Supplemental Materials
for
Attack of the Killer Fungus: A Hypothesis-Driven Lab Module

Brian K. Sato
Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697

Table of Contents
(Total pages 26)

Appendix 1: Primer for primary literature and hypothesis construction
Appendix 2: Instructor information regarding necessary equipment, reagents and recipes for the module
Appendix 3: Nematophagous fungi background information
Appendix 4: Protocols for Week 2 and Week 3 experiments
Appendix 5: Pre-/posttest taken by students in lecture to assess achievement of learning objectives
Appendix 6: Exam questions pertinent to the nematophagous fungi experiment.
Appendix 7: Rubric for nematophagous fungus lab report

Corresponding author. Mailing address: Department of Biochemistry and Molecular Biology, 2238 McGaugh Hall MC3900, University of California, Irvine, CA 92697. Phone: 949-824-0661. Fax: 949-824-8014. E-mail: bsato@uci.edu.

©2013 Author(s). Published by the American Society for Microbiology. This is an Open Access article distributed under the terms of the a Creative Commons Attribution – Noncommercial – Share Alike 3.0 Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/), which permits unrestricted non-commercial use and distribution, provided the original work is properly cited.
Appendix 1: Primer for primary literature and hypothesis construction.

Primary Literature Background

Primary literature in biology consists of journal articles that are written by experts in the field. These articles consist of research projects that produce novel information, which adds to the existing knowledge base. Primary literature is peer reviewed by other individuals in the same field to ensure that the conclusions made in the paper are supported by the presented data.

A typical scientific article consists of the following sections:

**Title** – Briefly and accurately details the contents of the research paper.

**Abstract** – Highlights the main question(s) of the paper, how they were tested and the main conclusion(s). By reading the abstract, one will have a solid idea of the contents of a given paper.

**Introduction** – Provides the audience with the background information necessary to understand the results of the study. The introduction includes the larger context of the research topic, more specific details pertaining to the particular research question, and the specific purpose or hypothesis that the authors examine in the results section.

**Materials and Methods** – This is a detailed description of how the experiments in the paper are performed. The level of information should be sufficient so that any reader can perform the same experiments described in the results section and obtain the same conclusions.

**Results** – The results section introduces the experiments performed by the author, displays the data as figures or tables, and states the conclusions from the given results. The figures or tables are presented in a logical manner to allow the audience to easily come to the same conclusions as the authors, and will be accompanied by titles and figure legends.

**Discussion** – While a brief summary of the results will be included, the primary function of the discussion is a more in depth analysis of the results in the context of previously published data in the field. It is here that the authors will revisit the larger context mentioned in the introduction and explain how their understanding may now be different. The discussion can also present future experiments to be performed based on the results of the study.

**References** – A list of citations used by the authors throughout the paper. This allows the reader to easily find more relevant information regarding the given topic.

How to Search for Primary Literature

Searching for primary literature is similar to searching for any information that one requires in daily life, although in this case the database the search is performed in tends to be more specialized.

It is key that when using search terms; one is as specific as possible. For example, when searching for background information for this module, searching for “fungi” or “worms” will present you with nearly limitless results, the vast majority of which will not be relevant. Instead, if those terms were replaced with “Arthrobotrys oligospora” or “nematophagous fungi”, the resulting research articles will be fewer and more useful.

One of the primary databases which houses relevant biology literature is PubMed (http://www.ncbi.nlm.nih.gov/pubmed). PubMed allows you to search for articles and provides the title, abstract and link to the entire paper. Once a search is performed, a list of results is produced. It is possible to filter these results by the type of article (journal article, review article, government publication, etc), publication date, species involved, and availability of the text, among others. After clicking on a given article, PubMed will provide a list of related publications that may be of interest.
In addition to PubMed, other commonly used search engines include Google Scholar (http://scholar.google.com/), Faculty of 1000 Prime (www.f1000.com), and BIOSIS Previews (http://thomsonreuters.com/biosis-previews/). Another option is to search specific journals, such as Nature, Science and Cell for relevant information.

**Hypothesis Construction**

A hypothesis is a key component of the scientific method. This statement is a predication regarding the effect of an experimental variable on a given phenomenon. A scientific hypothesis must be:

1. **Testable** – It is essential that an experiment can be designed that will demonstrate that a given hypothesis is correct or incorrect.

2. **Evidence-based** – The prediction contained in the hypothesis is not a random guess, but based on information found in the scientific literature or previous observations.

3. **Concise** – A hypothesis should be a statement and not a paragraph or a group of ideas spread throughout the introduction of a paper.

For the nematophagous fungus module, the hypothesis will include the specific variable being tested and the predicted impact it will have on *C. elegans* survival.
Appendix 2: Instructor information regarding necessary equipment, reagents and recipes for the module.

Equipment and Reagents

- Micropipettors (P20, P200, P1000) – one set per group of four students
- Appropriate micropipettor tips
- Bench top centrifuge – one per section of 20 students (capable of 400 RCF centrifugation)
- 1.5ml microcentrifuge tubes
- Microscope slides
- Compound or dissection microscope – one or two per group of four students
- Sterile water

- 6 cm diameter Cornmeal agar (CMA) dishes with and without *A. oligospora*
  - CMA plates are seeded with fungus one week prior to experiment
  - Inoculation is accomplished by streaking the fungus onto the plate with a sterile metal loop/wooden stick
  - When streaking the fungus, streak over the entire CMA plate to uniformly cover the entire surface

- 6 cm diameter Nematode growth media (NGM) dishes with *E. coli* (OP50) and *C. elegans*
  - NGM plates are seeded with *C. elegans* four days prior to the experiment
  - 50µl of an overnight *E. coli* (OP50) culture in Luria Broth is added to the center of an NGM plate
  - Using a sterile spatula, cut out a block of agar from an old NGM plate containing growing *C. elegans* and transfer to the new plate
  - *C. elegans* from the previous plate crawl off of the agar block, eat the *E. coli* and reproduce

All organisms can be obtained from the organizations listed in the materials section of the manuscript. Once obtained, all can be frozen:

*A. oligospora* can be stored according to the instructions from ATCC (http://www.atcc.org/~media/PDFs/Technical%20Bulletins/tb02.ashx). In between courses, cultures can be maintained by streaking the fungus on a new CMA plate roughly once a month or thawed from the frozen stock prior to the beginning of a new quarter/semester.

