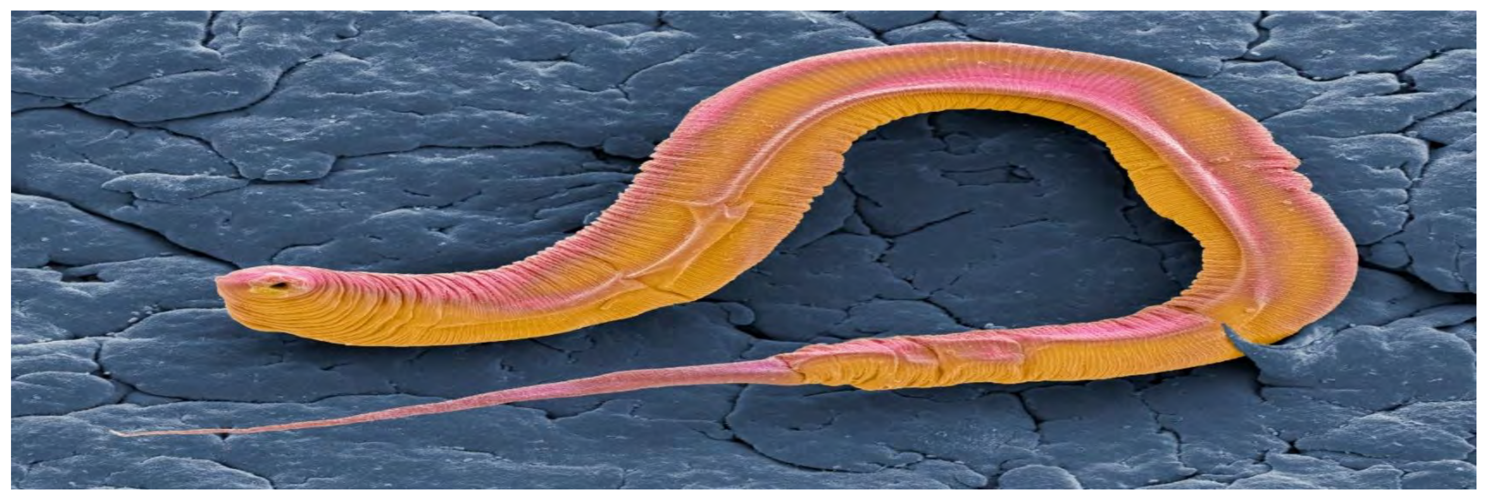


Worms in Research: A small powerhouse Giant.

OVERVIEW

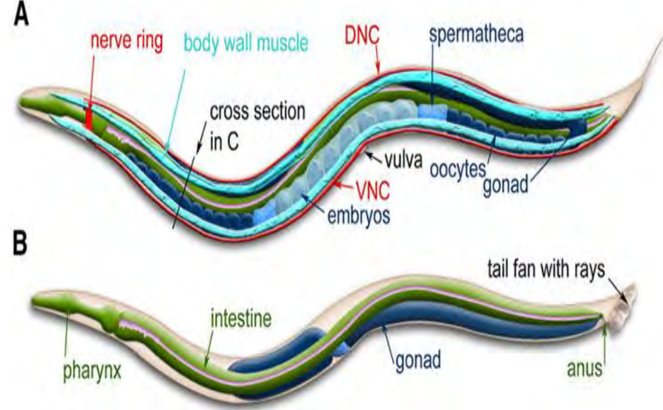
C. elegans is particularly useful in the study of the ageing processes because the organism passes through several distinct phases of life which can be observed physiologically and genetically using a dissecting microscope.

AUTHOR	GRADE LEVEL	CONTENT AREA
Edwin Davis	11th & 12th Grade	Academic Biology

ESSENTIAL QUESTIONS	TIME NEEDED	STANDARDS
The phylum Nematoda serves as an excellent model system for exploring how development evolves, using a comparative approach to Developmental genetics. Why is C. elegans a great model to use in the study of Human development and Diseases?	Ideally, this curriculum unit would begin on a Monday or Tuesday so that students could make their 15-minute, 24-hour and 48-hour worm observations on three consecutive school days. If the schedule does not allow for three consecutive days, the third observation can also be done at 72 hours.	**NCES.K.L.1** - Compare characteristics of animals that make them alike and different from other animals and nonliving things. **NCES.2.L.1.2** - Compare life cycles of different animals such as, but not limited to, mealworms, ladybugs, crickets, guppies, or frogs.

