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Activity 11 – DNA Replication Fork

Purpose:
Where does brand new genetic information come from?

Purpose:
You will be taking some notes today on DNA replication and then you will attempt to duplicate a simulated strand of DNA in a computer model.

Interactive Notes - The Structure of DNA.

Question
The notes and observations you take below will help you explore the question, "What is the structure of DNA?"

Predict: Chromosomes are made of DNA molecules. What do you picture of the structure of a DNA molecule will look like?

_____

_____

_____

Notes and Observations

Our Question	My Notes
What are chromosomes?	
Where are chromosomes found?	
How many chromosomes do humans have?	
How many chromosomes were in the karyotype for the **body cells** of the fish in the genetic drift model?	
How many chromosomes were in the karyotype for the **sex cells** of the fish in the genetic drift model?	

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Exploration 1:

Question

 "How is DNA replicated?"

Model Rules

Which enzyme will unwind or untwist the DNA strand? _____

Which enzyme will unzip the DNA strand? _____

Which enzyme must be on the DNA strand in order to attach a nucleoside to it? _____

When you use this model, find a partner or two to compete against for fun to see who can replicate their DNA more quickly.

Who all will be competing together? (include your names in this table):

Name:	Name:	Name:

Predict

Who do you think will be fastest replicator? _____

Test Your Predictions

1. Open the "DNA Replication Fork" model.

2. Set the initial values to:

Setting	Value
SUBSITUTIONS?	Off
DNA-STRAND-LENGTH	14
TIMER?	"None"

3. Remind everyone that they are about to compete against each other to see who can build two duplicate strands of the original DNA the fastest.   Remember to unwind and unzip the DNA molecule first!

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4. Press SETUP.

5. Once everyone is ready, say "GO" at the same time and then, and then GO/PAUSE to run the model.

6. Ask quickly as you can, unwind and unzip the DNA molecule and duplicate it.

7. Press DIVIDE THE CELL when you are done. Record your results and your partners results below from the user message that appears (similar to the one shown here):

User Message

You had 7 correct replications and 21 substitutions and 0 deletions. That replication process took you 18.594 seconds.

This was a rate of 0.3765 correct nucletoides duplicated per second.

Halt   OK
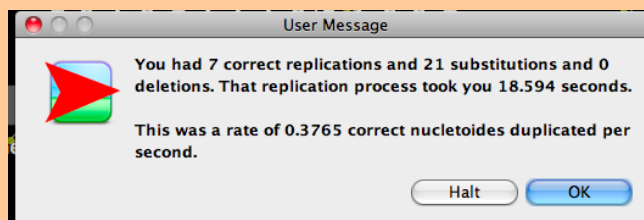
Record Your Observations:

Results of the competition

	Player 1	Player 2	Player 3
Name			
# correct replications:			
How long the replication took:			

Circle type of nucleotide that was paired up with "A" nucleotides in your DNA molecule:	Circle type of nucleotide should be paired up with "C" nucleotides in your DNA molecule:
A G C T	A G C T

Circle type of nucleotide that was paired up with "G" nucleotides in your DNA molecule:	Circle type of nucleotide should be paired up with "T" nucleotides in your DNA molecule:
A G C T	A G C T

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Making Sense of Your Data:

If someone had a fastest time to when the pressed DIVIDE THE CELL, but didn't finish duplicate all the DNA, should they be considered the winner of the competition? _____

Exploration 2:

Question
 "How do mutations occur through DNA replication?"

Model Rules

The same group as last time will be competing together. This table summarizes how to successfully duplicate the DNA strand exactly.

The original nucleotide	The nucleoside you should try to match ("fit") to it.
A	T
T	A
C	G
G	C

This time however, the model will allow you to substitute incorrect nucleoside letters when duplicating the DNA!

- Such substitutions will be considered a type of mutation (an incorrect replication).
- Any nucleotides that aren't matched at all (left unpaired) will also be considered a mutation (an incomplete replication)

Try to prevent or avoid both from occurring!

The winner is the person who has the highest # correct replications per second after a 2 minute timed competition.

Design Your Experiment

How many nucleotide base pairs do you want to try to duplicate in 2 minutes? Record the desired number (from 14 to 34) you want to use in the table below for DNA-STRAND-LENGTH

Setting	Selected Value (choose a # from 14-34)
DNA-STRAND-LENGTH	

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Predict

Who do you think will have the highest # of correct replications per second? _____

Do you think that person will also have no mutations in their duplicated DNA? _____

Test Your Predictions

8. Open the "DNA Replication Fork" model.

9. Set the initial values to:

Setting	Value
SUBSITUTIONS?	On
DNA-STRAND-LENGTH	*(the value you chose above)*
TIMER?	2 minutes

10. Press SETUP.

11. Once everyone is ready, say "GO" at the same time and then, and then GO/PAUSE to run the model.

12. Ask quickly and correctly as you can in 2 minutes, unwind and unzip the DNA molecule and duplicate it.

13. When the message that the competition has ended appears. Record your results and your partners results below from the user message that appears (similar to the one shown here):

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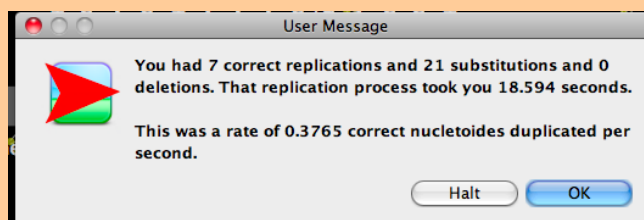
Record Your Observations:

Results of the competition			
	Player 1	Player 2	Player 3
Name			
Rate of correct replications per second:			
# deletions:			
# substitutions:			

Making Sense of Your Data:

Who had the highest rate of correct replications per second? _____

Find someone who had deletion mutations (either in your group or another group). Examine their model. What are deletion mutations and what caused them?

_____

_____

Find someone who had substitution mutations (either in your group or another group). Examine their model. What are deletion mutations and what caused them?

_____

_____

Follow-up:

DNA molecules in chromosomes are over 200 million nucleotides long. If you were asked to read only 1000 of 200 millions letters long for a specific combination of the four kinds of nucleotide bases (A, C, T, and G) about what percent of the letters do you think you would end up pairing correctly without any errors in reading the exact letter sequence? _____

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When cells make new cells (through mitosis) they try to make exact copies of chromosomes to pass on to the new cell. That process has other proteins that error check and proof-read the replication process. But it is not perfect. Sometimes these molecules do not detect the incorrect pairing or they miss a pairing completely. Sometimes they even add an extra nucleotide into the new DNA that shouldn't be there. This addition is called an insertion mutation.

