Supplemental Material

CBE—Life Sciences Education

Gouvea *et al.*

Appendix A. Lab Report Guidelines from each year

2014 Lab Report Guidelines

Below is a general guide for writing the antibiotic resistance laboratory report. It is not intended to be comprehensive. You will need to read through Pechenik's A Short Guide to Writing About Biology, for more detailed information. In addition to reading the appropriate sections of the book, especially "Writing Laboratory and Other Research Reports", we draw your attention to the checklist at the end of that chapter.

Knowing how to write a report does not come naturally but is a skill that you must develop. To do well, you must follow directions and work ahead so that you have time to proof-read your report before handing it in to your instructor.

Use the headings (Title, Methods, Results, Discussion) in your report.

TITLE

The title should clearly state the nature of the study. It should include:

1. The factor that was manipulated (independent variable)
2. The parameter of the organism that was measured (dependent variable)
3. The study species (use scientific name)

For example, the title, "The effects of temperature on the mating behavior of the domestic cricket, *Acheta domesticus*." fulfills the three criteria above because it specifies the factor that was manipulated (temperature), the parameter that was measured (mating behavior), and the organism (*Acheta domesticus*).

METHODS

Your goal is to include sufficient detail so that someone else could repeat the experiment. You must describe your:

- **Study System**
  Describe your study species and why it was selected for your study.
- **Experimental Protocol**
  Be sure to include concentrations, quantities of reagents, the number of organisms, replicates, etc. Omit details that are not essential. **State the rationale behind your methods** and define any unusual terms. This section will be very difficult to write if you did not take detailed notes when you were setting up and doing the study.

  Do not write this section like a recipe (as it is written in the lab manual).
  e.g. "Place three drops of solution A in...".

  Do write it in **past tense** (passive or active voice).
  e.g. "Three drops of solution A were placed" or
  "I placed three drops of solution A in..."
- **Data Analysis**
  Tell the reader what calculations you performed (growth rate, mean, standard deviation) and which statistical test you used (but do not describe the results of the statistical test).

RESULTS (1 paragraph)

Your goal is to describe the results (in past tense), **without interpreting** them. You will interpret the results in the Discussion section. Be sure to:

- Start with a **general statement** concerning the results.
- Use the rest of the section to describe the results in more detail.
- Use a figure to highlight your main findings. For this report, you should only have **2 figures**.
- Refer to the figure in the text. DO NOT say, "The figure shows..." Rather state the result, then reference the figure. For example you might write: "Lower temperatures resulted in fewer mating calls (Figure 1)."

Figure

Think carefully about the format that best conveys your results.

- Label your axes (including units)
- Include a complete caption so that the figure is self-sufficient.
  Caption should include:
  Study species
  Type of data presented (means? raw data?)
  Sample size (n= __)

DISCUSSION (1 paragraph)

Your goal is to summarize and interpret your results. Your discussion should include the following:

- A 1-sentence **summary** of what you found
- Provide **interpretation** of your results – what do they mean? If your results are unexpected, offer some explanations.
- Make suggestions for **future experiments** that test new ideas.

ACKNOWLEDGMENTS

Here you should thank people who helped you with the lab. People you might want to include are the Bio14 lab manager (Michael Grossi) and your lab partners.

LITERATURE CITED

If relevant, list in alphabetical order all articles that were cited in the text. Do not include articles read but not cited. Be sure citation format is correct.

OTHER COMMENTS

Be sure to include page numbers and, before you prepare the final copy of your report, go through the Pechenik checklist or exchange papers with a classmate and go through the checklist for each other.

2015 Lab Report Guidelines

Purpose of Lab Report

- The primary purpose of the lab report is for you to slow down and take time to think about what we have been doing and carefully write up your thoughts.
- The purpose is not to get to some particular "answer," but rather to make some sense of the data and support your ideas with logic and evidence.
- We want you to improve your skill in communicating scientific ideas clearly. Clean writing and formatting will help you communicate.
- But we will be primarily be grading for clear expression of ideas and evidence (not particular formatting conventions).

Purpose of Structured Review Cycle

- We want you to actually get better at this over the course of the semester!
- The TAs will quickly read the initial reports to get a sense for some of the major issues and list recommendations for peer reviewers to pay attention to.
- You will each receive a lab report from a peer (not in your lab group) that you will read carefully and provide feedback (not copyediting).
- You will get a better feel for what counts as good science writing by both reviewing and revising your own report.

