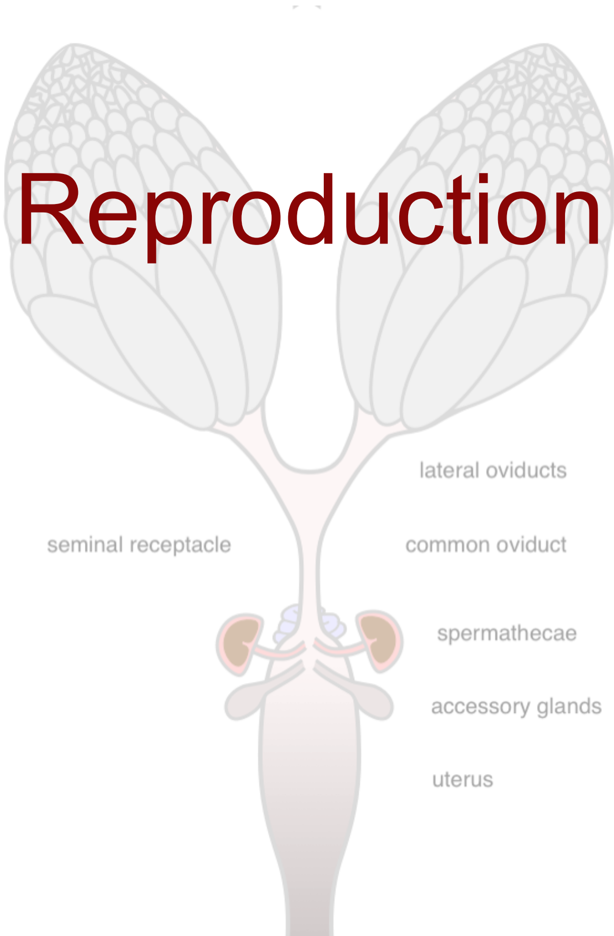
Insect Structure Function & Physiology

Reproduction



Presented and prepared by D Merritt

Reproduction

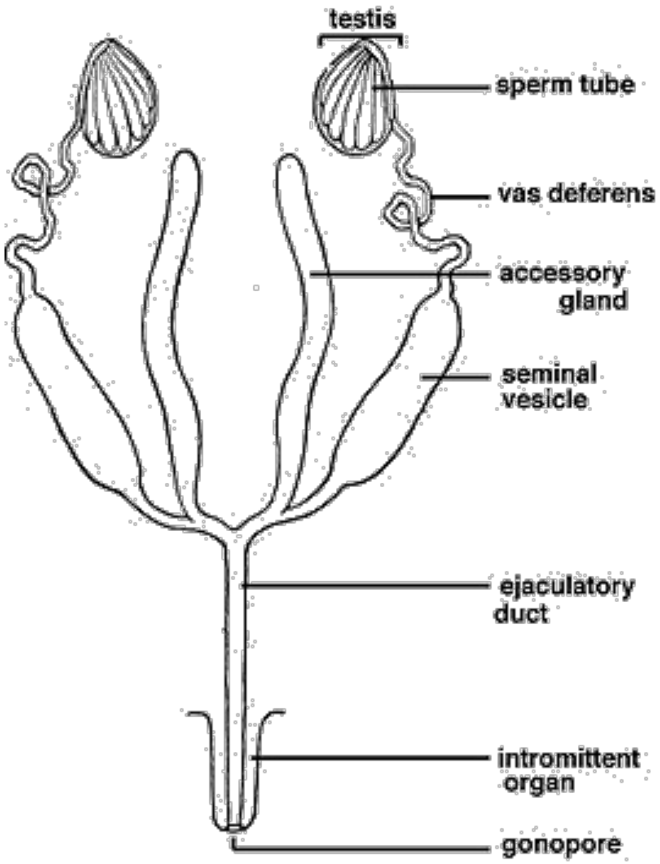
Morphology of generalized male and female
Prioritization of feeding, nutrition, orientation to allow reproduction
Reproduction and pest status
Mating
Egg development
Fertilization
Oviposition
Aggression

Examples:

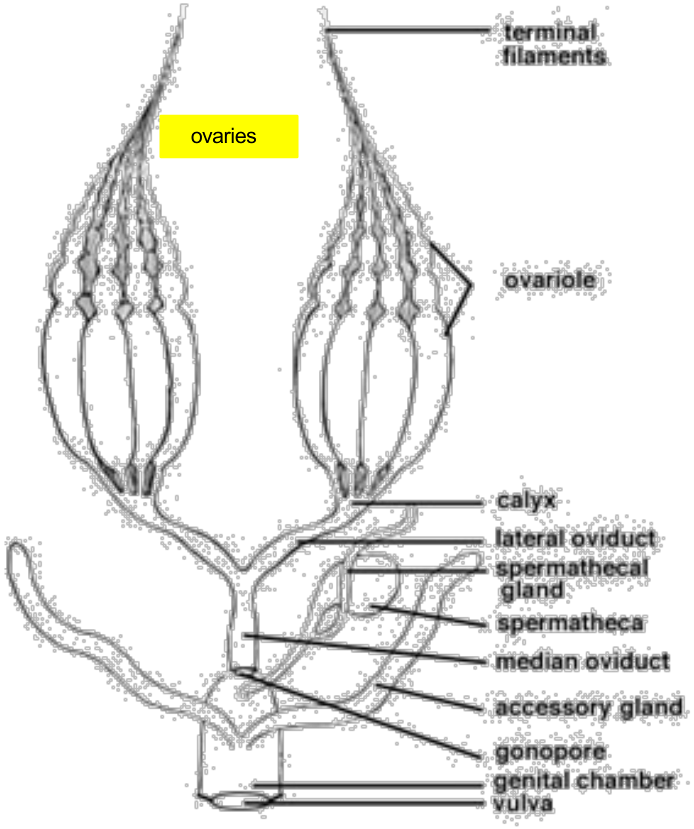
1. *Drosophila melanogaster*
2. Sheep blowfly *Lucilia cuprina*

