CHROMOSOME SMASH

Next Generation Science Standards
HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instruction for characteristic traits passed from parents to offspring.

Subjects
Introduction to Agriculture
Life Science (Biology, Botany)
Phylogeny
Plant Science

Grade Level
9th-12th

Time Required
45 minutes per activity

Overview
Chromosome number does not necessarily translate into organism complexity. However, chromosome number does give each organism its unique characteristics. Having multiple sets of chromosomes is a benefit to plants and scientists can utilize these distinctive benefits to make better, more genetically diverse plants. Using a computer simulation, students will explore the changes that occur in the cell's nucleus to prepare for cell division.

Materials
- Computer with internet access
- Lead rope made of colored twisted strands (1 lead rope per group of 4-6 students) find at farm store
- Tape measure

Objectives
1. Students will be able to classify cells taken from the tip of an onion root into appropriate phases in the cell cycle.
2. Students will be able to predict how much time a dividing cell spends in each phase.

Vocabulary
Chromosome: a threadlike structure of nucleic acids and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.

Histone: Any of a group of basic proteins found in chromatin.

Mitosis: A type of cell division that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus, typical of ordinary tissue growth.

Supercoil: A double helix (as of DNA) that has undergone additional twisting in the same direction as or in the opposite direction from the turns in the original helix.

CHROMOSOME SMASH

Background Information
The following websites are recommended to those wanting more information about the Wheat Genome Project and the Cell Cycle

The Biology Project: Cell Biology--University of Arizona
http://www.biology.arizona.edu/cell_bio/cell_bio.html

International Wheat Genome Sequencing Consortium
www.wheatgenome.org

Preparation
The teacher should assign lab groups of 4-6 students for the chromosome modeling activity. If individual student computers are not available, the teacher may need to reserve a computer lab.

Instructional Format
Students should begin by taking the lesson pretest.

Modeling the making of the chromosome
Student groups (4-6 students in each group) will twist lead ropes to model how chromosomes are formed from helical DNA.

First, have two students fold a lead rope in half and twist to make a helix. Explain to the students that the rope represents double stranded DNA.

Ask each student group to measure the twisted rope and record its length in their lab notebook or on the provided handout.

Next, have one of the students continue to twist one of the ends of the rope to model supercoiling. The rope will naturally bunch forming a supercoil.

Students should grasp the rope to hold the supercoil in place. (Note: The hands will represent the histone proteins found in chromosomes.) As more rope bunches are formed, then additional students will need to grasp the rope.

Continue to twist the rope until the entire rope has supercoiled. Each student group should measure the supercoiled rope and record its length in their lab notebook. Have students divide the length measurement by each other to determine the factor by which the rope decreased in size.

CHROMOSOME SMASH

Questions to ask students:

- What part of the model represents the double-stranded DNA?
- What part of the model represents the chromosome?
- What do the hands in the model represent?
- By how much did the supercoiled rope decrease in size? Since this is a model of chromosomes, speculate why would becoming more compact be beneficial for a cell?

The teacher should explain that a single fertilized cell will divide to form two cells. These two cells will each divide into two more cells. In time, millions of cells are produced. The division of nuclear material such that each new cell has the same nuclear code as the original cell is called mitosis. Mitosis occurs in four phases. There is an interphase between each mitosis.

The teacher should lead students in studying cells in reproduction by using the following procedure:

1. Log on to http://www.biology.arizona.edu/
2. Click on "cell biology"
3. Click on "cell cycle & mitosis"
4. On the left margin, you will be given an opportunity to go to DNA Basics. Click on this link.
5. Read each passage and take notes as necessary.
6. After each page, click on the box with the "next –>" through the screen giving information regarding "Mitosis".
7. Students should be silently reading the material about mitosis and making notes. You may choose to view the mitosis animation.
8. Return to the "Cell Biology" page
9. Click on "Online Onion Root Tips Activity" (upper left).
10. Review the information for each phase, and take notes as necessary.
11. Using the Student Sheet handout, proceed with the online lab. Notice that the chart assigned to draw is provided (Students will only complete the Number of Cells and Percent of Cells rows at this time).
12. At the end of the activity, complete the table and answer the questions on the attached page.

Conclusion Question

Using the provided handout of onion cell images, ask students to predict how many chromosomes they think are in a typical onion cell. How does that number differ from the beginning of mitosis to the end of mitosis? What makes it difficult to determine the exact number with the information you are given? (Note: onions have eight chromosomes.)

