

# Case study: Investigating a newly discovered muscle mutation in mice

## Preliminaries

If you are not already familiar with the structure of these exercises, read the Introduction first.

If you have not already worked through the first part of this exercise: Transmission of Genetic Information, begin with that first. That page has the background and information you need to fully understand this case study.

### Note

Reminder: Save your work regularly.

### Important

If you are using a Mac, we recommend that you use either Chrome or Firefox to complete these exercises. Some of the default settings in Safari prevent these exercises from running.

## Contact information

If you have questions about these exercises, please contact Dr. Kevin Middleton (middletonk@missouri.edu) or drop by Tucker 224.

## Introduction

In the early 1990's, Dr. Theodore Garland and colleagues began a long-term breeding experiment using house mice (*Mus musculus*). The goal of this experiment was to explore the inter-relatedness of traits involved in physiology, anatomy, brain function, immunology, behavior, and many others.

From a random, genetically diverse initial population, the research team established eight separate groups of mice. Four of the groups would be artificially selected for high levels of voluntary activity (measured using a running wheel with a sensor attached to the mouse's cage) and the other four would be bred randomly (these would also have a running wheel, but breeders were randomly chosen). Here is a link to a book section that describes the experiment. A movie

(Figure 1) shows the dramatic difference in activity between the control and selected mice.



[https://www.youtube.com/watch?v=RuqhC7g\\_XP0](https://www.youtube.com/watch?v=RuqhC7g_XP0)

Figure 1: A movie comparing the selected (left) and control (right) mice on the running wheels.

Because wheel-activity, the trait being selected, is heritable, the two sets of lines rapidly diverged so that by generation 10, all four of the selected lines of mice were running more, on average, than the four control lines (Figure 2).

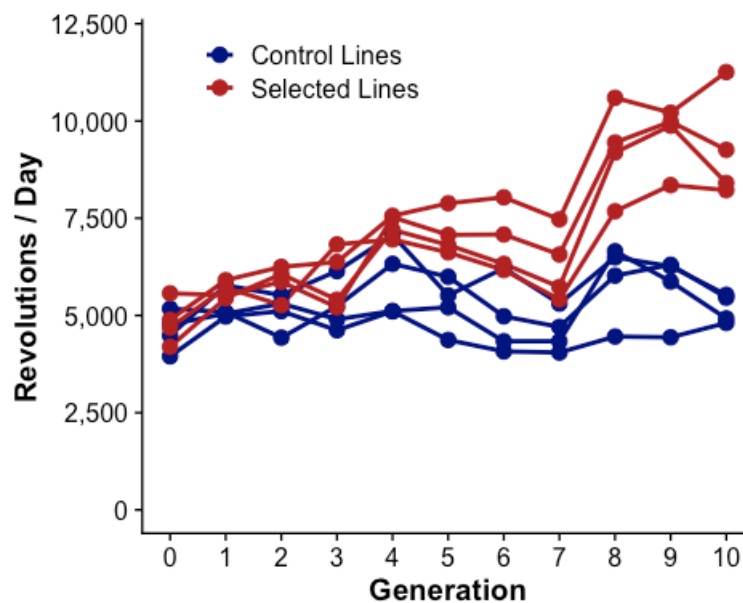


Figure 2: Total wheel revolutions per day among female mice for 4 selected (red) and 4 control (blue) lines of mice across the first 10 generations of selection. Data from Swallow et al. (1998).

Among other phenotypes, Garland and colleagues were interested in studying muscle physiology. Did the selected mice evolve to have different muscle

masses or muscle fiber types? They were routinely weighing the calf muscles of mice as part of their research and generated plots that looked like Figure 3.

