

## Stalking the Genetic Basis of a Trait

### OVERVIEW

This activity supplements the BioInteractive short film [Popped Secret: The Mysterious Origin of Corn](#). It focuses on the *tb1* gene, whose expression is related to phenotypic changes associated with the evolution of corn. Students analyze experimental results about the expression of the *tb1* gene and formulate an explanation as to how a specific difference in the teosinte version of the gene explains its more cornlike phenotype of less branching.

### KEY CONCEPTS

- In some cases, large phenotypic differences between species are caused by a small number of genes.
- Some phenotypic differences are caused by mutations in regulatory regions that affect the expression of genes.
- Similarities in amino acid and nucleotide sequences can support claims of common ancestry.

### STUDENT LEARNING TARGETS

- Analyze amino acid and DNA sequences to determine that differences in the coding region of *tb1* are not the cause of phenotypic differences between corn and teosinte but are evidence for a close relationship between the two.
- Construct an explanation based on evidence for how the structure of DNA in a regulatory region determines the expression of proteins that further affect the expression of other genes.

### CURRICULUM CONNECTIONS

Standards	Curriculum Connections
NGSS (2013)	HS-LS1-1, HS-LS3-1, HS-LS4-1; SEP1, SEP4, SEP6
AP Bio (2015)	1.A.4, 3.A.1, 3.A.4, 3.B.1, 3.C.1; SP3, SP5, SP6
IB Bio (2016)	3.1, 3.4, 7.2
AP Env Sci (2012)	II.C
IB Env Systems and Societies (2017)	3.2
Common Core (2010)	ELA.RST.9–12.7, ELA.WHST.9–12.1
Vision and Change (2009)	CC1, CC3; DP1

### KEY TERMS

corn, gene expression, gene regulation, *Hopscotch* insertion, maize, mutation, phenotype, regulatory region, *tb1* gene, teosinte

### TIME REQUIREMENTS

- One 50-minute class period if students have already viewed the film prior to the activity.
- Two 50-minute class periods for viewing the film in class and doing the open-inquiry option for Part 2 described in the "Teaching Tips."

### SUGGESTED AUDIENCE

- High School: Biology (Honors, AP/IB)
- College: Introductory Biology

### PRIOR KNOWLEDGE

Students should be familiar with:

- basic concepts related to the flow of genetic information, gene structure, and gene expression
- the role of promoters and regulatory regions
- sequence alignments and how they work

## BACKGROUND

For a full set of background information about content covered in the *Popped Secret* film, see the [Educator Materials](#) that accompanies the film. Below is the background section that is relevant to this activity.

### Genetic Evidence of Maize Domestication

As described in the short film, George Beadle conducted a massive experiment in the 1970s. He crossed teosinte with maize to produce F1 hybrids and then crossed the F1s to produce an F2 generation. Based on classical genetics and making some reasonable assumptions, Dr. Beadle developed a mathematical model for predicting how many genes differed between the plants based on the frequency of F2 offspring that looked like either parent.

He planted 50,000 F2s and once grown, he found that about 100 plants had an ear that looked like maize and 100 plants had an ear that looked like teosinte. From these data and his mathematical model, he concluded that four or five genes were responsible for the differences between teosinte and maize, supporting his claim that maize could have been domesticated rapidly from teosinte. In the 1990s, modern molecular genetics tools allowed geneticists John Doebley and Adrian Stec to reexamine Dr. Beadle's hypothesis. Dr. Doebley and Dr. Stec identified five genetic regions, which correspond to about five genes or blocks of genes, that together account for most of the variation between maize and teosinte, further supporting Dr. Beadle's hypothesis.

How could so few genes cause the dramatic differences between these plants? Scientists found that at least two of the genes are **regulatory genes** that code for proteins that turn other genes off or on. Thus, having a different version of a single regulatory gene can affect the expression of hundreds of others.

Dr. Doebley transplanted the *tb1* (*teosinte branched1*) gene across species. When the teosinte *tb1* gene was introduced into maize, the maize became more branched and developed many ears. When the maize *tb1* gene was transplanted into teosinte, the teosinte became less branched and had fewer, larger seed-containing ears. In this activity, students explore how changes to this one gene can cause such large phenotypic differences.

