

**TEACHER'S GUIDE**

# **DNA** to Darwin Case Study

## **Evolution of colour vision in primates**

**Jarosław Bryk**

Max Planck Institute for  
Evolutionary Biology, Plön

**Version 1.1**



*Charles Darwin*

# Evolution of colour vision in primates

In this activity students explore the molecular basis and evolutionary origin of trichromatic (red/green/blue) colour vision in humans and our close evolutionary relatives using nucleic acid sequences of opsins, key proteins involved in the process.

## Colour vision was lost by mammals

Colour vision evolved relatively early: most bony fish, reptiles and birds have excellent colour vision. The first mammals, however, are thought to have been largely nocturnal species. For them, the ability to see in low-light conditions was at a premium, rather than the ability to see in colour. The ancestors of modern mammals consequently lost colour vision at the time of the dinosaurs.

Some primates, including humans, have re-evolved colour vision. This ability applies mainly to Old World (African and Asian) primates. In contrast, with the exception of Howler monkeys, all male and some female New World primates lack colour vision.

In mammals, light is focused by the lens of the eye on a layer of rod and cone cells in the retina that contain visual pigments. These pigments are proteins called opsins or rhodopsins. Rhodopsin, the protein involved in low-light vision, is found in the rod cells. Each cone cell contains one of three sorts of opsins, each of which is sensitive to a different range of wavelengths of light. Usually these are referred to as red, green and blue receptors, although their peak sensitivities are actually to violet, green and yellow-green light.

One of the opsins, the 'blue' or short-wavelength-sensitive (SWS) variety, is encoded by a gene on chromosome 7. The genes encoding medium-wavelength-sensitive (MWS or 'green') and long-wavelength-sensitive (LWS or 'red') opsins are found next to each other on the X chromosome.

Humans usually have one copy of the *LWS* gene and several copies of the *MWS* gene. Their arrangement and proximity suggests that they have arisen by gene duplication. In this process, a gene is duplicated and one of the copies accumulates mutations and can become a 'new' gene encoding a protein with a different function. About 40% of the genes in the human genome may have evolved in this way. The two modern opsin genes are thought to have arisen from a single gene in a primate ancestor, similar to the opsin gene found in modern rodents and some other mammals.

Because most New World primates lack full-colour vision, the split to produce the *MWS* and *LWS* gene must have happened *after* continental drift

formed the separate continents of Africa and South America some 30–40 million years ago.

Selection pressure acts to maintain three-colour (trichromatic) vision in Old World primates — very few Old World primates are colour blind, although about 10% of humans are, presumably because selection does not operate on us in the same way as wild monkeys and apes.

Ecological studies suggest that there is an evolutionary advantage to trichromats when searching for fresh leaves in a forest environment. Significantly, howler monkeys in the New World are diurnal and have a leaf-based diet, and search for fresh red-tinged leaves which are nutritious and readily-digestible. The nocturnal South American ‘night monkeys’ (*Aotus* spp.) in contrast, lack not only the *MWS* and *LWS* genes but also the *SWS* sort. In their case, there is no advantage to seeing in colour, and selection pressure on their opsin genes is relaxed.

## Outline of the activity

There are five main parts to the activity:

- Comparing human *MWS* and *LWS* mRNAs to see how similar they are;
- Testing whether the *MWS/LWS* split is likely to be ancient or relatively recent;
- Comparing the mRNA sequences of Old World primates’ *MWS* and *LWS* opsins with those of the New World howler monkey, which is also a trichromat;
- Comparing the mRNA sequences of opsins of Old World and New World primates more generally;
- Looking for evidence of convergent evolution in the opsin genes of New and Old World primates.

## General reading

*The making of the fittest. DNA and the ultimate forensic record of evolution* by Sean B. Carroll (2009) Quercus Books (Paperback) ISBN: 978 1847247247.

*A popular account of some of the molecular evidence for evolution. Chapters four and six cover the evolution of colour vision in primates and other species in detail.*

*Reading the story in DNA: A beginner’s guide to molecular evolution* by Lindell Bromham (2008) Oxford University Press (Paperback) ISBN: 978 0199290918. *An engaging textbook on molecular evolution, which assumes no specialist mathematical knowledge and takes the reader from first principles. Although it’s aimed at undergraduates, this superb book contains sufficient detail for PhD students, yet parts will appeal equally to 16–19 year-olds.*

## Scientific publications

Some of these publications may require payment to access them.

Nathans, J. (1999) The evolution and physiology of human color vision: insights from molecular genetic studies of visual pigments. *Neuron*, 24 (2) 299–312. A useful review paper.

Yokoyama, S. (2008) Evolution of dim-light and color vision pigments. *Annual Review of Genomics and Human Genetics* 9, 259–82. doi: 10.1146/annurev.genom.9.081307.164228. Another review paper.

Hunt, D. et al (1998) Molecular evolution of trichromacy in primates. *Vision Research*, 38 (21) 3299–306. The paper that started this field of research.

Online Mendelian Inheritance in Man (search for ‘opsin’)  
[www.ncbi.nlm.nih.gov/omim](http://www.ncbi.nlm.nih.gov/omim)

## Requirements

### Software

The software required, *Geneious*, can be downloaded free-of-charge from: [www.geneious.com](http://www.geneious.com). The software is available for Windows, Macintosh and Linux operating systems. Only the free, ‘basic’ version of the software is required for this activity.

