

Stalking the Genetic Basis of a Trait

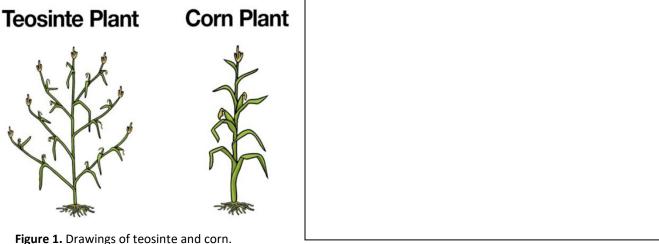
INTRODUCTION

The BioInteractive short film *Popped Secret: The Mysterious Origin of Corn* describes how the evolution of corn was mostly a mystery until George Beadle proposed a bold new hypothesis in 1939: corn evolved through artificial selection from a central American grass called teosinte. Researcher John Doebley continued Beadle's work and demonstrated, at the molecular level, how the differences between corn and teosinte were largely the result of just a handful of genes. One of these genes studied by Doebley is called teosinte branched1, or tb1.

In this activity, you will participate in a trail of discovery in which you will propose experiments, analyze data, and build an explanation for how changes in DNA cause corn to have a different phenotype than its ancestor, teosinte.

PART 1: How does the tb1 gene relate to corn having one main stalk?

1. Examine Figure 1, which shows drawings of corn and teosinte. List at least three differences in phenotype between the two plants.



2. The text below is part of the transcript from the film *Popped Secret: The Mysterious Origin of Corn*. Read the text and then write a short summary in your own words of the experiment Dr. Doebley performed and what the results indicate about the genetic control of branching in corn and teosinte.

Narrator: So, another really obvious difference between teosinte and corn is that teosinte produces dozens of these little tiny ears on a plant that branches a lot, and corn just produces a couple of ears on a plant that hardly branches at all. So what's going on there?

Dr. Doebley: There is one gene that we've identified that plays a central role in that process. And you call it the branching gene.

Narrator: Dr. Doebley explained how putting the teosinte version of the branching gene into maize made the maize plants more branched, like teosinte. And putting the maize version of the gene into teosinte made the teosinte plants less branched.

3. One of the genes studied by Dr. Doebley is called *teosinte branched1*, or *tb1*. This gene codes for a protein called TB1. Expression of the tb1 gene affects the number of the plant's side branches. In this activity, you

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will take on the role of a researcher whose goal is to determine the effect that specific changes to the *tb1* gene have on the phenotype of teosinte and corn plants to answer the overarching question: How did changes to the *tb1* gene influence the phenotype of teosinte and corn plants?

Before beginning your research, you should first review how genes affect proteins and ultimately phenotypes. Recall that the flow of genetic information starts with DNA, which is transcribed into RNA, which is then translated into protein. Summarize your understanding by making a sketch of the flow of genetic information in the box labeled Figure 2 and include a caption.

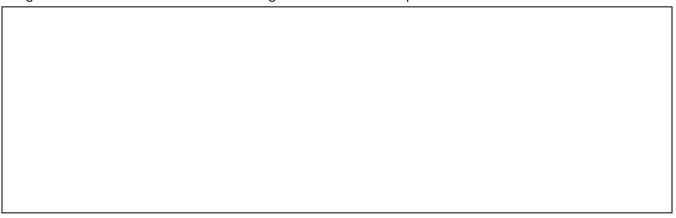


Figure 2. Caption:

4. There is a lot more to genes than just the region that codes for a protein. Figure 3 illustrates the basic structure of a gene that is being actively transcribed. Review your understanding of gene transcription and DNA regions that can affect gene transcription by labeling the boxes in Figure 3 with the letters a—e, which correspond to the terms listed below. For example, write the letter "a" in the box pointing at the coding region of a gene.

a. Protein-coding region

d. mRNA

b. Regulatory region

e. RNA polymerase

c. Promoter

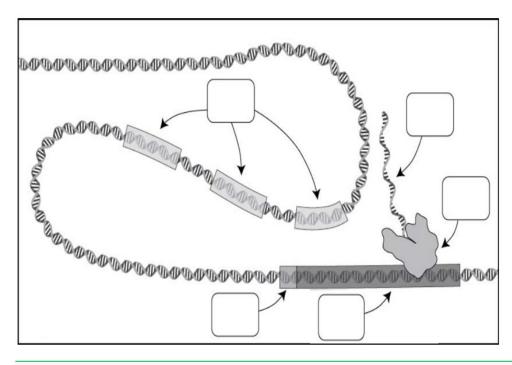


Figure 3. An actively transcribed gene and regions of DNA that affect the gene.

