ARTICLE
Exploration of Gerontogenes in the Nervous System: A Multi-Level Neurogenomics Laboratory Module for an Intermediate Neuroscience and Behavior Course

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In this paper, we describe and assess a laboratory module that we introduced into an intermediate-level undergraduate course in Neuroscience and Behavior (NEUR201) in order to expose students to the new and rapidly developing neurogenomic and bioinformatics approaches to neuroscience research.

The laboratory accompanies a topics-based, highly process-oriented course that explores research methodologies and integrative approaches to particular topics in the field. The laboratory comprises multi-week modules that expand upon the topics being covered in class. In the class for which this module was developed, a key topic under discussion is the role played by the nervous system in aging and/or lifespan. This laboratory module focuses on the model organism, *Caenorhabditis elegans* (*C. elegans*), which has been studied extensively. There is a large and ongoing literature elucidating a number of genes involved in determining or modulating lifespan in *C. elegans*. Students choose a candidate gerontogene expressed in neurons in *C. elegans* from a provided list for which we have mutant strains. Students use available databases to become experts on their candidate gene and design, carry out and analyze a behavioral experiment. In addition, students use available bioinformatics and genomic tools to conduct a protein sequence phylogenetic analysis of their candidate protein across at least 10 different taxa of animals. The laboratory module thus focuses on the integration of behavioral, genetic and bioinformatics approaches, as well as on the evolutionary considerations of the role played by gerontogenes in different organisms.

Key words: behavior, bioinformatics, Caenorhabditis elegans, neuroscience, gerontogene

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The goals of this laboratory module are to increase student understanding of the role of genes in nervous system function and behavior by introducing students to the use of neurogenomics and bioinformatics approaches. The inquiry and discovery-based module integrates behavioral, genetic, statistical and bioinformatics skills and also emphasizes student-generated hypotheses and experimental design.

The module is part of a required, intermediate-level course for Neuroscience and Behavior majors. The Neuroscience and Behavior major at Vassar College, founded in 1985, emphasizes an integrative, experimental analysis of primary literature in topics of neuroscience, neurobiology, physiological psychology, learning and memory, and animal -- including human -- behavior. This intermediate-level course is designed to bring Neuroscience and Behavior students together (a) to examine selected relevant topics intentionally and purposefully from a variety of perspectives, and (b) to integrate analyses across a wide spectrum of levels including evolutionary, organismal, systems (emphasizing but not limited to neural), cellular, and sub-cellular levels. The goals of the course are more process-related than content-driven, although of course ultimately, the two are not mutually exclusive.

The readings come almost exclusively from the primary scientific subfields that constitute the broad field of 'Neuroscience and Behavior.' Students examine underlying assumptions and hypotheses being tested, methods and techniques used to address research questions, interpretations of study results, and possibilities of integrating across levels of study and analysis.

After a brief review of fundamental concepts in neurobiology and neuroanatomy that students have encountered from the prerequisite coursework in introductory biology, introductory psychology and an intermediate course in physiological psychology, we explore in ecological and behavioral contexts some basic models of co-evolutionary processes and comparative neuroanatomy. We then turn to a consideration of chemosensory behavior in a model organism, *C. elegans*, and discuss uses of model organisms to understand more complex organisms and behaviors. These introductory weeks prepare us to consider 'Genes, Theories and Mechanisms of Aging,' the first integrative topic of the course. We explore different theories of aging and consider how the nervous system may be involved in or participate in the aging process. In the laboratory, we investigate putative gerontogenes in *C. elegans*, using bioinformatic, genomic, and evolutionary and behavioral approaches. This paper is primarily concerned with this laboratory module that is five to six weeks in length.

This laboratory module is designed to enhance the education in neuroscience and behavior by emphasizing student independent exploration and critical thinking, while at the same time introducing key biological concepts. This kind of integrative, discovery-based approach has been recommended by the Bio2010 report (Comm. Undergrad.