Making Connections

Why choose *C. elegans*?



In addition to being a powerful system for genetic studies, *C. elegans* has many inherent advantages as a model for eukaryotic biology. These features include its small size, large brood size, ease of cultivation, low maintenance expense, long-term cryopreservation, quick generation time, transparency, invariant cell number and development, and the ability to reduce gene activity using feeding RNAi. *C. elegans* are greatly aided by the transparency of the animal, which allows researchers to examine development and changes due to mutations or altered environments at the level of a single, identified cell within the context of the entire living organism.

Before students design their worm research projects, they learn and practice several research techniques with the model organism, *Caenorhabditis elegans*. This nematode is an ideal choice for experimentation in an Academic and Honors lab due to its powerful genetics, ease and low cost of maintenance, and amenability for basic research training. Students are challenged to characterize an instructor-assigned "mystery mutant" *C. elegans* strain. The "mystery mutant" strain has a defect in cholinergic synaptic transmission. Students are well poised to experimentally test *how* the mutation impacts synaptic transmission. For example, students design experiments that address questions including: Does the affected gene influence acetylcholine neurotransmitter release? Does it inhibit postsynaptic cholinergic receptors? Students must apply their understanding of the synapse while using their recently acquired research skills (including aldicarb and levamisole assays) to successfully design, execute and analyze their experiments. Students prepare an experimental plan and a timeline for proposed experiments. Students work collaboratively in pairs and share their research findings in oral and written formats.

Background

After distinguishing himself with major discoveries in molecular biology while at Oxford in the early 1960s, Sydney Brenner turned his attention to developmental biology. Working in Cambridge, he and his colleagues wondered how multicellular organisms develop. He looked for an animal that was simple but that had a nervous system and other distinct, differentiated tissues, including epidermis (skin), muscle, and intestine. After careful research, he selected a microscopic roundworm called C. elegans.

Although some roundworms are parasitic, C. elegans is free-living. These worms grow quickly—from embryo to adult in 3 days—are easy to culture and can be stored in a freezer. The embryos, larvae, and adults are transparent, making observations of morphology and development in living worms easy. C. elegans worms have 2 sexes: self-fertile hermaphrodite and male. Males are rare in wild-type populations, but they allow for genetic crosses. However, because the hermaphrodites are self-fertile, most strains can be maintained without time-consuming crosses. Remarkably, almost every cell division during development is invariant, occurring the same way in every worm. Isolating genes and introducing foreign DNA are much easier in worms than in more complicated animals. All of these features make C. elegans an excellent model for research on how cells divide, develop, and take on specialized tasks in higher (eukaryotic) organisms.

Brenner and John Sulston showed that C. elegans also has a small, compact genome, which proved to be especially important once large-scale sequencing became possible. The C. elegans genome, which is approximately 1/30th the size of the human genome, was the first genome of a multicellular organism to be entirely sequenced. Once the human genome was also completed, it became clear that 40% of C. elegans genes have human matches, and that in spite of their difference in size, both the human and C. elegans genomes contain approximately 25,000 genes.

In 2002, Sydney Brenner, John Sulston, and Robert Horvitz were awarded the Nobel Prize for their pioneering work on C. elegans and the discovery of genes that control cell death. In humans, these same genes are often mutated in cancer cells. Other C. elegans researchers have made numerous important discoveries, including those for genes controlling aging, obesity, diabetes, and developmental patterning. They have also made important discoveries in cell biology and neurobiology.

Materials

- 30 OP50-Seeded NGM-lite Plates
- 2 BLI-1 Mutant *C. elegans*
- 2 DPY-11 Mutant *C. elegans*
- 2 Wildtype (N2) *C. elegans*
- 8 Spatulas
- 1 Bunsen Burner
- 1 Stereomicroscope with Transillumination (minimum 30x)
- 1 Beaker with 95% Ethanol
- Incubator
- Thermometers
- Paper towels
- Distilled water

Students observing C. elegans under the microscope at Needham Broughton High School.