- 5. Keeping in mind the information illustrated in Figures 2 and 3, discuss some different ways that a mutation could affect the expression of the *tb1* gene. Record your ideas.
 - How could mutations in the protein-coding region affect tb1 expression?
 - How could mutations in regulatory regions affect tb1 expression?
 - How could you design an experiment to determine whether the mutation that led to corn's shape is in the coding region or the regulatory region?

PART 2: Analyze Investigations of the tb1 Gene

Researchers have conducted a number of experiments to decipher how specific changes in *tb1* led to changes in the branching pattern in corn to answer the overarching question:

How did changes to the tb1 gene influence the phenotype of teosinte and corn plants?

In Part 2, you will review the questions guiding different aspects of the research and analyze and interpret the results by answering the discussion questions. Make sure to include the evidence that supports your answer.

RESEARCH QUESTION A: Is the TB1 protein different in corn and teosinte?

Experiment A: Compare the amino acids in teosinte TB1 and corn TB1.

Result A: 120 of the amino acids from the TB1 protein in corn and teosinte are shown below.

ICTAGGMRDRRMRLSLDVARKFFALQDMLGFDKASKTVQWLLNTSKSAIQEIMADDASSE TB1 amino acids corn ICTAGGMRDRRMRLSLDVARKFFALQDMLGFDKASKTVQWLLNTSKSAIQEIMADDASSE TB1 amino acids teosinte

CVEDGSSSLSVDGKHNPAEQLGGGGDQKPKGNCRGEGKKPAKASKAAATPKPPRKSANNA TB1 amino acids corn CVEDGSSSLSVDGKHNPAEQLGGGGDQKPKGNCRGEGKKPAKASKAAATPKPPRKSANNA TB1 amino acids teosinte

Figure 4. A comparison of 120 amino acids from the TB1 protein in corn and teosinte. Different amino acids are represented by letters of the alphabet.

Discussion questions:

- 1. How would answering the research question "Is the TB1 protein different in corn and teosinte?" help you determine the role specific changes to the tb1 gene play in affecting the phenotype of teosinte and corn plants?
- 2. Carefully examine the amino acids found in the TB1 protein from the two species. What do you observe?
- 3. Do you predict that the two proteins function differently? Why or why not?
- 4. Based on the amino acid data, what do you predict about the similarity in the DNA sequences in the coding region of the gene in the two species?
- 5. Do the data from the amino acids making up TB1 support the claim that teosinte and corn are closely related? Why or why not?

RESEARCH QUESTION B: Is the coding region of the *tb1* gene sequence different in corn and teosinte?

Experiment B: Compare the DNA sequences of the teosinte *tb1* and corn *tb1* coding regions.

Result B: 120 nucleotides from the *tb1* gene in corn and teosinte are shown below.

GGAGGAGGAGGAGATCAGAAGCCCAAGGGTAATTGCCGCGGCGAGGGGAAGAAGCCGGCC	tb1 sequence from corn tb1 sequence from teosinte
AAGGCAAGTAAGGCGGCCACCCCGAAGCCGCCAAGAAAATCGGCCAATAACGCACAC	tb1 sequence from corn
AAGGCAAGTAAGGCGGCCGCCCCGAAGCCGCCAAGAAAATCGGCCAATAACGCACAC	tb1 sequence from teosinte

Figure 5. A comparison of 120 nucleotides from the tb1 gene in corn and teosinte.

Discussion questions:

- 6. How would answering the research question "Is the coding region of the *tb1* gene sequence different in corn and teosinte?" help you determine the role specific changes to the *tb1* gene play in affecting the phenotype of teosinte and corn plants?
- 7. Carefully examine the nucleotides found in the tb1 gene from the two species. What do you observe?
- 8. Recall that Dr. Doebley put the teosinte version of the branching gene *tb1* into corn and it caused the corn plants to have more branches. He also put the corn version of the gene into teosinte, which caused the teosinte plants to have fewer branches. Do the data for Result B support the claim that the difference in branching in the two species is caused by differences in the coding region of the *tb1* gene? Why or why not?
- 9. Do the data from the nucleotides in the *tb1* gene from the two species support the claim that teosinte and corn are closely related? Why or why not?