Educ, 2003).

The key learning goals for the module are for students to:

- *Engage in critical thinking, especially as applied to issues of experimental design;*
- *Perform and understand appropriate statistical analysis of behavioral data;*
- *Understand the use/limitations of model organisms in studying complex physiological phenomena like aging;*
- *Integrate and synthesize across taxa and levels of analysis;*
- *Gain confidence in the use of computational and bioinformatics approaches to explore evolutionary relationships at the gene and protein level;*
- *Distinguish basic relationships among genes and protein sequences like paralogy and orthology;*
- *Integrate, synthesize and present behavioral, bioinformatic and comparative genomic approaches.*

Gerontogenes in the Nervous System

Almost all animals have a finite lifespan punctuated by phases of growth, development, reproduction and senescence. Despite this universality, lifespans vary enormously among animals. Why do lifespans vary? How are lifespans regulated? Recent work has uncovered a surprisingly small number of genes that regulate lifespan in *C. elegans*, *Drosophila* and rodents (Kim, 2007). The genes are widely expressed in tissues, particularly in the nervous system. Intriguingly, manipulation of some of these genes exclusively in the nervous system dramatically affects lifespan (Wolkow, 2002; Taguchi, 2005), suggesting a key role for the nervous system in aging and lifespan.

As a pedagogical tool, this complex topic incorporates fundamental concepts like cell signaling, neural control of reproduction and development, hormonal regulation and metabolism. For example, *daf-2* is a gene that, when mutated in neurons, increases lifespan in *C. elegans* and mice (Wolkow, 2002; Taguchi, 2005); *daf-2* is a key component of the insulin-like receptor signaling pathway (Partridge and Gems, 2006). This pathway is modulated by neurotransmitter signaling as well as neurohormones (Mattson, 2002). In addition, many of these genes play a role in stress responses like reaction to heat stress, UV exposure and other environmental stressors, and also may be involved in other aspects of neural function (Braeckman et al. 2001; Mattson, 2002).

C. elegans is an ideal model organism for undergraduate projects investigating this topic. Not only are the nematodes easy to maintain in a lab, much of the current work on the genetics of aging and lifespan has been conducted in this organism (Kim, 2007), yielding a rich literature for students to read and use as the basis for developing independent research questions. Further, the availability of numerous mutant strains and the vast databases of information about the nervous system afford the opportunity to emphasize the power of and diversity within model organisms for studying complex behavioral and physiological systems.

MATERIALS AND METHODS

Materials and supplies for this laboratory module are readily available from major scientific supply companies. Nematode strains are available for a nominal fee for educational use through the *Caenorhabditis elegans* Genome Center (CGC) via their website, http://www.cbs.umn.edu/CGC/. We used the following mutants for this particular laboratory module: CB4876 (*clk-1*); TJ1052 (*age-1*); DR1568 (*daf-2(e1371)*); CF1038 (*daf-16*); RB1215 (*old-1*); CB1370 (*daf-2*); BA793 (*spe-26*), N2 (Bristol wildtype strain). General worm maintenance procedures can be found at: http://www.wormbook.org.

Nematode strains are grown on agar-filled Petri plates streaked with a small amount of liquid culture of OP-50 *E. coli* bacteria (Sulston and Hodgkin 1988; http://www.Wormbook.org). Worm cultures are maintained in a 20°C incubator. A small number are transferred weekly to fresh plates, once the food is exhausted. It is quite straightforward to time the culture growth so that many worms are available for student experiments. While we did not do this for our lab module, it is also possible to have the students be responsible for their own culture maintenance. Worm strains can be frozen at -80°C for long-term storage.

The bioinformatics and comparative genomic studies can be carried out using readily available software. For the study reported here, the following software was used: ClustalX (http://www.clustal.org/), TreeView X (http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/), MEGA, CLC Sequence Viewer (http://www.clcbio.com/index.php?id=28). All of these are freeware and are easily downloaded and used. Websites consulted included NCBI (including MapViewer; (http://www.ncbi.nlm.nih.gov)) and WormBase http://www.wormbase.org.

