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Born to run: Experimental evolution of high voluntary exercise in mice

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ABSTRACT:

Students rarely get opportunities for inquiry-based learning when they study evolution. Most of their hands-on learning experiences are simulations or involve reviewing data that has already been collected. In this lesson, students examine the changes in leg bones of mice that have been artificially selected in the laboratory for high levels of wheel running. Wheel running by laboratory rodents can be viewed as a model of human voluntary exercise or as a model of the daily movements that other animals exhibit in nature, so it has relevance for both applied and basic science. As the wheel-running behavior of the "High Runner" lines of mice has evolved across tens of generations, many other changes have also been observed in the mice, encompassing other behaviors, physiology, and morphology. Students develop hypotheses about how the thigh bones (femurs) of animals that are good runners might be different from those that are not. They develop a protocol for testing their hypothesis by using digital photographs to measure the bones of selected and control animals (taken from generation 11), and then analyze their data to determine if their hypotheses were supported. This lesson, including supporting resources, can be accessed on The Evolution and Nature of Science Website (2012).

INTRODUCTION:

Evolution is a "unifying theory" of biology and is a key component of the science curriculum in many states. However, teachers are challenged in how to teach this complex concept to the point of full student understanding when student learning experiences are limited to the historical study of Darwin's voyage, his observations of the organisms in the Galapagos Islands and inferences dran from them, and discussion of the concept that natural selection is a primary process causing evolutionary (genetic) changes in populations across generations. Moreover, this type of instruction falls short in reaching the goals of an inquiry-based classroom where students collect and analyze data to understand how organisms change in response to selection. A variety of activities that can be used in the classroom model the process and outcomes of natural selection; however, these can fail to engage students as they are often disconnected from "reality."

The importance of an inquiry-based approach cannot be underestimated, given the demands of Common Core standards (2012), new assessment strategies, and the importance of STEM (Science, Technology, Engineering, and Math) instruction. The recent adoption of the Next Generation Science Standards (2013) put additional demands on instructors of evolution. How can students explore evolutionary change in response to selection using real organisms rather than simulations? By the end of this lesson, students will have interfaced with research being conducted by scientists and designed their own investigations using samples produced in a research laboratory. They will have developed their own protocols, collected data, and had the opportunity to compare their results with those published in the scientific literature by the original research laboratory. In essence, this lesson develops complex thought, problem solving, and creativity in students, thus supporting Common Core standards while simultaneously addressing the Next Generation Science Standards (Standard: Natural selection MS-LS-4-4 and MS-LS-4-5 and Adaptation MS-LS 4-6 pg 55). Note that the activity is intended for use after students have been introduced to natural selection and it presumes that they have been assessed on these concepts prior to beginning the activity.

Evolutionary biologists use multiple strategies to study how the phenotypic characteristics of organisms (their behavior, morphology, and physiology) evolve over time. These include phylogenetic comparative approaches which infer changes that have occurred in the past (Rezende and Diniz-Filho 2012), as well as studies of the variations that occur in existing populations in the wild (Linnen and Hoekstra 2009). An additional strategy uses artificial selection experiments to model natural selection in a controlled and reproducible environment (Garland and Rose 2009).

Dr. Theodore Garland, Jr. (University of California, Riverside) and colleagues have developed four replicate lines of mice that have been selectively bred for a particular behavior: high levels of voluntary wheel running (Swallow et al. 1998, Swallow et al. 1999; Careau et al. 2013). As wheel running has increased across many generations of mice, the structural and physiological changes that co-occur with the selected behavior are being studied. The results of selection experiments like this can be adapted for inquiry-based instruction in the classroom (a direct application of NGSS life science disciplinary core idea MS-LS-4-5 showing that artificial selection can influence the characteristics of organisms.). In the present case, a database containing digital photographs of the femurs (thigh bones) of selected and control animals from generation 11 (the same animals as studied by Garland and Freeman 2005) has been created as a result of an American Physiological Society Fellowship to the first author and funding from a National Science Foundation grant to the second author. These photographs can be easily measured by students, either as printed hard copies or as digital images using Image J, a measurement tool developed by the National Institute of Health, which can be downloaded free of charge. Using this flexible resource, in conjunction with a spreadsheet program, students are able to collect and analyze the data graphically (or with statistical procedures) and test a variety of hypotheses, while simultaneously strengthening their skills in data analysis and graph interpretation.

