

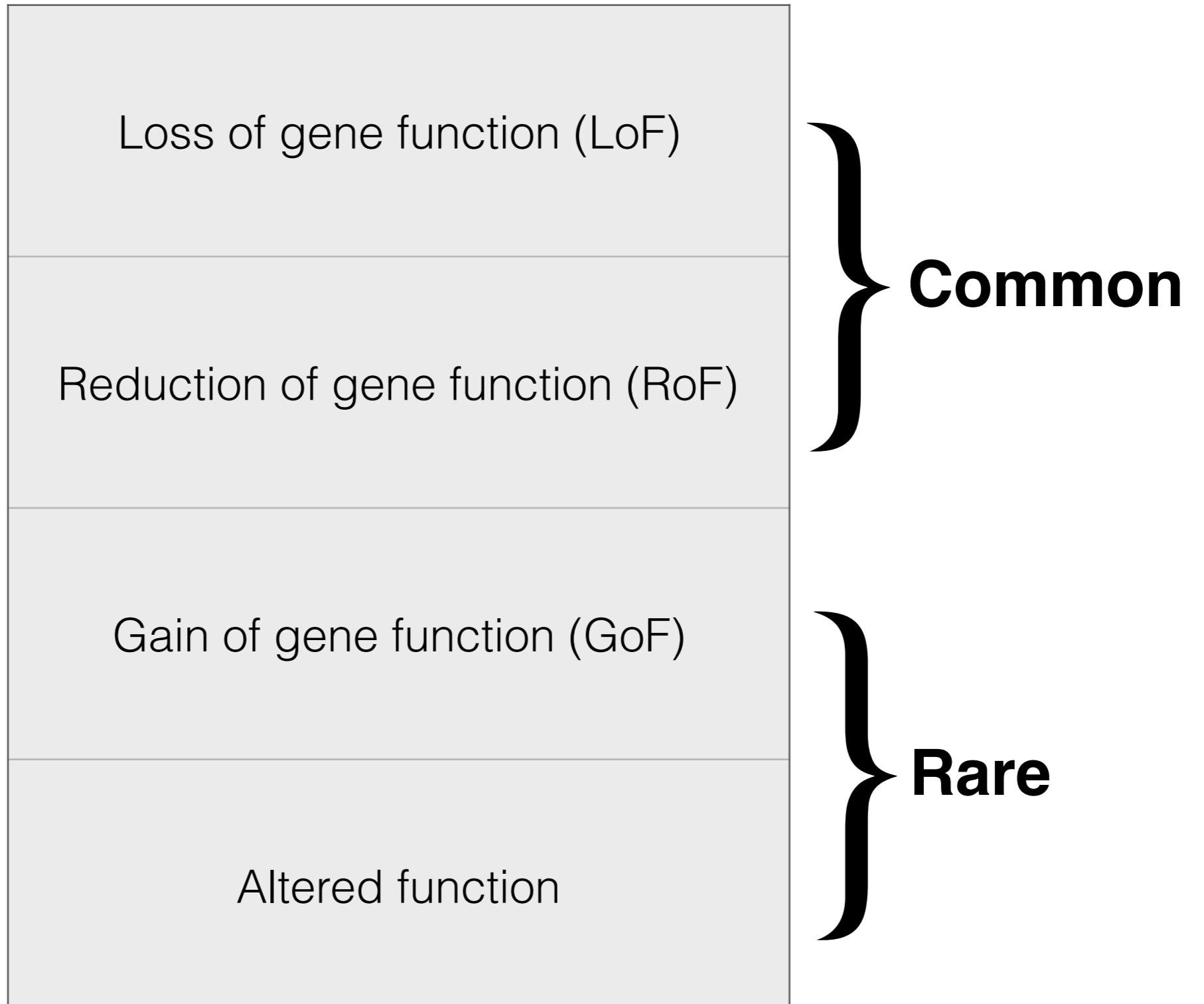
# Bio393: Genetic Analysis

Gene dosage and Complementation



Lexus CT 200h

**What does a mutation do to gene function?**



**Dominant or recessive  
correlates with mutation type most times**



**Hermann Muller**



# Muller's morphs - gene dosage tests

Loss of gene function (LoF)	amorph, nullomorph
Reduction of gene function (RoF)	hypomorph
Gain of gene function (GoF)	hypermorph
Altered function	neomorph, antimorph

m = mutation of gene

△ = deletion of gene

+ = normal allele of gene

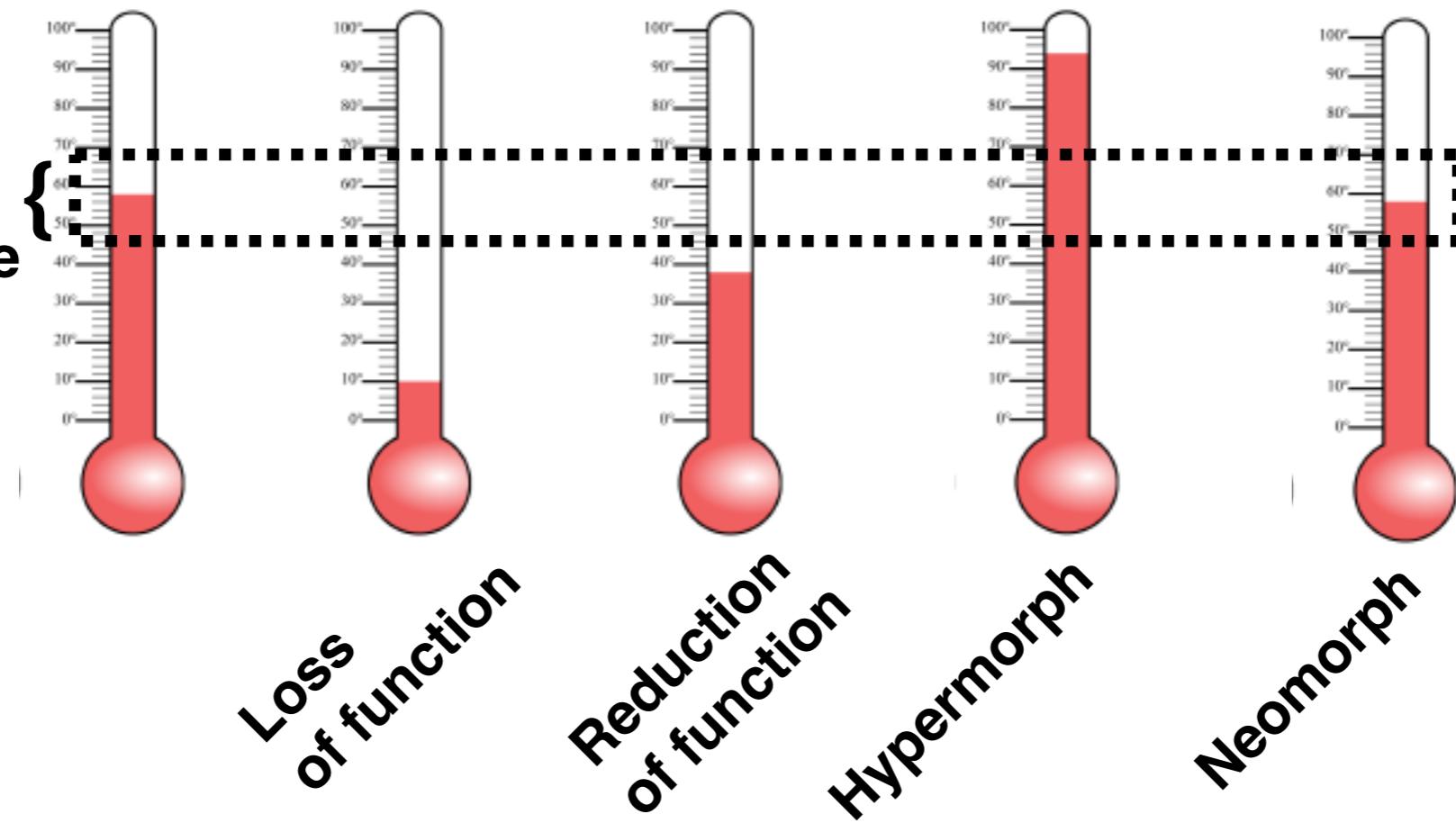
= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

# A wild-type phenotype is a reading of the amount of gene function

Range of gene function that confers a wild-type phenotype



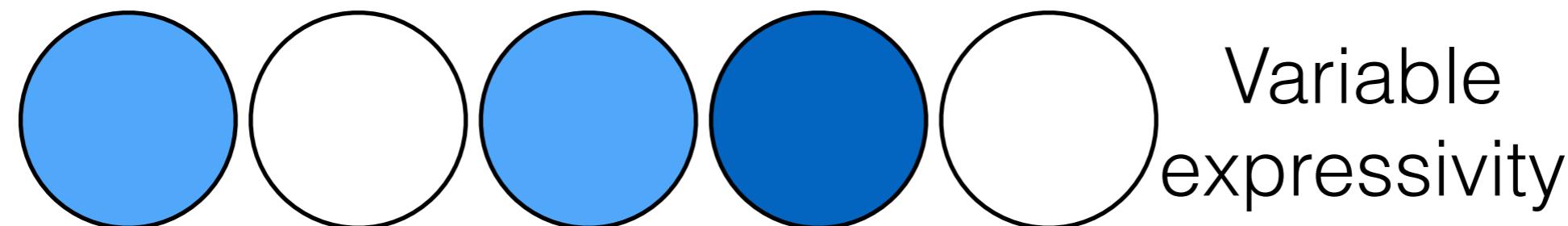
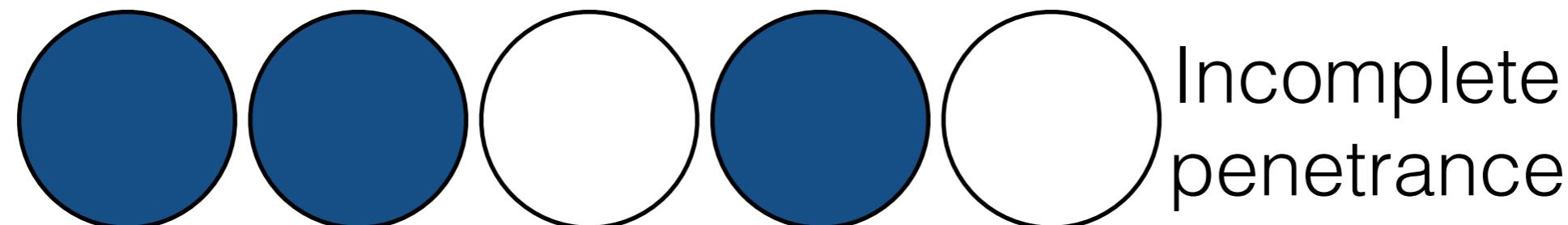
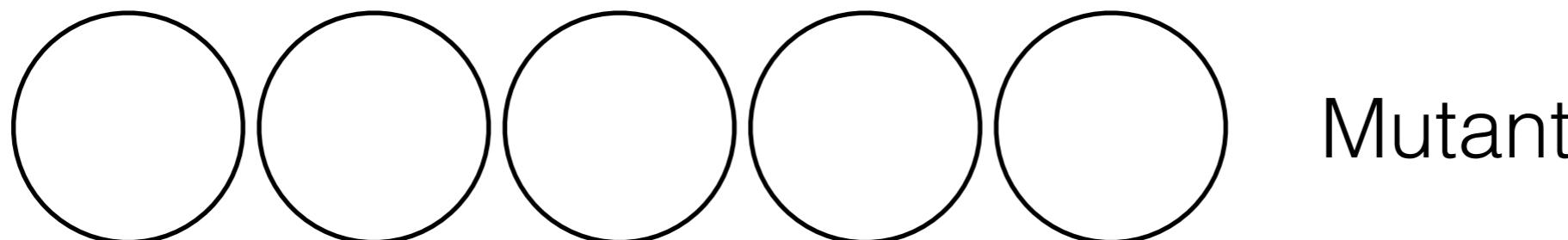
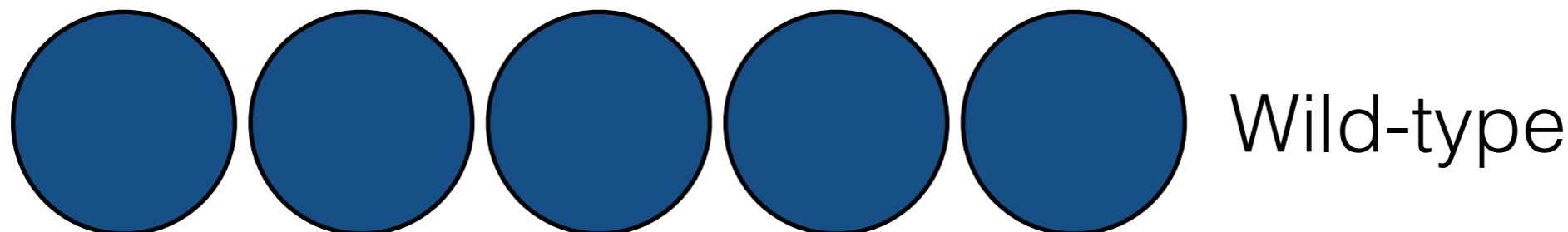
# **How do you get strains that are more or less mutant?**

## **Incomplete penetrance**

Even when a mutant has the mutant allele, it expresses the wild-type phenotype.