Students identifying the life cycle of C. elegans with Dr. Amy Maddox at Needham Broughton High School.

The Activity

Part 1: Introduction - Caenorhabditis elegans as a model organism.

- Students learn about the organism's life cycle.
- Students prepare media for C. elegans culture.
- Students prepare the incubator, set incubator temperature, and pour media plates for C. elegans.

Part I. *C. elegans* developmental timing is temperature sensitive. At higher temperatures, development will proceed faster, making timing difficult to predict. You may want to test the timing for your conditions by chunking one plate of wild-type worms prior to carrying out the activity. Ensure that the worms are kept at temperatures ranging from +11°C to +20°C. Brief periods above or below this temperature are tolerated but not ideal. Long periods outside this temperature range can kill or sterilize *C. elegans*. Note that Part II and Part III can be carried out on the same day or on separate days. If more than one day has passed between Parts II and Part III, chunk wild-type worms to at least one of the extra included plates to provide students with a control.

Part 2: Preparation – Preparation of bacterial food source & NGM petri plates for C. elegans.

- Students learn the 'chucking' method of transferring C. elegans onto a culture plate with E. coli.
- Students transferred C. elegans onto new plates.

"Chunking" is a way to keep worms alive and maintain growth – Cut a 1 cm square "chunk" of agar with worms in it from a plate with growing worms. Use a sterile scalpel dipped in alcohol and flamed before and after each use – Transfer "chunk" to new seeded plate. When to use this technique: – When you don't care exactly how many worms are transferred plate. Fig. 1

Making the first cut

A ~1 cm ² area

Figure 1

Dr. Amy Maddox and Edwin Davis at Broughton High School in Raleigh, NC.

NC STATE

Lessons

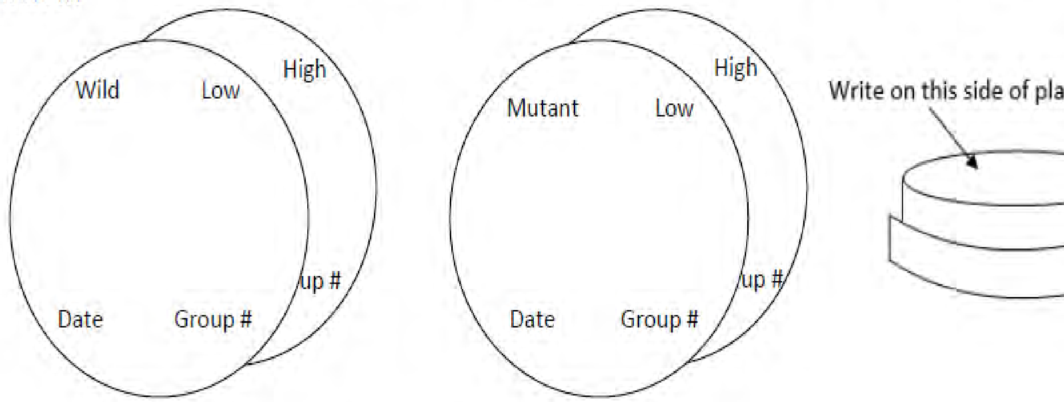
Lesson	Description	Time to present	Nematode activities	Conceptual activities
Lesson 1. Getting to know your worms: Observing wild and mutant *C. elegans*	Students discuss familiar examples of organisms that respond to environmental changes. They learn about nematodes through a PowerPoint presentation and then observe and compare two nematode strains under a microscope.	50 min.	Students observe and draw wild type and mutant worms.	Students learn about *C. elegans* as a model organism and learn "worm facts" through a presentation and observation.
Lesson 2. Worms in a changing environment: How does high salt affect *C. elegans*?	Through a PowerPoint presentation, students learn a few more basics about *C. elegans* and the experiment they will be doing. They "chunk" (transfer) both wild type and mutant worms to low and high salt plates. After 15 minutes, students record their observations for both worm strains.	90 min.	Students transfer wild type and mutant worms to low and high salt plates and observe them after 15 minutes.	Students learn more "worm facts" through direct observation.
Lesson 3: How does *C. elegans* keep from drying up in high salt?	Students use dialysis tubing to model what might be occurring with their worms on low and high salt. Students also make 24-hour observations of the two worm strains on low and high salt.	90 min.	Students make 24-hour observations of worms on low and high salt plates.	Students set up a model system using dialysis tubing and solutions containing low and high glycerol and test the effect of salt.
Lesson 4: Using evidence to develop an explanation for worm observations	Students examine worms after 48 hours and record observations. They analyze data from the scientific literature to develop an explanation for their observations of wild type and mutant worms on low and high salt plates.	50 min.	Students make 48-hour observations of worms on low and high salt plates.	Students examine graphs from the scientific literature comparing glycerol content and production in wild type and mutant worms.
Final Assessment Developing a model to show the effect of salt on C. elegans	Students build a model that describes what is occurring during the experiment, and they provide evidence for their claims.	90 min.	--	Students summarize their worm observations and inferences in a paper model

