Activity #7. Human Genomics

Learning Goals:

- To review the vocabulary of genomics
- To understand that different alleles of a gene will give proteins with different functions, which may confer selective advantages in specific geographic environments
- To appreciate that most human traits are NOT inherited in classical Mendelian fashion
- To appreciate levels of variation in specific phenotypes, such as skin, hair, and eye color, color vision, PTC tasting, ABO blood typing, and Rh antigens
- To understand how blood typing is performed
- To search the Online Mendelian Inheritance in Man (OMIM) database for the genetic basis underlying these traits
- To understand how a single-gene mutation could have multiple phenotypic effects
- To understand how mutations in different genes could have the same effect

Overview

Let us begin by defining terms:

- **Mutation** - a heritable change in a DNA sequence
- **Allele** – different form of a gene or chromosomal locus
- **Polymorphism** – Variation in DNA sequence
- **Single nucleotide polymorphism (SNP**) - a difference of a single base within a DNA sequence
- **Haplotype** – a set of polymorphisms that tend to be inherited together (linked)

Last week, we examined the inheritance of kernel color and kernel shape traits in corn. In that relatively simple example, we focused on just two alleles for each of 2 genes. There are undoubtedly additional alleles and additional genes that influence these traits as well. As we begin to examine inheritance in humans, it is important to recognize that throughout human evolution, many mutations have occurred, creating many different alleles for the ~25,000 genes in our genome. Some of these mutations have been disadvantageous and have been lost due to natural selection. Others have conferred an advantage and have increased in frequency within the human population. Most have been neutral and have remained at low frequencies. These mutations have been passed on throughout the generations to us.

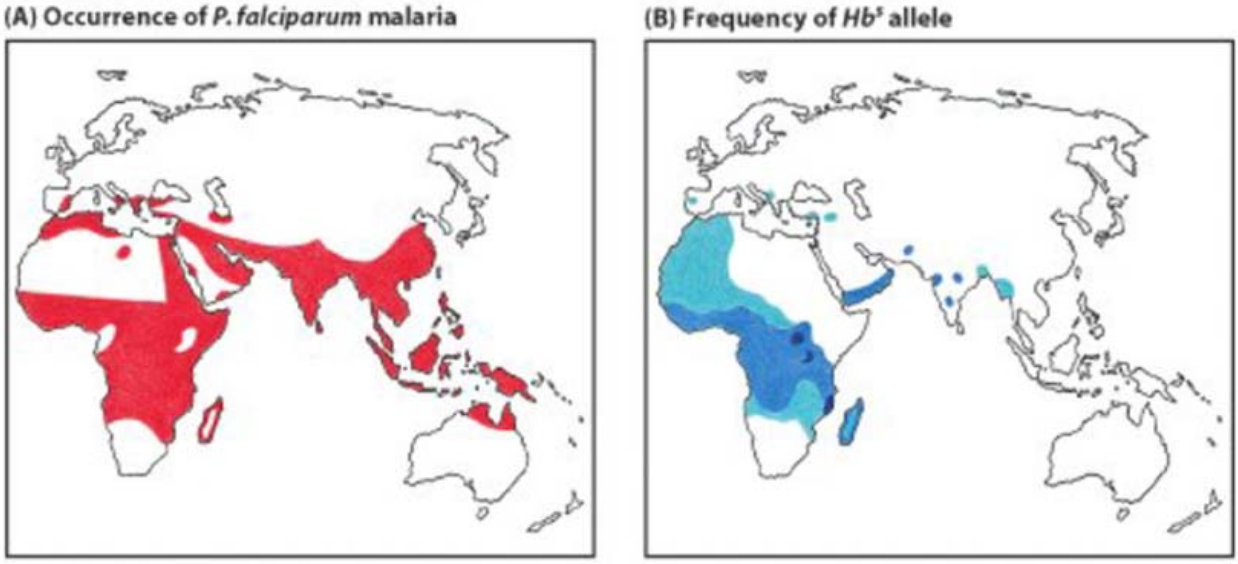
Connecting DNA to RNA to protein to function to selection to population genetics

Before we jump in to manipulating databases of human genome information, it's important to understand the relationship between changes in our genome, individual traits, and the distribution of genes within a population.