The reproductive system

Males have paired testes



Females have paired ovaries



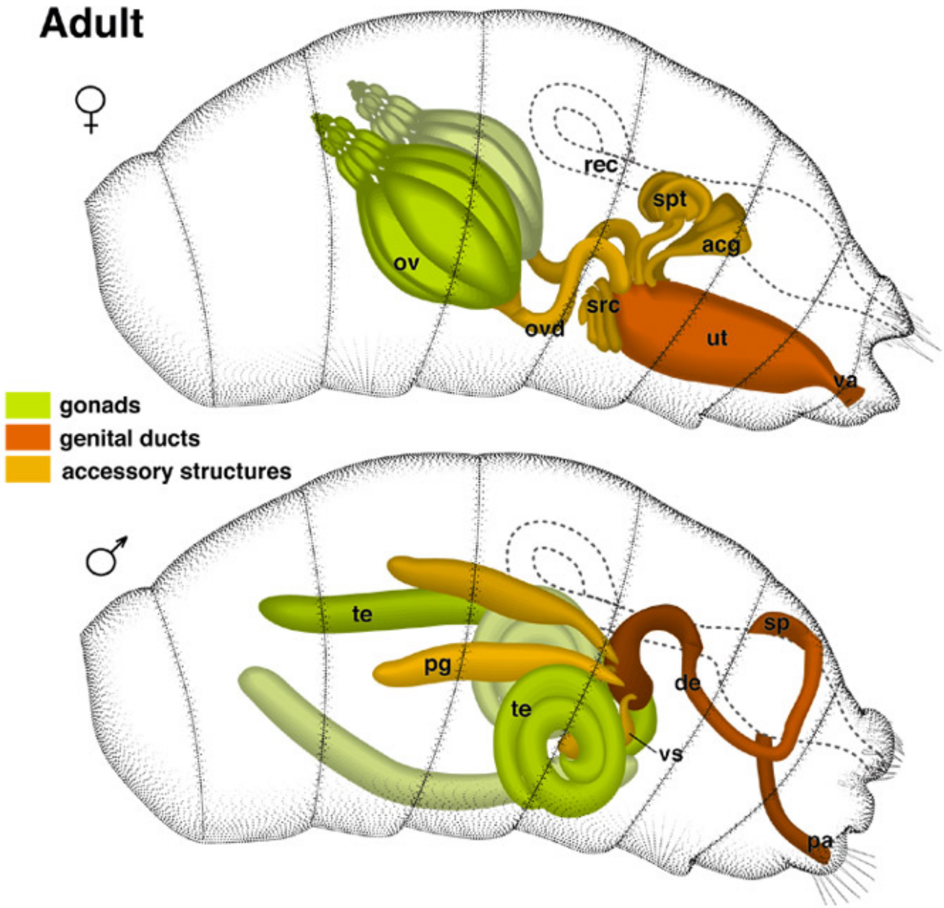
Autogenous: can produce eggs without first feeding, e.g. many Lepidoptera

Anautogenous: need to consume nutrients to develop eggs, e.g. many flies *Drosophila*, *Lucilia*, mosquitoes

The reproductive system

Females have paired ovaries

Ov: ovaries
Ut: uterus
Ovd: oviduct
Spt: spermatheca
Acg: accessory gland
Src: seminal receptacle



Males have paired testes

Te: testes
vs: seminal vesicles
de: ejaculatory duct
Sp: sperm pump
pg: paragonia=accessory glands

From "Atlas of Drosophila Development"
by Volker Hartenstein
published by Cold Spring Harbor Laboratory Press, 1993
Available online

Male – female differences

Genetics

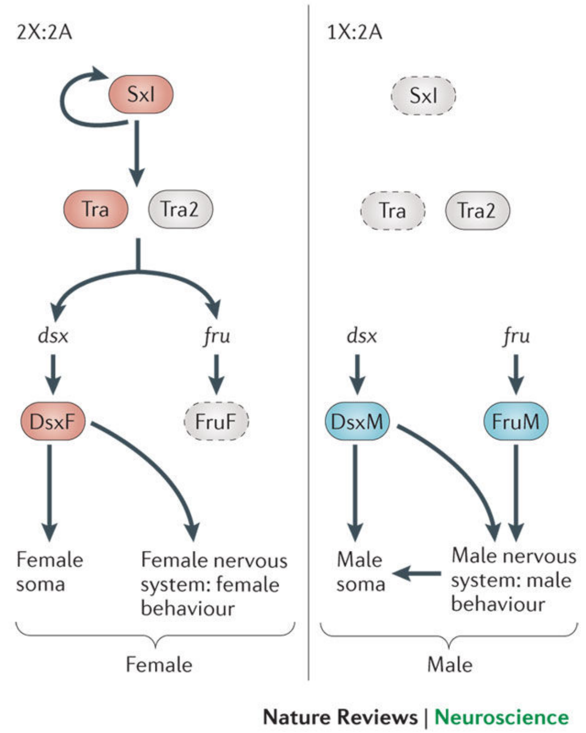
The gene *sexlethal* is transcribed only in future females
It regulates *transformer* alternative splicing
Doublesex: the female version *Dsx*^{F}
Fruitless expressed primarily in males

Nervous system

Sensory system: different numbers and types of sensilla and Or, Gr and Ir
Neurons in the brain: male isoform Fru^{M} is expressed in c. 1,500 neurons
Abdominal ganglia control centres associated with male and female reproductive structures

