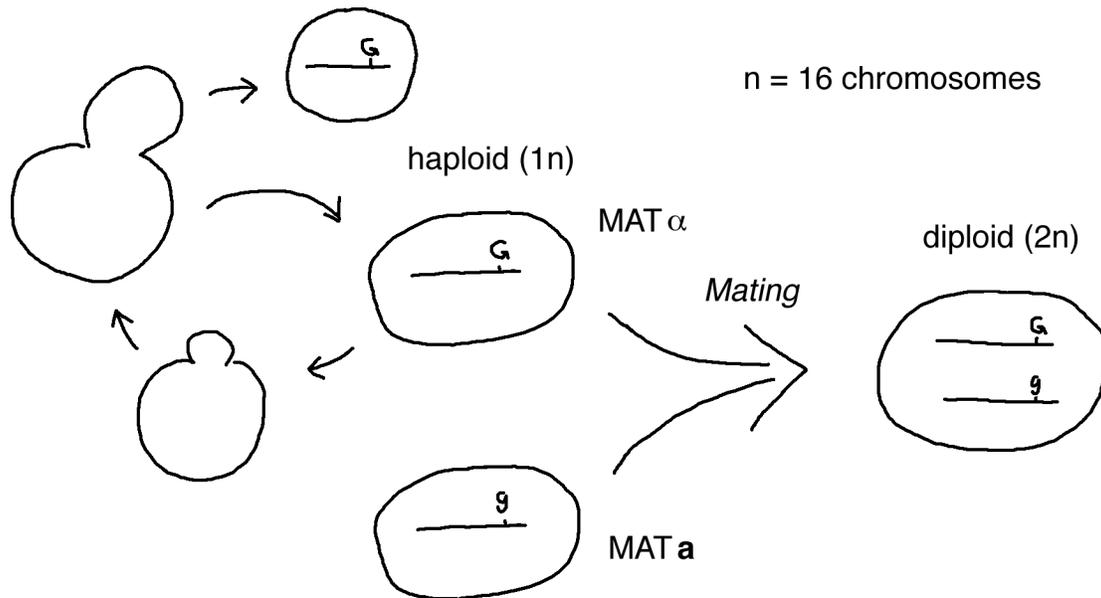


## Lecture 2

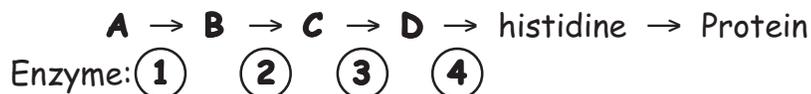
In this lecture we are going to consider experiments on yeast, a very useful organism for genetic study. Yeast is more properly known as *Saccharomyces cerevisiae*, which is the single-celled microbe used to make bread and beer. Yeast can exist as haploids of either mating type  $\alpha$  ( $\text{MAT}\alpha$ ) or mating type  $a$  ( $\text{MAT}a$ ). Haploid cells of different mating type when mixed together will mate to make a diploid cell.



Haploids and diploids are isomorphic - meaning that a given mutation will cause essentially the same change in haploid and diploid cells. This allows us to look at the effect of having two different alleles in the same (diploid) cell.

All yeast needs to grow are salts, minerals, and glucose (minimal medium). From these compounds, yeast cells can synthesize all of the molecules such as amino acids and nucleotides that are needed to construct a cell. The synthesis of complicated molecules requires many enzymatic steps. When combined, these enzymatic reactions constitute a **biochemical pathway**

Consider the pathway for the synthesis of the amino acid histidine.



Each intermediate compound in the pathway is converted to the next by an enzyme. For example, if there is a mutation in the gene for enzyme 3 then intermediate C can not be converted to D and the cell can not make histidine. Such a mutant will only grow if histidine is provided in the growth medium.

This type of mutation is known as an **auxotrophic mutation** and is very useful for genetic analysis.

	growth on minimal	growth on minimal + histidine
His <sup>+</sup> (wild type)	+	+
His <sup>-</sup>	-	+

**Phenotype:** All traits of an organism (with an emphasis on trait under investigation)

**Homozygote:** diploid with two like alleles of same gene

**Heterozygote:** diploid with two different alleles of same gene

**Recessive Allele:** trait not expressed in heterozygote

<u>genotype</u>	<u>phenotype</u>	<i>Mate to :</i>	<u>diploid genotype</u>	<u>diploid phenotype</u>
MAT $\alpha$ His3 <sup>-</sup>	His <sup>-</sup>	MAT $\alpha$ His3 <sup>-</sup>	His3 <sup>-</sup> /His3 <sup>-</sup>	His <sup>-</sup>
MAT $\alpha$ His3 <sup>-</sup>	His <sup>-</sup>	MAT $\alpha$ His3 <sup>+</sup>	His3 <sup>-</sup> /His3 <sup>+</sup>	His <sup>+</sup>

Based on the His<sup>-</sup> phenotype of the His3<sup>-</sup>/His3<sup>+</sup> heterozygote, we would say that His3<sup>-</sup> is recessive to wild type.

Let's consider a different kind of mutation giving resistance to copper that occurs in a gene known as CUP1.

<u>genotype</u>	<u>phenotype</u>	<i>Mate to :</i>	<u>diploid genotype</u>	<u>diploid phenotype</u>
MAT $\alpha$ Cup1 <sup>r</sup>	copper resistant	MAT $\alpha$ Cup1 <sup>+</sup>	Cup1 <sup>r</sup> /Cup1 <sup>+</sup>	copper resistant

**Dominant Allele:** trait is expressed in heterozygote

Cup1<sup>r</sup> is dominant to wild type (Cup1<sup>+</sup>).

