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Supplemental Materials

for

A Single, Narrowly Focused CREATE Primary Literature Module Evokes Gains in Genetics Students' Self-Efficacy and Understanding of the Research Process

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Appendix Ia: CREATE Module Script for 4-class period Dog Module

The following is a script for teaching the "Dog Module" based on Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellersh CS, Parker HG, Ostrander EA. (2007). A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. PLoS Genet 3(5): e79. doi:10.1371/journal.pgen.0030079 http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.0030079

Core Genetics Concepts

Genotype
Phenotype
Allele
Central dogma: DNA, mRNA, protein
Transcription, Translation
Mutation (types)
Codons
Protein structure, function
Ploidy/Diploid/Haploid
Transmission
Gene regulation
Evolution
Haplotype

Supporting Optional Popular Press Articles:

A Very Muscular Baby Offers Hope Against Diseases by Gina Kolata
http://www.nytimes.com/2004/06/24/science/24muscle.html?pagewanted=print

Use the script as a guide to teaching the paper. While you may stray from the script for the needs of your class and your personal style, please include all of the CREATE elements. Please remember NOT to tell the students that they are now entering the "experimental part" of the semester. Try to incorporate the module as a logical extension of the course material and the development of scientific skills. Time estimates are shown in blue below each section.

Day 1:

1. Introduce Concept Mapping

- Discuss concept mapping as a way to make connection among concepts/terms and a way to develop an understanding of concepts beyond definitions. It is not until students understand how concepts relate to other terms that they truly understand them. Indicate that concept maps makes an excellent study tool and that they go beyond using "flashcards". Yes, flashcards can be useful for memorizing definitions, but concept maps requires students to use the definitions and thus improve their learning.

- Don't spend too much times on the technical aspects of making concepts maps or the theories behind them. Post the "How to Construct a Concept Map" handout on Blackboard and post a link to an electronic concept mapping tool. Let each student decide if they want to draw their concept map by hand or use an electronic tool (http://cmap.ihmc.us/).

- Pick an easy topic/familiar topic and produce a concept map on the board. The topic could be non-science (e.g. Life in College) or a review of a topic that they understand well. You do not want the students to struggle with the concepts for the mapping demonstration, so make it EASY.

- If you have time have students work in groups to produce a concept maps in small groups. For this, pick a topic related to the one demonstrated on the board (e.g. Life after College or Summer Break).

~15 minutes

2. Introduce the paper and homework assignments (HW 1 and HW2)

- Explain that the students will use the concept-mapping tool to read the introduction to a scientific paper.
- Explain that one of the skills that you will develop in the Biology core is reading scientific papers-you get a bit in Bio 2, more in Bio 3.
- Explain that reading scientific papers can be challenging, but that you will be helping them to read the paper by breaking it up into small parts. Suggestion…"First, you will read the introduction, then you will figure out what questions the scientist are asking and how the study is designed. " "Only after you understand the experimental design, can you understand the results".
- Explain that there are TWO parts to their homework. One part is a concept map of important terms in the introduction (HW1). The second is an assignment to review important genetic concepts they will need to understand the paper (HW2).

~5 minutes

Day 2:

1. Brief discussion of concept mapping

- Ask if it was challenging, not challenging; how many used cmap tools (online version vs doing it by hand). Remind students that concept mapping takes practice to get comfortable.
- Just to acknowledge their effort, and reiterate that it takes practice to get comfortable with concept mapping. Remind them that this is "How Learning Happens" (relating concepts) and that it can help studying and retention (so says science education research); this info was in the posted homework but it is worth reiterating so they see there is a good reason for it

~5 minutes

2. Get a few (~3) volunteers to say what they titled their maps (i.e. how they paraphrased the paper title) in the homework.

Write these titles down as they are provided. Ideally have the "real" title prewritten either on the board to which you will add their titles; or do it on PPT and type their titles in.

Compare the three titles:

- See if they all say 'the same thing' or if any omit part of what is in the actual title, or if any conflict with each other. React and ask a couple questions to the class in relation to these issues.
- For example, ask "are the first and third titles here [the ones you just wrote down; that students provided] the same? How are they different?" [you are asking people in the class to respond]
- If no one will respond (to this or any question) except front row "A+" students, then for next question say: "talk to the person next to you for 30 seconds about this; then I will ask you again". And literally give them 30 seconds; it is enough for a simple question like this; you have to break the ice on getting them to talk if they haven't been doing this in class up to now.

~5-10 minutes

3. Ask what additional terms from the introduction people added to their maps (ie they were supposed to pick three of their own; plus use the 14 we provided)

Note: Spending a little time on the maps says that doing the maps mattered; and also gets them back into thinking of the paper, the issues and the topic area.