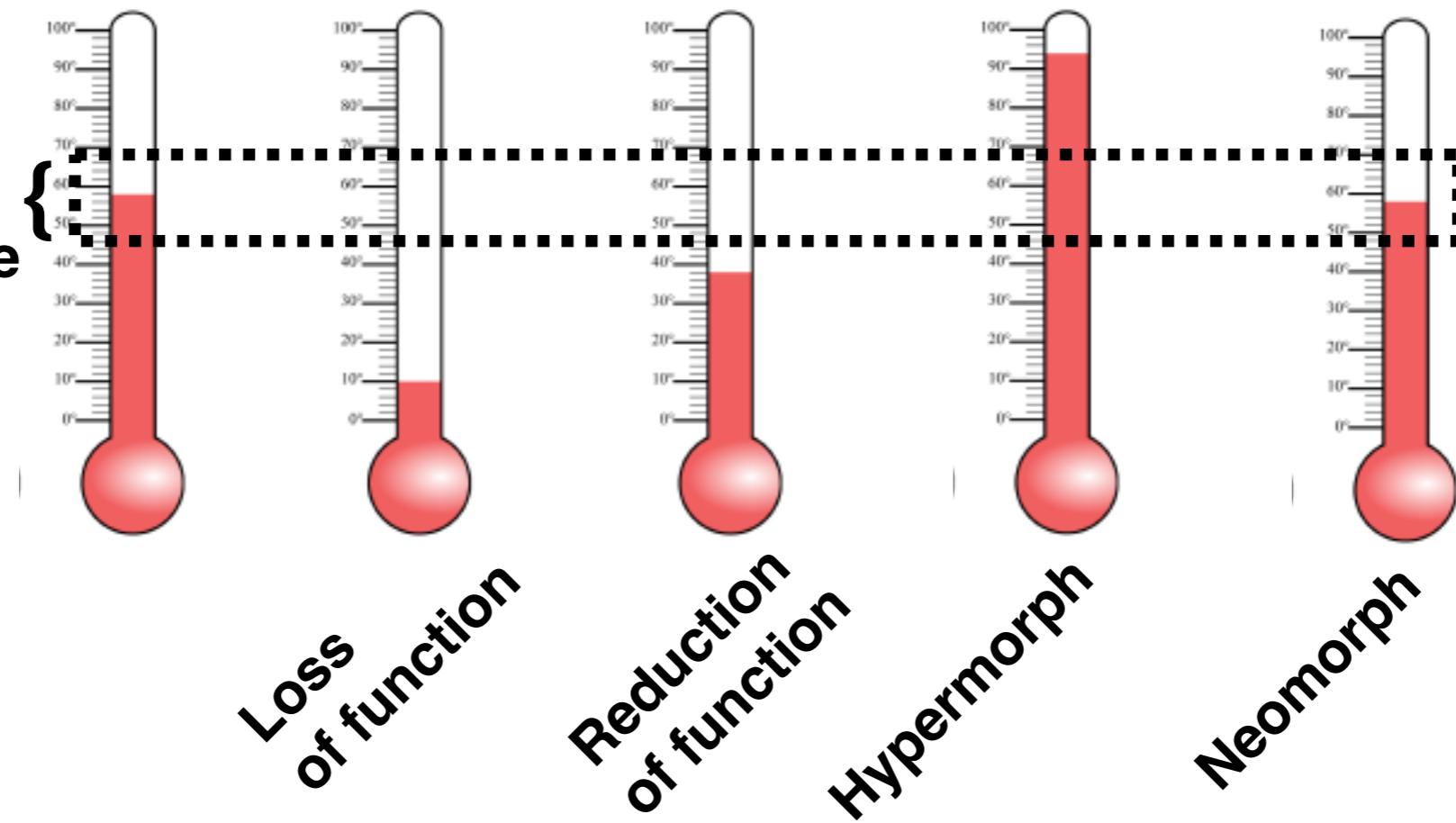
## **Variable expressivity**

If the mutant expresses the mutant phenotype, the severity of the mutant phenotype differs from individual to individual.



# A wild-type phenotype is a reading of the amount of normal gene function

Range of gene function that confers a wild-type phenotype





Wild-type worms have one vulva



Multivulva mutant worms have multiple vulvae

**Incomplete penetrance is when not every mutant animal has the mutant phenotype**

117/129 animals are multivulva  
91% penetrant



Wild-type worms have one vulva



Multivulva mutant worms have multiple vulvae

**Variable expressivity is when each mutant animal is not completely mutant**

A mutant only has two extra vulvae instead of three.

# Muller's morphs - gene dosage tests

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# Recessive mutant phenotypes

$$\frac{m}{m} > \frac{m}{+} = \frac{+}{+}$$

*amorph*, *null*, or *nullamorph* = mutant causes a complete loss of gene function

$$\frac{m}{m} = \frac{m}{\Delta} > \frac{m}{+} = \frac{\Delta}{+} = \frac{+}{+}$$

*hypomorph* = mutant causes a partial loss of gene function

$$\frac{m}{\Delta} > \frac{m}{m} > \frac{m}{+} = \frac{\Delta}{+} = \frac{+}{+}$$

$m$  = mutation of gene

$\Delta$  = deletion of gene

$+$  = normal allele of gene

$=$  = Phenotype is equivalent

$>$  = Phenotype is more mutant than

$<$  = Phenotype is less mutant than

# Muller's morphs - gene dosage tests

Loss of gene function (LoF)	amorph, nullomorph
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# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*haploinsufficient* = two wild-type copies are required for normal function

$$\frac{\Delta}{+} \geq \frac{m}{+} > \frac{+}{+}$$

m = mutation of gene

$\Delta$  = deletion of gene

+ = normal allele of gene

= = Phenotype is equivalent

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# Muller's morphs - gene dosage tests

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# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*hypermorph* = mutant causes an increase in wild-type function

$$\frac{m}{m} > \frac{m}{+} > \frac{m}{+} ? \frac{+}{+} > \frac{+}{+}$$

m = mutation of gene

Δ = deletion of gene

+ = normal allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

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# Hypermorphic mutations cause an increase of wild-type function



Wild-type



Too much  
signaling

# Muller's morphs - gene dosage tests

Loss of gene function (LoF)	amorph, nullomorph
Reduction of gene function (RoF)	hypomorph
Gain of gene function (GoF)	hypermorph
Altered function	neomorph, antimorph

m = mutation of gene

△ = deletion of gene

+ = normal allele of gene

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# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*neomorph* = mutant causes function unrelated to normal gene function (abnormal function)

$$\frac{m}{m} \geq \frac{m}{+} = \frac{m}{\Delta} = \frac{m}{\begin{matrix} + \\ - \end{matrix}}$$

m = mutation of gene

Δ = deletion of gene

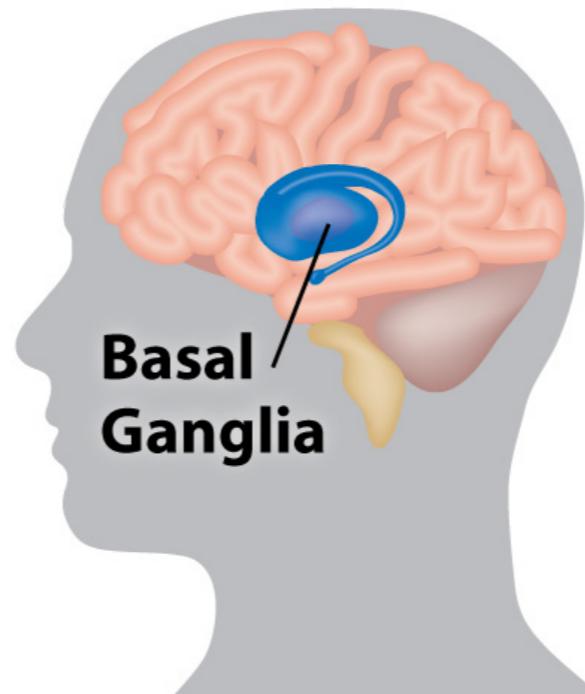
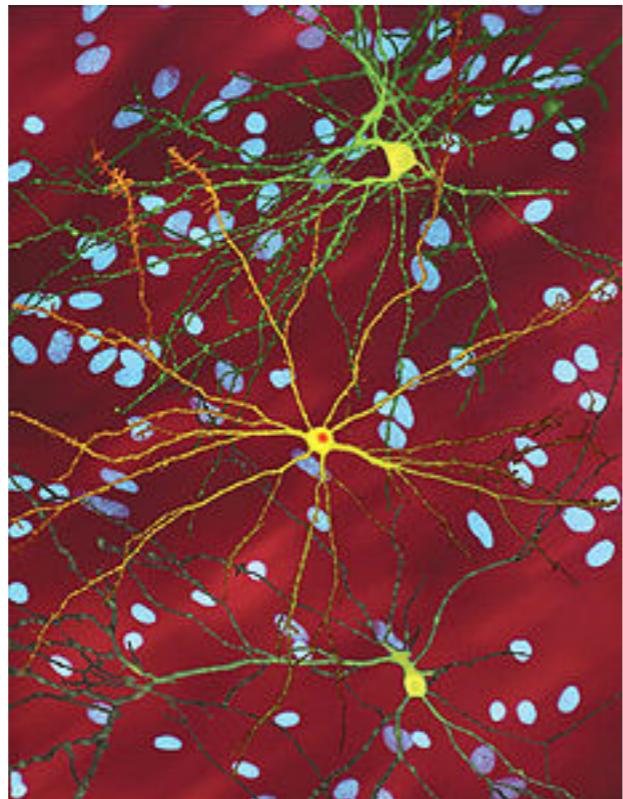
+ = normal allele of gene

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< = Phenotype is less mutant than

# Huntington's disease is caused by a neomorphic gain of function



The pathogenic increase in glutamine repeats causes protein aggregation. This phenomenon has nothing to do with normal protein function.

# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

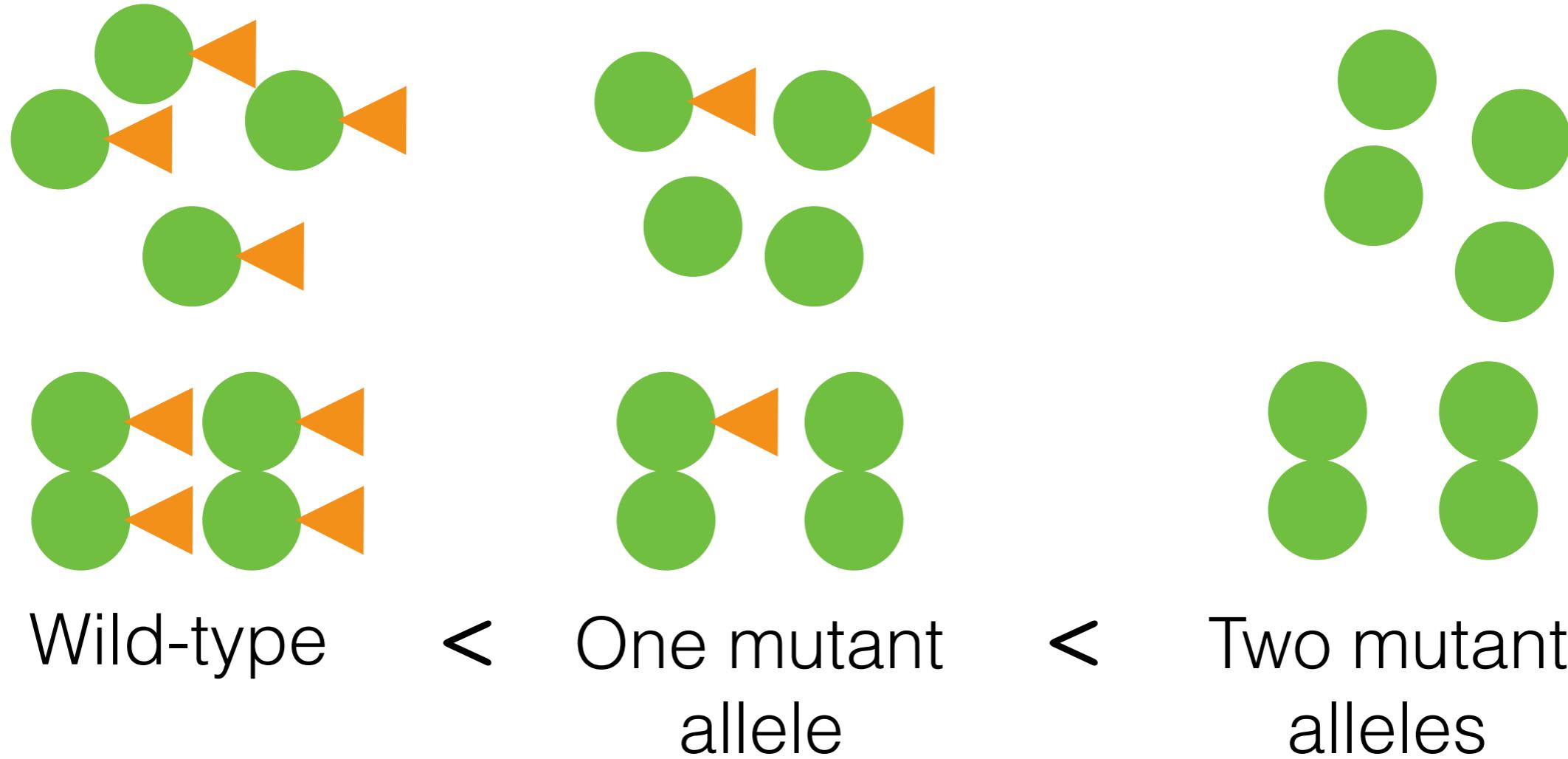
*antimorph* = mutant causes dominant loss of gene function  
dominant negative

$$\frac{m}{+} < \frac{m}{+} < \frac{m}{m} \leq \frac{m}{\Delta}$$

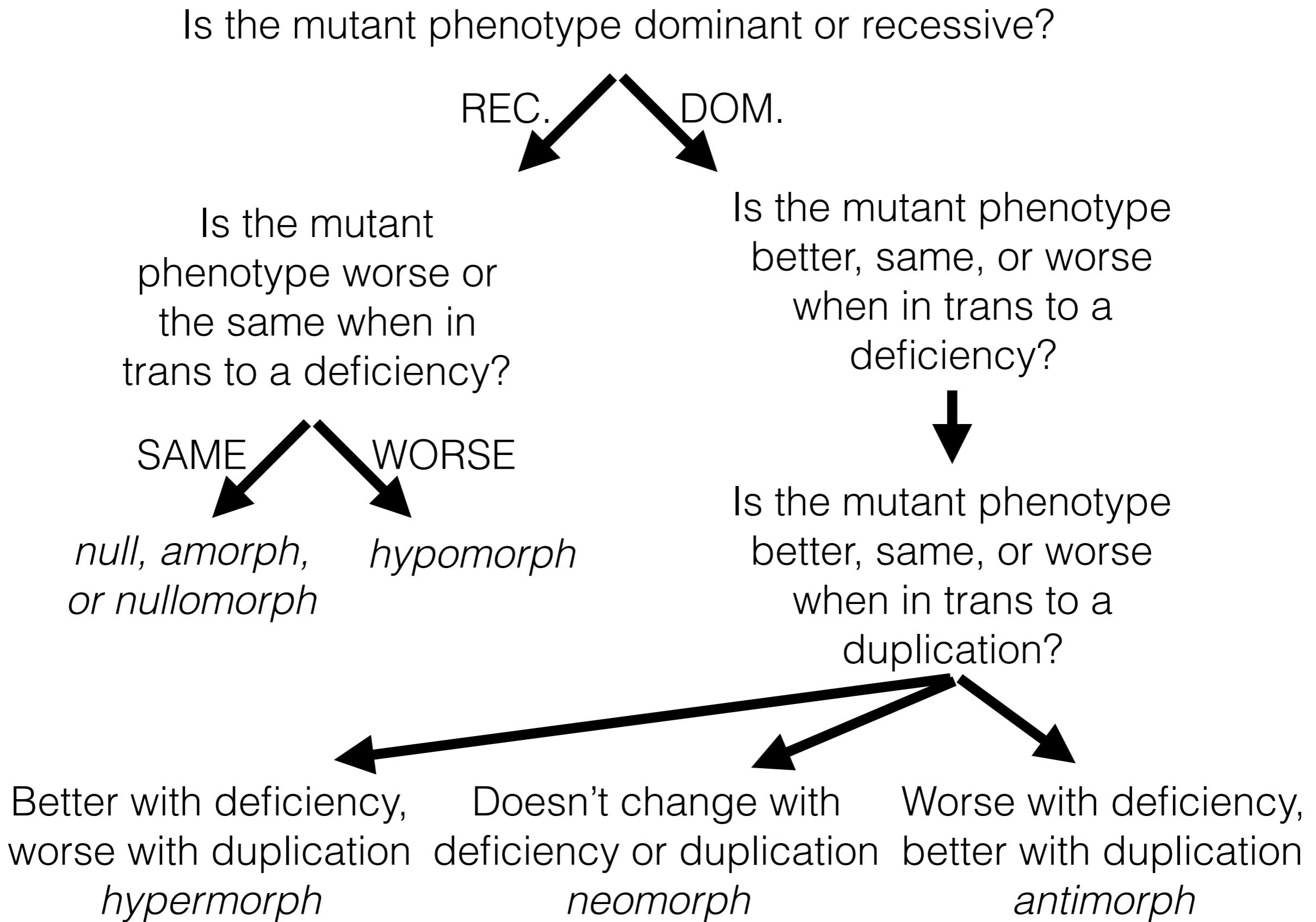
$m$  = mutation of gene  
 $\Delta$  = deletion of gene  
 $+$  = normal allele of gene

= = Phenotype is equivalent  
 $>$  = Phenotype is more mutant than  
 $<$  = Phenotype is less mutant than

# Antimorphs or dominant negatives compete with wild-type function



# Flow chart for gene dosage studies





Lexus CT 200h





Lexus CT 200h mutant 1  
Can't drive, doesn't start



Lexus CT 200h mutant 2  
Can't drive, engine dies

# Complementation: a test of gene function



Lexus CT 200h mutant 1  
Can't drive, doesn't start



Lexus CT 200h mutant 2  
Can't drive, engine dies

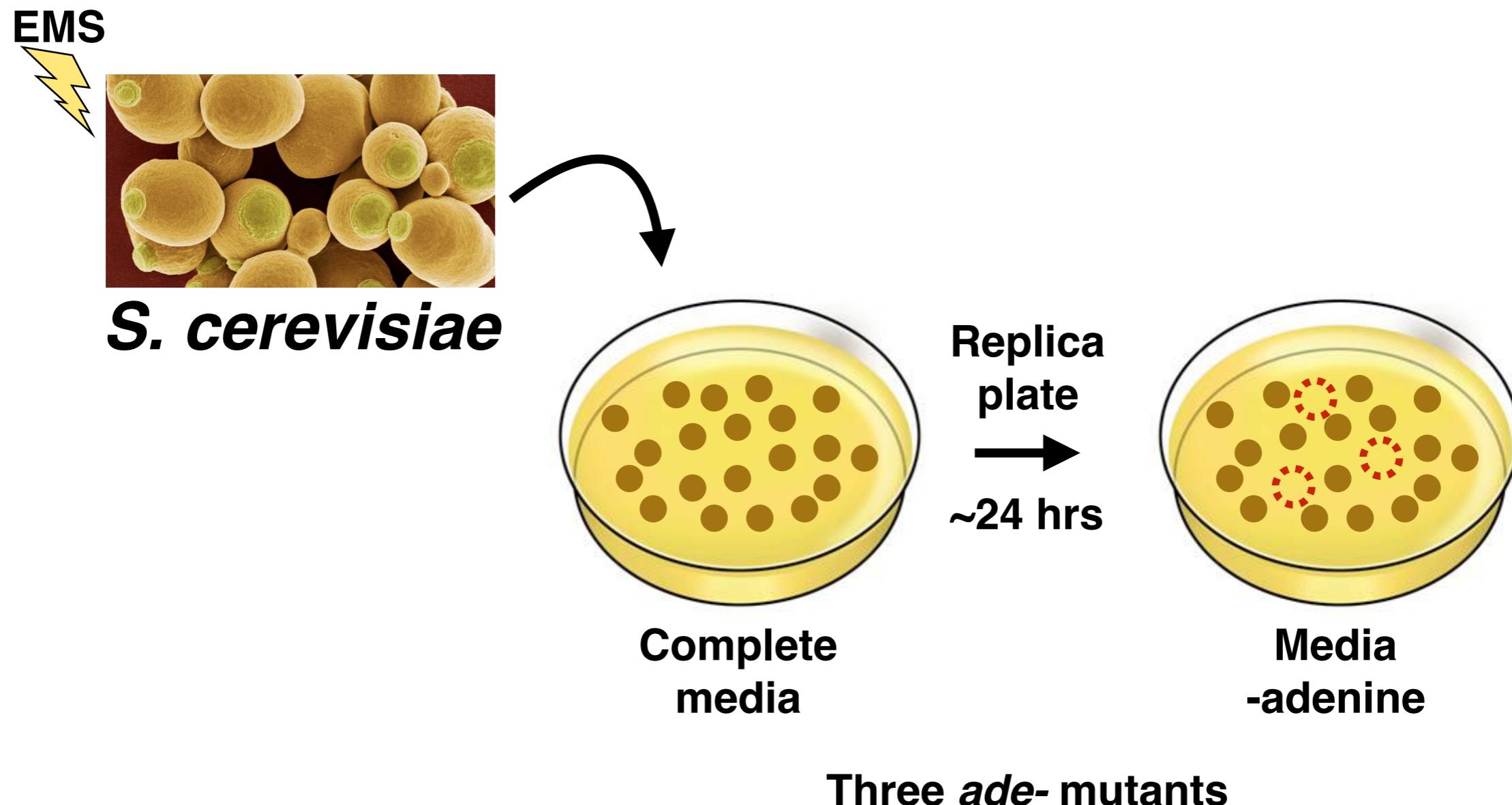
**“Cross” the two mutants and get what?**

**What is the trait we are testing?**



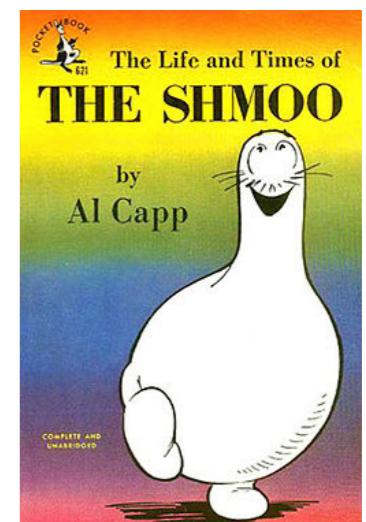
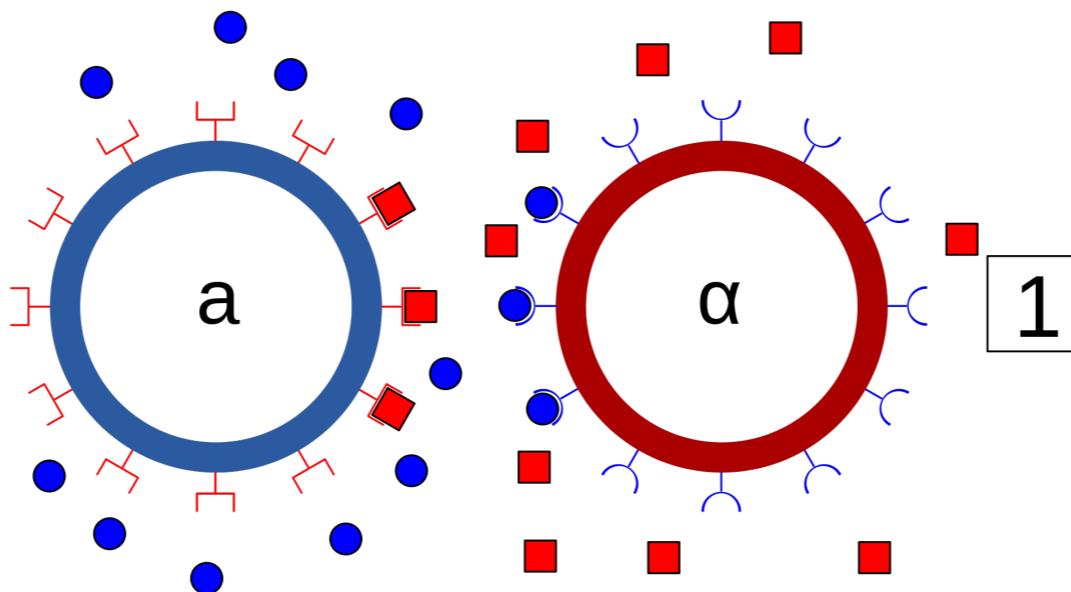
# Screen for mutants that require adenine to grow