### DNA sequence data

Students will need the *Geneious* documents containing various mRNA sequences of opsins from primates: **human\_green\_red.geneious**, **origin\_green\_red.geneious**, **origin\_green\_red\_howler.geneious**, **origin\_new\_old\_world.geneious** and **convergence561.geneious**.

### Students’ worksheets

Students will require copies of Student’s Guide, pages 2–12.

### Presentations

Teachers may find the *QuickTime* animations in the *Introductory Activities*, which are in a separate document, useful here. *QuickTime* may be downloaded free-of-charge from the Apple web site: [www.apple.com/quicktime](http://www.apple.com/quicktime). The *PowerPoint* or *Keynote* presentations that accompany this *Case Study* should be helpful for introducing this exercise.

## Educational aims

This activity provides an introduction to the topic of colour vision, including the genetics of colour blindness. It reinforces students’ understanding of DNA and RNA structure and function, introduces the principle of alignment and generating evolutionary trees from molecular data, and looking at sequence data for evidence of convergent evolution. It can also be used to introduce biogeographical and ecological evidence for evolution.

## Prerequisite knowledge

Students will need to understand the structure of DNA (*i.e.*, that DNA is comprised of nucleotides) and that it is translated into mRNA. They will need to be taught the principle of sequence alignment (the *Introductory Activities*, which are in a separate document, will be useful.)

It would be helpful if they were familiar with the structure and function of the mammalian eye.

## Answers to the questions on the worksheets

### Page 5

- a. The two sequences are almost 98% identical.
- b. It's not possible to tell from the mRNA data which changes may be responsible for the different properties of the proteins, although looking at changes in the amino acid sequences might provide useful clues.
- c. As suggested on page 3, opsin proteins can be synthesized *in vitro* and their properties investigated.

### Page 6

- d. The answer to this question is suggested in the text and in the following exercise on page 7. It involves building a phylogeny using sequences of MWS and LWS opsins and studying the pattern of relationships in the tree.

### Page 8

- e. The tree indicates that all MWS sequences are most similar to one another and not to LWS sequences (and *vice versa*).
- f. This supports the hypothesis that the MWS and LWS genes formed following an ancient duplication event. It suggests that MWS and LWS opsin genes originated *via* duplication before the divergence of the extant Old World monkeys.

### Page 9

- g. In this tree the howler monkey's MWS and LWS opsin sequences are most similar to each other, and not to MWS or LWS sequences from other species. It suggests that trichromatic colour vision in howler monkeys is the result of either an independent duplication or possibly howler monkeys' MWS and LWS opsins are just variants of a single gene.
- h. Since both MWS and LWS opsin genes from howler monkey are more closely related to the MWS opsins from Old World species, it suggests that the MWS opsin is the ancestral sequence that duplicated in the Old World species. The two sequences (the 'old' MWS and its newly-duplicated copy) subsequently diverged; one of the sequences acquired mutations that influenced its light sensitivity and formed what we now call LWS opsin.
- i. Howler monkeys have three opsin genes, not two.

- j. This is explained on the page and in the diagram on page 10.
- k. This is partly explained in the diagram on page 10. Female New World monkeys can be trichromats if they have different alleles on their two X chromosomes; if they have the same allele, they are, like all the males, colour blind. In humans, the situation is slightly different. Both males and females are usually trichromats as they have three opsin genes. If however, one or more of the *MWS* or *LWS* opsin genes on the males' X chromosome is lost or mutated, they will be colour blind as they do not have another X chromosome with a functional gene. Human females are unlikely to lose the genes on both of their X chromosomes, so are far less likely to be colour blind.
- l. Rare forms of colour blindness can occur in those who have different alleles of the opsin genes (rather than, as in Daltonism, lacking functional genes entirely). About 1% of women have a fourth colour opsin (and therefore four types of cone cell) and can be considered tetrachromats although it is not clear whether this provides an advantage in colour discrimination.

#### Page 10

- m. Human females have two X chromosomes; males only one. Therefore a mutation in a gene on a male's X chromosome will not be compensated for by a corresponding functional allele on a homologous chromosome. For a female to be colour blind, the genes on both X chromosomes must be mutated or absent.

#### Page 11

- n. These species are shown in the slide presentation.
- o. The tree shows that New World monkey opsins form a distinct branch in the tree and are all more closely related to one another than they are to the opsins of Old World Monkeys.
- p. The relationships between different opsins from different species is less clear in New World monkeys than in the Old World species. For instance, spider monkeys, who are closely related to howler monkeys, have only two variants of opsins, 553 and 538, and they form a distinct group (clade), just like howler monkeys. Other species, which have three variants of opsins (usually about 530, 550 and 560 nm), are scattered throughout the tree. But 561 opsins from the capuchin, marmoset and squirrel monkey are more closely related to one another than to other opsins from their respective species — similar to the pattern found in *LWS* and *MWS* opsins in the Old World species.
- q. The New World monkey's opsins are similar to *MWS* opsins from the Old World, confirming our finding from earlier.

#### Page 12

- r. A small number of amino acids in the opsins appear to be critical in affecting the sensitivity of the protein molecule to light. Other changes to the protein either disrupt its function entirely by altering its structure or have no significant effect upon the wavelength of light absorbed.
- s. It is thought that in primates trichromacy offers an advantage to diurnal, largely herbivorous species in food selection.