- Call on someone in the back; ie they need to know they are "in" the class and expected to engage their brains (and this is an easy question).
- List the additional terms; ask how others linked the terms…discuss similarities, differences; maybe ask what else they linked to (e.g. if they added 'highly conserved' what did they link that to)
- **Or** take one term from the map (e.g. 'exon'; a required term) and ask a couple people what they linked it to; if they only say one other thing; ie intron; then prompt for other potential links
- Another more student-interactive option: choose three maps from the class (that students volunteer to show) and briefly project each on the doc cam, overhead projector, etc. The point is "These are all different and all FINE maps " assuming that they are; the only map phenotype you want to avoid is the 0-0-0-0-0-0- style (long chain without interlinking; or the "daisy" with one central word and a single link to a bunch of words in a circle around it (also not thoughtfully interlinked enough). The point of doing this is to say "there are a lot of different ways to do this assignment successfully".
- *Note: Doing this "in the moment" can be tricky; one idea would be to compare treatment of the same terms (e.g. Exon, Intron) on each of three maps. Similarities, differences.*
- Ask if students have any specific questions about any of the concepts; use their queries as a jumping-off point for a mini-lecture on the topic.
    - Example: a question about the differences between introns and exons might result in my drawing a gene on the board, and then the mRNA and mRNA processing.

~10 minutes

4. How did the authors come up with the idea that "myostatin" was the relevant gene and protein? IE What are other important muscle proteins? (See what they come up with)

Note: This question is the kind of thing I (SH) tend to spend some time digressing on; but it does take time. I like it because it says to the students that scientists had to think things through; that they weren't just following a playbook that said "it will be myostatin"; but that they did have several reasons, from previous research, to think it would be.

And also to make the point that even if you don't have that kind of background info (like a myostatin knockout mouse with a similar phenotype) there are other ways to get at differences between one phenotype and another……ie that science asks questions and then comes up with ways to answer them; rather than being limited by existing methods. You may not want to digress in this way, but it can help them consolidate their understanding of evolution as well, if you at least ask them about this whole "conserved" issue and what it means at a deep level.

Possible Follow Up for Discussion:

- Why not assume the phenotype is due to a change in myosin?
- Why work on myostatin rather than myosin or actin or tropomyosin?[Relates to the paragraph starting "The myostatin protein….."]
- Did they have ANY info on myostatin in dogs when they started? [no]
- Why assume myostatin was a good candidate? [gets into what 'conserved' means; can connect knockout mice and how those results migth relate to dogs]
- How are mice like dogs? [get them to list multiple reasons]; How are mice UNLIKE dogs?
- What does this conservation relate to? (Common ancestor; evolution; any reinforcement of this from Biology 1 would be good). This also relates to use of mice as "model systems" for humans; or the use of model systems in general, if you choose to digress in this direction.
- After the discussion above, we understand why it seemed reasonable to look at myostatin.

~5-15 minutes depdending on direction taken

5. Analysis of Figures 1 and 2 (starts in class, continues with HW)

Ask the class:

- **What is the question(s) asked in Figure 1?**

  See what they suggest; get a couple responses—not from the same people who have been talking already

- **Instructor writes these questions above the figure.**

  Technically this can be done by making overheads of the figures or opening figures in a program that allows you to write text.

- **How did the authors get the data for Figure 1 ?**

  Tell students that "I want to sketch the experimental plan. Tell me what to draw".

  They should look in the methods and guide your drawing of the experimental plan on the board (this sets students up for their challenge to illustrate what went on to get Figure 2 data, as part of their HW for the next class). Students who have read the methods should be able to prompt you:

  - Investigators asked owners to characterize their dogs.
  - Asked owners if the dogs had "sired or whelped" any bullies (This is worth asking students about; first; what does that mean; second, why did they need to know? (i.e. they are chasing down the phenotype/genotype of heterozygotes).
  - Then they sequenced DNA of 22 of these whippets; compared sequence difference (discovery of the deletion) with phenotype.
  - You should have developed a sketch/flowchart (this one doesn't totally lend itself to a sketch; but a stick figure of a dog and owner would help. Some of their data collection is just word of mouth (what owners say the puppies looked like); other data collection is DNA sequence data.

~15 minutes

- **Figure Annotation. Part of the "read" step of CREATE involves 'annotating' figures, which means relabeling them with info from the caption, methods, and/or paper narrative, to make them more informative.**

- **Annotate** (label) Figure 1 by getting the class to tell you the following:

  - Which phenotypes are associated with "wildtype", "mutant", and "heterozygous" genotypes?
  - Of the original 22 whippets sampled, how many dogs of <u>each phenotype</u> were homozygous for the wildtype allele? How many were homozygous for the mutant alleles? How many were heterozygous?
  - Which phenotypes are associated with full length and truncated myostatin proteins?
  - What do they mean: "all photos represent unique individuals….." What aspect of that needs to be noted on the figure? [ie your annotation should reflect that two pictures are of the same dog]

  Note: Possible question for class: How does annotation affect your sense of the figure?—[Ideally, they notice that they have looked more closely at it by the time it is done getting annotated; also that they had to get more relevant info from elsewhere in the paper; like "n"; it's not all in the caption]. Side issue: why does "n" matter? What if they only showed you one bully whippet and only had ever encountered 2 in the world? Does that matter? Why or why not?

- **Before moving on, make some summary statement of "where we are" in this story;**
  - We noticed a phenotype (in Figure 1),
  - Came up with a candidate gene that might be involved, sequenced some wildtypes, heterozygotes, homozygotes; defined the genetic change (in Figure 2)
  - Now we want to characterize the phenotype beyond "It's a big dog" (Figure 3)

~10 minutes

6. Deciding on what to measure. For this exercise have students work in groups of 4 and put all their names on the sheet of paper.

Have student work on these questions in their small group:

If you had discovered the phenotype of the "Bully Whippet", what might you measure, if you wanted to convince yourself (and others) that these dogs are really different from standard (wildtype) whippets?

