

Laboratory 2: Artificial Selection Experiment, Week #1 -
Selection for “Fast” *C. elegans*

The Fourth Annual Bowdoin College Worm Derby

January 29 and 30, 2002

Introduction

Over the next three weeks, each student will conduct an artificial selection experiment on a population of nematode worms: *Caenorhabditis elegans*. The goal of this experiment is to allow you to empirically examine the process of natural (or in this case, *artificial*) selection, and its role in the evolutionary process. Throughout this experiment, keep in mind the evolutionary concepts and terminology that you will be exposed to in both lecture and lab over the next few weeks. When you look into a culture dish of *C. elegans*, you are not merely looking at a collection of nematode worms. Rather, that culture dish contains a **gene pool** upon which selection can be imposed. In this gene pool are a variety of **alleles** (alternate forms of a gene) that are combined in a variety of ways. As worms mate with each other and with themselves (remember that *C. elegans* is a selfing hermaphrodite), different combinations of alleles at different gene loci will result in individuals having unique combinations of alleles throughout their entire genomes. Each individual worm has its own genetic constitution, or **genotype**. You will not know what the underlying genotypes of the different worms are, but you can select for different **phenotypes** (the physical manifestation of the genotype) - in this case, the phenotype you are selecting is “fast” mobility towards a volatile chemical attractant. Individuals with the phenotype you are selecting for will then be allowed to mate, and we hypothesize that this will result in a high proportion of progeny containing a genetic background for that selected phenotype: “fast” worms.

This week, the class will perform the initial selection for “fast” nematodes. The population of *C. elegans* you start with today is a genetically variable *parental* stock created by crossing males and hermaphrodites of several different populations from around the world. You have two tasks to perform in lab this week:

1. To measure the frequency distribution of different “speed” phenotypes in the parental population, and;
2. To select parents that will produce the *next* generation of your *C. elegans* population.

During the next two weeks, the class will repeat this procedure – performing additional selection events. Data from the entire class will then be pooled, and we will discuss how to analyze and interpret the results you obtain.

Procedure

A. Background Information

In this experiment, you will select for “fast” mobility in *C. elegans* - a phenotypic character that is likely to be determined (at least in part) by the genetic makeup of the worms. However, unlike some other genetically determined characters you have encountered in lecture so far, “fast” mobility is a **quantitative trait** - it is probably determined by *many* different genes rather than by a single gene locus. This topic will be discussed in lecture on March 6, and which is described in Chapter 7 of *Evolutionary Analysis, Second Edition*.

When designing and performing an artificial selection experiment such as this, it is important to consider the many kinds of genes and the different phenotypes that are being selected for. In order to assess the mobility phenotype in *C. elegans*, we use a volatile organic compound to attract worms across a “racetrack”. Bargmann and others (1993) found that *C. elegans* exhibited a positive chemotactic response (i.e., were attracted) to **diacetyl (2, 3-butanedione)** at a 10^{-2} concentration. How might this particular method of attracting worms across a racetrack have an effect on (or contribute to) the phenotype being selected for? In addition, we use **sodium azide** to anesthetize the worms once they have successfully crossed the racetrack. How might *this* compound affect the “trait(s)” being selected for? Think about these and other questions as you perform your experiment today and in the following weeks.

B. The Initial Artificial Selection Experiment for Fast *C. elegans*

The parental population of *C. elegans* has been maintained at room temperature on 60x15mm culture dishes containing Nematode Growth Medium (NGM) and inoculated with *E. coli* strain OB50 (the worm’s primary food source in the lab). One hour before lab, the parental *C. elegans* population was starved by transferring them from a food-rich culture dish to one containing only NGM. In order to select for fast *C. elegans* mobility, each student will construct a “racetrack” at the beginning of today’s experiment. After doing so, you will assess the speed of your *C. elegans* population – that is, the average speed of the parental population before “selecting” the parents of your next generation.

C. Experimental Set-up

The artificial selection experiment involves two different experimental groups: a **selection** and a **control group** of students. During each lab period, a fraction of the class will act as “controls”, while the other students will act as “selection” group members. We will ask for volunteers to serve as the control group - there is a *slight* amount of additional work involved. After reading the protocols below, consider what experimental conditions are being “controlled” in this experiment. What conditions are *not* being controlled in this experiment?

D. Protocols

☠ **Caution:** Please wear protective gloves for step 1 of this experiment ☠

1. **Creating the “Racetrack”:** In order to assess mobility speed and select for the fastest worms in your *C. elegans* population, you must construct a “racetrack”. Using a pre-marked NGM culture dish that contains no food, place 1 μ L of the anesthetic **1M sodium azide** - (in green tubes) on the media directly over the black dot at one end of the culture dish. (Note: Sodium azide is a poison. Please be careful!) After this drop has dried, place 1 μ L of **diacetyl (2, 3-butanedione)** - (in orange tubes) on the media directly over the same black dot. Since diacetyl is a volatile organic compound, immediately cover the dish in order to create a concentration gradient across the inside of the dish. Make sure that the tubes containing the 1M sodium azide and the diacetyl are securely closed once you are finished.