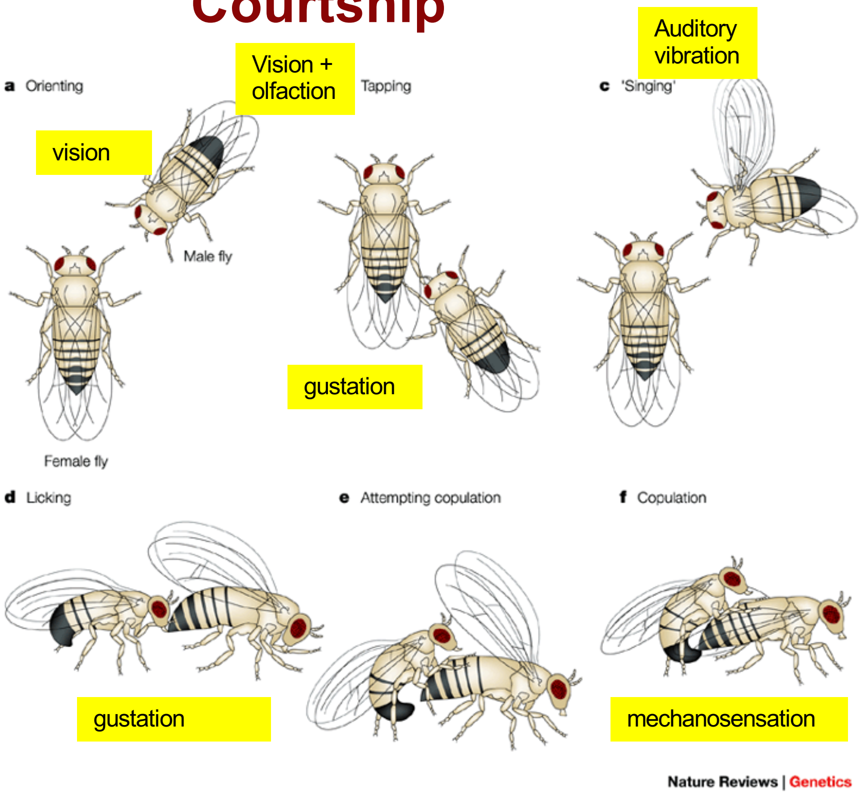
Pheromones

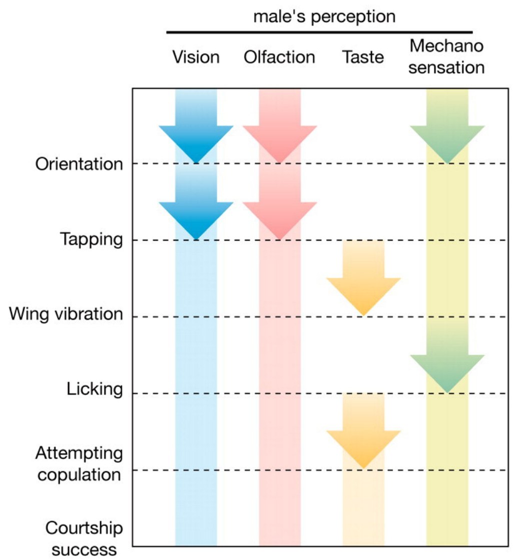
Male pheromone is cVA (cis-vaccenyl acetate)
cVA is deposited onto females during mating: allows males to discriminate virgin from mated females

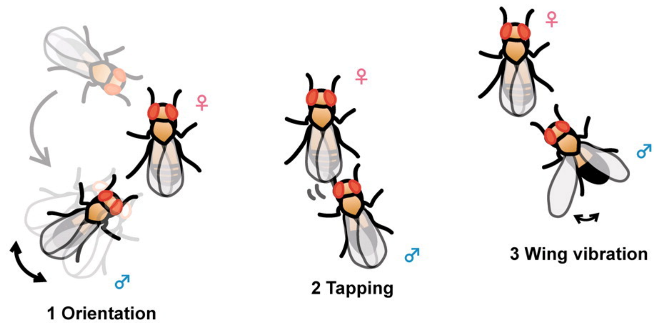


Sex lethal (*Sxl*) is transcribed only when the X/A ratio (the X chromosome-to-autosome ratio) equals or exceeds 1