The *E. coli* feeding strain can be stored according to instructions from Thermo Scientific (http://www.thermoscientificbio.com/uploadedFiles/Resources/preparation-ecoli-culture-glycerol-stocks.pdf). In between courses, cultures should be thawed from the frozen stock just prior to use.

*C. elegans* can be stored according to instructions from Worm Book (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html). In between courses, cultures can be maintained by adding worms to a new NGM + *E. coli* plate once every few weeks or thawed from the frozen stock prior to the beginning of the new quarter/semester.
Reagents per experiment

Week 2 practice experiment (performed in pairs)

Media (per pair):
- 1 NGM plate with *C. elegans*
- 1 CMA plate with *A. oligospora*
- 1 CMA plate without *A. oligospora*

Worms are added to the CMA plates in lab. At a given time point later (2 to 48 hours), worms are removed from the CMA plates and counted to calculate the percent survival.

In preparation for this week’s experiment, instructors will streak fungus on 1 CMA plate for each set of 4 students one week before the module begins. During week 1 of the module, each student will use this plate to streak a new CMA plate (4 students will share 1 starter plate). This means each pair will streak 2 CMA plates in week 1. One of these will be used for the week 2 experiment while the other will be used to streak plates for the week 3 experiment.

In preparation for this week’s experiment, instructors will add worms to one new NGM + *E. coli* plate (per pair) roughly four days prior to the start of the week 2 experiment.

Equipment (per pair):
Micropipettor tips – roughly ½ box of P20/P200 tips, and ¼ box of P1000 tips
Microcentrifuge tubes – 4
Microscope slides – 6
Sterile water – 10ml

Week 3 experiment (performed in group of 4) – Control versus variable condition

Media (per group of 4):
- 3 NGM plates with *C. elegans*
- 4 CMA plates with *A. oligospora*
- 4 CMA plates without *A. oligospora*

Worms are added to the 8 CMA plates. For each time point (6 hours, 24 hours), 4 CMA plates (2 control, 2 variable) are rinsed and worms are counted to calculate percent survival. Survival in the control condition and variable condition are then compared.

Using an extra (not used during week 2’s experiment) CMA plate inoculated with fungus during week 1, students will streak 4 new CMA plates (per group) during week 2. **Keep in mind, if the variable tested is a modified CMA plate, 2 of the CMA plates struck out will be control CMA while the other 2 will be the variable CMA.**

In preparation for this week’s experiment, instructors will add worms to three new NGM + *E. coli* plates (per group of 4) roughly four days prior to the start of the week 2 experiment.

Equipment (per group of 4):
Micropipettor tips – roughly 1 box of P20/P200 tips, and ½ box of P1000 tips
Microcentrifuge tubes – 8
Microscope slides – 10
Sterile water – 20ml
**Recipes**

**Cornmeal Agar Plates**

- Add 17g cornmeal agar (Becton Dickinson #211132) and water up to 1L. Autoclave for 20 minutes.
- Pour into 6cm petri dishes
- Store at 4°C once plates are dry

**Nematode Growth Media Plates (2.3% NGM)**

- Add 23g nematode growth media (Bioworld #30620040) and water up to 1L. Autoclave for 20 minutes.
- To this, add:
  - 1ml 1M MgSO$_4$
  - 1ml 1M CaCl$_2$
  - 25ml 1M phosphate buffer pH6 (KH$_2$PO$_4$/K$_2$HPO$_4$)
- Pour into 6cm petri dishes
- Store at 4°C once plates are dry

- Another option to make NGM plates not utilizing the commercial NGM is as follows (taken from Worm Book [http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e214](http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e214)):

  - Add 3g NaCl, 2.5g peptone, 17g agar and water up to 1L. Autoclave for 50 minutes.
  - Cool flask to 55°C.
  - To this, add:
    - 1ml 1M MgSO$_4$
    - 1ml 1M CaCl$_2$
    - 1ml 5mg/ml cholesterol in ethanol
    - 25ml 1M KPO$_4$ buffer pH 6
  - Pour into 6cm petri dishes
  - Store at 4°C once plates are dry

**Luria Broth (for OP50 *E. coli*)**

- Add 10g tryptone, 5g yeast extract, 10g NaCl, and water up to 1L. Autoclave for 20 minutes.
- Store at 4°C
The Nematophagous Fungi *Arthrobotrys oligospora* and its Ability to Capture *Caenorhabditis elegans*.

Pei H. Chan, Vahan Martirosian, Nathan D. Mih, Jason P. Olver, Abigail A. Radaza, Darany K. Tan, Brian K. Sato

University of California, Irvine, June 2012

Nematophagous fungi are soil dwelling organisms that have a number of mechanisms to trap worms. This is a means of sustenance for the fungi, is important for the ecological balance in the environment, and provides agricultural benefits. This review has gathered literature regarding nematophagous fungi and the regulation of worm capture.

I. *Arthrobotrys oligospora*

Background information

Fungi are eukaryotic microorganisms that are abundant across the globe and inhabit many different types of niches. They have a variety of benefits, both for society and the environment. These organisms are consumed for nutrition and used for food preservation purposes, for example through the addition of specific microbes to cheeses (11, 29). They also naturally produce antibacterial substances like penicillin (3). In addition, fungi are essential members of the ecosystem. Mycorrhizal fungi associate with plant roots, which increases the surface area of the roots, allowing greater uptake of water and nutrients. They also protect plant roots from infection by harmful pathogens. Saprotrrophic fungi colonize dead material through hyphal penetration of materials such as wood, leaves and manure, which are rich in cellulose. As animals are incapable of metabolizing cellulose, it is essential that fungi are able to break these items into simple compounds like sugars and carbon dioxide.

Fungi also have uses in the agricultural community as potential control agents for parasites in farm animals (9). Although most species of fungi forage on decaying matter, some species, which include the fungi of interest, are carnivorous and use predatory techniques to capture their prey. Of the many predacious species currently under study, *Arthrobotrys oligospora* holds great promise in the agricultural industry for its potential as a pest control agent for both crops and animals.

*A. oligospora* belongs to the class Leotiomycetes, and the family Orbiliaceae. It is mostly found on compost and decomposing wood, as well as animal excrements and metal polluted soils (9, 12, 38). It is broadly dispersed across the globe, found in both terrestrial as well as marine environments. Moreover, *A. oligospora* is considered the most abundant predacious fungus in the environment. In nutritious environments, *A. oligospora* survives through a saprotrophic mechanism (12), by processing dead and decaying matter. However, in low-nutrient environments, this species has an increased potency for capturing prey, which leads to the formation of traps.