RESULTS

Laboratory Module Format and Organization

The laboratory module and all supporting materials can be accessed electronically (http://serc.carleton.edu/genomics/units/25231.html). The laboratory module is organized into week-long parts, described as follows.

Gerontogenes I: Exploration of chosen candidate gerontogene gene

With public access to the vast information being gathered about particular genes of interest, the first place to begin to understand the role of a particular gene in aging/lifespan is to explore the database storehouses of information. For this opening session of the laboratory module, students use the databases at the NCBI (National Center for Bioinformatics), WormBase (a database maintained by the *C. elegans* research community) and other databases. Based on readings done in the lecture portion of the course and working in pairs or small groups, students select a candidate gene/allele to investigate, from the following list (Table 1).

Each of these worm strains is available from the CGC and can be easily maintained in a 20°C incubator.

Students are first asked to identify several (up to 5) relevant research articles that will shed light on the

following questions:

- What is the putative function of your gene in *C. elegans*?
- In what cells is the gene expressed?
- Is there a GFP image of your gene's expression pattern in WormAtlas or WormBase?
- What mutant phenotypes occur in mutants of your gene?
- Based on the expression pattern of the gene, speculate about how your gene might influence aging or lifespan?

Candidate gene	Worm strain name
Clk-1	CB4876
Age-1	TJ1052
Daf-2(e1371)	DR1568
Daf-16	CF1038
Old-1	RB1215
Daf-2(ts)	CB1370
Spe-26	BA793

Table 1. Candidate Gerontogenes for Student Experiments

To get started, students go to the WormBase home page (easily navigated by typing WormBase in a Google search field) and type in the gene name (example: *age-1*) in the Search field. This takes them to pages that hold all of the information known about this gene in *C. elegans*. To broaden the search to include other organisms that might have a homologous gene, students expand their search by going to the NCBI home page.

In addition to discovering initial information about their candidate gene, its mutant phenotype in worms and the primary literature published on their gene, the database searching yields several references to articles that can inform them as they design and plan to carry out a behavioral experiment. Students design experiments to compare the behavior of worms that have a mutation in their chosen gerontogene with that of wildtype worms. Following submission of the experimental designs as a written assignment, the groups consult the instructors to revise the plans and ensure that adequate materials can be provided, and that there has been sufficient consideration of appropriate controls and statistical analyses.

Gerontogenes II: Conducting independent experiments

Following revision of their experimental designs, student groups conduct their experiments independently. Depending on the particular experiment, students may need to come in to lab at other time periods, on their own. The goal for each group is to gather sufficient data to conduct statistical analyses of the data and to be able to construct either a figure or a table of data to include in a final presentation and manuscript. Most student groups performed their experiments in one week, while two groups conducted experiments that took two weeks. The students worked very independently and required very little to no guidance from the instructors after the initial day of experimentation, which occurred in all cases during the regularly scheduled laboratory time. The outside of laboratory time was spent mainly monitoring worm survival or counting progeny. The student grades depend in part on the quality of the experimental data and analyses, so it is in the students' interest to carefully plan and conduct their experiments, with adequate sample size for statistical analysis using the appropriate test (t-test, one or two-way ANOVA, etc.).

Students can be quite creative in coming up with their independent experiments depending on available resources and instructor guidance. In our experience, each student group designed a different experiment based on a handout we gave them providing suggestions, as well as the primary literature students consulted. Several groups chose to explore the role of their gene in fertility, while other groups examined the role of their gene in resistance to environmental stressors including heat shock, UV light exposure and anoxia. The experiments were challenging to prepare for; adequate resources, particularly Petri plates, timed cultures of worms and incubators were needed. If budget or time constraints exist, it is possible to allow students to choose from a more restricted list of possible experiment topics without compromising the independence of the experimental design.