Courtship

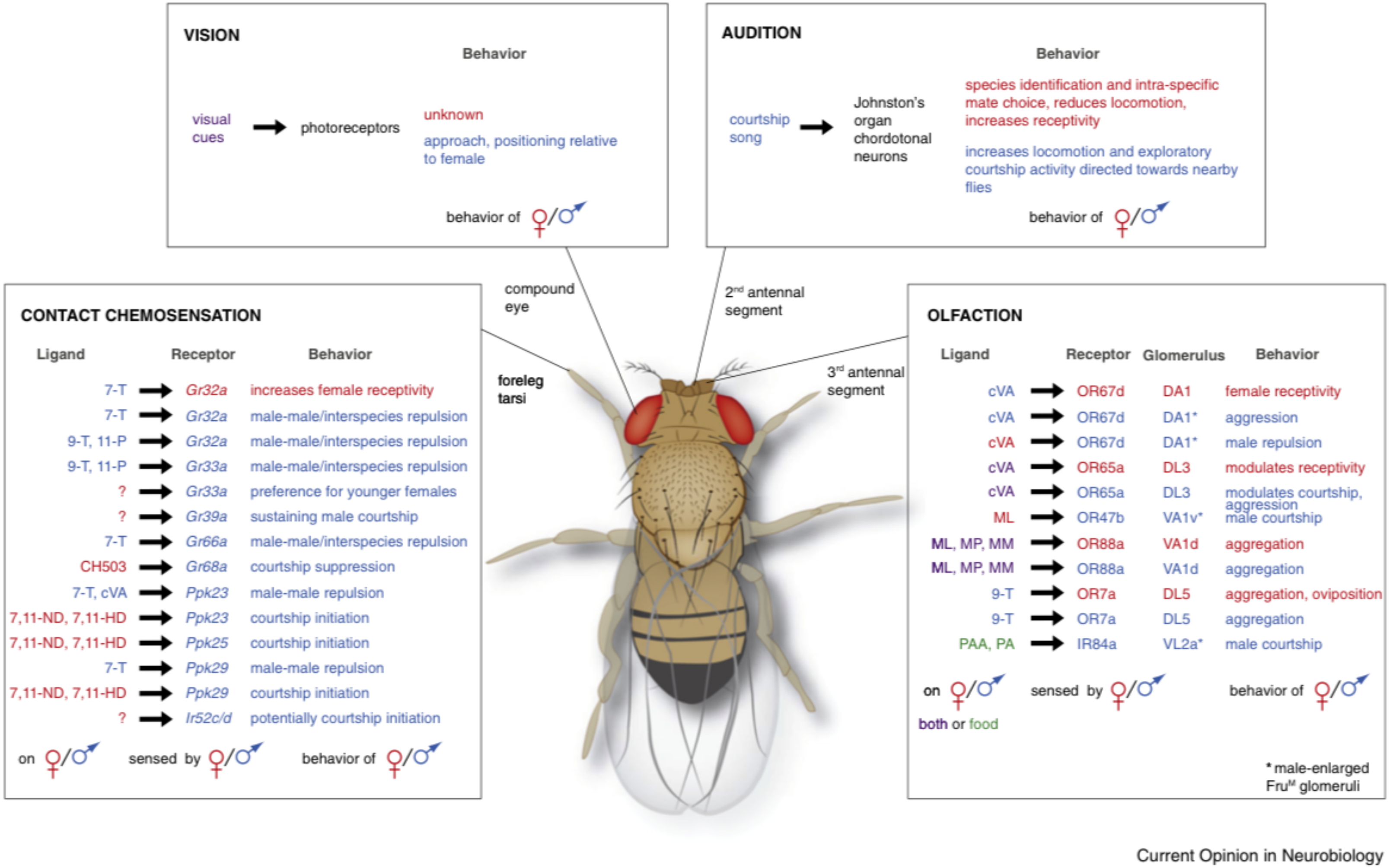






Ejima, A. and Griffith, L.C., 2007. Measurement of courtship behavior in Drosophila melanogaster. *Cold Spring Harbor Protocols*, *2007*(10), pp.pdb-prot4847.

Courtship



Auer, T.O. and Benton, R., 2016. Sexual circuitry in Drosophila. *Current opinion in neurobiology*, *38*, pp.18-26.

The Fruitless gene

Sexual orientation, as well as courtship behavior in *Drosophila*, is regulated by *fruitless*

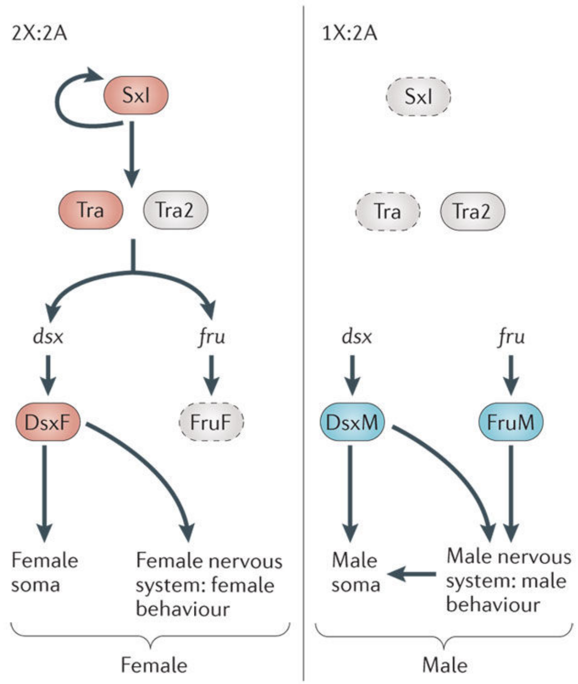
The “neural sex” is just as important as the morphological sex

Fru encodes proteins of the BTB-zinc-finger family of transcription factors

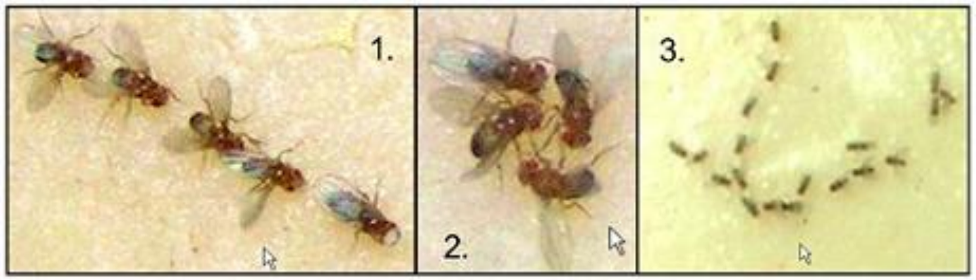
Originally identified on the basis of the aberrant courtship behaviour by *fru* mutant *Drosophila* males

- *Fru* mutants (loss of function allele) do not distinguish between male and female flies while courting
- When housed together, males form mating chains that result from males following each other while showing courtship behaviour

Sex lethal (*Sxl*) is transcribed only when the X/A ratio (the X chromosome-to-autosome ratio) equals or exceeds 1