When not capturing prey, the microscopic anatomy of this fungus is similar to other non-predacious species (Figure 1a). This includes hyphae and spore-forming apparatuses with the spore stalks growing up (Figure 1b). However, under the influence of particular stimuli, including nematodes or other microscopic worms, this fungus can develop circular traps (Figure 1c). It is not clear whether trap formation and capture of the worms is mediated through hormonal or physical contact signaling (20). Figure 1 in the research article by Yang et al. illustrates the entire life cycle of this species, from its saprotrophic stage to its predatory phase.
Figure 1. *A. oligospora in the presence and absence of C. elegans.* (A) *A. oligospora* prior to *C. elegans* addition. Notice the absence of trap formation. (B) *A. oligospora* spore stalks. The microscope is focused above the hyphal structures. (C I and II) *A. oligospora* that has been exposed to *C. elegans* for 24 hours. Arrows highlight some of the traps evident in each image. The dead *C. elegans* in the images have been ensnared in traps. Images taken by Lanny Gov and Alexandra Meiser (UC Irvine).
Current Uses in Society and the Lab

Parasitic worm larvae cause infection of grazing animals, such as cattle, horses, sheep and pigs. Health of the farm animals typically is controlled through the use of antibiotics or pesticides, to which there are an increased number of resistant nematode species (30). Several studies have explored the use of *A. oligospora* for controlling a variety of animal parasitic nematodes by applying them to farm land or directly feeding the animals fungal spores to inhibit larval growth (17).

In conjunction with this, scientists are devising methods to manipulate *A. oligospora* in the lab. A transformation protocol has been established along with plasmid vectors that are capable of being stably maintained in transformed *A. oligospora* isolates (32). This protocol has also been utilized to create knock-out strains (1). In addition, tools have been developed to study the mechanism of trap formation and nematode capture. Transmission electron microscopy has been used to observe *A. oligospora* traps as well as worms captured by these traps (33). Fluorescence microscopy is a useful alternative to electron microscopy because it allows the observation of live *A. oligospora* (14). The expansion of techniques used to study *A. oligospora* in the lab will allow for a greater understanding of this organism and result in increased applications beneficial to society.
II. Caenorhabditis elegans

History of Caenorhabditis elegans

Caenorhabditis elegans are small, bacteriovorous nematodes that were originally discovered by French zoologist Émile Maupas in 1900 (7). However, greater notice of C. elegans did not come about until the 1960s as biologist Sydney Brenner decided to use C. elegans as a model organism for his experiments in genetic research (4). With the goal of branching out into novel territories of molecular biology, Brenner chose C. elegans for its rapid life cycle, simple reproductive cycle, and relatively small size (4). His intent was to further observe the connections between genetics and behavior and development. Brenner, along with other fellow biologists, mapped the C. elegans nervous system, a total of 302 cell nuclei (7). Furthermore, Brenner’s work with mutant strains of C. elegans ultimately led to a greater understanding of genetics and how it applies to development, neurosensation, and aging (7). The utility of C. elegans is demonstrated by the fact that it is connected to many fields of biology.

Background information of C. elegans

C. elegans come from the genus Caenorhabditis, a line of nematodes that extends to 23 different species. C. elegans inhabit the soil and eat the microorganisms which live there (16). As an adult, C. elegans follow the typical nematode body plan, which is unsegmented, cylindrical, and tapered at both ends. The body is separated by a pseudocoelem into two tubes: an outer tube that consists of the cuticle, hypodermis, nervous system, muscle system, and excretory system and an inner tube that includes the pharynx, intestine, and reproductive system. Homeostasis is maintained through an internal hydrostatic pressure (2). Images of C. elegans anatomy can be found in the Worm Atlas (2).

The two sexes of adult C. elegans are self-fertilizing hermaphrodites (XX) and males (XO). Hermaphrodites possess 959 somatic cells while adult males have 1031 cells, however, adult males tend to be slimmer and shorter. The major difference between the two lies in the latter’s development of male-specific sexual organs in the posterior half of the body. Hermaphrodites, in contrast, also possess an egg-laying apparatus and undergo a 3-day reproductive life cycle. While self-fertilizing hermaphrodites can produce about 300 progeny, those that mate with males breed 1200 to 1400 offspring (2).

The developmental stages of C. elegans are the embryonic stage, the four larval stages L1 through L4, and adulthood, as illustrated in the Worm Atlas Introduction Figure 6 (2). Egg-laying and subsequent embryogenesis and hatching occurs within roughly nine hours. Development through the four larval stages is triggered by feeding post-hatching. Molting occurs between each stage as C. elegans develop. The time course of this process at 25°C is as follows: beginning when the egg is laid, L1/L2 molting occurs after approximately 18 hours, L2/L3 molting after 25.5 hours, L3/L4 molting after 31 hours, and L4/adult molting after 39 hours. In the L1 larva stage, five of the eight classes of motor neurons are developed, along with somatic gonad precursors and the formation of the germ line. At this stage, males are already distinguished. In the L2 larva stage, the nervous and reproductive systems continue to develop. Of note, in the event of unfavorable environmental conditions such as high temperatures, C. elegans at the end of the L2 stage can go into the dauer stage, an arrested state, which is maintained until growth conditions are favorable again. The L3 stage begins the more concrete formation of the parts of the reproductive system in hermaphrodites and males, which is then completed in the L4 stage. The completion of these larval stages at 45 to 50 hours after hatching marks the adult stage and the reproductive life cycle.

C. elegans is known as a colonizer of environments rich in microbes, such as decomposing plants (10). While the natural habitat of C. elegans is unknown, this organism is mostly found in human-made habitats: compost, mushroom beds, and garden soil in Europe, North Africa, Asia, North America, Hawaii and Australia (16). Within its community, C. elegans shares their environment with arthropods, mollusks, and other nematodes. C. elegans find their food source through a complex olfactory chemosensory system that recognizes the by-products of microbes. As such, potential predators include pathogenic microbes that use this system to their advantage (26). Additionally, C. elegans are preyed upon by fungi that adhere to the cuticles of nematodes or uses trapping devices to paralyze its movement and puncture the organism (10).
Laboratory uses of *C. elegans*

*C. elegans* are considered model organisms for numerous reasons, some of which include the following: inexpensive and easy to grow, well mapped genome, short generation time (2 to 3 days), short lifespan (2 to 3 weeks), small size with 959 somatic cells, set development stages, transparency, ability to be frozen, and homology to human genes (13). As such, these convenient properties of *C. elegans* allows for several uses in the laboratory. In addition to studies in neurobiology and cellular development, *C. elegans* can also be used to observe the interaction between known chemical reactions and living organisms (13). The genetic overlap between worms and humans can be used to study biochemical pathways in greater detail. Several laboratory techniques have been used when studying *C. elegans*, including antibody-specific labeling, GFP-gene construction, genetic crosses and screening, mutagenesis, transformation, gene mapping, and RNA interference (RNAi).

