Genetics IV: Biochemical Genetics

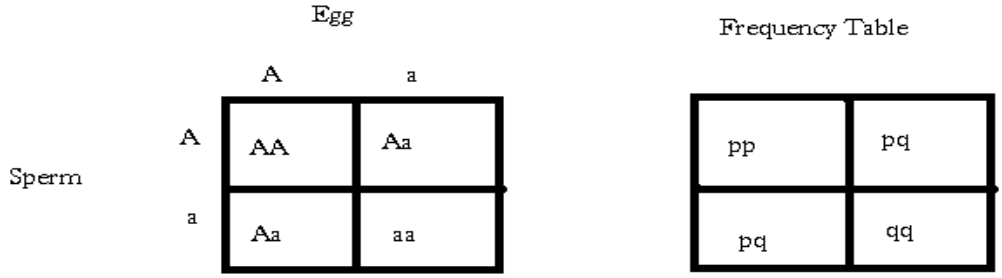
§1. Population Genetics

This field was advanced by laws proposed by two people Hardy and Weinberg (1908)

Suppose in a population there are 2 alleles for a given gene, A and a.

Alleles: A a
Frequency: p q there are only 2 alleles for gene so p + q =1

How often an "A" allele will be in the egg (in female) = p
How often an "a" allele will be in the egg = q



$AA = p^2$
$Aa = 2pq$
$Aa = q^2$

AA	:	Aa	:	aa
p^2		$2pq$		q^2

This is the equilibrium distribution – called the Hardy-Weinberg equilibrium

If you know Allele Frequencies, you can calculate genotype frequencies:

$$(p+q)^2 = 1 \rightarrow p^2 + 2pq + q^2 = 1$$

At Hardy-Weinberg Equilibrium:

$AA = p^2$
$Aa = 2pq$
$Aa = q^2$

Assuming equilibrium, allele frequencies (and therefore genotype frequencies) do not change over time. Allele frequencies tend to "stay constant" in populations.

Example: cystic fibrosis (autosomal recessive disease)
Frequency of individuals in a population with cystic fibrosis is 1/2000

Let A = normal or wild type allele and a = cystic fibrosis (disease) allele

q^2 (affected individuals) = 1/2000 so $q = \sqrt{1/2000}$ = 1/44 therefore p = 43/44

Frequency of Carriers: $2pq = 2(43/44)(1/44) \sim .044 = 1/22$

Therefore about 5% of population is carriers

Why are diseases like cystic fibrosis (cf) still prevalent in certain populations?

Heterozygotes carry mutant allele – may have a certain advantage, say increased resistance to cholera infection therefore "a" allele is still in the population.

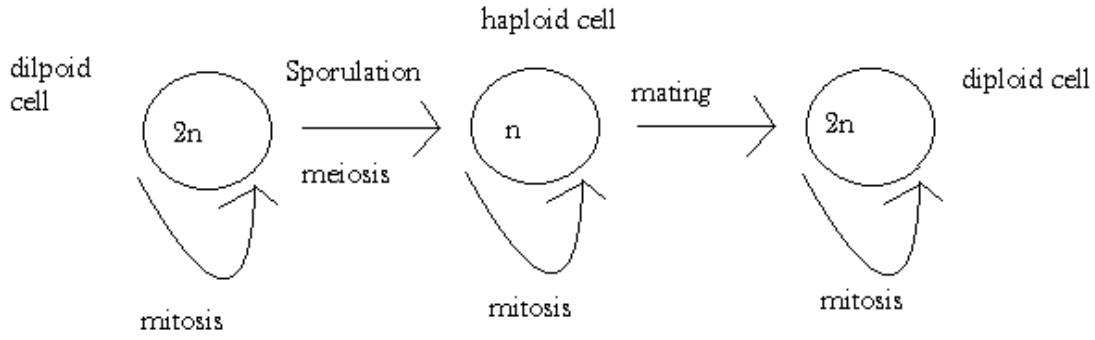
§2. Biochemical Genetics

-Archibald Garrod united a genetic defect with an enzymatic defect.
-Biochemical Genetics uses experimental genetics to dissect biochemistry

Experimental System:
Yeast: single celled fungus, eukaryote (has a nucleus)
Take a suspension of yeast cells and spread on a solid medium of agar in a petri dish, which contains nutrients. Over time these single cells grow on the agar into visible clumps called colonies. Each colony represents a pile of identical cells, each the descendant of the single cell that was plated on that position.

