**FRS 146**
**What is a Great Experiment?**

## Syllabus

**Week 1 What are the ingredients of an experiment?**

The first class will introduce you to the theme of the course – what makes a great experiment? We will start with a consideration of what constitutes *any* experiment, and why each element is critical to its overall success and impact. We will consider whether there are different kinds of experiments – those that begin with a hypothesis and those that do not.

**Week 2 Mendel's Discovery of the Laws of Inheritance**

- Gregor Mendel. Experiments in Plant Hybridization (1865) English translation by William Bateson (http://www.mendelweb.org/Mendel.html)

We begin this course with the founder of the field of genetics, Gregor Mendel, a 19th century Austrian monk, who worked in relative obscurity, and whose fundamental discoveries were ignored and forgotten until they were rediscovered in the 20th century. Most of you have probably learned about his experiments in your biology classes, but few students – not to mention working scientists - have actually read his original paper. It is truly a classic example of a great experiment, and so we will begin with Mendel. The paper is remarkably easy to read, in part because it could be as long as Mendel needed, so he can lay out his thinking and his conclusions very clearly. We will see that as we get to the late 20th century, papers become more terse as journals apply page and word limits, and consequently they become harder to follow. Quelle domage! It will help you if you start with a review of the paper, and Mendel's great discoveries, as a refresher course before tackling the original paper. This can be found in:

- John A. Moore. Mendelism. In Heredity and Development, Second Edition. Oxford University Press pg. 50-69 (http://www.nap.edu/catalog.php?record_id=13199)

**Week 3 Darwin's Theory of Natural Selection**

- B. Rosemary Grant and Peter R. Grant. (1993) Evolution of Darwin's Finches caused by a Rare Climatic event. Proc. R. Soc. Lond. B 251, 111-117.

The foundations of modern biology rest on the work of the two great 19th century giants, Gregor Mendel and Charles Darwin. Darwin's magnum opus, *The Origin of Species,* was first published in 1859, in response to his discovery that another scientist, Alfred Russel Wallace, had independently proposed natural selection as the underlying cause of evolution of species. The reason the theory is attached to Darwin's name in the 21st century is largely due to the publication of *Origin,* which laid out with intellectual rigor the evidence for the theory. Discussion of this extraordinary work could be the subject of an entire freshman seminar. Consequently it is difficult to pick out the key chapter for you to read as the long manuscript is a sustained argument that cannot be atomized. For those of you who are interested in the history of science I strongly recommend it to you. Instead we will read a modern paper by two emeritus Princeton faculty members, Peter and Rosemary Grant, who spent their entire careers studying Darwin's finches on a rocky outcrop in the Galapagos Islands. This is truly a classic in evolutionary biology, as it demonstrates evolution in real time. For background reading about the paper and the Grants, I am providing three chapters from the Pulitzer Prize-winning book *The Beak of the Finch*, by Jonathan Weiner. The first gives you a glimpse of what field work in the Galapagos is like; the second talks about Darwin's own experience on those islands and the third (entitled Princeton!) gives you a sense of the data analysis entailed in these studies. I commend the whole book to anyone interested in modern evolutionary biology.

- Jonathan Weiner. *The Beak of the Finch,* Vintage Press. Chapters 1, 2 and 8

Finally we will briefly discuss the paper by Almen et al., which provides you with an up-to-date perspective on using whole genome sequencing to begin to analyze the evolution of Darwin's finches, and to identify genes that affect the beak shape and size. YTou should not worry about understanding each and every word, but get a sense of how far the Grants' story has come in the age of the genome.

- Almen et al. Adaptive radiation of Darwin's finches revisited using whole genome sequencing. (2015) Bioessays 38, 14-20.

**Week 4 The Mechanism and Initiation of DNA Replication**

- Matthew Meselson and Frank Stahl (1958) The Replication of DNA in *Escherichia Coli*. Proc. Nat. Acad. Sci. (USA) 44, 671-682.

- Kathleen J. Danna and Daniel Nathans (1972) Bidirectional Replication of Simian Virus 40 DNA. Proc. Nat. Acad. Sci. (USA) 69, 3097-3100.

This week we will read two of the most beautiful experiments in molecular biology. Shortly after the proposal by Watson and Crick of the double helical nature of DNA, the crucial molecule that transmits information from one generation to the

next, Meselson and Stahl tackle the question of how such a molecule might be replicated at each cell division. Their experimental design was elegant, and their conclusions definitive. Fourteen years later Danna and Nathans were asking a new question about the replication of DNA - how does it begin and how does it proceed. Their strategy is also a classic, and illustrates how a well-designed experiment can reach unequivocal conclusions.