Note: Gregor Mendel had a similar challenge with his peas…..

How could you quantify the difference(s) that you observe in Figure 1? Each group should be able to come up with at least 5 measures.

Note: I would project the questions or write them on the board so you don't have to repeat them 100x.

- After a few minutes, get them to call out things they would measure
- Write them on the board; informally ask how many groups had "that measure"
- If people only have physical things, prompt them to think of what else they could look at.
- Keep going until you have a big list (note if some are behavioral; like "how far can they run"; "how fast can they learn tricks*") Note: In HW they will learn which ones the authors looked at; but don't tell them that now.*

The point here is a) for them to think creatively; b) for them to realize phenotypic differences are not all physical; c) for them to realize that the authors measured three things but again, they had to DECIDE what to measure; it is not written down in some "Phenotype Analysis Handbook". This is an important aspect of the nature of science, and also relates to one of the HW questions that asks them to find out what the authors did measure (Fig 3; but they have to seek it out).

~10 minutes

7. Discussion of HW 2 (Figure 2 related)

1. Compare the mutant and normal alleles and have students tell you how to sketch the myostatin alleles found on chromosome 37 for each of the following:

  - A heterozygous whippet
  - A wildtype whippet
  - A homozygous mutant whippet

Note: This is an important time to emphasize genetic concepts of genes, alleles and chromosomes as well on what it means to be homozygous or heterozygous.

2. Compare normal and mutant proteins by having students tell you how to draw a sketch that compares wildtype and mutant proteins. Or have students come up and do it on doc cam or whiteboard; could have three pairs of students do it on board; see if all do same; ask class to compare with their own drawings.

3. List possible explanations for why the mutant protein functions differently than the wildtype protein. Get students to make suggestions; if time allows, get them to talk to the person next to them for a few minutes; and then make suggestions; ie give them a little more time to think about it.

4. Discuss loss-of-function mutation (ie no myostatin gene function) leading to "gain" of muscle mass….can they speculate on how that could happen? Compare to Drosophila "hairy" or other mutants.

~10 minutes

Wrap Up:

1. Remind them they now know:
    How to concept map
    How to paraphrase
    How to sketch an experimental procedure
    How to annotate a figure
They will be practicing these tools in HW

2. Assign HW 3

Note: I'm assuming that the above is more than enough for the 75 minutes. You may not get to #7 (the questions on HW 2). Depending on the pacing of the class, you may simply collect HW2 or you may spend time on HW2 in the next class session.

When you collect these sheets, I would say "I'm not 'grading' these; but you get credit for being here and participating".

Day 3:

1. Experimental Design

Tell students…Share your illustration of the experimental design for Figure 2 with a partner (or at your table). While you compare your illustrations, add experimental details that you may have missed to your illustration and add the measurements that were taken for Figure 3.

- Instructor: walking around; looking at illustrations; addressing questions.
- Instructor: Select 2 or 3 illustrations to show using document camera or use as a guide to draw the experimental plan for the class. Ultimately, use student input to explain how the experiments were conducted.

~10 minutes

Parts of experiment to include in discussion:

1. Dog owners were asked if their dog was "normal", "bully", or had sired or whelped a "bully".
2. Dogs were measured by owners or scientists: height, mass, neck, chest. *Note: could expand discussion to include how phenotypes are characterized*.
3. DNA samples were collected from the dogs
4. Myostatin gene was sequenced.
    a. Ask why they targeted the myostatin gene.
        i. Why target myostatin?
        ii. Why not the whole genome?
    b. Highlight sequencing of introns and exons
5. Can discuss sequencing technique if appropriate for the class. However, consider that providing a detailed description of sequencing will take time away from understanding the experimental design. *Note: Can show animated clip of sequencing or figure from textbook or have students find an animation, etc.*

~15 minutes

2. Results

Students list results for Figures 1, 2, and 3 (students work in small groups; 2-4 students). (~10 minutes).

Instructor: Lead a discussion of the findings (~15 minutes).

Figure 1:

- There is a correlation between phenotype and genotype
- Bully whippets are homozygous for mh allele; normal whippets are homozygous for wt allele; intermediate phenotype are heterozygous
- All dogs that have sired or whelped a bully are heterozygous.
-

Figure 2:

- The mh (bully) allele has a deletion of two nucleotides (nucleotide # 939 (T) and 940 (G)).
- Deletion results in a stop codon.
- Stop codon results in truncated protein.
-

Note: This is an important time to focus on genetics concepts (genes, alleles, proteins, etc.). Be sure to illustrate/describe how alleles relate to chromosomes, how DNA relates to mRNA relates to protein. Talk about introns/exons, codons, deletions, etc). This directly relates to Question 4 of their homework.

Figure 3:

- Discuss mass/height ratio instead of just weight or just height.
- Discuss plot type and median-showing distribution
- Bully highest mass/height ratio and the larger largest variation (Figure 3A)
- Heterozygotes have an intermediate phenotype (Figure 3A)
- Discuss why there is a variation in the data-students can offer reasons
    - Environmental differences (feeding/training/racing)
    - Other potential genetic differences that contribute to size (polygenic traits)
    - Measurement accuracy (scale type, owner vs. scientist)

3: What's next?

