Exploring medfly reproductive biology
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Background. Ceratitis capitata, the Mediterranean fruit fly (medfly), has become a global invader due to several biological traits, including the extremely high reproductive potential. Wild females are polyandrous and display sperm precedence. Achieving a better understanding of the mechanisms controlling sperm dynamics and use in this species is important not only from an evolutionary perspective, but also for their impact on medfly control strategies.

Our research aims at unraveling medfly ejaculate composition, function and regulation, using an interdisciplinary approach that take advantage of a wide range of molecular tools.

Transgenic sperm
We developed sperm-specific marking systems based on the spermatogenesis-specific medfly b2-tubulin (CdB2t) promoter (Scolari et al., N Biotechnol. 2008 Jun;25(1):76-84). Using these transgenic lines we could trace the fate of fluorescent sperm in the female reproductive tract, including the spermathecae, the spermathecal duct and the fertilization chamber.

We found that sperm are stored in a stratified fashion, which from an evolutionary point of view, may initially favour the fresher ejaculate from the second male. However, as the second male’s sperm gradually becomes depleted, the sperm from the first male becomes increasingly available for fertilization. The accumulation of sperm from different males will increase the overall genetic variability of the offspring and will ultimately affect the effective population size.

Transcriptomes of the testes and accessory glands from adult male medfly
We developed high quality expressed sequence tags (ESTs) to advance our understanding of the male reproductive system and the transcriptional profiles, putative roles of secreted candidates that may regulate female reproductive processes.

5914 ESTs from testes and accessory glands were assembled into 334 contigs, of which 33% may represent novel medfly sequences. 400 transcripts encode putative secretory polypeptides

After excluding transcripts clearly not directly involved in reproduction (i.e. ribosomal and mitochondrial sequences) 206 transcripts that encoded putative secreted proteins remained, and these were considered for expression profile analyses related to their tissue-specificity and mating-responsiveness.

Transcripts from 10 genes with MAG-specific/enriched expression were analysed by Real-Time qPCR to assess changes in their abundance after 1, 2 or 3 matings, respectively. Two examples are reported.

This functional genomics analysis allowed us to identify transcripts showing mating-induced changes in abundance in medfly males, probably related to replenishment of their seminal fluid protein (SFP) products after multiple matings.

Acknowledgements

Transgenic sperm

The availability of red and green fluorescent sperm allowed us to investigate patterns of spermuse in the fertilization chamber of twiced-mated females.

Confocal merged images of fertilization chambers (fc) of twiced-mated females.

Annotation of Predicted Seminal Fluid Protein (SFP) Genes in the medfly genome

To annotate putative SFP genes in the medfly genome we queried (tBLASTn; e-value<0.05) the medfly genome scaffolds using the aa sequences of the 146 characterized D. melanogaster SFPs. 64 Drosophila SFPs gave no significant hits to the medfly genome, whereas the remaining sequences resulted in multiple hits to the scaffolds.

We annotated 493 genes that were then grouped into 17 functional classes based on the categories defined for Drosophila SFPs.

These data will enable proteomics-based analyses aimed at identifying/quantifying the peptides in the male ejaculate.

Understanding the identity and functional roles of the medfly SFPs will allow their exploitation for manipulating female reproductive physiology, behavior and fertility.

Papanicolaou et al. 2016, Genome Biol. 17(1):192