Open-inquiry projects are easy to support; students already have some prior knowledge of some of the physical characteristics of good runners (e.g., Olympic athletes, greyhounds versus other dogs, cheetah versus lion), from which they can extrapolate and then test these extrapolations on the mouse model. However, an important learning outcome would be for students to realize that the changes in body structure in these lines of mice are the result of selection applied to a behavioral trait; hence, the morphological changes epitomize correlated responses to selection, i.e., evolutionary changes that occur in traits that are not themselves necessarily subject to direct selection. Another important outcome would be instilling in students the fact that evolution can occur rapidly, not only in laboratory systems (Garland and Rose 2009; Barrett et al. 2010) but also in wild populations of plants and animals (Reznick et al. 2004; Herrel et al. 2008), not to mention such microorganisms as the viruses causing flu (http://www.livescience.com/7745-swine-flu-evolution-action.html; http://evolution.berkeley.edu/evolibrary/news/051115_birdflu). This dispels the common misconception that evolution is always slow, requires millions of years to effect observable changes in populations.

Developing a Hypothesis

In my 7th-grade classroom, students are introduced to the project by viewing video-clips of the selected High Runner mice available at http://www.youtube.com/watch?v=RuqhC7g_XP0, and we discuss the method that Dr. Garland's lab has used to develop these lines of mice. Mice from the selected lines run more on the wheel because their populations have been selected, not by nature, but artificially by the researchers (Fig. 1) (NGSS MS-LS-4-5). Specifically, the mice that run the most over days 5 and 6 of a 6-day period are chosen to breed, while mice from the non-selected control lines are bred randomly with respect to how much they run. The behavior of daily amount of wheel running is heritable; offspring of the high-level runners tend to run more than the controls (Fig. 2). I then ask groups of students to list features that they think are likely to distinguish “good runners” from average (or even poor) runners, and I extend these ideas to changes that might occur in selected mice to better adapt them for wheel running.

Dr. Garland and his colleagues have prepared a set of skeletons from one hundred thirty three individual mice, including both selected and control lines, which have been used in a variety of studies to explore the skeletal changes that occur with the selection process. Right and left femurs from these skeletons have been digitally photographed (Fig. 3) along with accompanying scale bars and identification tags that permit tracking of sex, body mass, and whether the individual is selected or control (Fig. 3). Students readily provide a variety of ideas and suggestions when they are asked to propose the ways that the legs might be different in individuals from lines bred for high levels of wheel running as compared with controls. However, they often need assistance in deciding how to connect their proposals to the femur images by asking focusing questions. For example, how would the femurs be different if the legs are stronger, longer or more flexible? Which parts of the leg bones would be expected to be the most different in selected versus control animals? Would there be any differences in leg symmetry? The use of guiding questions eases the transition for students to arrive at their own testable hypothesis, for which they can collect data in the following steps.

Data Collection

The images of the femurs from selected and control mice offer great flexibility for data collection, either manually by direct measurement of the printed images or electronically using software such as Image J, which can be loaded onto school computers. For schools with limited technological resources, the images of the bones can be printed and students can measure the photographs directly with a ruler, using the scale bar (Fig. 3) to adjust for magnification. Cross-curricular reinforcement of scale and proportion in math skills becomes an added benefit using this technique, although students are much more motivated by using electronic measurement techniques. Although several software options are available, I have had good success using Image J, developed by the National Institute of Health, which is available for free download (http://rsbweb.nih.gov/ij/). The images of the samples can be downloaded from the published lesson (Garland and Radojcic 2012). The images are resident in folders that organize them into selected and control by sex. However, teachers may reorganize the images so that students can extend their investigations, for example, to explore other questions, such as the degree of difference (asymmetry) in right versus left femurs in selected and control animals (Garland and Freeman 2005).

