

Research Program on *Caenorhabditis elegans*

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Characteristics of *C. elegans*



- Non-parasitic nematode
- ~ 1 mm in length
- Transparent
- Easily grown in the laboratory
- Animals synchronously develop through four distinct larval stages into adults
- *C. elegans* can be grown in sufficient quantities for biochemical studies

Anatomy



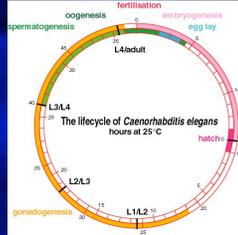
- 959 somatic cells
- Highly differentiated digestive, reproductive, muscular and nervous (chemo- and mechano-sensory) systems

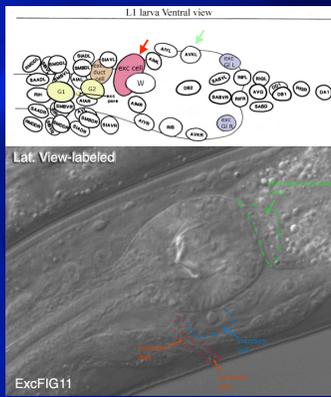
C. elegans Development



- 10 day life span
- 3.5 day developmental cycle

- Cell and developmental biology are understood in exceptional detail.
- Cell lineage's are known for the entire developmental program





ExcFIG11

C. elegans Neurobiology

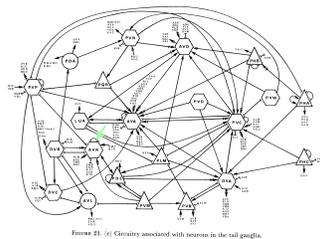
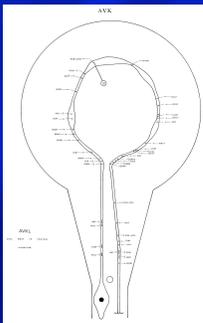
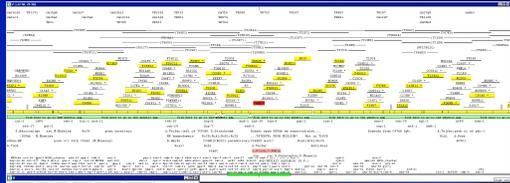
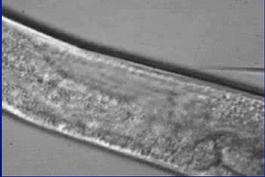


FIGURE 21. (c) Circuitry associated with neurons in the tail ganglia.



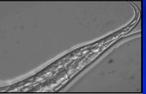
- Amenable to classic and molecular genetic analysis
- Small genome (2 x10⁹ base pairs)
- 21,700 predicted ORFs
- *C. elegans* genome completely sequenced

Transgenic *C. elegans*



- Gene knockout (RNAi, ribozymes, transposon)
- Knockout library (16K)
- Phenotype rescue
- Transgenic nematodes (*lacZ*, GFP)

RNA Interference

	Head	Tail
RNAi + Cd		
Cd		
RNAi		

Knockout genes by feeding bacteria expressing dsRNA

Typical Rodent Study

- Animals: 10,000 - 20,000
- Time: 1 - 2 years
- Cost: \$2,000,000 - 3,000,000

Typical *C. elegans* Study

- Animals: 100 - 200,000*
- Time: 3 - 5 days
- Cost: \$100's

Advantages of Alternative Species

- EPA requiring multiple species in toxicological test
- Most agencies are encouraging the use on non-vertebrate species
- Fewer or no animal welfare concerns
- Genetics/transgenics
- Rapid assays
- Lower cost

A Few Conservations Between *C. elegans* and Mammals

- Basic metabolic proteins
- Stress response
- Cell cycle control
- Signal transduction pathways
 - Insulin
 - Retinoic Acid
 - MAPK/Ras
 - Toll
 - p53
 - TGF
 - WNT
- Neurotransmitters
 - Dopamine
 - Acetylcholine
 - GABA
 - glutamate
 - serotonin
 - nitric acid
- Diseases
 - Cancer
 - ALS
 - Lysosomal storage disease
 - Polycystic kidney disease
 - Huntington's disease

What can you monitor in medium throughput format?