**Week 5 The Reversibility of Developmental Decision-Making**

- John Gurdon (1962) The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cell of Feeding Tadpoles. J. embryo. Exp. Morph, 10, 622-40.

The one-celled fertilized egg contains all the genetic information that is necessary to form the entire future organism. As the fertilized egg begins to divide, the daughter cells begin to "learn" their ultimate developmental fate, and to express only the genes that are necessary to execute that fate. Gurdon asked a fundamental question in this 1962 classic – is the decision-making that cells undergo as they progress through development irreversible, or is it possible to take a fully mature differentiated cell and reverse that decision-making to bring it back to its embryonic state? The dogma at the time was that it is irreversible. Before anyone had a clue as to what "decision-making" actually entailed at a molecular level Gurdon goes to great lengths to test that premise using cells and eggs from the frog Xenopus laevis. A good introduction to the topic, and a fast forward to today comes from the Nobel committee's summary, who awarded Gurdon the Nobel Prize in Physiology and Medicine for this work in 2012. Sir John Gurdon's Nobel lecture gives a great deal of background on his discovery, and again brings the topic up to date with the work of his co-Nobelist, Shinya Yamanaka for his work on induced embryonic stem cells.

- Mature Cells can be reprogrammed to become pluripotent. (2012) https://www.nobelprize.org/nobel_prizes/medicine/laureates/2012/advanced-medicineprize2012.pdf
- Sir John B. Gurdon (2013) The Egg and the Nucleus: A Battle for Supremacy. Nobel Lecture https://www.nobelprize.org/nobel_prizes/medicine/laureates/2012/gurdon-lecture.pdf

**Week 6 The Non-Equivalence of the Parental Mammalian Genomes**

- James McGrath and Davor Solter. (1984) Completion of Mouse Embryogenesis Requires both the Maternal and Paternal Genomes. Cell 37, 179-1983.

This week we will read a classic paper that asked a different question that is relevant to development in mammals: does development require the contribution of both a dogma on this question, but there was a single report from one group that claimed that the two

parental genomes were equivalent. This paper is a good example of the difficulty of proving a negative. I am including in your reading this week a chapter that chronicles the story behind this discovery – including a cautionary tale about a scientist who fabricated his results. It can be found as a PDF on Blackboard.

- Gina Kolata. Three Cloned Mice in Clone: The Road to Dolly and the Path Ahead. William Morrow. (1997)

The following is a more recent review of the topic, with information about the implications of Solter's findings.

- Randy L. Jirtle and Jennifer R. Weidman. Imprinted and More Equal. American Scientist March April 2007, pg. 143-149.

**F A L L B R E A K**

**Week 7 A Morphogen Gradient Determines the Body Plan**

- Wolfgang Driever and Christiane Nusslein-Volhard (1988) The *bicoid* protein determines position in the Drosophila embryo in a concentration-dependent manner. Cell 54, 95-104.

One of the oldest but most elusive ideas in developmental biology is the influence of morphogen gradients – thought to be small molecules that affect cell fate in a concentration-dependent manner. Despite the interest in such molecules, it was hard to identify and verify their existence. This paper by Driever and Nusslein-Volhard is a landmark in the field – establishing for the first time that a protein called bicoid can act as a morphogen. The authors use two methods to assess how changes in the concentration of the morphogen affect the fate of cells in the early *Drosophila* embryo. One of the strategies involves mutants that affect cell fate to test whether bicoid protein is affected; the other is to change the concentration of bicoid protein in the embryo by altering the dosage of the gene. Both approaches illustrate the power of genetics to dissect a critical biological phenomenon.

To get comfortable with the topic, you should start your reading with a Scientific American article by Christiane Nusslein-Volhard:

- Gradients that Organize Embryo Development. Scientific American pg. 54-61. August 1996

A more recent review of the history of morphogen gradients, written for a more scientific audience, has sections directly relevant to the paper as well:

- Anne Ephrussi and Daniel St. Johnston. Seeing is Believing: The Bicoid Morphogen Gradient Matures. Cell 116, 143-152 (2004).

**Week 8 Cell-cell interactions During Development**

- Geraldine Seydoux and Iva Greenwald. Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans* Cell 57, 1237-1245 (1989).