Gerontogenes III: Comparative genomics sequence analysis

During this laboratory period, the student groups download the protein sequence of their candidate gene using NCBI's MapViewer. They then perform a number of BLASTp procedures to find the best "hit" for the protein sequence in at least 10 different animal taxa. They collect these sequences in a text file formatted to work with available software tools like ClustalX, ClustalW, TreeView, Genious, CLC or MEGA to perform a multiple sequence alignment (Fig. 1). The alignment serves as the basis for the construction of a protein sequence similarity "tree."

Students are given a homework assignment to perform a similar analysis on their own time of the 16S prokaryotic rRNA gene, a sequence that is widely considered to be an excellent example of a gene with a robust "phylogenetic signal." We also discuss the advantages and limitations of this kind of approach to addressing evolutionary questions of conservation of gene sequence and function (Baldauf, 2002).

BLASTp searching

MapViewer (accessible from the NCBI home page) is a user-friendly interface that allows students to download the protein sequence of their candidate gerontogene. An easy way to get started is to select the organism (*Caenorhabditis elegans*) and then type in the gene name (example: *age-1*) in the Search field. From the resulting graphical representation of the chromosome containing the gene, students can select "WG" to go to the Wormgenes section in the NCBI site. Much of this information will overlap with what the students discovered in the first week of the laboratory module, but there may be additional information provided by NCBI's curators. The protein sequence can be downloaded to the laboratory computer and saved as a text file.

Using this protein sequence, students select "protein BLAST" and copy the protein sequence into the field. Students choose the SwissProt data base search set and, in the "Organism" field, type in an organism or taxa designation to search a particular organism's genome. We suggest that students span many different animal groups to have as wide a search as possible. For each taxon, students conduct a separate BLASTp search and select the "best hit," the one with the lowest e-score. Using this somewhat labor-intensive and iterative procedure, students collect 10 protein sequences from 10 different animal taxa (i.e. not all mammals) and organize them into a single text file.

Sequence Alignment

There are numerous web-based tools that can be used to construct a sequence alignment to compare sequences from different taxonomic groups. We used several straightforward software tools to perform multiple sequence alignments as well as prepare sequences for construction of a phylogenetic tree, or, in our case, a protein sequence similarity tree. Figure 1 illustrates an example protein sequence alignment produced by a student group.

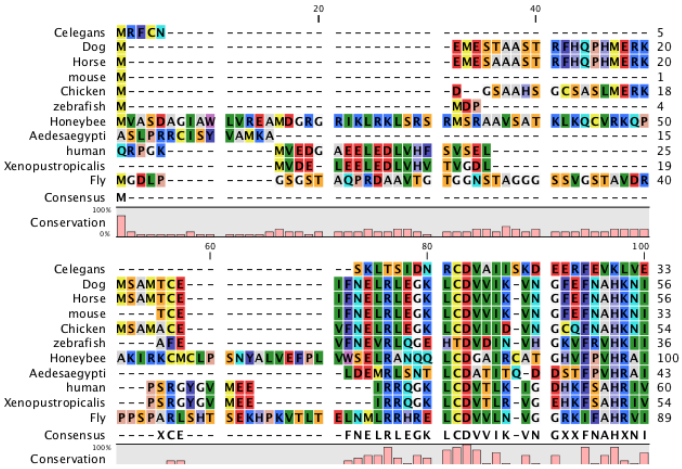

Figure 1. Example of multiple protein sequence alignment. This student-generated alignment was obtained using CLC Sequence Viewer software.

Students consider the following questions when evaluating their alignments:

- Are there regions of the protein sequences with strong alignment?
- Are there regions that seem quite different among the sequences?
- Are there gaps in alignment (i.e. where one or more sequences are not aligned with others)? Do these gaps seem to group organismally? In other words, are there organisms that seem to substantially diverge, either at the beginning or end of the sequence, that seem to be shared by organisms more closely related evolutionarily?
- Are there regions with substantial sequence identity (same exact amino acids), with sequence similarity (same TYPE of amino acid: acidic, neutral, etc)?