- Growth
 - Movement
 - Feeding
 - Reproduction
 - Size
 - Shape
 - Gene expression
 - Development
 - Whole organism
 - Specific cells
- Screen using
 - Wild-type *C. elegans*
 - Genetic mutations
 - Transgenic nematodes
 - Knockout Library

WormTox

Development of a high throughput toxicant screening system using *C. elegans*

Project Tasks

Task 1. Develop methods to measure the toxicity of developmental and neurological toxicants. This task involves the development of computer and image analysis software for monitoring growth, size, reproduction and movement. It also requires development of a 96-well format for growth, dosing and toxicity testing.

Task 2. Expose *C. elegans* to at least 200 known or suspected developmental and/or neurological toxicants and determine changes in phenotypic characteristics (survival, size, growth, reproduction and movement).

Project Tasks

Task 3. Create and/or obtain GFP-based, stress-responsive transgenic *C. elegans* for improving sensitivity and specificity of toxicity screens. This task will also include the development of multi-dimensional (3-D, 4-D) computer imaging software to quantitatively measure the effects of toxicant exposure on nervous system development.

Task 4. Use *C. elegans* microarray analysis and test a subset of chemicals from Task 2.

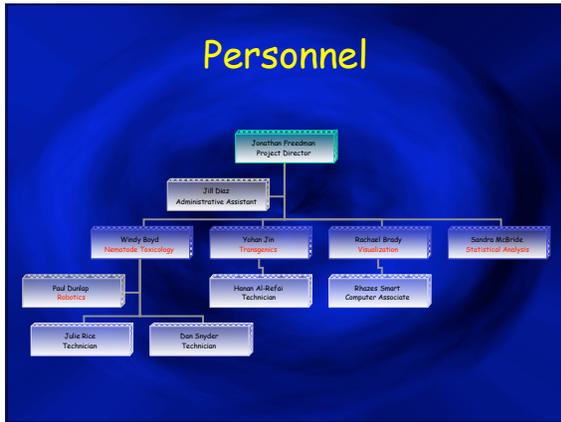
Task 5. Adapt methods for high throughput analysis to assess the toxicological responses in *C. elegans* in which each gene has been inactivated using RNAi

Toxicological Parameters

- LD₅₀ ✓
- EC₅₀
 - Motion ✓
 - Fecundity ✓
 - Feeding ✓
 - Growth rate
 - Reporter gene expression
 - Other
 - Size
 - Shape

Infrastructure

Personnel



Titertek MAP C2 Agar Dispenser

- Fill 96-well plates with precise volumes ($\pm 1\%$)
 - Agar
 - Liquid growth medium
 - Bacteria (*C. elegans* food)



96-well sample preparation

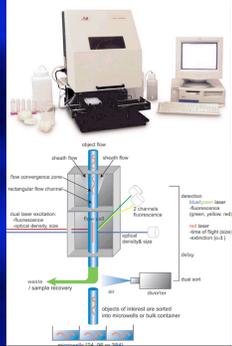
Liquid Handling Robots

- Biomek FX
 - Toxicant addition
 - Bacterial colony replication
- Biomek 2000
 - Toxicant dilution
 - Master plate preparation



COPAS Biosort (Complex Object Parametric Analyzer and Sorter)

- Dispense *C. elegans* (exact numbers at specific developmental stages)
- Count/Sort nematodes
 - 96-well format
 - Live versus dead
 - Developmental stage
 - GFP-expressing versus non-expressing
- Mutant screens
- Growth rates
- Population distributions
- Level of stress-responsive gene expression