One example of a mutation that confers an advantage under specific selective conditions is the sickle cell anemia allele. Recall from class that the sickle cell allele is a single nucleotide change in the DNA sequence of the gene that encodes the hemoglobin beta chain protein. The normal, "wild-type" allele has a T, while the mutant sickle cell allele has an A in its place. This change in the DNA causes a change in one nucleotide of one codon in the corresponding mRNA, from CTT to CAT. When the mutant RNA is translated, a mutant hemoglobin β protein is made, replacing the normal Glutamate with a Valine. Normally the Glutamate amino acid's charged, hydrophilic R group would be located on the outside of the protein, facing the aqueous environment. However, Valine is not charged and is hydrophobic, so the mutant amino acid's R group tends to be buried in the hydrophobic interior of the protein, distorting the protein's overall structure. This change in protein structure causes the hemoglobin molecules to stick together under low-oxygen conditions.

This change in protein stickiness creates large arrays of aggregated hemoglobin, causing the affected red blood cells to form a rigid sickle shape, instead of the normal flexible curved shape. Sickled red blood cells cause reduced blood flow through tiny capillaries, often leading to a sickle cell crisis that includes organ failure and even death. So why would a mutation that causes such serious disease be maintained in the population?

The answer lies in a special property of blood cells from people who carry one copy of the sickle cell allele: they are less efficient at supporting the life cycle of the malaria parasite *Plasmodium falciparum* than normal red blood cells. So in regions of the world where malaria occurs, humans who carry one sickle cell allele are protected from the most serious forms of malaria. The tradeoff is the risk of two such heterozygous protected humans producing homozygous offspring that carry two sickle cell alleles. So although two copies of the sickle cell allele produce potentially lethal sickle cell disease, one copy protects humans against malaria. This explains why human populations from tropical Africa, where the malaria parasite is common, exhibit high frequencies of the sickle cell allele, up to 25% or more.



from Minkoff and Baker (2004) Biology Today, 3e, Garland Science Publishers, NY, NY.

This example demonstrates the significance of small changes in the human genome: even a single nucleotide polymorphism (SNP) like the sickle cell mutation can have consequences for individuals and for worldwide populations.

Some History of Human Genome Sequence studies:

Early studies of human genetics began with observations of the inheritance of phenotypes (usually genetic disorders) and then painstakingly compared the inheritance of the trait with the inheritance of testable markers scattered widely throughout the genome. When a general chromosomal area was identified, this area of the DNA was cloned from individuals with the phenotype, and the DNA sequence was compared to that of people who did not show the phenotype to identify differences. Additional studies were then needed to show how the sequence difference could have caused the observed phenotype. While these studies were effective in identifying common and simple genetic mutations causing disorders such as sickle cell anemia, they were less effective for identifying the many different alleles in different genes that contribute to complex traits, such as skin color, height, intelligence, and predisposition to diseases such as heart disease or hypercholesterolemia.

For the human genome sequencing project, a pool of DNA was used from several different volunteers with ancestries from different parts of the world. Of course, their genomes were not identical. A reference sequence corresponding to the most common variant for each locus was generated and **single nucleotide polymorphisms (SNPs)** were noted. The distribution of these SNPs is not uniform across the human population. As some groups of humans migrated away from others, mutations that occurred in the migrating group were not present in the group that was left behind. Studying the distribution of these SNPs can give us insight into large-scale human population movements over time, and into the ancestral relationships between different groups of human populations.

Subsequent efforts to sequence more human DNA have identified many additional variants (122,786,837 as of 11/04/2014 - http://www.ncbi.nlm.nih.gov/snp/?term=txid9606[Organism:noexp]). Many of these were generated by identifying DNA sequence variations in 270 ethnically diverse individuals participating in the International HapMap Project, which aims to document the different haplotypes found in distinct human populations around the world (http://www.hapmap.org/index.html).

A. Variation in phenotype - Determine your phenotype for each of the following traits.

_____ **Skin Pigmentation** – Examine the pigmentation of the skin on your arm and rate the extent of pigmentation on a scale from 1 to 10 with 1 representing extremely fair (albino) and 10 representing exceptionally dark skin. Describe your skin shade using one of the following terms: milky, amber, copper, peach, olive, brown, black. President Barack Obama's pigmentation is about a 6.
--Does the range of skin color in our class support a simple Mendelian inheritance pattern for human skin color, or not?

_____ **Hair Pigmentation** - Examine the pigmentation of your hair and rate the extent of pigmentation on a scale from 1 to 10 with 1 representing extremely fair (white) and 10 representing exceptionally dark hair (black). Describe your hair color using one of the following terms: white, blonde, dirty blonde, sandy, light brown, dark brown, brunette, black, red, auburn.
--Does the range of hair color in our class support a simple Mendelian inheritance pattern for human hair color, or not?

