NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

To Pick a Peck of Orange Peppers:

From Molecular Variation to Macroscopic Change

by

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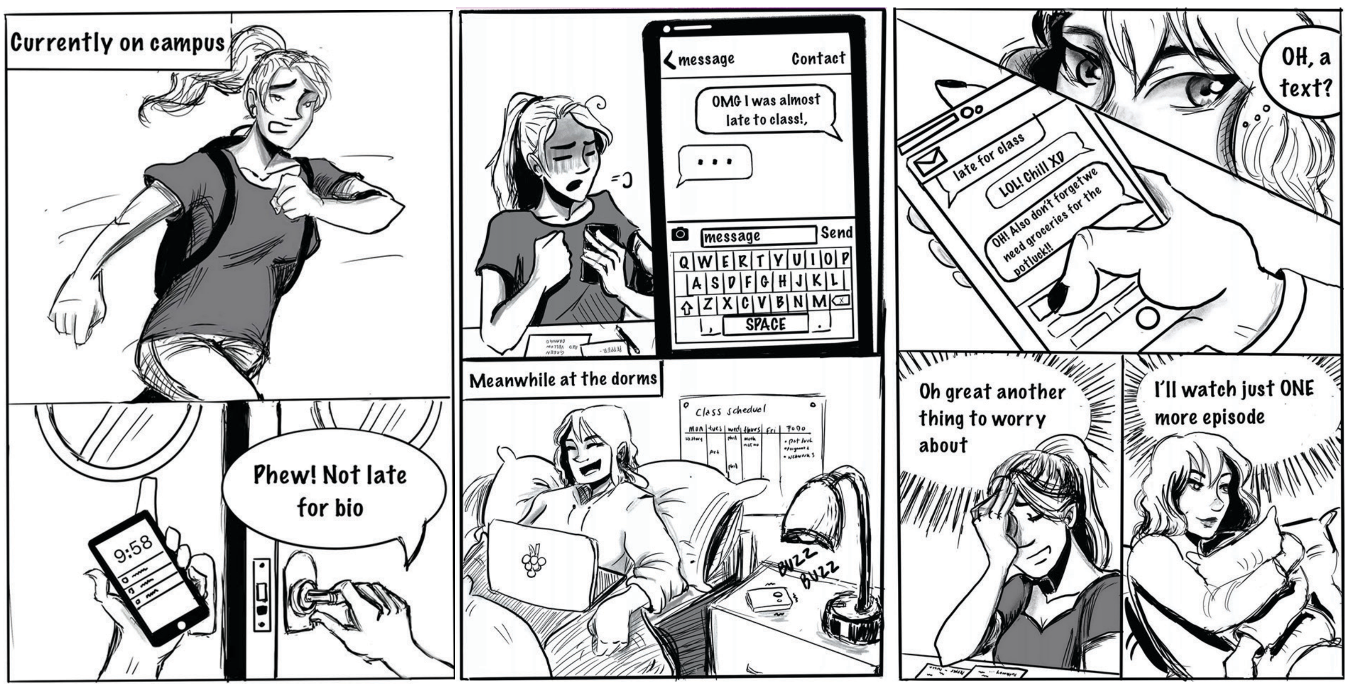
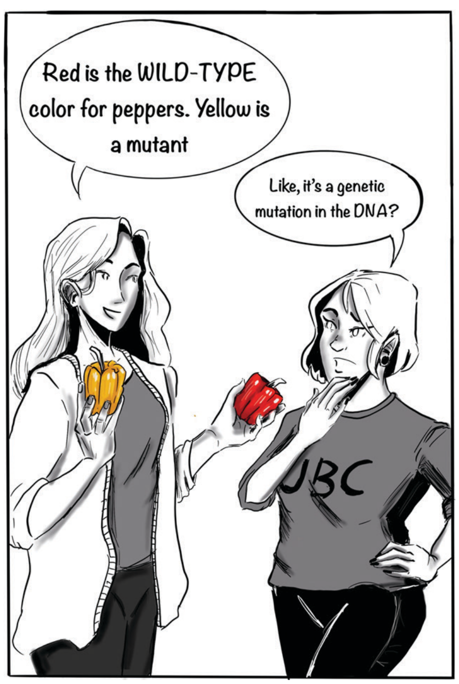
Part I – Mutant Peppers!