The paper by Seydoux and Greenwald describes the elegant use of both genetics and laser ablation to study the role that cell-cell interaction plays in a critical cell fate decision in the soil worm *C. elegans*. The authors knew at the outset the product of a specific gene, lin-12, was required for an either-or the cell fate decision of two cells in the early embryo, but not how. The model which they propose at the end of the paper has held up very well over time. The importance of this work became clear when its findings were generalized for many cell-cell interactions in many organisms, including humans.

**Week 9 The Molecular Basis for the Sense of Smell**

- Linda Buck and Richard Axel (1991) A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition. Cell 65, 175-187.

One of the most beautiful collection of studies in modern biology comes from the laboratory of Richard Axel, who together with his postdoctoral fellow Linda Buck, were awarded the Nobel Prize in 2004. The paper we will read this week describes their effort to unravel the molecular basis for our sense of smell. How is it that humans can detect and discriminate among over 10,000 odorants? How is this capacity encoded in the genome? At the time of this study it was assumed that there were one or more receptors in the nasal epithelium that detected the presence of small molecule odorants, but no one had a strategy for identifying them, much less knowledge of how they might translate a molecular structure (the odorant) into a cognitive reaction (the sense of smell). This paper describes the clever and laborious approach that broke open the field.

To provide a background to the field, as well as an update on the work that Axel continued to understand how odorant receptors transduce the knowledge of smell, you should start by reading his Scientific American article. A more recent review which is more detailed is provided in his Nobel Lecture.

- Richard Axel. The Molecular Logic of Smell. Scientific American. October 1995 pgs. 154-159. (PDF on Blackboard)

- Richard Axel. Scents and Sensibility: A Molecular Logic of Olfactory Perception. Angew. Chem. Int. Ed. 44, 6111-6127 (2005). (http://www.nobelprize.org/nobel_prizes/medicine/laureates/2004/axel-lecture.pdf).

**Week 10 Proteins as Infectious Agents**

- Stanley B. Prusiner. Novel Proteinaceous Infectious Particles Cause Scrapie. Science 216, 136-144 (1982).

This landmark paper by Stanley Prusiner provides an example of two phenomena in science – most importantly the amount of evidence that is needed if you are going to overturn dogma. It also illustrates the challenges that biochemists face when they begin to characterize a biological entity, whether the target is a protein, nucleic acid, organelle or small molecule. Biochemistry is messy until you have a purified substance. There are a lot of specific techniques used in this paper, the details of which are not so important for our discussion. What you should concentrate upon is the logic of each approach and the care with which the author interprets his results.

To put this paper into perspective you should start with a more recent paper by Prusiner in Scientific American, which tells the story and brings it up to date. We will also discuss the advances in the understanding of prion diseases that Prusiner writes about in this excellent review. For more up-to-date implications for human health, two other reviews are provided. The one by Heller is intended for a general audience. The other in Science is more technical.

- Stanley B. Prusiner. The Prion Diseases. Scientific American pg. 48-57 January 1995
- Danielle Heller. The Spreading confusion: Rethinking Alzheimer's disease. Harvard University School of Graduate Arts and Sciences. (2015)http://sitn.hms.harvard.edu/flash/2015/the-spreading-confusion-rethinking-alzheimers-disease/
- Michael Goedert. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled A□□ tau and □-synuclein. Science 349, 601 (2015)

**Week 11 RNAi – A New Mechanism for Gene Control**

- Andrew Fire, SiQun Xu, Mary K. Montgomery, Steven A. Kostas, Samuel Driver and Craig C. Mello. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806-811.

This paper provides an example of scientists making a major discovery unexpectedly. Fire and Mello were interested in developing tools to suppress the expression of genes in the small soil worm Caenorhabditis elegans. What they discovered in the

process was an entirely new mechanism for gene regulation in all plants and animals. No one was more surprised than they by their findings, which they pursued doggedly and rigorously, as they were describing something no one had expected. This paper is a great example of the power of "following your nose" when a scientist taken by surprise. The work was awarded the Nobel Prize in 2006, a shockingly short time by Nobel standards following their discovery.

A good summary of their findings and their significance can be found in the Nobel committee's summary of their work.

http://www.nobelprize.org/nobel_prizes/medicine/laureates/2006/popular-medicineprize2006.pdf

A more detailed summary is also provided in the following: http://www.nobelprize.org/nobel_prizes/medicine/laureates/2006/advanced-medicineprize2006.pdf

**Week 12 Summing up – What Makes a Great Experiment?**

In this class students will be assigned to review the experiments that have been discussed, emphasizing the aspects in each that were exemplary, and which could have been improved.