- Students work in small groups (2-4 people) to list questions that are still remaining related to this study. Ask them what they would do next? (~5 minutes)
- Have class discussion about possible extensions (~10 minutes)

Possible responses that students might come up with:

- Other racing breeds (actually in paper)
- Racing speed (actually in paper)
- How does mystatin work? How does losing part of a protein led to more muscle?
- Can I cure muscular dystrophy? Is this mutation found in humans?
- Can we manipulate the gene for meat production?
- Statistical analysis!

4: Life as a researcher-questions for authors:

- Have students talk in small groups about life as a scientist; "What do you envision life as a scientist would be like?" Provoke students to think about: What does it take to be a scientist; what motivates people to go into research; What motivates people to pursue specific types of studies, etc. (~5 minutes)
- After their brief discussion, ask each group to write 3 questions they would like to ask of research scientists. Have them write on index cards or use an electronic response system (if already used in class). (~5 minutes) (Alternatively, this can be homework).
- SH has conducted a phone interview with Elaine Ostrander using questions that students often ask. You can post the transcript of this interview and ask students to read it as homework. Alternatively, AK and SH have e-mail responses from authors of different papers that we can provide that may address specific student questions more directly. Contact AK if you would there are questions that your students would really like addressed that are not included in the Ostrander interview.

Day 4:

Spend a few minutes discussing the interview and/or author responses.

Appendix Ib: CREATE Module Student Assignments

A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs

Assignment 1

1) **Read the Introduction** (copied below) to *A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs* by Dana S Mosher, Pascale Quignon, Carlos D Bustamante, Nathan B Sutter, Cathryn S Mellersh, Heidi G Parker, Elaine A Ostrander. Refer to Figure 1 on page 3 of this handout.

Introduction

The wide variety of behaviors and morphological types exhibited among dog breeds and the overall low genetic diversity within each breed make the dog an excellent genetic system for mapping traits of interest [1,2]. Recently, owners of whippets, an established racing-dog breed, have reported a phenotype of heavy muscling occurring within the breed (http://www.k9community.co.uk/forums/index.php). The typical whippet is similar in conformation to the greyhound, a medium-sized sighthound, weighing about 9 kg and characterized by a slim build, long neck, small head, and pointed snout (Figure 1A) [3]. Heavily muscled dogs, termed "bully" whippets by breeders, have broad chests and unusually well-developed leg and neck musculature (Figure 1C). "Bully" whippets are easily distinguished from their normal littermates based on physical appearance alone (compare Figure 1A and 1C). Owners report that "bully" whippets do not have any health abnormalities other than muscle cramping in the shoulder and thigh. However, the dogs are often euthanized at an early age as they do not conform to the American Kennel Club breed standard. In addition, about 50% of "bully" whippets have a distinctive overbite.

The "bully" whippet phenotype is reminiscent of the double muscling phenotype seen in other species that is caused by mutations in the myostatin *(MSTN)* gene. Such variants have been observed in mice [4], cattle [5,6], sheep [7], and human, the latter described once in a German boy [8].

The myostatin protein has been shown to affect both the amount and composition of muscle fibers. For instance, the muscle mass of *Mstn* knockout mice is two to three times greater than that of wild-type mice [9]. Furthermore, the sequence of the *MSTN* gene is relatively conserved across species [9]. Therefore, we chose to interrogate the *MSTN* gene for possible mutations resulting in the "bully" whippet phenotype.

We sequenced the three exons and the majority of introns of the *MSTN* gene in an initial set of 22 whippets. A 2-bp deletion was discovered in the third exon of the *MSTN* gene (Figure 2). This deletion removes nucleotides 939 and 940 within exon three and leads to a premature stop codon at amino acid 313 instead of the normal cysteine, removing 63 aa from the predicted 375-aa protein. The lost cysteine is one of several highly conserved cysteines known to form disulfide dimers required for protein function [9].

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2) Produce a concept map of important terms in the Introduction

Used what you have learned about concept mapping in class and on the information in http://www.udel.edu/chem/white/teaching/ConceptMap.html, which is also posted on Blackboard, to complete your concept map.

Step 1: Produce a title for your concept map by, paraphrasing the title of the paper. That is, rewrite the paper title in your own words, creating a title that has the same meaning but is expressed in an alternative way. Do not look up each word and replace it with a synonym. Rather, think about the idea(s) expressed in the title and then re-express them in your own words.

Step 2: Look up all of the terms that you do not fully understand.

Step 3: Produce a concept map using the following terms:

Genotype, phenotype, mutation, myostatin gene, myostatin protein, musculature, bully whippets, typical whippet, morphological types, exon, intron, stop codon, plus three more that you choose.

Notes on concept mapping:

1. You can either do maps with paper/pencil (and/or Postit notes as outlined in the posted article about cmaps), or by using free "Cmap tools" software available at http://cmap.ihmc.us/cmaptools/

2. Concept maps are based on the idea that "learning" involves forming relationships between concepts, and research indicates that learning to use concept maps, and using them while studying helps students retain information. Therefore, you need to keep the following in mind:

- Think carefully about how you use unidirectional or bidirectional arrows to link the terms, and especially about what you write on the linking lines.
- Many of the concepts provided can and should be linked to more than one other concept.
- If your concept map takes only 5 minutes to make, you are not giving it enough thought.
- No two concept maps will be alike. Your map should reflect your understanding of the included concepts and their relationships. So don't worry about trying to reproduce the map that anyone else would make. Make your own map.
- Maps will be collected at the end of class.
- It takes practice to get comfortable making concept maps, so you probably will not feel like an expert the first time you try this approach.