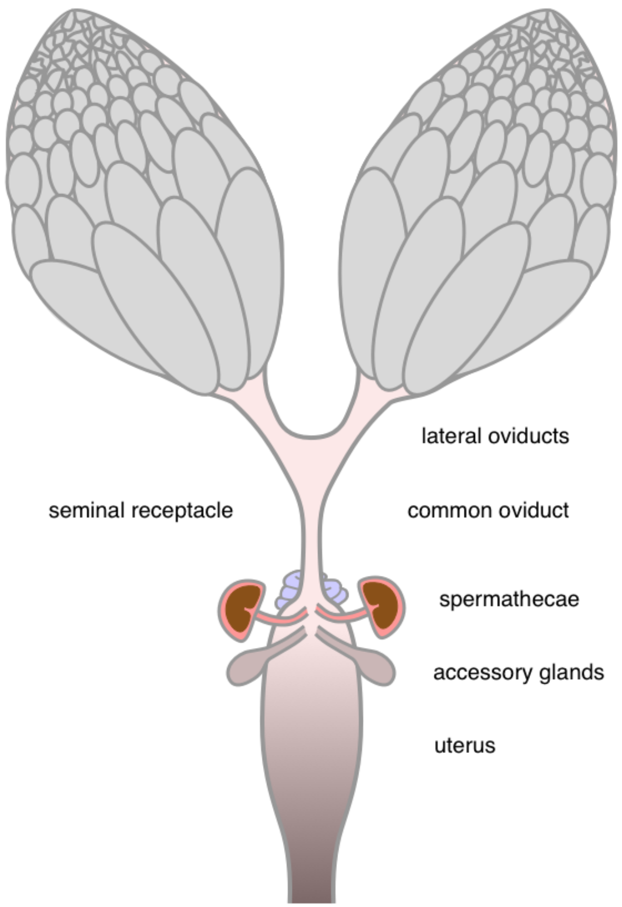


Nature Reviews | Neuroscience



Post-mating responses in female Drosophila

- Sex peptide (36 amino acids) originates in the male accessory glands
- SP binds to sperm tails and is gradually released within female from stored sperm.
- Binds to sites in brain, thoracico-abdominal ganglion and oviduct
- Detected by small number of sensory neurons in the female reproductive tract

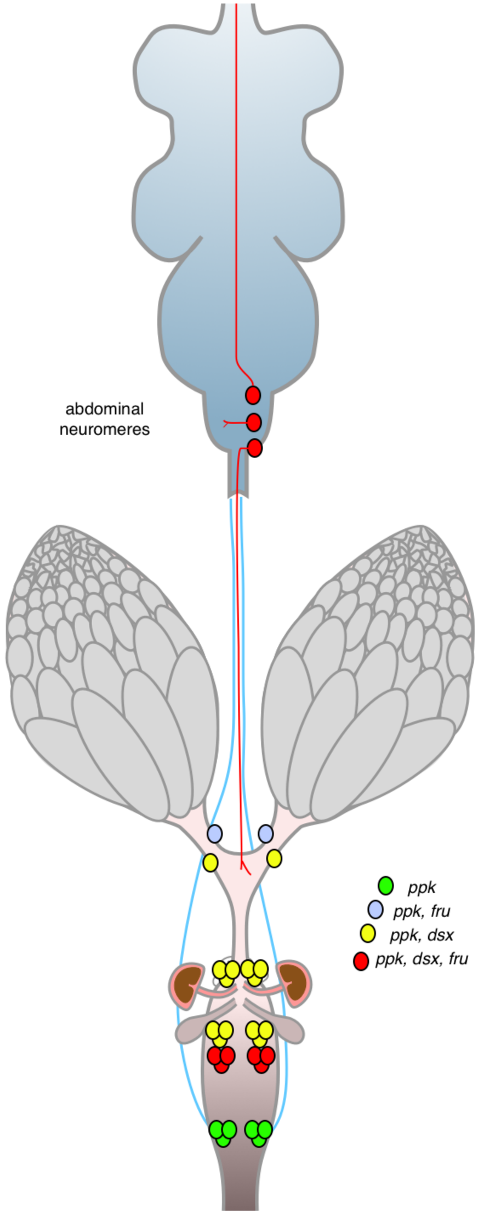


Drosophila female reproductive system

Rezával, C., Pavlou, H.J., Dornan, A.J., Chan, Y.B., Kravitz, E.A. and Goodwin, S.F., 2012. Neural circuitry underlying Drosophila female postmating behavioral responses. *Current Biology*, *22*(13), pp.1155-1165.

Post-mating responses in female Drosophila

- Multiterminal sensory neurons that express *fruitless doublesex* and/or *pickpocket (ppk)*
- Decreased receptivity and increased oviposition due to sex peptide
- Mutant females lacking sex-peptide receptor (a G-coupled receptor) remain receptive to mating postmating
- Neurons in the abdominal neuromeres send signals to brain, connect with each other, or connect with muscles overlaying the oviducts



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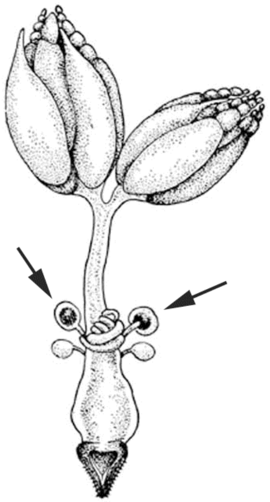
Sperm storage

Across insects, females have 1-3 spermathecae

Sperm remain viable for years in some cases (bees).

Spermatheca frequently lined with sclerotised cuticle (protect sperm from UV radiation?)

Usually surrounded by dermal glands or with an adjacent spermathecal gland



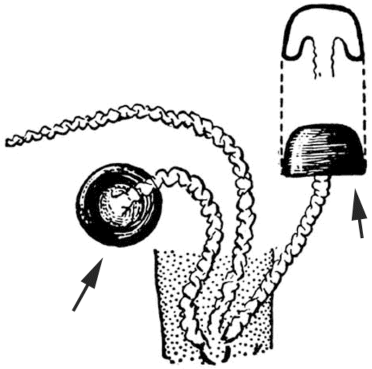
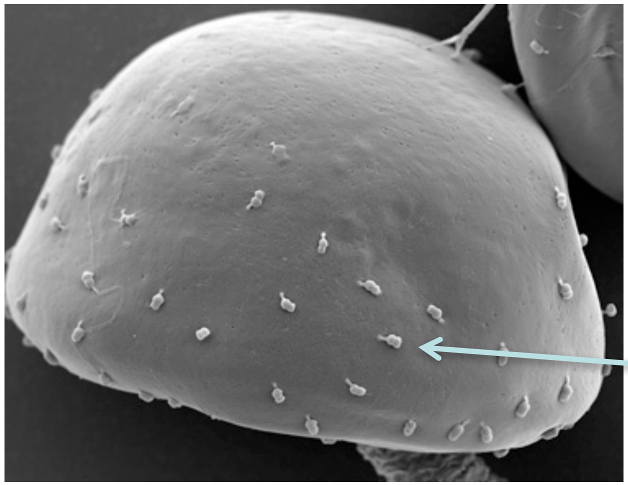


