# 2 Master projects – When survival is not enough

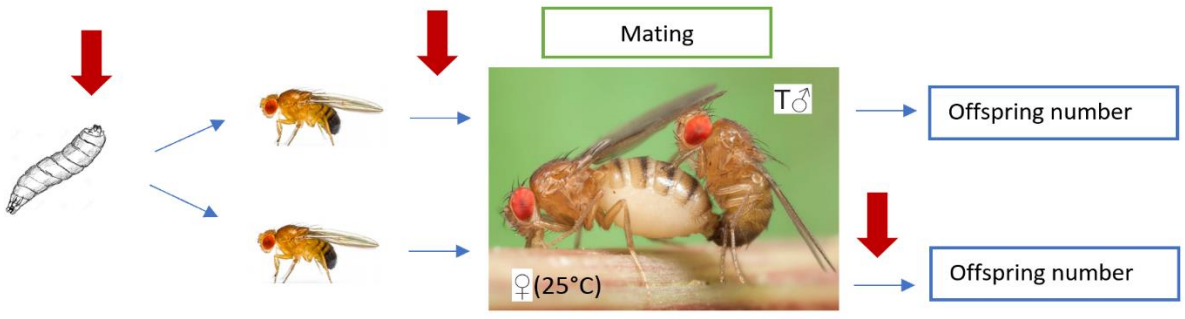
We offer two master projects focused on sex differences in the effects of high temperature on fertility. The predicted increase of temperatures due to climate change will result in more frequent occurrences of heat waves, with expected negative effects on species reproduction and persistence. Previous research has shown low reproductive success in the face of a single heat shock episode in *D. melanogaster* males, but little is known about the fate of sperm when it is stored inside the females. Further, the effects of repeated exposure are unknown. A first exposure might prepare and harden an animal for subsequent exposure episodes with less detrimental effects. Thus, in the two projects we want to evaluate these questions and investigate the consequences of repeated exposure episodes on male and female fertility (Project 1). Additionally, we want to investigate sperm fate by evaluating sperm storage processes after either the male or the female experienced a heat wave.

**Project 1. Effect of repeated heat shocks on *D. melanogaster* reproductive traits. Has an exposure to high temperatures during development a beneficial effect when facing a second exposure as an adult?**

Both males and females will be exposed to a series of heat waves in separated experiments and the effects on reproductive success will be investigated.

By exposing males and females to a heat shock, we will be able to compare whether and how their thermal sensitivity differs. Often spermatogenesis is more thermosensitive than oogenesis (David et al., 2005) and even though there are some insights in the effects of elevated temperatures on male fertility little is known about females. By applying heat shocks at various stages, we can test whether hardening occurs and whether this protects animals from the detrimental effects experienced after a single exposure and they can remain fecund and successfully reproduce.

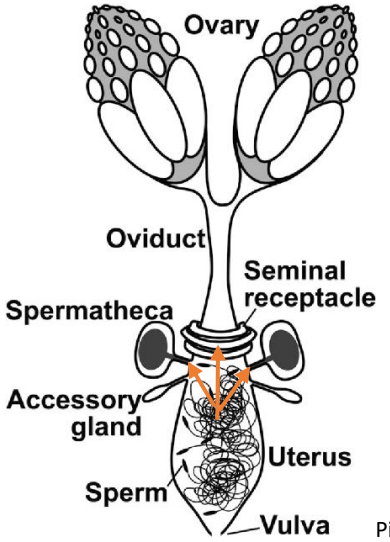
**Box 1**. **Scheme mating experiment**. The red arrows indicate heat shock exposure, while T♂ indicates male exposure treatment. The same procedure is planned for females.



**Project 2. Sperm transfer and sperm storage at high temperatures. Effects on sperm transfer and the sperm storage processes under challenging conditions.**

To further understand the impact of elevated temperatures on male and female fertility we want to study the fate of sperm inside the female reproductive tract and study sperm transfer and storage after heat shock. Previous research has demonstrated that high temperatures affect the male reproductive tract (e.g. accessory glands and testes), causing cytological abnormalities, damaging sperm, altering the transfer of seminal fluid proteins during mating and causing sterility at high temperatures (David et al., 2005). All these factors are essential for reproductive success. Even though males exposed to high temperatures are able to mate, we have few information about the processes involved during and after mating. Thus, with this project we want to determine if males exposed to elevated temperatures can transfer sperm successfully to the female (grown and kept at standard conditions) during mating. We will identify and count sperm after mating in the lower female reproductive tract by using the methodology described in Lüpold et al., 2011, see also Box 2 for more details.

**Box 2. Drosophila melanogaster female reproductive tract**




Picture from Schnakenber et al. (2012)

Methodology used: We will allow pairs to mate and 90 minutes after the mating the females will be frozen for analysis. This time reduces the risk of sperm ejection and allows the sperm to migrate from the uterus to the female's sperm storage organs (spermatheca and the seminal receptacle) (Lüpold et al., 2011).

Interested candidates please contact:

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Further reading:

David, J. R., Araripe, L. O., Chakir, M., Legout, H., Lemos, B., Pétavy, G., … Moreteau, B. (2005). Male sterility at extreme temperatures: A significant but neglected phenomenon for understanding Drosophila climatic adaptations. *Journal of Evolutionary Biology*, *18*(4), 838–846. https://doi.org/10.1111/j.1420-9101.2005.00914.x

Lüpold, S., Manier, M. K., Ala-Honkola, O., Belote, J. M., & Pitnick, S. (2011). Male Drosophila melanogaster adjust ejaculate size based on female mating status, fecundity, and age. *Behavioral Ecology*. https://doi.org/10.1093/beheco/arq193