Untangling Reproductive Success: *Caenorhabditis* Nematodes as a Model System for Fertilization

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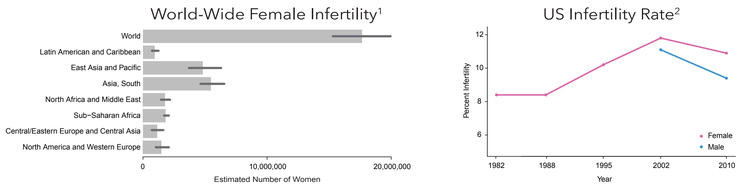
Reproductive Health is a World-Wide Goal



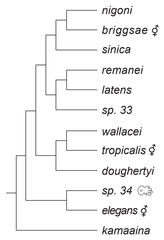
- Reproductive health includes the "capability to reproduce ... and the freedom to decide if, when, and how often" (WHO)
- In US, 90% of infertility examinations are initiated in female partners alone, while males account for ~33% of all infertilities[2,3]
- Impaired fecundity and infertility are characterized by abnormal sperm and poor semen quality
- These same sperm traits are related to male-specific health issues, such as testis and prostate cancers[3]
- CDC now considers infertility a disease

Understanding the molecular basis of infertility is important for diagnosing and treating not only infertility, but also male-specific health issues.

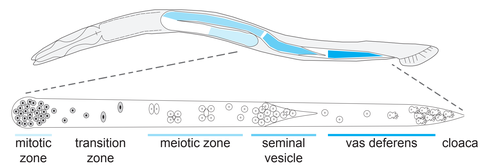
[1]Mascarenhas et al. (2012) PLoS Medicine. [2]National Center for Health Statistics (2013). [3]CDC (2013).

Caenorhabditis Nematodes as a Model System

Caenorhabditis nematodes are a model organism for aging, developmental biology, evolutionary genetics, molecular genetics, and neurobiology.



- Free-living life-history with a preference for rotting fruit and vegetation
- Natural variation in the strength of sexual selection
  - Three independent lineage transitions to self-fertilizing hermaphroditism
  - One lineage transition to sperm gigantism
- High quality genome assemblies available for most of the *Elegans* supergroup



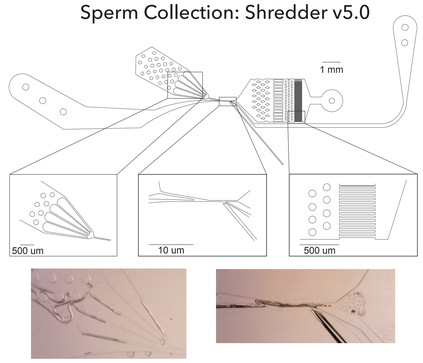
- Mate choice is determined by pheromone signaling
- Mating is initiated by the insertion of the male spicule into the female, triggering the ejaculation reaction
  - Immature spermatids are activated to form mature, motile spermatozoa
- Sperm crawl up the female reproductive tract to the spermathecae and wait to fertilize released oocytes
- The proteomic components of sperm and seminal fluid are largely unknown as well as the genetic basis for fertilization

Nematodes are an excellent model system for untangling the components of reproductive success and analyzing how they evolve both experimentally and in natural populations.

What Comprises the Male Reproductive Proteome?

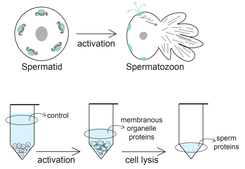
Goal: To characterize the complete set of sperm proteins (sperm proteome) in *C. elegans* and *C. remanei* and analyze the molecular evolution.