Structure of Lab Report

Introduction

- Describe why we did this lab from your perspective. We talked about the bigger ideas about mutation rates in class. Report on your own thinking about these questions. What do you think is interesting about mutation rates biologically?
- How do you see that larger conversation as connected to the experiments we did in lab?
- This section will be graded primarily for a logical and coherent "storyline." As you are writing, ask yourself, what is the interesting biological story here? Does this make sense? Could a reader follow this story and understand my train of thought?

Methods

- Explain what we did in a clear and concise manner.
- You *don't* need to recount every single detail, but you *do* need to give your reader enough of a sense for what you did that they can make sense of the results.
- Methods will be graded primarily for clarity.

Results

Report on what you found.

- Specifically, choose to focus on one or two interesting patterns in the data (at least one from the whole class data). Describe in words what you see in the data and appropriately use figures to help you communicate and summarize what the data say.
- Provide a 2-3 sentence justification for why you chose to highlight these results and what they mean to you in the bigger picture.
- Results will be graded for clarity and justification. The primary thing here is that someone else can make sense of what you have described and why.

Discussion

- Come back to the story you told in the introduction and describe how this experiment adds (or does not add) to the story. What does the experiment tell you about some of the questions you raised in the introduction, and what does the experiment *not* tell you?
- This is also place to include some of the insights and open questions from our exploration in the NetLogo simulation environment (if you want).
- This section, like the introduction, will be graded for the logical flow and for evidence of your own thinking. Don't be afraid to say things that are "incorrect," but be sure to fully explain your thinking and how the story fits together for you.

Formatting Guidelines:

- Include a coverpage with your name, the names of your lab group, your lab TA, and the Title of your write-up
    - Tip: Decide on your title after the lab report is written so you can choose a title the clearly communicates to a reader what they can expect to read about
- Include page numbers and a running header that has your name
- Make section headings clear and double-space your report so it's more readable
- Include citations in your text (if appropriate) and a **References Cited** section at the end of the write-up
- Acknowledgements (if appropriate)

Additional Tips for Good Scientific Writing

- Use simple, precise language. If there is a vocabulary word that will help you communicate your idea, then use it. But avoid using excessive jargon (especially if you aren't sure what it means).
- Read your lab report aloud or to your roommates. It should flow and make logical sense.
- Revise your writing! (All writing gets better with revision)

2016 Lab Report Guidelines

Purpose of Lab Report

The primary purpose of the lab report is for you to slow down and take time to think about what we have been doing and carefully write up your thoughts. The purpose is not to get to some particular "answer," but rather to make some sense of the data and support your ideas with logic and evidence. We want you to improve your skill in communicating scientific ideas clearly. Clean writing and formatting will help you communicate. We will primarily be grading for clear expression of your ideas and evidence (not particular formatting conventions).

Purpose of Peer Review Cycle

We want you to practice noticing good scientific arguments so that you will get better at making your own. You will have time in lab to discuss feedback from both a peer and your TA. You will have time to ask questions/get clarification before the final lab report is due

Structure of Lab Report

Introduction

Introduce the problem or question you will be writing about. What is known about the problem? What is unknown? What will you be writing about in this report? As you are writing, ask yourself,

what is the overarching “story” here? Does this make sense? Could a reader follow and understand my train of thought?

Methods

Explain what you did in a clear and concise manner.
You don’t need to recount every single detail, but you do need to give your reader a sense for what you did that will impact how they make sense of the results. What was the experimental setup? What was the data you were collecting? How did you collect it?

Results

Report on what you found. Specifically, choose how you want to represent the data. Decide what data is appropriate to include and what data it may be appropriate to leave out. Describe in words what you see in the data and appropriately use figures to help you communicate and summarize what the data say. You may add footnotes to explain or justify your choices.

Discussion

Come back to the question/problem you posed in the introduction and help your reader make sense of the results. Do the results answer any of the questions posed in the introduction? Do they raise new questions? What does it all mean?