*C. elegans* were crucial to the development of RNAi. RNAi refers to the silencing of gene activity through the use of double-stranded RNAs (dsRNA), which in turn are processed into single-stranded small interfering RNAs that become guides to target specific mRNAs (39). A key property of *C. elegans* is its ability to easily uptake dsRNA, particularly through ingestion (39). The connection between RNAi and dsRNA provided an easier method to induce gene silencing in organisms and therefore was a gateway to understanding other developmental pathways (19). This led to similar discoveries in other organisms, such as *Drosophila*, that demonstrated specific gene silencing through dsRNA triggering. Furthermore, RNAi was found to be involved in chromatin regulation in yeast and chromosomal rearrangement in the protozoa *Tetrahymena* (19).

Current uses in society

Due to the wide range in which *C. elegans* can be manipulated in the laboratory, this organism can be used to benefit society in many ways. The ability to readily alter the *C. elegans* genome allows for the examination of the relationship between mitochondrial mutations and aging, in which there is a possible connection between decreased mitochondrial activity and the extension of its lifespan (13). *C. elegans* can also be used to further understand the properties of certain volatile anesthetics by examining their effects on worm coordination and mobility (23). Additionally, *C. elegans* are being used as a model to examine obesity and fat metabolism by exploring the conserved genes related to fat regulation (5). This organism can even be used in studies concerning agriculture. Root-knot nematodes are one of many pests that cause billions of dollars in damages to crops annually (6). Homology with *C. elegans* genes enables for a greater understanding of its mode of infection (6).
III. Nematode-Capturing Fungi

Importance of nematode-capturing fungi

Nematodes can infect and destroy plant roots, resulting in reduced nutrient uptake and increased susceptibility to diseases. Use of pesticides is only a short-term solution to nematode infestation of agricultural crops and has resulted in a surge of resistant nematodes. Several nematode-trapping fungi have been identified and studied for their potential role in controlling nematode growth. This would provide an alternative to the use of toxic nematicides, which are potentially dangerous to our food supply and the environment.

The three primary consumers in the soil food web are bacteria, fungi, and nematodes, and all of these serve as food sources for other organisms (31). Some nematodes, such as C. elegans, utilize bacteria as their food source, and nematophagous fungi can consume these bacteria-eating nematodes. Bacteria and fungi often co-inhabit areas, known as the bacteria-fungus interface. Interestingly, in vitro experiments have shown that the presence of soil bacterial strains increases A. oligospora trap induction (18). This suggests bacteria and fungi may share a symbiotic relationship in controlling the nematode population.

There have been two models suggested for explaining the relationship between nematophagous fungi and nematodes and the importance of trapping for these fungi (31). The numerical response model suggests that nematode-trapping fungi are obligate parasites that use nematodes as a carbon and nitrogen source. This plays an important role in the ecological cycle because during the decomposition process, the population of microorganisms increases. This increase in food would result in an increased nematode population, but nematophagous fungi can control growth by capturing these nematodes as their own energy source. On the other hand, the supplemental nitrogen model suggests nematodes serve only as a nitrogen source for the fungus. Thus, fungi are facultative parasites that degrade nematodes for nitrogen in order to survive in a nitrogen-poor environment while they exploit other organic matter as a carbon source and for energy.

Classification of nematode-trapping fungi

Nematode-preying fungi are classified as “nematophagous fungi” or “endophytic fungi”. Endophytic fungi grow within plant tissue without causing diseases and are important for preventing parasitic nematodes from growing on plant roots. On the other hand, nematophagous fungi, a diverse group of fungi, colonize and parasitize nematodes for exploitation of nutritious substances (21).

Nematophagous fungi are subcategorized into facultative parasites or obligate parasites. Facultative parasitic fungi utilize trapping structures and secrete antimicrobial and nematicidal compounds. They produce adhesive spores or develop specialized hyphae to penetrate the nematode. Trapping structures are usually complex three-dimensional nets with the branches covered with adhesive material. The adhesive knobs are composed of adhesive polymers produced on the apex of a slender hyphal stalk. The trapping structure also consists of constricting rings that swell to trap the worm. Toxins are secreted to immobilize the nematodes before penetration of the hyphae through the nematode cuticle. Scanning electron micrographs of A. oligospora traps can be found in Nordbring-Hertz et al. (1986).

Obligate parasitic fungi release spores that are ingested or adhere to the nematodes. Ingested spores germinate inside the intestine or adhere to the cuticle of the nematode and sporulate on the surface, generating an infection on the nematode. Zoospores are also used by parasitic fungi to infect nematodes that inhabit inside of the surface of plant roots as cysts or root knots.

Classification of nematodes

Nematodes are generally classified as “free-living nematodes” or “plant-parasitic nematodes”. Free-living nematodes can move freely through the soil and rhizosphere and are captured by fungi that form trapping networks of constricting rings or adhesive hyphae. Plant-parasitic nematodes are sedentary and characterized by the formation of cysts (egg masses). They localize near the roots of plants, form a root-knot and feed and reproduce permanently on the roots of the infected plants. The larvae that grow from the egg masses are capable of infecting the plant roots and draining the plant’s photosynthate and nutrients. Fungi that prey on plant-parasitic nematodes colonize the rhizosphere and grow into cysts on the nematodes (15). The C. elegans we are using are classified as free-living nematodes that are preyed on by nematophagous fungi.
**Actions of *A. oligospora* following worm capture**

A schematic of the events described below can be found in Figure 18 of the article by Veenhuis et al. (1986).