INSTRUCTOR PLANNING, PREPARATION, AND LAB FINE POINTS

The following table will help you to plan and integrate the four parts of the experiment.

Part	Day	Time	Activity
I. Chunk Wild-type and Mutant *C. elegans*	1	30 min.	**Pre-lab:** Set up student stations. **Lab:** Chunk wild-type and mutant worms to OP50-seeded NGM-lite plates.
II. Observe the *C. elegans* Life Cycle	3-4	20 min. 45 min.	**Pre-lab:** Set up student stations. **Lab:** Examine worms under the dissection microscope. Study the morphology and behavior of wild-type worms and identify the different developmental stages of the *C. elegans* life cycle.
III. Observe *C. elegans* Mutants	3-4	30 min. 45 min.	**Pre-lab:** Set up student stations. **Lab:** Examine wild-type and mutant worms. Identify differences in morphology or movement.

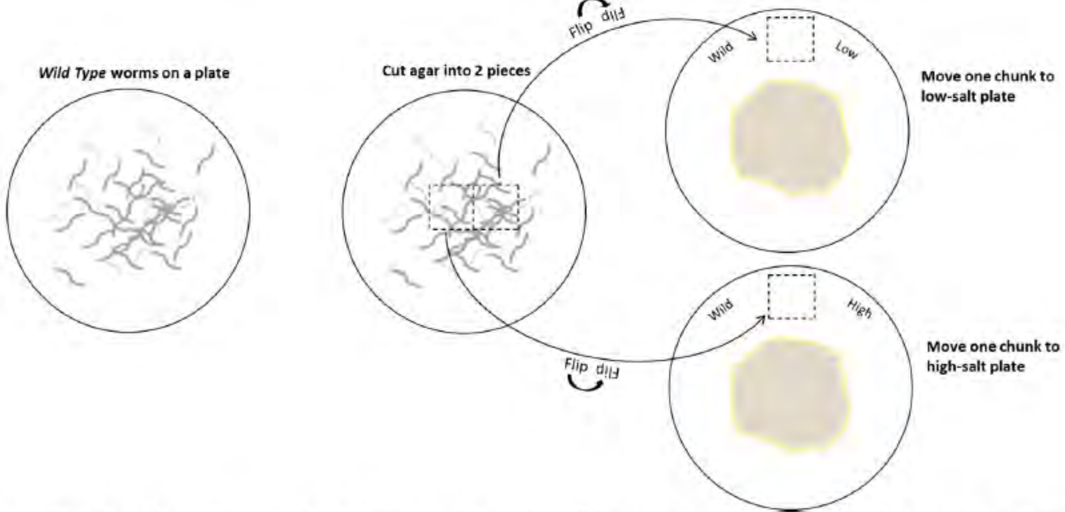
1. Label the **four** new plates with: the kind of worms that will be transferred (**wild** or **mutant**), the salt concentration in the plate (**low** or **high**), the **date**, and **your group number**, as shown below.



2. Remove the lid from the plate of wild type worms, place the plate under the microscope at low power, and find where most of the worms are.

