Matters arising

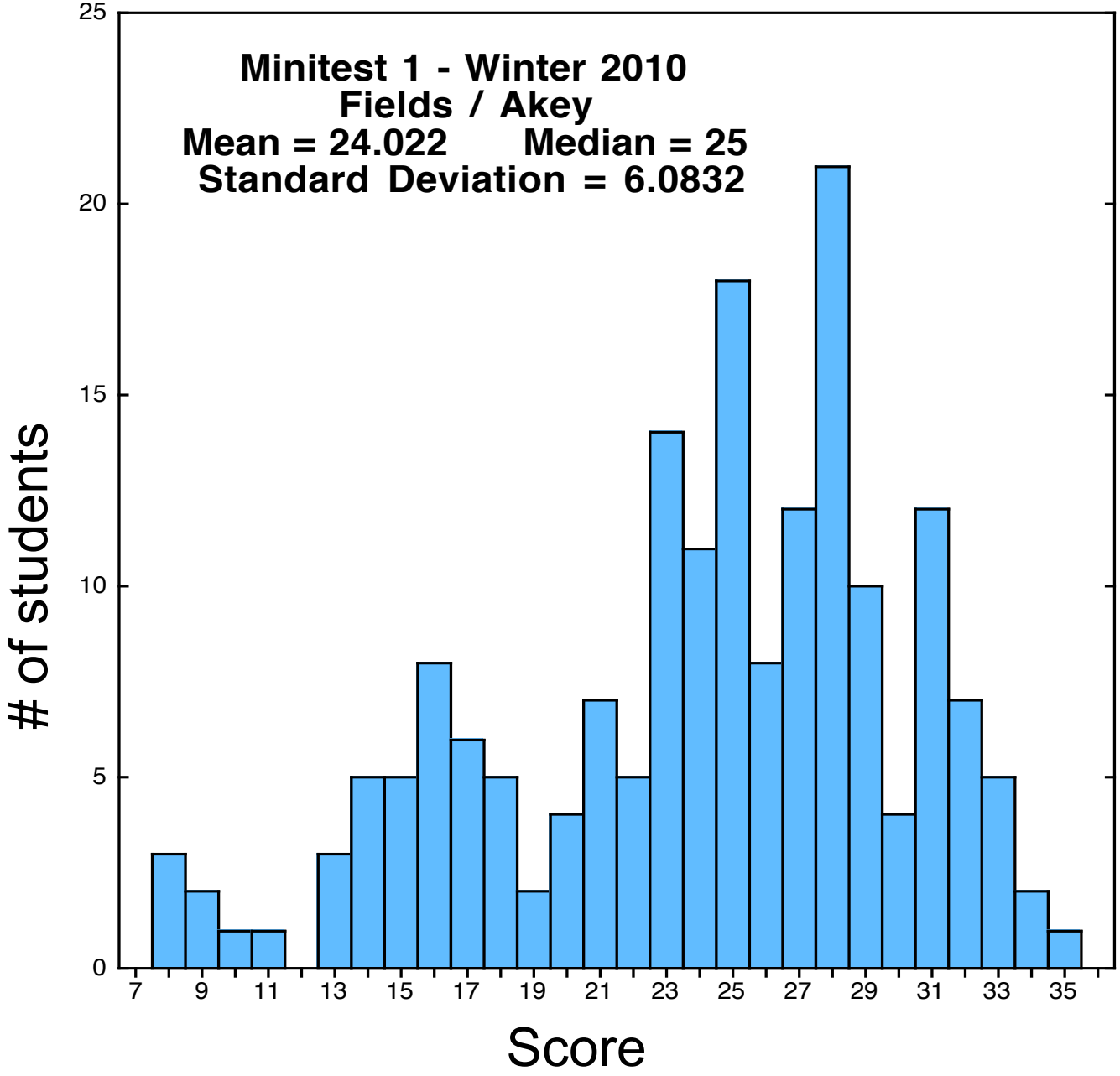
- Minitest

  Pick up from your TA after class

  Answer key posted next week

  Re-grade requests in writing to Anne Paul by next Friday, please

- Mini-survey just before break


Minitest 1 - Winter 2010
Fields / Akey
Mean = 24.022 Median = 25
Standard Deviation = 6.0832
# of students
Score
0
5
10
15
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35

Page last updated at 10:27 GMT, Tuesday, 12 January 2010

By Jonathan Amos
Science correspondent, BBC News

Shar-pei wrinkles explained by dog geneticists

"There was probably a mutation that arose in that gene that led to a really wrinkly puppy and a breeder said, 'hey, that looks interesting, I'm going to try to selectively breed this trait and make more of these dogs'," explained Joshua Akey from the Department of Genome Sciences at the University of Washington, Seattle, US.

Science express, December 31, 2009

PRDM9 Is a Major Determinant of Meiotic Recombination Hotspots in Humans and Mice

F. Baudat,[1*] J. Buard,[1*] C. Grey,[1*] A. Fledel-Alon,[2] C. Ober,[2] M. Przeworski,[2,3] G. Coop,[4] B. de Massy[1†]

Drive Against Hotspot Motifs in Primates Implicates the *PRDM9* Gene in Meiotic Recombination

Simon Myers,[1,2*†] Rory Bowden,[1,2*] Afidalina Tumian,[1] Ronald E. Bontrop,[3] Colin Freeman,[2] Tammie S. MacFie,[4‡] Gil McVean,[1,2§] Peter Donnelly[1,2§]

***Prdm9* Controls Activation of Mammalian Recombination Hotspots**

Emil D. Parvanov, Petko M. Petkov,* Kenneth Paigen*

Meiotic recombination events cluster into narrow segments of the genome, defined as hotspots. Here, we demonstrate that a major player for hotspot specification is the *Prdm9* gene.

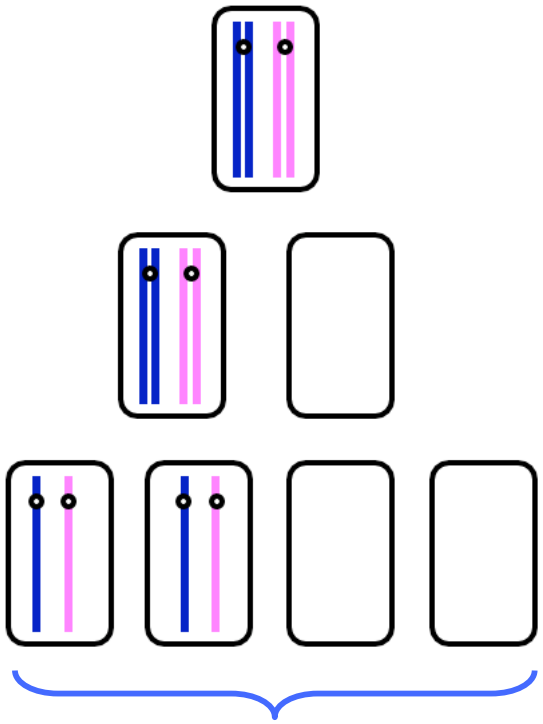
Our results provide a molecular basis for the distribution of meiotic recombination in mammals, where the binding of PRDM9 to specific DNA sequences targets the initiation of recombination at specific locations in the genome.

Aneuploidy (cont'd)

Major cause of aneuploidy—meiosis nondisjunction

failure to separate chromosomes correctly

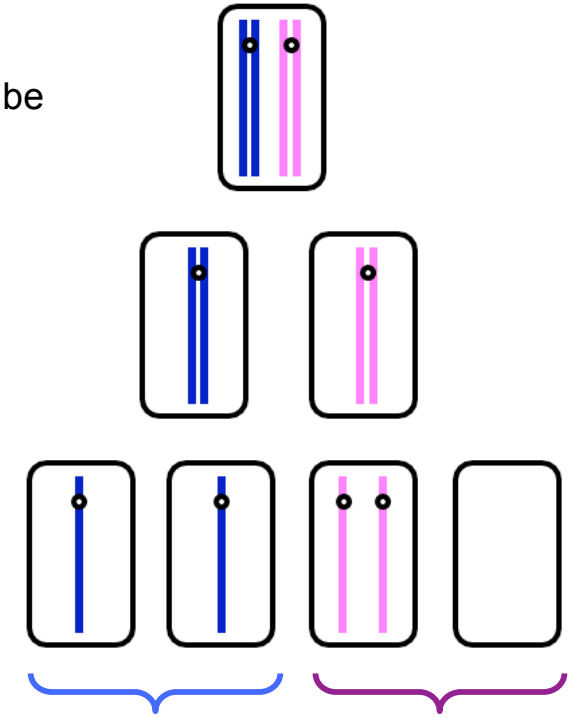
Meiosis I nondisjunction



All 4 products defective

(only showing the problem chromosome… others could be perfectly normal)

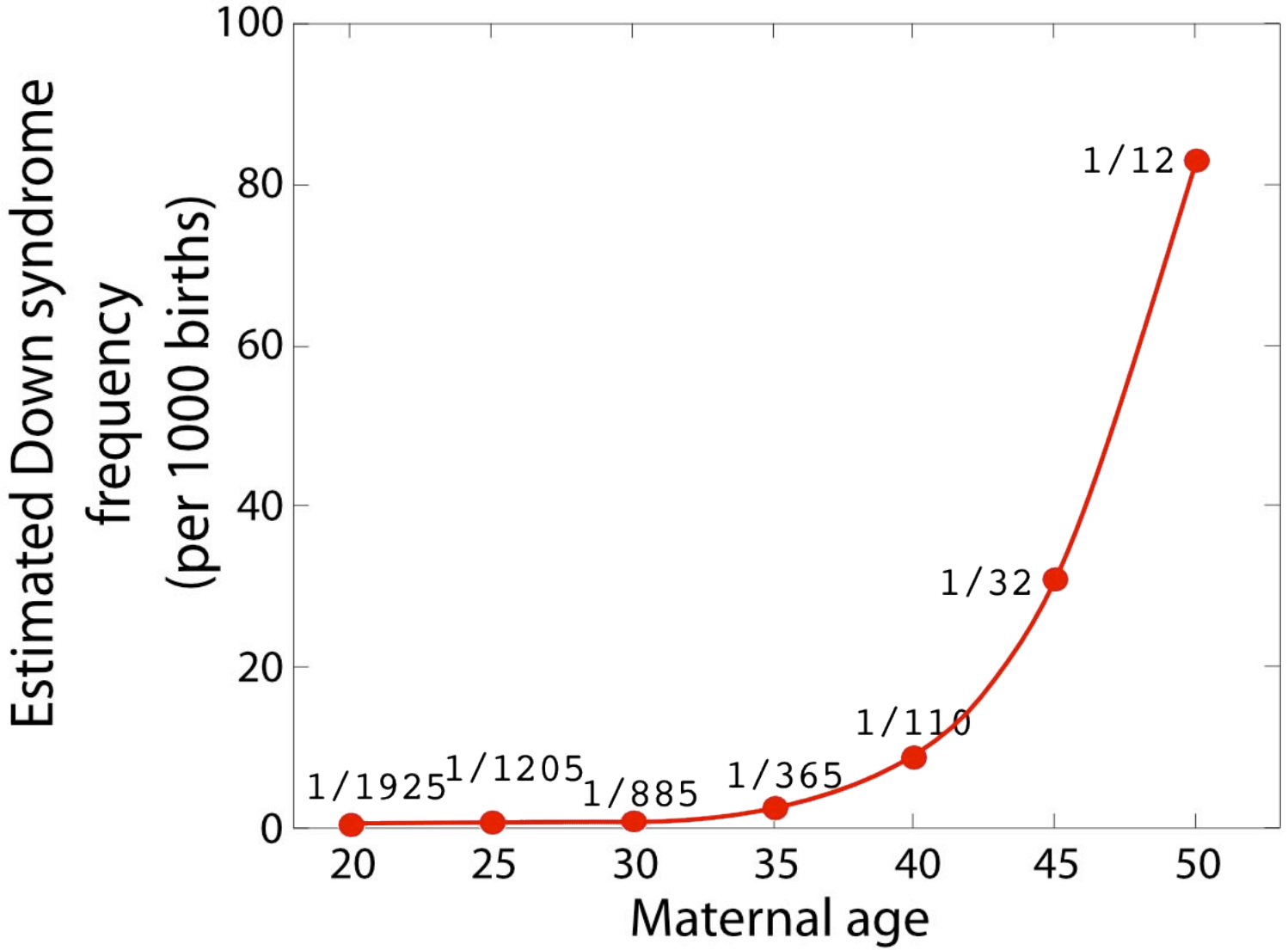
Meiosis II nondisjunction



2 normal

2 defective

Aneuploidy and maternal age



Nature Genetics **37**, 1351 - 1355 (2005)
Published online: 30 October 2005; |
doi:10.1038/ng1672

cohesin subunit

SMC1$_\beta$-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction

Craig A Hodges[1], Ekaterina Revenkova[2], Rolf Jessberger[2, 3], Terry J Hassold[4] & Patricia A Hunt[4]

Aneuploidy and maternal age (cont'd)

Why the increase in ND with age?

Keep in mind...

- Humans... oocytes begin meiosis before birth
- Arrested in prophase I of meiosis until ovulation

- checkpoint loss in older oocytes?
- less robust spindle?
- "good" oocytes used first?