Formatting Guidelines:

- Include your name, the names of your lab group, your lab TA.
- Decide on your title after the lab report is written so you can choose a title that clearly communicates to a reader what they can expect to read about (i.e. not just “Lab Report 1).
- Include page numbers and a running header that has your name.
- Make section headings clear and double-space your report so it’s more readable
- Include citations in your text (if appropriate) and a References Cited section at the end of the write-up
- Acknowledgements (if you want)

Additional Tips for Good Scientific Writing

- Use simple, precise language. If there is a vocabulary word that will help you communicate your idea, then use it. But avoid using excessive jargon (especially if you aren’t sure what it means).
- Read your lab report aloud or to your roommates. It should flow and make logical sense.
- Revise your writing! (All writing gets better with revision).

Appendix B. Examples of Discussion sections for each level.

The three dimensions of the modified SOLO Taxonomy are indicated in each example. Claim structure in bold. Scope of evidence in italics. Consistency and closure underlined.

Level 1

The results of this experiment supported our hypothesis **that the E938 strand would be able to mutate more to become more resistant to antibiotics and to adapt to a lactose filled environment. A less effective DNA repair system would mean that more mutations would occur because of unseen errors in DNA replication**. **The E938 strand mutated more often because of its less effective DNA repair system (mut-)**. *This is why it was able to grow more colonies in the presence of the antibiotics (+rif) and was able to produce more colonies with red papillae (indicator of lac+) in a lactose filled environment.* **The E939 strand was not able to mutate because of its functional DNA repair system (mut+) and therefore less colonies grew in the +rif (antibiotics) and less colonies had red papillae**. With regards to these results, it'd be interesting to conduct other experiments on other strands of E. coli containing other mutations

Level 1.5

Our hypothesis was that E938, which is Mut-, would show increased mutation over strain E939. *This would be visualized by the E938's growth of more colonies on the agar with rifampicin and more red papillae on the MacConkey agar than the E939 strain with an intact DNA repair mechanism*. **This hypothesis was supported** by *both our individual and class data, which showed increased growth of the E938 over the E939 on rifampicin treated plates. E938 also developed more red papillae per colony on the MacConkey agar than did the E939.* **The increased mutation rate and survival of E938 can be attributed to the faulty DNA repair of the Mut- strain.** Increased mutation rate allows more bacteria of this strain to gain the traits necessary for survival on the rifampicin plates. Likewise, E938 also adapts better for the use of lactose as an energy source on the MacConkey agar.

Some uncertainty was present in the class data for the E938 strain. The standard deviations were high for 1-2 and >2 papillae per colony (30% and 40% respectively). This may be attributable to problems while performing the serial dilution. Groups may not have allowed for the proper dispersion of the bacteria in each solution before performing further dilutions. This may result in initially lower concentrations of bacteria on some plates. This can easily be remedied by allowing proper dispersion of the bacteria in each dilution step. Further studies of these E. coli strains may examine the prevalence of deleterious mutations in each of the two strains. This may [be] accomplished by further diluting the bacterial solutions and plating the two strains on LB agar. An incubation time less than two days may be necessary to observe countable colonies of the bacteria. Despite the high standard deviation for the class E938 data, the experiment supported our hypothesis that E938 would show increased mutation due to the lack of a functional DNA repair mechanism.

Level 2

It was found that on average in Parts I and II of the experiment the E938 strain of E. coli was capable of growing more colonies that were more adept at finding a food source than the E939 strain of E. coli. *In Part I of the experiment both strains were capable of creating a "lawn" covering of the –rif agar plates (Table 2). This was not the same for the +rif agar plates. The mean number of colonies of*

E938 on these plates was more than 8 times the number of colonies of E939 (Table 1). Although the standard deviation for the average number of E938 colonies is large the results are not invalidated because no trial returned more E939 colonies than E938 colonies and the standard deviation for the E939 colonies is comparatively larger than that for E938 as it is almost equal to the mean (Table 1).

In Part II of the experiment, my group found that more E938 colonies grew than E939 colonies (Table 5). The same cannot be said for certain for other groups, as this data was not shared. **Part II demonstrates that the E938 was better than the E939 at evolving.** A greater percent of colonies of E938 had 1-2 papillae than E939 and no E939 colonies had more than 2 colonies, whereas an average of 47.2% of E938 colonies had more than 2 colonies (Tables 3-4). The papillae indicate a different type of waste product than usual, meaning that the colonies with papillae would have developed the ability to consume lactose, becoming lac+ as opposed to lac- as they were at the beginning ([Lab manual] 7). **Because the E938 colonies had more papillae on average than the E939 colonies and a greater percent were capable of developing papillae, one can infer that the E938 are better at evolving than E939**. In Part I and II E938 was able to grow more colonies on plates containing an antibiotic and in Part II E938 was able to adapt to using lactose as a food source.

