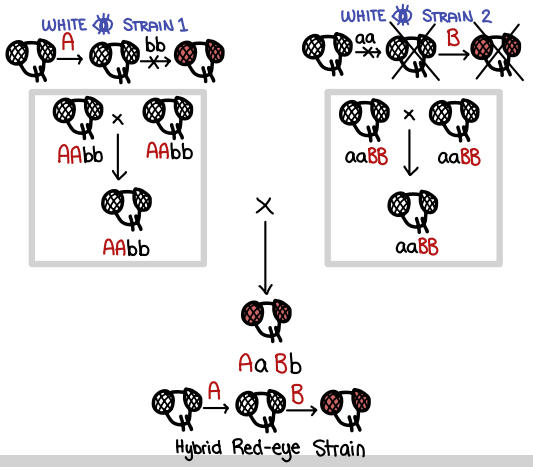
# Chapter 5 ~ The Complementation Test

## Introduction:




A particular phenotype is usually the result of the biochemical products of multiple genes acting in a pathway

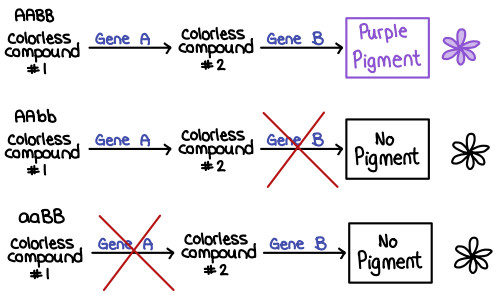
### Polygenic Inheritance

- Occurs when one characteristic is controlled by two or more genes
- Often the genes are large in quantity but small in effect
- example of human polygenic inheritance:
  - Height
  - Skin colour
  - Eye colour
  - Weight
- A mutation in any one given gene of the set governing a phenotype, can result in an alteration of the manifested trait

### Complementation Test

- Used to determine if two mutants which have the phenotype carry their mutation in the same gene or in different genes
- Consists of classical Mendelian genetic cross to determine if one mutant can complement another or in other words, produce the wild type phenotype

## Complementation Test : Allelism

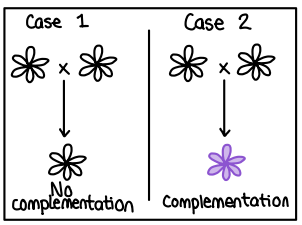


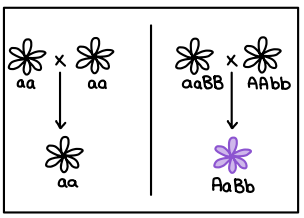

The pigment in a purple flower could depend on a biochemical pathway

A diploid plant that lacks the function of gene A (genotype aa) would produce mutant white flowers that phenotypically looked just like the white flowers of a plant that lacked the function of gene B (genotype bb)

Both enzymes A & B are in the SAME pathway that leads from a colourless compound #1, through colourless compound #2, to the purple pigment

- Blocks at either step result in mutant white flower & NOT the wild type purple flower




Strains w/ mutants in gene A (aa) × Strains w/ mutation in gene B (bb)

→ AaBb

- Have wild type, functional A & B gene and will thus have a pigmented flower
- Mutations are in different genes therefore are NON allelic mutations

### Ask Yourself...

- Whether or NOT the mutant phenotype is due to a loss of function in the same gene, or are they mutant in different genes that both cause the same phenotype (e.g in the SAME pathway)
- Are they...
  - Allelic mutations
  - Non-allelic mutations

*Can be answered using the complementation test*

If the $F_1$ progeny all have the same phenotype then we interfer that the same gene is mutated in each parent

- These mutations would then be called allelic mutations - mutants would then be called allelic mutations - mutant in the same gene locus
- These two mutations fail to COMPLEMENT one another (still mutant)
- These could either be exactly the same mutant alleles (same base pair changes) or different mutations (different base pair changes, but in the SAME gene - allelic)

Third pure-breeding, independently derived white-flower, mutant strain, we won't initially know if it is mutant in gene A, gene B or some other gene altogether