_____ **Iris Pigmentation** - Examine the pigmentation of your irises and rate the extent of pigmentation on a scale from 1 to 10 with 1 representing extremely fair (pale blue) and 10 representing exceptionally dark eyes (black). Describe your eye color using one of the following terms: light blue deep blue, grey, green, hazel, light brown, dark brown, black.

_____ **Color Vision Testing** – Examine the charts available in the laboratory and record whether you have normal, protan or deutan color vision. The first 4 plates are to calibrate your eyes. up to plate 14 will study your red/green color vision. Plates 15-19 will distinguish between several distinct red/green mutations.

_____ **Phenylthiocarbamide (PTC) tasting** – Place a strip of paper impregnated with PTC on your tongue and rate the extent of your ability to taste the bitterness on a scale of 1-10 with 1 representing no taste at all, and 10 representing the most unpleasantly bitter taste you've ever experienced. Also indicate whether you like broccoli.

_____ **ABO blood type** – Follow the blood typing procedure described below to determine whether the A and/or B blood group antigens are present on your red blood cells. Indicate "A" if agglutination occurs with only the anti-A antibody, "B" if agglutination occurs with only the anti-B antibody, "AB" if agglutination occurs with both antibodies, and "O" if agglutination occurs with neither antibody.

_____ **Rh antigen** - Follow the blood typing procedure described below to determine whether the Rh antigen is present on your red blood cells. Indicate "+" if agglutination occurs with the anti-Rh antibody, "-" if agglutination does not occur with the anti-Rh antibody.

B. Serological Agglutination to determine Blood Type

Serological **agglutination** is an easy and routinely performed in vitro immunological procedure. It involves mixing a cellular antigen with its homologous antibody either in a tube or on a microscope slide. When the antibodies react with the cellular antigens a clustering of the cellular antigens result. This is because the antibodies are at least divalent, that is they have at least two active sites that can combine with antigenic determinant sites (epitopes). Consequently, antibodies can react with more than one cellular antigen thus binding or crosslinking them together. The cellular antigen is multivalent with regard to antigen epitopes and therefore can react with many antibody molecules. When a suspension of the cellular antigen is mixed with an antiserum (a serum that contains antibodies), **a lattice between antigen and antibody is built up** as the reaction proceeds, and the cells are "glued" together into a cluster or clump. The word “agglutinate” comes from the Latin word agglutinare which means to stick together.

The agglutination reaction can be used to determine **cellular identity**. For example, a known antisera can be used to identify cells of an unknown type. Since antibodies are relatively specific for antigens only homologous systems should show agglutination. This type of testing has been used to determine red blood cell (RBC) type. By mixing RBC's with specific antisera and observing the agglutination patterns it can be determined if the cells are type A, B, AB or O.

The Rh factor is also determined by an agglutination reaction. Rh-positive individuals produce the Rh antigen on the surface of their red blood cells, while Rh-negative individuals do not. An Rh-negative mother may mount an immune response against her Rh-positive fetus’s developing red blood cells if she breeds with an Rh-positive partner.

Agglutination reactions can also be used to test for **food contamination** with bacteria that can cause disease, as in the case of the contaminated hamburger meat in the Pacific northwest some years ago. In this example the unknown cell is a bacterial cell. Bacteria cultured from meat can be reacted with antiserum known to contain antibodies specific for the pathogen. If the bacteria from the meat are agglutinated it indicates the meat is contaminated with the pathogen, If agglutination does not occur it indicates the meat is not contaminated with the pathogen.

Agglutination reactions are used in the **diagnosis of infectious disease**. If a person becomes infected, the immune system will respond by producing antibodies that are specific for the infecting pathogen. The antibodies reside in body fluids including serum. To determine if a person has been infected by a particular pathogen one collects a blood specimen, and allows the blood to coagulate. One then collects the fluid that remains after clotting, which is called serum. The serum contains antibodies for all antigens to which the person has been exposed. If one mixes the serum with a suspension of the cellular antigen, agglutination will occur if antibodies are present. This of course means the person has been infected, and the infection resulted in the

production of serum antibodies. If on the other hand agglutination does not occur, the interpretation is that the serum does not contain antibodies for the antigen at the time of testing. A variation of this theme is used as an initial screening test to determine if a person has been infected with the HIV virus that causes AIDS. If a person tests HIV positive, it means that the person has been infected with the Human Immunodeficiency Virus and the immune system responded to this infection by producing antibodies. If a person tests HIV negative it means that the test did not detect antibodies to the HIV and it is assumed that the person has not been infected. Re-testing several months later is necessary to confirm that this person was not simply in the early stages of HIV infection, prior to antibody production.