Screen:

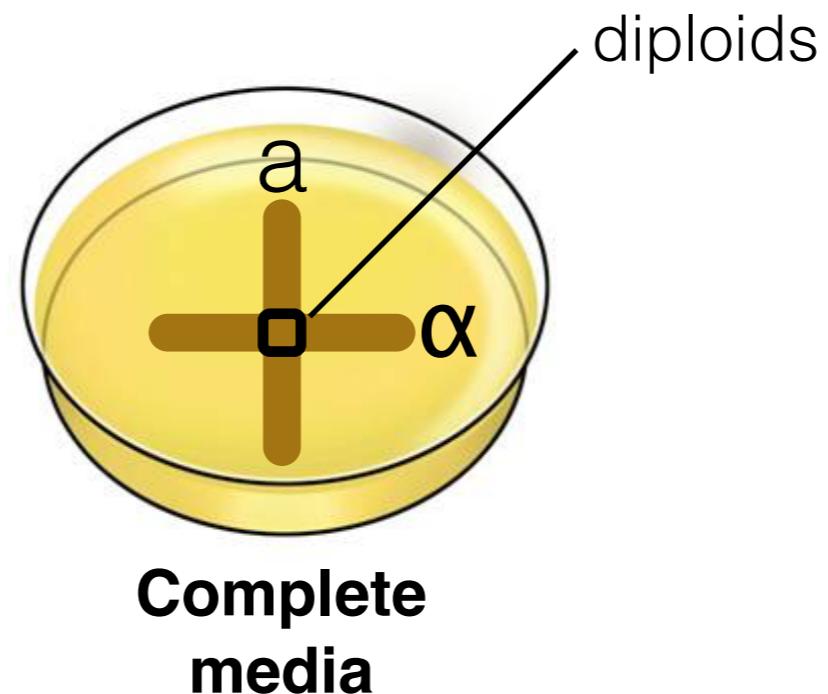


Are all three mutants deficient in the same function  
(i.e. mutants in the same gene)?

# Yeast mating



# Yeast mating tests

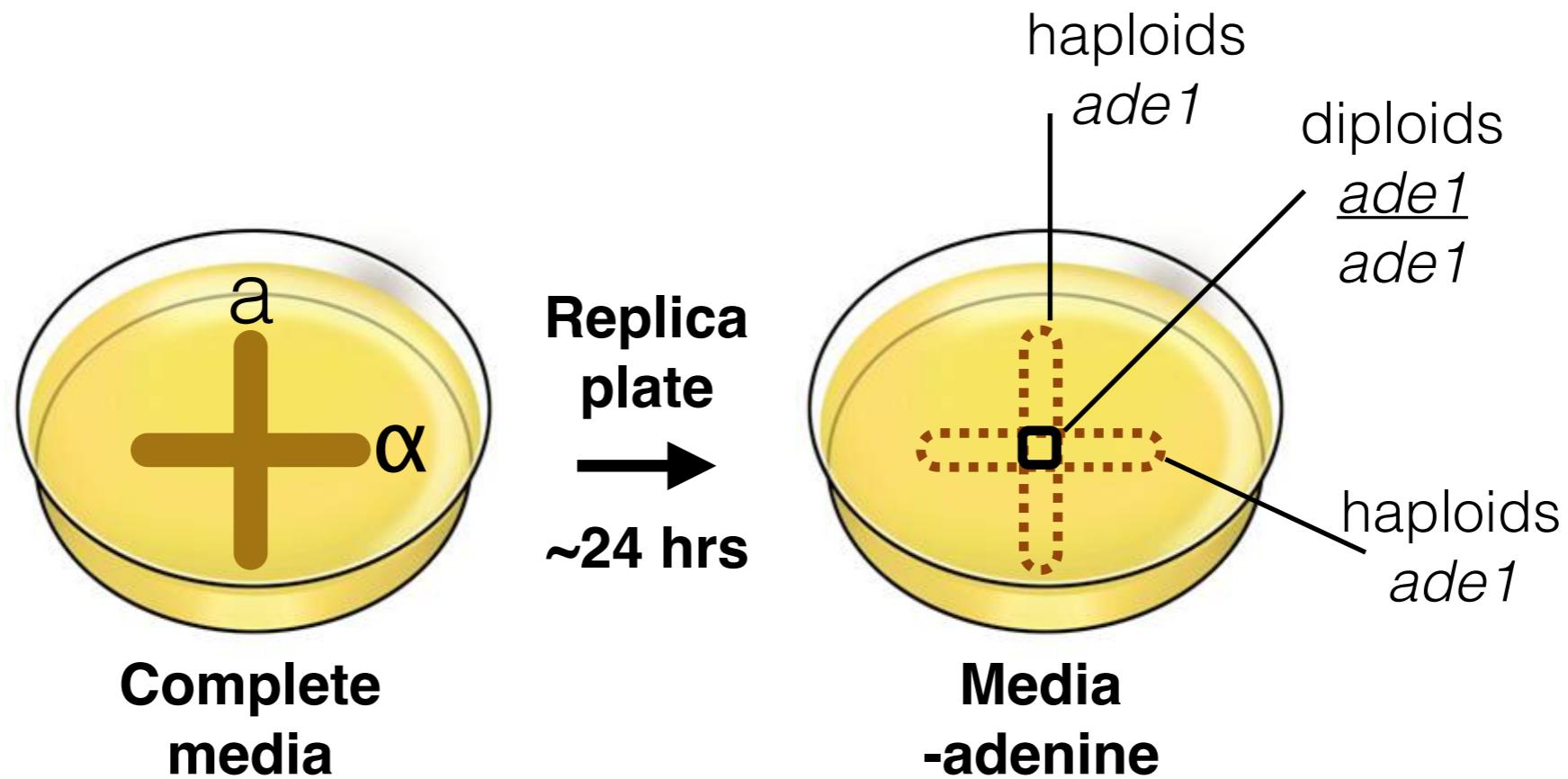


*ade1* mutant

*a* cells are *ade1* mutant

*α* cells are *ade1* mutant

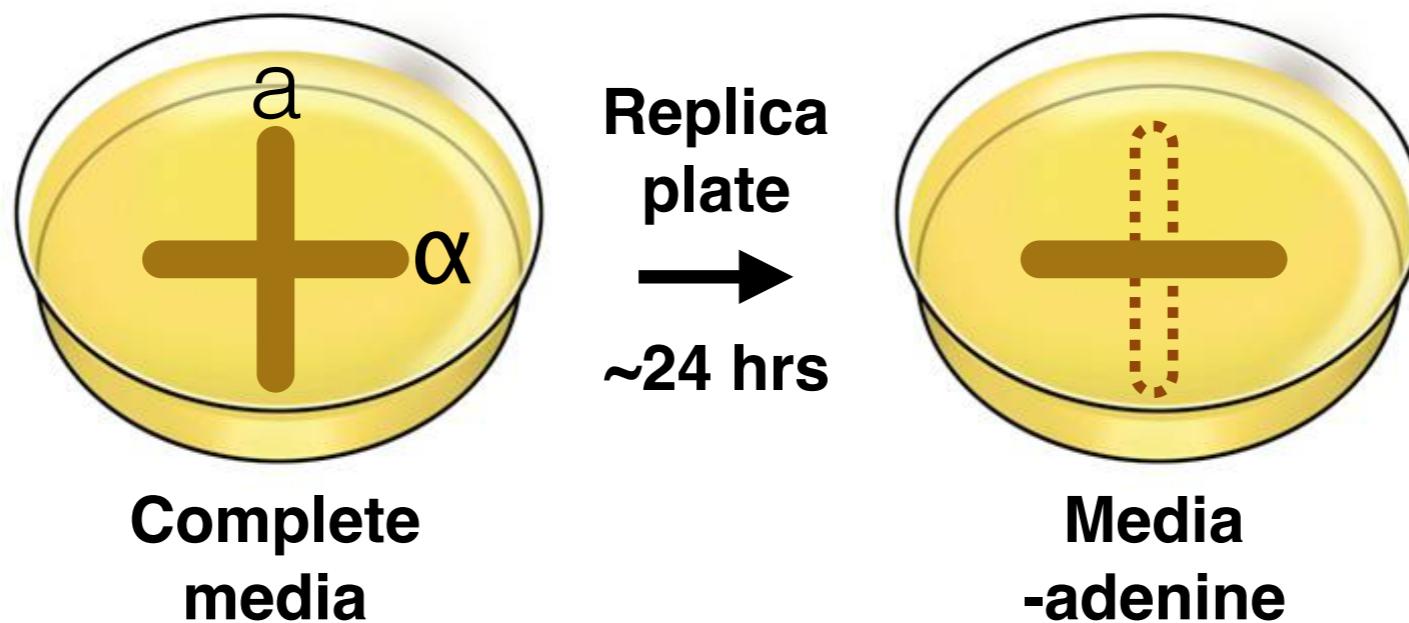
# Yeast mating tests



a cells are *ade1* mutant

$\alpha$  cells are *ade1* mutant

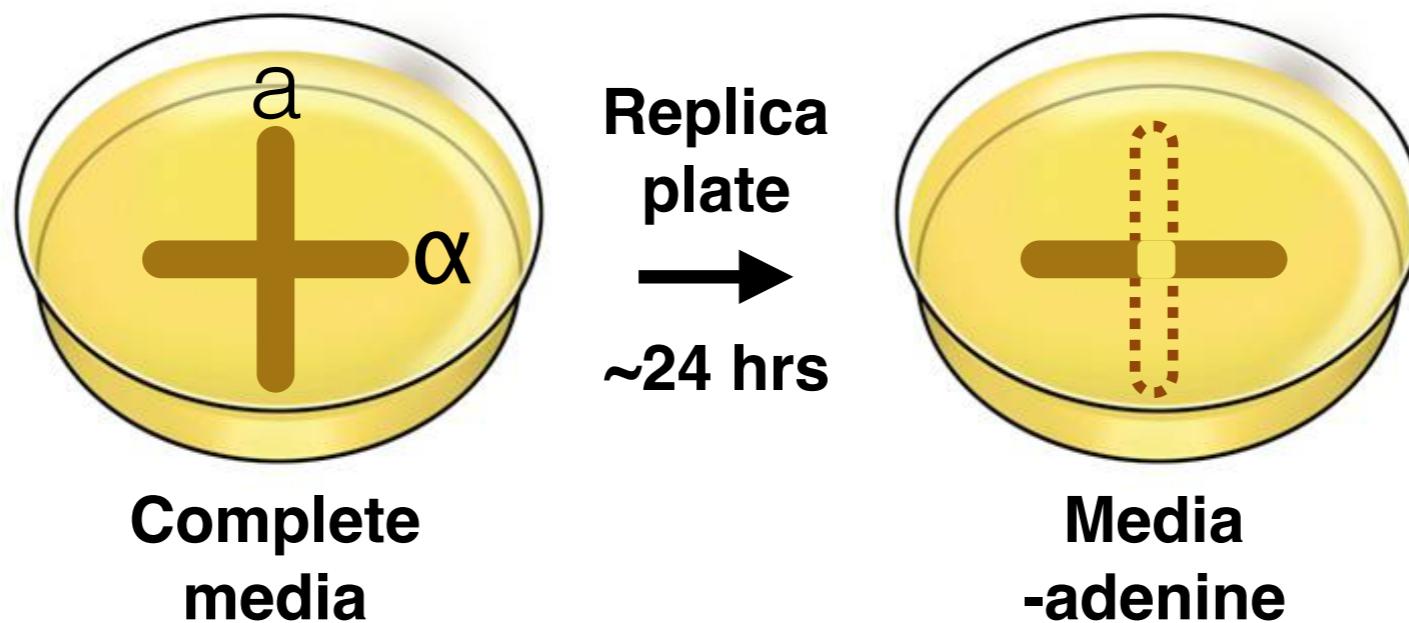
# Dominance testing comes before complementation testing



$a$  cells are *ade1* mutant  
 $\alpha$  cells are wild-type

Is the mutant phenotype dominant?