3. Heat the flat end of the spatula in the Bunsen burner for a few seconds. Let the spatula cool for a few seconds.

4. Use the flat end of the spatula to cut the part of the worm plate that contains most of the worms into two pieces, each with about the same number of worms, as shown below.



5. Heat and cool the end of the spatula again. Slide spatula under one of the two chunks of agar and place the chunk, worm side down, onto the fresh **low salt plate** near the edge of the plate. Use your Sharpie to draw a circle on the outside of the plate at the place where the chunk landed (the "drop site"). Let the chunk of agar sit on the new plate for 3-4 seconds, and then use the clean spatula to flick the chunk of agar into the waste container.

6. Record the time that you transfer the worms on the data table.

7. Look at the plate under the microscope to make sure that you have transferred some worms.

8. Repeat steps 5-7 to transfer wild type worms onto the **high salt** plate.

9. Repeat steps 3-8 with the mutant worms.

10. **Look at the plates and record on the data table what you see *15 minutes*** after you transfer the worms to each plate. Here are some things for you to observe and record:
    - **Who:** How many adults, larvae, and eggs were transferred?
    - **Where:** Are the worms at the drop site or have they moved away? Are they on the food or the agar?
    - **What:** What are the worms doing? Are they moving or still? Have they moved from the drop spot or are they still in the same place? Do they seem to be eating?

WRAP UP AND ACTION

Explain here how you will assess the students' knowledge of the activity. Formally or informally?

As a follow up, students will be able to perform the following task.

- Get hands-on experience with a model eukaryotic organism.
- Observe and study the life cycle of *C. elegans.*
- Utilize their microscope skills.
- Learn how to subculture C. elegans.
- Learn about genetics and its effect on behavior.

Experimental Technique Assessment

1. Students will be able to use experimental nematodes such as C. elegans cultured in the laboratory on 6 cm Petri plates containing Nematode Growth Medium (NGM). Students will be accessed on their technique on transferring C. elegans to new culture plates. C. elegans worms are typically kept at 20°C; but can be incubated at a range of temperatures (between 4°C - 25°C), depending on the nematode species and experimental design.