- Complementation Test is used to determine which gene is mutated
- To perform a complementation test two homozygous individuals w/ similar mutant phenotypes

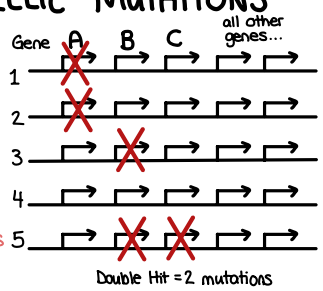
## Complementation Groups = Groups of Allelic Mutations

### Example: Double Hit Strain

| # | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | − | | | | |
| 2 | − | − | | | |
| 3 | + | + | − | | |
| 4 | + | + | + | − | |
| 5 | + | + | − | − | − |

#5 has mutations in 2 different genes

- Double hit strain is normally a very rare event
  - May appear to belong in 2 different groups
  - In this case mutants #3 & 4 complement (diff genes) but #5 fails to complement both #3 & #4 indicating it has mutations in both the mutant genes in #3




With the 3rd mutant strain above, we could assign it to be allelic w/ either gene A or gene B, or some other locus, should it complement both gene A and gene B mutations

### Example: Multiple Mutant

| # | a | b | c | d | e | f |
|---|---|---|---|---|---|---|
| a | w | | | | | |
| b | p | w | | | | |
| c | p | w | w | | | |
| d | p | p | p | w | | |
| e | p | p | p | w | w | |
| f | p | w | w | p | p | w |

It all depends on HOW MANY mutations you have in a gene.

For Example:

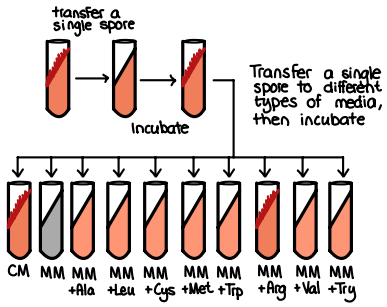
- White gene in Drosophilia has >300 different mutations within the white gene
  - If you obtain & cross all these mutations to themselves, you would find they all belong to the same complementation group or same white gene
  - Each complementation group = a gene
- However, if you obtain a different mutation, vestigial for example, which affects wing growth and cross it to a white eye-colour mutation, the double heterozygous would result in red eyes and normal wing (w/t) so the two would complement & represent two different complementation groups
  - ① White
  - ② Vestigial

] Same would be true for other eye colour mutations

### Complementation Groups

- When allelic mutations are organized into groups
  - These are groups of mutation that FAIL TO COMPLEMENT one another (a group of NON-complementing mutations) and are assumed to have mutations in the SAME gene; hence they are grouped as complementation group
  - A group can consist of as few as one mutations in the same gene (allelic)
  - # of complementation groups represent the number of genes that are represented in total collection of mutations

# Transformation Rescue




## Beadle & Tatum

- Built on the connection b/w genes and metabolic pathways
  - led to "One gene, one enzyme/protein"
    - ↳ States that each enzyme that acts in a biochemical pathway is encoded by a different gene (There are some exceptions)
- used the fungus Neurospora crassa
  - Good model organism
  - Protrophic = can grow on minimal medium
    - ↳ Can synthesize amino acids, vitamins, etc for normal growth
  - Exposed to x-rays = randomly induced mutation
    - ↳ Most spores could still grow (prototrophic)
    - ↳ Some spores phenotype changes from prototrophic to auxotrophic and can no longer grown on MM

## Important

- For conceptual advances in understanding genes
- Demonstrated the utility of screening for genetic mutants
- Useful for investigating biological process like the metabolic pathways that produce amino acids

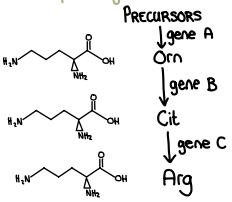
- Minimal media lacks most nutrients besids a few minerals, simple sugars & biotin
- Auxotrophes grow on complete media as it is supplemented w/ nutrients, amino acids and vitamins
- Implies that each auxotrophic mutations was blocked at a specific step in a biochemical pathway, and that by adding an essential compound (arginine) that block could be circumvented