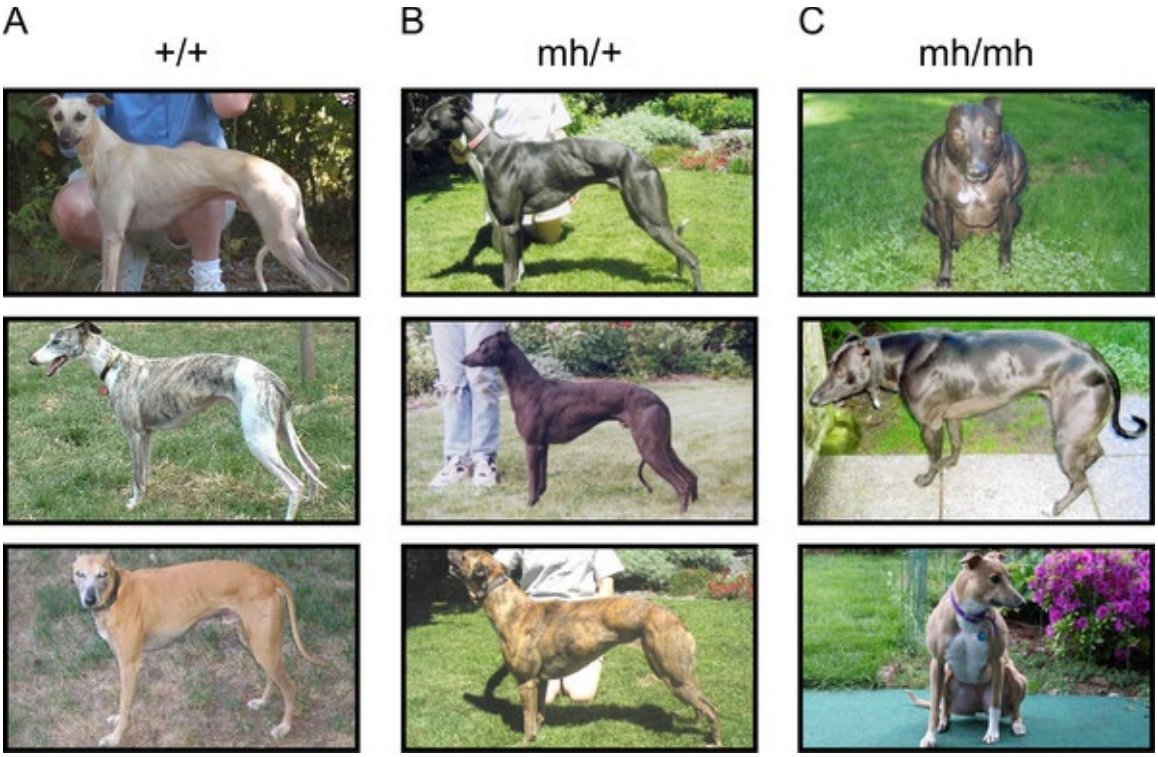

Figure 1. Comparison of Whippets with Each of the Three Potential Genotypes
(A) Dogs have two copies of the wild-type allele (+/+).
(B) Dogs are heterozygous with one wild-type allele and one mutant cys → stop allele (*mh/+)*.
(C) Dogs are homozygous for the mutant allele with two copies of the cys → stop mutation *(mh/mh)*.
All photos represent unique individuals except for the top and middle panels in the righthand column.

A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs

Assignment 2

Below are the sequences of wildtype and mutant alleles of the myostatin gene discussed in the paper introduction

Wildtype Allele: AATTACTGCTCTGGAGAGTGTGAATTTGTG

Mutant Allele: AATTACTGCTCTGGAGAGTGAATTTGTGTT

1) Using a genetic code table and single-letter code for amino acids, write the amino acid sequences of both predicted proteins.

2) What might have happened, to generate the mutant allele?

3) How does the wildtype protein compare to the mutant protein? Produce a sketch that compares them.

4) In dogs (which are diploid), the myostatin gene is on chromosome 37.

Sketch the myostatin alleles found on chromosome 37 for each of the following:

A heterozygous whippet

A wildtype whippet

A homozygous mutant whippet

5) Given the differences between the normal and mutant proteins, and the phenotypes seen (as described in the introduction that you concept mapped), suggest and illustrate (in simple diagrammatic sketches) two different possible explanations for why the mutant protein functions differently than the wildtype protein. Add a short (1 or 2 sentence) caption to each sketch.

Keep in mind that these hypothetical explanations should be based in your understanding of proteins. That is, just saying "they function differently because they are different proteins" is not an 'explanation'.

6) This homework will be collected along with your concept maps.

A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs

Assignment 3

Figures 1, 2 and 3 and the text relevant to these figures are found below on the next pages.

1. What experimental question is asked in Figure 2? Write it above the figure.

2. How did they do the experiment whose results are shown in Figure 2? **Illustrate (yes, draw a picture; not a flowchart)** of the experimental procedure used to produce Figure 2's data. To understand the experimental plan, you will need to refer to the figures and their legends, and the portions of the results and methods that refer to the figures (see text following the figures).