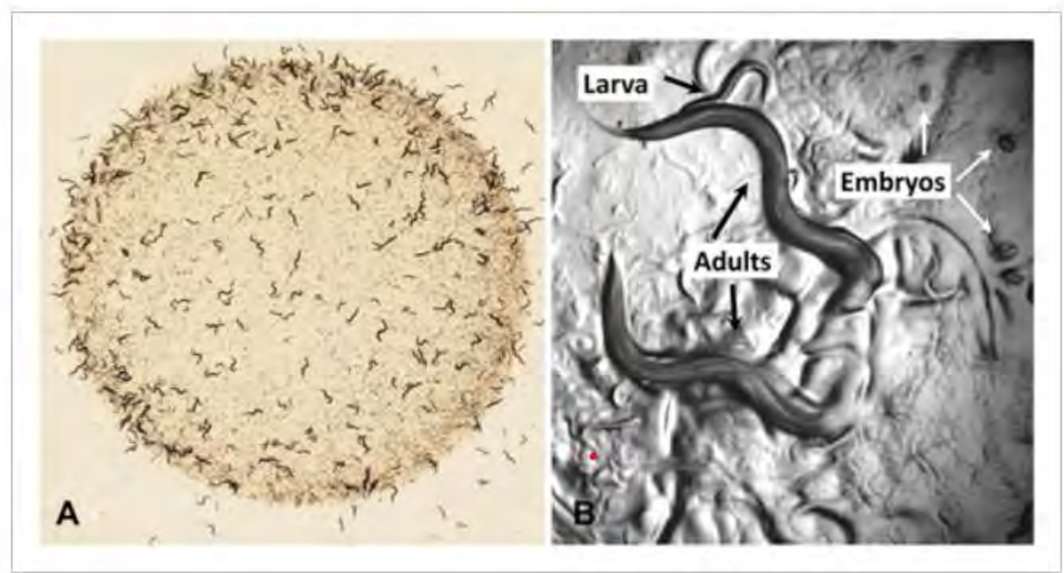


Figure 1 Open in figure viewer | PowerPoint

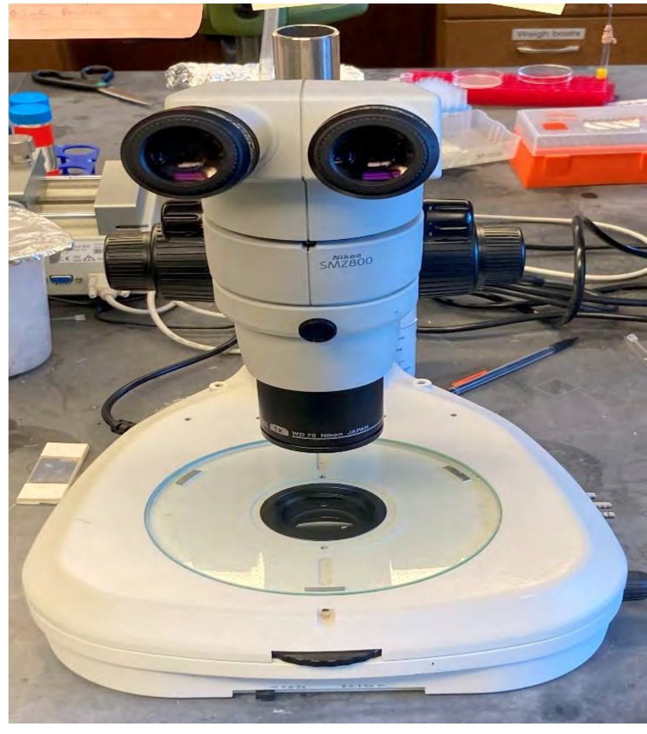
(**A**) A population of *C. elegans* feeding on a lawn of bacteria in a petri dish. Eggs and animals of all ages can be observed using sub-stage transmitted light on a stereo microscope. (**B**) A close up of animals at different developmental stages feeding on a similar lawn of bacterial. Courtesy of *WormBook.org* and used by permission.

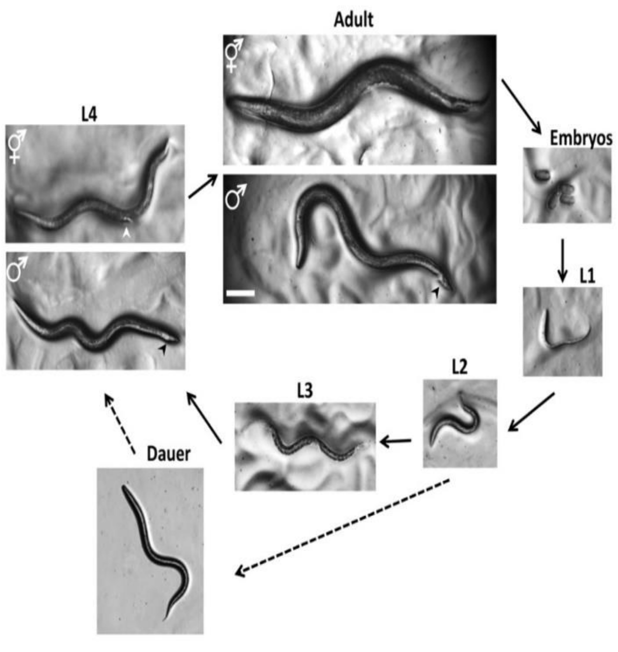
2. Students will be accessed on their “Chucking” technique. Students must use the “Chucking,” technique to transfer C. elegans worms to culture plates. A quick and convenient method is "chunking", wherein a sterilized scalpel or spatula is used to move a chunk of agar from an old plate to a fresh plate. The worms will crawl out of the chunk and spread out onto the bacterial lawn of the new plate.

Making the first cut

A ~1 cm ^{2} area

3. Students will be assessed on their ability to identify the various stages of C. elegans on an agar plate using a dissecting microscope.





Dissecting Microscope (20X, 40X)

Various growth phases of C. elegans.

Students using dissecting microscopes too make observations on the various stages of C. elegans.