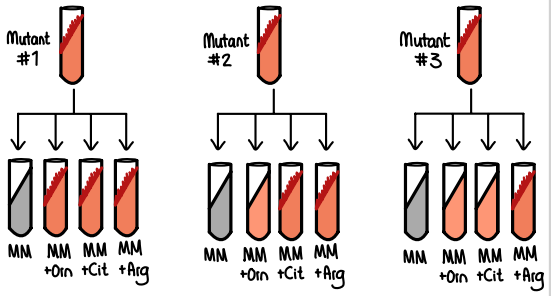
| Mutants in | MM+Orn | MM+Cit | MM+Arg |
|---|---|---|---|
| Gene A | Yes | Yes | Yes |
| Gene B | No | Yes | Yes |
| Gene C | No | No | Yes |

ex: Arginine

- If a mutagenized spore could grow on MM only when supplemented w/ arginine, then auxotroph must bear a mutation in the Arg biosynthetic pathway
  - "Arginineless" strain
- Arginine has two biochemical intermediates
  - Ornithine (Orn)
  - Citrulline (Cit)

] mutation anywhere in this path could turn Neurospora into an Arg auxotroph (arg-)

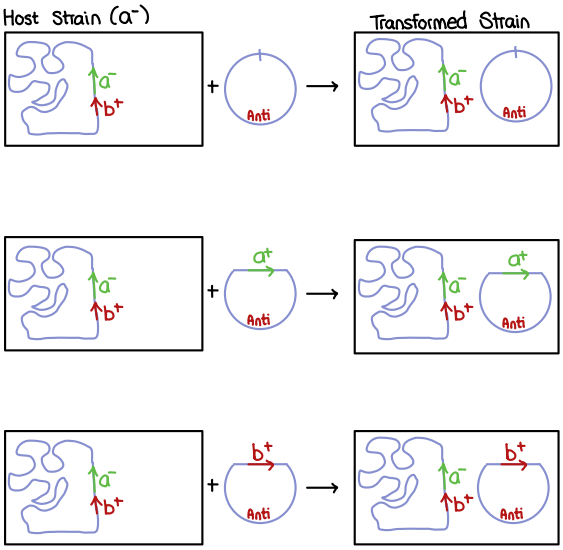



| E.Coli Strain | MM(Minimal medium) | MM+ supplement |
|---|---|---|
| a- | Auxotrophic (no growth) | Growth |
| a+ | Growth | Growth |

To transform the autotrophic strain & rescue we need to...

1. Make E.Coli auxotrophic cells competent so that it can incorperate foreign DNA molecules. We can form a competent cell via heat shock or electroporation
   - Slightly damages membrane = passage for DNA molecules into the cell
2. Extract DNA molecule from a wild type E.Coli then break it down into smaller fragments
3. Insert the short fragments of E.Coli DNA into a DNA vector, which is a DNA molecule than can contain amplify and transfer the inserted DNA into the cell
   - Recombinant DNA = combined DNA
4. After the E.Coli DNA fragment are inserted into DNA vectors, we have collection of recombinant DNA molecules, which when transformed, can be called a DNA library (3 possibilities)
   - I DNA clones that contain gene a
   - II DNA clones that don't contain gene a, which will be collectively presented by letter B
   - III DNA clones that don't contain any foreign genes
5. Combine the recombinant DNA molecules and host E.Coli strain together, so that the auxotrophic strain can incorperate those DNA molecules through transformation
6. The host strains genotype is "b+". It will need wild type "a+" to grow on minimal media.
   - Plasmids that have a+ allele would grow (prototrophic)
   - Strains w a plasmid w/ no transgene or have a plasmid w/ gene b+ would still be auxotrophic




## Plasmids

- Small circular DNA molecules that are mostly found in bacteria and are suitable as DNA vectors
- The vector + DNA insert molecule can be replicated and the result would be multiple clones of the original DNA insert