When these type of mistakes occur in a real cell is it mostly the result of random errors or intentional selection? Explain

_____

_____

_____

_____

_____

Discoveries and Insights:

Summarize any important discoveries you made related to either "How is DNA replicated?" or "Where does new genetic information come from?"

_____

_____

_____

_____

How might mutations be related to the driving question for the unit?

_____

_____

_____

_____

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Reading 11 – Mutations

Jumpstart: You learned about three types of mutations in class: insertions, substitutions, and deletion Below are three models of DNA replication of a chromosome showing a different type of mutation. In the each model, the chromosome on the left was the original and the one on right is the replicated chromosome.   Label each one: which one would be considered an insertion, which one a substitution, and which one a deletion mutation?
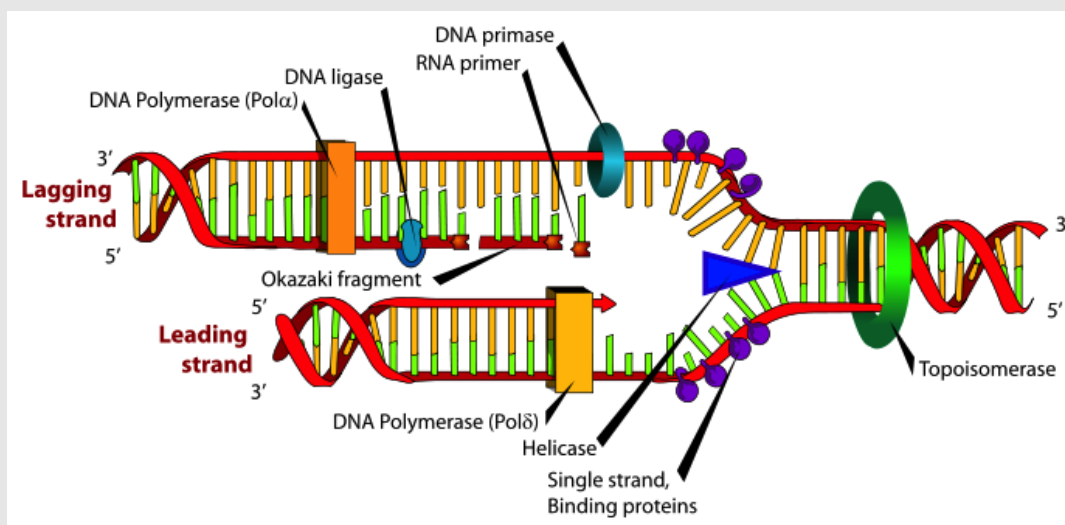

Images from http://en.wikipedia.org/wiki/Mutation
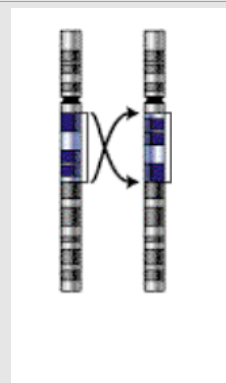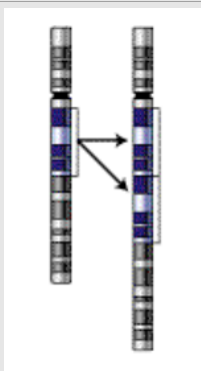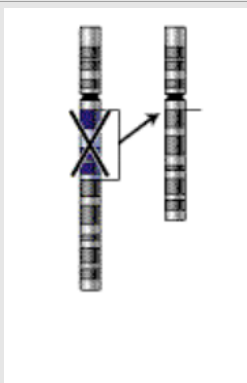
Though you can think of all mutations as either removing, adding, or replacing nucleotides, scientists sometimes describe certain types of mutations in greater depth. For example, here on the right is a model showing a mutation where some of chromosome 4 ended up in chromosome 20. That type of mutation might be thought of as a deletion mutation on one chromosome and a corresponding insertion mutation on another chromosome.

Replicating DNA is a process that involves the interactions of lots of molecules. In class you used four protein complexes to coordinate that process (topoisomerase, helicase, primase, and polymerases). On the next page is a diagram of how these proteins aid in DNA replication. It also shows two other types of proteins involved in this process (ligase and binding proteins).
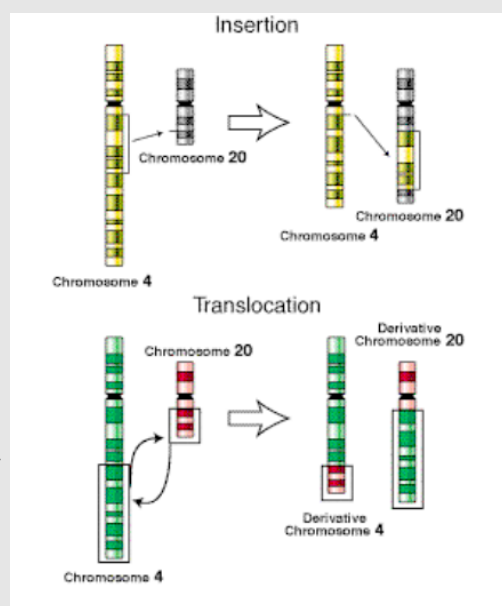
Because of the complexity of interactions of molecules, some molecules sometimes interfere with the operations of others. Sometimes, the DNA twists and folds in such a way that it gets kinked up. Sometimes not all the DNA is untwisted or unzipped. It may be helpful to think of it similar to when a zipper gets stuck on your coat. Sometimes the parts get jammed, stuck, or misaligned and its hard to get the entire zipper to zip or unzip.

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Some of the proteins involved in the DNA replication process

Question 1   Which protein appears to unwind the DNA? _____

Question 2   Which protein appears to unzip the DNA? _____

Such "jams" can result in mutations occurring.   Sometimes those mutations affect only a few nucleotides. Sometimes it can affect large sections of chromosomes (millions of nucleotides).   And sometimes it can cause an entire extra chromosome to duplicated or deleted.

Cells contain some proteins that help detect and repair mutations as they occur. Many mutations are corrected before the duplication process is completed. But every time new cells are made from old cells and chromosomes are duplicated, the number of mutation that the offspring cells have grows. Copies of copies of copies tend to accumulate more mutations over time.   In an adult human, scientists estimate that every cell in the human body incurs thousands of mutations in them! This is a relatively small percent of our genes in our cells that are mutated, since we all have millions of non-mutated genes in our cells.