After contact with the nematode cuticle, *A. oligospora* utilizes a fibrillar matrix, approximately 0.1μm thick, to attach the nematode to the hyphae (8). This adhesive is not found on vegetative hyphae. The fibrillar matrix becomes reorganized in one direction targeting the site of capture on the worm, and indentations of the nematode cuticle begin forming (34). Then, the hyphae begin penetrating the nematode at the site where the fungal cells have the greatest adhesion to the cuticle, which usually occur within 2 to 4 hours after capture. This begins by the accumulation and association of small vesicles at the cellular membrane of fungal trapping cells.

A new cell wall begins forming on the cytoplasmic side of the fungal cell, and the original nematode cell wall begins to degrade. This leads to the release of small vesicles into the fibrillar matrix and emergence of the newly formed cell wall, creating a penetration tube that indents the nematode cuticle. The cuticle begins thinning and eventually, the fungal hyphae penetrate the nematode creating an infection bulb with large numbers of electron-dense microbodies. New trophic hyphae then develop from the infection bulb inside of the nematode within a few hours of capturing (36).

Mycelium growth outside of the nematode body is not observed until 10-24 hours later. This mycelium only develops from trapping cells that had captured the nematode. Trophic hyphae are rarely seen growing and developing outside of the nematode, so this indicates that trophic hyphae are mainly utilized for the digestion of nematodes in order to support the growth of vegetative mycelium. Approximately 6 hours after penetration, low levels of lipid droplets and microbodies accumulate in the trophic hyphae (35). Lipid droplets are used for storage of nutrients derived from the nematode. In the later stage of infection, lipid droplets begin increasing in number and fusing into larger droplets while microbodies also develop and undergo fission. Microbodies were found to contain catalase and thiolase, and are often associated with the lipid droplets. Eventually, the lipid droplets begin disappearing while growth of vegetative mycelium accelerates. Veenhuis *et al.* suggest that the catalase and thiolase from the microbodies provide enzymes for the β-oxidation pathway of fatty acid metabolism to provide the carbon source that supports the mycelium growth (35). These organelles eventually disappear, and only the nematode cuticle, filled with trophic hyphae, remains after digestion.
IV. Regulation of nematode capture by nematophagous fungi

Below are results accumulated from a variety of published articles that examined fungus-dependent worm capture. This is by no means an exhaustive list of the available literature.

Time dependence of trap formation

After introducing the nematodes to the fungal plates, researchers have attempted to determine the timescale of trap formation. The traps were observed at three-hour intervals over a 27 hour period in a study by Nansen et al. (24). Another approach was to observe the traps at 17 hours after worm addition (37). A third study reported that no traps were observed until 24 hours, and after that, trap formation ceased within 5 days (28). Thus, there seems to be variability on the observed timing of trap formation. This may be due to differing factors such as media used, strain of the fungus, or temperature of incubation. Furthermore, the age of the traps seemed to have no impact on their ability to capture the worms, as young developing traps had the same efficacy as already existing traps (35).

Larval density

There is a correlation between increasing concentrations of nematode population and the number of traps formed (28). Researchers added 50, 100, and 200 worms to fungal plates, and observed a much higher number of spores and rings in the first day in the 200 nematode condition. This was also seen in another study testing suspensions containing 50 to 3200 L3 nematodes, as increased nematode trapping was seen at higher larval densities (22).

Temperature

Incubating the fungal plates with nematodes at multiple temperatures from 7 to 37°C elucidated the optimum temperature required for trap formation with the nematode Heligmosomoides polygyrus. A. oligospora was found to have a peak growth rate between 20 and 25°C. Predacity of the fungus was highest between 25 and 28°C. The fungus was found to be significantly slower in capturing nematodes at much lower temperatures (22).

Media conditions

Morgan et al. tested the effect of using corn meal agar diluted with water. Increased trapping occurred on plates with a lower CMA concentration implying that nutrient poor conditions forced the fungus to obtain more of its nutrients from the worms. Scholler and Rubner analyzed carbon and nitrogen sources and concluded that the fungus is more predacious when either is lacking (27). Additionally, they found that a specific concentration of carbon and nitrogen was sufficient to prevent the fungus from forming traps at all. It has been concluded that nematodes may not even be necessary for trap formation and that a combination of a low nutrient medium and small peptides will be sufficient to induce traps to form (25).
References

Appendix 4: Protocols for Week 2 and Week 3 experiments.

Week 2 - Practice Nematophagous Fungi Experiment

Purpose: To measure the fungus dependent capture of *C. elegans* after 48 hours of co-incubation.

Reagents (per pair): 2 Corn Meal Agar Plates (1 with fungus, 1 without), 1 Nematode Growth Media Plate with *C. elegans*, Micropipettors, Micropipettor Tips, Microcentrifuge Tubes, Sterile Water, Microscope Slides, Compound Microscope

Media (per pair): Corn meal agar (CMA) is the growth media for *A. oligospora*. You struck out the fungus on a CMA plate last week. In addition, you will be using a CMA plate lacking fungus. Nematode growth media (NGM) is the growth media for *C. elegans* and contains *E. coli*, the worm food source. *C. elegans* are added to the plate 4 days prior to use in the experiment.

Procedure:

The protocol involves the addition of *C. elegans* to plates with and without fungus. The “no fungus” plate is a control condition to take into consideration worms that die naturally during the course of the experiment. At set times, worms are rinsed off of the control and fungus plates and are counted. From these values, the percent of worms that survive the fungus is calculated.

\[
\text{Worms on Fungus Plate} \quad \text{Percent Survival} = \quad \frac{\text{Worms on No Fungus Control Plate}}{}
\]

Prior to adding the worms to the fungus plate at the beginning of the experiment, a rough count is taken to approximate how many *C. elegans* are added to each plate. Equal numbers will be added to the fungus and control plates.

