Teacher Resource for:

The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations

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GENERAL USE OF *Science* in the Classroom

Student Learning Goals:

"One fundamental goal for K-12 science education is a scientifically literate person who can understand the nature of scientific knowledge."[1]

The U.S. National Academy of Sciences defines science as: "Any new finding requires independent testing before it is accepted as scientific knowledge; a scientist is therefore required to honestly and openly report results so that they can readily be repeated, challenged, and built upon by other scientists. Proceeding in this way over centuries, the community effort that we call science has developed an increasingly accurate understanding of how the world works. To do so, it has had to reject all dogmatic claims based on authority, insisting instead that there be reproducible evidence for any scientific claim."

An important student learning goal, central to any understanding of "the nature of scientific knowledge," is to give each student an appreciation of how science is done.

This includes knowing why:

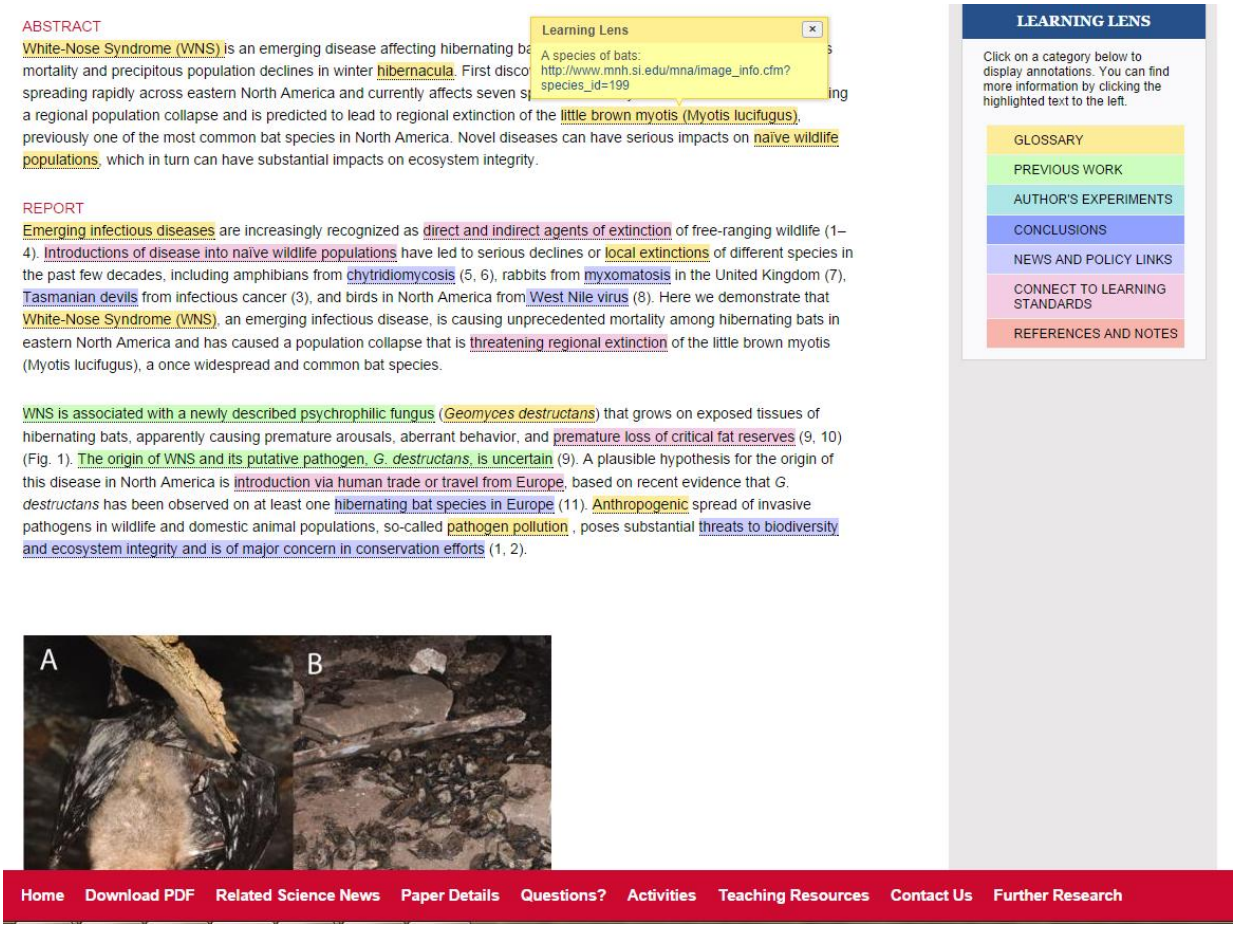
- Scientists must be independent thinkers, who are free to dissent from what the majority believes.
- Science can deal only with issues for which testable evidence can be obtained.
- All scientific understandings are built on previous work
- It is to be expected that one scientist's conclusions will sometimes contradict the conclusions of other scientists.
- Science is a never-ending venture, as the results from one study always lead to more questions to investigate.