Once students have developed their hypotheses, they must decide how to make measurements on the photographs. Whether this is done electronically or directly on printouts, students will need to ensure that their measurements are consistent between student pairs and from image to image, providing a concrete exemplar of good experimental technique. Students can discover the importance of consistency when they compare the results of duplicate or triplicate measurements, or when they compare the measurements taken by two students on the same sample. In addition, for students using Image J, a day spent practicing using the software not only increases the accuracy and consistency of their measurements but also allows them to become more familiar with use of the software. Students can be provided with a brief summary of the use of Image J (Fig 4) or a teacher-directed demonstration. Students often discover various shortcuts that they can use to increase speed of data collection, and this experience encourages independent and innovative thinking.

Students can record their data in a spreadsheet (Fig. 5) that summarizes other information about the selected and control individuals (provided with the lesson plan at Garland and Radojcic 2012). This includes identification (ID) numbers of the individuals (which are also recorded on the images), the sex, and the body mass of the individual at death. Teachers can customize how the data are collected – students can record the measurements independently or in small groups. Using Google Drive (formerly Google Docs) applications to share the spreadsheet with a whole class makes it possible for students to work collaboratively on the same data set by adding the measurements taken from assigned photographs. (When developing the spreadsheets, students should be sure to add a column used to indicate the person making the measurements.) A potential shortcoming of this technique is that all students have access to the data and may be able to (accidentally) make alterations to the measurements taken by other students. However, this issue can be ameliorated by having students create their own spreadsheets initially, and then merge them at a later time.

Data Analysis

Data analysis using the spreadsheet program affords an ideal math-science connection for students, allowing them to rapidly generate a variety of graphs of a data set and to use these graphs to reveal patterns. Students may complete this component either independently or collaboratively. It is interesting to note that for students choosing to examine the effect of selective breeding on femur length, that the average femoral length of selected individuals is shorter than those of controls.

Swallow et al. (1999) have found that body size is in fact smaller in selected mice, and this fact can be readily confirmed by student's graphs of the lab’s data on body mass (e.g., see Fig. 7). Scatter plots of average femur length versus body mass reveal that femur length increases with body size (Fig. 8) and the overlap between selected and control animals indicate that there is no appreciable difference between the femoral length of control and selected animals. This confirms the findings obtained for these animals by Garland and Freeman (2005) using direct measurement of the bones with hand-held calipers.

Class discussions can explore the questions that are generated by these data: Does it make sense that the smaller selected animals have shorter legs? Might there be an evolutionary advantage of a smaller body size to running? For the latter question, students might discuss the differences in the body size of Olympic marathoners as compared with other types of athletes.

Assessment

With the advent of Common Core standards, teachers must not only strive to develop critical thinking skills in their students but also to infuse more writing into their curricula that support language arts standards. Using a lab report assessment is an optimal strategy for assessing the depth of student understanding as well as to support the writing component of the Common Core standards. Although teachers may create their own rubric to evaluate the reports, , a suggested rubric appears in Figure 9. Student report handouts are available on the Evolution and Nature of Science website (http://www.indiana.edu/~ensiweb/lessons/BornToRun.html).

Student presentations are also an effective way to develop students' communication skills. Teachers might consider incorporating student presentations as a culminating activity to this lab, especially for groups of students that have investigated diverse questions. Complex thinking skills are increased when students are exposed to a variety of approaches and perspectives of their peers.

Possible Extensions

The digital photographs have great flexibility as a resource for students to conduct open-ended inquiry projects and test a variety of student-generated hypotheses about the skeletal differences between selected and control animals. These may include differences in the femoral head, the knee joint, as well as other features of the femur that students observe on the photographs. Leg symmetry can be explored by comparing measurements of the right and the left femur, and can be compared with the results obtained in the laboratory indicating that leg length symmetry increases in selected animals (Garland and Freeman 2005). Are other parts of the femur also more symmetrical in selected versus control animals?