Control Group: In addition to the steps described above, individuals in the control group should also create a “control racetrack”. This is done by placing a line of 2 X 1 μ L drops of **1M sodium azide** (indicated by black marks on the dish) into the center of a pre-marked NGM culture dish that contains no food. Make sure to clearly mark this as a “control racetrack”.

2. **Transferring Worms to the Racetrack:** Once the racetrack has been constructed, make sure to flame-sterilize the circular metal loop so as to prevent contamination from other *C. elegans*. Use this metal loop to scoop out a 2-2.5cm long trough of media from your parental culture dish. Place the loop of media **face down** over the straight black line on the end opposite the diacetyl target. Start your stopwatch immediately - the experiment has just begun! Gently press down the trough of media to ensure a transfer of worms to the racetrack. Cover the culture dish. After one minute, carefully remove the trough from the racetrack - if left on the racetrack, the worms tend to remain under the trough to avoid desiccation. Replace the cover to the culture dish.

Control Group: Repeat the above procedure for your “control racetrack” - placing the 2-2.5cm loop along the black line indicated.

3. **Measuring the Mobility Phenotype in the Parental Generation (Both the Selection and Control Groups Perform this Procedure):** Under a dissecting microscope, watch the nematodes move across the plate to the diacetyl target. Keep your racetrack covered - this helps to maintain the concentration gradient of diacetyl and prevents desiccation of your *C. elegans* (if the cover starts to fog up, wipe the moisture off with a Kimwipe). Watch closely as the worms approach the attractant - once the head of a worm crosses the “finish line” record the **elapsed time to the nearest second** on your data sheet (do not attempt to record to the tenth of hundredth of a second – it’s very difficult to be that accurate). When possible, record the Life

History Stage (as “larvae” or “adult”) and Sex (as “hermaphrodite” or “male”) of each worm as it crosses the finish line. In an effort to control as many variables as possible, strive to be very consistent in the manner that you sample each worm - this makes for meaningful comparisons between different generations, as well as between the data collected by your classmates. Measure the elapsed time for each worm to cross the finish line for at least **45 minutes** - in this way you can collect a more accurate measurement of the average mobility speed for the parental population, (including the very fast and relatively slower worms).

4. Establishing the F1 Generation

a. Selection Groups: To establish your next generation (F1) of fast *C. elegans*, you must select the fastest worms from your race and transfer them to a new culture dish – these will be the parents of you selected F1. Before transferring any worms, make sure to label **the bottom** of your new culture dish with the following information: (1) your name, (2) your lab day (i.e., Tuesday, or Wednesday), (3) the date, (4) the generation created by the progeny of the worms being transferred (in this case, “F1”), and (5) that you are in the “selection” group. To select parents that will create the next generation, you must transfer the **first 25 to 30** hermaphrodites to cross the finish line. In order to facilitate the transfer, the worms are anesthetized by the 1M sodium azide at the finish line. Once 25 to 30 worms have crossed the finish line and are anesthetized, use a sterile platinum probe to **carefully** move these worms to an area away from “slower” worms that cross the finish line afterwards (we recommend shifting them to the center of the target). In order to transfer the worms, carefully use a sterile toothpick to cut out the media underneath your “selected” worms. Place this excised media **face up** on a new culture dish containing no food.

b. Control Groups: Before transferring any worms, make sure to label **the bottom** of a new culture dish with the following information: (1) your name, (2) your lab day (i.e., Tuesday, or Wednesday), (3) the date, (4) the generation created by the progeny of the worms being transferred (in this case, “F1”), and (5) that you are in the “control” group. To establish your next generation of control *C. elegans* (F1-control), transfer **25 to 30** hermaphrodites from the two sodium azide puddles on the “control racetrack”. **Carefully** use a sterile toothpick to cut out the media underneath these control worms, and place the excised media **face up** on the new plate containing no food.

5. Once you have completed step 4, your lab instructor will de-anesthetize the *C. elegans*, and place them on a culture dish with food. Your worms will be maintained for you until next week (this is 2 generations for *C. elegans*). Before leaving, please place your used racetrack and your dish of parental *C. elegans* in the plastic disposal tub on the center table.

6. Before leaving lab, hand in your data sheet to your lab instructor. The data will be entered into a spreadsheet, and we will begin analysis of these data in the next few weeks.

References

Bargmann CI, Hartwig E, Horvitz HR. 1993. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell*. 74: 515-527.

Freeman S, Herron JC. 2000. *Evolutionary Analysis, 2 ed.* Prentice Hall. 698Pp.