[1] *A Framework for K-12 Science Education*, National Research Council, 2012

Using This Resource

Learning Lens:

The Learning Lens tool can be found on the right sidebar of each resource and is the source of annotations. Click on the headings to highlight portions of the text of the corresponding research article. A subsequent click on the highlighted text will produce a text box containing more information about that particular piece of text. Below is an example of the Glossary function of the Learning Lens.



An example of the resource with the Glossary, Previous Work, Author's Experiments, News and Policy Links, and References and Notes tools turned on. The Glossary tool is in use.

Learning Notes:

Learning Notes accompany each figure and are designed to help students deconstruct the methods and data analysis contained within each figure.

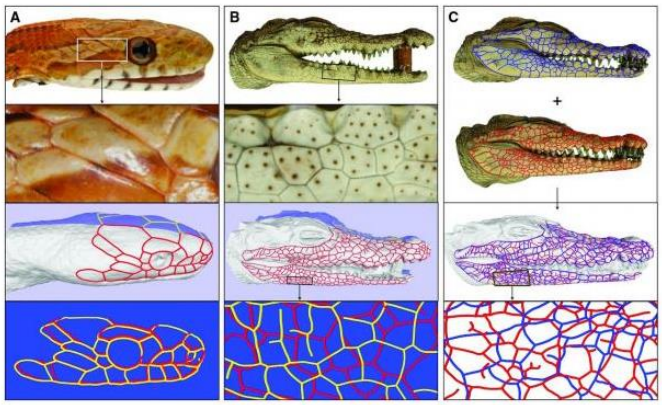


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). (B) Polygonal head scales in crocodiles have a largely random spatial distribution without symmetrical correspondence between left and right. (C) Head scales from different individuals have different distributions of scales' sizes and localizations (blue and red edges from top and bottom crocodiles, respectively).

Method: 3D geometry and color-texture reconstruction | Panel A | Panel B | Panel C

The authors took 120 color pictures of each animal to create detailed, three-dimensional models of reptile heads. Watch this video in which the authors further explain their modeling methods:

http://www.sciencemag.org/content/suppl/2012/11/29/science.1226265.DC1/1...

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

- GLOSSARY
- PREVIOUS WORK
- AUTHOR'S EXPERIMENTS
- CONCLUSIONS
- NEWS AND POLICY LINKS
- CONNECT TO LEARNING STANDARDS
- REFERENCES AND NOTES

References:

The Reference section of each resource is annotated with a short statement about how or why each reference relates to the current research study.