Life Cycle of the Yeast

Yeast can grow as diploid (2n) or haploid (n) n=16 chromosomes in yeast



Growth Requirements

-Yeast can grow on a minimal medium: liquid or solid media that contains: a carbon source (a fermentable sugar such as glucose, fructose or lactose), nitrogen, phosphorous, salts.

-Yeast has biosynthetic pathways in which they can make all of the biological components required from the simple nutrients provided in a minimal medium

-Yeast can also grow in rich medium: liquid or solid media that contains complex nutrients and macromolecules. Yeast cells will take these nutrients from the medium in lieu of synthesizing them.
Yeast can switch off its own synthetic pathways and use what is available in the medium by regulating enzymes involved in pathways

-yeast can synthesize what it needs
-yeast can also take up nutrients from medium

To understand these biosynthetic pathways we need to characterize yeast that lack certain enzymes, and thus cannot grow under certain conditions. For example…

How would you isolate genes/proteins that are involved in amino acid synthesis (e.g synthesis of arginine)?

You could find yeast that cannot synthesize arginine. You would go on a mutant hunt (look for mutant yeast that cannot grow without arginine added to the growth medium

Mutant Hunt Strategy
-start with wild-type yeast cells
-mutangenize these cells with chemicals or radiation to damage DNA
-plate mutangenized cells on rich medium
-replica plate (transfer) yeast colonies to minimal medium
-look for colonies that cannot grow on minimal medium – this is a mutant that has some kind of defect in a biosynthetic pathway
-the way you design a mutant hunt will determine what mutants you will find

Once you obtain a collection of mutants that cannot grow on minimal medium without a supplement, need to determine which component (nutritional supplement) the mutants require for growth on minimal medium. For example, look for a mutant strain that cannot synthesize arginine:
Plate on rich medium → transfer to minimal medium→ transfer to minimal medium + arginine
All cells grow on rich medium. All colonies except ones that are deficient in a biosynthetic pathway grow on minimal. Ones that are deficient in an arginine synthetic pathway can grow on minimal medium + arginine. (You could also do selection on rich medium – arginine. Colonies that do not grow on this medium would be an arginine synthesis mutant)

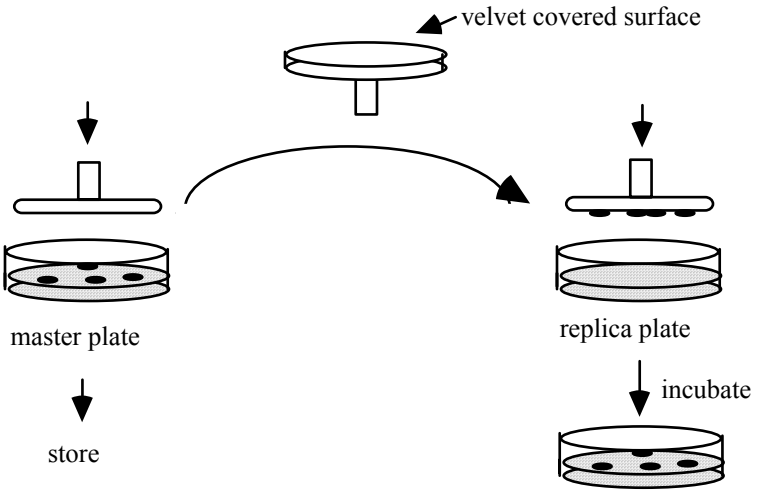
If we tested growth of yeast on rich vs. minimal medium, we could obtain a variety of mutants called auxotrophs

Definitions:
Prototrophs: wild type strain that can grow on minimal medium
Auxotrophs: Mutant strain that has lost the ability to grow on minimal medium

The mutants that were isolated above that do not grow on minimal medium, but grow on minimal medium + arginine are called arginine auxotrophs. But to analyze a large number of cells we need a better way to test growth on conditions and screen for mutants.