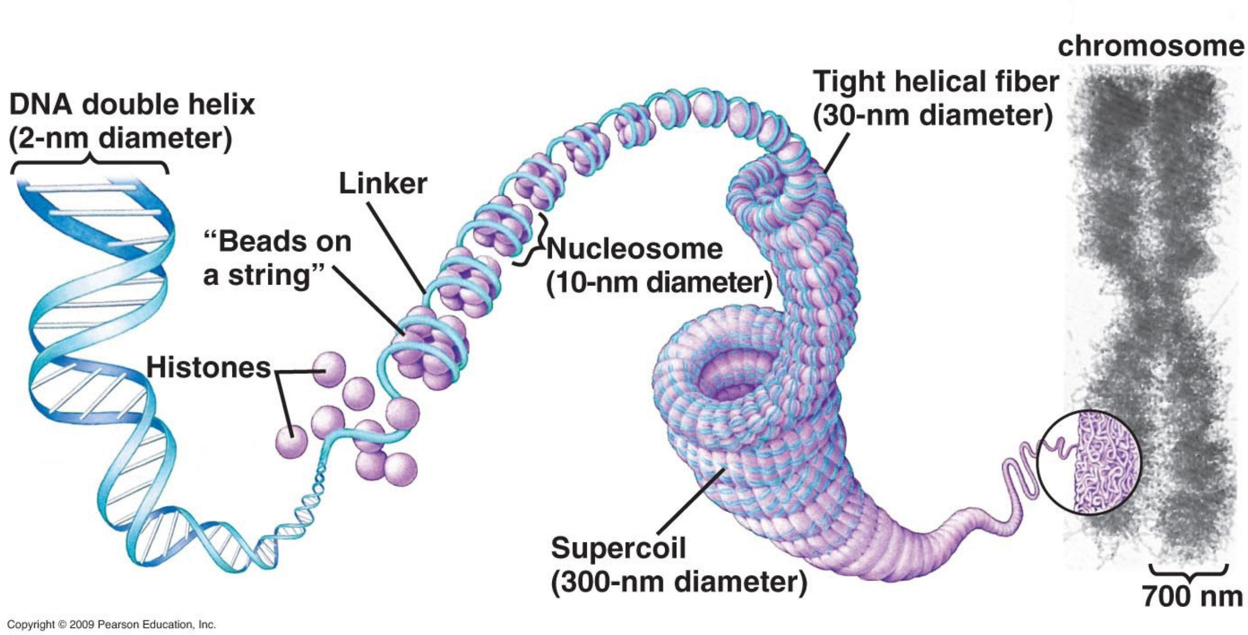
Student Handouts:

Lesson Pre-test

Directions: Before starting this set of lessons, indicate your agreement or disagreement with each of the statements by writing an A for agreement or a D for disagreement in the Before column. At the end of these lessons, you will revisit your answers and use evidence from the lessons to refute or support your initial thoughts.

Before	After	Statement
		All living things have only two sets of chromosomes.
		Multiple sets of chromosomes cause all organisms to have health problems and can lead to death.
		There is no useful purpose to know the exact DNA sequence of a living thing.
		Speciation by hybridization might be more common than scientists once thought.

Modeling the making of a chromosome



https://thecandidscientist.com/2015/06/02/dna-101/

Chromosome Structure	Length of lead ropes (meters)	Observations of rope appearance
At beginning as double stranded DNA		
At end as a supercoiled chromosome		

Questions to consider after the activity

What part of the model represents the double-stranded DNA?

What part of the model represents the chromosome?

What do the hands in the model represent?

By how much did the supercoiled rope decrease in size? Since this is a model of chromosomes, speculate why becoming more compact would be beneficial for a cell?

Name ___________________ Date _____________________ Period __________________

Online Onion Root Tips
Determining time spent in different phases of the cell cycle

Directions

1. Log on to http://www.biology.arizona.edu/
2. Click on "cell biology"
3. Click on "cell cycle & mitosis"
4. On the left margin, you will be given an opportunity to go to DNA Basics. Click on this link.
5. Read each passage and take notes as necessary.
6. After each page, click on the box with the "next –>" through the screen giving information regarding "Mitosis".
7. Students should be silently reading the material about mitosis and making notes. You may choose to view the mitosis animation.
8. Return to the "Cell Biology" page
9. Click on "Online Onion Root Tips Activity" (upper left).
10. Review the information for each phase, and take notes as necessary.
11. Using the Student Sheet handout, proceed with the online lab. Notice that the chart assigned to draw is provided (Students will only complete the Number of Cells and Percent of Cells rows at this time).
12. At the end of the activity, complete the table and answer the questions on the attached page.