Question 3   Which organism would have typically incurred more mutations in its lifetime and older dog or a young puppy? _____

Question 4   Which organism would have typically incurred more mutations in its lifetime a hundred year old oak tree or a just spouted oak sampling?   _____

Mistakes in copying DNA isn't the only way mutations occur. Mutations can also occur through interactions with certain substances, certain forms of light, and viruses. Such external environmental interactions that increase the rate of mutations are called **mutagens**.

Exposure to certain types of substances in the environment can increase the chances of a cell getting a mutation.   Some of these substances include Benzene, Bromine, DDT, Asbestos, and Chloroform. It is believed such types of substances readily enter the cell and react with stuff in the cell that impact

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DNA replication.

Exposure to certain types of light can increase the chances of a cell getting a mutation.   Ultraviolet light (UV) is the most common type of light that people are exposed to that causes mutations. Many people take precautions to apply sun screen or wear UV blocking sunglasses to decrease the amount of this type of light that reaches their cells when they are out in the sun.   Other types of non-visible light (also called radiation) that cause mutations include x-rays and gamma-rays (released from radioactive decay and other nuclear reactions).

Exposure to viruses in another way cells get mutations. Viruses insert their genetic material (either DNA or RNA) into the cells they infect. That infected cell then incorporates that extra DNA into its existing DNA. This causes the cell to build new virus parts when it translates the genetic code. Viruses then, end up causing insertion mutations in all cells they infect.

The experiences an organism has in its lifetime then can indirectly influence the number of mutations they incur if those experiences expose them to some of the mutagens listed above. radiation, substances, and viruses. All such mutations can be thought of random processes since whether an exposure actually affects a cell, which cells are affected, what portion of the DNA is affected, and the type of mutation that occurs can't be predicted.

Up until recently, the only way a human could change his or her DNA was by incurring random mutations.

Then in 1973, two scientists, Huber Boyer and Stanley Coher directly manipulated the DNA of another organism for the first time. They used proteins to cut the DNA in a bacteria cell and inserted another strand of DNA in the gap. Both bits of DNA were from the same type of bacteria. But this process was the first technique developed in genetic engineering (purposeful deletion, insertion, and substitution of DNA in cells).

In the past forty years, scientists have discovered many other genetic engineering technologies that now allow them to purposefully identify, remove, and replace specific parts of DNA from cells. It is hoped that these and other new technologies will soon lead to efficient ways to identify and cure thousands of genetic diseases by replacing missing genetic information through insertion and substitution of nucleotide bases into a person's DNA.

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Activity 12 – DNA Protein Synthesis

Purpose:
How do mutations in DNA affect the proteins that cells make?

Exploration 1: Notes on the structure and function of proteins

Question
"How do new genes encode for making new proteins?"

Prior Knowledge: What do you already know about proteins and how they are made?

_____

_____

_____

Notes and Observations

Our Question	My Notes
What are proteins made of?	
What do proteins look like?	
What do proteins do?	
Where are proteins made?	

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A cell encodes for what proteins it should make (and all cells it produces should make) using DNA. The DNA is made up of combinations of A, G, C, and T nucleotides only. You saw this in the previous computer model you used.

Brainstorm :   How might it be possible that only 4 different types of nucleotides in the DNA could be used to encode for 20 different possible amino acids used in protein construction?

_____

_____

_____

Exploration 2:

Question
 "Does all DNA encode for protein production?"

Predict
Do you think that all DNA will encode for protein production? _____

Design Your Experiment

Using only the four letter representation of the nucleotides found in DNA (A, T, C, or G), construct a sequence of 12 nucleotide letters to represent a small strand of DNA.

You will be able to test six different possible combinations to see whether they all encode for protein production in the next computer model.   Fill in the values you want to test for each trial in the table below. The value to test for trial 1 has been chosen for you.

Trial	Setting	Value to test
1	USER-CREATED-DNA-CODE	ATTATGTGGTAG
2	USER-CREATED-DNA-CODE	
3	USER-CREATED-DNA-CODE	
4	USER-CREATED-DNA-CODE	
5	USER-CREATED-DNA-CODE	
6	USER-CREATED-DNA-CODE	

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Test Your Predictions

14. Open the "DNA Protein Synthesis model.

15. Set the initial values to:

Setting	Value
INITIAL-DNA-STRING	"From Input Box"
USER-CREATED-DNA-CODE	In this box type in the 12 letters you wrote for trial 1 in the table above

16. Press SETUP. You will see the following DNA sequence appear on the screen:

17. Now press the GO/STOP button.

18. Press TRANSCRIBE button in the section labeled ORIGINAL DNA. *You will see an mRNA molecule appear under some of the DNA.*
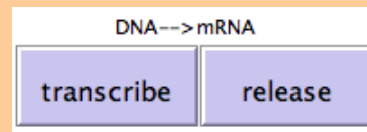
19. Press the RELEASE button to the right of the TRANSLATE button. *You will see the mRNA molecule detach from the DNA and move upward.*

20. Now that the mRNA is released you will press the TRANSLATE button in the ORIGINAL DNA section.
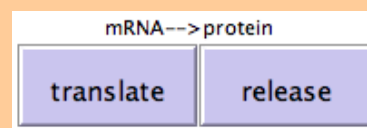
21. Lastly, Press the RELEASE button to the right of the TRANSLATE button (this is a different release button than the one you pressed before)

You will now see the monitors that keep track of GENES and PROTEINS MADE for the Original DNA both show the value of 1.

22. Now repeat all these steps changing the value for the INITIAL-DNA-STRING in step 2 to the next value you intended to test for trial 2. After each trial record your results below.

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Record Your Observations:

Trial	Number of Genes	Proteins Made
1	1	1
2		
3		
4		
5		
6		

Making Sense of Your Data

Based on the evidence you collected, make a claim to answer this question:
 "Does all DNA encode for protein production?"

_____

_____

Exploration 3:

Question
 "How do mutations affect the number of genes in a strand of DNA?"

Design Your Experiment

Choose one of these strands of DNA to explore the effects of mutations on. Circle the one value you intend to use in your experiment

Setting	Possible Values to Use
INITIAL-DNA-SETTING	no genes (long DNA strand)
	no genes (short DNA strand)
	1 long gene
	1 short gene
	2 sequential genes
	2 nested genes
	3 sequential genes

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Next choose the type of mutation you wish to explore. Circle your selected value.