Combining the data from Part I and II, it can be deduced that the E938 strain's status as mut- allows it to better survive. In the future, it would be interesting to test these two strains by introducing a substance or environment that would harm most strains of E. coli but does not affect these two and see if the mutations to the E938 strain are still helpful in this situation.

Level 2.5

With regards to the concept of **how mutations can have beneficial impacts on a species' ability to survive,** this experiment strengthens that claim by giving multiple instances of mutational success. The mutations in the wildtypes, both red colonies and total colonies, caused the e. Coli cells to survive and consume the Lactose present in their environment. In lactose environments, e. Coli would normally not survive, but by mutating, a small amount of colonies adapted and were able to live. **The survival and adaptation of the mutants in this experiment demonstrates how mutations can be beneficial and neutral, not simply detrimental to a species' survival.** The higher mutation rate of the e. Coli populations dropped on the lactose plates also appeared to influence their mutations and survival.

The experiment makes an effort to compare populations with low mutations with those of higher rates. While this does occur, it does not really answer the question of how mutants would fare in normal environments. Seeing how E938-103 and E939-103 would fare in an environment without lactose would show how well populations with high mutations rates can survive in normal environments compared with those of lower mutation rates. **Ultimately, this would demonstrate how mutations can have positive, negative, or neutral impacts on certain species.**

The NetLogo simulation provided an interesting view on the impacts of mutations in on a simulated population. By altering the mutation rate, rate of fatality, and the presence of poison, several unique situations were created. **Naturally, the species with the higher mutation rate and lower rate of fatality survived over the others**. *The poison would usually kill off the weaker of the two groups, but sometimes both survives, while other times saw both groups dying*. While interesting, the simulation did not really relate to the experiment, nonetheless it provided another perspective on the effects of mutation.

Level 3

The questions we asked revolved around mutation rates and how different environments selected one E. coli culture over the other. *In the LB nutrient agar only plates both E. coli types were able to thrive*. We expected a different scenario with the rifampicin plates. Theoretically, the rifampicin would kill off the bacteria unless a mutation present allowed the bacteria to live despite the rifampicin, as should have been the case with the mutated E938. Accordingly, we predicted the E939 would have a minimal showing with the rifampicin present. **Our data showed us that we over-anticipated the extent rifampicin would take away from colony growth.** *Not only did the mutated E938 produce on that plate but the E939 was present as well, and both in fairly large quantities. There was a large amount of the either culture present on the plates considering we expected to see very few colonies.* However, *there was a greater number of E938 colonies,* which fulfills our expectation.

The presence of the red dots on the MacConkey plates showed exactly how much mutation was happening. The way MacConkey plates work is to have lactose which is not normally metabolized by the bacteria culture so that when mutations are present the bacteria gain the ability to metabolize the lactose. *Our expectation was that very few E939 colonies would appear on the MacConkey plate while many would appear on the E938 because of its mutation. While the number of colonies did not necessarily agree with that statement, E938 had more papillae than the E939.*

From this experiment we can draw conclusions about when natural selection favors certain traits, specifically, mutation rates. **The stable environment of the LB plate would favor the E939 because it has functional mismatch repair. The rifampicin plate would (and did) favor the E938 with its higher mutation rate. Thus we see a major factor in determining mutation rates: the environment.**

Level 3.5

For this lab, we examined how different strains of E. Coli reacted under extreme conditions. We dealt with two slightly altered strains—939, the original, and 938, a strain that was prone to high mutation rates. 938 was defective in regards to an enzyme that checked for errors during replication. As a result, the organism was less likely to fix potential mutations. *When both were plated on agar, the two strains reacted similarly,* which seemed to indicate that **under normal circumstances, there was little difference between both populations.**

The first extreme condition the E. Coli populations underwent was exposure to rifampicin—a deadly substance. *When plated on surfaces with rifampicin, the mutator strain outperformed the normal strain about 50 to 1*. As expected, **the strain that can mutate more easily exceeded the regular strain as it was more likely to contract the beneficial mutation**. Moreover, we were able to aggravate a mutation rate that proved the two strains performed as we expected them to. The rate was the number of surviving cells divided by the cells plated, as all of the cells on the plates would have developed the mutation. We found that the E939 mutation rate was 8.95E-9, while the E938 mutation rate was 4.54E-7, significantly higher. **We can conclude that under extreme conditions, the mutator strain has the advantage over the normal strain because it is easier for that strain to adapt to their new environment.**