6. L. Lewejohann, B. Zipser, N. Sachser, "Personality" in laboratory mice used for biomedical research: A way of understanding variability? Dev. Psychobiol. 53, 624 (2011).
7. G. Kempermann, The neurogenic reserve hypothesis: What is adult hippocampal neurogenesis good for? Trends Neurosci. 31, 163 (2008).
8. Lewejohann et al., Behavioral phenotyping of a murine model of Alzheimer's disease in a seminaturalistic environment using RFID tracking. Behav. Res. Methods 41, 850 (2009).
9. B. Steineret al., Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46, 41 (2004).
10. Garthe, J. Behr, G. Kempermann, Ad… flexible use of spatially precise learning strategies. PLoS ONE 4, e54…
11. G. Kempermann, New neurons for 'su… 27 (2012).
12. G. Kempermannet al., Why and how … brain plasticity. Front Neurosci 4, 189 (2010).
13. K. Fabelet al., Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Front. Neurosci. 3, 50 (2009).
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16. C. Crabbe, D. Wahlsten, B. C. Dudek, Genetics of mouse behavior: Interactions with laboratory environment. Science 284, 1670 (1999).
17. Lewejohann et al., Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes Brain Behav. 5, 64 (2006).
18. D. W. Bailey, How pure are inbred strains of mice? Immunol. Today 3, 210 (1982).
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21. R. P. Talenset al., Epigenetic variation during the adult lifespan: Cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11, 694 (2012).
22. P. B. Baltes, J. R. Nesselroade, S. W. Cornelius, Multivariate antecedents of structural change in development: A simulation of cumulative environmental patterns. Multivariate Behav. Res. 13, 127 (1978).
23. M.E. Raijmakers, P. C. Molenaar, Modeling developmental transitions in adaptive resonance theory. Dev. Sci. 7, 149 (2004).
24. K. Friston, M. Breakspear, G. Deco, Perception and self-organized instability. Front. Comput. Neurosci. 6, 44 (2012).
25. Van de Weerd et al., Effects of environmental enrichment for mice: Variation in experimental results. J. Appl. Anim. Welf. Sci. 5, 87 (2002).
26. D. P. Wolferet al., Laboratory animal welfare: Cage enrichment and mouse behaviour. Nature 432, 821 (2004).
27. K. Lewin, Dynamic Theory of Personality (McGraw-Hill, New York, 1935).
28. K. L. Jang, R. R. McCrae, A. Angleitner, R. Riemann, W. J. Livesley, Heritability of facet-level traits in a cross-cultural twin

Learning Lens

This paper showed that while both physical activity, like running, and living in an enriched environment can result in the generation of new hippocampal neurons in mice, a combination of the two activities leads to even greater rates of neurogenesis.

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

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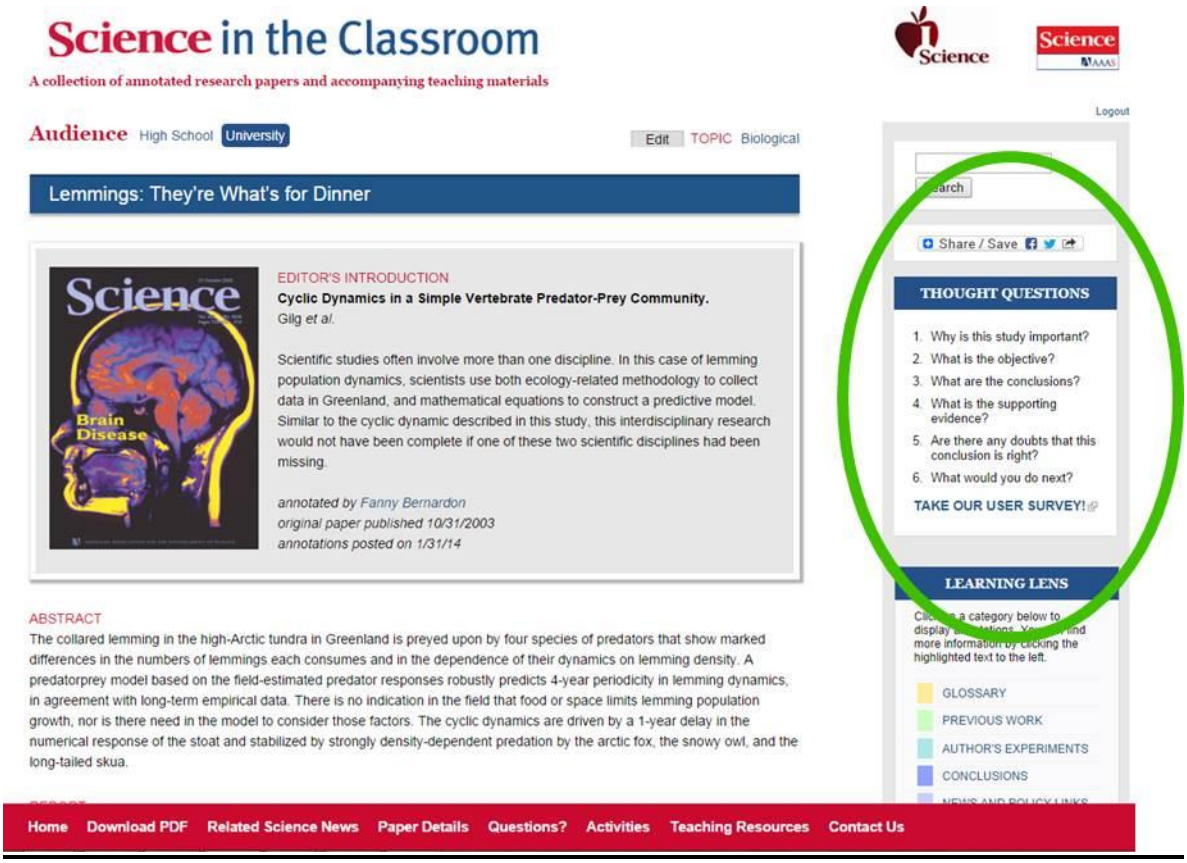
Thought Questions