Setting	Possible Values to Use
MUTATIONS-IN-DNA-COPY?	deletion
	insertion
	substitution

Choose how many nucleotides will be affected by this mutation. Fill in your selected value.

Setting	Possible Values to Use *(any value 1 to 6)*
#-NUCLEOTIDES-AFFECTED	

Predict
How do you think the number of genes will be affected by this mutation?

Test Your Predictions

1. Set the initial values to what you recorded you wanted to use for this experiment (from the 3 tables above).

2. Press SETUP. Press GO/STOP.

3. Press the REPLICATE button.

4. Record the number of genes in the Original DNA and the number of genes in the Replicated DNA in the Observation section below.

5. Repeat the experiment 20-30 more times to determine an answer to test your prediction by simply pressing REPLICATE again. *You do not need to press SETUP or GO/STOP again.*

6. Record any patterns you see in what causes a gene to start or stop

Record Your Observations:

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Making Sense of Your Data

Can mutations affect the number of genes in DNA? _____

What three nucleotide letters are always used to indicate the start of a gene? _____

Exploration 4:

Question
 "How do mutations affect the structure of proteins?"

Design Your Experiment
Choose the type of mutation you wish to explore. Circle your selected value.

Setting	Possible Values to Use
MUTATIONS-IN-DNA-COPY?	deletion
	insertion
	substitution

Choose how many nucleotides will be affected by this mutation. Fill in your selected value.

Setting	Possible Values to Use *(any value 1 to 6)*
#-NUCLEOTIDES-AFFECTED	

Predict
How will your selected mutation affect the type of amino acids used to construct the protein a gene codes for?

_____

_____

Test Your Predictions

1. Set the initial values to what you recorded you wanted to use for this experiment (from the tables above).

Setting	Possible Values to Use
INITIAL-DNA-SETTING	1 long gene

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2. Press SETUP. Press GO/STOP.

3. Press the REPLICATE button.

4. For the original DNA press these buttons in this order:
   - TRANSCRIBE
   - RELEASE (to the right of TRANSCRIBE)
   - TRANSLATE
   - RELEASE (to the right of TRANSLATE)

   The PROTEINS MADE monitor will now show "1"

5. Now repeat this process in the replicated DNA section of the model (below the set of buttons you pressed above). Press this new set of buttons in this order:
   - TRANSCRIBE
   - RELEASE (to the right of TRANSCRIBE)
   - TRANSLATE
   - RELEASE (to the right of TRANSLATE)

6. Compare the structure of each protein made and record the abbreviated amino acids names used in the construction of each protein in the table below.   *For example, the amino acids used in the construction of the original protein produced was:*

   Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu

If no proteins were made in the Replicated DNA, because the mutations eliminated the gene, then simply record "none" in the observation table.

7. Repeat the experiment five more times recording your results each time, by simply pressing REPLICATE again. *You do not need to press SETUP or GO/STOP again.*

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Record Your Observations:

Trial	Differences in The Amino Acid Construction Order For the First Protein Translated from the First Gene				
	in the first protein from the first gene in the Original DNA		In the first protein from the first gene in the Replicated DNA		# of Amino acids that are different between these two proteins
	# of amino acids	Amino acid order	# of amino acids	Amino acid order	
1	12	Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu			
2		Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu			
3		Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu			
4		Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu			
5		Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu			
6		Met-Asp-Thr-Leu-Ser-Phe-Ala-Ser-Asp-Gln-Phe-Glu			

Making Sense of Your Data

- When averaging out all six trials, about what percent of the time was the amino acid order different in the two proteins (from original DNA vs. replicated DNA)? _____
- When averaging out all six trials, about what % of time were additional amino acids added to the protein in the replicated DNA? _____
- When averaging out all six trials, about what % of time was no protein produced in the replicated DNA? _____

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Discoveries and Insights:

Summarize any important discoveries you made related to How do changes in DNA affect the proteins that cells make?

_____

_____

_____

_____

_____

How might the **outcomes** of mutations be related to the driving question for the unit - "How does the Distribution of Traits in a Population Change?"

_____

_____

_____

_____

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Reading 10 – Mutations

Do you know anyone who has trouble eating or drinking dairy products?

If you can drink milk, eat cheese, or other dairy products without getting sick, you have a gene that produces a certain kind of protein to thank for it. Between 30 and 50 million North Americans have two copies of a gene mutation (one inherited from each parent) that makes them lactose intolerant. This means that they develop nausea, cramps, bloating, gas, and diarrhea if they eat dairy products.

Their bod reacts this way because they do not have DNA that encodes for constructing lactase. Lactase is the protein that breaks down lactose in dairy products. If lactose isn't broken down then it can't be digested.

Question 1:   Do you know someone who may have a gene mutation that makes them intolerant to other types of foods? _____

From: http://www.americanscientist.org/issues/id.3724,y.0,no.,content.true,page.7,css.print/issue.aspx

Whippets are usually sleek, trim dogs (*left*), but a variant called a "bully" is overly muscled (*right*). The difference between the two dogs is due to a single nucleotide base-pair difference. The mutation affects the gene for construction of a signaling protein (myostatin). Myostatin limits the buildup of muscle tissue. Because the mutation results in that protein no longer being constructed myostatin is no longer constructed. With this mutation, cells in the dogs body don't receive a clear "stop" signal for building muscle.

Individuals that carry two copies of the mutation are "bullies," but those dogs that carry only one copy are only somewhat more muscular and are also often faster racers.   One copy of the the gene provides instructions to build some myostatin, while two copies of the gene provide an extra set of instructions to build myostatin.

As you learned in an earlier lesson, mutations are the result of random unintended chemical reactions that alter the genetic information in organisms. The most noticeable mutations are the ones that occur in the genetic information of cells that controls heredity (e.g. pollen, ovules, sperm, egg cells, etc..). This altered genetic information is more noticeable since it ends up being passed on to all of the cells of the offspring and become a part of the genetic information expressed as a trait in all of the cells of the individual.

Typically when such a mutation occurs, it only affects a small portion of the genetic information of the organism.   Most mutations change the genetic code in such a way as to only change the genetic information for construction of a particular protein. But sometimes, the mutation ends up generating a different outcome, affecting multiple proteins and multiple traits.

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A mutation can have a variety of possible effects on an organism. A mutation can be lethal (removing a gene that produces a protein needed for immediate survival) or non-lethal (not having an immediate impact for survival).