Femur lengths and widths can be determined in a variety of ways depending on how and where students choose to measure the photographs. Discussions about the procedures that will be used to measure the bones should emphasize the importance of consistency in experimental protocols. Is it important to measure the bones in the same way each time? Are the results obtained by the class consistent with the measurements obtained in the original research laboratory using a different way of measuring the bones? Which would be more likely to be accurate? Photographs of leg bones from later generations of mice can be obtained from Dr. Garland and could be used to explore whether any further changes have occurred with continued selection.

Multiple cross-curricular opportunities are inherent in this project. Successful interface with Math classes, Language arts classes, and computer-essential classes can be arranged within schools, achieving a high level of integration in students' science experiences. This strategy is a key feature that meets the demands of Common Core, Next Generation Science Standards, and also STEM approaches in general. Students' data can be used to illustrate concepts discussed in their math classes. For example, using direct measurements of the bones and calculating actual bone length using the scale provides an application of ratios and proportions, a critical area of focus for seventh-grade mathematics. In their computer classes, students can be instructed as to the use of Excel spreadsheets to analyze and graph their data. Their presentations and lab reports can be refined in Language arts classes, supporting Common Core Standards in writing.

Conclusion

The ability to gather and interpret data is a vital skill for science students; however, the limitations of time and resources curtail practicing these skills in the context of many classrooms. This is especially true in the study of evolution, where the possibility for students to conduct experiments is virtually absent. Lesson plans based on the High Runner mouse resource can fill this need, allowing students to develop their mathematical, investigation, reasoning, and experimentation skills within the context of the study of biological evolution.

A major consideration in the modern classroom is the need to develop students' technology skills. The use of imaging technology (Image J) to measure the femurs as well as using spreadsheets in a more sophisticated way to analyze the results and graph the data has a substantial ability to address this need. However, as many school sites have limited availability of technological resources – especially the availability of student computers – flexibility is an important consideration in lesson design. Teachers are easily able to tailor this project to fit the resources they have at hand. Photographs can be downloaded and printed for students to measure by hand and use repeatedly. Teachers can create and print a variety of graphic representations ("graphing tasks") of the data on a teacher computer and distribute them to student groups to interpret and present. The adaptability of this resource to classrooms with a wide spectrum of technological resources fosters accessibility and equity to all schools.

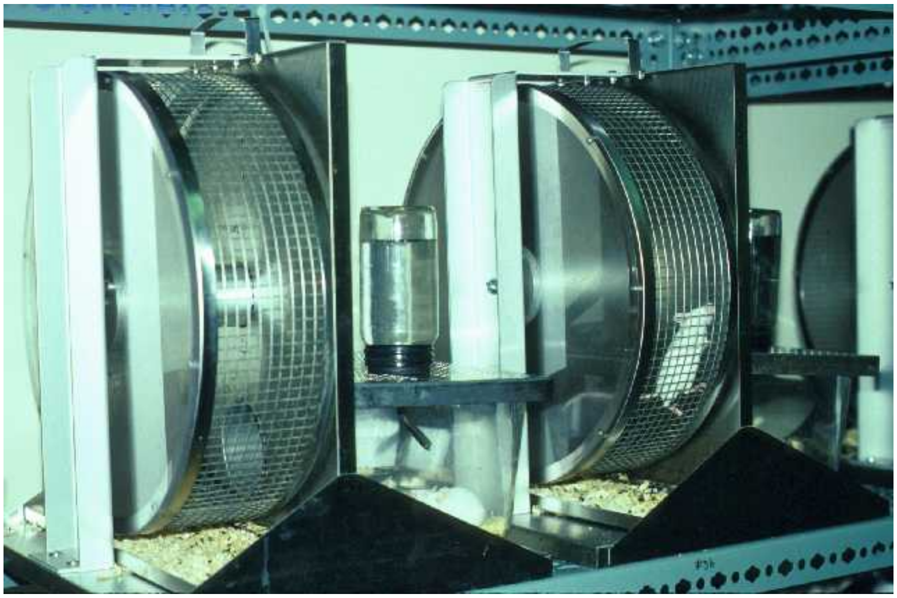

Figure 1. Mice are housed in cages and given free access to food and water. The wheels are attached to computers that collect data on the number of revolutions of the wheel over a 6-day period.