Microscopy

- Microscopes
 - Inverted motorized
 - GFP dissecting
- Automated 96-well measurement
- Motion tracking
- Size distribution
- Z-series
- 3-D rendering
- Phenotype characterization



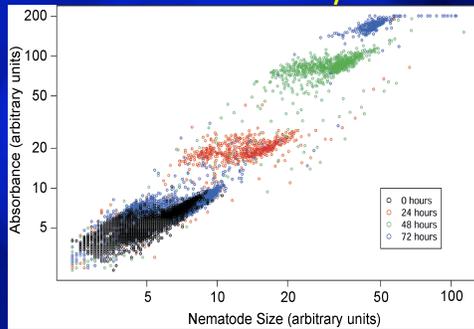
Progress

- Find appropriate concentration of bacteria (*OP50 E. coli*) for each endpoint
- Determine experimental design for each endpoint
 - Number worms/well; number wells/treatment
 - Exposure duration
 - Problem with lethality and Sytox staining
- Standardize chemical testing to optimize time and reproducibility
 - Range finding, vehicle tests, chemical dilution, etc.
- Design and implement statistical analyses programs in S-plus
- Write programs to partially automate movement and 3D image analysis

Chemicals Currently Being Studied

- Cadmium ✓
- Chlorpyrifos ✓
- Vitamin E (Non-Toxic) ✓
- Standard Solvents ✓
 - α-cyclodextrin
 - β-cyclodextrin
 - DMSO
 - Methyl Cellulose
 - Polyethylene glycol
- Ethanol
- Diquat
- Acetaminophen ✓
- Iron
- Sodium Arsenite
- MMNG ✓
- Chromium Oxide ✓
- Silver Nitrate ✓
- Zinc Sulfate ✓
- "On Deck"
 - Fumonisin
 - Fipronil
 - Caffeine
 - Nicotine
 - Sodium Metam (Dithiocarbamate)
 - Methyl Isothiocyanate
 - Ascorbic acid

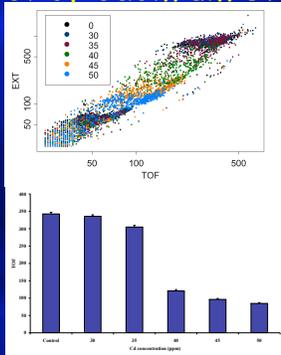
Growth Study



Protocol for Growth Study

1. Prepare 96-well plate with the Biomec 2000 S-medium, toxicant, and *E. coli*
2. Load 50 L1 stage nematodes to each well using COPAS Biosort. Read OD
3. Incubate at 20 C for 72 hours. Read OD.
4. Count nematodes using the COPAS Biosort.

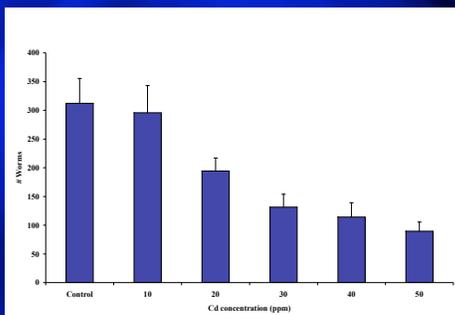
Effect of Cadmium Growth



Reproduction Protocol

1. Prepare 96-well plate with the Biomec 2000 S-medium, toxicant, and *E. coli*
2. Load 5 L4 stage nematodes to each test well using COPAS Biosort. Read OD.
3. Incubate at 20 C for 48 hours. Read OD.
4. Count nematodes using the COPAS Biosort.

