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Live Q&A: **Monday, 4/27 2-2:30 pm**

Octopamine is required for the female modulation of sperm competition in *Drosophila melanogaster*

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Introduction

In polyandrous species, competition between rival male sperm for opportunities to fertilize a female's eggs (i.e. sperm competition) is an important aspect of postmating sexual selection. In *Drosophila melanogaster*, sperm properties and seminal fluid proteins are important for a male's competitive ability, but the mechanisms by which females sense and respond to male qualities are less well understood. We previously showed that when three developmental or neural function genes (*caup*[1], *hid*[2] and *Rab2*[3]) were each knocked down in octopaminergic *Tdc2*[+] neurons in doubly-mated females, the outcome of sperm competition was altered[4]. We hypothesize that these genes' knockdowns affect *Tdc2*[+] neurons and octopaminergic (OA) signaling, which in turn affect sperm competition. **Here, we investigate the role of the female's OA signaling in modulating sperm competition.**

Method

Two main types of assays performed in this study are outlined below.

Mating plug ejection assay:

Mating plug ejection status is scored as 3 categories based on the location of sperm and/or mating plug:

Status	Sperm and mating plug location
Unejected	Sperm mass and plug in bursa
Partial	Mating plug partially outside ovipositor
Complete	Sperm stored in storage organs; no sperm mass or plug in bursa

n=13.9±1.43 for each treatment

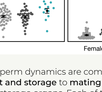
Fertility and sperm competition assay:

In each plot below, *n* is indicated as the number of dots.
Stored sperm is counted manually and/or with a custom Fiji script.

Statistical analysis is performed in R. Statistical significance at the 0.05 level between pairwise comparisons is shown with compact letter display (groups that share a letter are not significantly different from each other).

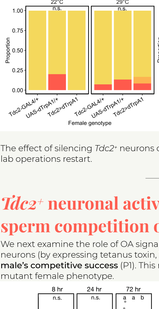
Octopamine and tyramine are required for a wildtype sperm competition outcome

In *Tdc2*[+] octopaminergic neurons, OA is synthesized from a tyrosine precursor by the following biosynthetic pathway:

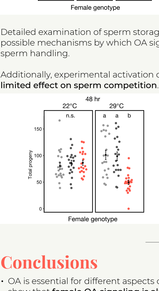


To ask if OA and TA are involved in sperm competition, we first compare **sperm storage and release dynamics** between **doubly-mated OA- and TA-less** *Tdc2*[RO54]*/Df(2R)42* mutant females[5] and control females at 1, 4 and 10 days after mating. The figures below show first- and second-male sperm counts in each sperm storage organ and the total.

Tdc2[RO54]*/Df(2R)42* mutant females store **more first-male sperm** at **one day after second mating**, resulting in a higher PI (defined as first male progeny / total progeny).



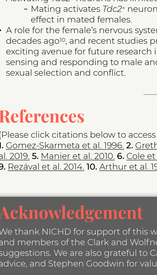
At **10 days after second mating**, *Tdc2*[RO54]*/Df(2R)42* mutant females **deplete first-male sperm** to control levels but **retain more second-male sperm**. They also retain more sperm overall, consistent with the sperm retention defect previously reported in singly mated females[5].



Postmating sperm dynamics are complex and involves multiple steps, from **sperm displacement and storage** to **mating plug ejection**, and later to gradual **sperm release** from storage organs. Each of these steps can influence the outcome of sperm competition[5,6]. Which steps are affected in *Tdc2*[RO54]*/Df(2R)42* mutants? In addition, are these effects attributable to *Tdc2*[+] OA neurons?

Experimental activation of OA neurons might have limited effect on mating plug ejection

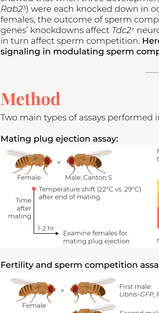
Activating *Tdc2*[+] neurons (with the temperature sensitive cation channel *dTrpA1*) does not have detectable effects on mating plug ejection 1 or 2 hours after mating. This might be because *Tdc2*[+] neurons are activated after mating, and expressing *dTrpA1* has little additional effect.



The effect of silencing *Tdc2*[+] neurons on mating plug ejection will be examined once lab operations restart.

Tdc2[+] neuronal activity affects fertility and sperm competition outcome

We next examine the role of OA signaling in reproductive output. Silencing *Tdc2*[+] neurons (by expressing tetanus toxin, *TNT*) reduces fertility[9] and **increases the first male's competitive success** (PI). This result recapitulates the *Tdc2*[RO54]*/Df(2R)42* mutant female phenotype.



Detailed examination of sperm storage and release dynamics will shed light on possible mechanisms by which OA signaling modulates sperm competition through sperm handling.

Additionally, experimental activation of *Tdc2*[+] neurons also reduces fertility, but has **limited effect on sperm competition**.

Conclusions

- OA is essential for different aspects of female reproduction in insects; we further show that **female OA signaling is also involved in sperm competition**.
- Both OA mutant and *Tdc2*[+]-neuron-silenced females have altered sperm competition outcomes, offering the first male higher competitive success (PI).
  - Future experiments will examine OA's role in sperm handling dynamics in greater detail.
  - This will reveal possible mechanisms by which females' OA signaling modulates sperm competition.
- Activating *Tdc2*[+] neurons has limited effects on sperm competition.
  - Mating activates *Tdc2*[+] neurons, and *Tdc2>dTrpA1* likely has little additional effect in mated females.
- A role for the female's nervous system in sperm competition was proposed two decades ago[10], and recent studies provide experimental support[4,11]. This opens an exciting avenue for future research into the female's neuronal architecture for sensing and responding to male and ejaculate quality, and how it evolves under sexual selection and conflict.

References

(Please click citations below to access respective article.)
1. Gomez-Skarmeta et al. 1996. **2.** Grether et al. 1995. **3.** Sasamura et al. 1997. **4.** Chen et al. 2019. **5.** Manier et al. 2010. **6.** Cole et al. 2005. **7.** Avila et al. 2012. **8.** Lüpold et al. 2013. **9.** Rezával et al. 2014. **10.** Arthur et al. 1998. **11.** Chow et al. 2013.

Acknowledgement

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