Construction of a protein sequence similarity tree

Using Clustal X and Tree Viewer X, students construct protein sequence similarity trees from their alignments, using a neighbor-joining algorithm and boot-strapping that ignores gaps in the alignments. Figure 2 illustrates an example protein similarity tree constructed by a student group.

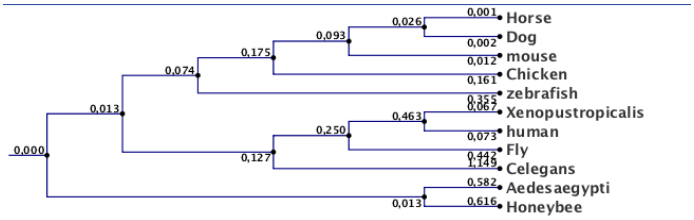

Figure 2. Example protein sequence similarity tree. Student-generated tree constructed using Tree Viewer X software. Numbers on the branches represented bootstrap values. Bootstrap values above 0.3 are considered a more accurate representation of the relationship between the two branches after the nodes than are values less than 0.3.

Students consider the following questions:

- What does your tree tell you about changes in the protein sequence across the taxa you explored?
- Which sequences are most similar to that of *C. elegans*?
- Do you think your protein sequence has evolved in ways similar to or different from other phylogenetic characters? Why?

The last question relates to discussions we have had in class.

Gerontogenes IV: Student presentations

The five-week laboratory module culminates in student group oral presentations. Because the several (eight in our lab) student groups' experiments and candidate genes differ, the presentations are organized in the form of a research symposium, complete with abstract booklet. Each presentation is separated by a question/answer period where the "audience" is required to pose questions. A lively discussion often ensues as students begin to see the ways in which the different data inform each other. Depending on class size and time availability, a poster presentation could substitute for the oral presentations.

DISCUSSION AND ASSESSMENT

Summary of goals of the laboratory module

This laboratory module has students explore the role of the nervous system in aging and lifespan using genetic mutants of *C. elegans*. Gerontogenes are genes that influence lifespan in many organisms, including nematodes, insects and mammals. An intriguing question in evolutionary biology is what function is served by gerontogenes. Are these genes actually regulating aging? Or, are they involved in other cellular or physiological processes and influence aging/lifespan only indirectly (via pleiotropic effects)?

Students use a bioinformatic approach to identify candidate gerontogenes in *C. elegans*. They select a gene

to become "expert" on, based on primary research articles that we have discussed in class. They then take a comparative genomic approach by identifying orthologs of candidate genes in other organisms and explore evolutionary relationships by sequence alignment and phylogenetic tree construction. We discuss in laboratory, based on assigned readings, the differences between phylogenetic trees using data with a strong phylogenetic signal (like mitochondrial DNA or ribosomal DNA) and those constructed more to understand protein sequence similarities and differences. Students design and carry out a behavioral experiment, such as a thermotolerance test, based on literature-based exploration, that tests aspects of candidate gene function in behavior and conduct a behavioral screen of mutant nematodes. The laboratory culminates in a presentation, along with a scientific manuscript, that integrates the students' behavioral data, their literature-based work, as well as their protein sequence similarity analysis.

This module introduces students to the powerful tools and resources available to learn about genes involved in behavior and neural systems. Bioinformatic and comparative genomics approaches are relatively new to the field of neuroscience. Introducing students to these approaches to studying important biological questions, when combined with the more familiar behavioral experimentation and the powerful nematode model organism enhances the learning of current issues in neuroscience and behavior. The comparative genomic exploration allows students to study a system from multiple levels of analysis (a key goal of the entire course)--from cellular/molecular to organismal to behavioral to comparative to genomic to evolutionary.