Student Sheet: Online Onion Roots Activity

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
Number of Cells						36 minutes
Percent of Cells						100 minutes
Time spent in phase (minutes)						720 minutes

Assume that the number of cells in a phase indicates the time spent during mitosis of that phase. Time spent in a mitotic phase and in interphase can be calculated based on the percentage of cells found in each phase. If onion cells require 12 hours (720 min) to complete mitosis (from interphase to interphase), then:
time for a phase = percentage of cells in given phase (as a decimal) X 720 minutes

Calculate the time required for each phase of mitosis using your data and add this to your chart (heading would be "time for phase to occur"—don't forget units and decimals!) Show your work!

Which phase requires the longest time for completion?

What important changes are occurring in the nucleus and cell during the longest phase?

Why do you think so much time was spent in this phase?

Which phase requires the next longest time for completion?

Which phase requires the shortest time for completion?

EXAMPLE OF COMPLETED STUDENT SHEET: Online Onion Roots Activity

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
Number of Cells	(20)	(10)	(3)	(2)	(1)	36 minutes
Percent of Cells	(55.6%)	(27.8%)	(8.3%)	(5.6%)	(2.8%)	100 minutes
Time spent in phase (minutes)	(400)	(200)	(60)	(40)	(20)	720 minutes

Assume that the number of cells in a phase indicates the time spent during mitosis of that phase. Time spent in a mitotic phase and in interphase can be calculated based on the percentage of cells found in each phase. If onion cells require 12 hours (720 min) to complete mitosis (from interphase to interphase), then:
time for a phase = percentage of cells in given phase (as a decimal) X 720 minutes

Calculate the time required for each phase of mitosis using your data and add this to your chart (heading would be “time for phase to occur”—don’t forget units and decimals!) Show your work!

Which phase requires the longest time for completion? (interphase)

What important changes are occurring in the nucleus and cell during the longest phase? (metabolic activity and performing its duty as part of a tissue. The DNA replicates during interphase to prepare for mitosis)

Why do you think so much time was spent in this phase? (ensure the proper information is duplicated)

Which phase requires the next longest time for completion? (prophase)

Which phase requires the shortest time for completion? (telophase)

CHROMOSOME SMASH

Activity 2 - Using the Acetocarmine Protocol to View Onion Chromosomes

Next Generation Science Standards

HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instruction for characteristic traits passed from parents to offspring.

Materials

- Root tip from a green onion
- Test tube (13x100mm) fixative (Carnoy's solution 1:1 part glacial acetic acid and 3 parts 95% ethanol or absolute (100%) ethanol) (order or obtain from Chemistry lab)
- Water bath (50 degrees C)
- Heat source for water bath
- Watch glass
- Tweezers
- Beaker, pyrex (150mL, approximately)
- Scissors
- Eyedropper
- Razor blade
- Metal spatula
- Aceto-orcein stain (get from chemistry lab)
- Unsharpened pencil with unused eraser
- Microscope slides (four for each student pair)
- Cover slips (four for each student pair)
- Microscope (400X)
- Paper towels or chemwipes

Overview

By using specific staining protocols, chromosomes of plants can be viewed. Students will make a chromosome squash of onion root tips.

Objective

1. Students will prepare a slide of the chromosomes of onions and determine the number of chromosomes found in the typical onion cell.

Vocabulary

Chromatin: The material of which the chromosomes of organisms other than bacteria (i.e., eukaryotes) are composed. It consists of protein, RNA, and DNA.

CHROMOSOME SMASH

Background Information

The following websites are recommended to those wanting more information about the Wheat Genome Project and protocols for staining chromatin and chromatin.

International Wheat Genome Sequencing Consortium:
www.wheatgenome.org
Ice Water Pretreatment—Wheat Genetic Resource Center:
https://www.k-state.edu/wgrc/electronic_lab/pretreatment.html
Fixatives—Wheat Genetic Resource Center:
https://www.k-state.edu/wgrc/electronic_lab/fixatives.html
Acetocarmine Staining--Wheat Genetic Resource Center:
https://www.k-state.edu/wgrc/electronic_lab/aceto_stain.html
Complex Bread Wheat Genome Cracked--National Geographic:
http://news.nationalgeographic.com/news/2014/07/140717-wheat-genome-bread-crop-science/

Preparation

The teacher will need to ensure each lab group has a set of the reagents for the acetocarmine staining protocol.

Students should use the acetocarmine protocol found at the Wheat Genetic Resource Center Website.