Genome 371, 15 Jan 2010, Lecture 4

Mutation and Complementation

Types of mutations

- Dominant/ Recessive
- Gain of function/ Loss of function

Complementation analysis

Phenotypes in diploid organisms

Phenotype = physical or observable characteristic

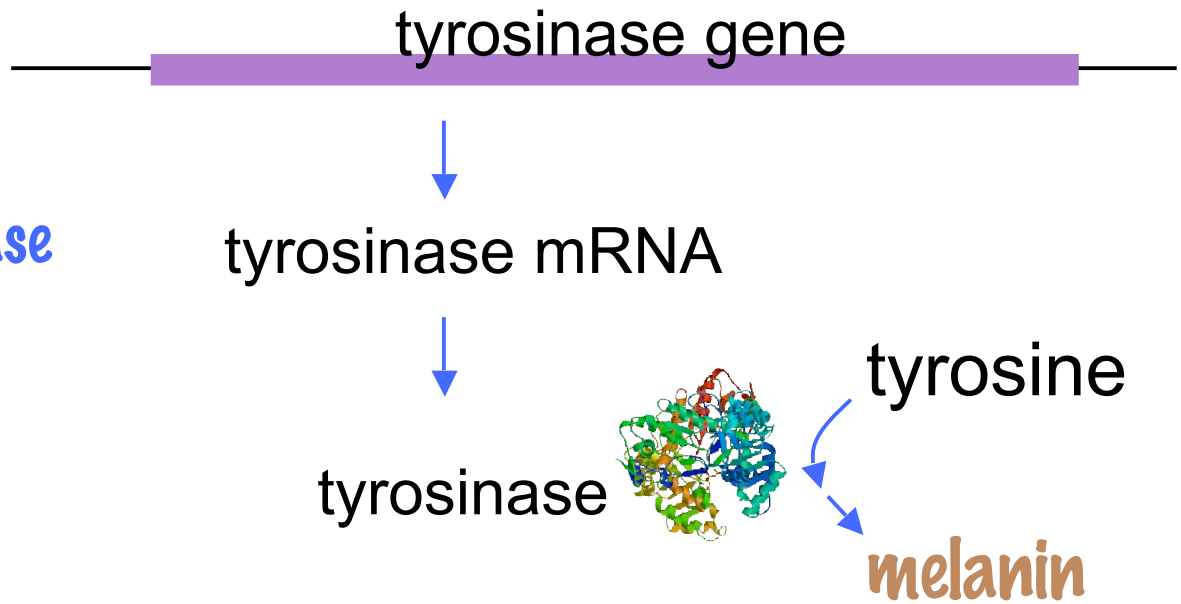
e.g., eye color

hair type

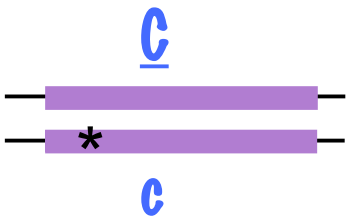
ability/inability to digest lactose

ability to synthesize melanin (pigment)

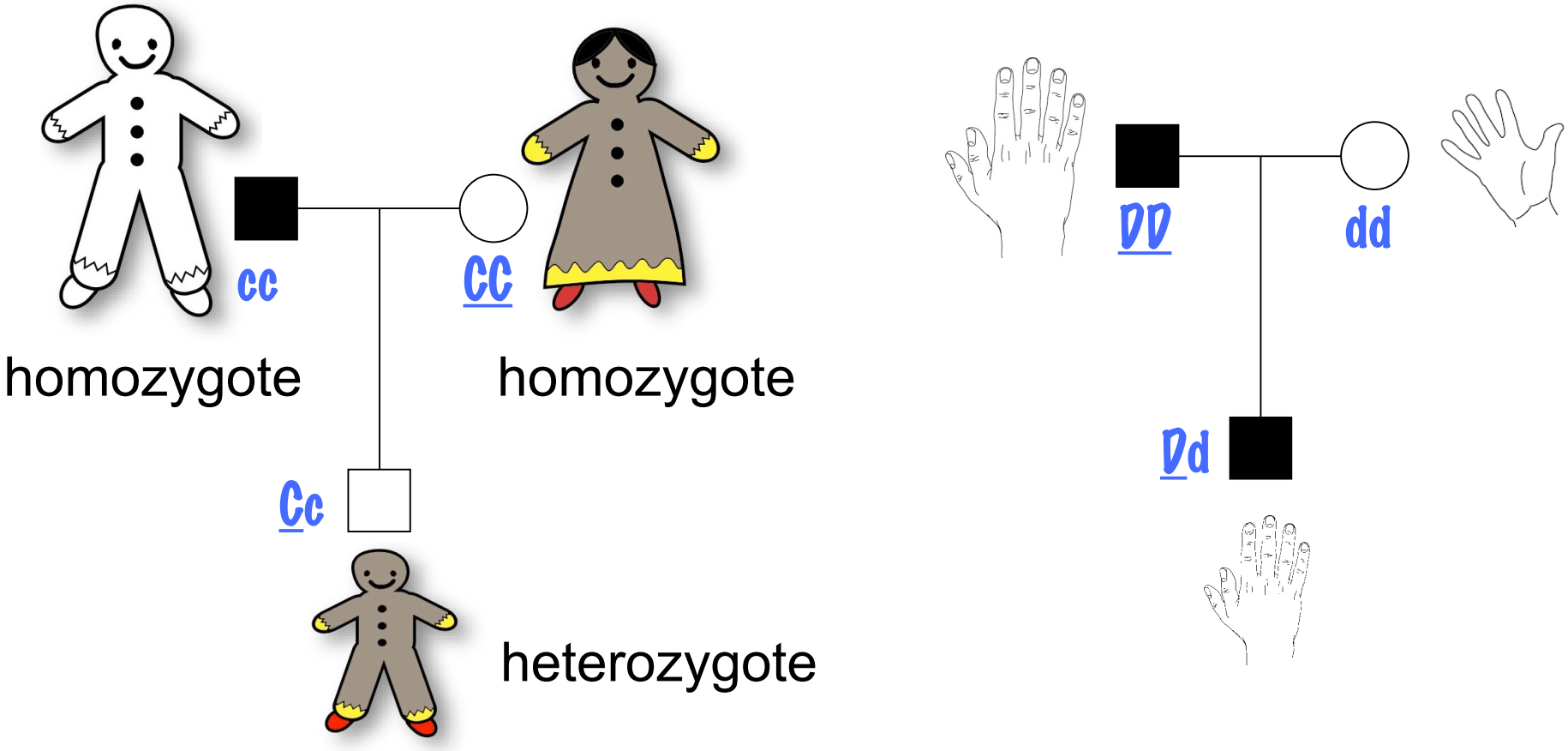
Alleles of a gene are variant forms of the gene



C gene encodes tyrosinase



Phenotypes in diploid organisms

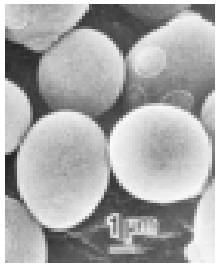


Why is a Cc individual just as pigmented as CC?

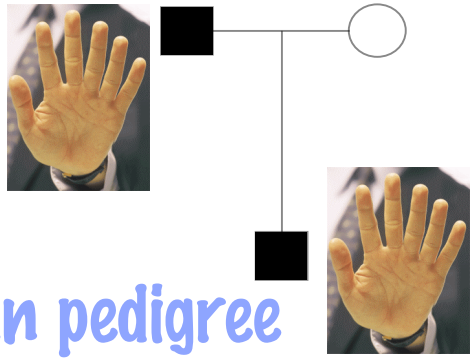
What makes an allele dominant or recessive?

To think about

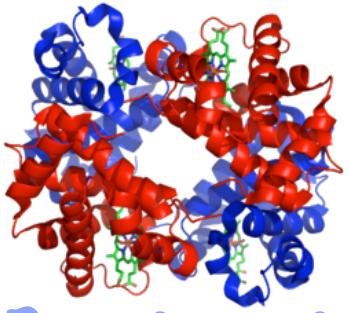
Linking genotype & phenotype: sequence analysis



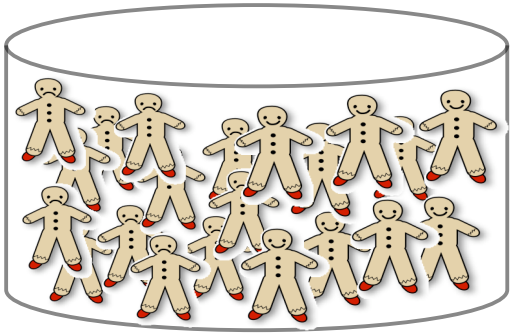
Mutant identified
in a model organism



Human pedigree
segregating a trait



Protein acting in
a biological process



Association study

```
 946  ATT GTC TGT AGC CGA TTG GAG GAG TAC AAC AGC CAT
1009  GGA CCT TTA CGG CGT AAT CCT GGA AAC CAT GAC AAA
1072  GCT GAT GTA GAA TTT TGC CTG AGT TTG ACC CAA TAT
1135  AAT TTC AGC TTT AGA AAT ACA CTG GAA GGA TTT GCT
1198  TCT CAA AGC AGC ATG CAC AAT GCC TTG CAC ATC TAT
1261  GGA TCT GCC AAC GAT CCT ATC TTC CTT CTT CAC CAT
1324  TGG CTC CGA AGG CAC CGT CCT CTT CAA GAA GTT TAT
```

Sequence analysis

If I were a mutagen…

Randomly pick a base in the coding sequence and change it to any other base:

Because alleles are sequence variants…

» A given gene can have many different alleles

Terminology

Mutation = heritable change in the DNA

Wild type = allele that is commonly found "in the wild"

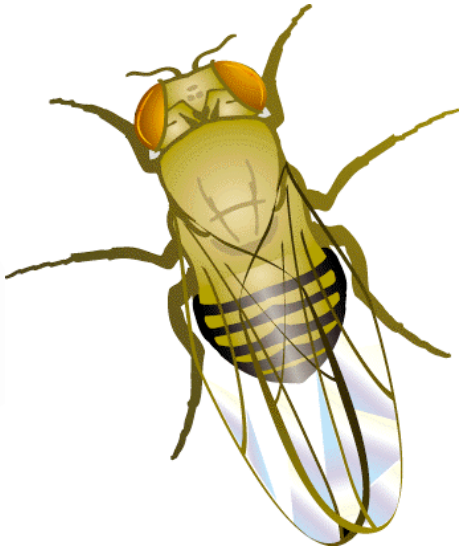
Polymorphism = variant of a gene or noncoding region (*i.e.* locus) within a population that has two or more alleles

» Different alleles of the gene may have different phenotypic outcomes

Different alleles with different outcomes… an example

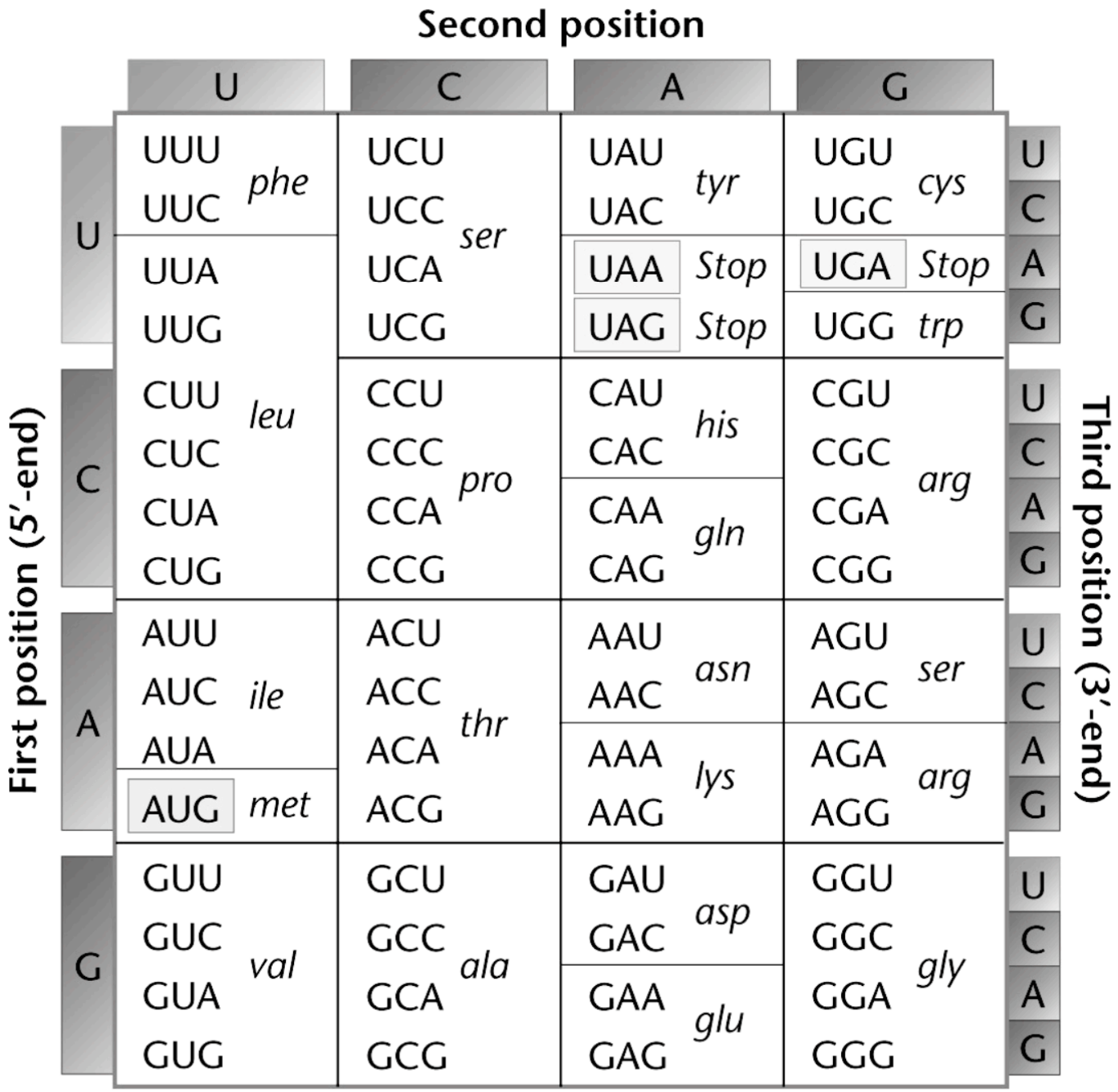
Drosophila melanogaster with:

wild-type
white gene



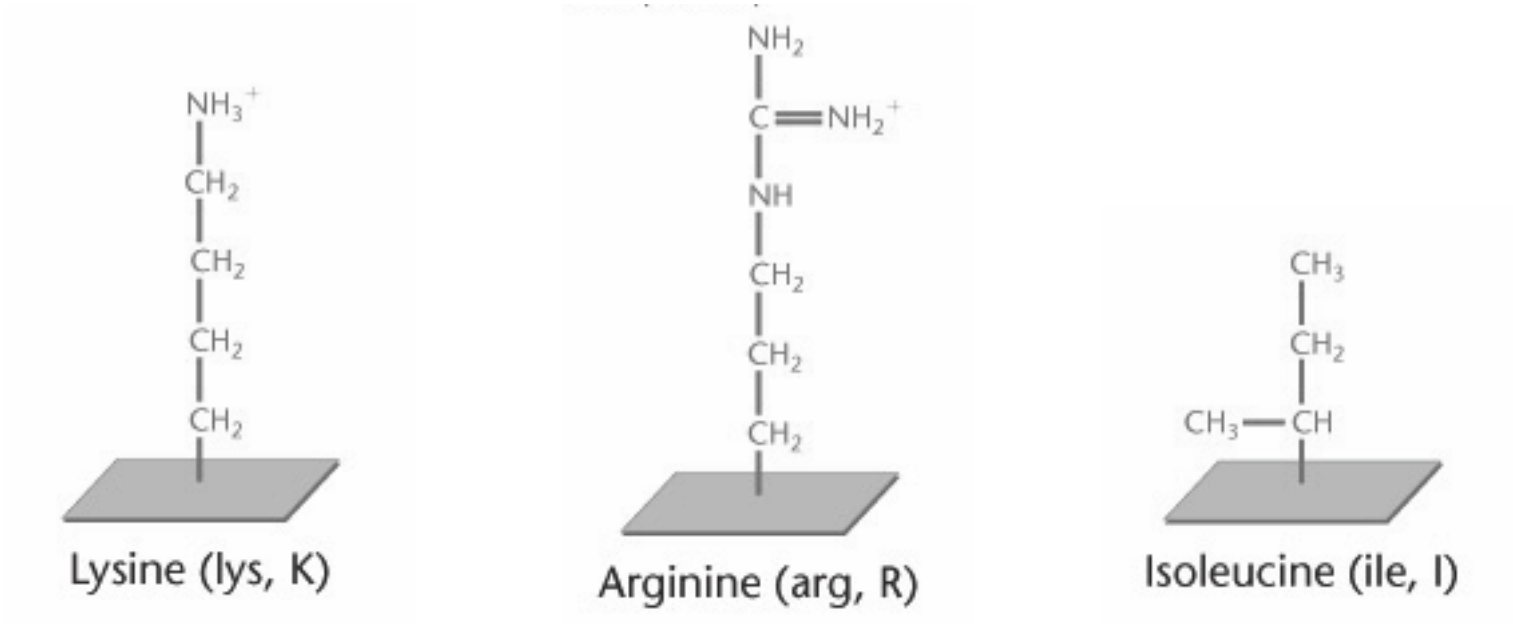
partially
defective
white gene

completely
defective
white gene


Second position
U
C
A
G
First position (5′-end)
Third position (3′-end)
UUU phe
UUC
UUA leu
UUG
UCU ser
UCC
UCA
UCG
UAU tyr
UAC
UAA Stop
UAG Stop
UGU cys
UGC
UGA Stop
UGG trp
CUU leu
CUC
CUA
CUG
CCU pro
CCC
CCA
CCG
CAU his
CAC
CAA gln
CAG
CGU arg
CGC
CGA
CGG
AUU ile
AUC
AUA
AUG met
ACU thr
ACC
ACA
ACG
AAU asn
AAC
AAA lys
AAG
AGU ser
AGC
AGA arg
AGG
GUU val
GUC
GUA
GUG
GCU ala
GCC
GCA
GCG
GAU asp
GAC
GAA glu
GAG
GGU gly
GGC
GGA
GGG

Amino acid replacements vary in their effects

AAA ⟵ AGA ⟶ ATA

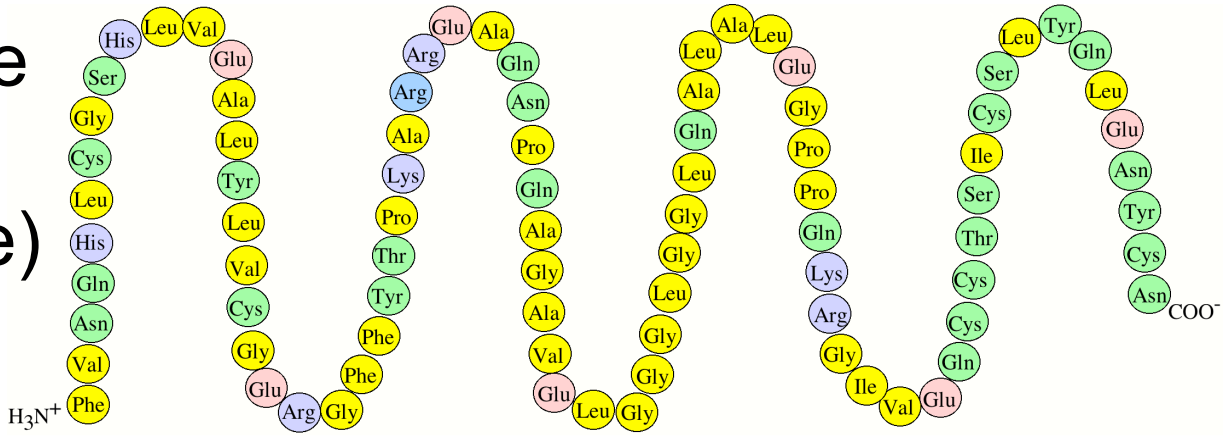


not so different

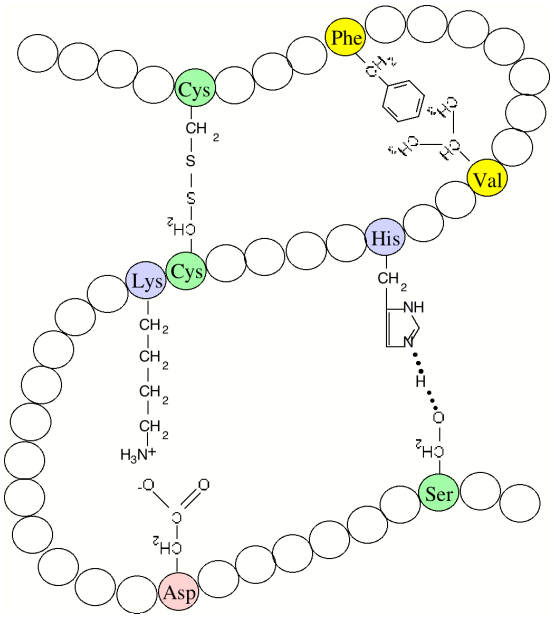
very different!

DNA sequence dictates protein structure

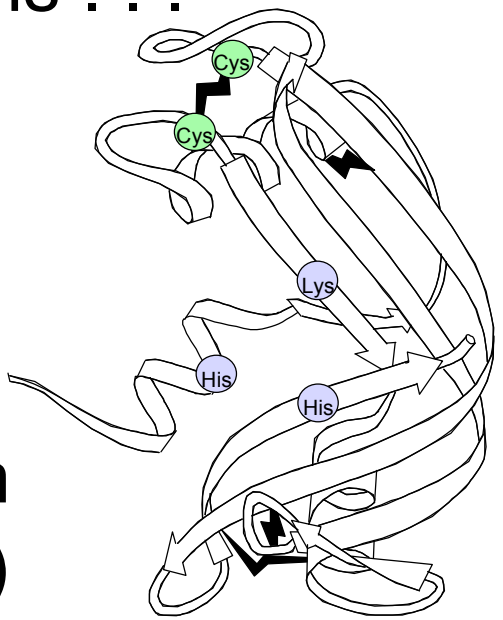
Genes encode the order of amino acids (1˚ structure)



. . . which sets up amino acid interactions . . .



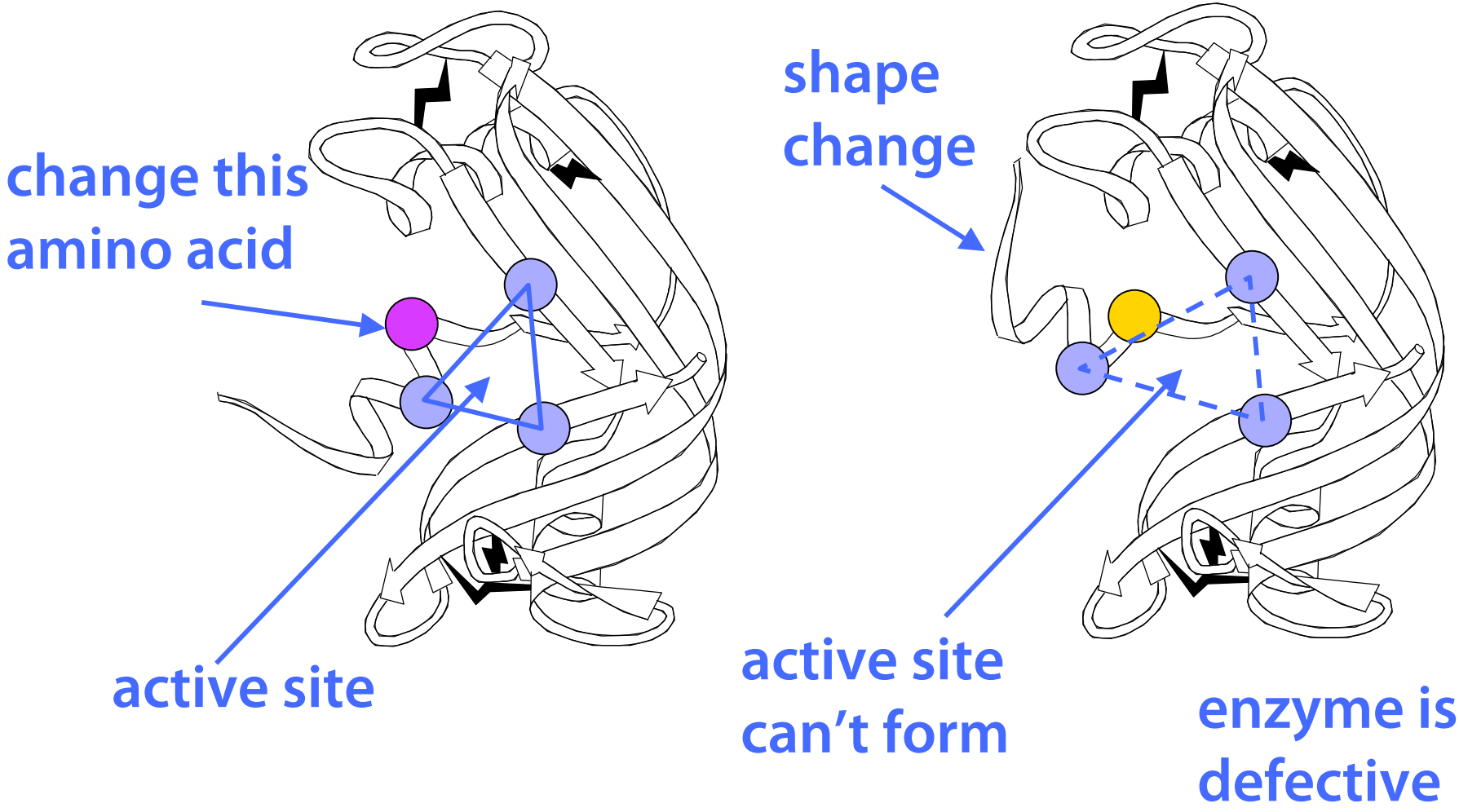
. . . that dictate protein conformation (3˚ structure)



Changes in the primary structure of proteins can change folding and alter function of a protein

wild type

mutant



Consequences of "point" mutations in coding sequence

A sequence change that does not result in an amino acid change?

→ silent mutation

A sequence change that **does** result in an amino acid change?

→ missense mutation

A sequence change that causes a premature STOP?