Thought Questions are located above the Learning Lens in the right sidebar of each resource. These questions were written to be universal and applicable to any primary research paper. Thought questions do not have a single answer, or a correct answer for that matter, and can be used to stimulate discussion among students.



Suggestions for Classroom Use:

In addition to the thought questions discussed above, other resources are provided for use in the classroom. These can be found toward the end of the teacher guides associated with each specific article and include:

1. Discussion questions specific to the article, related to the standards, and/or associated with the figures.

2. Activities tied to the articles.

Some ways to use the *Science* in the Classroom articles:

1. Assign to student groups to read and discuss during class.

2. Assign small sections of the article to student groups to read and discuss during class, with the expectation that they will present or use jigsaw to teach the entire class what is in their part of the article.

3. Assign to individual students to complete during class or as homework.

4. Assign reading as an extra credit project.

Some ideas for interactive student engagement after reading the article:

1. Students write answers to discussion questions (for example, those linked to the standards or those linked to the diagrams).

2. Go over the abstract, as well as information about the purpose and structure of an abstract, and have students write their own abstracts for the articles in language that could be understood by their peers.

3. Have students edit the article, or parts of the article, to a simpler reading level.

4. Have students, alone or in small groups, use the annotated list of references to explain how the scientists who wrote this article built on the published work of at least one independent group of scientists in making their discoveries. In the process, did they produce data that supports the findings of the earlier

publication that they have cited in the text? In what way does this article support the statement that scientific knowledge is built up as a “community effort”?

5. Use the article and discussion questions linked to the standards and the diagrams for a teacher-led classroom discussion. The discussion can focus on the nature of science and scientific research, as well as on the science in the article itself.

6. Have students give a classroom presentation about the article, parts of the article, or their answers to discussion questions.

ARTICLE-SPECIFIC MATERIALS

Connections to the nature of science from the article

- Why do scientists want to genetically manipulate model organisms?
- How does the research in this paper help scientists better achieve this goal?

The importance of this scientific research

- An existing genetic tool is repurposed in a novel way to powerfully control inheritance of desired genetic material.

The actual science involved

- *Drosophila* genetics
- DNA plasmid design
- Genotyping

Connect to Learning Standards:

The Next Generation Science Standards

- Science and Engineering Practice 1: Asking questions
- Science and Engineering Practice 3: Planning and carrying out investigations
- Science and Engineering Practice 4: Analyzing and interpreting data

The AP Biology Standards

- Essential knowledge 3.A.1: DNA, and in some cases, RNA, is the primary source of heritable information.
- Essential knowledge 3.A.3: The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.