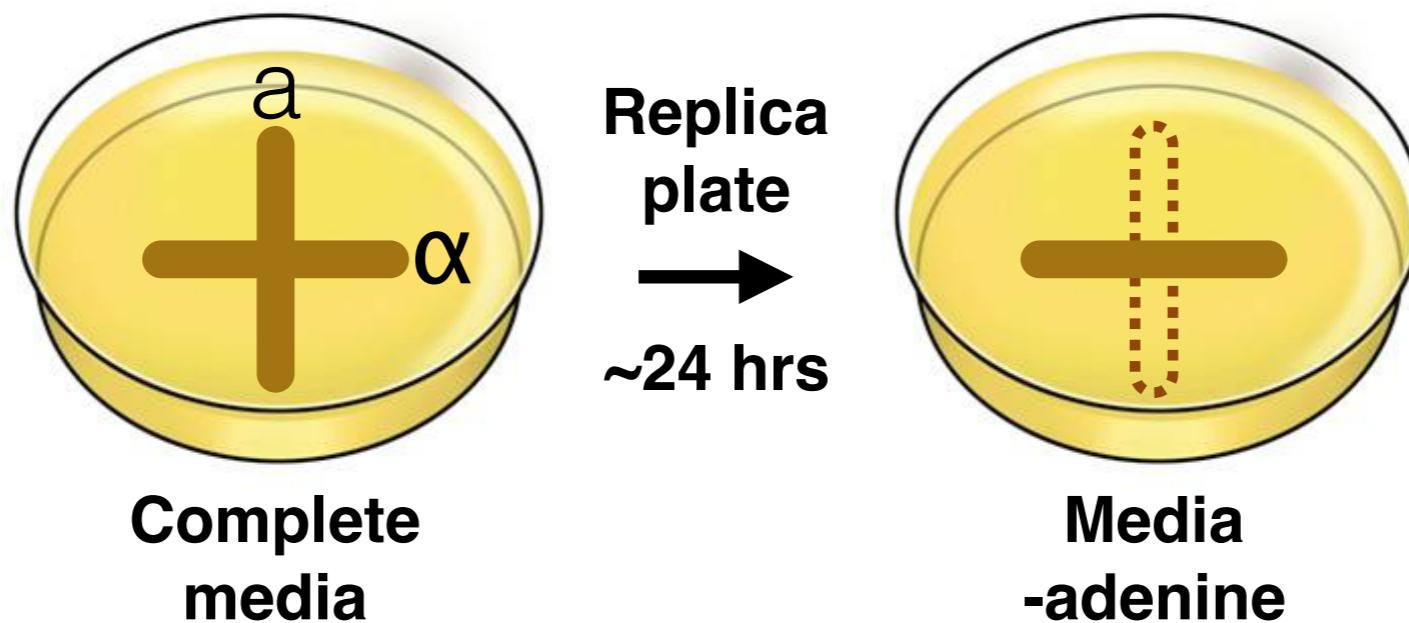
# Dominance testing comes before complementation testing



a cells are *ade2* mutant  
α cells are wild-type

Is the mutant phenotype dominant?

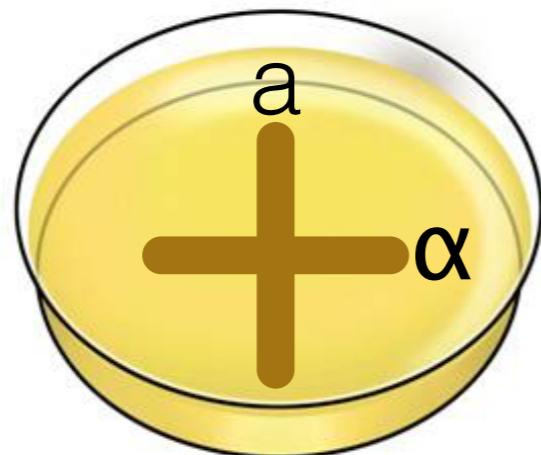
# Dominance testing comes before complementation testing



a cells are *ade3* mutant  
α cells are wild-type

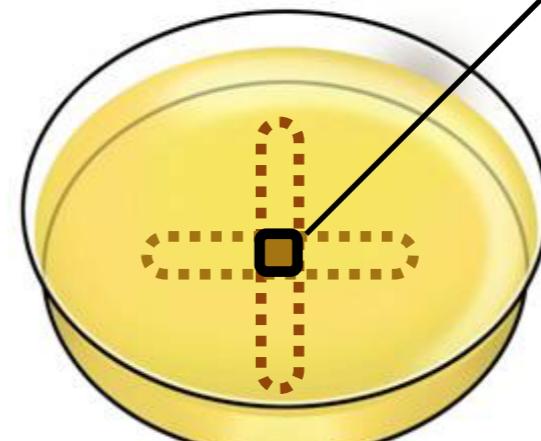
Is the mutant phenotype dominant?

# The complementation test



Complete  
media

Replica  
plate  
→  
~24 hrs



Media  
-adenine

diploids  
 $\underline{ade1} +$   
+  $ade3$

Gene	Phenotype
$ade1$	Rec.
$ade2$	Dom.
$ade3$	Rec.

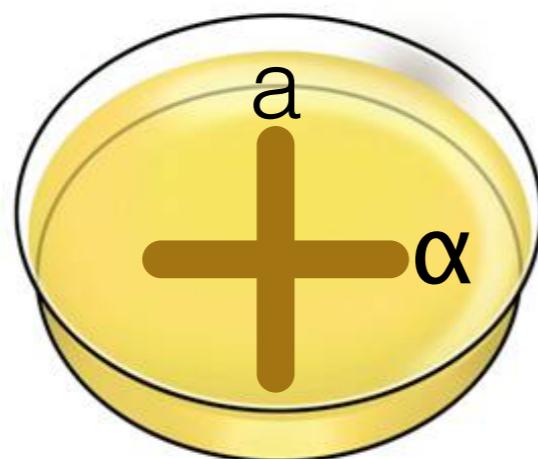
a cells are  $ade1$  mutant

$\alpha$  cells are  $ade3$  mutant

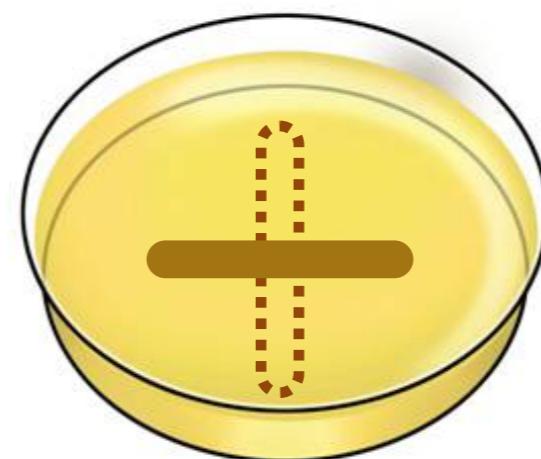
**$ade1$  and  $ade3$  mutations are in different genes**

**They are deficient in different functions!**

# Dominance testing comes before complementation testing



Replica  
plate  
→  
~24 hrs



Complete  
media

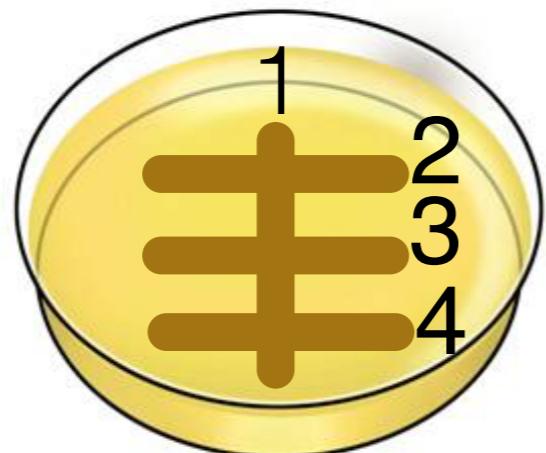
Media  
-adenine

a cells are *ade4* mutant  
α cells are wild-type

Gene	Phenotype
<i>ade1</i>	Rec.
<i>ade2</i>	Dom.
<i>ade3</i>	Rec.

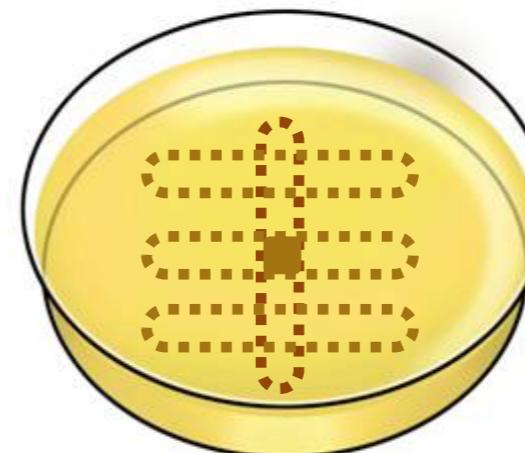
Is the mutant phenotype dominant?

# The complementation test



Complete media

Replica plate  
→  
~24 hrs



Media -adenine

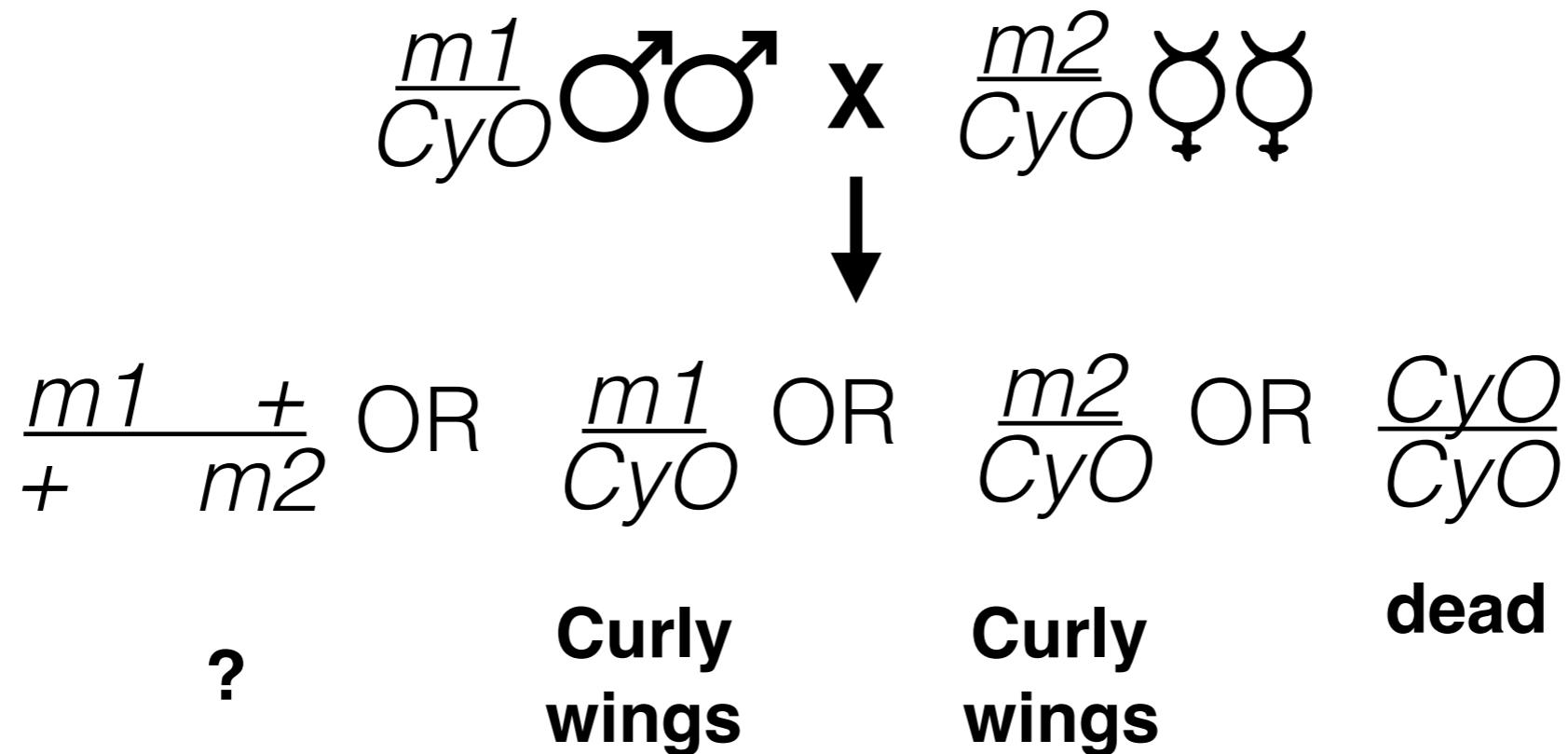
- a cells are *ade1* mutant
- $\alpha$  cells are *ade2* mutant
- $\alpha$  cells are *ade3* mutant
- $\alpha$  cells are *ade4* mutant

Gene	Phenotype
<i>ade1</i>	Rec.
<i>ade2</i>	Dom.
<i>ade3</i>	Rec.
<i>ade4</i>	Rec.

