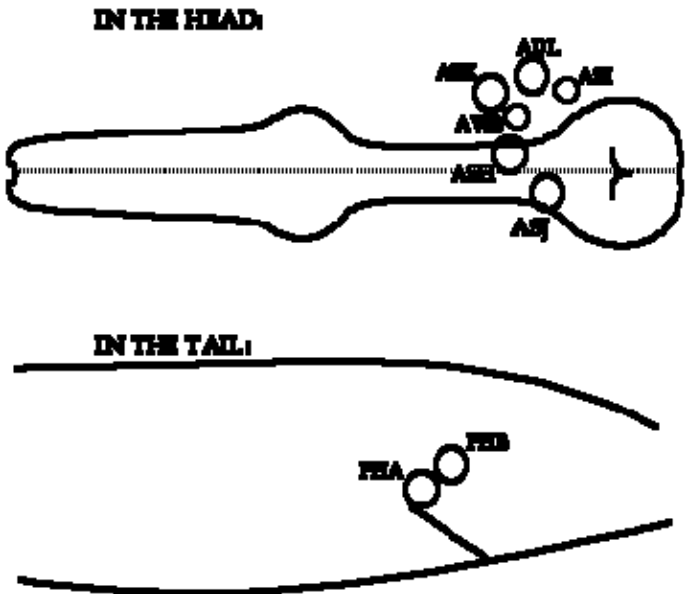
Experiment 2 Mutant and Mating

In *C. elegans* research, we express proteins tagged with a fluorophore (GFP or RFP for example) that enables us identify the precise location of the protein in the living worms. We can compare the location between wild-type and mutant worms then which gives us rise to the function of the protein (for example in a specific signaling pathway). Many mutant strains have obvious phenotypes, for example, uncoordinated movements (unc), dumpy i.e. shorter and fatter body length (dpy), limited chemotaxis etc. Also coinjection markers can lead to new phenotypes as *rol-6*, with rolling and twisted shape phenotypes, or *odr-1* leading to strong RFP expression in sensory neurons.

Today you will learn: 1. Observe important mutant strains, ex: Unc, Dpy, Vul, and Coiler, and worms with coinjection marker, *rol-6*, and understand the meaning behind these abbreviations 2. Dye filling experiment. 3. Worm mating. 4. Genomic DNA extraction.

Dye filling experiment

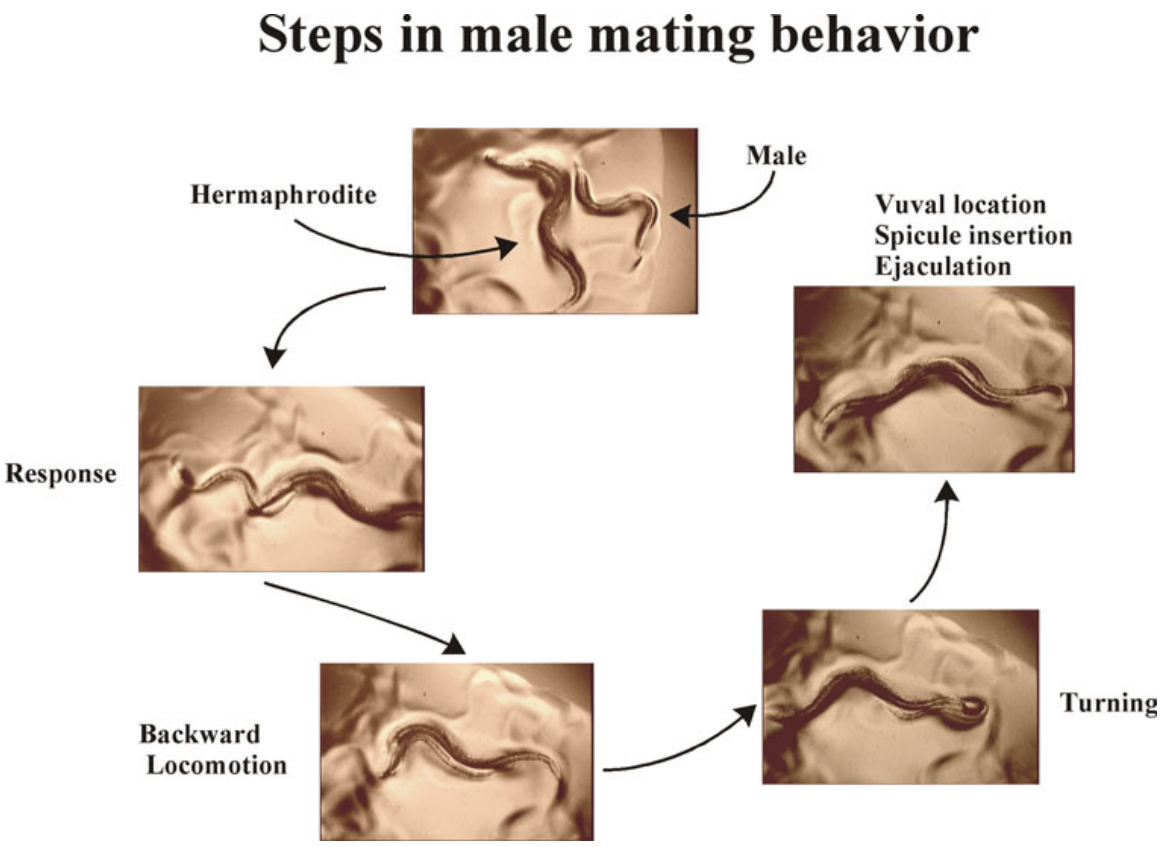
Cellular structures and components can be labeled with a variety of fluorescent dyes that can be visualized using a fluorescence microscope. In *C. elegans* amphid and phasmid neurons (both are sensory neurons) can take up lipophilic dyes (DiI, DiO) from the environment. Thus, these dyes can be used to visualize sensory neurons. If *C. elegans* has abnormalities in the quantity, structure or organization of the components of the amphid and phasmid neurons, it might exhibit dye-filling defect (Dyf). In this experiment, you will use wildtype (N2) and mutant worms (PR802) to do the DiI and DiO staining; then compare the staining outcomes.



