Mating frequency and sperm oxidative stress in *Drosophila*: a cross-species comparison

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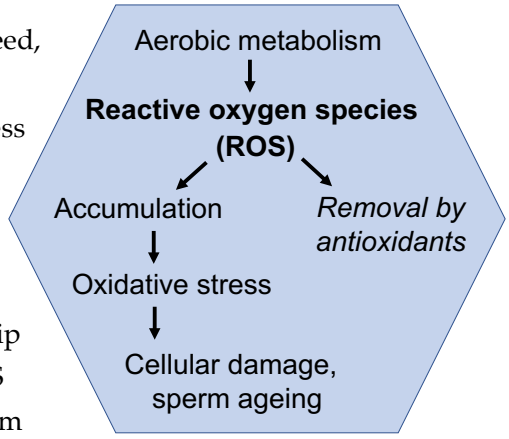
Background: Sperm ageing and sexual selection

- Variance in fertilization success is often unexplained by sperm number, speed, morphology, etc.
- Sperm age is an overlooked character that may influence fertilization success
- The cellular ageing process is accelerated through the over-production of **reactive oxygen species (ROS)** during aerobic metabolism
- Decreasing ROS production in stored sperm should delay its ageing



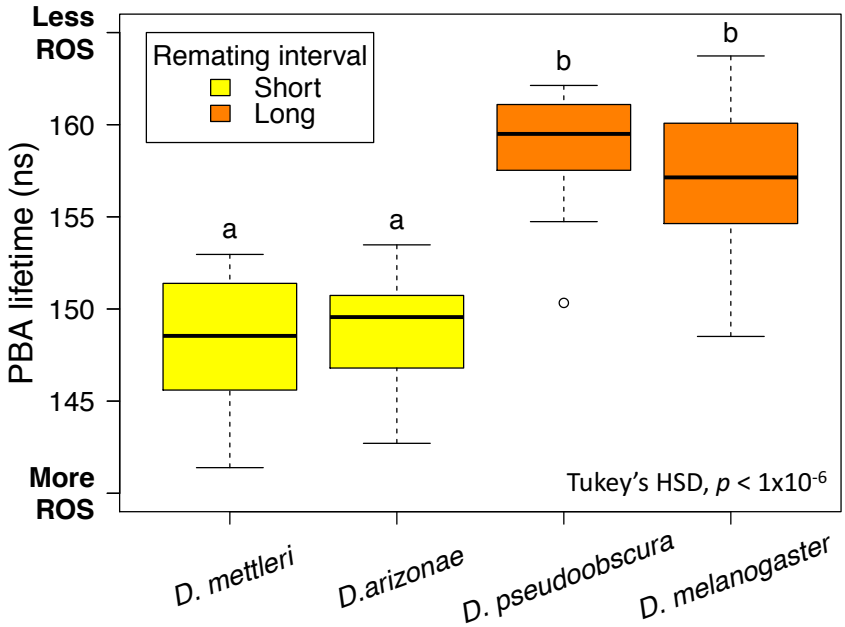
Hypothesis: Species with longer remating intervals are under selection to decrease sperm ROS production

Prediction: Positive relationship between mating rate and ROS production in male-stored sperm

Species	Female remating frequency*	Days fertile (wild-caught females)*
D. mettleri	2x day	1
D. arizonae	daily	6
D. pseudoobscura	3-4 d	15
D. melanogaster	5 d	8

*Data from Markow *et al*. 2012

Result: Lower ROS levels in male-stored sperm among species with longer remating intervals

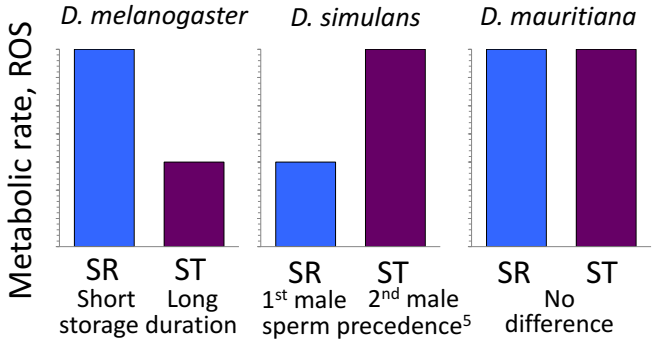


Discussion

- Long remating intervals lead to longer sperm storage in males and females
- Longer sperm storage likely selects for lower ROS levels in order to delay sperm ageing
- Future direction: How do ROS levels change in female storage?

Methods: time-resolved microfluorimetry

- Fluorescent probe: 1-pyrene butyric acid (PBA)
- Fluorescence lifetime decreases with increasing oxygen radical concentration
- Sperm taken from seminal vesicles of virgin males (3-6 d old, n = 15 per species)



Future work: Metabolic rate (MR) and ROS in female-stored sperm in *Drosophila*

- In both crickets[2] and bedbugs[3,4], MR and ROS are lower in female-stored than in male-stored sperm
- Hypothesis: Differential MR and ROS explains differential sperm use patterns in three closely related species

Predictions: seminal receptacle (SR) and spermatheca (ST)

Literature cited: [1]Markow, T.A., *et al*., Fly 6:98-101 (2012); [2]Ribou, A.-C., & K. Reinhardt , Proc. R. Soc. Lond. B 279:2196–2203 (2012); [3]Reinhardt, K., & A.-C. Ribou, Sci. Rep. 3:2888 (2013); [4]Reinhardt, K., *et al*., J. R. Soc. Interface 12:20150609 (2015); [5]Manier, M., *et al*., Curr. Biol. 23:1853-1862 (2013)