→ nonsense mutation

Insertion or deletion of 1 or 2 base pairs?

→ frameshift mutation

Mutations within the ORF… summary, illustration

"Wild type"	→	ONE BIG FLY BIT THE DOG AND HIS MAN
substitutions - Missense	→	ONE BIG FLY HIT THE DOG AND HIS MAN
substitutions - Nonsense	→	ONE BIG FLY BIT .
substitutions - Silent	→	ONE BIG FLY BIt THE DOG AND HIS MAN
-Frameshift (insertion)	→	ONE BIG FAL YBI TTH EDO GAN DHI SMA
-Frameshift (deletion)	→	ONE BIG FLY BTT HED OGA NDH ISM AN

Wild type alleles…

- usually code for functional proteins
- usually dominant

Mutant alleles…

- usually code for defective proteins
- often recessive

DOMINANT alleles…indicated by CAPITAL letters

recessive alleles…indicated by lower case letters

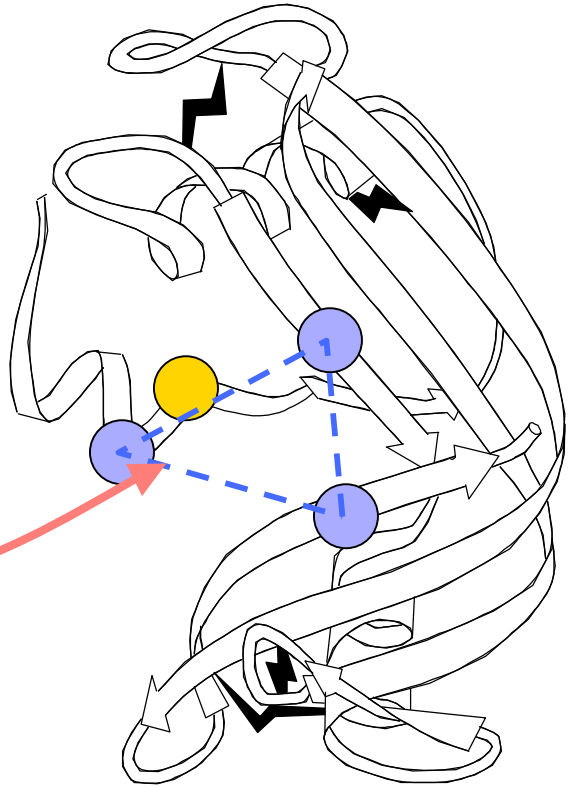
Loss of function mutations

LOF: Loss Of Function mutations result in a protein that has little or no enzymatic activity.

Most mutations associated with a phenotype are LOF.

Why?

Many changes that affect the normal 3° structure would disrupt the active site (even if the mutation affects an amino acid that is far away from the active site).

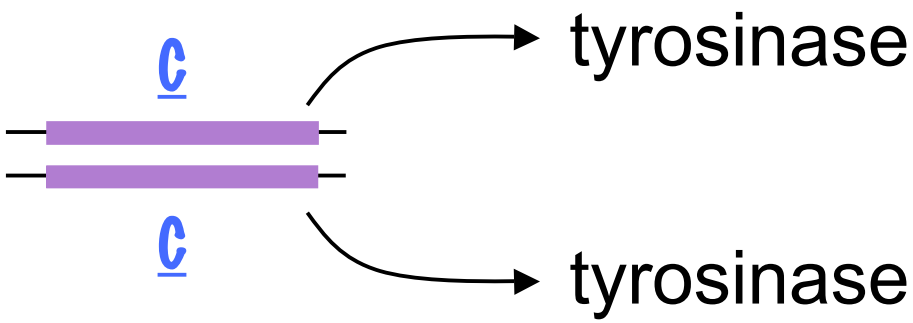


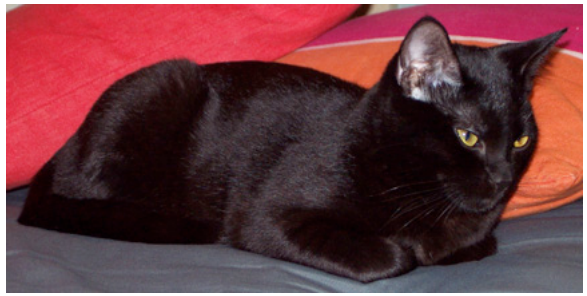
Loss of function mutations

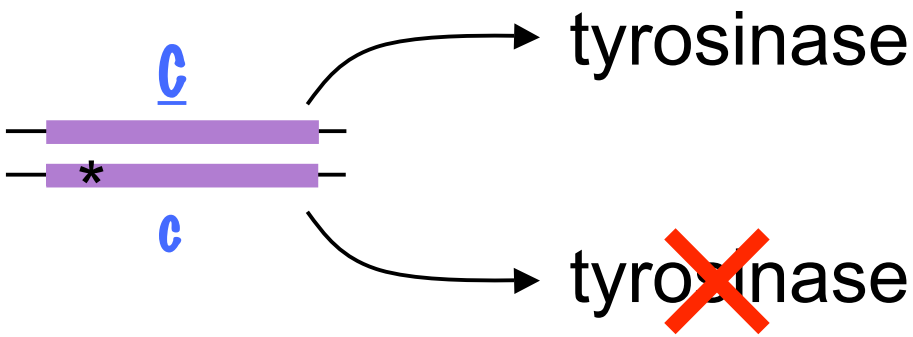
Most LOF mutations are recessive.

Why?

Half the amount of wild type gene product is usually sufficient to give a wild type phenotype.







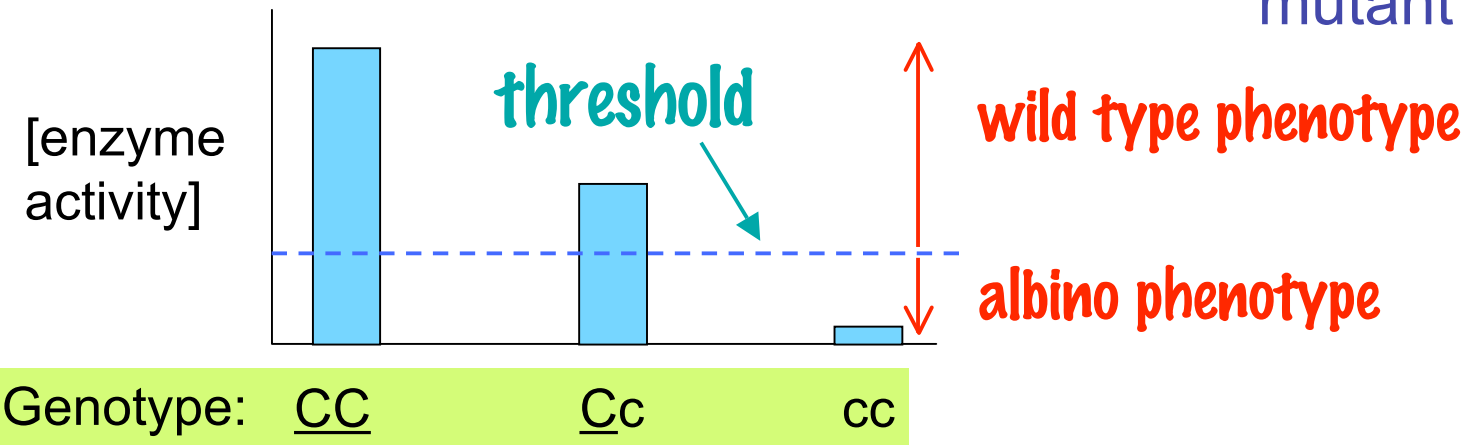
Why does Cc look as pigmented as CC?

General rule for LOF mutations…

Half the amount of wild type gene product is sufficient to give a wild type phenotype

Example : Tyrosinase

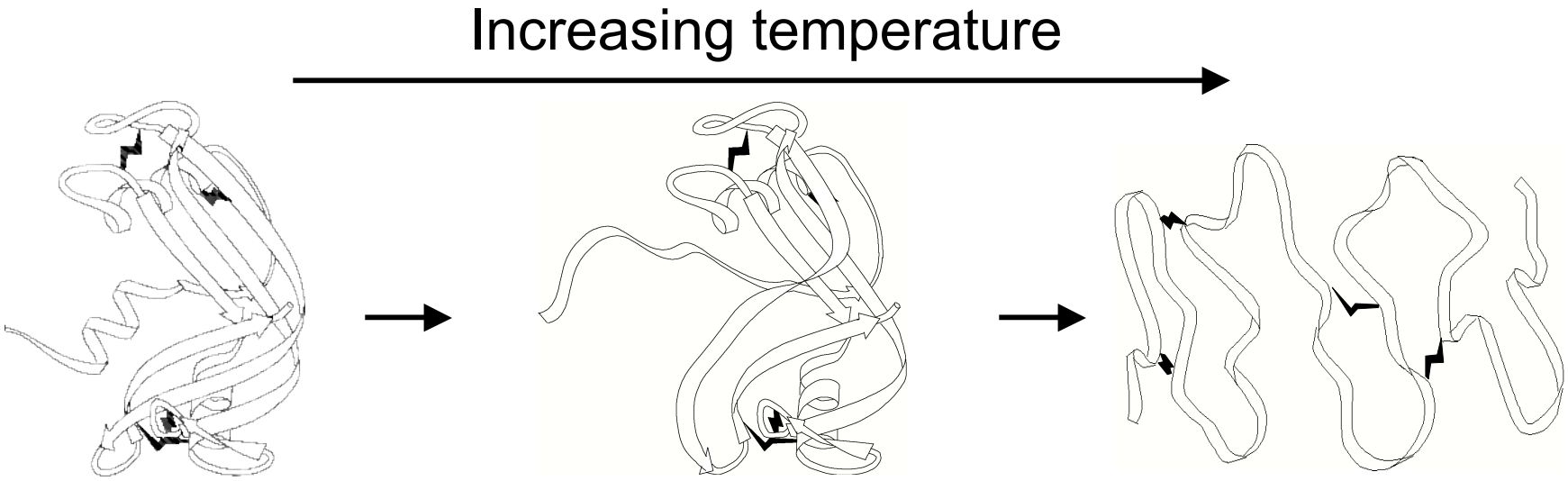
wild type allele = C
mutant = c



- 1 wild type copy → enzyme activity above threshold needed for normal pigmentation, so carriers unaffected (mutant allele → recessive)

Temperature-sensitive proteins

Proteins unfold upon heating.



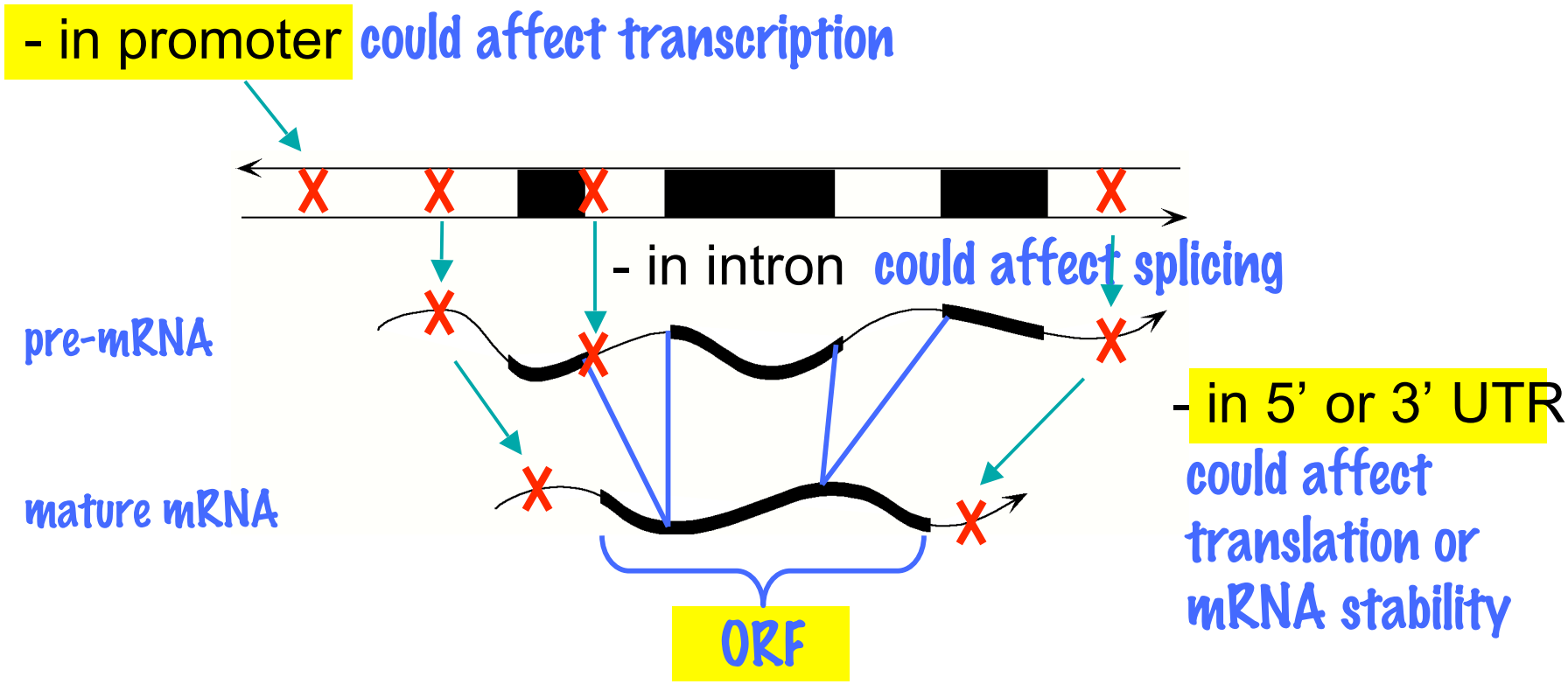
Missense mutations can destabilize 2˚ and 3˚ structures so the protein unfolds at lower than normal temperatures.