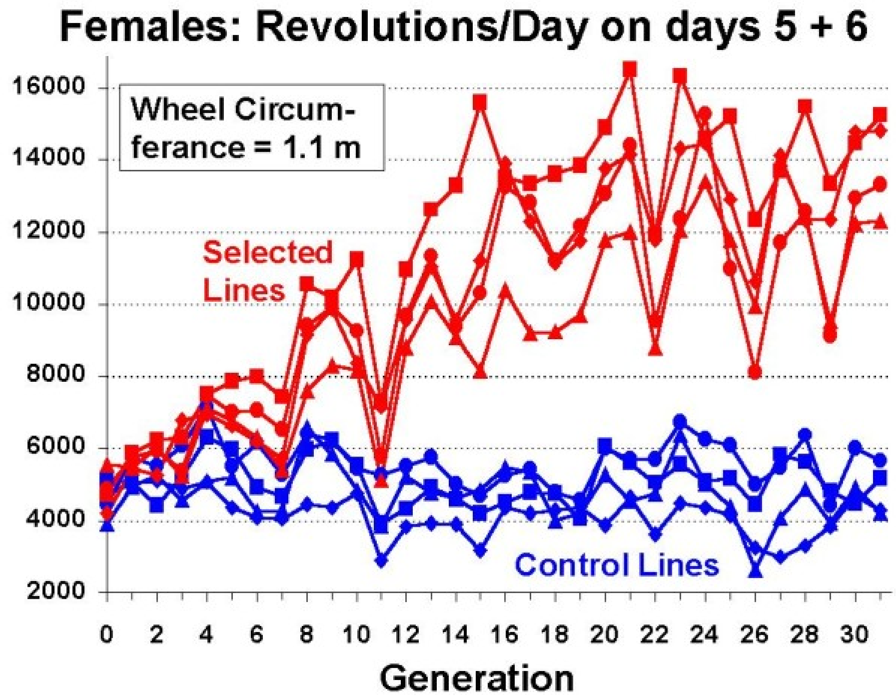

Figure 2. Average value of wheel running for four selectively bred High Runner lines and four non-selected control lines across the first 31 generations of the experiment.

