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

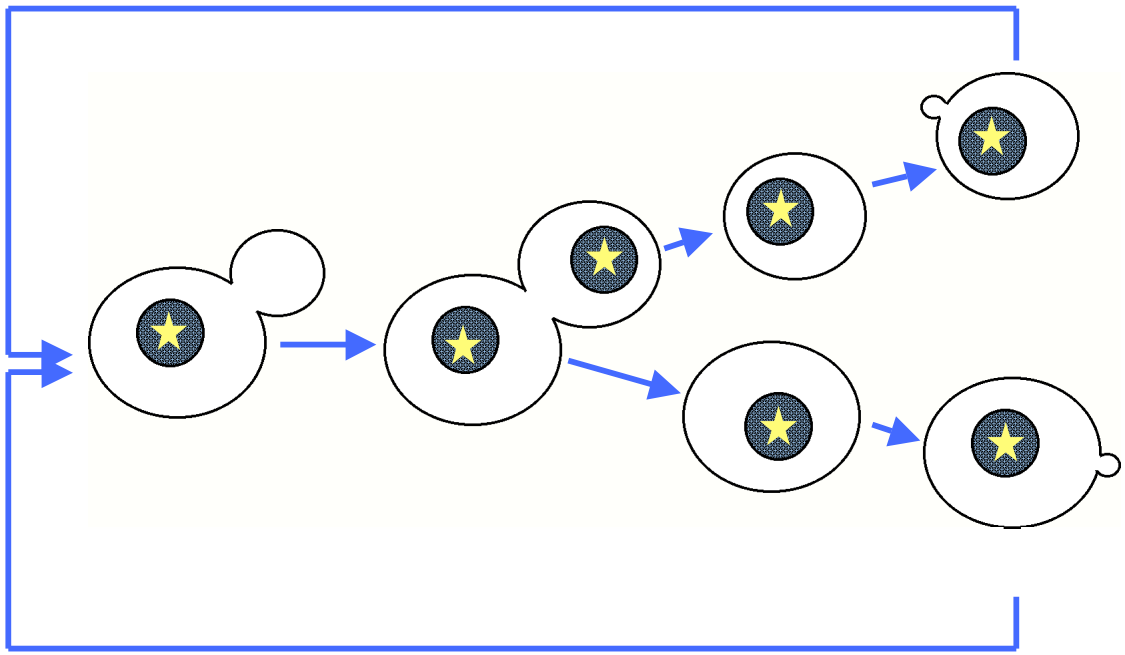
Recessive mutations in genes that act on the same process...

- 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

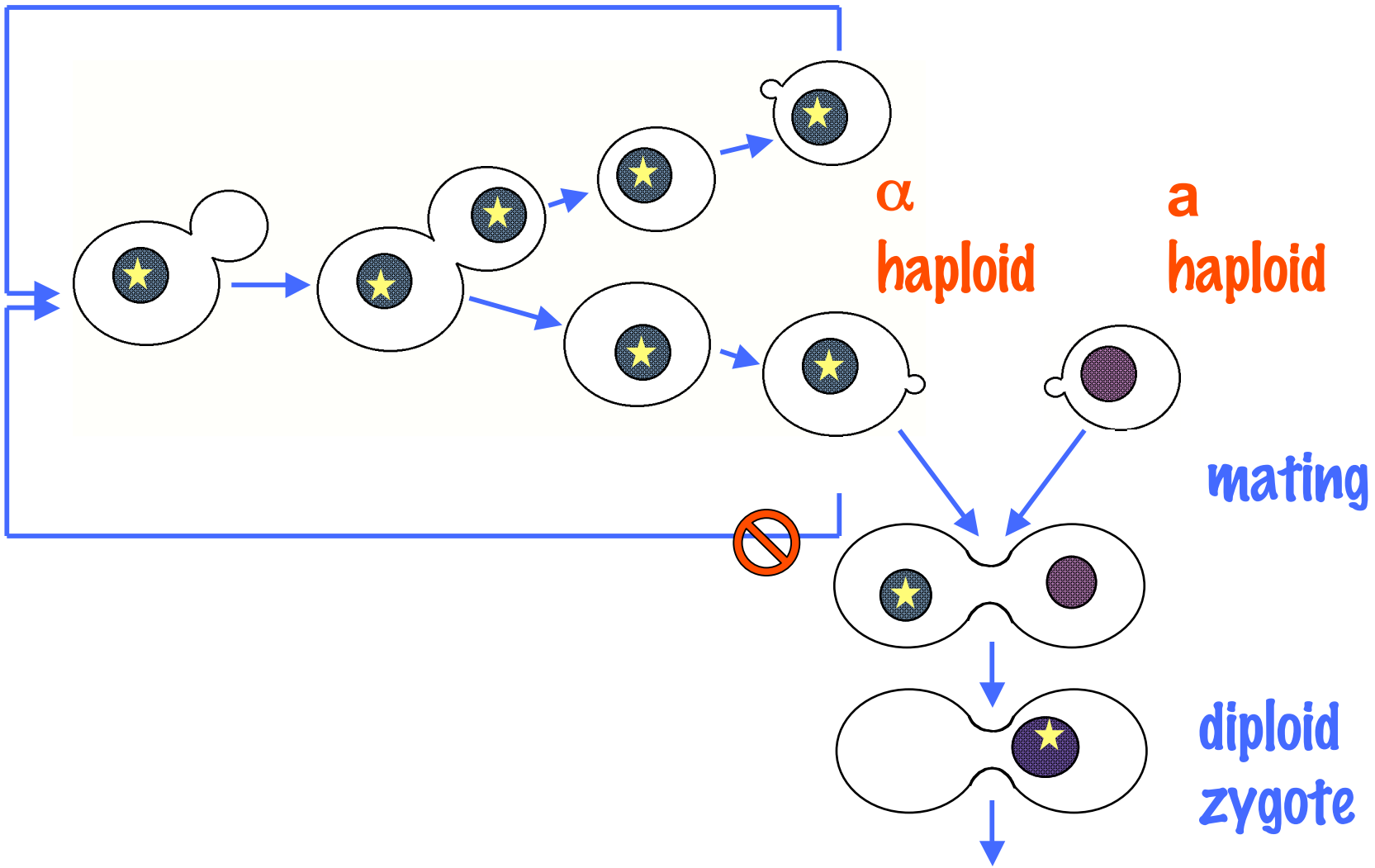
Example: Budding yeast—a single-celled fungus that divides by budding

Haploid life cycle:

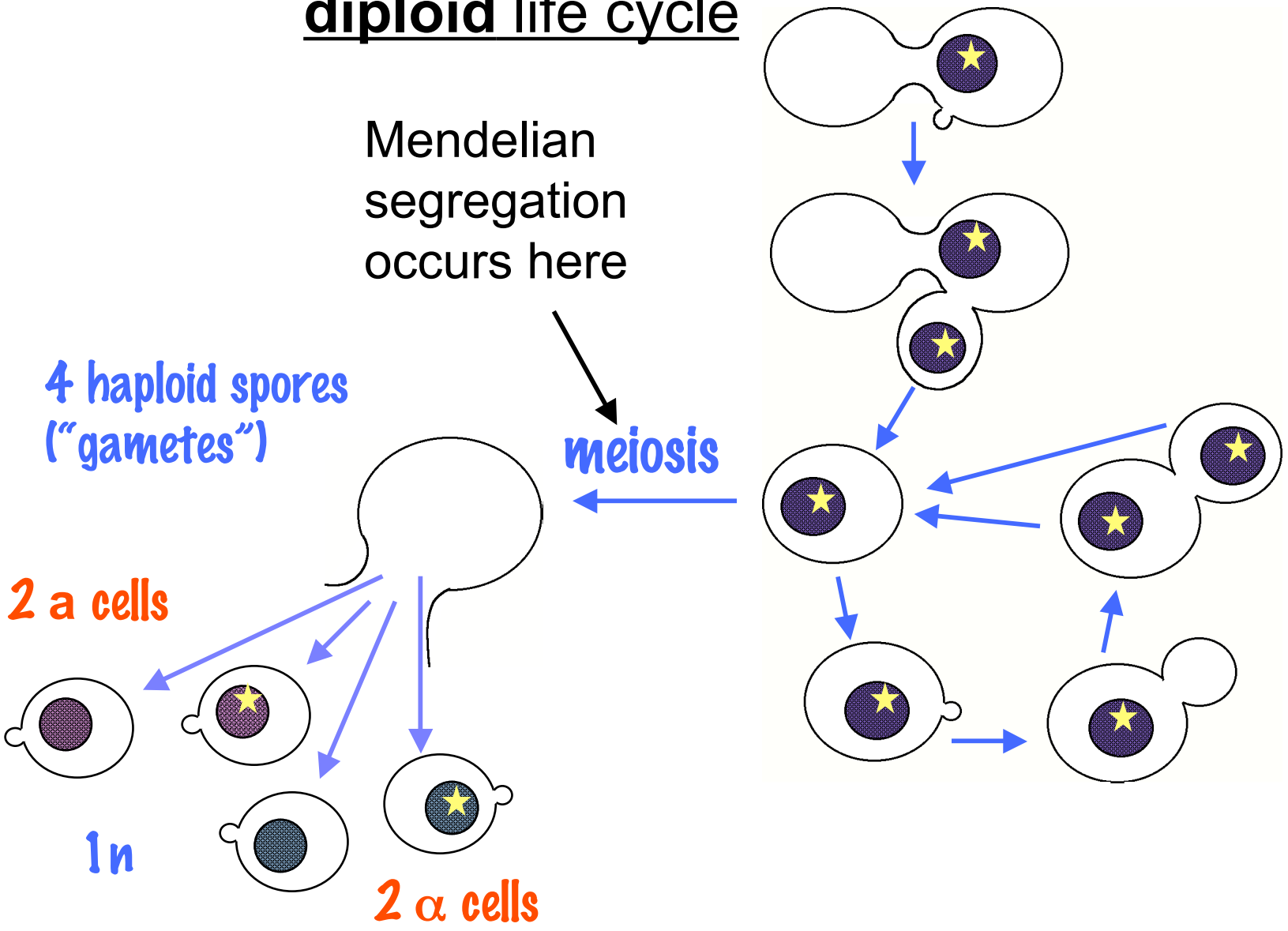


Yeast cells can exist as haploids...

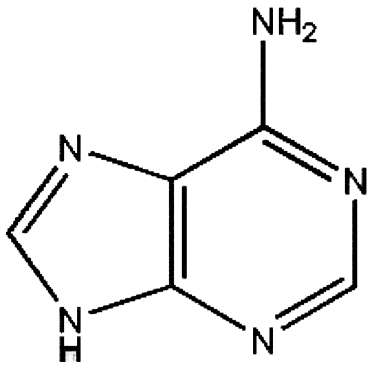
... and as diploids



diploid life cycle



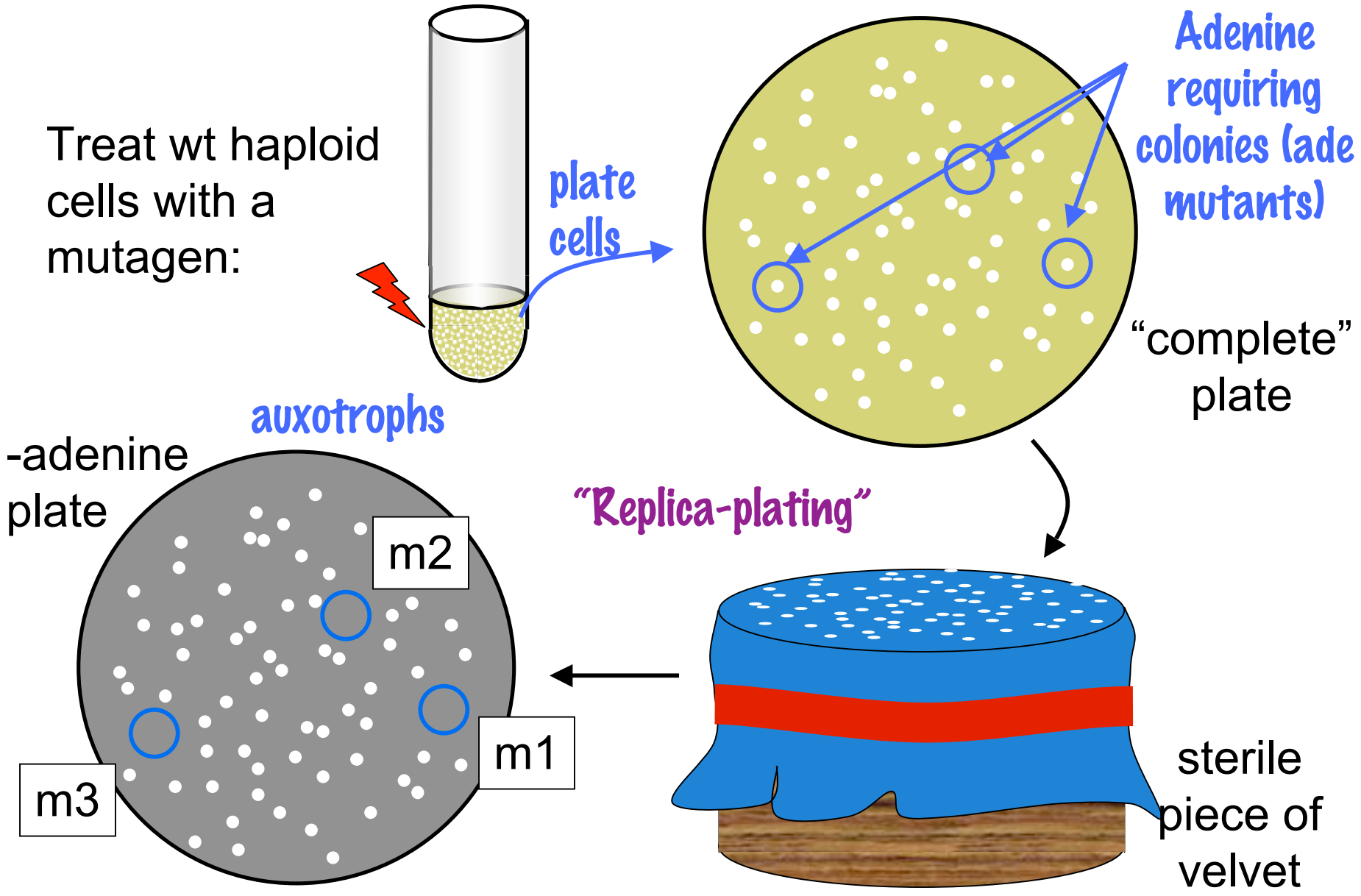
Case study: genetic dissection of adenine biosynthesis in yeast