Determination of RBC Type by Slide Agglutination.

When working with human body fluids, it is essential that universal precautions are used. Always assume that anything in contact with such fluids is infectious and therefore must be disposed of in the beaker of bleach or biohazard bag. Wear gloves when handling such materials and handle only your own bodily fluids.

1. Clean a microscope slide and draw three circles on the slide with the wax marker. Label one circle "A" , another "B", and the third “R”.

2. Disinfect fingertip with an alcohol pad and use a lancet to harvest three drops of blood, placing one drop into each circle. Apply an adhesive bandage to the harvest site.

3. Place one drop of “anti-A" in the blood in circle "A", and one drop of “anti-B" in the blood in circle "B", and one drop of “anti-Rh” into circle “R”.

4. Mix with a toothpick and place slide on a warm (37^{o}C) surface. Do not allow the preparation to dehydrate. Add one drop of water if it appears that dehydration is occurring.

5. It may take 15 min for agglutination to occur. Observe agglutination patterns with white light illumination from beneath. Agglutination in anti"A" only indicates type A RBC, agglutination in anti"B" only indicates type B RBC, agglutination in both anti"A" and anti"B" indicates type AB RBC and no agglutination in either indicates type O RBC. Agglutination by “anti-Rh” indicates that the blood is Rh positive, failure to agglutinate indicates that it is Rh negative.

6. Discard slide in beaker of disinfectant.

C. Genetic basis for traits

First, your Professor will introduce the concept of odds ratios and the principles behind chromosome banding. You will also discuss melanosomes (pigment granules).

1. Visit the National Center for Biotechnology Information (NCBI) website, Online Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/OMIM)

2. Enter "skin pigmentation" in the search box and review some of the top scoring entries and enter the appropriate data below.

OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
609802				
606202				

Click on the allelic variants associated with normal variation in skin pigmentation, scroll down to the Population diversity section to examine the frequency of the alleles in different populations.

- Which is the ancestral allele?
- Which groups carry the derived allele?
- How is the gene product of the derived allele different from that of the ancestral allele?
- Under what circumstances might this allele be advantageous?
- Under what circumstances might this allele be disadvantageous?
- Is this inheritance pattern Mendelian, or not? How can you tell?
- What is oculocutaneous albinisim?

3. Enter “hair pigmentation” in the search box and review some of the top scoring entries and enter the appropriate data below.

OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
155555				
609734 176830				

- Is hair color a Mendelian trait in humans? How can you tell?

- How can a mutation in a single gene like POMC have multiple phenotypic effects?

- How can mutations in different genes (like MC1R and POMC) have the same effect?

4. Enter “color vision” in the search box and review some of the top scoring entries and enter the appropriate data below. Your Professor will discuss the concept of the visible spectrum of light: ROYGBIV, from long wave (R) to short wave (V).

OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
303800				
303900				

- How many color vision opsins are required for full color vision? Why?

- Explain the evolution of these two genes and color vision in primates.

5. Enter “ABO blood group” in the search box and review some of the top scoring entries and enter the appropriate data below.

OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
110300				

- What is co-dominance?

- What phenotype is associated with each possible genotype?

- Examine the maps on the next page to determine the geographic distribution of the different blood types.