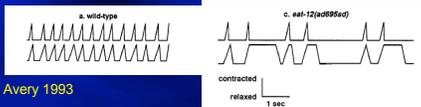
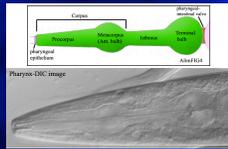
Effect of Cadmium on Reproduction



Protocol for feeding

1. Prepare 96-well plate with the Biomec 2000 S-medium, toxicant, and *E. coli*
2. Load 25 adult nematodes to each test well using COPAS Biosort. Read OD.
3. Incubate at 20 C for 24 hours. Read OD.
4. Add rhodamine-conjugated microsphere to each well.
5. Incubate for 15 minutes.
6. Count nematodes and measure the fluorescence using the COPAS Biosort.

Contraction of corpus and terminal bulb



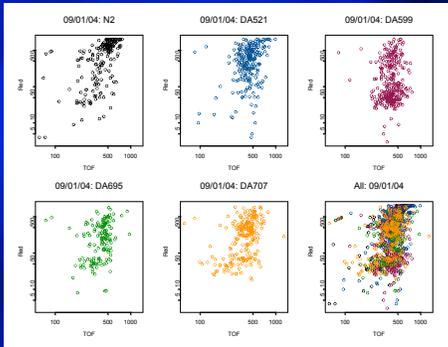
Avery 1993

Feeding Tests

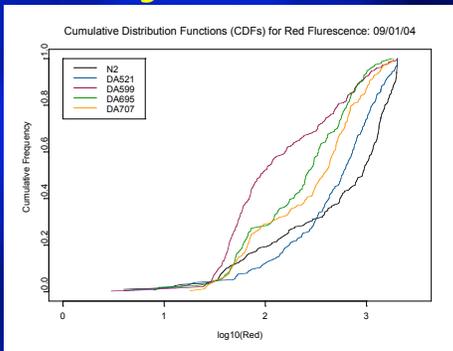
EAT mutants (Abnormal Pharyngeal Pumping)

- *eat-7* (DA521) "mysterious and amusing mutation"; "it is very difficult to study a phenomenon that vanishes when looked at" (Avery 1993). Falls asleep. While asleep they do not move or pump, and probably don't crap. Disturbing them wakes them up, and while awake they act fairly normal.
- *eat-8* (DA599) Brief, rare pumps. Slight coiler Unc.
- *egl-19* (DA695) Previously called *eat-12*. Relaxation defective. Smallish. Males don't mate. Weak Eat: terminal bulb stays contracted for longer than normal, sometimes > 1 s
- *eat-17* (DA707) Uncloned locus that affects feeding by affecting posterior movement of bacteria in the pharyngeal corpus and isthmus, and the timing of terminal bulb contractions. Displays clumping behavior; isolated in an RC301 background, so it's likely to have the RC301 *bor-1* mutation.

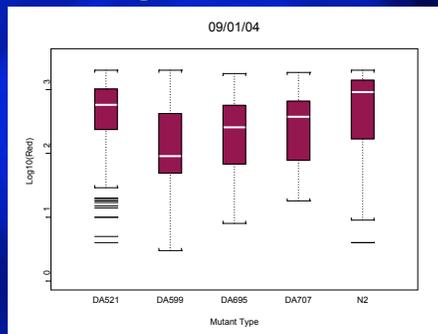
Feeding (Dye Accumulation)