RESEARCH QUESTION C: Is there a difference in the regulatory region for the *tb1* gene in corn and teosinte?

Experiment C: Compare the DNA sequences of the regulatory regions of the teosinte *tb1* and corn *tb1* genes.

Results C: The sequence of the regulatory region of the *tb1* gene differs between corn and teosinte.

- There are differences in the upstream regulatory region of the gene.
- The regulatory region for the corn *tb1* gene has an insertion of 4,900 nucleotides (called *Hopscotch*) compared to the regulatory region for the teosinte *tb1* gene.
- The insertion is estimated to have occurred about 28,000 years ago.



Figure 6. Illustration showing the regulatory region of the *tb1* gene in corn and teosinte.

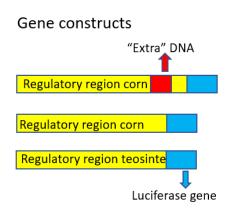
Discussion questions:

- 10. How would answering the research question "Is there a difference in the regulatory region for the *tb1* gene in corn and teosinte?" help you determine the role specific changes to the *tb1* gene play in affecting the phenotype of teosinte and corn plants?
- 11. What are possible functions of the regulatory region of a gene?
- 12. What are some ways that mutations in a regulatory region can affect the amount of protein product made from a gene? What ideas do you have for how you could further test this question?
- 13. Do the data for Result C support the claim that the difference in branching in the two species is caused by differences in the regulatory region of the *tb1* gene? Why or why not?
- 14. What is the significance of the date at which the Hopscotch insertion is thought to have occurred?

RESEARCH QUESTION D: Does the *Hopscotch* insertion in the corn *tb1* regulatory region affect the expression of the gene?

Experiment D: To answer this question, researchers made DNA regions that mimicked the regulatory region of the *tb1* gene from both corn and teosinte. They made the corn regulatory region both with and without the large DNA insertion (*Hopscotch*). They then created larger DNA regions that also contained the gene that gives off light when it is expressed (luciferase) and other elements needed for gene expression. Researchers put these three different gene constructs into the leaf cells of corn. They measured the amount of light given off for the three different types of regulatory regions. The amount of light is a measure of the degree to which the gene is expressed.

Results D:



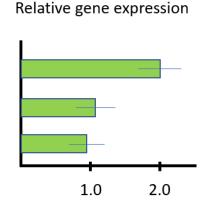


Figure 7. Gene constructs and the resulting gene expression levels. Error bars represent 95% confidence intervals (margins of error) on the means for each construct. Non-overlapping error bars suggest that the difference between means may be significant. Formal statistical tests confirmed that the difference in expression for the regulatory region from corn with the "extra" DNA is statistically different than the levels of expression from the other two constructs.

Discussion question:

15. Use the data in Figure 7 to answer the research question "Does the *Hopscotch* insertion in the corn *tb1* regulatory region affect the expression of a gene?"

RESEARCH QUESTION E: What is the function of the TB1 protein?

Experiment E: See whether the TB1 amino acid sequence is similar to sequences from proteins of known function.

Result E: The TB1 protein is a member of a family of proteins that bind to DNA. More specifically, the protein is a transcription factor.

Discussion questions:

- 16. Does the TB1 protein determine the phenotype directly, or does it work indirectly by influencing the expression of other genes? Explain.
- 17. For a plant to make a branch, many cellular processes must take place. For example, the plant needs to make new cells. With this in mind, brainstorm ideas for possible functions for genes that are regulated by the *tb1* gene.

Synthesis:

Reflecting on the original overarching question, "How did changes to the *tb1* gene influence the phenotype of teosinte and corn plants?", construct an explanation based on evidence for how the structure of DNA in a regulatory region determines the expression of proteins that can affect the phenotype. Your explanation should include a claim, evidence that supports the claim, and the scientific reasoning or logic that links the evidence to the claim.