Developmental and molecular analysis of sperm production in Drosophila

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Project description:

This project links developmental biology and determination of cell fate, through transcriptional controls that interpret that cell fate decision and control differentiation to evolution and optimisation of reproductive function.

Males from the Drosophila obscura group of flies make two types of sperm. Eusperm are long, and are capable of fertilising eggs, parasperm are short and protect eusperm from spermicide in the female reproductive tract.

The mechanisms underlying both the specification and differential morphogenesis of eusperm vs parasperm is not known. Many testis-specifically expressed genes identified as single copy genes in other Drosophila species (eg melanogaster) are duplicated in D. pseudoobscura. Most genes required for spermatid differentiation are transcribed in spermatocytes, we hypothesise that the spermatocytes destined to differentiate into eusperm have a different transcript profile from those that differentiate into parasperm. This would include both differential expression of single copy genes, and expression of different paralogues of multicopy genes. We have already found differential presence of mRNA from one paralogous gene pair in spermatids. Two testis-specific transcriptional regulatory complexes have been identified in D. melanogaster. Several subunits of these complexes have duplicated in the obscura group, so we propose that there are distinct versions of the transcriptional complexes, one to promote the eusperm transcript profile, the other to promote the parasperm profile. To test this hypothesis, we are conducting RNAseq on isolated individual cysts or individual spermatocytes. This will identify whether there are two distinct populations of spermatocytes, and which transcripts are differentially expressed.

You will analyse the RNAseq data sets and identify candidate genes for determining the differentiation into the two sperm morphs. You will apply bioinformatics tools to analyse evolution of both coding sequences and regulatory sequences of co-regulated genes. Q-RT-PCR on isolated cysts (early spermatocytes to spermatids) will validate and extend the analysis. You will experimentally test the roles of candidate genes you identified, in the broader context of understanding how two sperm morphs are generated. The methods applied will include primary culture of spermatogenic cysts; male fertility tests and sperm function assays; using CRISPR to knock out specific regulators and cytology and molecular analyses to determine the effects in mutant testes; generation of transgenic flies – a) expressing GFP-fusion proteins for cytology and ChIP; b) with reporter constructs to identify functional transcriptional elements; c) to over express specific genes; d) to employ clonal analysis to label progeny from single stem cells.