Sperm Collection: Shredder v5.0



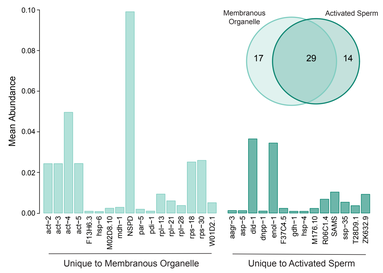
- Microfluidic technique that allows for worms to be manipulated on a micron scale
- Dissects 15-20 males per device
- Collect 100s of sperm cells per male

Sperm Activation



- Mature sperm requires activation
- Pseudopods form and membranous organelles (MO) fuse with cell membrane
- Function of MOs is uncharacterized
- Using *in vitro* activation separate MO proteins can be separated from activated sperm proteins

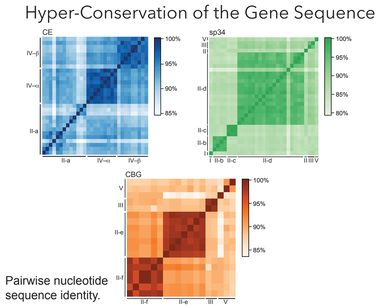
Sperm Proteome Composition



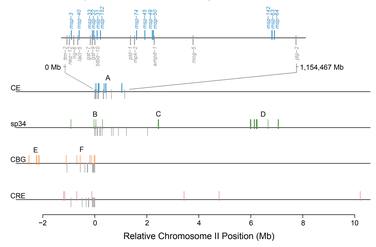
- Activated sperm and MO have unique proteomic signatures
- Major Sperm Protein most abundant
- Identified uncharacterized, nematode-specific proteins in MO
- Conservation of composition between species

Major Sperm Protein Evolution

Hyper-Conservation of the Gene Sequence



Rapid Gene Family Evolution



Colored up-ticks represent Chromosome II MSPs and gray down-ticks represent Chromosome II anchors.

Interplay of functional constraint on the gene sequence and evolution at the level of the gene family.

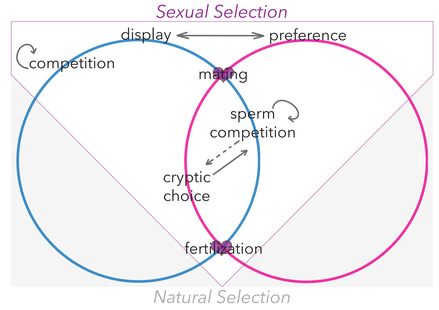
What Determines Fertilization Success?

Goal: To quantify the relative importance of post-insemination dynamics in determing total reproductive success using experimental evolution.

male reproductive success = successfully mate + successfully fertilize

female choice & male-male competition

cryptic choice & sperm competition

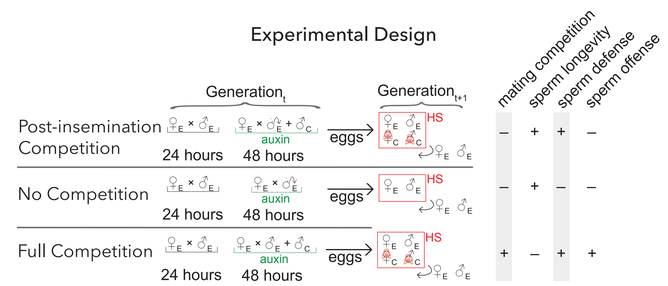


Experimental evolution is a powerful way to study the action of selection and its phenotypic and genotypic consequences.

Genetic Transformations for Inducible Sterility and Inducible Lethality

	Symbol	Molecular Mechanism	Phenotype
Experimental	*No Auxin*	degron *spe-44* / TIR1	normal spermatogenesis normal mating sperm transfered
	Auxin	TIR1 degron *spe-44* Ub Ub Ub	arrested spermatogenesis normal mating no sperm transfered
Competitor	*20C*		normal cellular function
	35C		ectopic expression of PEEL-1 muscle & epidermal breakdown death within 3 hours

Experimental Design



- Capitalizing on the genetic power of *C. elegans* to completely isolate post-insemination
- Select for sperm defensive capability and longevity
- Ancestral population is an outcrossed wild-isolate
- Evolve populations of thousands of worms for tens of generations and measure the phenotypic and genomic changes due to sexual selection

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