Assessment of skills and student learning

The knowledge that we hope students gain is a familiarity with the use of major databases, particularly NCBI, WormBase and others. We also expect them to gain substantial knowledge about their chosen gene, as well as experience in designing experiments and using appropriate statistics. We assessed this knowledge by having problem set style assignments. For example, students submitted experimental designs and then had faculty/student conferences to go over the designs and discuss other details important for planning and conducting a good experiment (issues like appropriate controls, sample size, types of statistical analysis, etc). Another assignment included a sequence alignment and phylogenetic tree construction using bacterial 16S rRNA sequence data from a database provided by the Joint Genome Institute 16S rRNA phylogenic homework assignment. Another assessment of student knowledge and mastery was the student presentation.

In addition to these more traditional forms of assessment, we also administered a skills questionnaire (Table 2) before the beginning of the laboratory module and again a few weeks after the conclusion of the laboratory module. This questionnaire allows students to report their confidence level in using bioinformatics and comparative genomics software tools and approaches. Figure 3 demonstrates that student confidence in these skills was substantially improved after the laboratory module. We found a substantial increase in student-reported confidence employing the skills we emphasized (Fig. 3). Fewer than 10% of the 23 students we surveyed reported confidence identifying analogous genes using Blast or constructing phylogenetic or protein sequence similarity trees before the lab module. By the end of the module, 80% or more students felt confident with these skills. We noticed in our own observations in lab and in evaluating the quality of the student data analysis a substantial improvement in student ability to describe and interpret these kinds of data. This, combined with the student performance on the other graded assignments, indicates to us that the laboratory module successfully enhanced student learning of these techniques and approaches.

Finally, we administered a Likert-based attitude questionnaire (Likert, 1932) at the end of the laboratory module to gauge student attitudes about the use of bioinformatics and comparative genomics approaches in neuroscience and behavior (Table 3, Figure 4). We asked students to rate the degree to which they agreed or disagreed with seven statements, with 5 indicating strong agreement and 1 indicating strong disagreement. The statements focused on the use of bioinformatics and comparative genomics approaches in neuroscience and behavior since these are new approaches that many students do not tend to associate with the field. Indeed, our initial observations of students at the beginning of the module suggested to us that a majority of the class was skeptical at first about the relevance of these approaches to their study of neuroscience. However, more than 80% of the students, surveyed after the laboratory module, agreed or strongly agreed that “genomics approaches are important for current neuroscience and behavior research” and 65% felt that the approach enhanced their overall understanding of the complex topic of aging and gerontogenes (Fig. 4). In response to a final question, 75% of the students surveyed would take another course that used these approaches to neuroscience and behavior, indicating the effectiveness of the module in achieving those particular course goals. Indeed, it has been documented that active engagement of students through development of student-generated data increases motivation and improves understanding (Prince, 2004).

Instructors’ observations of student activities

We find that student confidence in using bioinformatics and comparative genomic tools is substantially increased by the end of the laboratory module. Indeed, many of our students reported a better appreciation of the relevance of using these kinds of tools and analyses in the study of neuroscience and behavior. Based on the quality of the analyses of the student experimental behavioral data, as evidenced in the student presentations, we observed an improvement in student ability to use statistical tools to analyze behavioral data. For the students, perhaps the most satisfying aspect of this laboratory module was the opportunity to design, carry out and present the analysis

Please indicate your level of familiarity with each skill/concept.