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

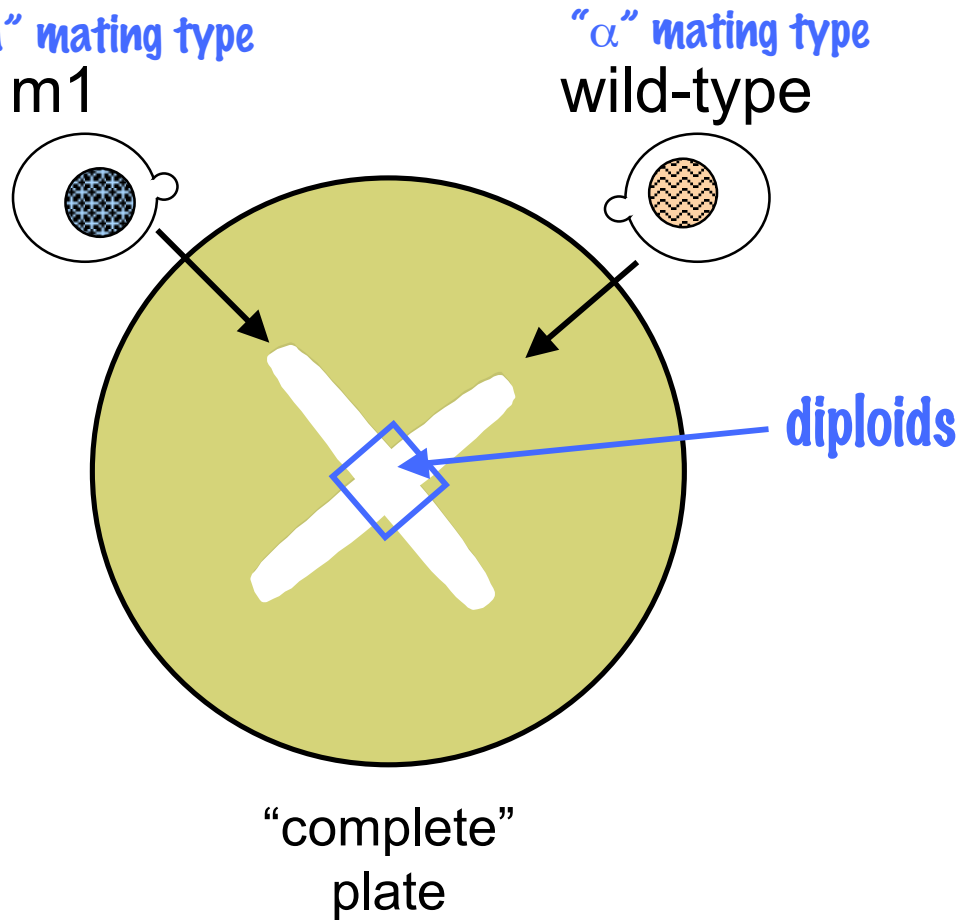
What genes are needed for ability to synthesize adenine?

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? ADE is dominant over ade

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

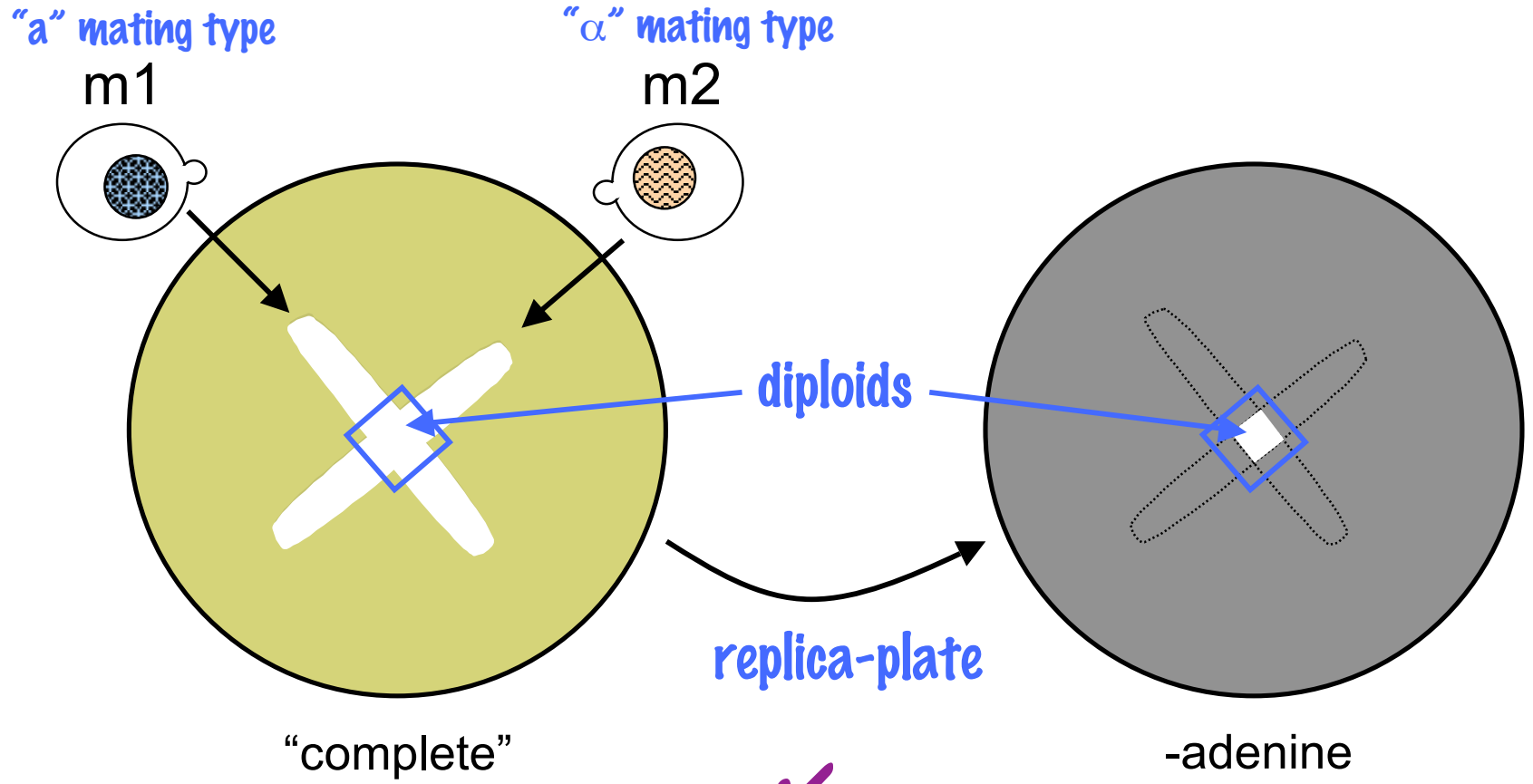
- many enzymes are needed for synthesis of adenine?