The next experiment tackled the question of how the two strains would do in competition with each other. There were three possible outcomes—939 could dominate, the mutator strain could dominate or the two strains could co-exist. To test this, we grew a test tube of equal parts 939 and 938. However, we could not see which strain was more successful by merely observing the tube as the two strains are virtually

identical to the naked eye. We decided to plate the mixed strains on a plate with rifampicin. If the 938 strain dominated, the number of colonies would be similar to the number that we observed on the plate with only 938 and would be high (near 500-800). The same goes for the 939: if it dominated, there would be a small amount of cultures, as that is what we saw on the control (about 1-15). If they co-existed, the number of colonies would be somewhere in the middle of the two extremes. When the experiment was done, *the average of number of mutations was about 80.* This is not low enough to suggest that the 939 completely dominated as it is significantly larger than the control. However, it is not enough in between the two cultures to suggest that there was no one strain that was more successful. In the end, it seemed as if **939 had a slight advantage over the 938 strain***,* going against what we had predicted.

An examination of the computer modeling can explain this result. The model allowed you to change the outcome of the mutations, making them lethal, beneficial or neutral. In the modeling, *if the amount of lethal and beneficial mutations were similar (or even if the latter was slightly higher) the normal strain far out-competed the mutator strain.* **The beneficial mutations were simply not good enough to make up for the fact that they were more likely to contract a mutation that would cause them to not survive. For this reason, the normal strain was able to outcompete the mutator strain in our experiment.**

Level 4

Our results somewhat supported the hypothesis, in that **the mutant E938 strain had a much higher survival rate on the rifampicin plates than the wildtype E939 strain**. However, *the results of the co-culture growth indicate that the strains actually co-exist with one another, rather than one strain completely dominating the other one.* As indicated in Figure 2, the mutation rate of the co-culture, 0.769, is almost directly between E939's mutation rate of 0.170 and E938's mutation rate of 1.390. In addition, the number of mutations of the co-culture, 76.889, is also almost directly between the number of mutations of E939, 17, and the number of mutations of E938, 139. Since the number of colonies and the mutation rate is an average of the two strains, we can't say whether or not one had a better survival rate than the other.

The strains were possibly able to co-exist with one another because, **although the strain with the higher mutation rate has a higher resistance to rifampicin, the wild-type strain is more stable altogether due to its functioning RNA polymerase and "proofreading" system. Therefore, it doesn't experience as many deleterious mutations. There's a chance that the strain with the higher mutation rate would eventually die out due to harmful and at times fatal mutations**. *This is something we saw firsthand during the computer simulation of bacterial growth – when the conditions were set so that one of the strains had a higher mutation rate and the lethality of that mutation was higher, the mutated strain eventually died out.* Since we only looked at their growth on a very short scale, however, we can't say for sure if this is happening or not. **It's important to consider the metabolic cost such an extreme mutation rate has on bacteria. Although the mutant E938 could survive better in an environment with rifampicin, it has to compensate for this adaptation in other ways, meaning that its ecological range is most likely reduced. That relates to why organisms have such varying mutation rates, especially bacteria in relation to humans and plants.** *Billions of E. coli bacteria are produced in our intestines every day – along with millions of mutations within their populations.* **Since bacteria have such a short lifespan and rapid reproduction rate compared to plants and humans, their mutation rates are much more significant.**

A way to further our study is to continue growing the co-culture for a longer amount of time than just a week to see what eventually happens, such as whether or not the higher mutation rate is overall harmful

to the mutant population than beneficial. Also, plating more samples would help to determine whether or not our results were accurate and consistent due to the extreme ranges in the data.

It's important to note that there were a lot of possible sources of error. For example, plating the petri dishes required the serial dilution of each of the strains multiple times and in very small dosages, which left a lot of room for human error. In addition, as mentioned in the results section, there were some data points we had to discard due to the fact that they were so ridiculously far off from the rest of the data, they weren't comparable. If we had more time and resources, we would re-do the experiment to account for these extreme outliers.