4. **Students will perform a statistical grid count to determine the number of C. elegans adults needed for the experiment.**

Extensions

Subject Area: C. elegans (Genes and behavior lab).

Learning Activity Description: Students will learn how sensory behavior is controlled by genes using the worm C. elegans.

- Students will observe that C. elegans is attracted to some odors (e.g., the smell of popcorn butter) and repelled by other odors (e.g., high concentrations of the odor of almond oil).
- Students will learn that C. elegans attraction to and repulsion by specific odors require the activity/expression of specific genes.

Lesson Activity Objective and Outcome:
Students will distinguish between C. elegans that are a wild type (normal) versus mutant for smelling of specific food odors.

Materials/Supplies Listed:
- Young adult C. elegans: wild type and mutant
- Four 10-cm agar plates
- Pipette and tips for transferring 2-20 microliter or 20-200 microliter volumes.
- Kimwipe tissues
- Chemicals: ethanol, sodium azide
- Dissecting microscope

Procedure:
Chemotaxis assays Record your observations of each plate at the end of this section.
To draw the right conclusions, it is especially important to work carefully, to label your samples/plates accurately and record your observations completely.

(1) There will be four plates: Plate 1 will have diacetyl at the (+) end of the plate. Wild-type C. elegans will be added to this plate at the origin.
(2) Plate 2 will have benzaldehyde at the (+) end of the plate. Again, wild-type C. elegans will be added to this plate at the origin.
(3) Plate 3 will have diacetyl at the (+) end of the plate. Strain A will be added to this plate at the origin.
(4) Plate 4 will have diacetyl at the (+) end of the plate. Strain B will be added to this plate at the origin.

You have to determine the following:
(a) Is the odor diacetyl attractive to C. elegans?
(b) Is the odor benzaldehyde attractive or repulsive to C. elegans?
(c) Which strain (A or B) is wild type and which strain (A or B) is mutant?

Resources

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About the Author

Growing up in the inner city of Newark, New Jersey I learned to overcome many academic and personal challenges. After pursuing my BS in Biology from Trenton State College, I completed my Master's Degree in Science Education in 1½ years. Upon graduation, I worked various jobs as an Environmental Chemist along with other jobs in the scientific fields. For 3 years under Muslim Affairs, I volunteered as Basic Skills Teacher at East Gate Maximum State Prison located in Rahway, NJ. (Formerly called Rahway Valley Prison.) The valve of passing your knowledge forward became a reality, since my Uncle was incarcerated at the facilities for 25 years to life. As a Basic Skills Teacher, I had the opportunity to interact with the inmates to teach them about the principles of remorse, compassion, responsibility, accountability, and results of decision making.

In 2003, after an industrial accident, my family relocated to Knightdale, NC.. Currently, I have been teaching for 20 years as a certified comprehensive High School science teacher in both Wilson and Wake counties. I am a dynamic science teacher. My catch phrase to my students: "I'm not a copycat, but a cat no can copy." Simply put, I strive to teach my student to think and be original about their pursuits and dreams, while observing a positive role model. I assist my students with creating a vision for their future whereby their academic actions and deeds become synonymous with excellence, innovation, honor, integrity, and outstanding quality and community service.

My Kenan Fellows experience starts with an awesome sense of thankfulness. As a Kenen Fellow I have served as a motivating supernatural agent of change. My minority students directly observed a highly motivated Afro-American male teacher with a strong appreciation of STEM education along with experimentation, innovations in STEM education inspiring future generations. Many of the lessons I teach as a Kenan Fellow go far beyond academic content and pedagogy. My uniquely lived experiences, as a Kenan Fellow teacher, creates a rich curriculum that contributes to student success in ways that can't be graded or easily quantified. Using methods that transcend traditional teaching and learning, Black male teachers can provide hope, inspiration, advice, compassionate listening and, sometimes, tough love to make a difference in their students' lives. As a Kenen Fellow, I spend more time mentoring and counseling students than teachers of any other demographic. For example, I mentor with a local community nonprofit called "Communities In Schools of Wake County," and as a result for Black boys from low-income households, exposure to a Black teacher for one year in elementary school reduces high school dropout rates by 39 percent.