Lab Period 1

Worm addition to fungus plates

1. Remove the agar block on the NGM plate with a sterile pipette tip. Dump in the biohazard waste.
2. Wash the NGM plate containing *C. elegans* with 1ml water by dispensing and drawing up the same 1ml with the P1000. After repeating this a few times and covering the entire NGM plate, add the liquid to a 1.5ml microcentrifuge tube. Repeat once and combine in the same tube. Although you added 2ml water total, it is not possible to remove all 2ml from the plate and thus the collected water will fit in a 1.5ml tube.
3. Spin the microcentrifuge tube at 2,000 rpm for 1 minute. Let the tube sit on the bench for 5 minutes to allow worms to settle.
4. Remove all but 100μl water in the tube, using the marking on the side of the tube. Keep the pipette tip at the meniscus of the liquid as you remove it to prevent from disturbing the worm pellet.
5. Wash #1 – Add 1ml fresh water to the worms and invert the tube to mix. These wash steps are necessary to remove the *E. coli* from the worms.
6. Spin 2,000 rpm for 1 minute and let sit for 5 minutes at the bench.
7. Wash #2 – Remove all but 100μl water using the 100μl mark on the microcentrifuge tube and add 1ml water. Keep the pipette tip at the meniscus of the water as you remove liquid to prevent it from disturbing the worm pellet at the bottom.
8. Spin 2,000 rpm for 1 minute and let sit for 5 minutes at the bench.
9. Remove all but 100μl water using the markings on the microcentrifuge tube. Keep the pipette tip at the meniscus of the water as you remove liquid to prevent it from disturbing the worm pellet at the bottom.
10. Add 100μl water (so 200μl total) and mix with the P200 set at 150μl.
11. Remove 25μl worms and add to 75μl water. Mix well using the P200 set at 75μl.
12. With this 1:4 dilution, pipette 10 5μl aliquots on to 2 microscope slides and count the number of live worms in each aliquot under the 4X objective. No cover slip is necessary. Note these values in your notebook.
13. Find the average number of worms in the 10 aliquots and divide by 5 to determine the average number of worms per 1μl.
14. Multiply this number by 4 to get the worm concentration in the original tube (due to the 1:4 dilution you performed).
15. Mix the original worm tube by pipetting up and down.
16. Add 75μl of the worms to the center of each CMA plate (fungus and no fungus). Take the number of worms per 1μl and multiply by 75 to determine the total number of worms added to each plate.
17. Keep the plates upright (lids on top) and incubate at room temperature until discussion.

Lab Period 2 (roughly 48 hours later)

At the appropriate time point (in this case 48 hours), you will rinse the worms off of the fungus and control plates. You will then count the number of worms on each plate and compare to determine the percentage of worms killed by the fungus.

1. For each plate, wash with 1ml water, rinsing the plate several with that 1ml as you did at the beginning of the experiment. Add the liquid to a 1.5ml microcentrifuge tube. Repeat once. Rinse gently as your goal is not to dislodge the fungus. Keep in mind you will have two tubes (one for CMA without fungus, one for CMA with fungus).
2. Spin the tubes at 2,000 rpm for 1 minute and let sit on the bench for 5 minutes.
3. Remove all but 100μl solution from each tube using the 100μl mark on the microcentrifuge tube. Keep the pipette tip at the meniscus of the water as you remove liquid to prevent it from disturbing the worm pellet at the bottom.
4. Resuspend the worms in the remaining 100μl by pipetting up and down. Add ten 5μl aliquots to two microscope slides and count the number of live worms in each aliquot.
5. Find the average number of worms per aliquot and divide by 5 to get the number of worms per 1μl.
6. Multiply this number by 100 to determine the total number of worms that were remaining on the plate.
7. Compare the fungus versus the no fungus plates to determine worm loss caused by the fungus. Percent survival will be calculated as worms on fungus plate divided by worms on no fungus plate.

You are now prepared to conduct your experiment next week, where you will be comparing your tested variable to the standard condition you performed this week. Keep in mind this means you will have twice as many plates to work with. Each group will also be collecting worms at two time points (6 hours and 24 hours), so the step performed today will be done twice next week. Discuss any issues you had with your group to better prepare yourself for the coming experiment.
Week 3 - Nematophagous Fungi Experiment

This week you and your group will conduct the nematophagous experiment while comparing the control conditions to a variable. This will be the basis of your lab report. Due to the timing of the experiment, 2 of the 3 time points will occur outside of normal class time. Be sure to coordinate with your group who will come in and when.

Below are the times and locations for your specific section to perform the experiment.

<table>
<thead>
<tr>
<th>Worm addition to fungus plate</th>
<th>Tues AM lab</th>
<th>Tues PM lab</th>
<th>Wed AM lab</th>
<th>Wed PM lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st time point ~6hr later</td>
<td>Monday 8-11a</td>
<td>Monday 8-11a</td>
<td>In lab</td>
<td>Wednesday 8-11a</td>
</tr>
<tr>
<td>2nd time point ~24hr later</td>
<td>In lab</td>
<td>In lab</td>
<td>Thursday 8-11a</td>
<td>Thursday 8-11a</td>
</tr>
</tbody>
</table>

**Purpose:** To test how a specific variable alters the ability of *A. oligospora* to capture *C. elegans*.

**Materials:** 8 CMA plates (4 with fungus, 4 without), 3 NGM plates with *C. elegans*, Micropipettors, Micropipettor tips, Microcentrifuge Tubes, Water, Microscope Slides, Microscope

This week you will use the same protocol as the previous week, except in an expanded capacity. Instead of one time point (48hr), you will perform 2 time points (6hr, 24hr) and instead of the control condition only, you will examine a variable to compare to the control.

Regardless of the changes, the protocol itself is the same. You will first rinse worms from an NGM plate and add them to the various plates used in the experiment. After roughly 6 hours you will collect worms on the plates with and without fungus, and repeat this after 24 hours. Follow the flow chart below:

**Worm addition to fungus plates** (changes from Week 2 protocol in bold).

1. Remove the agar block on the NGM plate with a sterile pipette tip. Dump in the biohazard waste.
2. Wash the NGM plate containing *C. elegans* with 1ml water by dispensing and drawing up the same 1ml with the P1000. After repeating this a few times and covering the entire NGM plate, add the liquid to a 1.5ml microcentrifuge tube. Repeat once and combine in the same tube. Although you added 2ml water total, it is not possible to remove all 2ml from the plate and thus the collected water will fit in a 1.5ml tube. **This will be performed 3 times, one for each NGM plate.**
3. Spin microcentrifuge tube at 2,000 rpm for 1 minute. Let the tube sit on the bench for 5 minutes to allow worms to settle.
4. Remove all but 100μl water in the tube, using the marking on the side of the tube. Keep the pipette tip at the meniscus of the liquid as you remove it to prevent from disturbing the worm pellet. **Using a P1000 tip, combine all worms into a single tube.**

5. Wash #1 – Add 1ml fresh water to the worms and invert tube to mix. These wash steps are necessary to remove the *E. coli* from the worms.