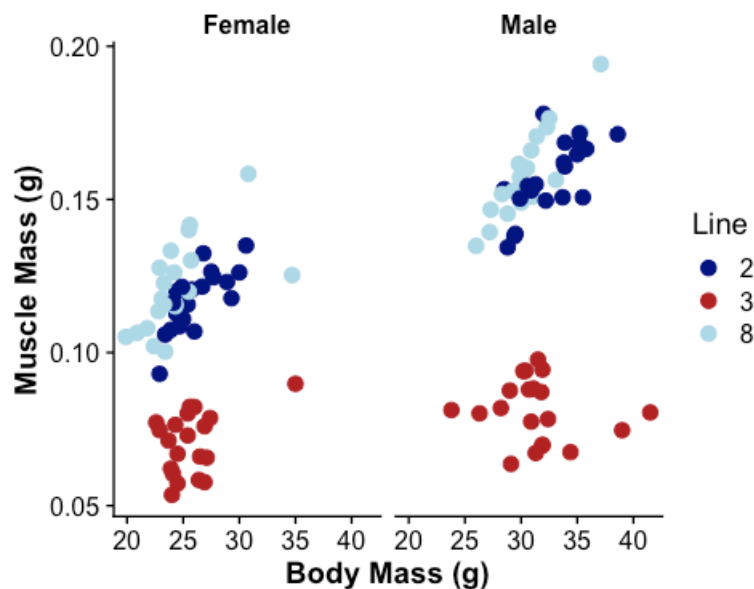


Figure 3: The mass of the calf muscles plotted against body mass for both female and male mice. In general you expect to find that larger mice will have larger muscles, just like an adult human has larger muscles than a child. But you do not expect that one of the lines of mice would have such disproportionately small muscles compared to the others (the red points above).

Generation after generation, they found that some of the mice had muscles that weighed only about 50% as much as they would be expected to based on their body mass (Figure 3). In other words, there appeared to be a subset of mice that had muscles which were only half as large as predicted. The pattern was the same in both female and male mice.

Overall, body mass was not that much lower in the selected lines of mice (as you can see in Figure 3, with the exception of one very large mouse, most of the red points fall toward the left side of the plot), however, the muscles were much smaller than predicted (the red points are all lower than the two sets of blue points). They called this small muscle phenotype “Mini-muscle”.

Through studying many generations of mice, they detected this small muscle phenotype in three different lines, and estimated the prevalence across generations (Figure 4). The researchers also found that the small muscle phenotype was being favored by selection in these mice (Houle-Leroy et al. 2003), because it appeared to be increasing in frequency across generations in lines 3 and 6, but just changing randomly in line 5, which was a control line.

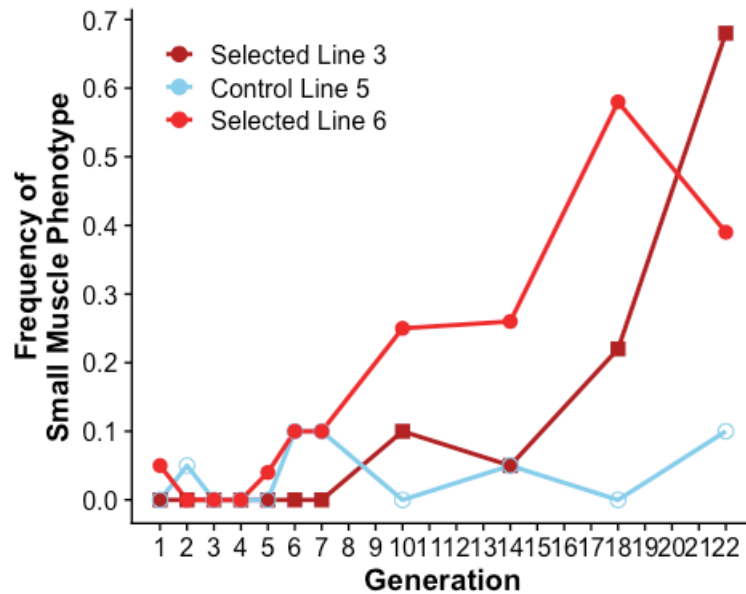
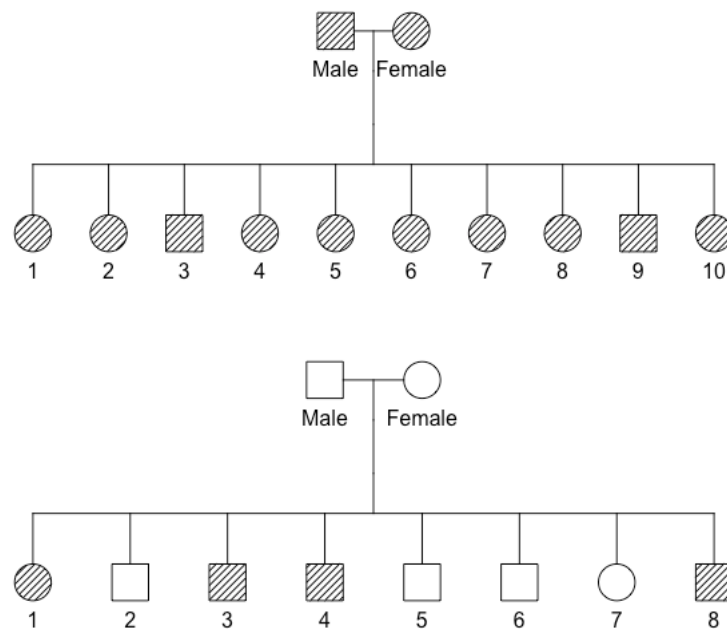