  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	o	+	+	+	o	+	o
m2		o					
m3			o				
m4				o			
m5					o		
m6						o	
m7							o

Conclusion?

m1, m5, m7 are mutations in the same gene

o = 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?

Four complementation groups

x	m1	m2	m3	m4	m5	m6	m7
m1	o	+	+	+	o	+	o
m2		o	+	o	+	+	+
m3			o	+	+	+	+
m4				o	+	+	+
m5					o	+	o
m6						o	+
m7							o

Conclusion?

m1, m5, m7 are mutations in one gene

m2, m4 are in one gene

m3

m6

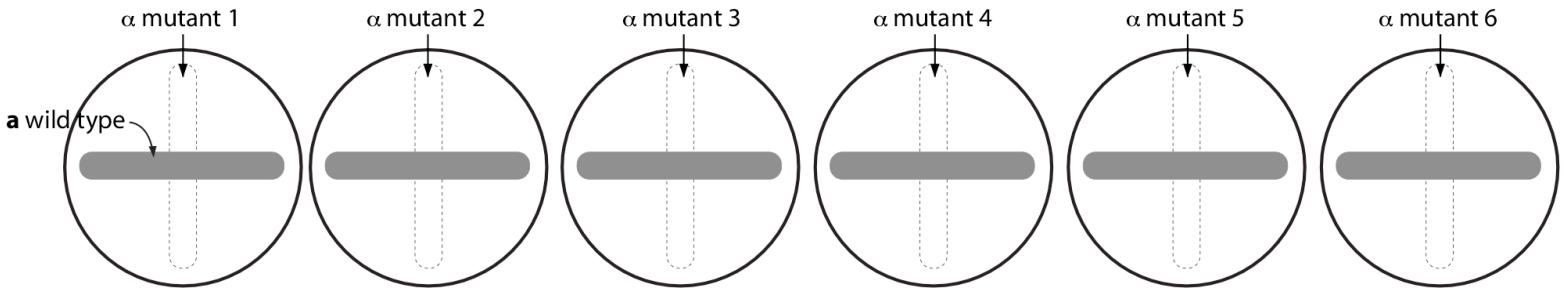
o = no growth on -ade

+ = growth on -ade

Practice question

Yeast cells can normally grow on a sugar called galactose as the sole carbon source. Seven mutant "α" 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.

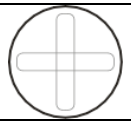
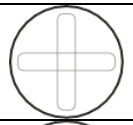
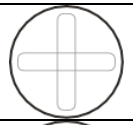
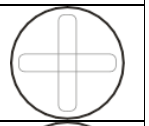
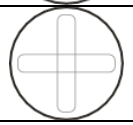
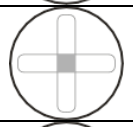
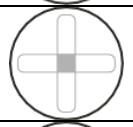
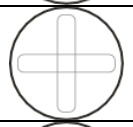
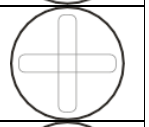
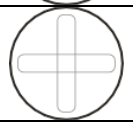
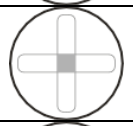
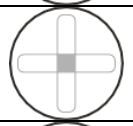
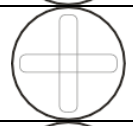
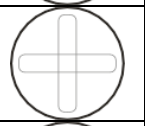
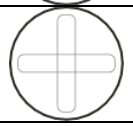
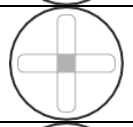
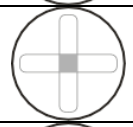
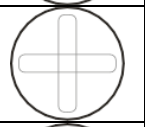
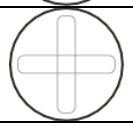
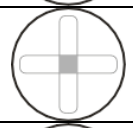
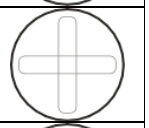
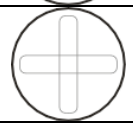
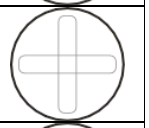
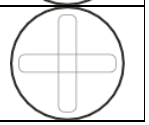


What is the mode of inheritance of mutant phenotype in mutants 1-6? How can you tell?

Diploids grow on gal plate... so, wild type is dominant

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							

m1, m2, m5

m3, m6

m4

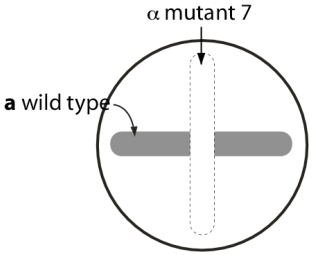
Looking **just at mutants 1–6** for now... group these six mutants by complementation group.

Practice question (cont'd)

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

Fails to complement any of the others... how could it be an allele of 3 different genes?

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?

Complementation test fails with a dominant mutation... heterozygote will always show the mutant phenotype

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

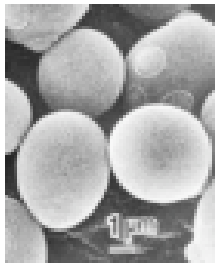
At least 3 genes (can't tell about m7)

Genome 371, 22 Jan 2010, Lecture 5

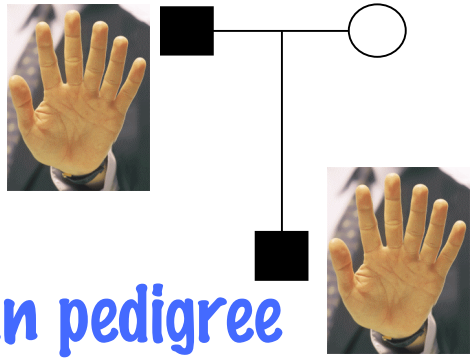
Mendelian Genetics and Probability

- The relationship between genotype and phenotype
- How phenotypic variation is passed on from one generation to the next
- Basic concepts of probability relevant to genetics

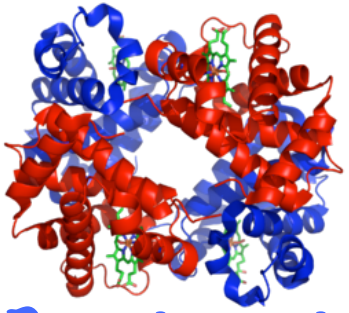
Common theme: linking genotype & phenotype



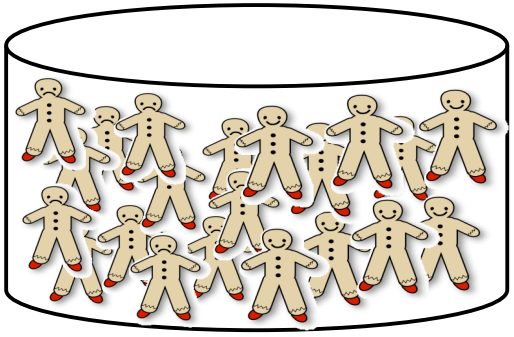
Mutant identified
in a model organism



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

Patterns of Inheritance

What are the rules of inheritance?