The terms dominant and recessive are simply shorthand expressions for the results of particular experiments. If someone says a particular allele is dominant that means that at some point they constructed a heterozygous diploid and found that the trait was expressed in that diploid.

Note: Sometimes an allele will have more than one phenotype and may be recessive for one and dominant for another. In such cases, the phenotype must be specified when one is making statements about whether the allele is dominant or recessive. Consider for example, the allele for sickle cell hemoglobin in humans designated Hb<sup>s</sup>. Heterozygous individuals (Hb<sup>s</sup>/Hb<sup>a</sup>) are more resistant to malaria, thus Hb<sup>s</sup> is *dominant* for the trait of malaria resistance. On the other hand, Hb<sup>s</sup>/Hb<sup>a</sup> heterozygotes do not the debilitating sickle cell disease, but Hb<sup>s</sup>/Hb<sup>s</sup> homozygous individuals do. Therefore, Hb<sup>s</sup> is *recessive* for the trait of sickle cell disease.

Once we find out whether an allele is dominant or recessive, we can already infer important information about the nature of the allele. The following conclusions will usually be true.

**Recessive** alleles usually cause the loss of something that is made in wild type

**Dominant** alleles usually cause increased activity or new activity

It turns out that the Cup<sup>r</sup> allele actually carries more copies of the gene for a copper binding protein and therefore increases the activity of the gene.

Last lecture we defined the gene *structurally* as the DNA needed to encode a protein. We can now define a gene in a new way based on its *function*. Using the phenotypic difference between wild type and a recessive allele we can use a **Complementation test** to determine whether two different recessive alleles are in the same gene.

Say you isolate a new recessive histidine requiring mutation that we will call HisX<sup>-</sup>. In principle, this mutation could be in His3 or it could be in any of the other genes in the histidine biosynthetic pathway. In order to distinguish these possibilities we need a test to determine whether HisX the same as His3.

To carry out a complementation test, one simply constructs a diploid carrying both the His3<sup>-</sup> and HisX<sup>-</sup> alleles.

An easy way to do this would be to mate a MAT<sup>α</sup> HisX<sup>-</sup> strain to a MAT<sup>a</sup> His3<sup>-</sup> strain.

<u>possibility</u>	<u>genotype of diploid</u>	<u>phenotype of diploid</u>	<u>complementa-</u> <u>tion</u>
HisX= His3	His3 <sup>-</sup> /His3 <sup>-</sup>	His <sup>-</sup>	<b>No</b>
HisX≠His3	His3 <sup>-</sup> /His3 <sup>+</sup> , HisX <sup>-</sup> /HisX <sup>+</sup>	His <sup>+</sup>	<b>Yes</b>

Having performed this test, if the two mutations *don't* complement we conclude that they are in the same gene. Conversely, if they *do* complement we conclude that they are in different genes.

This test only works for recessive mutations. Think about what the outcome would be if HisX<sup>-</sup> were dominant.

The complementation test can be thought of in the following way. If I have an allele with an observable phenotype whose function can be provided by a wild type genotype (i.e., the allele is recessive) — I can ask whether the function that was lost because of the recessive allele can be provided by another mutant genotype. If not, the two alleles must be defective in the same gene. The beauty of this test is that the trait can serve as a read-out of gene function even without knowledge of what the gene is doing at a molecular level.

## Definitions from the Language of Genetics

**Gene:** The fundamental unit of heredity, which can be defined in three ways: i) A gene can be defined in molecular terms as a segment of DNA carrying the information necessary to express a complete protein or RNA molecule, including the promoter and coding sequence. ii) A gene can be defined by function with a group of recessive mutations that do not complement each other. iii) A gene can be defined by position with a single-locus segregation pattern in a cross between lines with different alleles. Examples are a 1:3 phenotypic ratio in the F<sub>2</sub> generation in a cross between diploid organisms or a 2:2 segregation pattern in yeast tetrad analysis.

**Alleles:** Distinguishable versions of the same gene.

**Locus:** The site on a chromosome where a gene is located. Usually defined by recombinational mapping relative to neighboring loci.

**Genotype:** The allelic constitution of an individual, usually with emphasis on the gene or genes under examination.

**Phenotype:** All of the traits or characteristics of an organism, usually with emphasis on traits controlled by the gene or genes under examination.

**Wild type:** A standard genotype that is used as a reference in breeding experiments. Note that for human crosses there is no standard genotype and the concept of wild-type is therefore not meaningful.

**Haploid:** A cell or organism with one set of chromosomes (1n).

**Diploid:** A cell or organism with two sets of chromosomes (2n).

**Homozygous:** The condition of having two like alleles in a diploid.

**Heterozygous:** The condition of having two different alleles in a diploid.

**Dominant allele:** An allele that expresses its phenotypic effect or trait in the heterozygous state.

**Recessive allele:** An allele whose phenotypic effect or trait is not expressed in a heterozygous state.

**Incomplete dominance:** The case where a heterozygote expresses a phenotype intermediate between the corresponding homozygote phenotypes.

**Complementation test:** A test of gene function where two genotypes with recessive alleles are combined by a cross to test whether the genotype of one parent can supply the function absent in the genotype of the other parent.

**F<sub>1</sub>:** First generation produced by interbreeding of two lines.

**F<sub>2</sub>:** Generation produced by interbreeding of F<sub>1</sub> individuals.

**Incomplete penetrance:** Cases where certain alleles are not always expressed to give observable traits because of other environmental or genetic influences.

**True-breeding:** Refers to a line of individuals that on intercrossing always produce individuals of the same phenotype. This can almost always be taken to mean that the individuals are *homozygous*