Tyrosinase protein (for melanin) can be encoded by alleles so that it folds properly only in the coolest parts of the skin.

Burmese

Siamese

Mutations in regulatory regions and introns



Any of these changes could change when, where, or how much protein is made

Practice question

Wild type yeast cannot grow in the presence of canavanine, a drug that mimics the amino acid arginine. If present **even in small quantities** in the cell, canavanine can be used in place of arginine (an amino acid) during translation, causing defects in **all** proteins being made.

However, canavanine can get into a cell only if the cell is making a transporter protein (allele **T**). A mutant allele (**t**) results in non-functional transporter which cannot import canavanine.

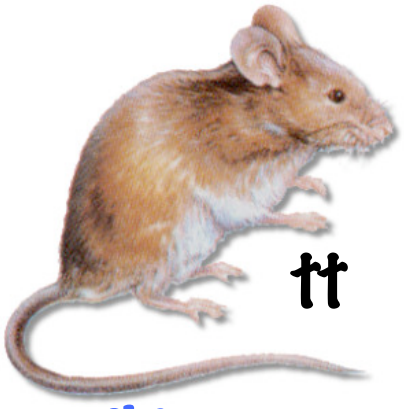
(1) Would a **tt** homozygote be resistant to canavanine or sensitive? resistant

(2) Which is dominant, resistance or sensitivity to canavanine?

“Half the amount of wt gene product is sufficient for wt phenotype”

Exceptions?

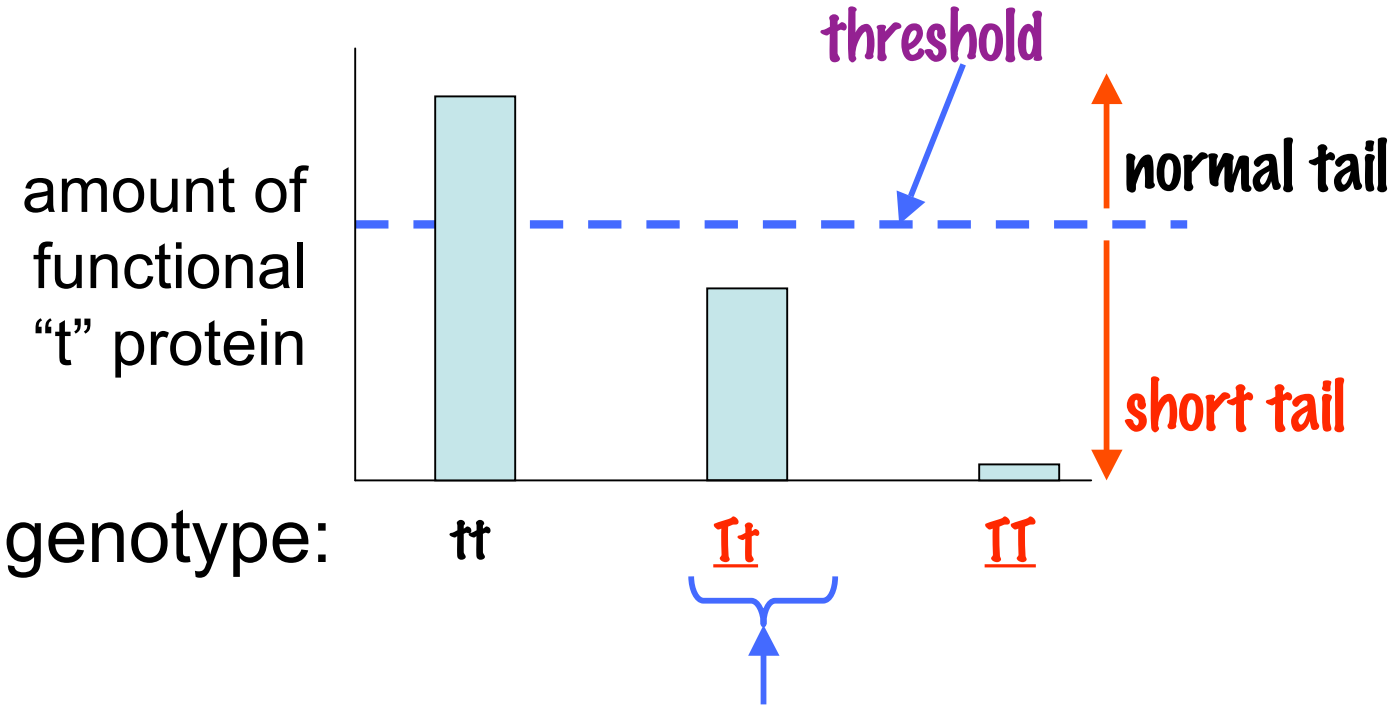
Rare exception #1—haploinsufficiency



wild type

short tail mutant

Half the number of t protein molecules is not sufficient to maintain normal tail length

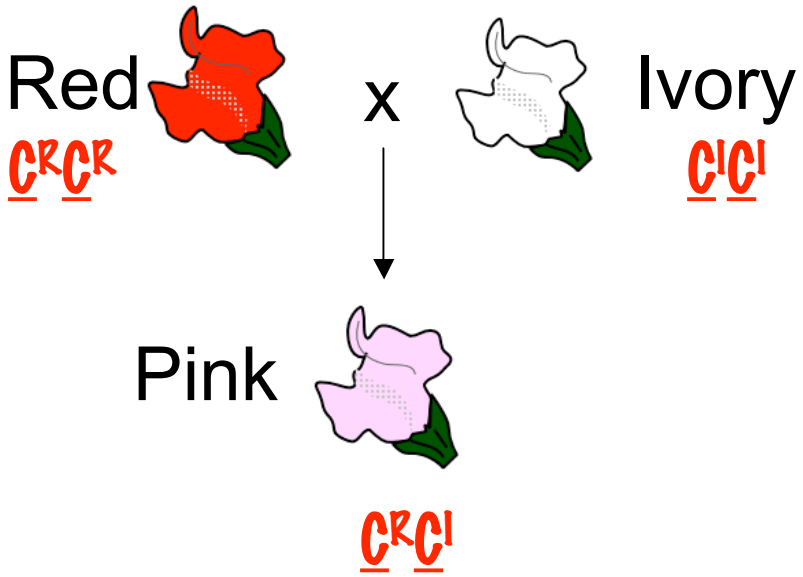


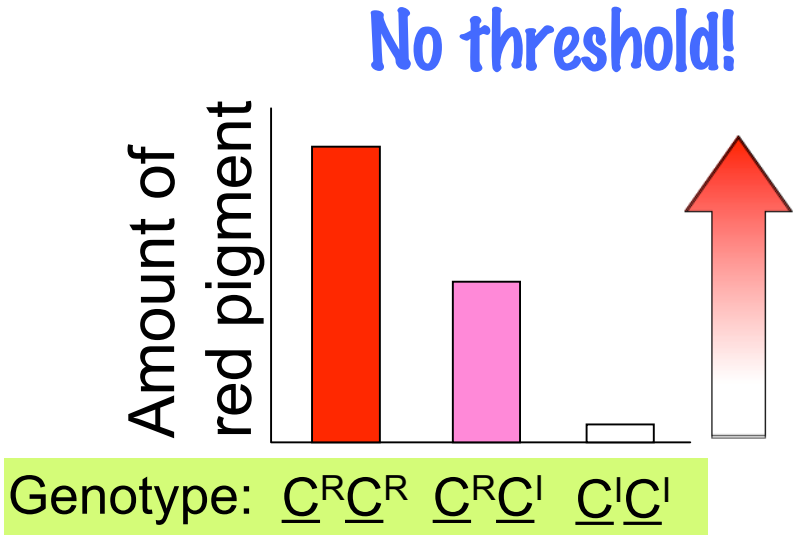
Rare exception #2—*no* threshold

e.g., snapdragon flower color

C^R: enzyme that makes red pigment

C^I: no enzyme activity

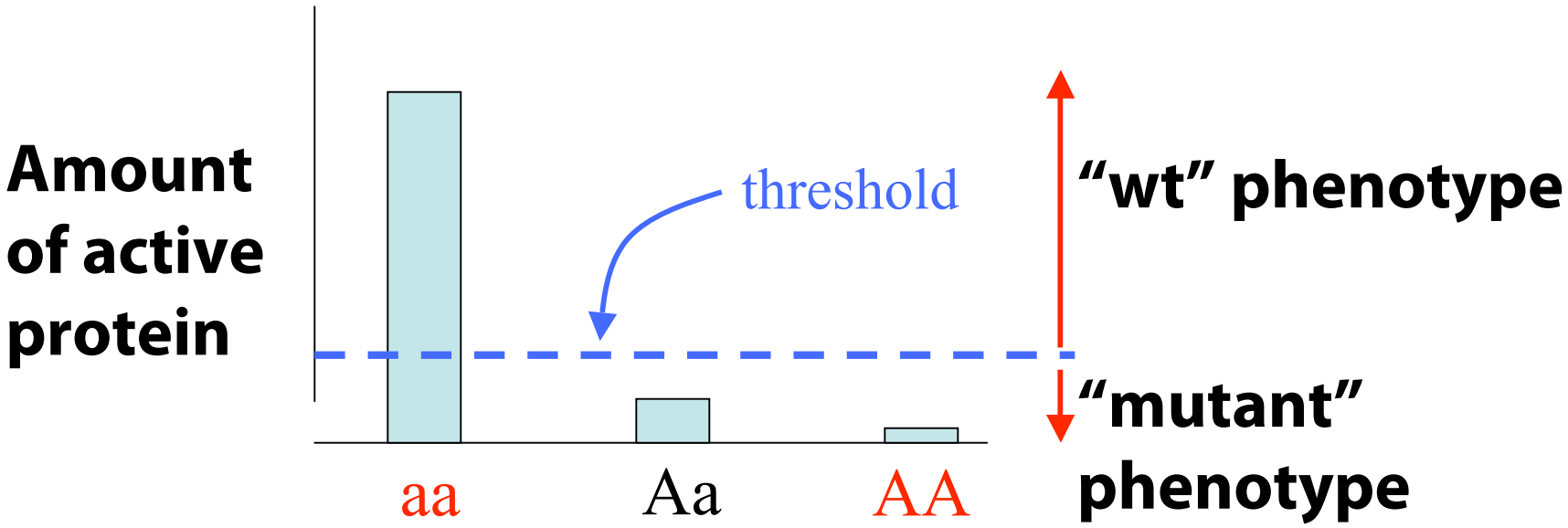




Heterozygote has intermediate phenotype... incomplete dominance

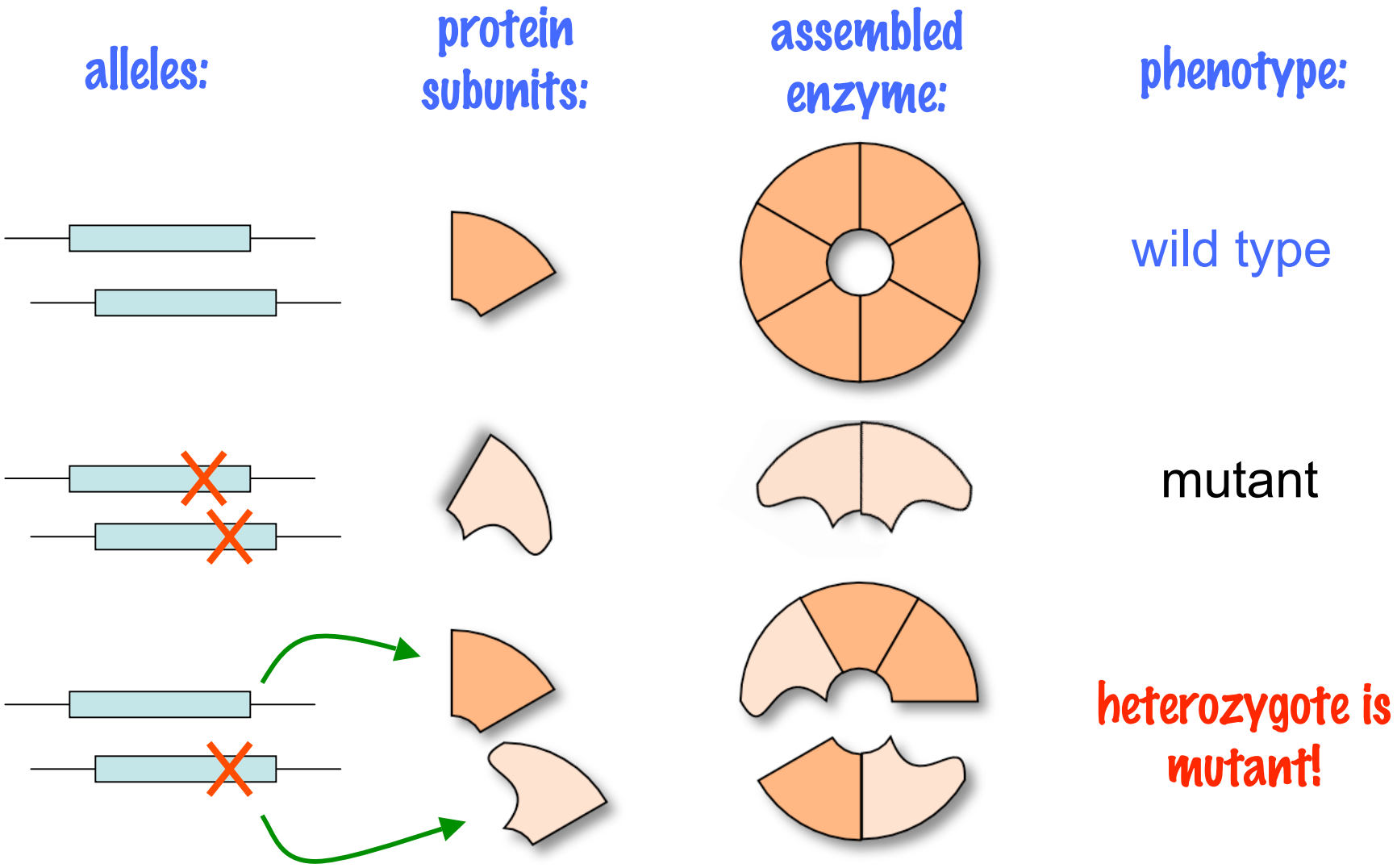
Rare exception #3—"poisonous" subunits

…also called "dominant-negative" mutations



Why does Aa have so little activity?