Two good friends share a room at their university's dorm. Invited to a potluck, they decide to bring a dish of stuffed peppers—but before they know it, they find themselves investigating the genetics of *Capsicum annuum* fruit color.



Before going any further, do you have any questions about the content of the preparatory readings? Individually or in pairs, please make a list of all the points that are still unclear to you:

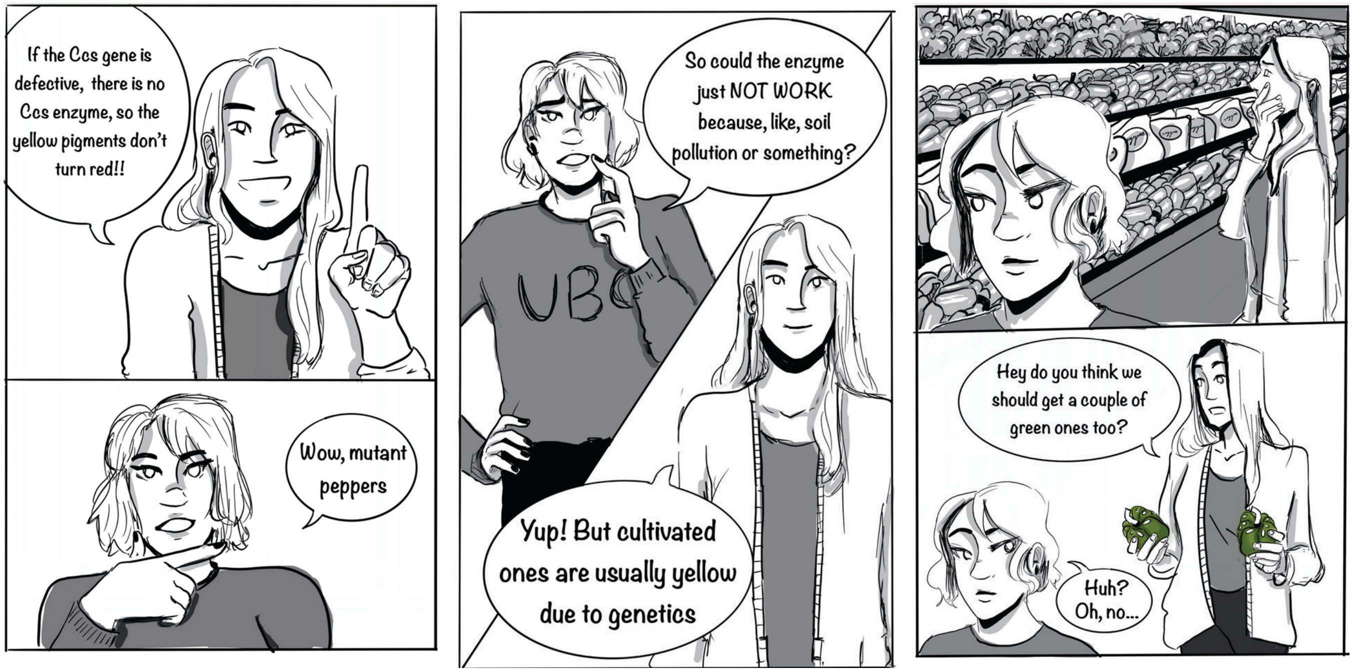


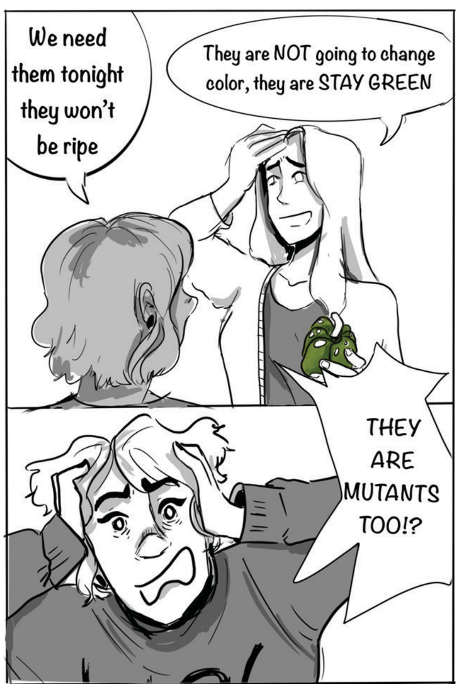
Question 1

What could be a genetic cause, and a non-genetic cause, for the yellow color of a bell pepper fruit?