Since domestication, humans have continued to change maize through artificial selection; one recent study showed that 1,200 genes of the maize genome have been affected in some way. Changes in these genes resulted in more subtle differences in the evolution of early maize plants to present-day maize; for example, present-day maize has much bigger ears and sweeter seeds.

### Assays of Gene Expression

The experiments summarized in this activity (Studer et al. 2011) used a common gene expression reporter construct to determine the extent to which regulatory regions with and without a large DNA insert affect gene expression levels. The reporter construct included the minimal promoter from a virus (the cauliflower mosaic virus), the luciferase open reading frame (coding region) from fireflies, and a terminator region from an enzyme called nopaline synthase. Aside from the large *Hopscotch* insertion, the regulatory region for *tb1* differs between teosinte and corn by only one nucleotide.

One limitation of the experiments reported in this study is that direct expression of the *tb1* gene was not assayed. A later set of experiments (Studer et al. 2017) provided additional data using quantitative PCR to confirm that

indeed, the *tb1* allele from corn results in an approximately twofold higher expression of the *tb1* gene compared to the teosinte allele.

### Selection on a Gene Network

In the film, Dr. Doebley discusses how changes to regulatory genes can have a large effect on phenotypes because they influence the expression of many other genes. Further work by Dr. Doebley and colleagues (Studer et al. 2017) has identified a number of other genes that lie downstream of *tb1* in a gene network that affect the architecture of plant branches and the structure of plant inflorescences, including cell cycle genes and many other transcription factors. In fact, it appears that the gene network that changed in corn was a preexisting developmental gene network associated with a shade-avoidance phenotype.

### TEACHING TIPS

- Consider having students work with a partner to complete the activity.
- An [interactive version of the film with embedded quiz questions](#) can be used if students need to complete viewing as homework.
- Reference/use the [“DNA Transcription \(Advanced Detail\)”](#) animation with students in Part 1, Question 4 of the student handout to show RNA polymerase binding to a promoter.
- For students unfamiliar with sequence alignments, examples and an explanation of how they work will need to precede Part 2 of the activity. Sequence alignment is discussed in the [Creating Phylogenetic Trees from DNA Sequences](#) Click & Learn.
- Students might need an explanation of how corn reproduces.
- For more information about gene switches, see BioInteractive resources about the role of gene switches in the evolution of sticklebacks, such as the short film [Evolving Switches, Evolving Bodies](#) or the activity [“Modeling the Regulatory Switches of the Pitx1 Gene in Stickleback Fish.”](#)
- For a more inquiry-based activity, students can design their own experiments to address the investigative research questions in Part 2. To do this, print and distribute only Part 1 of the student handout. After students complete Part 1, post or distribute the five research questions for students to develop ideas for how they would proceed with designing and setting up an experiment to investigate the research questions. After this is complete, distribute Part 2 of the student handout.

### PROCEDURE

1. Distribute one copy of the student handout to each student. If students will work in pairs, have them start working together from the beginning of the activity.
2. Instruct students to read the introduction and list their ideas about mutations and the expression of the *tb1* gene as directed in questions 1–5.
3. After students have completed their tasks, conduct a class discussion that summarizes the students’ ideas.
4. If conducting an open-inquiry lesson, the five research questions to distribute to students after Part 1 are:
  - A. Is the TB1 protein different in corn and teosinte?
  - B. Is the coding region of the *tb1* gene sequence different in corn and teosinte?
  - C. Is there a difference in the regulatory region of the *tb1* gene in corn and teosinte?
  - D. Does the *Hopscotch* insertion in the corn *tb1* regulatory region affect the expression of the gene?
  - E. What is the function of the TB1 protein?
5. For Part 2, instruct the students to first read the research questions, experiments, and results, and then discuss and answer the discussion questions. Decide whether you want students to complete all of Part 2 or if

they should complete the discussion questions for one research question and follow up with a class discussion. The experiment associated with research question D (Does the *Hopscotch* insertion in the corn *tb1* regulatory region affect the expression of the gene?) is likely to present the greatest challenge to students.

- After students have analyzed all the research questions, conduct a class discussion to summarize answers to the discussion questions. Students should be able to briefly describe how a mutation in the regulatory region of the *tb1* gene led to teosinte acquiring a more corn-like phenotype.

## ANSWER KEY

### PART 1

- List at least three differences in phenotype between the two plants.