http://www.biology.ucr.edu/people/faculty/Garland/TotRevsLinesXGenF_640_Q9.jpg

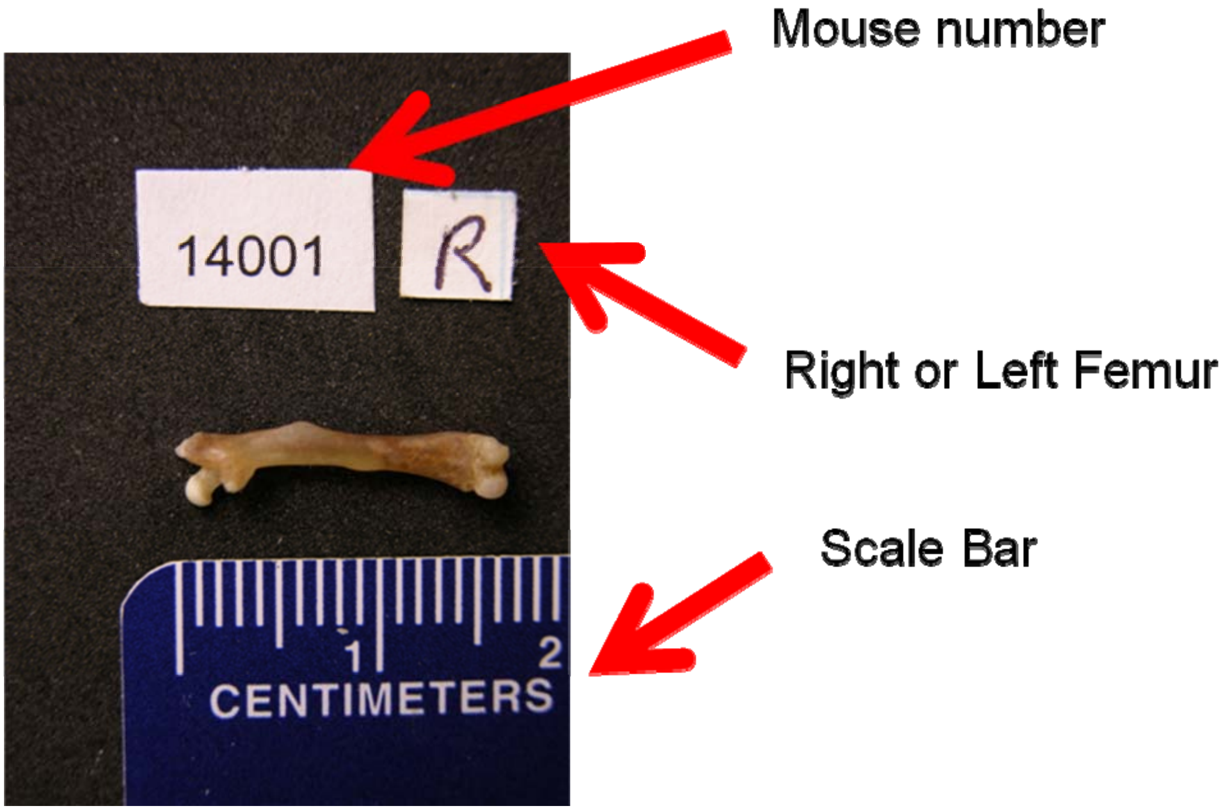

Figure 3. Image of a mouse femur, as would be provided to students for measurement

Using Image J to measure bone (femur) lengths:
Student Directions

1) ***File – open***: Select image
2) Select line tool on the tool bar
3) Draw line on the ruler that is 10mm long(1 cm)
4) ***Analyze – set scale***
5) Enter 10 in “Known scale” and mm in “Unit of length”. You can choose to display in cm also and set the number of decimals of accuracy.
6) Click “Global” This will apply the scale measurement to each picture that you measure thereafter.
7) Draw a line at the dimension which will be measured. The scale bar will display the measurement as well as the angle of the line. This will disappear when you click the end point of the line.
8) ***Analyze-measure***: A data table will appear with the first measurement
9) ***File – open next*** : The next image to be measured appears
10) Draw a new line on the new femur picture.
11) ***Analyze – measure***: The next length will be displayed on the data table
12) When your measurements are complete, select ***Analyze – summarize***.
13) Record the average from the displayed information. You will be graphing this data later.
14) Repeat steps 1-13 for the control animals.

Figure 4. Student directions for using Image J to measure selected and control femurs

ID	sex	Body mass	Measurement 1 (mm)	Measurement 2 (mm)	Measurement 3 (mm)	Average (mm)
14001	0	37.68				
14004	1	39.17				
14006	0	36.00				
14007	1	50.00				
14011	0	41.98				
14018	0	35.20				
14024	1	44.46				
14027	0	30.03				
14028	1	40.40				
14029	1	44.04				
14039	0	41.17				
14043	1	44.92				
14045	1	46.47				
14047	0	31.33				
14048	0	38.86				
14056	1	47.08				
14057	1	48.13				

Figure 5. Excerpt from Student Handout Master Data Sheet: Selected versus control mice