Figure 4: Frequency of mice exhibiting the small muscle phenotype in three lines across the first 22 generations of selection. The squares in this plot (Line 3) are the same line as the red points in Figure 3. Data from Garland et al. (2002)

### Developing the hypothesis

Garland and colleagues examined pedigrees of mice that showed the small muscle phenotype. They found patterns like the ones below. In the pedigrees, hatching indicates that the mouse has the small muscle phenotype.



*What kind of inheritance pattern do you think could explain the observed distribution of phenotypes (dominant vs. recessive, sex-linked vs. autosomal)? Briefly explain your reasoning.*

The first pedigree shows that when both parents have the small muscle phenotype, all of their offspring do, both male and female. The second pedigree shows that when neither parent shows the phenotype, they can still have both female and male offspring that have the phenotype. In the first case, it is clear that both parents have at least one mutant allele. We don't know yet whether the trait is dominant or recessive. The second suggests that the trait is recessive. Both parents must be carriers of the allele. In their offspring that have the phenotype, one copy must have come from each parent. Because both sexes are equally represented, the gene is probably autosomal. So, this is likely an autosomal recessive allele.

### Testing the hypothesis

Garland and colleagues hypothesized that the small muscle phenotype was caused by an autosomal recessive allele. They set out to determine if their hypothesis was correct by designing a test cross.

The original strain of mice that had been used to produce the 8 lines was genetically outbred, meaning that it had high genetic variability.<sup>1</sup> For the test cross, they chose to use a standard inbred strain of mouse: C57/Bl6 ("C57 Black 6"). This mouse had never been known to show the small muscle phenotype, which the researchers confirmed through dissection.

For the other parental line, they chose all mice that had the small muscle phenotype.

*Using a piece of paper, set up a Punnett square with a homozygous dominant C57/Bl6 father (MM genotype) and a homozygous recessive small muscle phenotype mother (mm genotype). What genotypes and phenotypes do you predict if the hypothesis is correct? Describe your results below.*

Your Punnett square should look something like this:

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<sup>1</sup> The specific strain *ICR* was chosen because it had a similar level of genetic variation as found in humans. If there were no genetic variation, then selective breeding would not have worked.

	M	M
m	Mm	Mm
m	Mm	Mm

The father can make M and M alleles. The mother can make m and m. So all combinations of these alleles will be Mm. If the hypothesis is correct that the allele is recessive

The F<sub>1</sub> generation should all be *Mm* (all combinations of alleles will be *Mm*). The resulting muscle phenotypes will be normal if the allele is recessive (a single copy of *M* is enough to result in the normal phenotype).

Garland and colleagues found the expected phenotypic pattern (but still didn't know the underlying genotypes). Next they set up a second cross between the F<sub>1</sub> mice resulting from the first cross and the small muscle phenotype parental female mice (this is called a "backcross").

*Using a piece of paper, set up a Punnett square with a heterozygous father (Mm genotype) and a homozygous recessive small muscle phenotype mother (mm genotype). What genotypes and phenotypes do you predict if the hypothesis is correct? Describe your results below.*

Your Punnett square should look something like this:

	M	m
m	Mm	mm
m	Mm	mm

Half of the mice should have the *Mm* genotype and half the *mm* genotype. So, the resulting phenotypes should be equally split between normal and small muscle.

In this cross, genotypes of half of the mice should be heterozygous (*Mm*) and half homozygous for the small muscle allele (*mm*). The phenotypic ratio will also be 1:1, because the heterozygous mice will have the normal muscle phenotype.

## Results

In total, there were 404 mice in the  $F_2$  backcross generation (Hannon et al. 2008). The authors weighed the calf muscles of all the mice (called “triceps surae” here). The results are shown in Figure 5.