	Confident	Have Tried	Never Tried
1. Use of NCBI databases for literature researching			
2. Use of WormBase or NCBI MapViewer to investigate a gene			
3. Downloading a gene sequence in FASTA format			
4. Downloading a protein sequence in FASTA format			
5. Using BLAST to identify homologous genes or proteins from other organisms			
6. Formatting sequences for sequence comparison			
7. Using bioinformatics tools to align gene or protein sequences			
8. Interpreting sequence alignments			
9. Using genomics tools to construct a phylogenetic tree			
10. Interpreting phylogenetic trees constructed from gene or protein sequences			

Table 2. Skills Assessment for Genomics

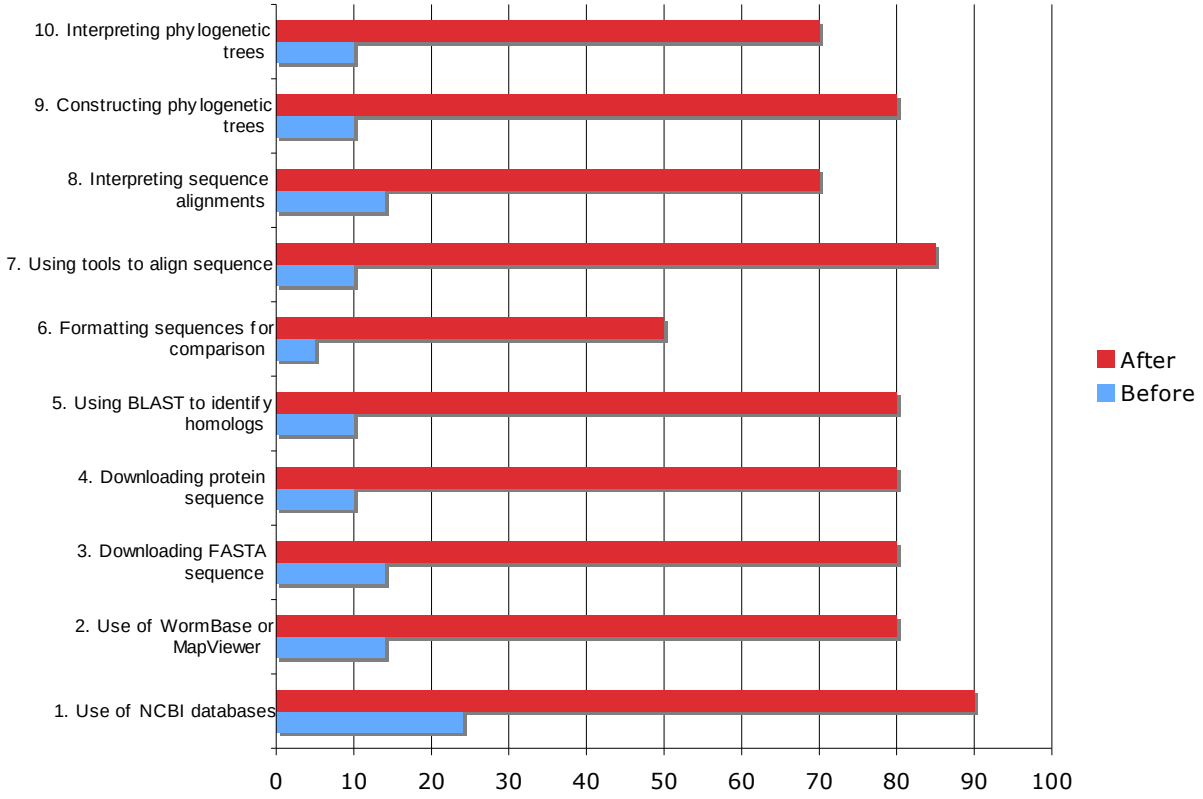

Figure 3. Student confidence in the use of bioinformatic approaches increases as a result of laboratory module experience. The percent of students (n=23) indicating a high level of confidence with each skill is indicated before (blue) the module and at the end of the module (red).

from an experiment that was entirely their own design, based on careful reading of the primary literature related to their candidate gene.