Can we predict the outcome of matings?

How does genotype determine phenotype?

The genetic makeup of an organism (usually with respect to one or a few traits)

The physical or observed form

(1822 - 1884)

I have two laws:

1. Random Segregation

2. Independent Assortment

- "The Father of Genetics"
- Born 1822 in what is now Czech Republic
- Catholic priest, science teacher, monk
- Tended the monastery garden which housed peas

What Mendel Did

In his own words (http://www.mendelweb.org/Mendel.html):

> "observe these variations (in the progeny of *hybrids*) in the case of each pair of differentiating characters, and to deduce the law according to which they appear in successive generations."

Mendel's Experiment

Establish true-breeding lines each of which exhibit clear character differences

crosses within the true-breeding population yield progeny that show the same trait as the parent

⇩

Make crosses between each pair of lines

⇩

Identify and count the progeny traits (phenotypes)

round X wrinkled

⇩

??

⇩

Make crosses between the progeny…

...are the progeny phenotypes like one parent or the other? How many of each class are there?

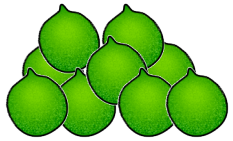
Pea Traits Mendel Studied

- Wrinkled and round peas
- Green or yellow peas
- Inflated or bumpy pod shape
- Green or yellow unripe pods
- Flowers at tip or along stem
- Purple or white petal

The Results

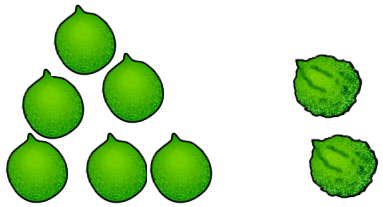
P_1
Parental

F_1
1st filial generation



$F_1 \times F_1$

F_2
2nd filial generation



5474 : 1850

= 2.96 : 1

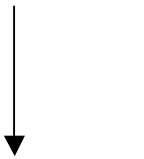
Where did the wrinkled peas go?

Wrinkled trait re-appeared intact in F_2!!

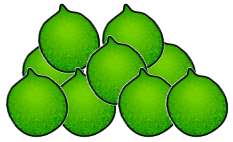
What Did Mendel Conclude?

P_1  x

Parental

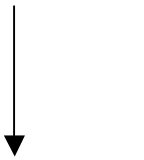


F_1

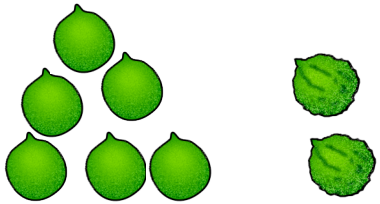


1st filial generation

$F_1 \times F_1$



F_2



2nd filial generation

5474 : 1850

= 2.96 : 1

Wrinkled trait re-appeared intact in F_2

- it was present in F_1 but not seen... wrinkled trait is recessive (withdraws temporarily), round is dominant

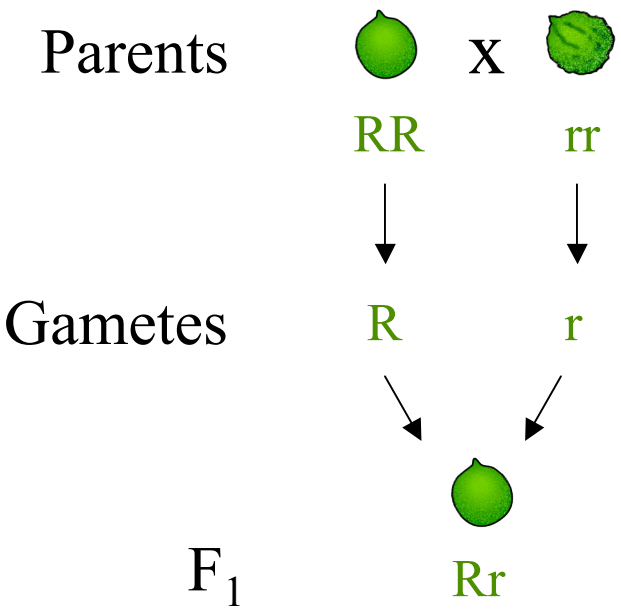
- F_1 plants must have 2 copies of seed shape determinant—so P and F_2 must too

- gametes must have only one copy each

The 3:1 ratio was no fluke…

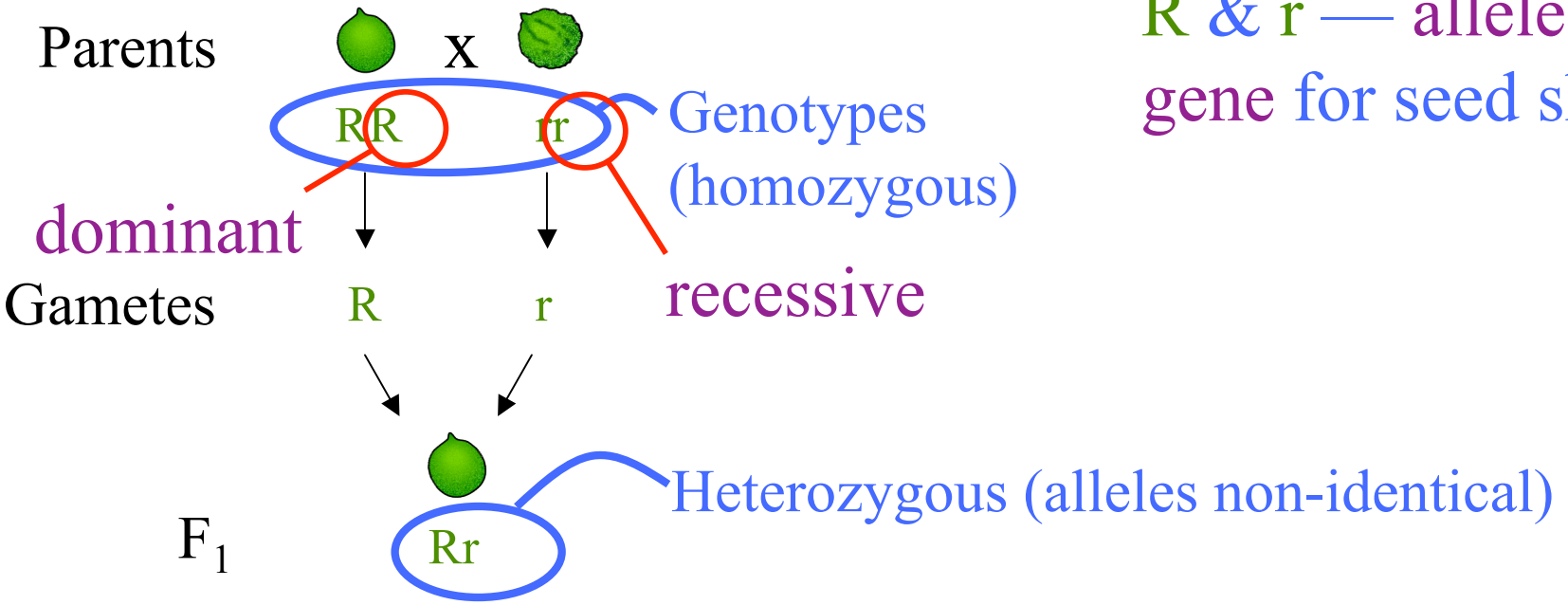
PARENTAL GENERATION PHENOTYPES			F_2 GENERATION PHENOTYPES			
DOMINANT		RECESSIVE	DOMINANT	RECESSIVE	TOTAL	RATIO
Spherical seeds	×	Wrinkled seeds	5,474	1,850	7,324	2.96:1
Yellow seeds	×	Green seeds	6,022	2,001	8,023	3.01:1
Purple flowers	×	White flowers	705	224	929	3.15:1
Inflated pods	×	Constricted pods	882	299	1,181	2.95:1
Green pods	×	Yellow pods	428	152	580	2.82:1
Axial flowers	×	Terminal flowers	651	207	858	3.14:1
Tall stems (1 m)	×	Dwarf stems (0.3 m)	787	277	1,064	2.84:1