Common Core English Language Arts

- 11-12.3: Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks; analyze the specific results based on explanations in the text.
- 11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11-12 texts and topics.

Summary of the Article for the Teacher:

It is recommended that this not be used by students in place of reading the article.

General Overview:

In recent years, the CRISPR-Cas9 gene-editing system has been used as a molecular tool to allow researchers to quickly and effectively create specific DNA mutations in a variety of organisms. This system was originally discovered in *Streptococcus pyogenes* as a way for the bacteria to fight off viral attacks. Here, the authors describe a way to use CRISPR-Cas9 to simultaneously mutate the same gene on both chromosomes, creating a homozygous mutant in a single generation, a process called mutagenic chain reaction (MCR). This technique has many potentially beneficial applications, such as enhancing our ability to create models of human disease, rendering pest populations less threatening, and serving as a delivery system for gene therapy. However, if left unsupervised, MCR is a potentially dangerous new tool.

Topics Covered:

- Mendelian inheritance
- Homology-directed DNA repair
- CRISPR/Cas-9 genome editing
- X-linked inheritance

Why this research is important:

The immediate importance of this study is the discovery of a new tool that will dramatically increase the efficiency of generating mutant organisms for biological and biomedical research. Homozygous mutations are often needed to study the function of a specific gene or the progression of a disease. This study introduces a technique that allows the acquisition of the desired homozygous mutation in half the time as traditional techniques and with 100% efficiency. This technology will greatly enhance the rate of research that uses animal models. The more long-term importance of this new tool is its potential use in global health and in therapy for genetic diseases. The authors suggest that MCR could help us rid the population of disease-harboring insects. It could also be used in gene therapy to treat human disease, because of its power to mutate DNA.

Methods used in the Research:

- Generation of DNA constructs (using the polymerase chain reaction [CR] and restriction enzymes)
- *Drosophila* embryo injection
- *Drosophila* breeding
- PCR
- DNA sequencing

Conclusions:

- The authors designed an MCR construct that, when injected into *Drosophila* embryos, used the process of homology-directed repair to create a homozygous mutation.

- The authors tested their new system by mutating the X-linked y locus in flies. Males injected with the MCR construct were mated with y+ (wild-type) females, which resulted in y- (mutant) females, a result that could not occur by Mendelian inheritance of a recessive allele.

- F_2 progeny of females expressing the MCR allele with wild-type males had a full-bodied y- phenotype at a rate of 97%, much greater than the expected 50% transmission of an X-linked allele (only in males).

Areas of Further Study:

- Can this technology be applied to other organisms? Mice? Humans?
- How could this be used to treat human disease?
- How can we engineer this technology to protect against accidental release of MCR organisms into the environment?

Discussion Questions:

- CRISPR/Cas9 was originally discovered in bacteria as a mechanism to protect the bacteria against viruses. Why would it be advantageous for bacteria to have a genetically encoded mechanism like this? How might this mechanism work to protect the organism?

- Can you think of examples of human diseases that could be modeled using MCR to create homozygous mutations of a specific gene?

- Why do you think the authors decided to use MCR to mutate the *y* locus in *Drosophila*?

- In the current paper, the authors use MCR to create an insertional mutation in the region of interest, but they postulate that creating deletions of genes is also possible with this technology. Can you design a construct that would allow for deletion of a specific gene?

- Why do you think it would be dangerous if MCR organisms were unintentionally released into the environment?

Discussion questions associated with the figures

Fig. 1:

- Why was it so important for the authors to include homology arms in their DNA construct?

- Authors take advantage of an endogenous DNA repair mechanism called homology-directed repair. What is another mechanism of DNA repair? What are the advantages and disadvantages of both mechanisms?

Fig. 2:

- Panels E and F: How would the results of the fly crosses have been different if the mutated gene had been somatic rather than X-linked?

- Panel E: Why do you think the authors never saw mosaic males among the F_2 progeny?