Location of amphid and phasmid cell bodies in the head and in the tail of the worm.

Worm Mating

Male mating behavior is the most complex motor activity in *C. elegans*. The steps in mating include the response to contact, backward locomotion, turning, vulva location, spicule insertion, and sperm transfer (see figure below). In this experiment, you will let male exhibiting one phenotype (UNC-104::GFP) mate with hermaphrodite exhibiting another phenotype (N2) to cross these two strains ; then observe the crossing result.



Homework

1. Briefly explain the abbreviations and phenotypes, then draw the pictures of Unc, Dpy, Vul, Rol, Coiler.
2. Present the dye filling outcome in a graph.
3. Calculate the number of males in F1 after crossing and compare it with the number of males gained from your heat shock experiment.

Dye Filling Experiment

Strain: N2, PR802, Punc-104::UNC-104::GFP(e1265)

1. Pick some worms (~50) into the dye (10 microgram/1 ml DMF). (DMF is toxic. Wear gloves, mask and protective glasses.)
   N2, PR802 in DiO
   Punc-104::UNC-104::GFP(e1265) in DiI
2. Incubate 2 hours at room temperature. Keep the eppendorf cap from light.
3. Wash the worms 3 times with M9 Buffer.
4. Put the worms on agarose pad with 0.1M tetramisole.
5. Observe results under the fluorescent microscope.

Genomic DNA Extraction

Strain: N2, RB809

1. Pick 50 worms into the Worm Lysis Buffer (20 λ).
2. Incubate 2 hours at 65°C.
3. Incubate 15 minutes at 95°C.
4. Store at -20°C.

Mutant Observation

Strain: BC277, CB1265, NM440, MT105, MT4351

1. Observe mutants under the stereo microscope.

Worm Mating

Hermaphrodites: N2, Males:Punc-104::UNC-104::GFP

1. Pick males (Punc-104::UNC-104::GFP) into a NGM plate with OP50. Allow the worms crawl through the OP50 lawn.
2. Pick males from step 1. to a NGM plate with mating spot OP50 lawn. Pick L4 hermaphrodites (N2) to the mating plate. Male: Hermaphrodites = 3:1.
3. Observation.
4. Incubate 3 days at 15°C fridge.
5. Remove the males. Return to 22°C.
6. Observe mating results.

Reagents

DiI

Dissolve 2 mg DiI in 1 ml dimethly formamide as stock solution. Store the stock at -20°C in a foil wrapped tube. Dilute the stock 1:200 in M9 for dye filling experiment.

DiO

Dissolve 2 mg DiO in 1 ml dimethly formamide as stock solution (DMF is toxic. Wear gloves, mask and protective glasses). Store the stock at -20°C in a foil wrapped tube. Dilute the stock 1:200 in M9 for dye filling experiment.

Worm Lysis Buffer

Mix 5 µl 9% Tween-20, 25 µl 9% NP-90, 10 µl 10X Thermal Polymerase buffer, 1 µl 20mg/ml Proteinase K and 79 µl H2O.

Strain information

http://www.wormbase.org/