A genetic cause:

A non-genetic cause:





Question 2

What kinds of mutations, and at what *loci*, do “permanently green” peppers possibly have?

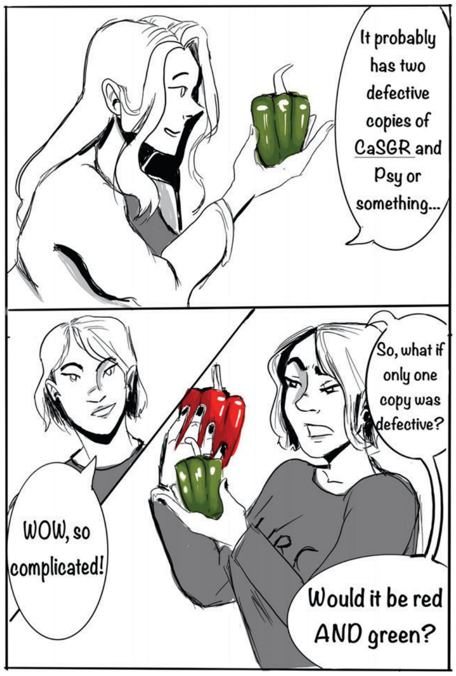
To help constructing and articulating your answer, please address the following points:

i. What pigments are present, and what pigments are missing, in ripe red peppers and in permanently green peppers, respectively?

ii. What proteins do you expect to be absent or non-functional in a plant that produces permanently green peppers?

iii. What kinds of mutations, and at what *loci*, would result in the protein defects you postulated in (ii) above ? What would this mutation (or these mutations) cause in terms of mRNA?

Part II – Defining Dominance



Question 3

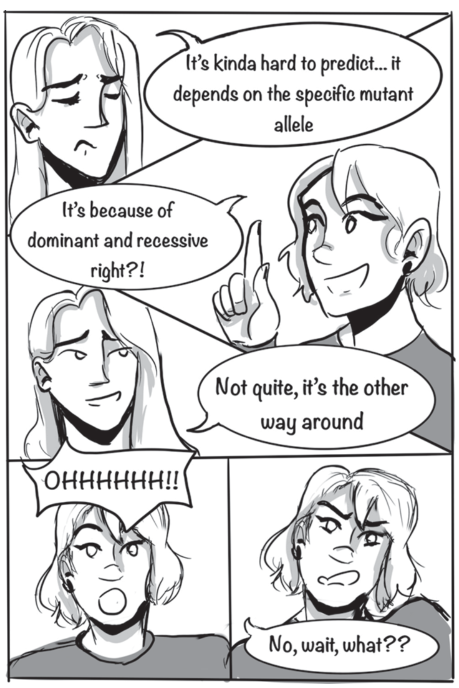
Right…what if one *CaSGR* allele was wild-type and the other had a large deletion in the promoter region, making it non-functional? What fruit color would such a plant produce? *Select the best answer.*

A. It depends on which of the two *CaSGR* alleles is dominant.

B. Most likely permanently green fruits.

C. Most likely purple-brown fruits.

D. Most likely red fruits.



If you are as confused as our curious friend, pay attention to the "Intermission" that will follow and take some good notes.

Question 4

Reconsider: A pepper plant has one *CaSGR* allele that is wild-type and one that has a large deletion in the promoter region, making it non-functional. What fruit color will such a plant produce?

A. It depends on which of the two *CaSGR* alleles is dominant.

B. Most likely permanently green fruits.

C. Most likely purple-brown fruits.

D. Most likely red fruits.

Question 5

A pepper plant has one *CaSGR* allele that is wild-type and the other has a large deletion in the promoter region, making it non-functional. What do you expect the dominance relationship between these two alleles to be?