Many mutations are harmful to cells, because the new sequence of nucleotides in the cell now code for genetic information that ends up creating new proteins in the cell that cause the cell to die or because necessary proteins for the survival of the cell are no longer being created. This then leads to the death of the offspring (which starts growing from a single fertilized sex cell). Other mutations, however, either lead to the creation or removal of substances, but do not cause cells to die. These type of mutations don't lead to the immediate death of the offspring.   In fact the creation of a new type of protein or removal of an old type of protein in the cells of the individual, may sometimes end up giving that individual a competitive advantage in some environments.

Some proteins that are created or removed from a mutation, regulate the order and placement of body parts and organ development. When mutations that affect production of these protein occur, radically new and different structures and functions cam emerge in offspring in a single generation.

Question 2:   A young boy is walking through a field of dandelions and notices something peculiar. There is one dandelion that looks very different than the rest. It looks like four dandelions in one – in that it has four co-joined stems and a flower head that has four nested sets of flower petals.   What type of mutation might have caused this dandelion to end up with a different physical structure than the rest of the population?

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Goldfish originated in China in the Sung Era, approximately 1000 A.D. The goldfish originated as a natural mutation in a population of carp, which was originally a drab olive color. The orange-red color is the result of a natural mutation, which was selectively bred-for over the years.

Other types of mutations occur in goldfish. Some common mutations affect fin size, fin shape, head shape, scale size, and body length.

In stickleback fish (shown on the right), mutations affecting the activity of three development-control genes have produced striking anatomical changes, including the complete loss of pelvic hind finds, large differences in bony armor, and much lighter skin color.

The difference between a stickleback with and without one of these mutations.

From http://www.sciencedaily.com/images/2004/04/040415012031.jpg

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Question 3: A lady keeps a goldfish population in a large pond in the backyard. For many years, she keeps a healthy ecosystem in the tank, but does not introduce new fish. Instead she lets the existing goldfish die and give birth on their own. One year she notices a small baby fish that is strikingly different than the other goldfish. It appears to be missing the gold coloring of the rest of the individuals, and is nearly completely white colored.   How might a mutation have caused this one fish to end up with different coloring than the rest of the population?

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If we look at populations of animals and plants we find that there are many genes that are multiple copies of other genes. In other words there is duplicated genes. On average, about 10%-20% of genes in a population are the result of mutations that added extra copies of the gene through extra duplication. Sometimes these extra genes result in the production of the same protein.

Question 4: A farmer grows corn each year, and keeps some of the offspring kernels from his crop in storage to experiment with selective breeding. He takes 10 kernels off of a single ear of corn that he pollinated with pollen from one other plant. All the kernels appear identical. Even though the kernels look identical, why might some of them still have mutations in their genetic information?

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The effects of many mutations are not immediately visible. Some mutations may produce proteins that do not have a noticeable effect the structure or function of the organism. Other mutations may produce substances that are only detectable in certain environments. For example, if one mutation causes a plant to produce a protein that decreases its drought tolerance and a different mutation causes a plant to produce a protein that increases its drought tolerance, it is possible that neither of these mutations would be detected in a year when there is no drought.

Though not every mutation creates an immediate effect on the structure or function of an organism in its current environment, every mutation does result in a change in the creation of one or more substances in the cells of the organism. Sometimes the effect of this change are noticeable and sometimes they are not, but at the very least 1 trait about the individual has changed – namely the production of one of the substances that the cells produce in the individual.

Scenario 1: Imagine a flock of birds which split apart and each group settled on two separate islands in the ocean. Imagine the islands are separated by quite a distance, so that it is very unlikely that birds from one island travel to the other island.

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You come to the islands after the birds lived on them for many generations And you compare both populations.   You find that each population has its own distinct feather color, head size, beak shape, and nesting locations.

Question #5: For Scenario 1, how could the mechanism of mutation contribute to why each population looks so different?

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Question #6: For Scenario 1, how could the mechanism of genetic drift contribute to why each population looks so different?

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Question #7: For Scenario 1, how could the mechanism of natural selection contribute to why each population looks so different?

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Scientists have experimented with artificially induce mutations in bugs by exposing them to radiation.

From http://evolution.berkeley.edu/evolibrary/article/_0_0/history_18

In 1908, Thomas Hunt Morgan bred fruit flies by the thousands, and his team tried to create mutant flies with x-rays, acids, and other toxic substances. Finally, in 1910, one unaltered lineage of flies, the researchers found a surprise. Every single fly in that line had been born with red eyes, until one day a fly emerged from its pupa with white eyes. Something had spontaneously changed in the white-eyed fly.

Morgan's experiment showed that mutations could be artificially induced in organisms. But how often do such mutations occur in organisms when not exposed to additional mutagens?

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Imagine you could permanently record the genetic information of an individual in every generation and observe how each generation of offspring differed from its parents. This would be a useful form of experimental design if you wanted to observe what type of mutations arise in each generation, how often mutations occur, and whether any of the mutations are resulting in noticeable trait changes. In this experiment you might even be able to detect trait changes that result in individuals that are more competitive and better suited to survive in their environment than their ancestors.

Researchers at Michigan State University have attempted such an experiment with bacteria. Their project, called "Experimental Evolution", involves the study of the evolution that results from the offspring of a single asexually reproducing bacteria. In their experiment, they make use of sealed petri dishes to enforce "geographic isolation" from other generations of offspring, freezers to store some of the generation of offspring into permanent storage and temporary hibernation, and microscopes, indicators, and genetic testing equipment to study both the genetic information and visible traits that have been evolving in the offspring generations through mutations, natural selection, and genetic drift.

Their findings show how often mutations occur in this population, and how combinations of mutations over thousand of generations can then lead to sudden and rapid advances in the progress of evolution of the population.