Interpreting Mendel's Monohybrid Cross



R & r — alleles of gene for seed shape

Interpreting Mendel's Monohybrid Cross



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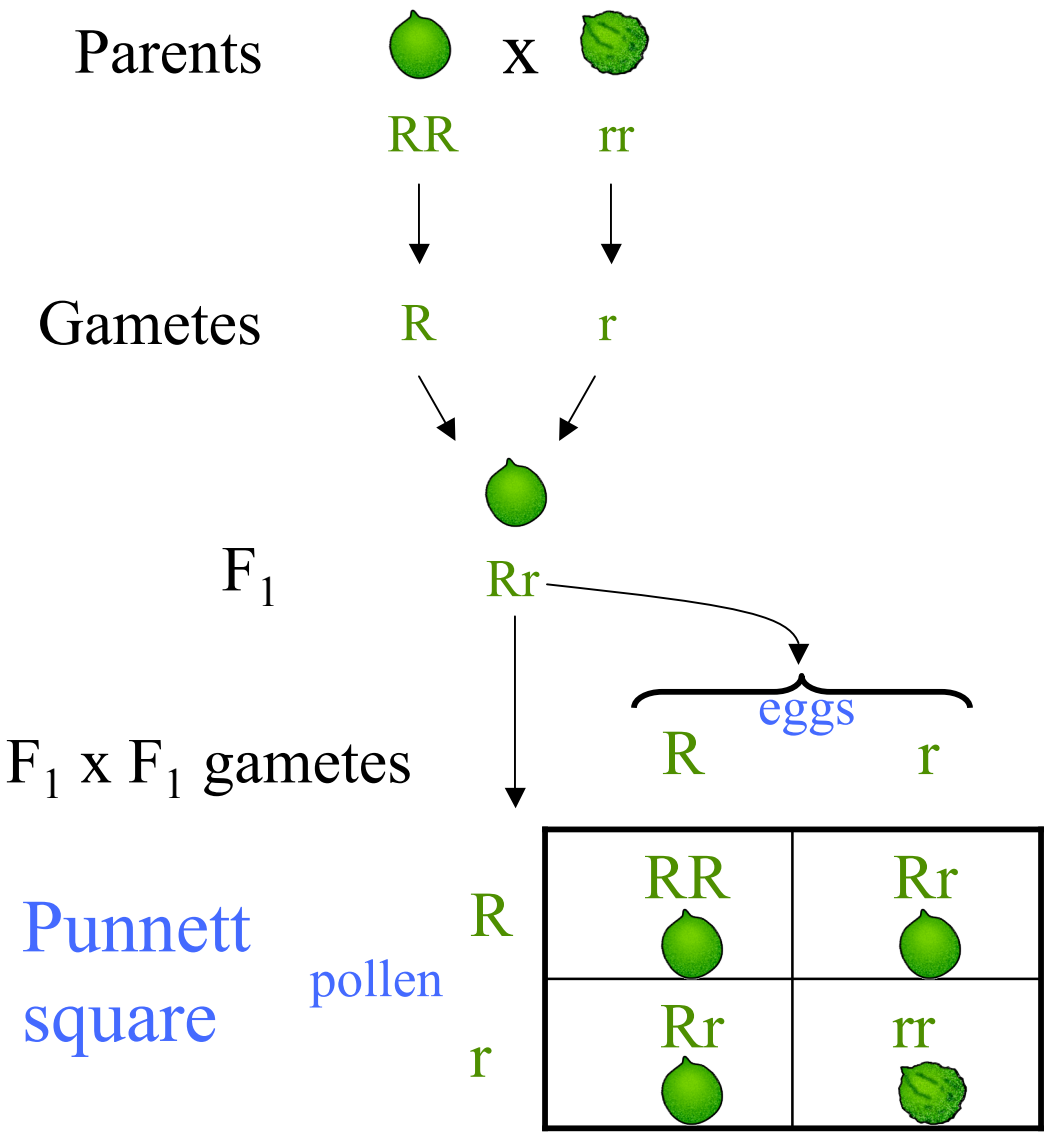