A. The wild-type allele is dominant to the "deletion" allele.

B. The wild-type allele is recessive to the "deletion" allele.

C. The wild-type allele and the "deletion" allele are neither dominant nor recessive to each other.

D. We do not have enough information to predict the dominance relationship between these two alleles.

Question 6

To help us rigorously predict the dominance relationship between the wild-type allele of *CaSGR* and our mutant allele of interest (deletion of the gene's promoter), let's start by predicting the following:

	Plant's genotype (*CaSGR* gene)		
Phenotype of interest	*Homozygous wild-type*	*Homozygous mutant*	*Heterozygous*
Presence of *CaSGR* mRNA	Yes, present		
Amount of *CaSGR* mRNA	Two "doses"		
Amount of CaSGR protein	Two "doses"		
Amount of chlorophyll in ripe fruits	None		
Color of ripe fruit	Red		

Question 7

Reconsider: A pepper plant has one *CaSGR* allele that is wild-type and one that has a large deletion in the promoter region, making it non-functional. What do you expect the dominance relationship between these two alleles to be?

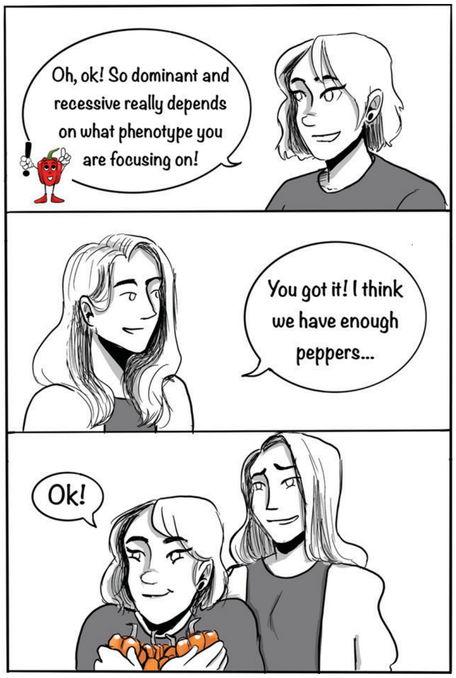
A. The wild-type allele is dominant to the "deletion" allele.

B. The wild-type allele is recessive to the "deletion" allele.

C. The wild-type allele and the "deletion" allele are neither dominant nor recessive to each other.

D. We do not have enough information to predict the dominance relationship between these two alleles.

Part III – What Makes a Pepper Orange?



Question 8

Form groups of about four. Then, in your groups, answer the following:

What could cause a plant to produce orange peppers? Propose at least two distinct hypotheses that would explain the production of orange peppers.

Please ensure that each of your hypotheses:

- is consistent with what you have learned about fruit color in bell peppers; and
- is testable, i.e., it makes one or more predictions that you could verify experimentally through measurements and observations.

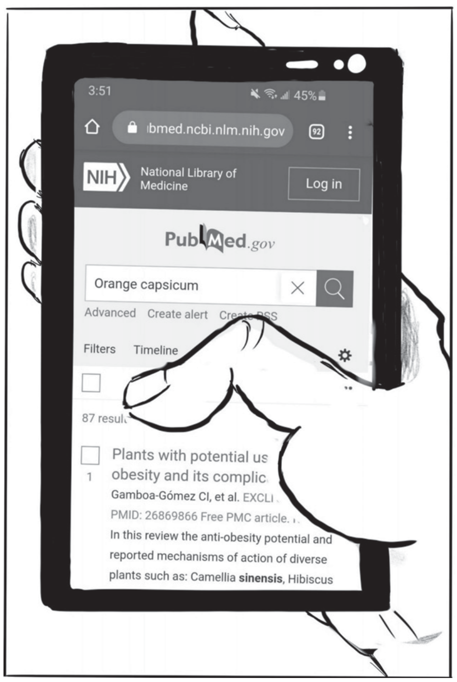
Hypothesis 1:

Hypothesis 2:

While dinner is in the oven, our friends find a very relevant article on orange peppers:

> Rodriguez-Uribe, L., I. Guzman, W. Rajapakse, R.D. Richins, & M.A. O'Connell. (2012). Carotenoid accumulation in orange-pigmented *Capsicum annum* fruit, regulated at multiple levels. *Journal of Experimental Botany* 63(1): 517–26. <https://doi.org/10.1093/jxb/err302>

Do the results reported in this article support one of your hypotheses? We will soon find out!