The day before this lab students should cut off four root tips from their green onion, each approximately 1.5 to 2 cm long. Place the root tips into a glass vial that contains 2-ml of tap water that has been cooled to 1ºC in an ice-water bath. Add a small piece of paper with the description of the material, cap tube, and return to the ice-water bath. The root material should be fixed for 24 hours. Using this method is preferred because cell division is stopped while in metaphase during the 24 hour period rather than only 3 hours as with other pretreatment procedures.

Procedure

Students should work with the lab partner on this protocol.

Next, they should fill a test tube 3 cm full of fixative. Students should place the four pre treated root tips into the test tube of fixative and incubate at 50 degrees Celsius for six minutes.

Then students should pour the heated fixative and tips into a watch glass.

Next, using tweezers, students should remove the root tips, one at a time, from the watch glass and place each on in the middle of a microscope slide. Using scissors, students should cut all of the excess from the root tips except for 2 mm at the bottom of the root (note: the end that was not cut from the plant).

Procedures Continued

Students should place two drops of acetocarmine stain on top of the 2 mm root tip. The stain should soak into the root tip for two minutes. (Note: for best results, freshly fixed material can be stained for 30 minutes and then analyzed using the squash method.)

Using the metal spatula, students should squash the root tip, on each slide, pressing straight down so there is not to overlap in the cells.

Students should apply two more drops of stain upon the root tip and wait for another two minutes. Next, students should place a coverslip flat upon the root tip, making certain not to move the cover slip horizontally.

Using a pencil eraser, student should press the coverslip gently straight down without moving the coverslip. Students should soak up extra stain from the slide around the coverslip with a paper towel without moving the coverslip.

Finally, students should observe and record the steps of cell division under microscope (at 400x). Students should make a sketch of what they see in their lab notebooks and label the different structures they see on the chromosomes. (Note: students will only see individual chromosomes with particular banding patterns. They will only be able to label the chromosomes and the chromosome bands.)

Next, students should determine the length of the stages of mitosis by first locating the meristem region of the root tip. Students should start by looking at the squash using the 10x objective and find the region of active cell division.

They should then switch to the 40x objective and begin observations at the lower end of this region. Student pairs should take turns as observer and recorder. The observer should call out the stage of mitosis of each cell that they observe for the first and second slide. These should be tallied by the recorder in the results table. Roles should be switched for the second and third slide. Since prophase and prometaphase are difficult to distinguish, classify these cells as prophase. Only count as prophase, cells that contain distinctly visible chromosomes.

The observer should systematically scan the root tip moving upward and downward through a column of cells. The recorder should tally each cell in a stage of mitosis that is observed (note: students should take careful not to record the same cell twice in the tally). For each slide, twenty mitotic cells should be counted. Tally the stages of those 20 cells mitotic in the table provided. Students should calculate the percentage of cells at each stage in mitosis by using the data collected from all four slides.

Procedures Continued

The teacher should bring the class back together and have all student pairs pool their raw data with that of the class. The teacher should record the class totals in the table on the board. Student groups should calculate the percentage of cells in each stage using the combined class data. (Note: the relative time span of each stage is equivalent to the percentage of cells found in mitotic stage.) In their lab notebooks, they should compare and contrast their group's percentages with the entire class percentages.

(Note: Activity is modified from Babich, H., Segall, M.A. and Fox, K.D. (1997). The Allium Test–A Simple, Eukaryotic Genotoxicity Assay. The American Biology Teacher. 59, 580-583.)

Students should then find five cells that are at metaphase and count the number of chromosomes in each cell. Students should record the number they counted in student data table. (Note: Students should find that onions have eight chromosomes and are considered to be diploid.)

Conclusion Question

After comparing different parts of the onion root, where on the onion did students view the most actively dividing nuclei? Ask them to speculate why this location would have more active cell division.

CHROMOSOME SMASH

Activity 3 - Comparing Wheat with Onions

NOTE: Obtain seeds for both wheat and barley, germinate the seeds on moist paper towels to ensure root tips can be harvested to use for chromosome smash comparison Allow 5 days for germination and root growth ahead of this activity.