Figure 6. Students from Bella Vista Middle School working on data collection

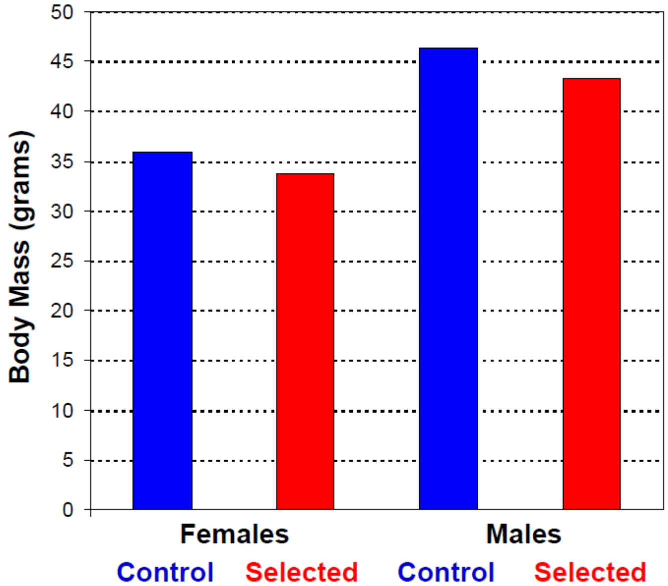

Figure 7. Sample graph of body mass in control versus selected animals – produced with graphing function of a spreadsheet program. Note that, on average, male mice are larger than females, and those from the selectively bred High Runner are smaller than those from the non-selected control lines.

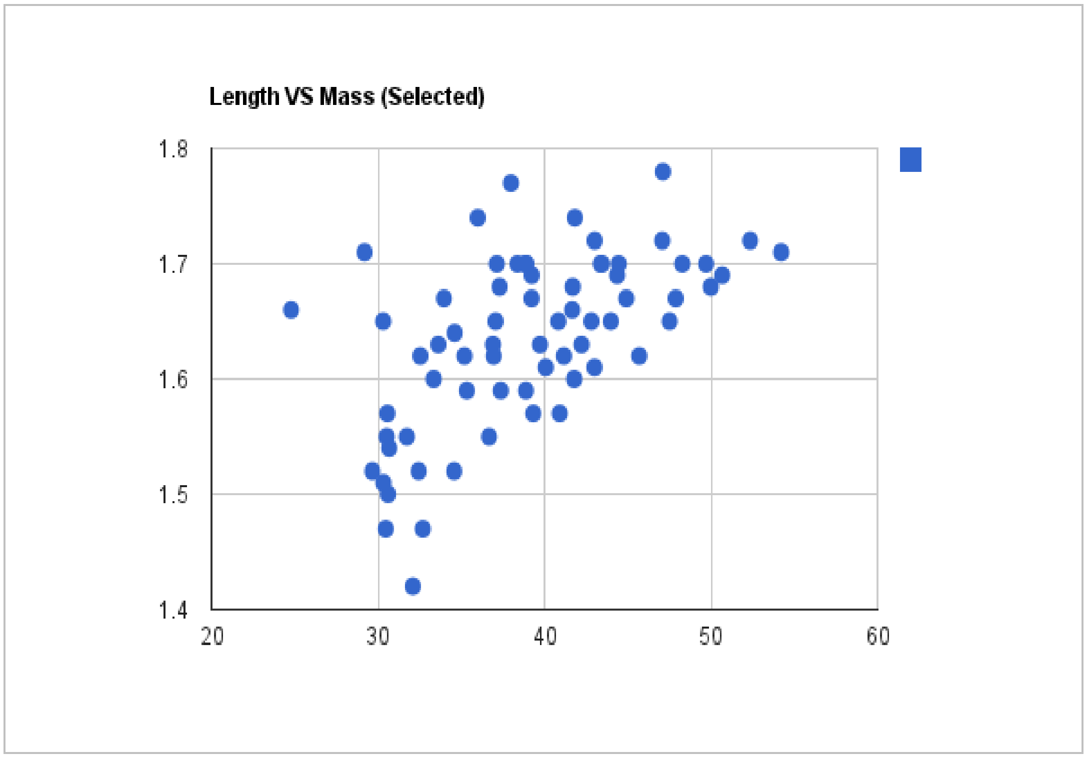

Figure 8. Student-generated scatter plot of femoral length (cm) (student data) versus body mass (g) (body masses are provided to the students)