Question 9

As part of their study, Dr. Laura Rodriguez-Uribe and her colleagues at New Mexico State University studied fruits from several orange cultivars, as well as one yellow and one red one. For most of them they examined levels (amounts) of several pigments present in the fruits, levels (amounts) and sizes (length in bases) of RNAs encoding carotenoid biosynthesis pathway enzymes, and DNA sequence of the *Ccs* gene promoter.

What would their results have been if your Hypothesis 1 was correct? What about if your Hypothesis 2 was correct? Discuss with your group and report your prediction in your Question 9 tables (see next page).

Table 9 (i). Results predicted if Hypothesis 1 is correct.

Phenotype (measurement)	*Red cultivar*	*Yellow cultivar*	*Orange cultivar*
amount of b-carotene*			
amount b-cryptoxanthin*			
amount of lutein*			
amount of zeaxanthin*			
amount of violaxanthin*			
amount of capsanthin*			
amount of *Psy* RNA*			
amount of *LcyB* RNA*			
amount of *CrtZ2* RNA*			
amount of *Ccs* RNA*			
length of the *Psy* RNA* (compared to length in WT red)			
length of the *LcyB* RNA* (compared to length in WT red)			
length of the *CrtZ2* RNA* (compared to length in WT red)			
length of the *Ccs* RNA* (compared to length in WT red)			
mutation(s) in *Ccs* gene promoter			
* in mature (ripe) fruits			

Table 9 (ii). Results predicted if Hypothesis 2 is correct.

Phenotype (measurement)	*Red cultivar*	*Yellow cultivar*	*Orange cultivar*
amount of b-carotene*			
amount b-cryptoxanthin*			
amount of lutein*			
amount of zeaxanthin*			
amount of violaxanthin*			
amount of capsanthin*			
amount of *Psy* RNA*			
amount of *LcyB* RNA*			
amount of *CrtZ2* RNA*			
amount of *Ccs* RNA*			
length of the *Psy* RNA*			
length of the *LcyB* RNA*			
length of the *CrtZ2* RNA*			
length of the *Ccs* RNA*			
mutation(s) in *Ccs* gene promoter			
* in mature (ripe) fruits			

For Questions 10–14, you will need to consult the relevant figures and tables from the article by Rodriguez-Uribe et al. (2012).

Question 10

The authors studied eight cultivars, pictured in Figure 2 of their article. We will focus on the following: NuMex Sunset (A), Valencia (B), Orange Grande (C), Oriole (D), NewMex Garnet (E), and Canary (H).

NewMex Garnet has the wild-type, red fruit color phenotype. How would you define the respective fruit colors of the other five cultivars of interest?

Question 11

Examine Table 1 from Rodriguez-Uribe and colleagues' paper, focusing only on mature (ripe) fruits.

i. What pigments are present in high amounts, in low amount, or absent in the yellow and red cultivars, respectively? Do the data match either of your hypotheses' predictions?

ii. What do you notice about the pigments present in high amounts, in low amount, or absent in the orange cultivars of interest? Are there any patterns?

iii. Do any of these orange cultivars match the predictions made by one of your hypotheses?

Question 12

Examine Figure 4 from Rodriguez-Uribe and colleagues' paper, focusing only on mature (ripe) fruits, denoted "2." The picture shows the results of a northern blot.

i. Do any of the yellow or orange cultivars of interest have RNAs that are different in size from those of NuMex Garnet? If so, which ones?

ii. In terms of the relevant RNAs size, do any of these cultivars match the predictions made by one of your hypotheses? If so, which ones?

iii. *Bonus:* The data for the *LcyB* RNA shows two distinct bands. What could they represent?

iv. *Bonus:* What is the 28S RNA? Why did the authors include it in the northern blot?