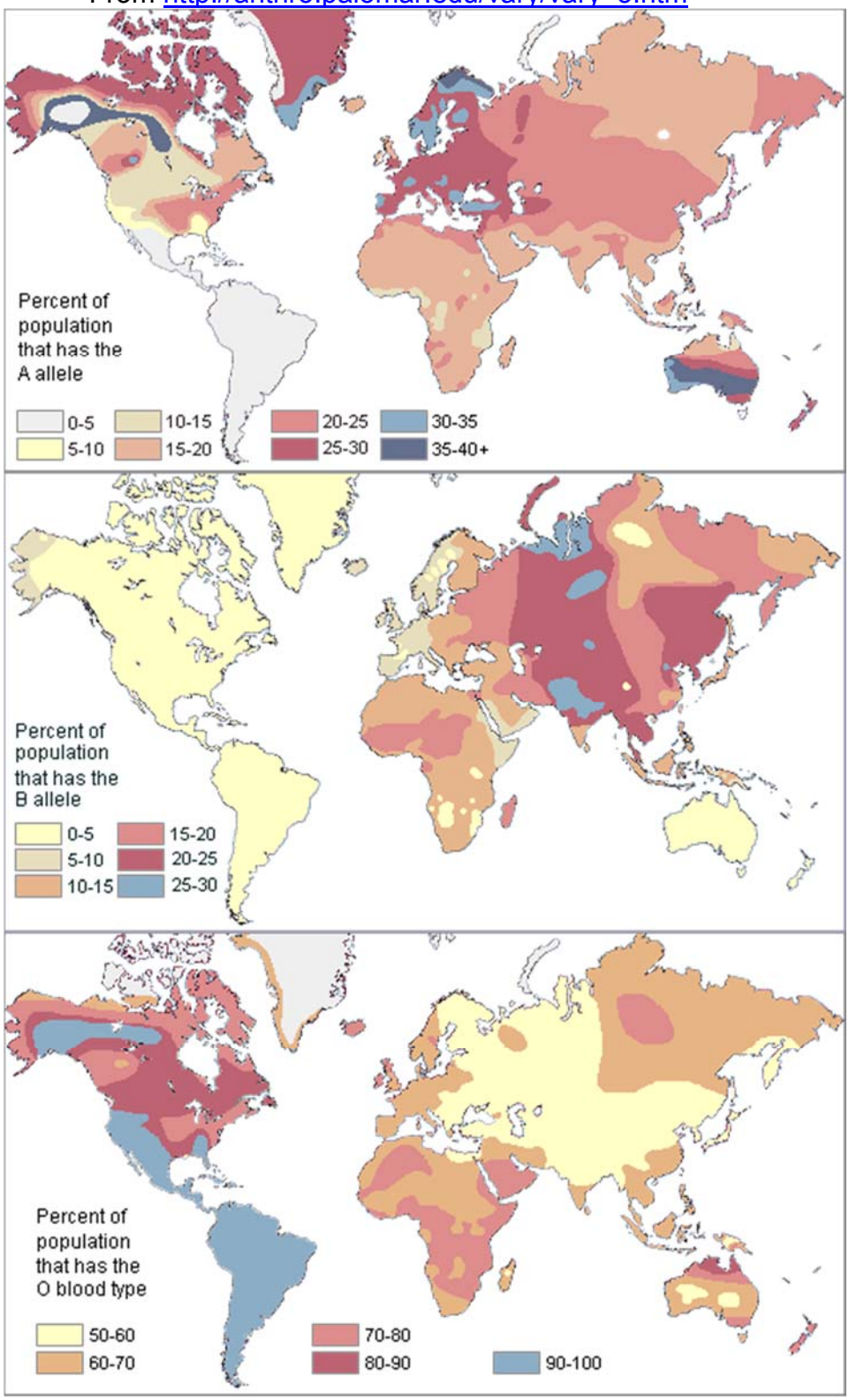
  Where is type A blood most common? …least common?

  Where is type B blood most common? …least common?

  Where is type O blood most common? …least common?

  Does your blood type match the geographic origins of your ancestors?

Distribution of ABO Blood Types Worldwide (note: display this in color if you can!)
From http://anthro.palomar.edu/vary/vary_3.htm



6. Enter “Rh antigen” in the search box and review some of the top scoring entries and enter the appropriate data below.

OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
111700				

- How could an Rh negative mother have an Rh positive child?
- Why does this cause hemolytic disease of the newborn?

7. Enter “eye pigmentation” in the search box and review some of the top scoring entries and enter the appropriate data below.

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OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
227220 611409				
227240				

8. Enter “PTC tasting” in the search box and review some of the top scoring entries and enter the appropriate data below. Be sure to click on the dpSNP entries.

OMIM #	Gene Name	Chrom. location	Gene/Protein Variants	Protein function
607751				

- Which is the ancestral allele?
- What are the frequencies of each haplotype?
- How might the AAV allele have originated?

What are the advantages and disadvantages of tasting PTC?

Summary Questions:

Review all of the questions in this lab activity packet.

Make sure you've accomplished the Learning Goals listed at the beginning.

Come to office hours for help with your review.