Image: Flybase



End apparatus of dermal glands

dermal glands

Sperm storage

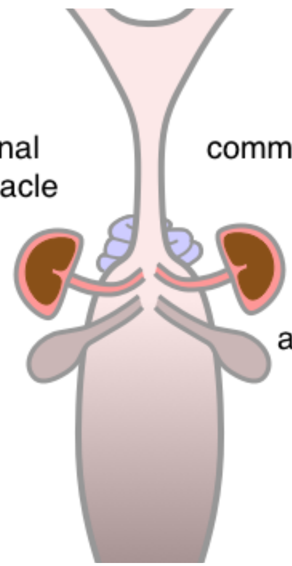
Secretions from spermathecal gland cells and accessory gland cells

- Required to attract sperm to spermathecae (via affecting uterine contractions or sperm motility?)
- Required to store sperm: sperm show morphological abnormalities in absence of secretions
- Required for ovulation

Seminal receptacle and spermathecae innervated by octopaminergic (OA) and tyraminergic (TA) neurons whose cell bodies are in the addominal neuromeres.

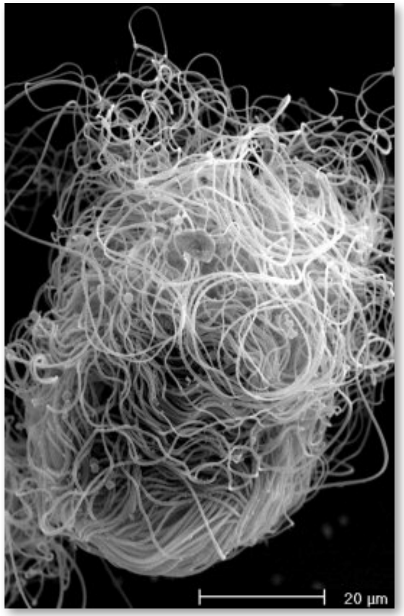
TA and OA regulate the release of sperm from storage

OA innervation of muscles required for ovulation



Copulation

Genitalic morphology is very diverse
Often used in species diagnostics so important in taxonomy and phylogenetics
"Lock and Key" concept: some controversy (theories of evolution and speciation)
Male and females are in a competitive race, placing great selective pressure on the genitalia
Males: onus is to be the only father (plugs, sperm removal, injection of accessory gland fluids)
Females: choose the fittest males



In *Drosophila melanogaster*, a Sex Peptide produced in male accessory glands binds to sperm tails and is carried into female
Suggested reason for extra-ordinarily long sperm tails in some Drosophila spp (3 mm male of D. bifurca has sperm tail length of 58 mm)

http://pitnicklab.syr.edu/Pitnick/proposal_movies.html
For movies of *Drosophila melanogaster* fluorescently tagged sperm travelling within the female tract.

Mating & Oviposition

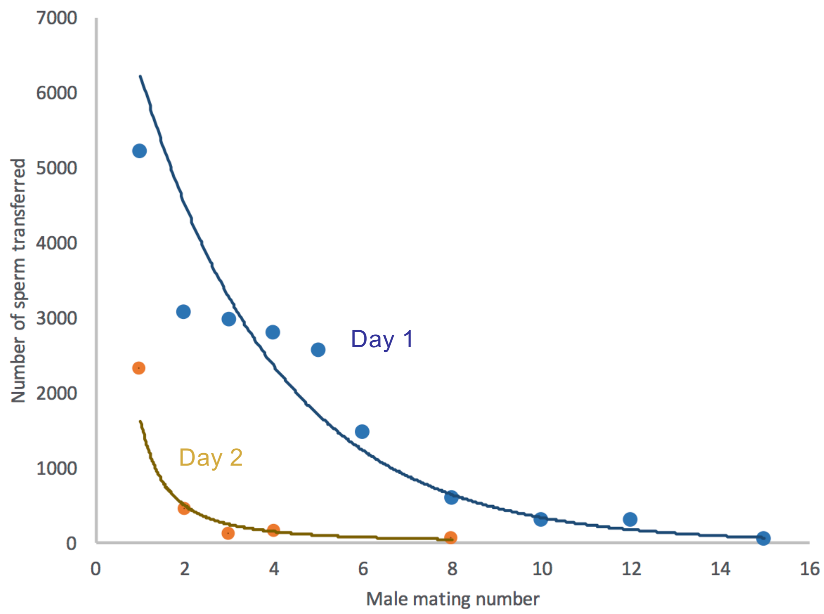
Lucilia cuprina females:

- anautogenous,
- undergo synchronous ovarian development
- lay eggs in batches of 250 or so.
- If inadequate protein is available, females resorb oocytes in the early vitellogenic stage.
- Females become receptive to males only after they have ingested protein.
- The amount of protein required for full receptivity is less than that required for oocyte maturation (Barton Browne et al ‘76).
- Protein feeding acts upon the ovaries and also initiates the onset of receptivity.

