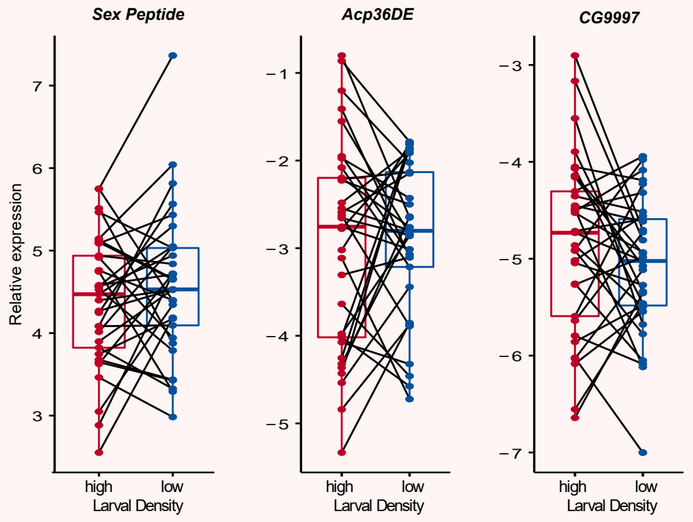
GEI for genes affecting CSP; *Acp36DE* and *CG9997* [3] may be variable in their effect on reproductive isolation depending on enviromental heterogentiy and genotypic variation.

Eggs were collected and reared either in high density (1-2 ml food for 350 eggs) or low density (50 ml food for 200 eggs) from each DGRP inbred line (34 lines in total)





We found strong genotype effect for expression of all genes, however, Neither CSP genes nor SP exhibit significant GEI, perhaps due to low statistical power, however their response to different environment changes.



CSP assays are ongoing...