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from http://en.wikipedia.org/wiki/E._coli_long-term_evolution_experiment

The long-term evolution experiment was intended to provide experimental evidence for several of the central problems of evolutionary biology: how rates of evolution vary over time; the extent to which evolutionary changes are repeatable in separate populations with identical environments; and the relationship between evolution at the phenotypic and genomic levels.[2]

The use of *E. coli* as the experimental organism has allowed many generations and large populations to be studied in a relatively short period of time, and has made experimental procedures (refined over decades of *E. coli* use in molecular biology) fairly simple. The bacteria can also be frozen and preserved, creating what Lenski has described as a "frozen fossil record" that can be revived at any time (and can be used to restart recent populations in cases of contamination or other disruption of the experiment). Lenski chose an *E. coli* strain that reproduces only asexually, without bacterial conjugation; this limits the study to evolution based on new mutations and also allows genetic markers to persist without spreading except by common descent.[2]

Methods
Each of the 12 populations is kept in Lenski's laboratory at Michigan State University in a minimal growth medium and transferred daily into a flask of fresh nutrients, with samples of each population preserved at 500-generation (75 day) intervals. The populations are also regularly screened for changes in mean fitness, and supplemental experiments are regularly performed to study interesting developments in the populations.[3] As of 2008, the *E. coli* populations have been under study for over 40,000 generations, and are thought to have undergone enough spontaneous mutations that every possible single point mutation in the *E. coli* genome should have occurred multiple times.[4]

The initial strain of *E. coli* for Lenski's long-term evolution experiment came from "strain Bc251", as described in a 1966 paper by Seymour Lederberg, via Bruce Levin (who used it in a bacterial ecology experiment in 1972). The defining genetics traits of this strain were: T6^r, Str^r, r m , Ara (unable to grow on arabinose).[1] Before the beginning of the experiment Lenski prepared a Ara$^+$ variant (a point mutation in the *ara* operon that enables growth on arabinose) of the strain; the initial populations consisted of 6 Ara colonies and 6 Ara$^+$ colonies, which allowed the two sets of strains to be differentiated and tested for fitness against each other. Unique genetic markers have since evolved to

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allow identification of each strain.

In the early years of the experiment, there were several common evolutionary developments shared by the populations. The mean fitness of each population, as measured against the ancestor strain, increased—rapidly at first, but leveling off after close to 20,000 generations (at which point they grew about 70% faster than the ancestor strain). All populations evolved larger cell volumes and lower maximum population densities, and all became specialized for living on glucose (with declines in fitness relative to the ancestor strain when grown in dissimilar nutrients). 4 of the 12 populations developed defects in their ability to repair DNA, greatly increasing the rate of additional mutations in those strains. Although the bacteria in each population are thought to have generated hundreds of millions of mutations over the first 20,000 generations, Lenski has estimated that only 10 to 20 beneficial mutations achieved fixation in each population, with less than 100 total point mutations (including neutral mutations) reaching fixation in each population.[2]

In 2008, Lenski and his collaborators reported on a particularly important adaptation that occurred in one of the twelve populations: the bacteria evolved the ability to utilize citrate as a source of energy. Normally, *E. coli* cannot transport citrate from outside the cell to the cell interior (where it could be incorporated into the citric acid cycle); the lack of citrate transport is considered a defining characteristic of the species. Around generation 33,127, the experimenters noticed a dramatically expanded population-size in one of the samples; they found that this population could grow on the excess citrate in the growth medium. They found that the ability to use citrate could spontaneously (although rarely) appear in cultures replicated from earlier frozen samples of that population, from before the citrate mutation appeared, but not in the other 11 populations or in samples before generation 20,000. According to the authors of the study, this suggests that the mutation depends on an earlier, perhaps non-adaptive, change—and more generally (following the argument of Stephen Jay Gould) "that historical contingency can have a profound and lasting impact" on the course of evolution.[4]

Question #8:   What are some important ideas you learned about mutation and how the bacteria changed over time?

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You read about how bacteria compete for limited resources in a petri dish, have the ability to reproduce offspring that inherit genetic information from a parent (asexual reproduction). Over time, as new individuals are produced in each population of bacteria in each petri dish, the mechanisms of competition, genetic drift, natural selection, and mutation are all at work. These are the mechanisms that lead to the evolution of bacteria and mechanisms that lead to the evolution of all life.

Question #9:   Genetic drift and natural selection makes populations of isolated individuals more like each other over time. Mutation adds new traits to a population over time, so that the individuals are less like their ancestors. Why would the combined effects of mutation, genetic drift, and natural selection keep making separate herds of animals of the same species less and less similar to each other over time?

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If the mutations that aren't detected by the cell, many only have a small or negligible effect on the ability of a cell to survive. Others are much more harmful to the cell (because some critical protein is no longer being produced that is needed for the cell to survive).

But even if a cell dies in a multi-cellular organism, due to mutated DNA no longer correctly encoding for proteins critical for the life of the cell, the body can make new cells out of other old cells that don't have those harmful mutations to replace cells that die off from harmful mutations.   So in general, mutated cells in the human body are largely undetected and of little consequence to the survival of the organism.

There is an important exception to this. If a cell in an organism gets a mutation that alters the genetic information for proteins that control and limiting how and when cell division occurs. Without such proteins a mutated cell may grow, divide and keep growing and dividing without ever stopping. When this type of mutation occurs in cells the resulting growth of mutated cells is called cancer. All cancers share this common outcome – they were mutated so that the cells no longer have the genetic instructions that tells them to stop or limit their cell division. Eventually this uncontrolled growth and division of cells can lead to the death of the organism.

And sometimes, a mutation results in the code for production of a protein that has never been produced before. This new protein may yield an innovative new trait variation that grants a competitive advantage for this organisms to outcompete others. If so, it is likely that mutation will be passed on to offspring, increase in frequency in the gene pool, and eventually become a characteristic new feature of the descendant population.

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Lesson 13:   How Do the Mechanisms of Evolution Change Populations?

Purpose:

To connect the concept of evolution to the mechanisms of population and trait variation change discovered so far.   To contrast the differences between evolutionary mechanisms (or causes) and evolutionary outcomes (end results).

Procedure:

You have studied many different mechanisms that cause populations to change over time. When these mechanisms interact together they often cause the trait variations in a population to change over time. This outcome is referred to as evolution.

In addition to this outcome, evolution also refers to the combination of processes that lead to this outcome. Evolution can involve multiple mechanisms working together and so understanding it as a process can be very complex.   Today you will review the mechanisms of evolution. And you will describe some of the outcomes that result from each.   **With a group, complete the following chart:**

	mechanism of evolution					
Outcomes of this mechanism	**competition for limited resources**	**new combinations of trait variations in offspring from sexual reproduction**	**genetic drift**	**natural selection**	**changes in environmental conditions**	**mutations**
is the result of random events or interactions	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)
can add brand new variations of traits into a population	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)
can remove existing variations of a trait from a population	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)
can contribute to a population becoming better adapted to survive a particular environment	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)
will generate the exact same outcome every time.	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)	(yes or no)

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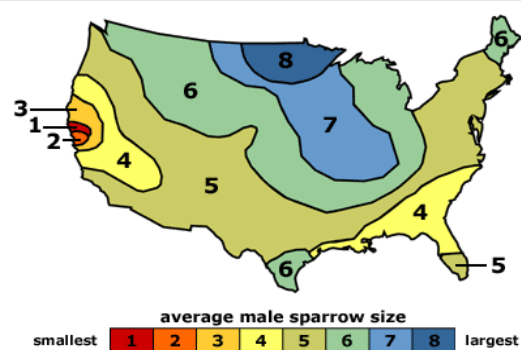## Scenario 1: Evolution of sparrows in North America

text and images from http://evolution.berkeley.edu/evolibrary/article/_0/evoscales_03

The Size of the Sparrow and the Color of Its Feathers Before: House sparrows were introduced to North America in 1852. Their average body size of that founding population was less than it is now. The color of the feathers of these sparrows showed less variation than they do now.