***ade1* and *ade4* mutations are in the same gene**

**They are deficient in the same function**

# The complementation test - *Drosophila*



It is easy to control crosses using obligate outcrossers (e.g. males and females)

# The complementation test - *C. elegans*



$\frac{m1}{m1}$  ♂ x  $\frac{m2}{m2}$  ♀



$\frac{m1}{+}$  +  
+  $m2$

# The complementation test - *C. elegans*


$$\frac{m1}{m1} \text{ ♂} \times \frac{m2}{m2} \text{ ♀}$$

↓                    ↓

$$\frac{m1}{+} \quad \frac{+}{m2} \quad \frac{m2}{m2}$$

# The complementation test - *C. elegans*



$\frac{m1}{m1}$  ♂ x  $\frac{dpy-5}{dpy-5}$  ;  $\frac{m2}{m2}$  ♀



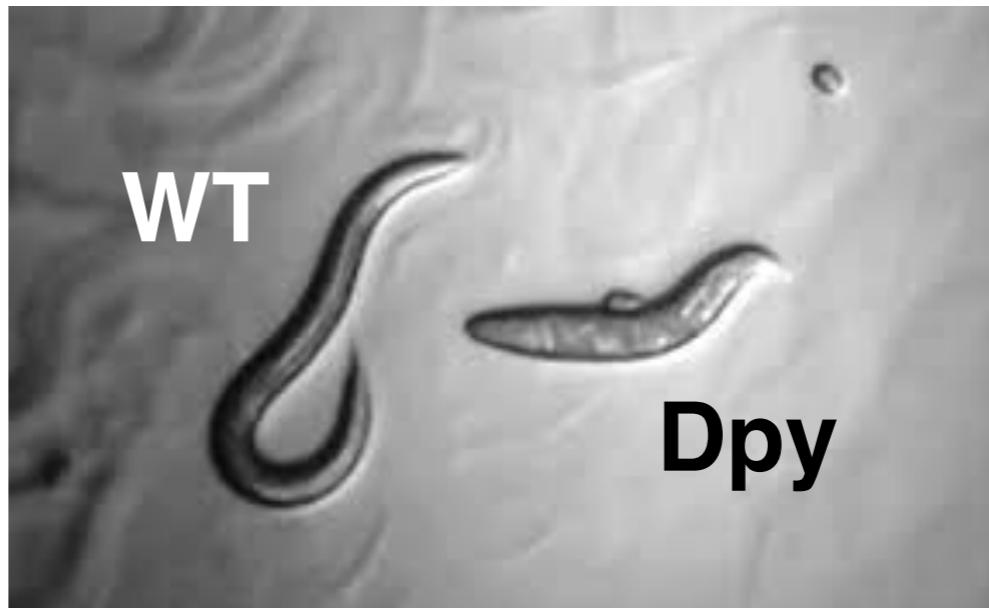
# The complementation test - *C. elegans*



$\frac{m1}{m1}$  ♂ x  $\frac{dpy-5}{dpy-5}$  ;  $\frac{m2}{m2}$  ♀



$\frac{dpy-5}{+}$  ;  $\frac{m1}{+}$  +  $\frac{m2}{m2}$        $\frac{dpy-5}{dpy-5}$  ;  $\frac{m2}{m2}$

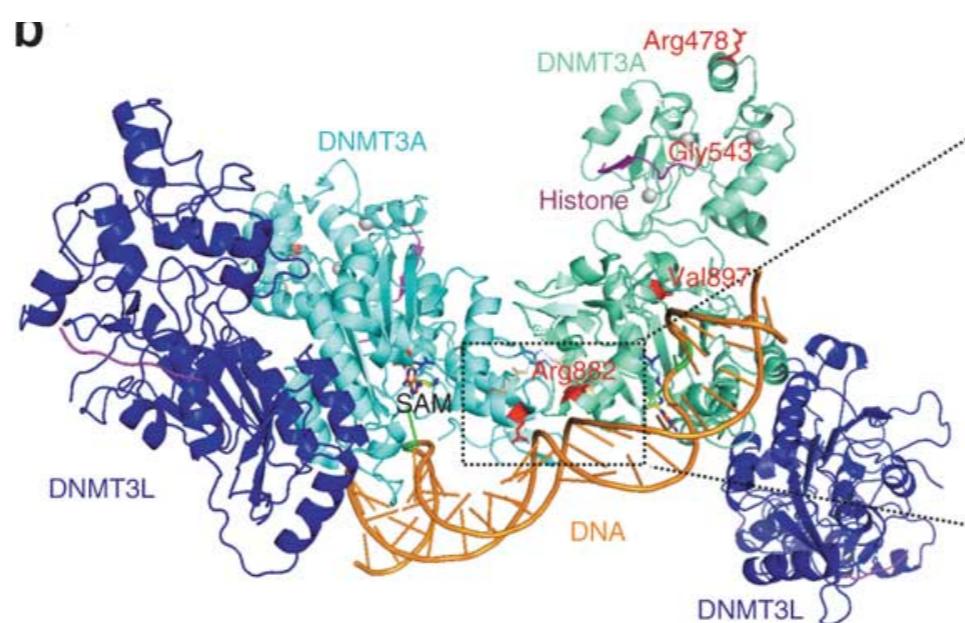


# Why do we care about complementation?

- Multiple alleles of a gene allow us to probe gene function



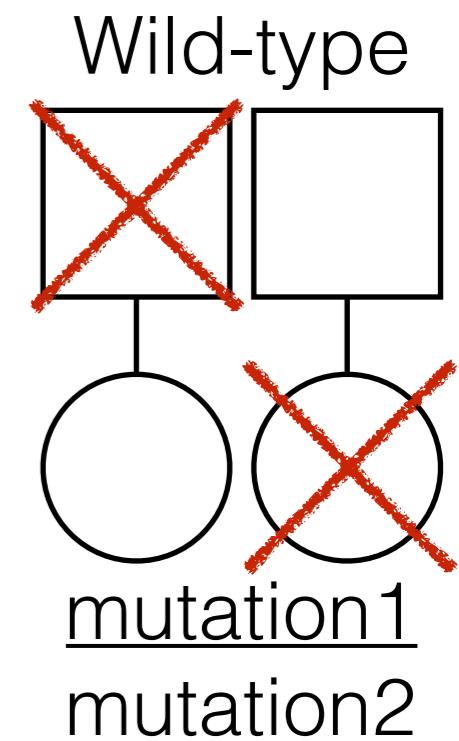
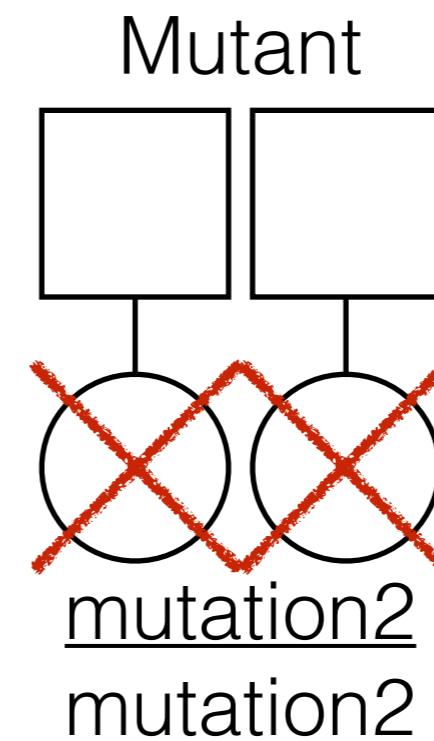
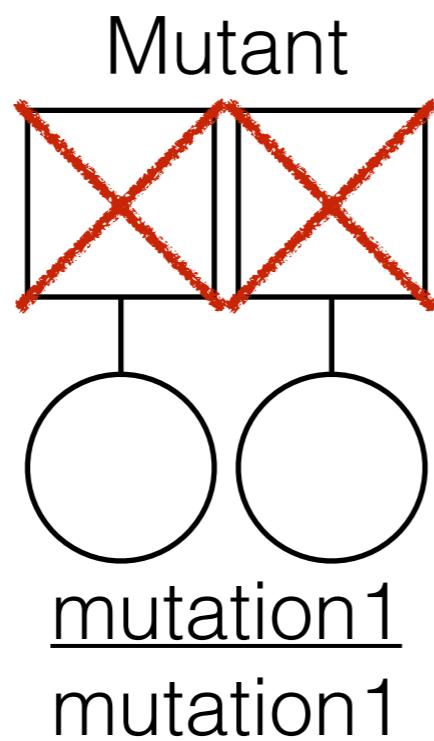
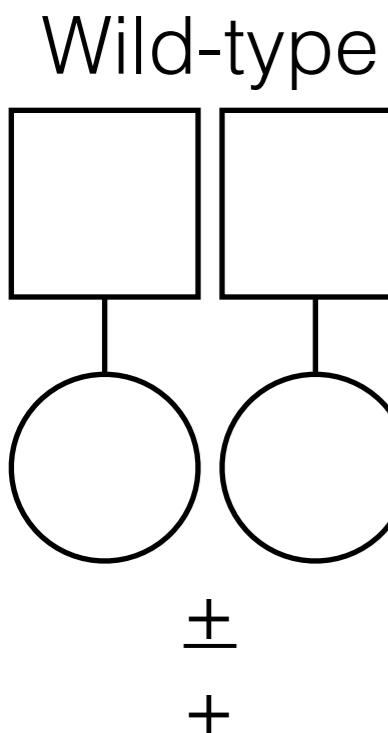
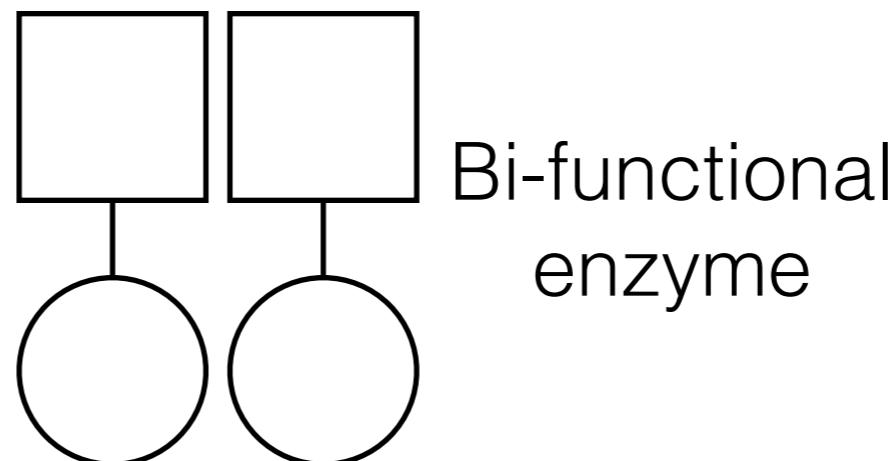
- Different alleles affect different parts of gene