6. Spin 2,000 rpm for 1 minute and let sit for 5 minutes at the bench.

7. Wash #2 – Remove all but 100μl water using the 100μl mark on the microcentrifuge tube and add 1ml water. Keep the pipette tip at the meniscus of the water as you remove liquid to prevent it from disturbing the worm pellet at the bottom.

8. Spin 2,000 rpm for 1 minute and let sit for 5 minutes at the bench.

9. Remove all but **500μl** water by using the 500μl mark on the microcentrifuge tube. Keep the pipette tip at the meniscus of the water as you remove liquid to prevent it from disturbing the worm pellet at the bottom. **Mix with the P1000 set at 400μl.**

10. Remove 25μl worms and add to 75μl water. Mix well with the P200 set at 75μl.

11. With this 1:4 dilution, pipette 10 5μl aliquots on to 2 microscope slides and count the number of live worms in each aliquot under the 4X objective. No cover slip is necessary. Note these values in your notebook.

12. Find the average number of worms in the 10 aliquots and divide by 5 to determine the average number of worms per 1μl solution.

13. Multiply this number by 4 to get the worm concentration in the original tube (due to the 1:4 dilution you performed).

14. Mix the original worm tube by pipetting up and down.

15. **Label CMA plates:**
   - 6hr – control (no fungus)
   - 6hr – variable (no fungus)
   - 24hr – control (no fungus)
   - 24hr – variable (no fungus)

16. Add **50μl** of the worms to the center of each CMA plate (8 total). Take the number of worms per 1μl and multiply by 50 to determine the number of worms added per plate.

17. Keep plates upright (lids on top) and incubate at room temperature until discussion.

**At 6 hour and 24 hour time points:**

At the appropriate time point, you will rinse the worms off of the fungus and control plates. You will then count the number of worms on each plate and compare to determine the percentage of worms killed by the fungus. **At each time point this will be done for 4 plates (2 control (w/ and w/out fungus) and 2 variable (w/ and w/out fungus)).**

1. For each plate, wash with 1ml water, rinsing the plate several with that 1ml as you did at the beginning of the experiment. Add the liquid to a 1.5ml microcentrifuge tube. Repeat once. Rinse gently as your goal is not to dislodge the fungus. Keep in mind you will have two tubes (one for CMA without fungus, one for CMA with fungus).

2. Spin the tubes at 2,000 rpm for 1 minute and let sit on the bench for 5 minutes.

3. Remove all but 100μl solution from each tube using the 100μl mark on the microcentrifuge tube. Keep the pipette tip at the meniscus of the water as you remove liquid to prevent it from disturbing the worm pellet at the bottom.

4. Resuspend the worms in the remaining 100μl by pipetting up and down. Add ten 5μl aliquots to two microscope slides and count the number of live worms in each aliquot.

5. Find the average number of worms per aliquot and divide by 5 to get the number of worms per 1μl.

6. Multiply this number by 100 to determine the total number of worms that were remaining on the plate.

7. Compare the fungus versus the no fungus plates to determine worm loss caused by the fungus. Then compare the control to the variable tested.

Percent survival will be calculated as worms on fungus plate divided by worms on no fungus plate. This value will be calculated with both the control and variable conditions.
Appendix 5: Pre/post-test taken by students in lecture to assess achievement of learning objectives.

The pre-test was given to students a week before the module was discussed while the post test was given following completion of the lab report. Students had 10 minutes to take each test. The grading rubric is included with each question.

Nematophagous Fungi Test

Name/ID: _______________________________

The following quiz will be used to measure the degree to which the fungus experiment you will perform in the next few weeks is successful as a teaching tool. While you should put your full effort into answer these questions, the quiz will not contribute to your class grade. As stated at the beginning of the quarter, your answers will be analyzed but only displayed in aggregate with the remainder of the class. No individuals will be identified.

*For any questions you do not know the answer to, write “Do not know.”

Answers in red

1. Name one positive contribution of fungi in nature.
   Possible answers: source of antibiotics, decomposition, food source, eliminate parasitic worms

2. Name the branching structures generated by fungi during vegetative growth.
   hyphae

3. Describe how fungi are maintained in the laboratory.
   On agar plates containing carbon/nitrogen source, room temperature

4. Describe how *C. elegans* are maintained in the laboratory.
   On agar plates with *E. coli* food source

5. Jack grows *E. coli* in liquid media for 24 hours. He then removes 1ml of the culture media to determine the amount of culture growth. This analysis consists of removing 10μl aliquots from that 1ml and counting the number of cells present. He counts the number of cells in 5 aliquots, and finds the average to be 750 cells/aliquote. How many cells are present in the original 1ml? Feel free to leave your answer in the form of the equation you would type into a calculator.
   75,000 cells

6. Jane the researcher would like to demonstrate that *C. elegans* are killed in a fungus dependent manner when placed on plates that contain the fungus. Describe a control experiment that she will need to perform to make this conclusion.
   Add *C. elegans* to a plate lacking fungus and see how many survive
7. Jane learns in the presence of worms, expression of a fungal serine protease, PII, is upregulated. Once she establishes her experimental protocol, Jane generates the following hypothesis:

Gene expression is important for the fungi to capture *C. elegans*.

Is this a strong hypothesis? **YES** or **NO**.

8. If no, re-write the statement.

**PII expression is important for the fungi-dependent capture of *C. elegans***.

9. Briefly describe how Jane could test the hypothesis.

**Inhibit/knock-out PII gene in the fungus, examine worm survival when added to fungi that do and do not express PII**

What volume are the following pipettes set to? Include units.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>14.1 μl</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

530 μl

Answer the questions below using the 7 point scale:

(1) Strongly disagree (2) Disagree (3) Slightly Disagree (4) Neutral (5) Slightly Agree (6) Agree (7) Strongly agree

12. I am very capable of using pipettors.

13. I am very capable of searching for primary literature using tools like Pubmed.

14. I see a connection to the material I learn in class and the work being performed in research laboratories.

15. I am very capable of **designing** an experiment to answer a scientific question.
Appendix 6: Exam questions pertinent to the nematophagous fungi experiment.