The Size of the Sparrow and the Color of Its Feathers Now: Since then the sparrows have evolved different characteristics in different locations they migrated to. Sparrow populations in the north are larger-bodied than sparrow populations in the south and have darker feathers.

The map on the right shows the average male sparrow size in the United States today.

Notice how the size of the sparrows is different in the northern part of the U.S. versus the southern part of the U.S.
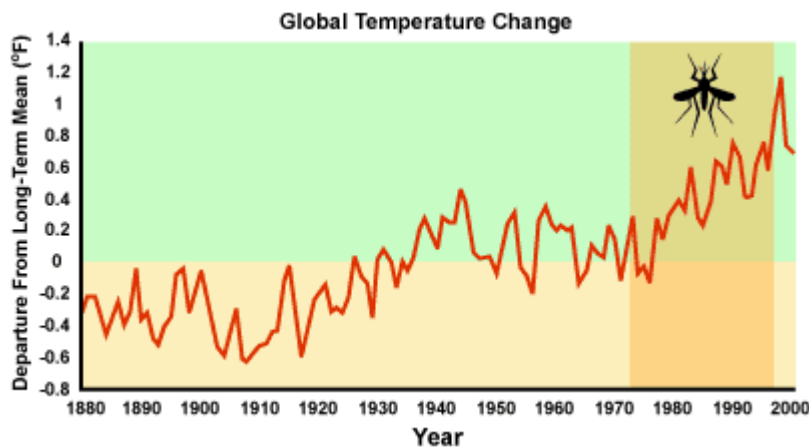

Draw a diagram or explain in words how the mechanisms of evolution may have interacted together to generate this outcome: Sparrows in the north are darker feathered and larger bodied than sparrows in the south.

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Scenario 2: Evolution of Mosquitoes and Global Warming

It is believed that the mosquito species (Wyeomyia smithii), has evolved in response to global warming. Mosquitoes use day length (not temperature) as a cue to tell them what time of year it is and when to overwintering (lay eggs to hatch after the winter). This "cuing" of when to lay their eggs for the winter is genetically controlled.

Researchers who have been collecting data on these mosquitoes for almost 30 years have observed that the mosquitoes now overwinter at a later day in the year than they did 30 years ago.

This graph illustrates changes in global temperature from 1880 to 2000.[3] Between 1972 and 1996 mosquito population has evolved in response to this rise in climate temperature

Draw a diagram or explain in words how the mechanisms of evolution may have interacted together to generate this outcome:

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Evolution can result from combinations of some or all of the mechanisms you studied: competition, recombination of genetic information in sexual reproduction, genetic drift, natural selection, environmental changes, and mutation. To observe its effects you must study populations in a changing environment over multiple generations. Even then, it may be very difficult to determine which mechanisms were specifically responsible for which changes in its evolutionary history and when. The more generations you can observe the more noticeable the effects of evolution are and the easier it is to isolate the interactions of these mechanisms at different points in time.

Compare: Rank order the following populations, based on which would be easiest to observe noticeable the effects of evolution in, within a 20 year period?

Rank order the easiest to observe noticeable effects of evolution in, within a 20 year period (1 = easiest, 7 = hardest)	population of	average time between new generations of offspring
	wolves	~ 1 year
	bacteria	~ 30 minutes
	dandelions	~ 2 months
	orangutang	~ 8 years
	aphids	~ 2 weeks
	guppies	~ 3 months
	mosquitoes	~ 1 week

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New Traits from Mutations.

Question
"How do mutations sometimes grant a competitive advantage?"

Procedure
In the chart below, consider the following possible scenario – a mutation creates or removes an allele in the related organism. The mutation in turn leads to a new trait variation. For this possible scenario, list a type of environment where the trait that results would give a competitive advantage and list an environment where the trait that results would give a competitive disadvantage

Organism	Outcome of Mutation is to produce a different protein in cells that cause the organism to:	An example environment that would give a **competitive advantage** for this mutation	An example environment that would give a **competitive disadvantage** for this mutation
Rabbit	Has whiter fur		
Bear	Produces more body fat		
Fish	Has a smaller body size		
Wolf	Grows shorter fur		
Bird	Has a pointer beak		
Butterfly	Detects the color red more clearly		
Tree	Builds deeper roots		

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Homework 13 – Reviewing the Mechanisms of Evolution

Evolution can be described both in terms of its outcomes and its mechanisms.

Its outcome is simply that changes in trait or gene frequency in a population of organisms occur from one generation to the next. This outcome is observable in any population of living organisms.

outcomes of evolution
changes in trait or gene frequency in a population of organisms occur from one generation to the next.

There are many mechanisms of evolution that can generate this outcome.

mechanisms of evolution					
competition for limited resources	recombination of genetic information (alleles) in sexual reproduction	genetic drift	natural selection	mutation	environmental changes

Question 1: Which one these mechanisms can add new variations of traits into populations?

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Question 2: Which mechanisms can remove variations of traits from a population?

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A single mechanism can lead to a given outcome of evolution. Or many mechanisms working together may have also led to the same outcome.

In this sense, there are many possible combinations of causes that you can propose for how the mechanisms of evolution are interacting together to generate evolutionary outcomes.

In class you heard different models proposed for which mechanisms interact together to generate the outcomes in mosquitos and sparrows that you studied in class.   As you continue to investigate evolution in this unit you will explore increasingly complex models, which have multiple mechanisms at work at once. Which mechanisms are responsible for the outcomes you see in the model might be different in different model runs.

As you encounter new case studies in class, try to apply the mechanisms of evolution (listed above) so that you can account for the outcomes in your case study. Applying the mechanisms of evolution to a scientific explanation should including linking these mechanisms together in your reasoning, and explaining how these mechanisms could generate the data or observations you are seeing.

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Scientists have discovered that if you don't take evolution into account for most biological problems in the world, proposed solutions to those problems are likely to fail.