Image: Museum Victoria

Mating & Oviposition

- Approximately 3000 sperm transferred at mating.
- 38% of the stored sperm is used at each oviposition event (egg batch deposition).
- Males can mate multiple times, 10 – 20 times per day.
- Experiments established that after 8 or so sequential copulations the number of sperm transferred begins to decrease (graph, right) and the fertility of egg batch laid by the mated female starts to drop.
- A day later (day 2) sperm numbers have not been replenished
- Probably have a sex peptide because injections of male accessory glands into receptive females causes them to become unreceptive



Redrawn from Smith et al., 1990

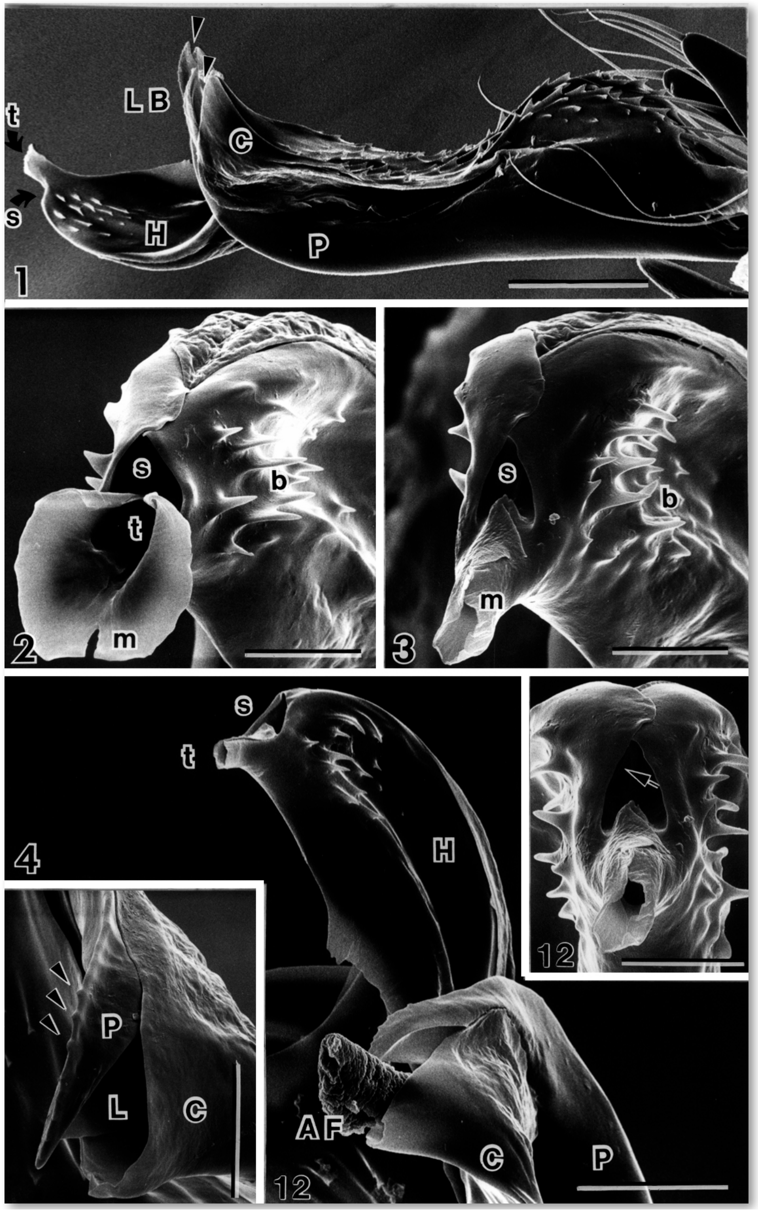
Each egg batch is approximately the same size, yet the females use a *constant proportion* of the stored sperm for each egg batch. Indicates that the efficiency of sperm use *increases* as the sperm store diminishes — 15 sperm per egg when 10,000 sperm in store, to 1.5 sperm per egg when 1000 in store.
However, when the sperm stores drop below about 600 the fertility of the batch reduces, suggesting there is an optimal number of sperm required in the spermathecae for successful fertilisation of each egg in a batch.

Copulation

In *Lucilia cuprina* (Australian sheep blowfly), males have evolved penis structures to deliver accessory gland material (AF) that probably includes the sex peptide into the female receptacles

Paired barbs (c) on either side of terminal opening (t) tear into the cuticle lining the internal female tract and release accessory gland fluid

Images: D. Merritt, UQ



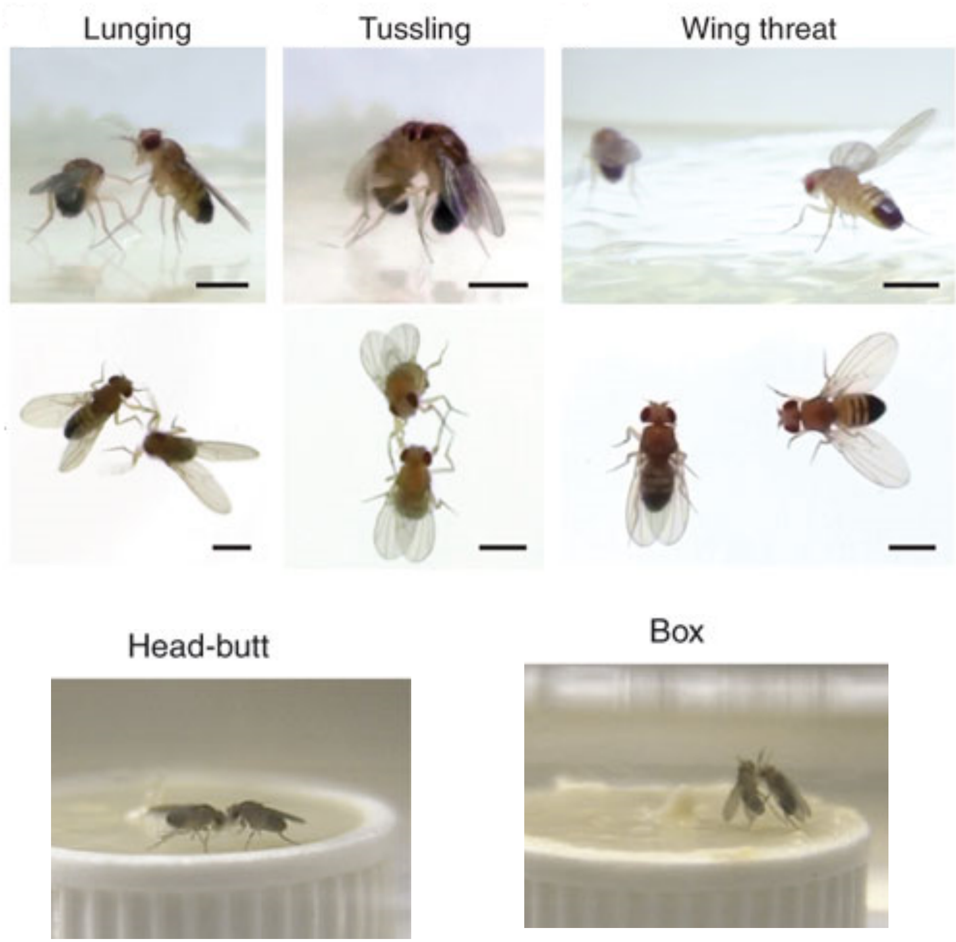
Aggression

Aggression is used by essentially all species of animals to gain access to desired resources, including territory, food, and potential mates