Question 13

Examine Table 2 from Rodriguez-Uribe and colleagues' paper, focusing only on mature (ripe) fruits. This table reports the results of their qRTR-PCR experiments.

i. What RNAs are present in high amounts, in low amounts, or absent in the yellow and red cultivars, respectively? Do the data match either of your hypotheses' predictions? If so, which ones?

ii. What do you notice about the RNAs present in low amounts, in high amount, or absent in the orange cultivars of interest? Are there any patterns?

iii. Do any of the orange cultivars match the predictions made by one of your hypotheses?

Question 14

The authors report DNA sequences of three critical regions of the *Ccs* gene promoter in several cultivars to the wild-type reference sequence (GenBank Y14165). Examine the data reported in Table 3.

i. What cultivars have a *Ccs* promoter sequence different from the wild-type one? What are these differences?

ii. In some cases, two different bases are reported for a position (e.g., T or C at position –711 for Valencia). What do you think this means about the *Ccs* genotype of those cultivars?

iii. Do any of these findings match with what is predicted by one of your hypotheses? If so, in what way?

iv. Review the qRT-PCR data for *Ccs* RNA in our cultivars of interest. Could the difference in promoter sequence explain any of them? If so, how?

Question 15

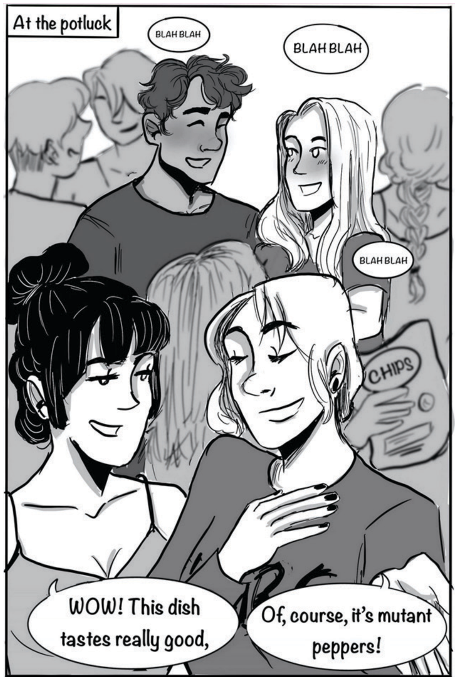
Consider these additional pieces of information provided in the article by Rodriguez-Uribe et al. (2012):

- Previous work by Guzman et al. (2010) has shown that the coding regions of the *Psy*, *LcyB, CrtZ-2,* and *Ccs* genes are wild-type (no mutations) in all the orange cultivars except for Fogo, which has mutant alleles of *Ccs* (nonsense mutation).
- A protein corresponding in size to CCS was detected in NuMex Sunset, Dove and Valencia, but not in Fogo, Orange Grande, Oriole, or Canary.

With your group, review all the information you have about NewMex Sunset and another orange cultivar of your choice (you may also want to review the data for NuMex Garnet and Canary, as comparisons).

Then, propose a comprehensive model that explains how your two orange cultivars come to produce orange fruits.

Your model should be consistent with the data discussed in your group and include a step-by-step mechanism (e.g., "a mutation in the promoter of gene X results in reduced production of transcript X, leading to reduced levels of enzyme X, so step Y in the carotenoid biosynthesis pathway occurs inefficiently…," etc.).



After your hard work, you and your group can finally join the potluck—and the conversation about mutant peppers!

In one sentence, how would you answer the question:

What causes pepper plants to produce orange fruits?

If you feel so inclined, illustrate a scene that incorporates your answer, to conclude the story presented in this case study.

References

Guzman, I., S. Hamby, J. Romero, P.W. Bosland, & M.A. O'Connell. (2010). Variability of carotenoid biosynthesis in orange colored *Capsicum* spp. *Plant Science* 179(1–2): 49–59. <https://doi.org/10.1016/j.plantsci.2010.04.014>

Rodriguez-Uribe, L., I. Guzman, W. Rajapakse, R.D. Richins, & M.A. O'Connell. (2012). Carotenoid accumulation in orange-pigmented *Capsicum annum* fruit, regulated at multiple levels. *Journal of Experimental Botany* 63(1): 517–26. <https://doi.org/10.1093/jxb/err302>