3. In order to more clearly understand the difference between the wildtype and mutant alleles, **annotate** Figure 2 (label or draw on the figure) to include the following:
    a. The difference in the nucleotide sequence between the wildtype and mutant alleles.
    b. What base pairs are missing in the mutant allele?
    c. How have the missing base pairs changed the codons in the mutant allele?
    d. How have the missing base pairs changed the amino acids encoded by the mutant allele?

4. **Illustrate** the differences between the wildtype and mutant alleles of the myostatin gene and the resulting changes in myostatin protein by drawing and clearly labeling the following:
    a. Draw the wildtype and mutant alleles of the mysotatin gene (including introns and exons) indicating how they differ.
    b. Draw the relative length of the mRNA transcribed from each allele. Indicate how they differ.
    c. Draw the relative length of the protein produced from each allele. Indicate how they differ.

5. Find the following sentence that is highlighted in the results section:

    "In order to quantify the allelic substitution and dominance effects of the deletion mutation we considered three measures…."

    a) What are the three measures used by the authors?

    b) How do these compare to the list of possible measures we came up with in class? Write 2-3 sentences comparing the authors' list and the class list.

    c) Paraphrase the whole sentence. You may use more than one sentence in your paraphrase. That is, rewrite the authors' "In order to quantify" sentence in your own words. Do not just rearrange phrases from that sentence. Write a new sentence or set of sentences that have the same meaning but are written in simpler language.

FIGURES 1, 2 and 3

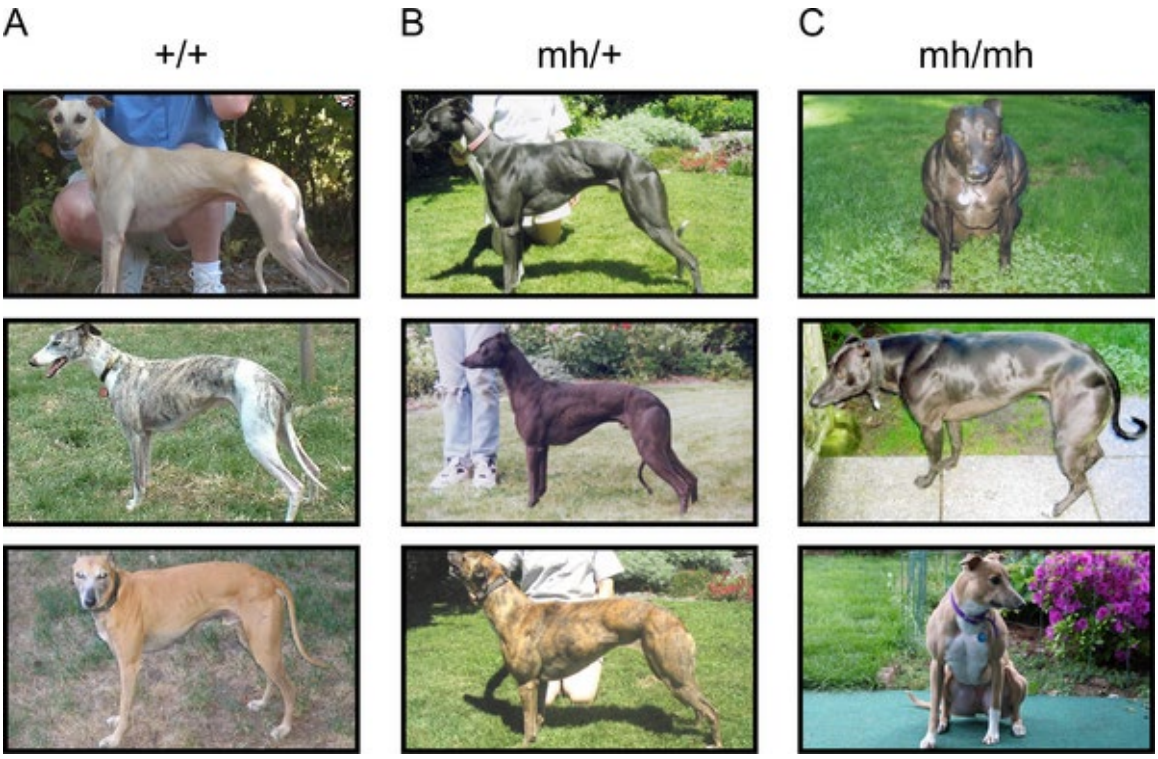

Figure 1. Comparison of Whippets with Each of the Three Potential Genotypes

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(B) Dogs are heterozygous with one wild-type allele and one mutant cys → stop allele (*mh/+)*.

(C) Dogs are homozygous for the mutant allele with two copies of the cys → stop mutation *(mh/mh).*

All photos represent unique individuals except for the top and middle panels in the righthand column.