Answers in red

1. *C. elegans* feed on _________ *E. coli_________ which is added to (CMA/NGM) plates. (1 point each)

2. TRUE or FALSE. *Arthrobotrys oligospora* form trap structures in all growth conditions. (1 pt)

3. Matt the microbiologist wants to make 80μl of a 1:20 worm dilution. How much worm solution and water would he add? **Feel free to leave your answer in terms of the equation you would type into your calculator.** (1 pt)

   Worm solution _______4μl_________
   Water _______76μl_________

4. After incubating worms and fungus together on a plate for 24 hours, you remove the worms. On the fungus plate, there are 3 worms per 5μl aliquot and on the no-fungus plate, there are 12 worms per aliquot. Before removing those aliquots, you originally had 100μl of worm solution from each plate (fungus or no-fungus plate).

   a. How many total worms were on each plate? (1 pt)

   With fungus _______60 worms________
   Without fungus _______240 worms_______

   b. What is the percentage of worms that survived the fungus? (1 pt)

   25%

5. Based on the information below, write a detailed hypothesis for a potential experiment. (2 pt)

   Yeast cells have a protein degradation pathway to eliminate misfolded proteins. Overexpression of proteins that play a role in this pathway, such as Hrd1, results in more rapid degradation of those misfolded proteins. On the other hand, increasing cellular stress by adding compounds such as tunicamycin (TM) results in an increased load of misfolded proteins, and yeast that have been struck on plates containing TM do not grow.

   **Hypothesis:**

   Potential answer: Overexpression of Hrd1p protein allows yeast cells to survive in the presence of tunicamycin.

   **Rubric**

   +2 – hypothesis is clear, concise, testable and relevant to the presented information
   +1 – hypothesis that only possesses 2 of the 3 characteristics (clear, concise, testable)

   OR

   +1 – hypothesis that is clear concise, testable but not relevant to the presented information
6. The following pipettes are used to add water to a microcentrifuge tube. What is the total volume of water that will be added to the tube? **Include units. Feel free to leave your answer in terms of the equation you would type into your calculator.** (2 pts.)

<table>
<thead>
<tr>
<th></th>
<th>P1000</th>
<th>P200</th>
<th>P10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

Total volume: \[1082.7\mu l\]

**Rubric**
+2 for correct answer
+1 for correct volume but incorrect units
Appendix 7: Rubric for nematophagous fungus lab report.

<table>
<thead>
<tr>
<th></th>
<th>Excellent</th>
<th>Good</th>
<th>Needs Work</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TITLE (+1)</strong></td>
<td>Concise, accurately describes the contents of the report</td>
<td>Lacks specificity, not clear about the exact purpose of the report</td>
<td>No title, or generic title (ex. Lab Report)</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate content (+3)</td>
<td>Includes specific background about nematophagous fungi, worm capture, basics of experiment</td>
<td>Includes items listed in the excellent category, but too much or too little information, such as details of experimental protocol or extensive background resembling a review article</td>
<td>Lacks most or all of the information found in the excellent category, or conversely reads like a methods section</td>
</tr>
<tr>
<td>Variable background (+3)</td>
<td>Background information regarding the tested variable clearly supports the hypothesis, uses outside sources</td>
<td>Logical train of thought but focuses on generalities and not specific pieces of evidence, little use of outside sources</td>
<td>No support for hypothesis</td>
</tr>
<tr>
<td>Organization (+1)</td>
<td>Introduction starts with general information such as the value of nematophagous fungi and gradually becomes more specific, ending with the hypothesis</td>
<td>Introduction starts general and ends with specifics, but varies in between</td>
<td>No clear train of thought evident</td>
</tr>
<tr>
<td>Hypothesis (+2)</td>
<td>A clear and concise hypothesis that is testable with the nematophagous fungi experiment performed in class</td>
<td>A testable hypothesis statement but not concise or clearly written</td>
<td>No obvious statement of hypothesis</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data Table(s)/Graph(s) (+8)</td>
<td>Data table or graph that displays group data (worms present on each type of plate at each time point along with corresponding percent survival), class data (average percent survival at each time point), Must have a title and figure legend</td>
<td>Data table or graph as described in excellent category, lacks accurate title or figure legend</td>
<td>Presented data is incomplete or presented in a manner that is difficult for the reader to follow</td>
</tr>
<tr>
<td>Results text (+3)</td>
<td>Text introduces the experiment, highlights main conclusions taken from the data and states whether hypothesis was correct</td>
<td>Minimal text that fails to do one of the requirements found in the excellent category, or excess text that includes in depth analysis of the results</td>
<td>No text included with table/graph</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>Analysis of results in the context of the hypothesis (+5)</td>
<td>Plausible, specific explanation for the results obtained in lab, scientific literature used to support claims</td>
<td>Plausible explanation for results obtained in lab, but lacks backing from literature</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Discussion of experimental error (percent survival values greater than 100%, differences in group v. class data, etc) (+2)</td>
<td>Specific reasons for errors in data, for example stating how a pipetting error resulted in an incorrect count of worms on one plate which resulted in an incorrect percent survival value</td>
<td>Generic reasons for errors in data, for example just stating that a pipetting error caused the error without elaborating</td>
<td>No reasons given for error</td>
</tr>
<tr>
<td>Future Directions (+4)</td>
<td>Description of an experiment that is relevant to the results obtained in lab, must provide information about how the experiment will be performed</td>
<td>An idea for an experiment to perform that is relevant to the results, but no discussion about how said experiment can be performed</td>
<td>A variation of “repeat the experiment” or no future directions stated</td>
</tr>
<tr>
<td>REFERENCES (+3)</td>
<td>At least 4 references (at least 2 must be scientific papers) with correct formatting, references are used to support hypothesis or analyze data</td>
<td>At least 4 references (at least 2 must be scientific papers) with correct formatting, references are used mainly for factual background information rather than hypothesis construction or data analysis</td>
<td>Fewer than 4 references, or fewer than 2 papers cited, includes Wikipedia as a cited source</td>
</tr>
<tr>
<td>GRAMMAR (+3)</td>
<td>Little to no grammar errors throughout the report, main issues are with correct nomenclature for organisms or genes, verb tense, spelling</td>
<td>Noticeable grammar errors throughout the report</td>
<td>Numerous grammar errors that make it difficult to focus on the message the author is presenting</td>
</tr>
<tr>
<td>FACTUAL ACCURACY (+2)</td>
<td>Little to no obvious incorrect statements throughout the entirety of the report</td>
<td>Noticeable inaccuracies throughout the report, some of which may result in contradictory statements</td>
<td>Multiple inaccuracies that make it difficult to trust the reliability of the information provided by the author</td>
</tr>
</tbody>
</table>