*This question is meant to serve as a quick review of some of the content from the film. Students will likely list differences in the number of branches, the size and number of ears, and the size of the leaves.*

- Read the text and then write a short summary of the experiment Dr. Doebley performed and what the results indicate about the genetic control of branching in corn and teosinte.

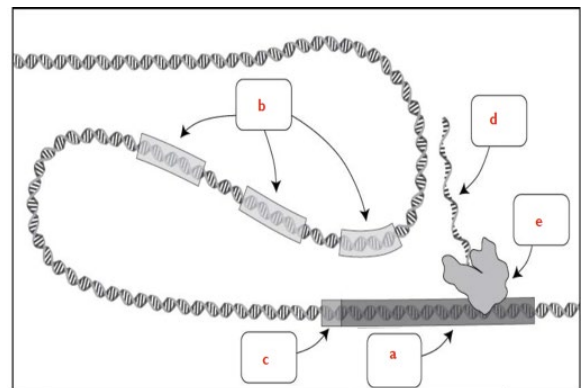
*Student summaries should include an explanation of how Dr. Doebley moved the corn allele for the “branching gene” into teosinte, which caused it to have fewer branches. Putting the teosinte allele for the same gene into corn caused the corn to have more branches. These data suggest that the branching gene plays a large role in the genetic control of this trait.*

- Summarize your understanding by making a sketch of the flow of genetic information in the box labeled Figure 2 and include a caption.

*This question is designed as a formative assessment of students’ understanding of the central dogma, which states that DNA codes for RNA, which codes for a protein. Proteins are the molecules that ultimately shape the phenotype of an organism. Look to see that their sketches reflect this flow of information and that their caption summarizes the process in their own words.*

- Review your understanding of gene transcription and DNA regions that can affect gene transcription by labelling the boxes in Figure 3 with the letters a–e.

*[See figure to the right.]*



- 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.

- How could mutations in the protein-coding region affect *tb1* expression?

*Students may respond that mutations in the coding region can:*

- alter the amino acid sequence of the protein, rendering it less effective (In rare cases, the mutation may help the protein work more effectively.)*
- cause the protein to terminate prematurely, effectively eliminating the protein*

- How could mutations in regulatory regions affect *tb1* expression?

*Students may respond that mutations in the regulatory regions can either increase or decrease the expression of the gene.*

- How could you design a study to determine whether the mutation that led to corn's shape is in the coding region or the regulatory region?

*Be open to a range of student answers. This question is meant to build in a "need to know" for Part 2 of this activity. The series of experiments students explore in Part 2 provide the answer to this question.*

## PART 2

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

#### 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?

*By answering this research question, researchers gain insight into whether the phenotypic changes might be caused by structural changes in the TB1 protein.*

2. Carefully examine the amino acids found in the TB1 protein from the two species. What do you observe?  
*There are no amino acid differences in the 120 amino acids shown from the TB1 proteins from corn and teosinte.*

3. Do you predict that the two proteins function differently? Why or why not?

*Because the amino acid sequences of the two proteins are the same, it would be expected that the proteins would function in the same way.*

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?

*This question is designed to help students think critically about the genetic code. Many are likely to answer that because the amino acids are the same, the DNA coding for the amino acids will be the same. However, just because the amino acid sequences of the two proteins are the same, it doesn't necessarily follow that the DNA sequences of the coding regions are identical. The genetic code is referred to as being degenerate. This means that the same amino acid can be specified by more than one codon.*

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?

*The similarity in amino acids in the TB1 protein support the claim that the two species are closely related. For advanced students, you may want to highlight that there are alternative explanations for the similarity in amino acids, including high evolutionary conservation. For example, some proteins have similar sequences across a large variety of organisms because there is strong selection against any changes at the amino acid level. To further support the claim of common ancestry, sequences from a broader range of organisms are needed.*

### RESEARCH QUESTION B: Is the coding region of the *tb1* gene sequence different 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?

*If differences in the *tb1* gene were discovered between the two species, researchers could do further experiments to see whether the differences in DNA sequence were linked to changes in phenotypes. Students may struggle with this*

question, so be prepared to help give feedback and challenge questions to support them as needed. For example, you may ask students to revisit the structure of a gene in Figure 3 and ask how exploration of the sequences for the coding region could help researchers evaluate the role of changes to different regions associated with a gene.