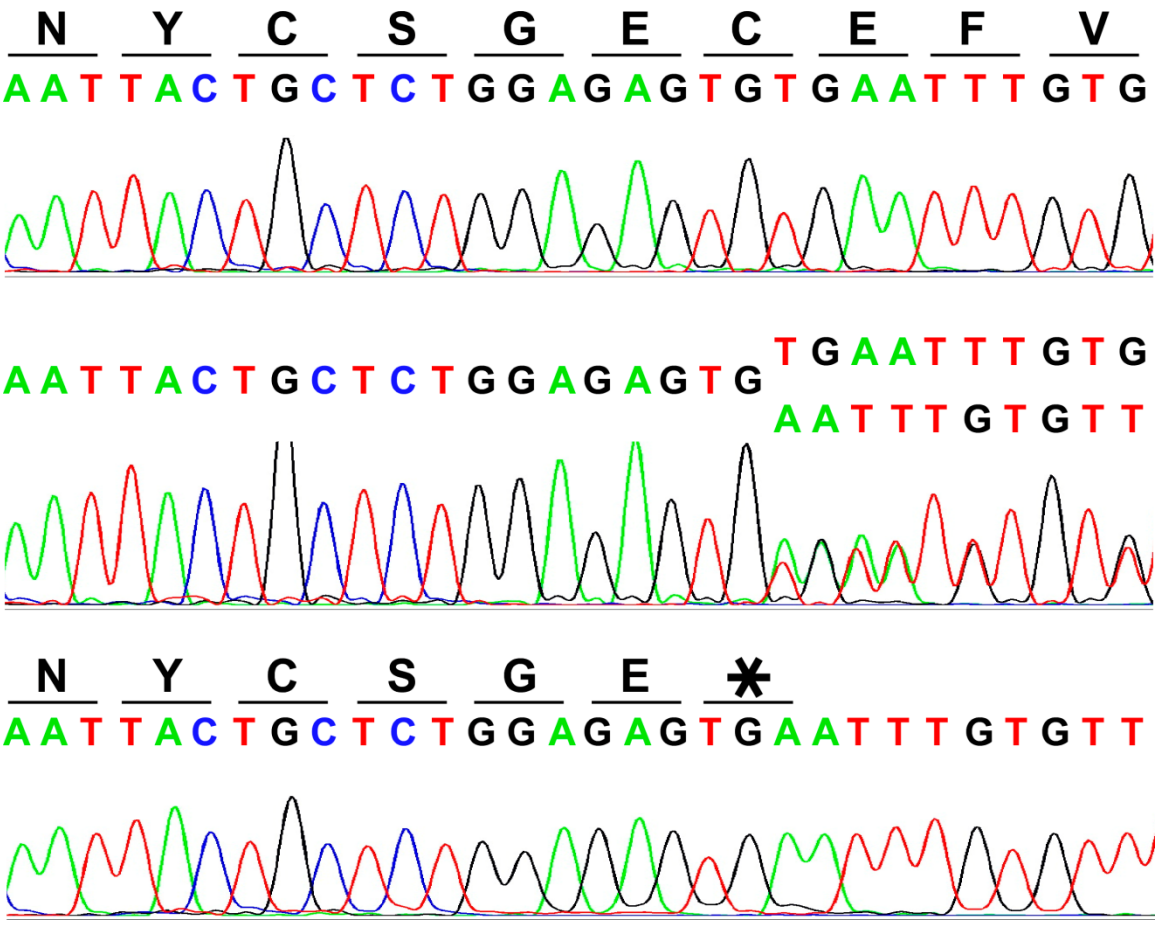

Figure 2. A 2-bp Deletion at Nucleotides 939 and 940 of the Canine *MSTN* Coding Sequence

This deletion results in a cysteine→stop codon change at amino acid 313. The top panel shows the sequence trace of a wild-type (+/+) individual in the region of the mutation. The middle panel shows the sequence trace of a *mh*/+ individual with a single copy of both the wild-type and mutant alleles. The bottom panel shows the sequence trace from a homozygote "bully" dog of the *mh/mh* genotype. The amino acid sequences for +/+and *mh/mh* individuals are located above each trace.