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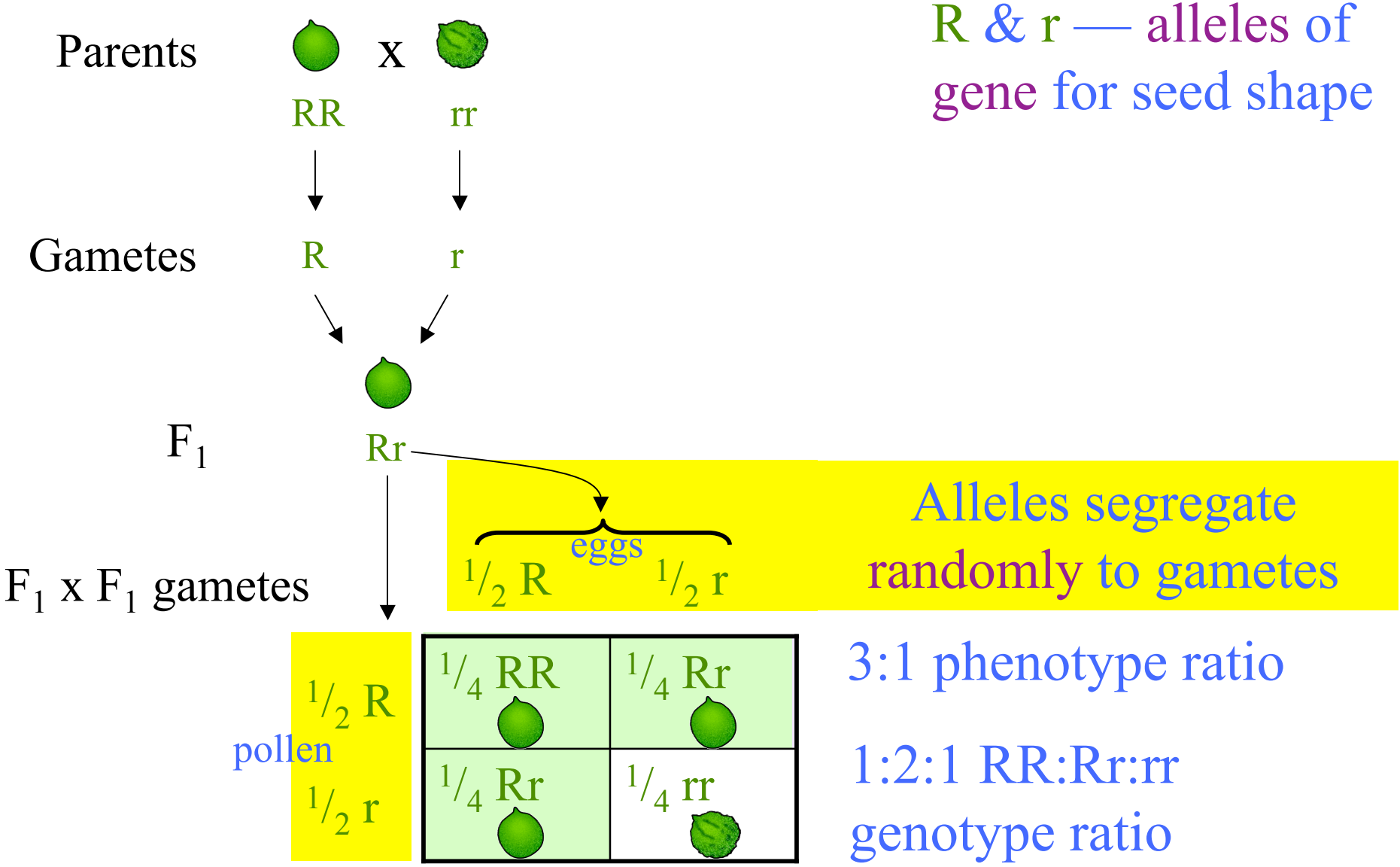


R & r — alleles of gene for seed shape

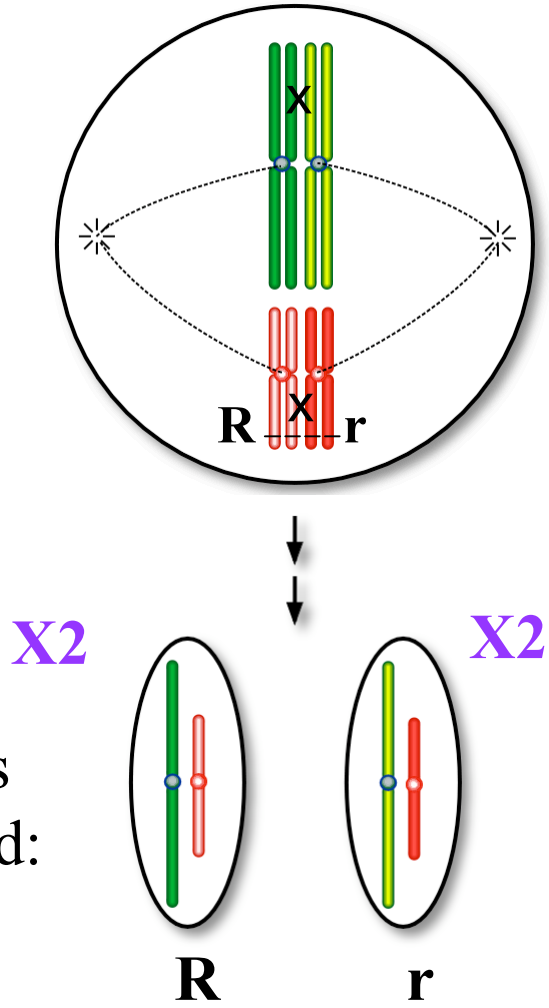
Alleles segregate randomly to gametes

3:1 phenotype ratio

1:2:1 RR:Rr:rr genotype ratio

Meiosis Explains Patterns of Inheritance

F1 genotype: **Rr**



Gametes produced:

Mendel's Conclusions Regarding Seed Shape

1. Seed shape is determined by one gene (determinant)

2. Two forms (alleles) of the determinant; Round is dominant over wrinkled

3. Alleles segregate randomly into gametes

   In heterozygotes, R segregates from r. Each gamete has 50:50 chance of getting R or r.

Mendel's law of random segregation

Generality of Mendel's Findings?

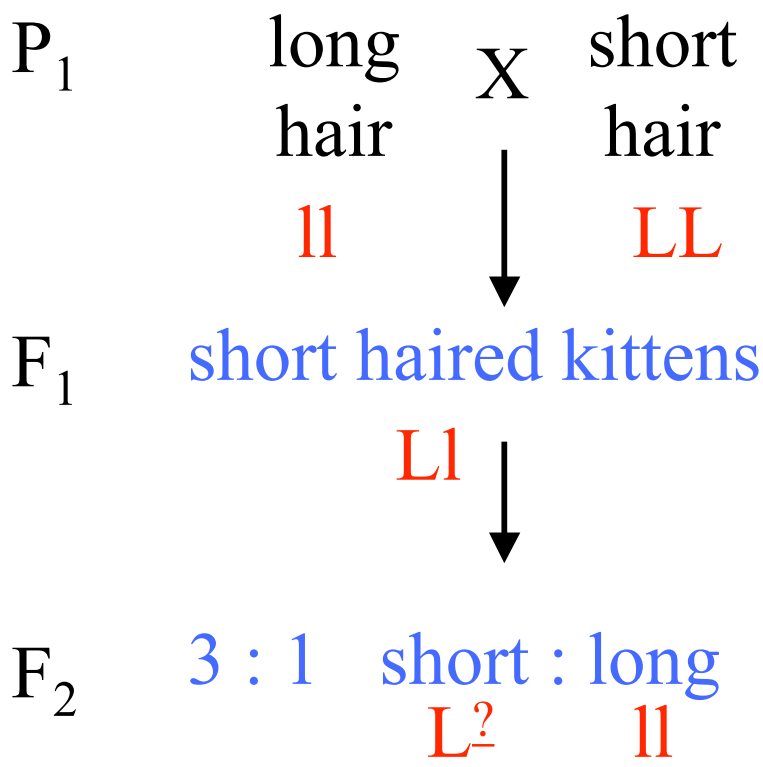
short haired tabby

X

long haired tabby

Assuming they are pure breeding, what kinds of F_1 and F_2 kittens would they have?

Back to the Cat Cross



Hypothesis?

one is dominant, the other is recessive

Conclusions and predictions?

Short (L) is dominant

F_2 are expected to be . . .

Over many litters, that is what is found!

Data are consistent with Mendel's hypotheses

The Testcross

Suppose you were given a short-haired cat…

How would you tell its genotype?

(What are the possibilities?)