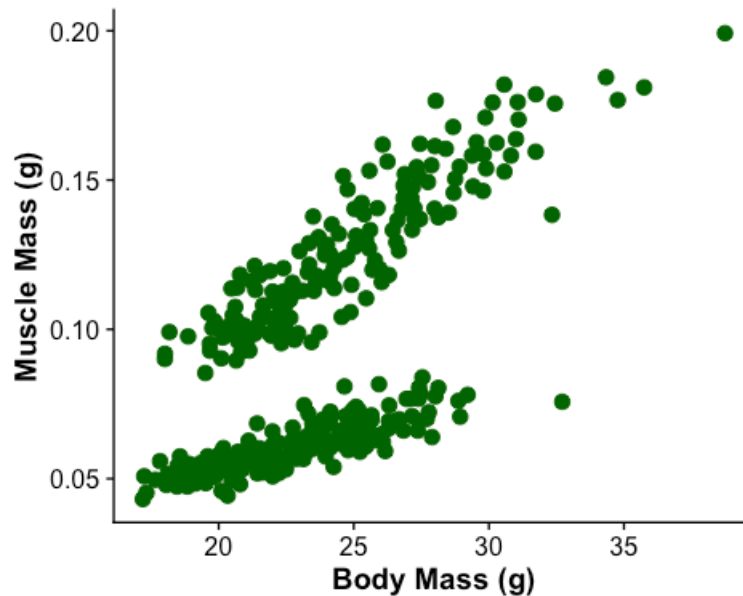


Figure 5: Observed muscle masses compared to body mass in a sample of 404  $F_2$  backcross mice. Data from Hannon et al. (2008).

*What is your prediction for the phenotypic ratio from looking at the plot above? Does it appear that there is a 1:1 ratio of normal to small muscle mice?*

It appears that there are two distinct groups of muscle masses. The clusters of points seem to be about the same size, suggesting that the groups are roughly equal. So, it does appear that there is a 1:1 ratio.

Based on the measured muscle masses, 201 mice were classified as having the small muscle phenotype and 203 were classified as normal.

Phenotype	Count
Normal	203
Small Muscle	201

## Testing the results

Now it's your turn. We will set up the data just like we did for the date of birth example.

In the block below, replace the 0s below with the observed counts: 203 normal and 201 small muscle.

```
MM <- tribble(  
  ~ Phenotype, ~ Count,  
  "Normal", 203,  
  "Small Muscle", 201  
)  
MM
```

```
# A tibble: 2 × 2  
  Phenotype    Count  
  <chr>       <dbl>  
1 Normal      203  
2 Small Muscle 201
```

*Based on the observed counts, what do you predict that the test will show? Will the counts differ from a 1:1 ratio (agreeing with the researchers' hypothesis) or will they not differ (the null hypothesis)?*

The counts are very close, and the sample size is quite large. The counts should not differ from a 1:1 ratio, which agrees with the hypothesis that this is an autosomal recessive allele.

In the code block below, use the counts above to carry out a chi-squared test for the data. You just need to add the column with the data.

```
chisq.test(MM$Count)
```

Chi-squared test for given probabilities

```
data: MM$Count  
X-squared = 0.009901, df = 1, p-value = 0.9207
```

*What are the  $\chi^2$  value, the degrees of freedom, and the associated P-value? What does this test tell you about the observed ratio of counts?*

The chi-squared value is 0.0099, with 1 degree of freedom (because there are 2 groups), and  $P = 0.92$ . So, the counts are not different from the expected 1:1 ratio.

*How do you think the results might have been different if the authors had only measured 40 mice instead of ~400? Feel free to change the numbers above and*



re-run the test. Just be sure to run the code block where the MM object is created before you rerun the chi-squared test.

The results would have been much more uncertain. For small sample sizes, it is more difficult to say whether the results are a real pattern or if they are the result of chance alone.

## Epilogue

The “mini-muscle” allele was confirmed as autosomal recessive, just as Garland and colleagues hypothesized. The full description of the experiment and a large amount of data and discussion can be found in the original publication (Hannon et al. 2008).

After some work on the genetics of the small muscle phenotype, it was mapped to a 2.6 megabase region on chromosome 11 that contained about 100 genes (Hartmann et al. 2008). After several more years of more research, the allele was finally identified as a single nucleotide polymorphism (SNP) located in the Myosin Heavy Chain 4 gene (Kelly et al. 2013). Homozygous mice with two copies of this allele (named *Myh4<sup>minimsc</sup>*) fail to produce one type of myosin, which results in small muscles that produce less contractile force but do not fatigue as quickly as unaffected muscles.

## Feedback

We would appreciate your anonymous feedback on this exercise. If you choose to, please fill out this optional 4-question survey to help us improve.

## References

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- Swallow, J. G., P. A. Carter, and T. Garland Jr. 1998. Artificial Selection for Increased Wheel-Running Behavior in House Mice. *Behav. Genet.* 28:227–237.