Example: "Poisonous" subunits



How mutations affect phenotype

GOF: **Gain Of Function** mutations result in a functional protein that…

…is made at the wrong place
…is made at the wrong time
…has a new activity

GOF mutations need not be beneficial

Very few mutations are GOF.

Why?

Only very specific mutations (e.g., specific amino acid changes) will have this effect

How mutations affect phenotype

Will GOF mutations be dominant or recessive? Can you predict?

depends on threshold!

Most GOF mutations are dominant

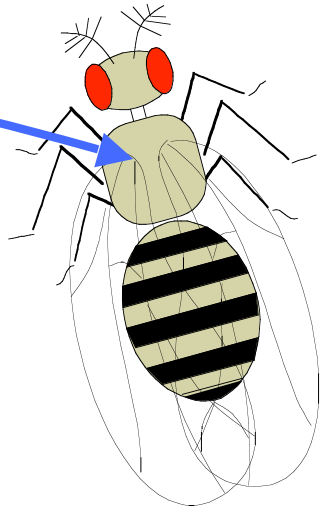
For example, only a small amount of the altered protein is sufficient to produce the mutant phenotype

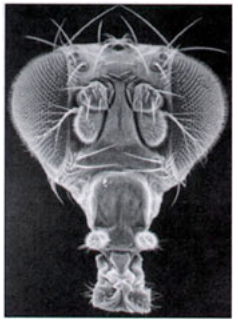
But there could be cases in which the altered protein in combination with the wt protein gives a wt phenotype

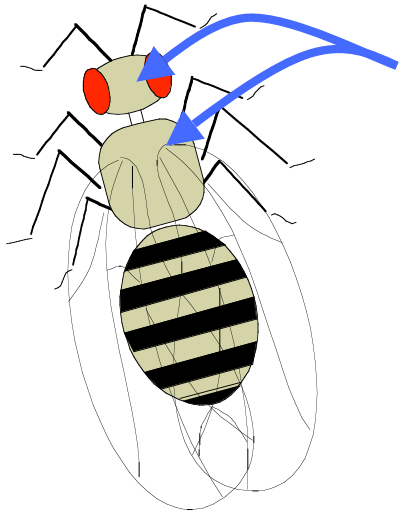
GOF example #1–normal protein in the wrong place

Antennapedia in Drosophila

Wild type Antennapedia gene is only expressed in the thorax; legs are made.







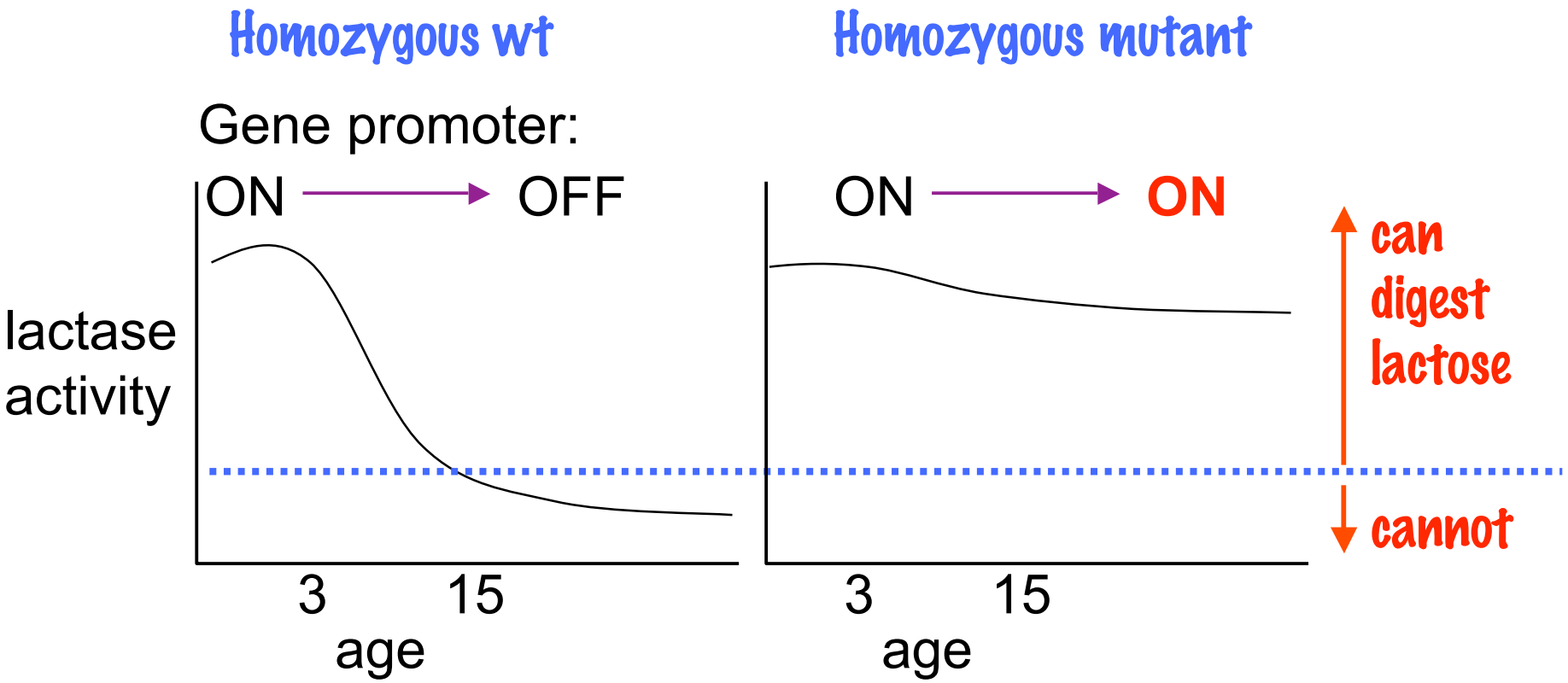
A mutation causes the Antennapedia gene to be expressed in the thorax and **also in the head**, where legs result instead of antennae!

What kind of mutation is this?

What phenotype would you predict for the heterozygote?

GOF example #2–normal protein at the wrong time

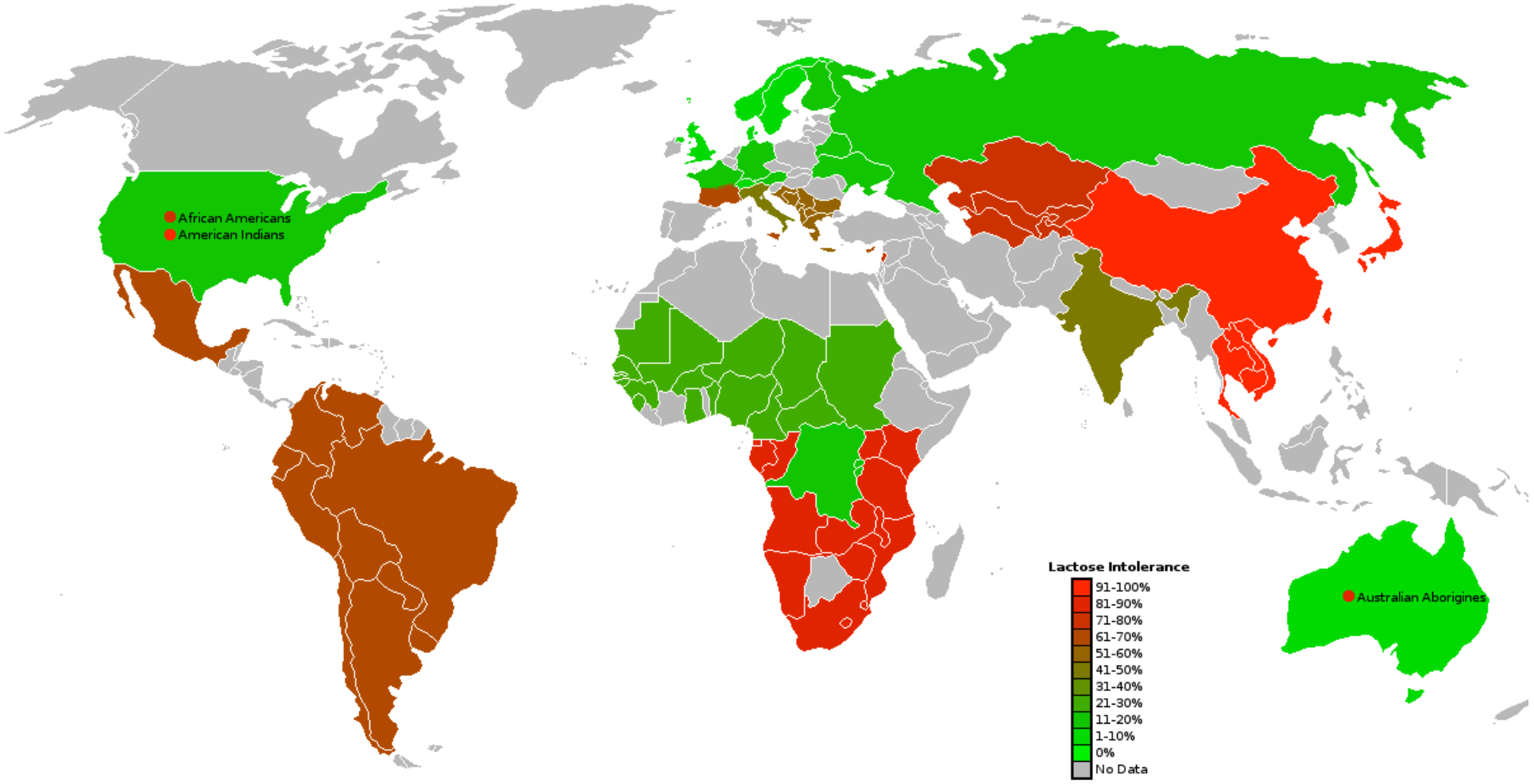
Lactose tolerance in humans



So which allele is dominant? What would be the phenotype of the heterozygote?

Mutant has gain of function... expect lactose tolerance to be dominant

Lactose Intolerance: Worldwide Distribution



GOF or LOF

Defined by comparison with the normal properties of the gene, not of the organism

Is it LOF or GOF?

GOF or LOF

Defined by comparison with the normal properties of the gene, not of the organism

Is it LOF or GOF?

Ask yourself what would happen if the gene were missing altogether

Complementation

- wild type copies of two genes needed to perform a function
- if either gene is not functioning → mutant phenotype

Complementation?

Why do we care?

Find mutant(s) with "interesting" phenotypes

How many genes have we mutated?

The complementation test

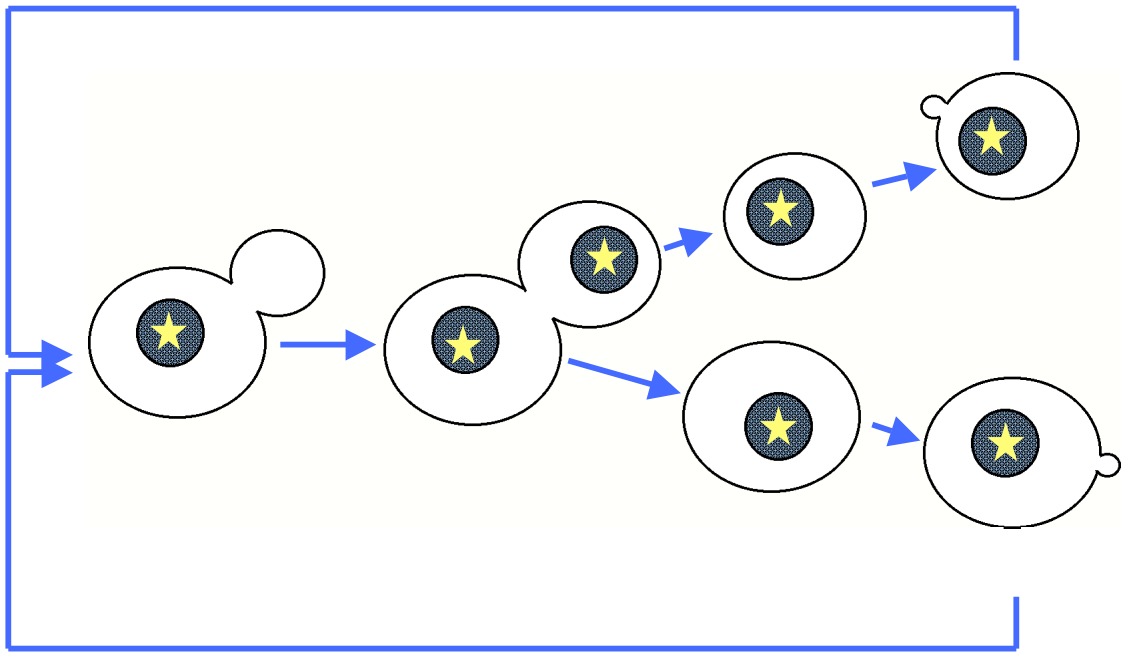
Recessive mutations in genes that act on the same process...