The Kenen Fellows program has allowed my students to experience STEM based labs using DNA Gel Electrophoresis, Microscopic Observations, and live specimen experimentation and genetic innovations.

About the Fellowship

Throughout the summer, working in Dr. Amy Maddox's lab became a daily adventure. Dr. Maddox's colleagues were simply amazing. Professors, graduate students, and lab personnel offered to speak with me about their research and topics related to my study of C. elegans flat worm. For instance, on many occasions, Dr. Maddox's graduate students provide me with research information, observational guidance on lab procedures related to C. elegans, and personal insight related to their research and its possible application toward uncovering, understanding, and possibly preventing Human Diseases.

During my internship, I had the privilege to attend weekly staff meeting, observe research updates provided by graduate students, and ask questions to researchers studying various components related to C. elegans. During the Fall semester of 2023, Dr. Maddox came to Broughton High School and co-instruct students with Mr. Davis. Dr. Maddox reviewed the life cycle of C. elegans and demonstrated the techniques of "Chucking" and transferring C. elegans into new agar plates. Dr. Maddox assisted me with the transfer of C. elegans to demonstrate the organism's use in studying human-based disease.

The selection of C. elegans was selected as a good model for humans because the C. elegans genome indicates that this 'simple' worm contains many genes with a high degree of similarity to human disease genes. For example, C. elegans can be grown in large numbers, can be easily screened for effects of novel drugs on complex processes involved in human disease. C. elegans is particularly useful in the study of ageing processes because the organism passes through several distinct phases of life which can be observed physiologically and genetically. The advantages of C. elegans in high school science research has advantages, such as its small size, short generation time with a high number of eggs, and short life cycle, has become a suitable candidate for the study of potential methods for new drug development from natural sources.

Student Pages

Glossary

1. Autosomes: Chromosomes not directly involved in determining the sex of an organism.
2. Dauer: When food is scarce, or colonies become crowded, young worms stop developing normally and enter the *dauer stage*. In this form they can live, without eating or reproducing, for months - about ten times longer than the worm's normal lifespan. When ideal conditions (including necessary resources) are available, the *dauer* finally develops into an adult and resumes its normal aging process.
3. Desiccated: Freed of moisture; dried out.
4. Diffusion: The movement of a substance from areas of high to low concentration.
5. Field of View: the area that is visible (as through an optical instrument like a microscope).
6. Genotype: Genetic makeup of an organism or combination of genes/chromosomes in an organism.
7. Larvae: The newly hatched, earliest stage of any of various animals that undergo metamorphosis, differing markedly in form and appearance from the adult.
8. Mole: The amount of a substance that contains as many atoms, molecules, ions, or other elementary units as the number of atoms in 12 grams of carbon 12. The number is 6.0225×10^{23}, or Avogadro's number.
9. Molt: To periodically shed part or all of a coat or an outer covering, such as feathers, cuticle, or skin, which is then replaced by a new growth.
10. Nematode: Any unsegmented worm of the phylum Nematoda, having an elongated, cylindrical body; a roundworm.
11. Osmolarity: Solute (e.g., salt or sugar) concentration expressed as molarity (moles/L).
12. Osmoregulation: Ability to sense and respond to changes in cell volume.
13. Spontaneous non-disjunction: Failure of two members of a chromosome pair to separate (disjoin) during meiosis so that both go to one daughter cell and none to the other.

The PBS educational site is a great, free resource for educators, but you must create an account to use the materials. The first time you log in to the PBS Learning Media website you will be asked to create an account and provide an email and password. Once you have logged in, select "keep me logged in" to avoid having to repeat the process.

• Interactive activity to learn about different model organisms:
http://mass.pbslearningmedia.org/resource/hew06.sci.life.gen.modelorg/model-organisms/

Other Resources:

• Brief introduction to C. elegans (video, 2:11):

https://www.youtube.com/watch?v=zjqLwPgLnV0

• Harlem Shake worms (video, 0:29)

https://www.youtube.com/watch?v=BWCm20gXnEs

• C. elegans movement (video, 5:27)

https://www.youtube.com/watch?v=GgZHziFWR7M

Appendix

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