From an instructors' viewpoint, we recommend guiding students in their experimental design, being mindful of the importance of adequate sample size and replication to ensure statistical analysis is possible. We think it is important to allow time and resources for groups to repeat or modify an experiment that does not succeed the first time. While the student independent experiments require

Please indicate the extent of your agreement with the following statements. 1= Strongly Disagree, 2= Disagree, 3= Neither Agree nor Disagree, 4= Agree, 5= Strongly Agree					
1. Computers are essential for conducting neuroscience and behavior research.	1	2	3	4	5
2. Genomics approaches are important for current neuroscience and behavior research.	1	2	3	4	5
3. I feel the comparative genomics approach helped my understanding of a mechanism of aging.	1	2	3	4	5
4. The use of the public databases increased my knowledge of the concepts we studied in class.	1	2	3	4	5
5. Combining computer-based research with behavioral research enhanced my understanding of the topic.	1	2	3	4	5
6. I feel confident using comparative genomics computer tools to address biological research questions.	1	2	3	4	5
7. The use of bioinformatics and genomics tools in the laboratory increased my interest in neuroscience/behavioral research.	1	2	3	4	5
8. Would you want to take another laboratory course that incorporates genomics?	Yes			No	

Table 3. Questionnaire about student attitudes towards bioinformatics/genomics in neuroscience.

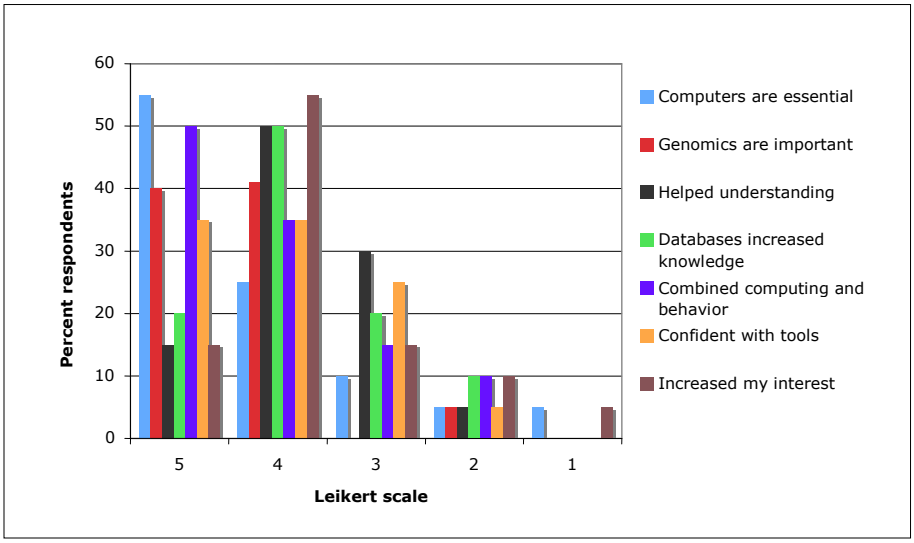

Figure 4. Student responses to questionnaire in Table 3.

substantial preparation time and materials, the value to the students in conducting experiments based on their own hypotheses support the importance of the time and energy devoted to laboratory prep. We were able to take advantage of our scheduled spring break to prepare the materials needed for our students to conduct their experiments. However, depending on how the course laboratory is planned, we could envision a week in the course schedule in which the students prepare materials themselves. Alternatively, students could have a restricted selection of types of experiments that can be carried out, should time or resources prove limiting.

The overall format of this module involved generation of literature-based hypotheses exploring functions of candidate genes, followed by student-designed experiments, bioinformatic and comparative genomic analyses, and student presentations. This format is readily adaptable to many topics of study and different organisms, to suit a wide variety of neuroscience and behavior courses. However, we note the advantage of using *C. elegans* as described in this paper to allow a complexity of designs and methods that would be difficult to implement in an undergraduate teaching lab sequence with most other commonly studied model organisms. In addition, the present module could be expanded to include a microscopic analysis of what cell types in *C. elegans* express candidate genes using GFP-tagged strains specific for the genes.

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