- If the mutations complement—they must be in separate genes
- If the mutations fail to complement—they must be in the same gene

Mutagenesis is easier in single-cell organisms with haploid lifestyles

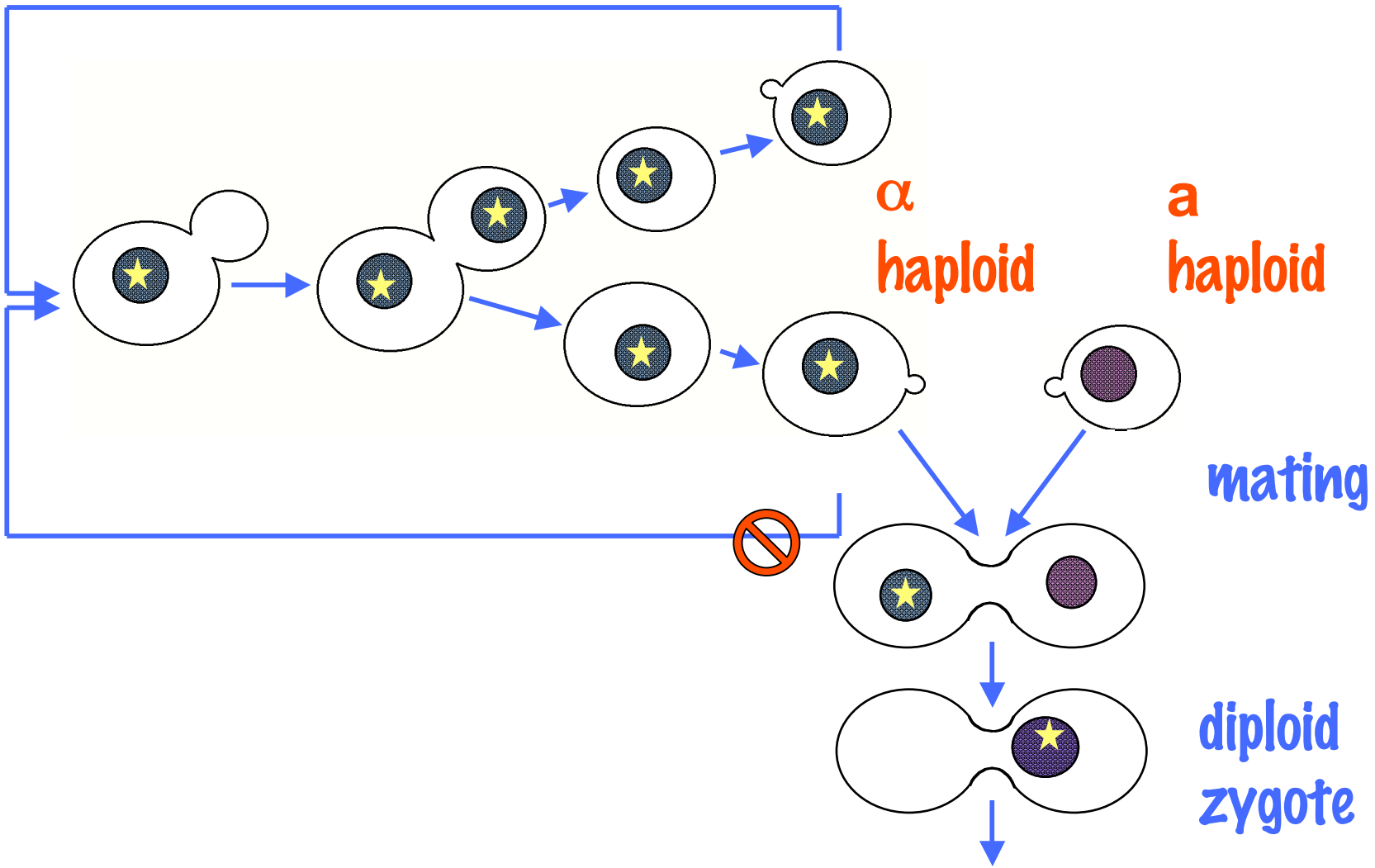
Example: Budding yeast—a single-celled fungus that divides by budding

Haploid life cycle:

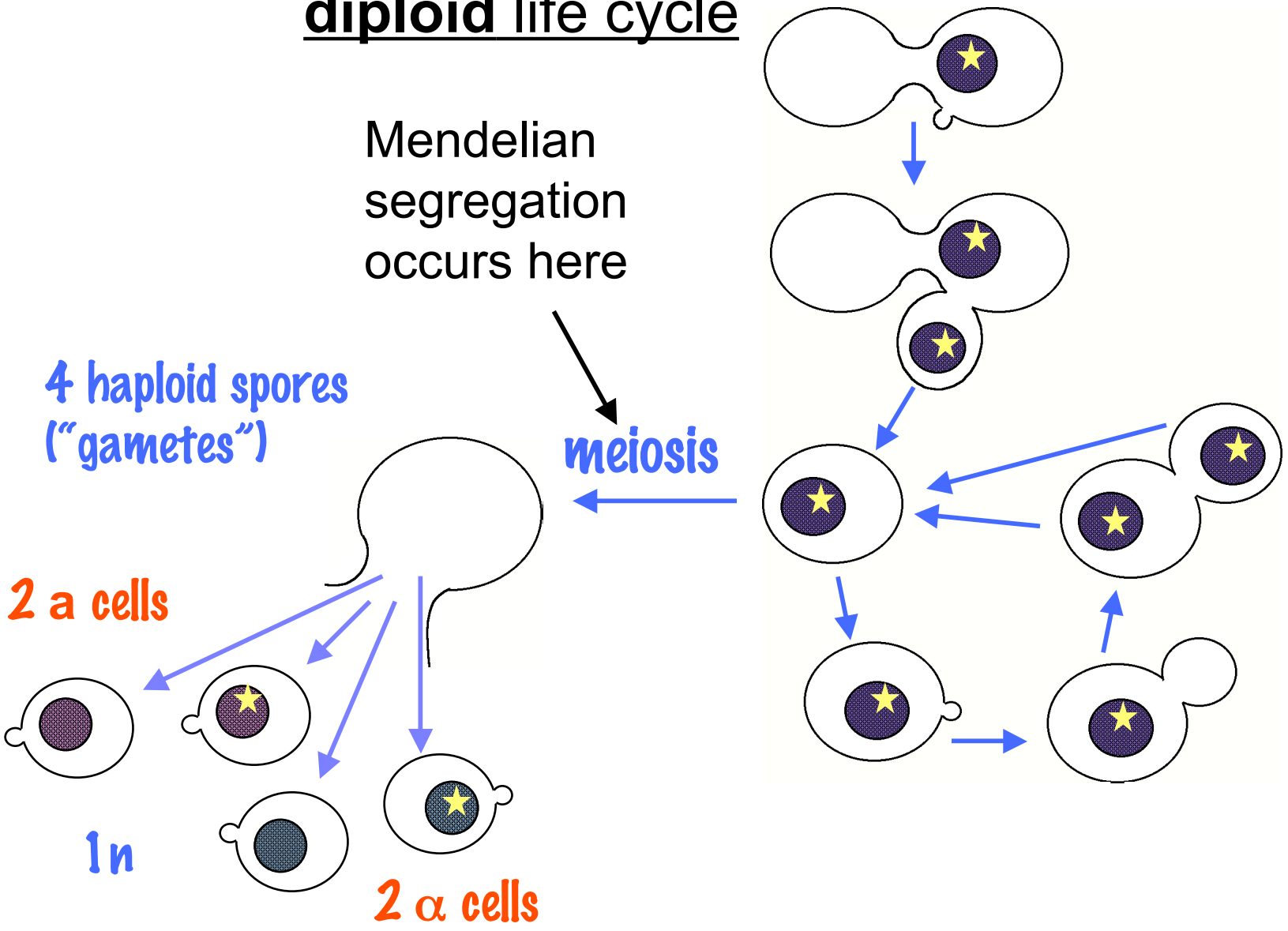


Yeast cells can exist as haploids...

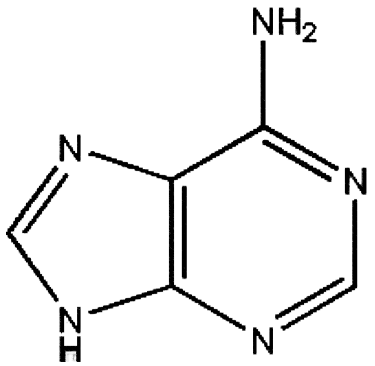
... and as diploids



diploid life cycle



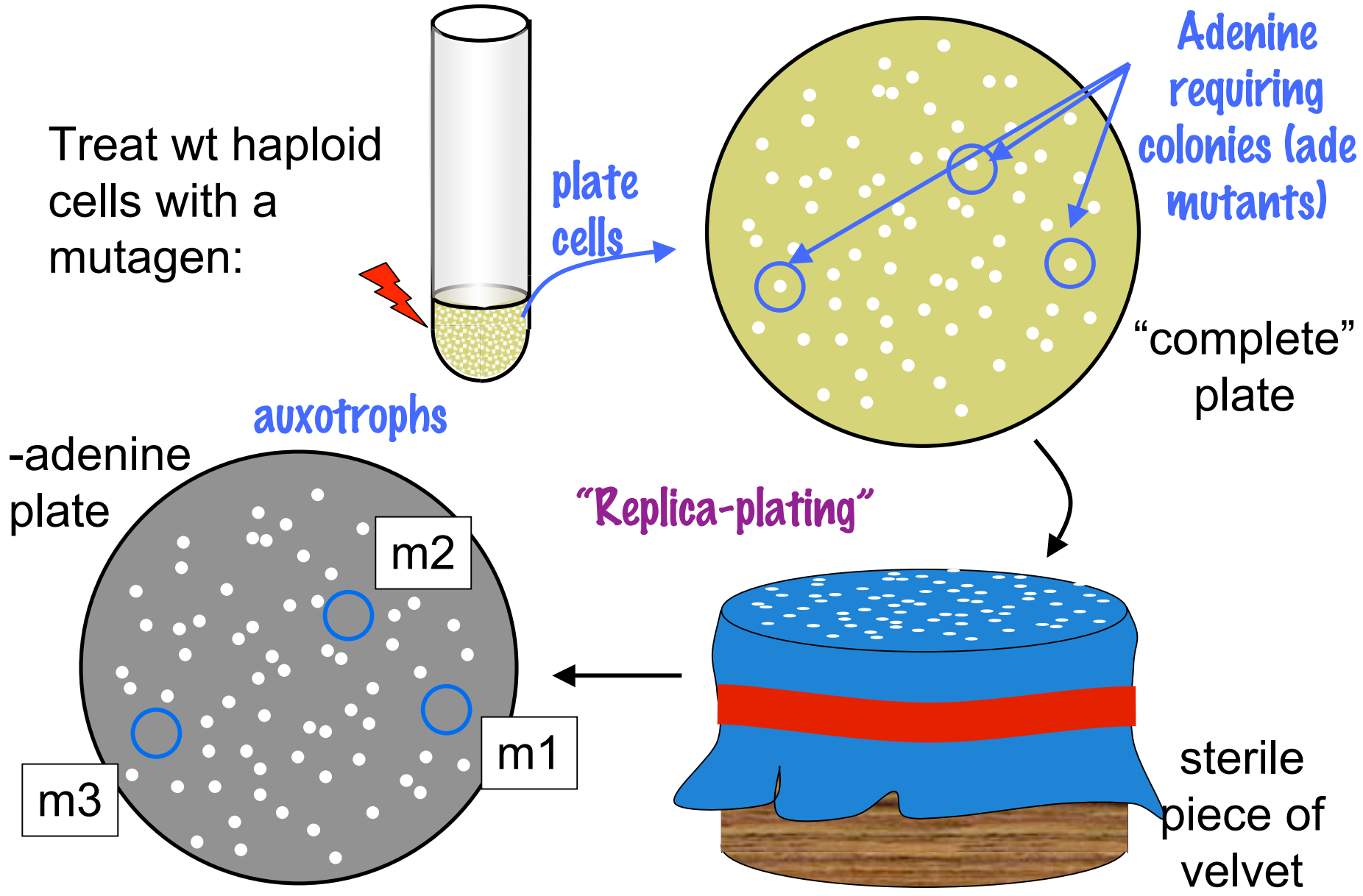
Case study: genetic dissection of adenine biosynthesis in yeast