A wide range of environmental, physiological, and genetic factors influence *Drosophila* aggression

Fighting Positions & Manoeuvres

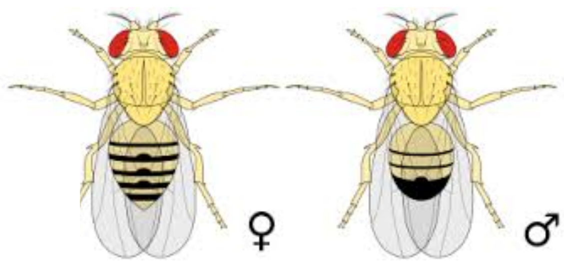
- Charging
- Tussling
- Wing threat
- Boxing
- Fencing
- Lunging
- Head Butting

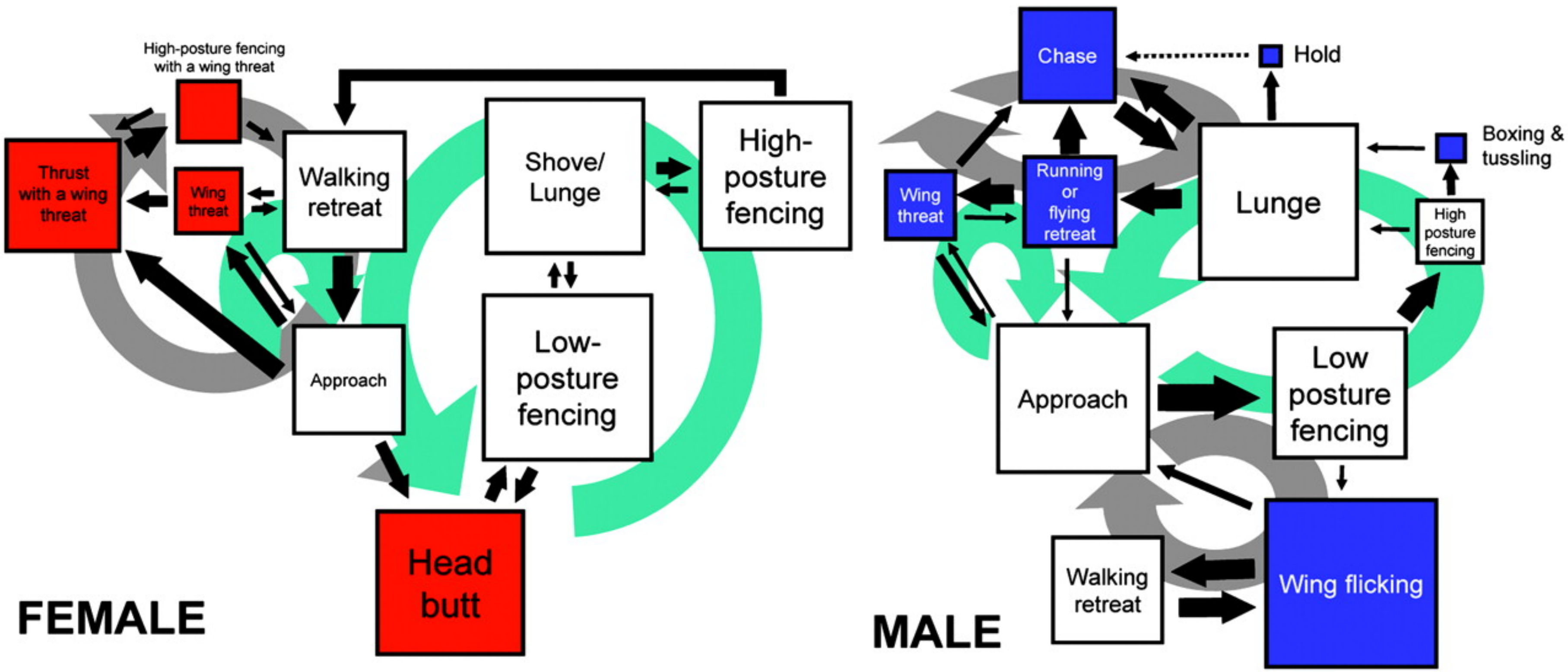


Differences Between Sexes

- Both sexes display gender-specific patterns of aggressive behaviour

- Males:
  - Aggression is motivated primarily by securing mates and territory
  - Display more territorial aggression patterns (e.g. more likely to protect a potential resources or territory)
  - Engage in higher-intensity components of fights (boxing, tussling, and holding)
- Females:
  - Aggression is primarily motivated by protecting food resources
  - Hierarchical (or dominant) relationship do not typically develop from female fights
  - Lower intensity and shorter aggressive bouts (head butting)





Common behavioural patterns in males and females (white boxes)

Gender-selective behavioural patterns (male: blue boxes; female: red boxes)

Fruitless

Are genes of the sex determination hierarchy involved in aggression?

Change brain sex by:

- Experimentally expressed *Fru* in female brains, where normally absent
- Prevent expression in male brains (3 forms of the protein normally present).

Changing the sex of brain neurons without changing the sex of the rest of the fly leads to animals fighting using patterns of aggression appropriate to the sex of the fru-expressing brain neurons and not to the genetic sex of the animals