A technique called replica plating was developed by Esther Lederberg (used her facial compact cloth)

Replica Plating is when you transfer all colonies in the same arrangement between two plates by pressing the plates lightly on a piece of velvet. You can do this type of screening to isolate loss of function mutants.



Two types of Assays to look for colonies that have a desired property or function

1) Genetic Screen: Look at all colonies that grow and then look for cones that cannot grow on a particular medium. Screening for mutants that have "lost a function" – their ability to grow under certain conditions.
2) Genetic Selection: Look for colonies that can grow on a particular medium. Selecting for mutants that have "gained a function" – ability to grow under certain conditions (conditions that would normally not permit growth, like drug resistance)

To study mutants that have an inability to grow on arginine – need to make a collection of arginine auxotrophs – mutants that need only arginine to grow on minimal medium.

To obtain these mutants – need to use haploid yeast cells – have only 1 copy (n) of each gene. Cannot use diploid (2n) yeast because a mutation in 1 of the 2 genes in not enough for auxotrophy. This is because the level of enzyme produced from 1 copy of the gene (half the normal levels) is sufficient for normal function

Look at haploid vs. diploid yeast:
Haploid: 1 mutation in arg gene leads to arg auxotrophy
Diploid: 1 mutation in arg gene does not lead to arg auxotrophy because other gene is wt

To be arg auxotrophic both copies of the arg gene need to be mutated; not common to find diploid yeast that has two mutations in the same gene

§3. Characterization of Mutants

Collect many mutant yeast strains auxotrophic for arginine – call them Arg1, Arg2, Arg3 etc.
Then ask, are these mutants in the same gene or in different genes?

In order to do this, first test whether the mutations are causing a recessive phenotype.

1) Test of Recessivity:

- loss of enzyme function is usually recessive to wild type phenotype
- usually 50% of a gene product is enough to show a wt phenotype for an enzymatic defect – usually only 1 wt copy of gene is enough to show wt phenotype.

Mate 2 haploid strains: Arg1 with wt to generate a diploid cell. If the phenotype of the resulting diploid cell is wild type than the phenotype associated with the Arg1 mutant is recessive to wt.

2) Test of Complementation

Do a complementation test to determine if the two different mutations are in the same gene or in different genes. Mate two haploid mutants to generate a diploid cell and look at phenotype of diploid.

a) If mutations are in the same gene,(*i.e.* the gene defective in the Arg1 mutant is the same gene that is defective in the Arg2 mutant) then the resulting diploid will be unable to grow on minimal medium. Because both mutants carry mutations in the same gene the diploid lacks a functional enzyme – the diploid can not grow without Arginine added to the medium.

**When Arg1 and Arg2 carry mutations in the same gene and the resulting diploid shows a mutant phenotype then the mutants Arg1 and Arg2 fail to complement each other. They are said to be in the same complementation group.
b) If mutations are in different genes,(*i.e.* the gene defective in the Arg1 mutant is a different gene then that which is defective in the Arg2 mutant) then the resulting diploid will grow on minimal medium. Each mutant has a mutation in a different gene, thus the diploid has all functional enzymes and can grow on minimal medium.

**When Arg1 and Arg2 carry mutations in different genes and the resulting diploid shows a wild type phenotype then the mutants Arg1 and Arg2 complement each other. They are said to be in different complementation groups.

In general, mutations in different genes complement each other, restores protrophy in the diploid (each mutant rescues the others defect).

One can do a complementation test between different haploids and make a table:

	Wt	Arg1	Arg2	Arg3	Arg4
Wt	+	+	+	+	+
Arg1	+	-	-	+	+
Arg2	+	-	-	+	+

Arg3	+	+	+	-	-
Arg4	+	+	+	-	-

"+" means complementation, diploid has wild type phenotype
"-" means no complementation, diploid has mutant phenotype
*Notice that All mutants are recessive to wild type
Mutants 1 and 2 fail to complement: therefore they are in the same complementation group and are assumed to be in the same gene
Mutants 3 and 4 fail to complement: therefore they are in different complementation groups and are assumed to be in the same gene.

Mutants 1 and 4 complement: therefore they are assumed to be in different genes.

The above table suggests that there are two complementation groups or likely two genes involved in arginine synthesis.