Wild type yeast can survive on ammonia, a few vitamins, a few mineral salts, some trace elements and sugar…
they synthesize everything else, including **adenine**
= prototrophs

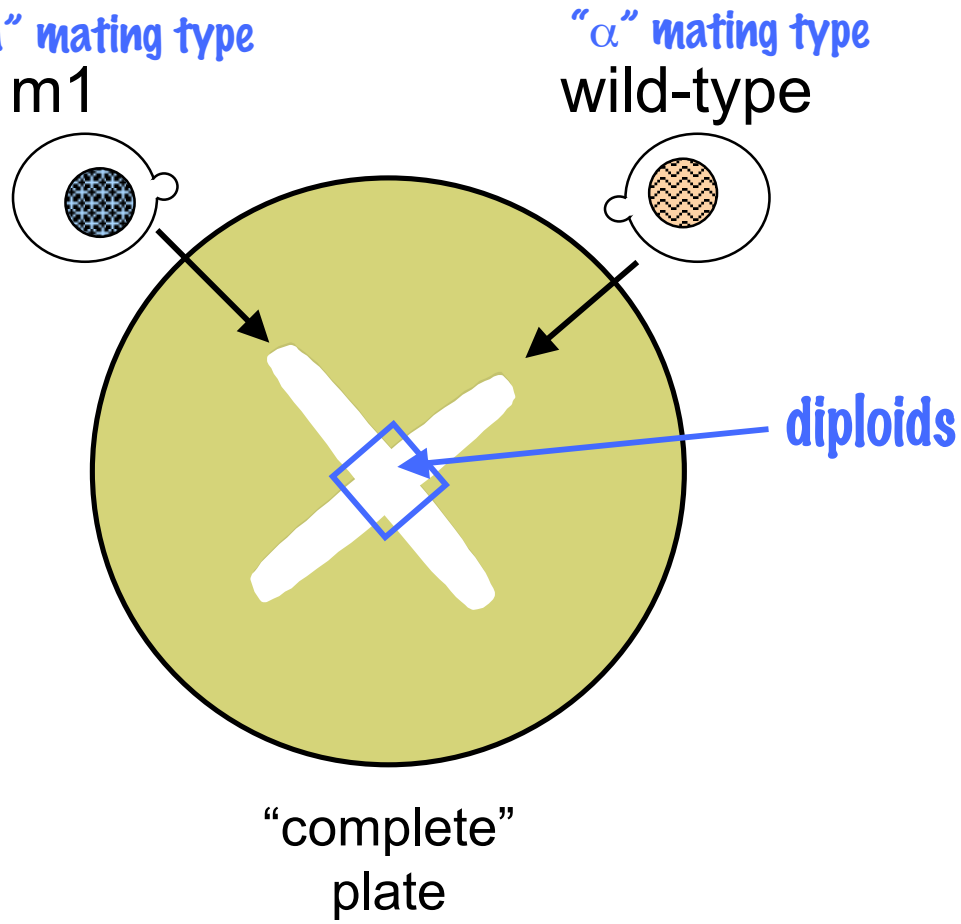
What genes are needed for ability to synthesize adenine?

Identifying yeast mutants that require adenine



Are the adenine-requiring mutations recessive?

That is, are they LOF mutations?



What do you conclude? What is dominant?

Are all the ade mutations in one gene?

Are m1 and m2 **alleles of the same gene**?

What would you predict if...

- only one enzyme is needed for synthesis of adenine?

  all mutants... alleles of one gene

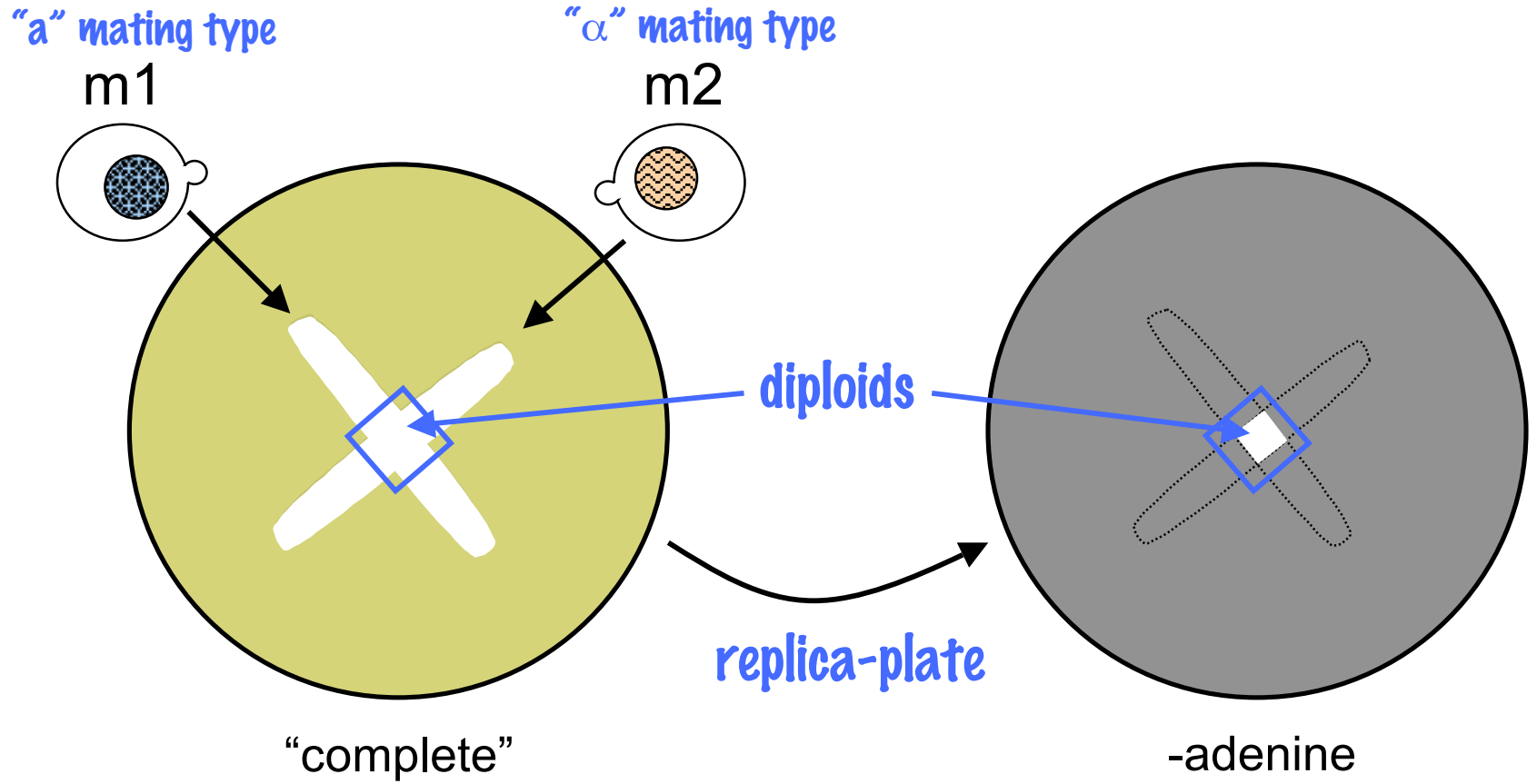
- many enzymes are needed for synthesis of adenine?

  more than one gene represented

How to find out whether our mutants are mutated in the same gene?

Do complementation test to ask: are the mutations alleles of the same gene?

One complementation test



Conclusion? Do m1 and m2 complement, or fail to complement?

Are m1 and m2 alleles of the same gene, or alleles of different genes?

Complementation tests with ade mutants

What do you conclude from the pair-wise crosses shown below?

X	m1	m2	m3	m4	m5	m6	m7
m1	0	+	+	+	0	+	0
m2		0					
m3			0				
m4				0			
m5					0		
m6						0	
m7							0

Conclusion?

0 = no growth on -ade

\+ = growth on -ade

Complementation tests with ade mutants (cont'd)

What do you conclude from the pair-wise crosses shown below?

X	m1	m2	m3	m4	m5	m6	m7
m1	0	+	+	+	0	+	0
m2		0	+	0	+	+	+
m3			0	+	+	+	+
m4				0	+	+	+
m5					0	+	0
m6						0	+
m7							0

Conclusion?

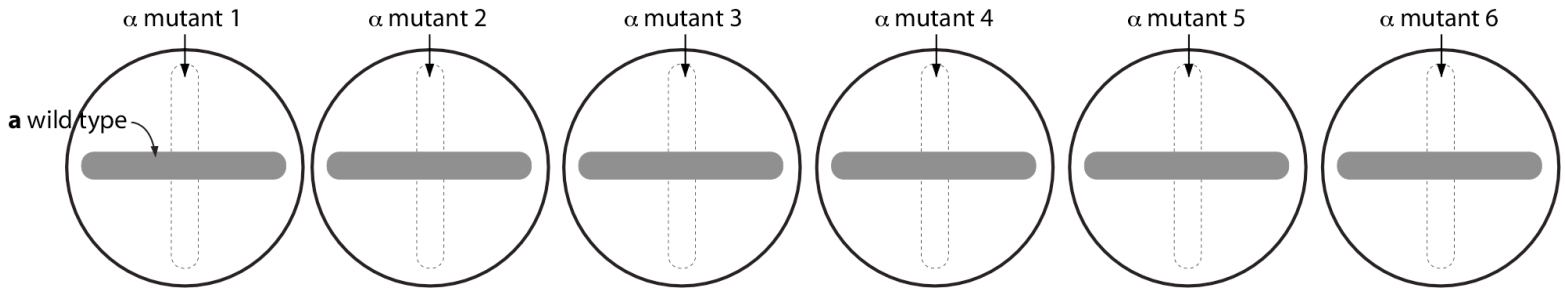
0 = no growth on -ade

+ = growth on -ade

Practice question

Yeast cells can normally grow on a sugar called galactose as the sole carbon source. Seven mutant "a" haploid yeast strains have been isolated that are unable to grow on galactose ("gal") plates.

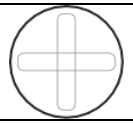
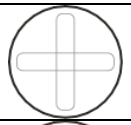
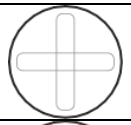
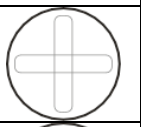
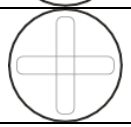
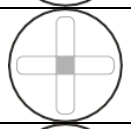
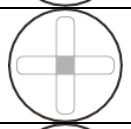
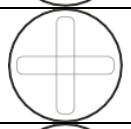
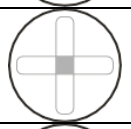
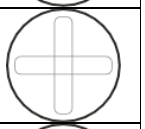
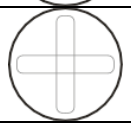
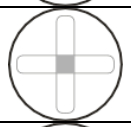
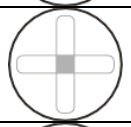
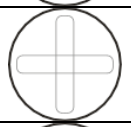
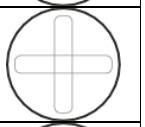
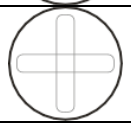
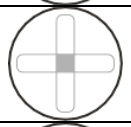
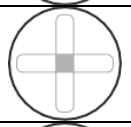
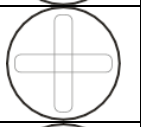
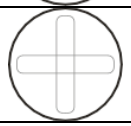
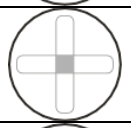
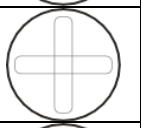
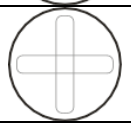
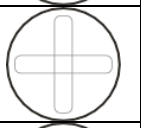
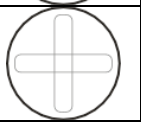
Six of these mutant strains were each cross-stamped on a gal plate with a wild type "a" strain. The resulting pattern of growth on the gal plates is depicted below (shading = growth). In all plates, the wild type strain is in the horizontal streak.



What is the mode of inheritance of mutant phenotype in mutants 1-6? How can you tell?

Practice question (cont'd)

Each of the seven "α" mutant strains was cross-stamped on gal plates against "a" versions of the seven mutants. The results are depicted below:

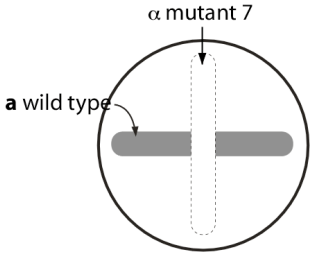
	Mutant 1	Mutant 2	Mutant 3	Mutant 4	Mutant 5	Mutant 6	Mutant 7
Mutant 1							
Mutant 2							
Mutant 3							
Mutant 4							
Mutant 5							
Mutant 6							
Mutant 7							

Looking **just at mutants 1–6** for now... group these six mutants by complementation group.

Practice question (cont'd)

Now consider mutant 7. What is surprising about the result in the complementation table?

Mutant 7 was cross-stamped on gal plate with wild type as you saw with the other six mutants earlier:



What do you conclude about the mode of inheritance of mutant 7? How does that help you explain the complementation test result for mutant 7?

What can you conclude about how many genes are represented in this collection of seven mutants?