Artificial selection Lab Report Rubric

	4	3	2	1
Hypothesis	1. Hypothesis is stated in "if..then.." format. 2. The measurement techniques are clearly connected with the question. 3. The reasoning is clearly explained.	1. Hypothesis is stated in "if..then.." format. 2. The measurement techniques are clearly connected with the question. 3. The explanation of the reasoning is unclear.	1. Hypothesis is stated in "if..then.." format. 2. The connection between the measurement techniques and the question are unclear. 3. The reasoning is unclear.	1. Hypothesis does not use the "if..then.." format 2. There is no connection between the question and the measurements made. 3. Reasoning is not explained.
Graphing	1. A bar graph is shown with labeled axes. 2. The measurement is shown on the y axis and the x axis is labeled with selected and control. 3.Measurements are shown in cm or mm and the values are rounded. 4. The scale selected clearly shows the differences (or lack thereof) in the measurements.	1. A bar graph is shown with labeled axes. 2. The measurement is shown on the y axis and the x axis is labeled with selected and control. 3.Measurements are shown in cm or mm and the values are rounded. 4. The scale selected clearly shows the differences (or lack thereof) in the measurements.	1. A bar graph is shown but axes are not labeled. 2. The axes are reversed. 3. Measurements are shown in cm or mm but the values are not rounded. 4. The scale selected yields a misleading representation of the data.	1. Graph type is incorrect and unlabelled. 2. Axes are reversed. 3. Measurements are not shown in metric 4. The scale is misleading or missing.
Presentation	1. Every person in the group speaks clearly and shows an in depth understanding	1. Some people in the group show in depth understanding of the results. 2. The results	1 Group members are unclear in their understanding of the results. 2. The results	1. There is no evidence that group members have any understanding of the results.

	of the results. 2. The results shown by the graph are clearly explained. 3. Members of the group are able to respond to questions.	shown by the graph are clearly explained. 3. Members of the group are able to respond to some questions.	shown by the graph are explained. 3. Members of the group are unable to respond to questions.	2. There is no explanation of the results obtained. 3. Group members are unable to respond to questions.
Conclusion	1. All elements of the conclusion are included. 2. Reference is made to the data shown in the graph to support the conclusion. 3. Sources of error are clearly and completely explained and extend beyond human error. 4. Further analyses are suggested, referring both to those listed in Part A of the lab handout and others that are not listed.	1. All elements of the conclusion are included. 2. Reference is made to the data shown in the graph to support the conclusion 3. Sources of error are lacking in depth. 4. Further analyses are suggested, referring to those listed in Part A of the lab handout.	1. Most elements of the conclusion are included. 2. Reference is made to the data shown in the graph to support the conclusion but the explanation is unclear. 3. Sources of error are restricted to human error. 4. There are no suggestions for further analyses.	1. Most elements of the conclusion are missing. 2. No reference is made to the data and the conclusion is not explained. 3. No reference is made to sources of error. 4. There are no suggestions for further analyses.

Figure 9. Sample rubric for evaluating student lab reports or presentations

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Video of selected and control mice running on wheels:

http://www.youtube.com/watch?v=RuqhC7g_XP0

http://www.biology.ucr.edu/people/faculty/Garland/Girard01.mov

Figure 2 showing wheel running in selected versus control animals.

http://www.biology.ucr.edu/people/faculty/Garland/TotRevsLinesXGenF_640_Q9.jpg

Resources

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http://rsbweb.nih.gov/ij/docs/pdfs/ImageJ.pdf

Information about Dr. Theodore Garland, Jr's. Research Lab

http://www.biology.ucr.edu/people/faculty/Garland.html

List of all publications in the High Runner lines of mice (with PDF files available for download)

http://biology.ucr.edu/people/faculty/Garland/Experimental_Evolution_Publications_by_Ted_Garland.html

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