# Exceptions to complementation of function

## Intragenic complementation

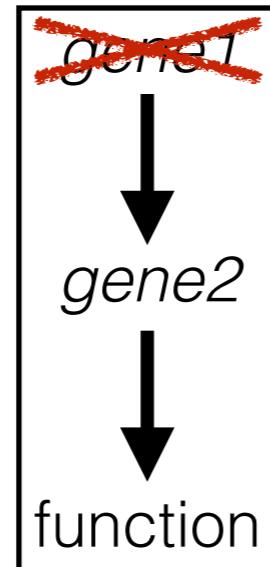
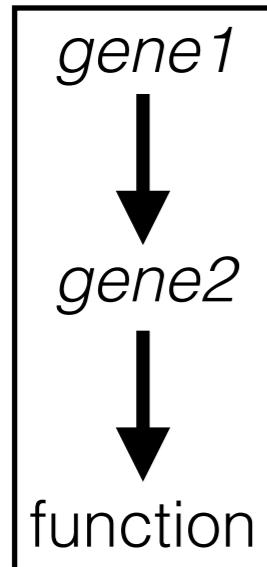
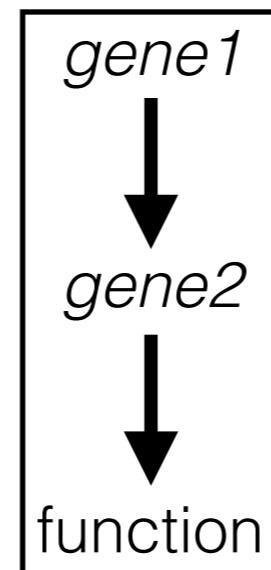
same gene but different functions



# Exceptions to complementation of function

## Intergenic noncomplementation

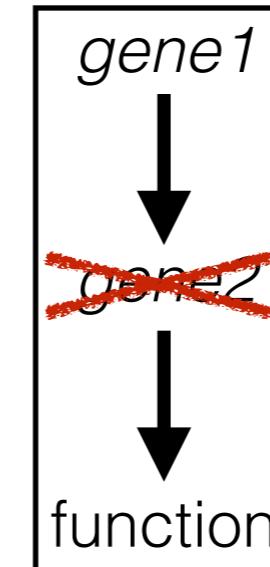
different gene but same function



-100%

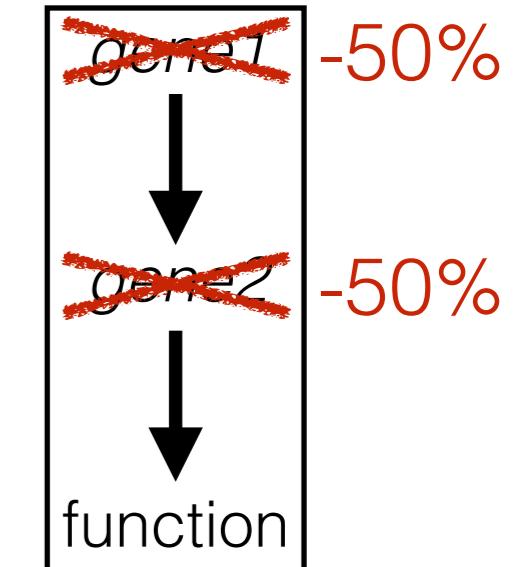
±  
+

mut. g1  
mut. g1



-100%

mut. g2  
mut. g2



-50%

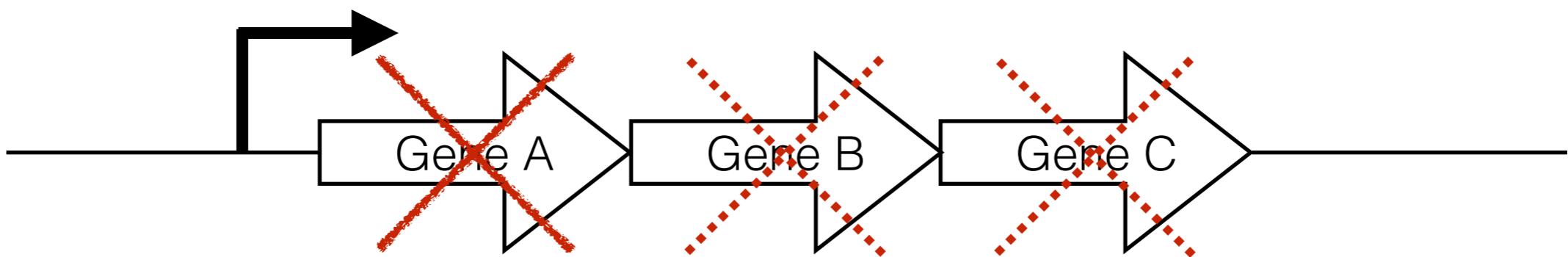
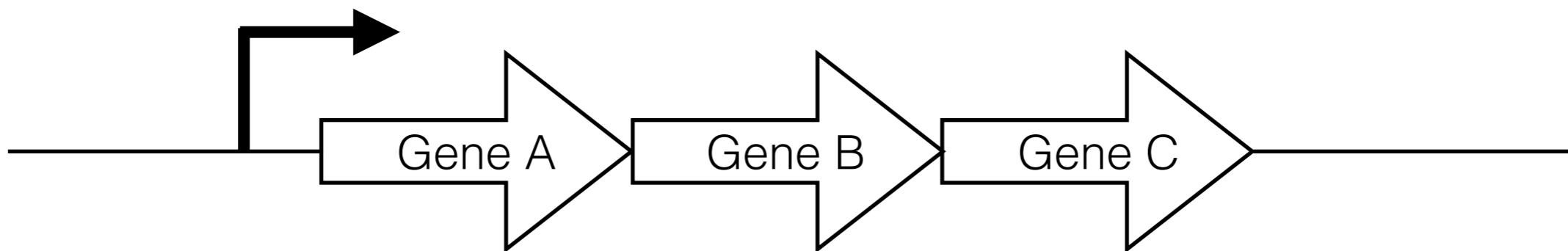
-50%

mut. g1 +  
+ mut. g2

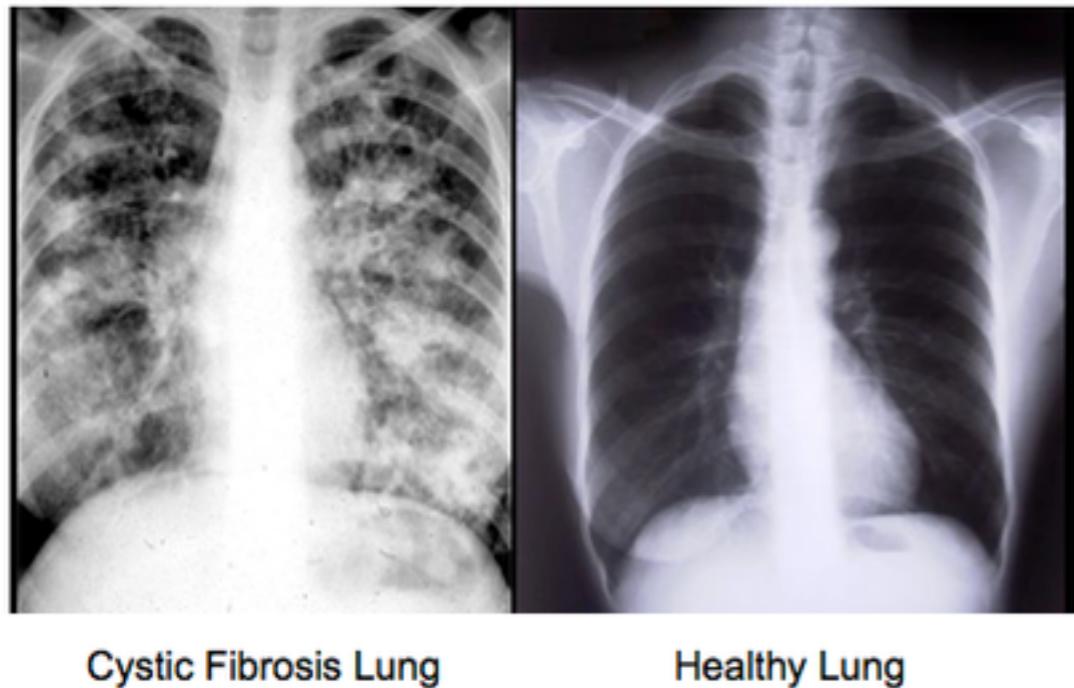
# Exceptions to complementation of function

## Polar effects

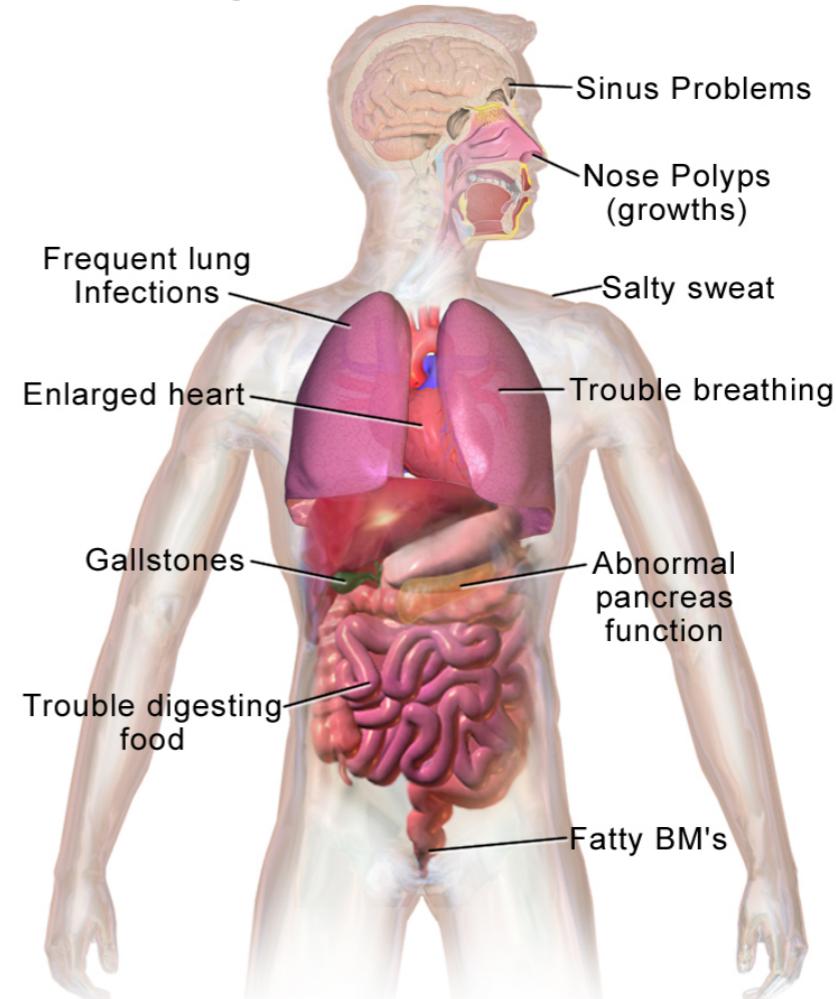
one mutation affects multiple genes/functions