Materials

- Root tip from wheat seedlings with true leaves, one of three variety (hexaploid, tetraploidy, or triploidy) (Five days old)(Five days after germination and root tips can be seen)
- Root tip from barley seedlings with true leaves (Five days old)
- Test tube (13x100mm) fixative (Carnoy's solution: 1 part glacial acetic acid to 3 parts 95% ethanol or absolute (100%) ethanol) Obtain from Chemistry lab
- Water bath (50 degrees C)
- Thermometer
- Heat source for water bath
- Watch glass
- Tweezers
- Beaker, pyrex (150mL, approximately)
- Scissors
- Eyedropper
- Razor blade
- Metal spatula
- Acetocarmine stain(Obtain from Chemistry lab)
- Unsharpened pencil with unused eraser
- Microscope slides (four for each student pair)
- Cover slips (four for each student pair)
- Microscope (400X)
- Paper towels or chemwipes

Overview

Chromosome number does not necessarily translate into organismal complexity. However, chromosome number does give each organism its unique characteristics. Having multiple sets of chromosomes is a benefit to plants and scientists can utilize these distinctive benefits to make better, more genetically diverse plants. Through a chromosome squash, students will observe the differences among several varieties of wheat. They will compare the number of chromosomes in the wheat with the onion root tip slide that were made in the previous lesson.

Objective

The student will prepare a slide of the chromosomes of wheat root tip and compare the number present in wheat to those of barley and onions.

CHROMOSOME SMASH

Background Information

The following websites are recommended to those wanting more information about the Wheat Genome Project and protocols for staining chromatin and chromosomes.

International Wheat Genome Sequencing Consortium - www.wheatgenome.org
Acetocarmine Staining--Wheat Genetic Resource Center: https://www.k-state.edu/wgrc/electronic_lab/aceto_stain.html
Jointed Goatgrass Genetics--Washington State University Extension: http://smallgrains.wsu.edu/wp-content/uploads/2014/03/JGG-Genetics.pdf
Complex Bread Wheat Genome Cracked--National Geographic: https://news.nationalgeographic.com/news/2014/07/140717-wheat-genome-bread-crop-science/

Preparation

The teacher will need to ensure each lab group has a set of the reagents for the acetocarmine staining protocol. The teacher should assign each lab group one of the varieties of wheat. (Activity 2 should be done before Activity 3 in order to compare.)

Students should then find five cells that are at metaphase and count the number of chromosomes in each cell. Students should record the number they counted in student data table.
The teacher should ask students to list grains that are consumed.

Teachers should give the number of chromosomes for *Hordeum vulgare* (barley) (14), *Secale cereale* (rye) (14), *Triticum urartu* (red wild einkorn) (14), *Aegilops speltoides* (goatgrass) (28), *Triticum aestivum* (bread wheat) (42), and durum wheat (28). The teacher should ask whether there is a relationship between the chromosome number and the different species. Give them some time to determine any mathematical relationships. After giving students several minutes, the teacher should ask students what the specific number (prime factorization/greatest common multiplier) that the entire numeric set has in common. Explain that greatest common multiplier is the haploid number (N). (In this case, the haploid number is 7) The multiplier for haploid number is the ploidy (for example 2N=diploid, 3N=triploid, 4N=tetraploid, 6N=hexaploid).

Ask students to determine the ploidy for each of the grains, if the haploid number is 7. Then explain how scientists use the connection between the number of chromosomes within the Tricetacae tribe (ancestral grains, barley, rye) to develop the phylogenetic tree that they constructed several days ago. Further explain to the students that it is this understanding of the wheat genome will enable insight into the genetic diversity for wheat improvement. This will enable genomics-assisted breeding on a level needed for developing high-yielding, climate-resistant wheat varieties. Scientists are finding that the size of the genome complicates things. If you compare rice, barley, and wheat in terms of genome size, there is a 35-fold increase in genome size from rice to wheat.
Barley has a genome that is 10 times larger than rice. Wheat has a genome that is three times larger than barley. The wheat genome is five times the size of the human genome.

CHROMOSOME SMASH

Elaborate

The teacher should explain to students what chromosome numbers tell us and explain the benefits of ploidy to plants. It is important to mention that plants have large areas of the genome made up of repetitive elements (80%). Currently, only two percent of the entire genome represent genes that we can identify. The teacher should focus on how to increase genetic diversity *(Note: repetitive gene sequences are not diverse).*

Evaluate

Students should take the post-test.

Conclusion Question

How has ploidy helped plants like wheat become successful?

What problems might ploidy cause in the future?

Student Handouts:

Lesson Post-test

Directions: After completing this set of lessons, indicate your agreement or disagreement with each of the statements by writing an A for agreement or a D for disagreement in the After column. In the space under each statement, cite information from these lessons that supports or refutes your original ideas.

Before	After	Statement
		All living things have only two sets of chromosomes.
		Multiple sets of chromosomes cause all organisms to have health problems and can lead to death.
		There is no useful purpose to know the exact DNA sequence of a living thing.
		Speciation by hybridization might be more common than scientists once thought.