Exploring medfly reproductive biology

Francesca Scolari, Grazia Savini, Mosè Manni, Giuliano Gasperi, Anna R. Malacrida

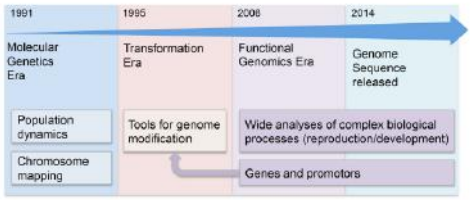
Department of Biology and Biotechnology, University of Pavia, Italy

Correspondence to: francesca.scolari@unipv.it

Reunión del grupo de trabajo MOSCAS DE LA FRUTA del hemisferio occidental — Meeting of Tephritid Workers of the Western Hemisphere — OCTOBER2016 BUENOS AIRES - ARGENTINA

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.



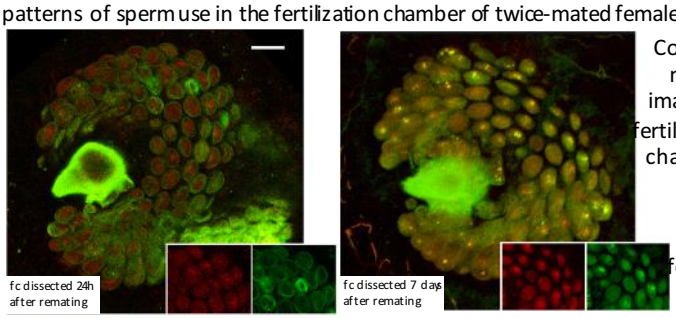
Scolari et al. 2014, BMC Genetics 15(Suppl 2):S11

Transgenic sperm

We developed sperm-specific marking systems based on the spermatogenesis-specific medfly b2-tubulin (Ccb2t) 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.

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



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

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.

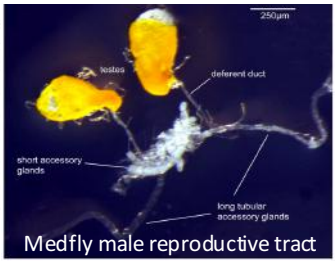
Scolari et al. 2014, BMC Genetics 15(Suppl 2):S10

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.

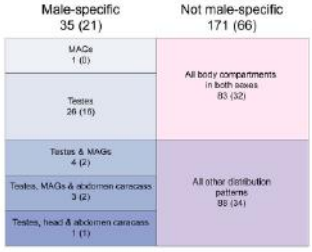
5914 ESTs from **testes and accessory glands** were assembled into 3344 contigs, of which 33% may represent novel medfly sequences. **400 transcripts encode putative secretory polypeptides**

Putative secreted	Number of contigs	%
Odorant Binding Proteins	40	10
Protease inhibitor domains	20	5
Antigen 5 proteins	14	3.5
Mucins	12	3
Immunity related	10	2.5
Proteases	22	5.5
Other enzymes	12	3
Other putative secreted	270	67.5
Total	400	100



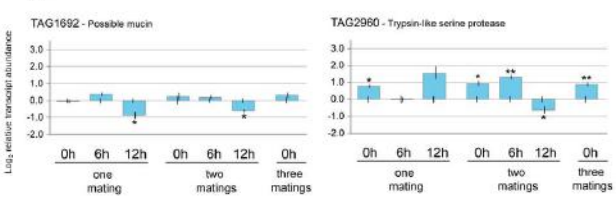
Medfly male reproductive tract

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.



Tissue distribution of 206 transcripts that putatively encode secreted peptides as determined by RT-PCR on male and female body compartments. Figures in brackets indicate the number of transcripts that share no significant similarity to known proteins.

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. Here 2 examples are reported.



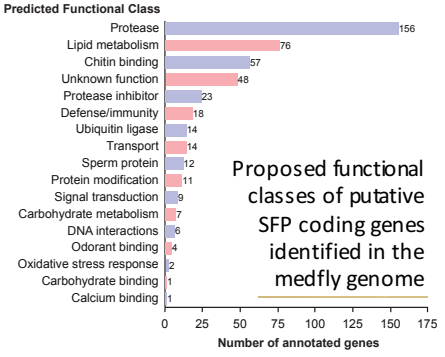
This functional genomics analyses allowed us to identify transcripts showing mating-induced changes in abundance in medfly males, probably related to replenishment of their seminal fluid protein (SFP) products after multiple matings.

Scolari et al. 2012, PLoS ONE 7(10): e46812

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<10^{-10}) 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.



Proposed functional classes of putative SFP coding genes identified in the medfly genome

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

Acknowledgements

IAEA · fondazione cariplo · Fondazione Banca del Monte di Lombardia — Progetto Professionalità Ivano Becchi