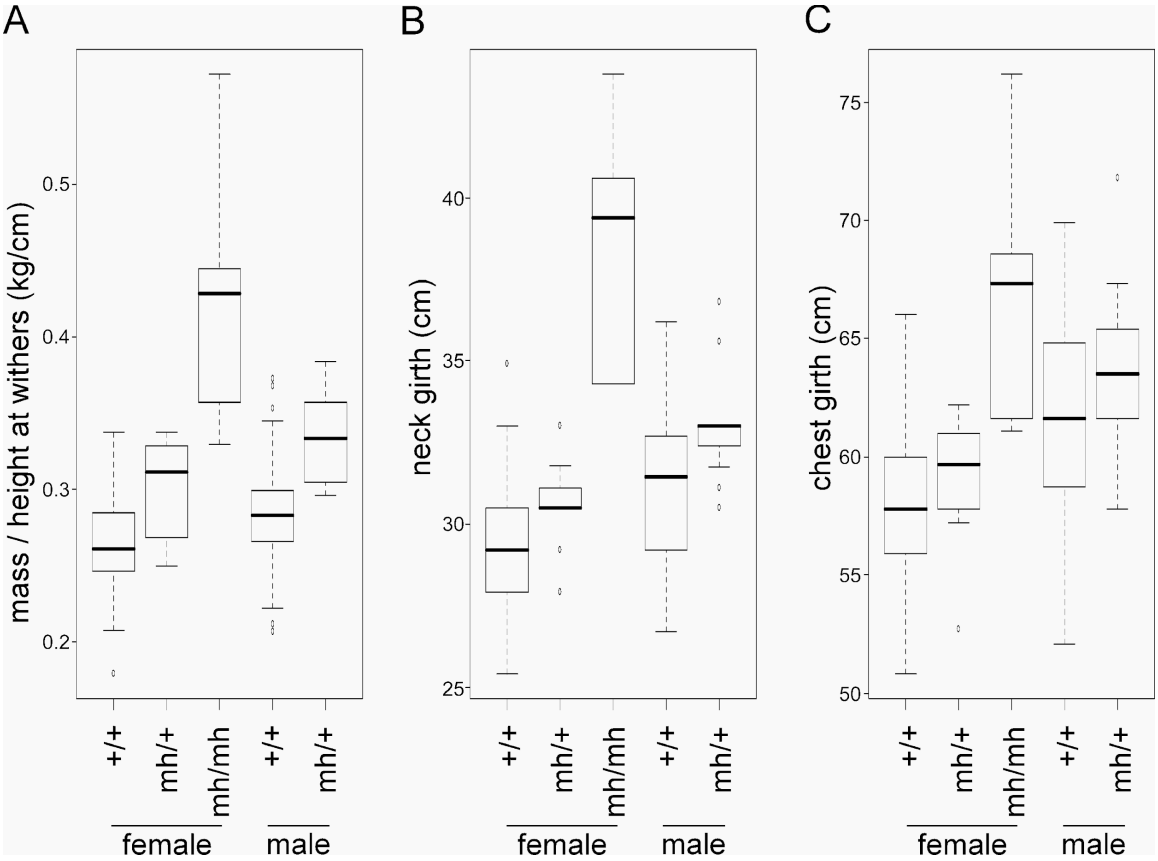

Figure 3. Variation in Musculature among Whippets of the Three Potential Genotypes

Whippets homozygous for the cys → stop mutation have a higher mass-to-height ratio (A), a larger neck girth (B), and larger chest girth (C) than wild-type or heterozygous individuals. Males and females are shown separately. +/+, wild-type individuals; *mh*/+, individuals heterozygous for the cys → stop mutation; and *mh/mh,* individuals homozygous for the cys → stop mutation. The center bar indicates the median value and the top and bottom edges of the box delimit the 75th and 25th percentiles, respectively.

Results-Excerpts Relevant to Figure 1, 2, and 3

We sequenced the three exons and the majority of introns of the *MSTN* gene in an initial set of 22 whippets. A 2-bp deletion was discovered in the third exon of the *MSTN* gene (Figure 2). This deletion removes nucleotides 939 and 940 within exon three and leads to a premature stop codon at amino acid 313 instead of the normal cysteine, removing 63 aa from the predicted 375-aa protein. The lost cysteine is one of several highly conserved cysteines known to form disulfide dimers required for protein function [9].

Of the 22 whippets sequenced, all "bully" whippets tested ($n = 4$) were homozygous for the deletion *(mh/mh)* while all dogs that sired or whelped a "bully" whippet ($n = 5$) were heterozygous for the deletion (*mh*/+). None of the initial set of 13 normal-appearing whippets that lacked a family history of the "bully" phenotype carried the deletion; these dogs were designated wild type (+/+). An additional set of DNA samples from 146 whippets (both racers and nonracers) were collected at racing events and through the mail without regard to the dogs' family histories of the "bully" phenotype. These were sequenced across exon three to determine the frequency of the 2-bp mutation among the dogs sampled. Of these, two were homozygous for the deletion, 20 were heterozygous, and the remaining 124 did not carry the deletion.

Mode of Inheritance and Heterozygote Phenotypes

The "bully" phenotype displays a simple autosomal recessive mode of inheritance, as all "bullies" resulted from the mating of carriers. The parents have a phenotype of intermediate musculature (Figure 1B). In order to quantify the allelic substitution and dominance effects of the deletion mutation we considered three measures of musculature: mass-to-height ratio, neck girth, and chest girth. For all three measures, heterozygous females (*mh/+)* were intermediate in musculature, *mh/mh* females had the highest measures, and female +/+ whippets had the lowest measures. Male *mh*/+ whippets were more muscular than wild-type males (Figure 3 andTable 1).

Appendix 2: SAAB substatements

SAAB survey substatements in selected categories; see Hoskins, Lopatto and Stevens (2011) for details of development of this survey. Statements labeled (R) were reverse-scored for analysis.

1--Decoding Primary Literature

The scientific literature is difficult to understand (R).

When I see scientific journal articles, it looks like a language I don't understand (R).

I am not intimidated by the scientific language in journal articles.

I am confident in my ability to critically review scientific literature.

I am comfortable defending my ideas about experiments.

3--Active Reading

I could make a simple diagram that provides an overview of an entire

experiment.

If I am assigned to read a scientific paper, I typically look at the methods section to

understand how the data were collected.

I do not know how to design a good experiment (R).

The way that you display your data can affect whether or not people believe it.

7--Knowledge is certain

If two different groups of scientists study the same question, they will come to similar conclusions. (R)

The data from a scientific experiment can only be interpreted in one way. (R)

Because scientific papers have been critically reviewed before being published, it is unlikely that there will be flaws in scientific papers. (R)

Because all scientific papers are reviewed by other scientists before they are published, the information in the papers must be true. (R)

Sometimes published papers must be reinterpreted when new data emerge years later.

Results that do not fit into the established theory are probably wrong. (R)