Question 3: Explain why understanding the mechanisms of evolution can help people understand how strains of bacteria might evolve over time and how would this help them develop more effective fight ways to treat infections from these bacteria.

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Question 4: Explain why understanding the mechanisms of evolution can help people understand how insect populations change over time and help them develop and administer more effective methods of pest control.

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From http://evolution.berkeley.edu/evolibrary/news/090401_biofuels

Better biofuels through evolution

April 2009

Engineers are using bacteria to produce biofuels from plant matter.

In celebration of the Year of Science's April theme, energy resources, we bring you a story from the frontiers of energy research that depends on evolution. Right now, most of us fill up our gas tanks with fossil fuels, the remains of plants and animals that died many millions of years ago and eventually became petroleum — but, of course, this can't last forever. Petroleum is a limited resource and will eventually run out. To help solve this problem, many scientists, policy makers, business people, and concerned citizens have placed their hopes in biofuels — fuel derived from plant matter that we can grow today. Biofuels might sound like an ideal solution to our energy problems, but there are still major kinks to work out — kinks that evolution can help us overcome.

Where's the evolution?

Part of the problem with the biofuels currently produced in the US is that they are mainly made from corn — a greedy crop that sucks nutrients from the soil and needs lots of fertilizers (which are often themselves derived from fossil fuels!). Even worse, since corn is also a food crop, using it to produce biofuel drives up the cost of this dietary staple. Furthermore, a tiny fraction of the plant, the kernels, can be used to make fuel. Biofuel production would be much more efficient if it used whole plants and if it could rely on less greedy plants that are not also used as food. Such a fuel might be a viable alternative to gasoline. Unfortunately, getting biofuel out of plant matter is no easy task; it requires a series of complex chemical reactions. Figuring out how to make those reactions happen without using up tons of energy in the process is the job of synthetic biologists — and they have turned to evolution to help crack this nut.

Evolution in the wild involves the survival and reproduction of organisms with traits particularly well-suited to their environments. Synthetic biologists, like Jay Keasling of UC Berkeley, Frances Arnold of Caltech, and many others, are trying to replicate this process in the lab — only instead of evolving organisms with particular traits, they aim to evolve molecules that are particularly good at transforming plant matter into fuel. The process is called directed evolution, and it works like this:

Begin with a gene that does something close to the job you have in mind. Perhaps it produces a molecule that can chop up cellulose — the substance that makes up most plant matter and the most abundant organic molecule on Earth.

Produce many copies of the gene with slight variations. Often, this is done by intentionally using a "bad" copying system — a process for copying the DNA that introduces lots of

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random mutations. You'll end up with a pool of similar genes with slight genetic differences from one another.

Insert the different gene copies into organisms — usually bacteria or yeast. This is done to find out what all the different gene versions do. Bacteria and yeast have all the cellular machinery needed to turn on the gene and let us see how it functions.

Screen the organisms to see which are best at performing the job you have in mind. In this case, you might be looking for the ones that are speediest at breaking down cellulose. As in evolution in the wild, most of the new mutations will be detrimental or neutral — that is, they will make the molecule worse at its job or will have no effect on it at all. However, if you look at enough mutants, you are likely to find a few that improve the molecule's functionality.

Select the best gene version and use it in Step 1. Repeat this cycle many times. It's unlikely that any one mutation would make a huge difference in the functioning of the molecule — but by selecting the best gene from each generation and using it as the basis for the variants produced in the next generation, you can identify a series of changes that, together, make a big improvement.

This process mimics evolution by natural selection. In natural selection, the organisms with the genes that perform best in a particular environment survive to pass those genes (possibly with new mutations) on to the next generation. As this cycle is repeated over many generations, organisms with genes for traits well suited to the environment evolve. Directed evolution works the same way, but the selection is focused on gene versions that are good at doing a certain job. The key difference between the two processes is that, in the wild, the environment determines which gene versions are most successful, and in the lab, the scientist does. While natural evolution has no goal, directed evolution can be channeled toward the ends we have in mind. Of course, the end that we have in mind — transforming plant material into fuel — will be easier to achieve if we start with genes that already have some of the functions we want. Genes like this do exist. After all, plant matter doesn't accumulate indefinitely in our gardens or on the forest floor. It is eventually broken down, mainly by organisms like microbes and fungi. While such life forms might not seem very exciting — just compare the number of nature documentaries on mushrooms to the number on meerkats — for synthetic biologists, these organisms are treasure troves of thrilling new genes that could spark a biofuels revolution.

Directed evolution is a key tool for synthetic biologists because those new genes (currently hidden away in bacteria on the rainforest floor or in the gut of an exotic termite) won't be perfectly matched to our biofuels needs. That mismatch can be chalked up to the genes' own evolution. Evolution shaped these genes in their complex natural environments — environments in which optimizing one trait often meant downgrading another. For example, improving a molecule's efficiency at chopping up cellulose might also mean putting more energy into producing the molecule or making the molecule less stable at different temperatures. Nature selection balances such trade-offs and tends to maximize the *overall* survival and reproduction of organisms — not the efficiency of *particular* molecules. The genes we find in nature are the product of a balancing act among evolutionary trade-offs and constraints — which means that they work well enough in the real world but probably aren't ideal for the mass production of biofuel. One of the advantages of directed evolution is that, in the lab, scientists can remove these trade-offs and constraints, optimizing the genes for performance in a particular environment.

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The alternative to optimizing genes through evolution is improving them through engineering — intentionally making particular changes in a gene for certain effects. We definitely have the technology to alter genes — in fact, these days, you can order a particular genetic sequence online and have the DNA shipped directly to you! The problem is that in few cases do we have enough knowledge to figure out exactly which changes will produce the desired results. That's why directed evolution is such an appealing way to attack the problem of building better biofuels. Evolution, after all, has no foresight — so when scientists use directed evolution, they don't need detailed knowledge of which genetic changes will help solve their problem. By screening millions of variants and building on the best of those, directed evolution lets science stumble onto engineering solutions that we might never have thought of — solutions that just might allow us to unlock the tantalizing, but still unrealized, promise of biofuels.

Question 5 Using the ideas suggested in the article, and your own understanding of the mechanisms of evolution, propose a way to use a "directed evolution" experiment that might lead to a bacteria that could be used to clean up oil spills, by rapidly "eating" oil.

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Question 6 What are some possible unexpected outcomes that might occur in a real world ecosystem, if either the bacteria you read about in the article or the fast "oil eating" bacteria was continually released into it?

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