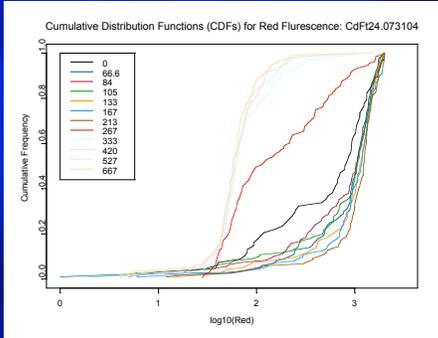
Feeding for Eat Mutants



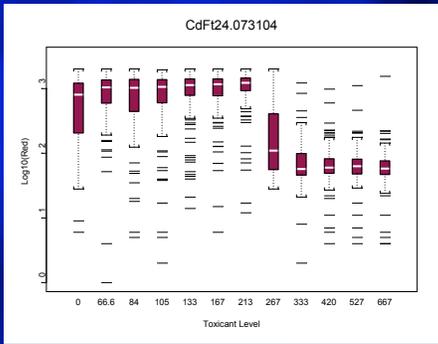
Feeding for Eat Mutants



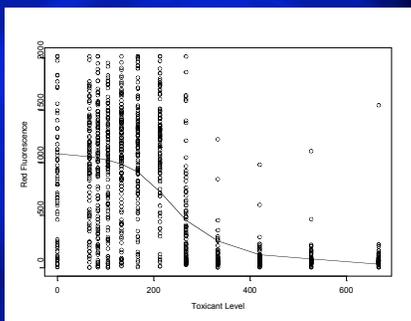
Effect of Cadmium on Feeding



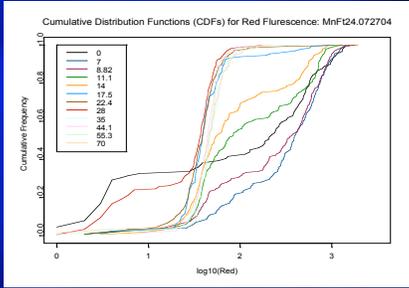
Effect of Cadmium on Feeding



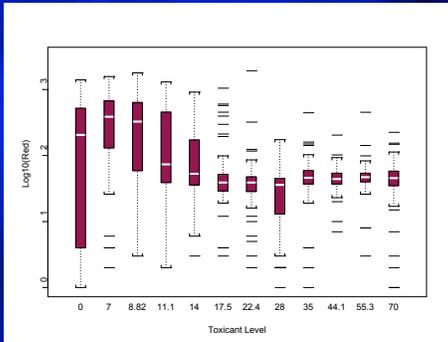
Effect of Cadmium on Feeding



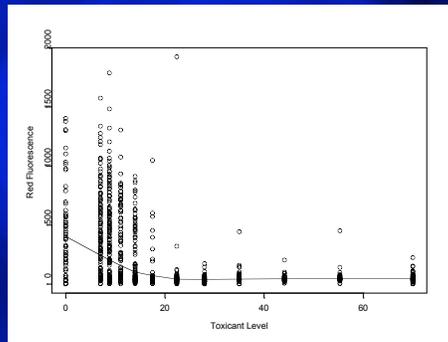
Effect of MMNG on Feeding



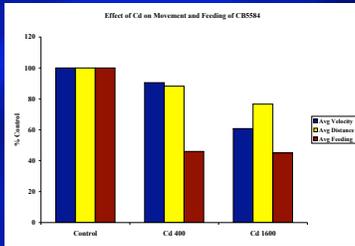
Effect of MMNG on Feeding



Effect of MMNG on Feeding



Effect of Cadmium on Movement



Transgenic Nematodes (GFP-based)

- Metallothionein (*mtl-1*, *mtl-2*)
- Glutathione-S-transferase (*gst-1*)
- Multiple cytochrome P450's
- Multidrug resistance gene (*mrp-2*)
- Low/high molecular heat shock protein
- Superoxide dismutase
- map kinase kinase (*mek-1*)
- p38 map kinase (*pmk-1*)
- heat shock protein 20 (*sip-1*)

Low Throughput

Imaging Nematodes

C. elegans neurons

- Hardware
 - Core Microsystems custom 3-D image analysis workstations
- Software
 - C-imaging
 - Data acquisition
 - Amira
 - 3-D rendering
 - 3-D modeling

Imaging Nematodes

- Neuronal GFP-transgenic
 - Touch/sensory neurons
 - Vulval neurons
- Male Tail

Genomics

- Agilent *C. elegans* custom microarrays
 - Based on ~21K predicted OFRs
- Agilent microarray scanner
- Agilent Bioanalyzer