7. Carefully examine the nucleotides found in the *tb1* gene from the two species. What do you observe?

*The nucleotide sequences shown for the *tb1* gene in the two species are identical.*

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?

*There are no differences in the *tb1* gene or *TB1* protein that would explain the results that Dr. Doebley obtained when he switched the *tb1* gene regions between the two species. This suggests that the difference may lie in other regions besides the protein-coding region of the gene.*

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?

*The similarity in nucleotides in the *tb1* gene further support the claim that the two species are closely related.*

**RESEARCH QUESTION C:** Is there a difference in the regulatory region for 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?

*If differences in the regulatory region of the *tb1* gene were discovered between the two species, researchers could do further experiments to see whether the differences in DNA sequence were linked to changes in phenotypes. Most random mutations that occur over time would not have a phenotypic effect.*

11. What are possible functions of the regulatory region of a gene?

*Regulatory regions of a gene can increase or decrease gene expression.*

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?

*In simple terms, mutations can increase, decrease, or leave gene expression unaffected, depending on the type and location of the mutation. [Ideas for how to test this may vary.]*

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?

*This question is designed to help students think critically about what the data do and do not say. In this case, the data simply shows that there is a difference in the regulatory region between the two species for the *tb1* gene, but at this point, the data do not say anything about whether the differences in the regulatory region affect gene expression or branching in the two species.*

14. What is the significance of the date at which the *Hopscotch* insertion is thought to have occurred?

*The Hopscotch insertion is thought to have occurred approximately 28,000 years ago. As mentioned in the film, the domestication of corn is thought to have begun about 10,000 years ago. This means that plants with the less*

*branched phenotype characteristic of the *tb1* gene mutation could have been available to the ancient people who began the domestication process. In genetics language, it means that the mutation that led to decreased branching came from standing genetic variation as opposed to new variation, supporting the idea that new mutations do not come about because organisms want or need them to change.*

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

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?”

*The data support the claim that the Hopscotch insertion increases the expression of a gene. The construct that included the Hopscotch insertion had twice as much gene expression compared to both of the constructs that lacked the Hopscotch insertion.*

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

Discussion questions:

16. Does the TB1 protein determine the phenotype directly, or does it work indirectly by influencing the expression of other genes? Explain.

*The TB1 protein is identified as a transcription factor. Transcription factors bind to the promoter or regulatory regions of multiple genes, affecting their levels of expression.*

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.

*Be open to logical suggestions students make for possible gene functions. For example, students may recognize that cells need to be made to form a branch, so genes that affect the cell cycle may be regulated. As described in the background section, some of the genes downstream of *tb1* in a gene network have now been identified and include cell cycle genes.*

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.

*Claim: The Hopscotch insertion into a regulatory region of the *tb1* gene in corn increases the expression of the gene and causes corn to have fewer side branches.*

*Evidence:*

- *Sequence comparisons of the *tb1* regulatory region in teosinte and corn show that corn has a large insertion called Hopscotch.*
- *Gene expression studies show that when the Hopscotch region is included in the regulatory region, gene expression increases.*

- *When Dr. Doebley moved the corn allele for **tb1** into teosinte, the resulting plants had fewer branches. When he put the teosinte allele for the same gene into corn, it caused the corn to have more branches.*

*Reasoning: If a regulatory region for **tb1** is involved in the difference in phenotype between corn and teosinte, there should be a difference in the DNA sequence for the regulatory region in the two species. This is the case, as reported in Result C. If the insertion affects the expression of the **TB1** protein, then including the insertion in a gene expression reporter system should show more gene expression activity. This is also the case, as reported in Result D. The prior experiments of Dr. Doebley showed that **tb1** is related to branching phenotypes.*

## REFERENCES

Studer, Anthony, Qiong Zhao, Jeffrey Ross-Ibarra, and John Doebley. "Identification of a functional transposon insertion in the maize domestication gene **tb1**." *Nature Genetics* 43, 11, (2011): 1160–1163. <https://doi.org/10.1038/ng.942>.

Studer, Anthony J., Huai Wang, and John F. Doebley. "Selection during maize domestication targeted a gene network controlling plant and inflorescence architecture." *Genetics* 207, 2 (2017): 755–765. <https://doi.org/10.1534/genetics.117.300071>.

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