# What about cystic fibrosis and today's topic?

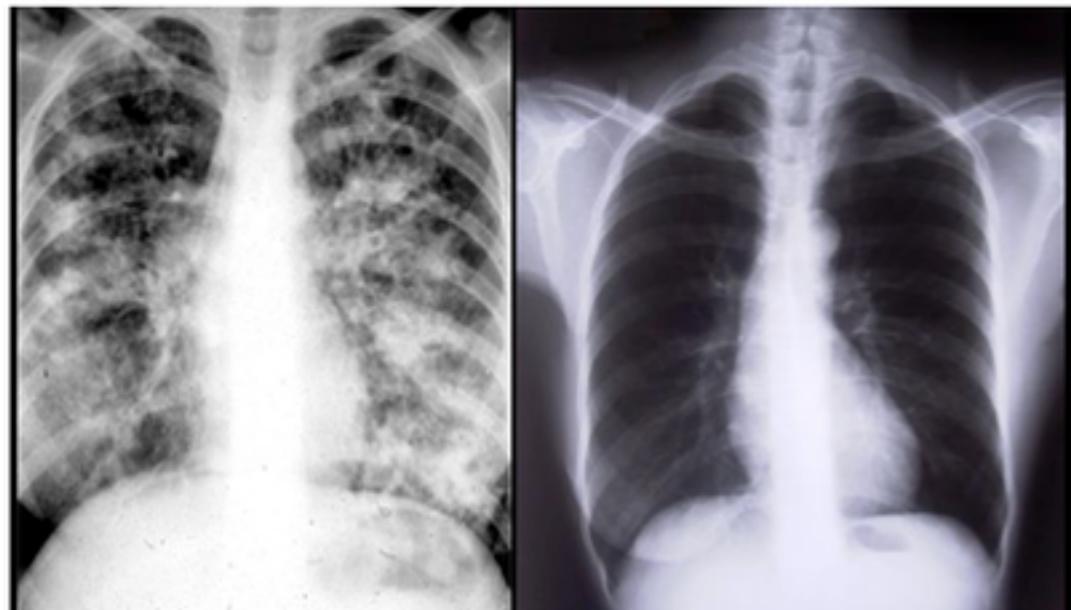


## Health Problems with Cystic Fibrosis



1. Autosomal recessive disorder
2. Not caused by chromosomal aberrations or meiotic NDJ
3. Mapped to chromosome 7

# CF is an autosomal recessive disorder



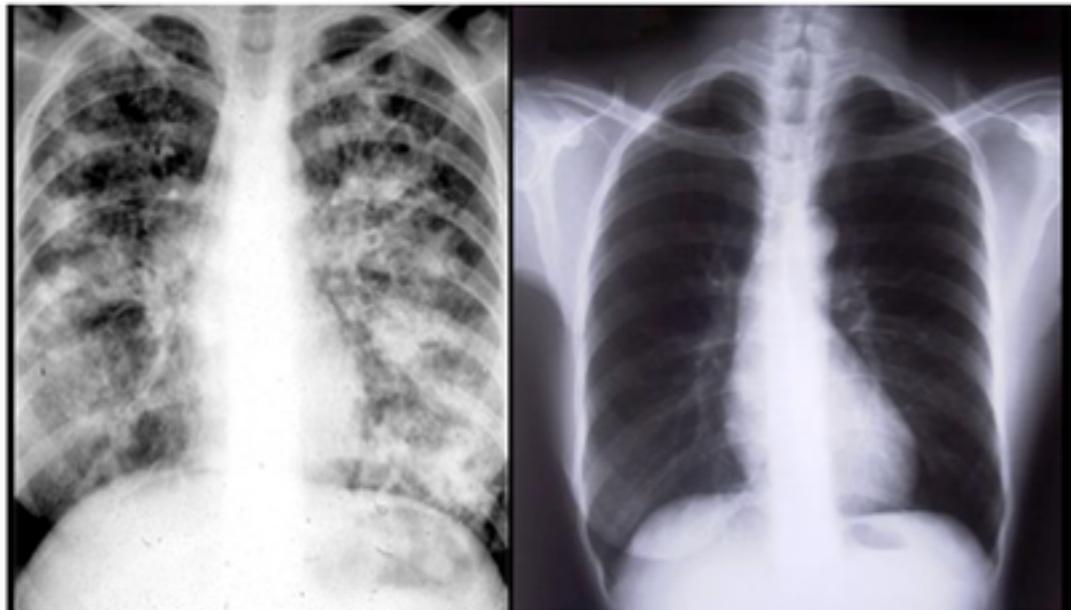
null or hypomorph?

Cystic Fibrosis Lung

Healthy Lung

CF allele	Severity	Survival (yrs)	Prevalence in pop.
F508del	High	36.3	~83%
G542X	High	36.3	~5%
I507del	High	36.3	~0.8%
R347P	Medium	50.0	~0.6%

# CF is an autosomal recessive disorder



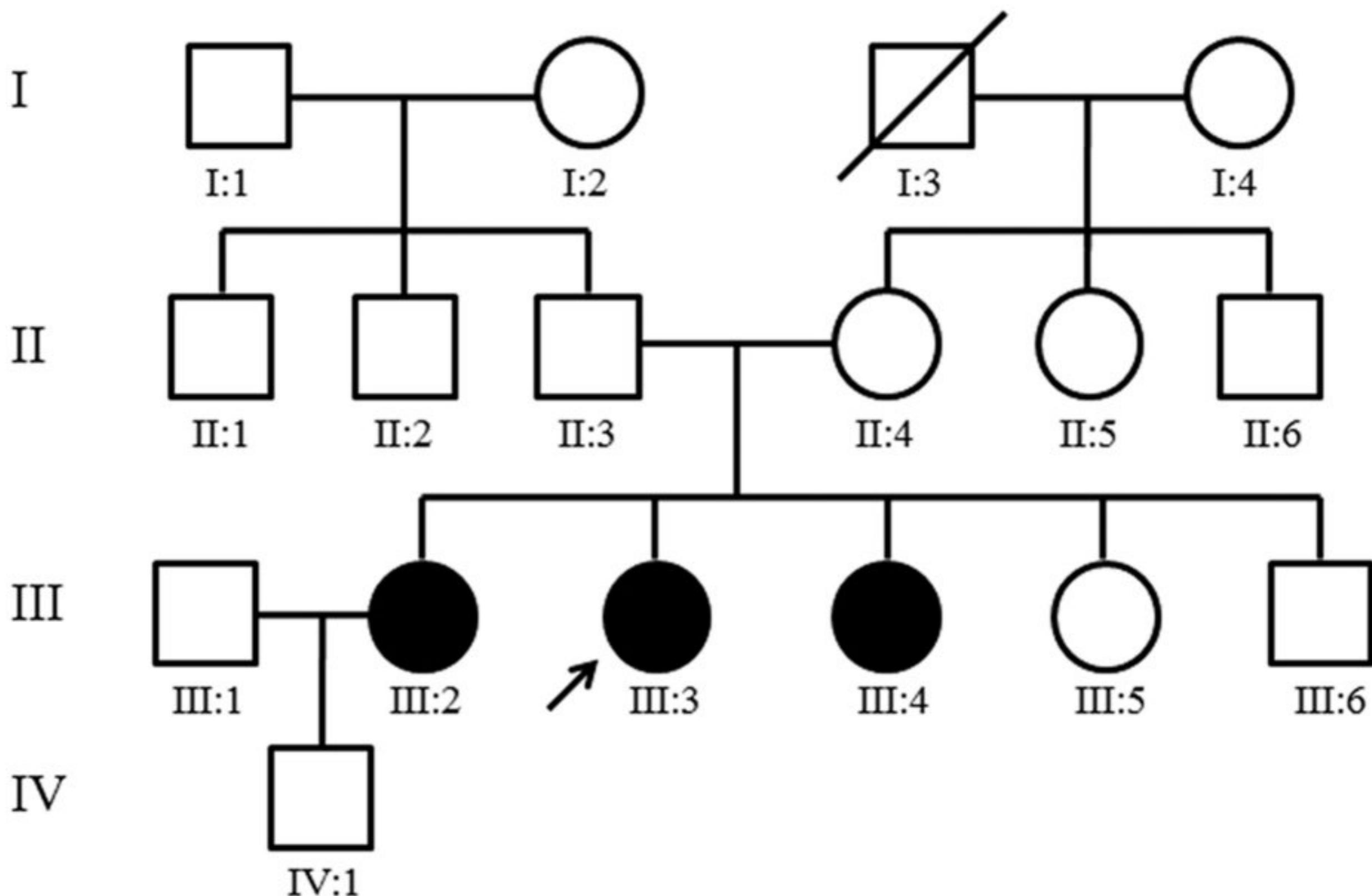
Cystic Fibrosis Lung

Healthy Lung

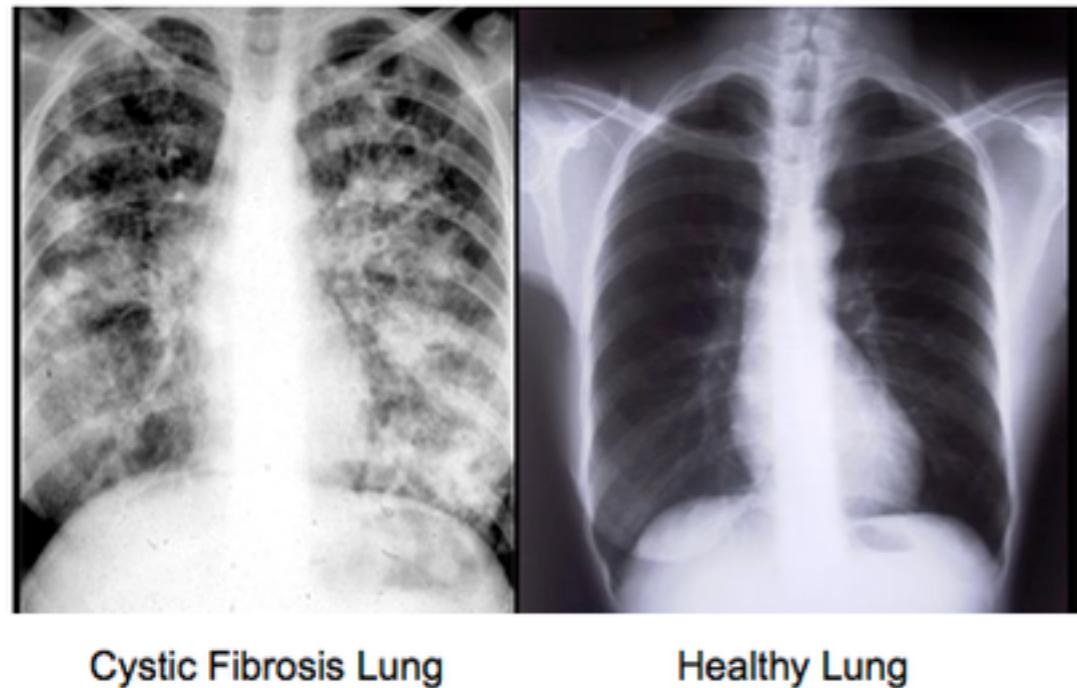
null or hypomorph?

How do we do gene dosage tests in humans?

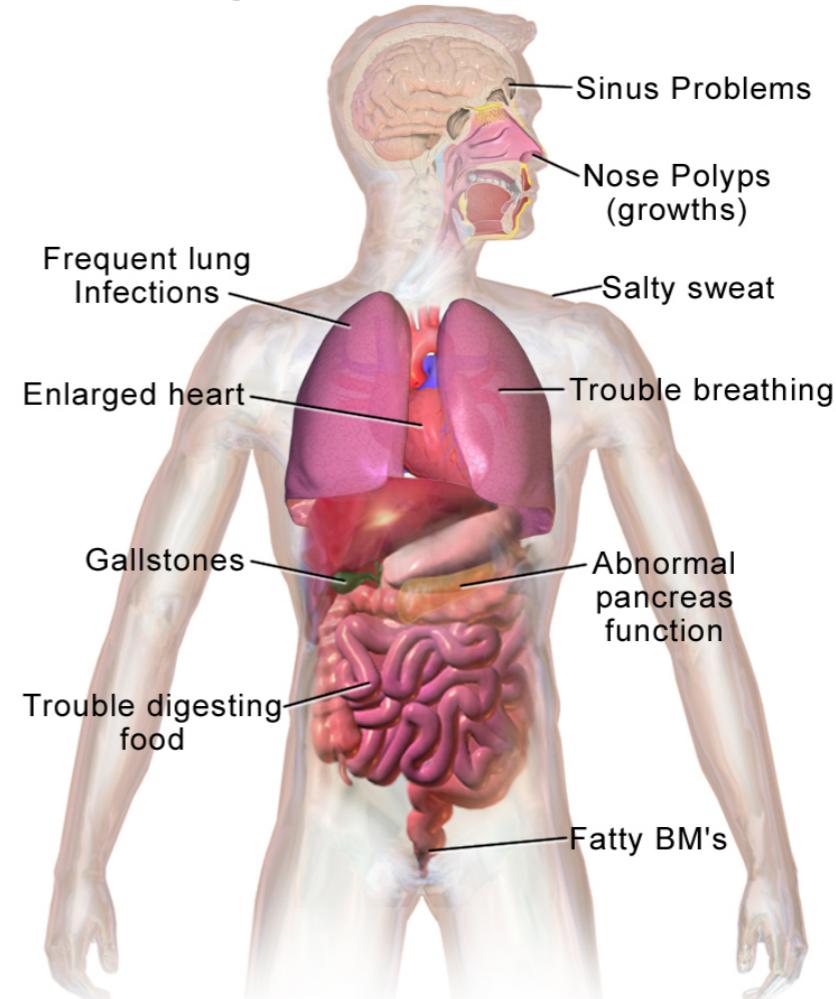
# Compound heterozygosity - failure to complement



# What about cystic fibrosis and today's topic?



## Health Problems with Cystic Fibrosis



1. Autosomal recessive disorder
2. Not caused by chromosomal aberrations or meiotic NDJ
3. Mapped to chromosome 7
4. Mutants in CF gene are null or hypomorphs
5. Mutants can be compound heterozygotes between two different mutations