LL or Ll — a.k.a., L? or L_

Do a testcross… mate it to a known ll cat…

If your cat is LL → all kittens shorthaired

If your cat is Ll → short and long hair kittens in equal proportions

Are There Mendelian Traits in Humans?

Traits for which there are distinct alternative forms

Tongue rolling
Arm crossing
Ear lobe shape
Mid-digital hair

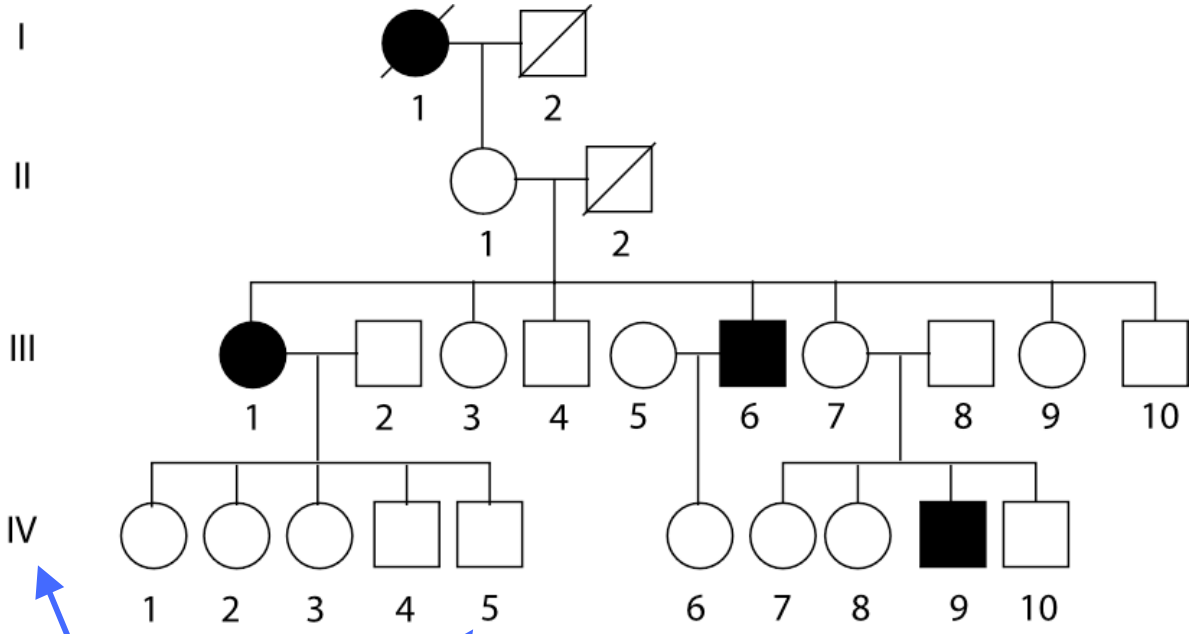
Suggests there are single genes in the human genome that govern these traits!!

Many Mendelian diseases (Cystic Fibrosis, Tay Sachs, etc.)

Most traits have a more complicated basis (height, skin color, etc.)

To test predictions for human traits, must rely on finding appropriate families

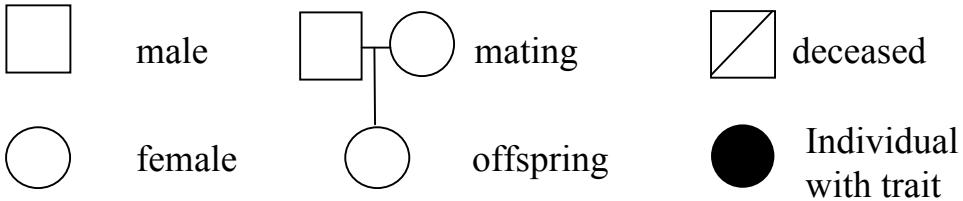
Transmission of Albinism in a Family



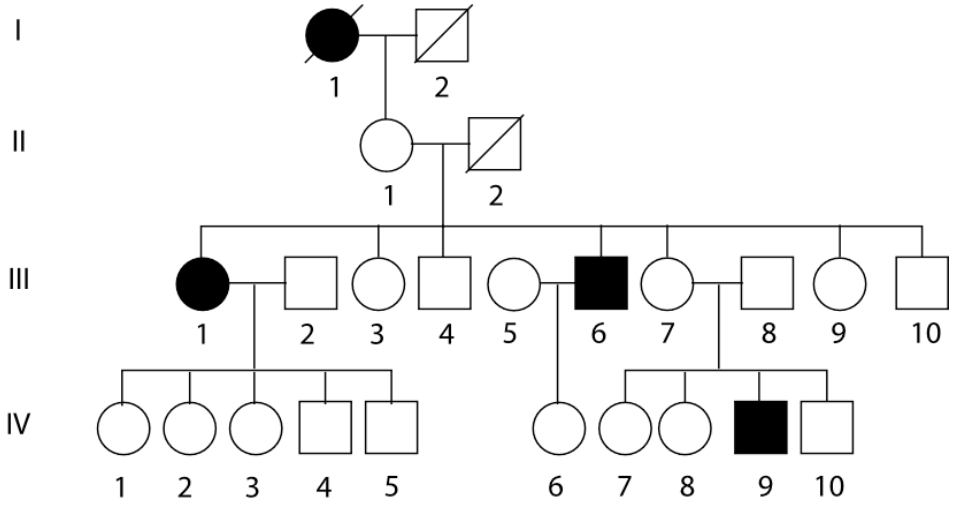
generation

individual (by age within family)

No pigment =



Transmission of albinism (cont'd)



Albinism dominant or recessive?

recessive

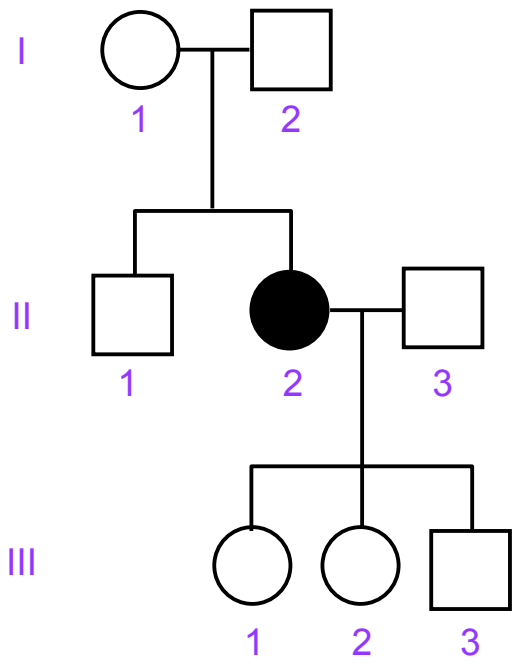
Summary

- Pedigrees show transmission of specific traits in family trees
- Helpful in inferring mode of inheritance and in gene mapping

Pedigree Basics — Practice Question

An example… a different genetic disorder…

Is the disease phenotype **dominant** or **recessive**?



Parents of an affected child don't show the trait... so, recessive

Can we deduce the genotypes of the individuals in this family?

Let...

D = unaffected (no disease)

d = affected (disease phenotype)

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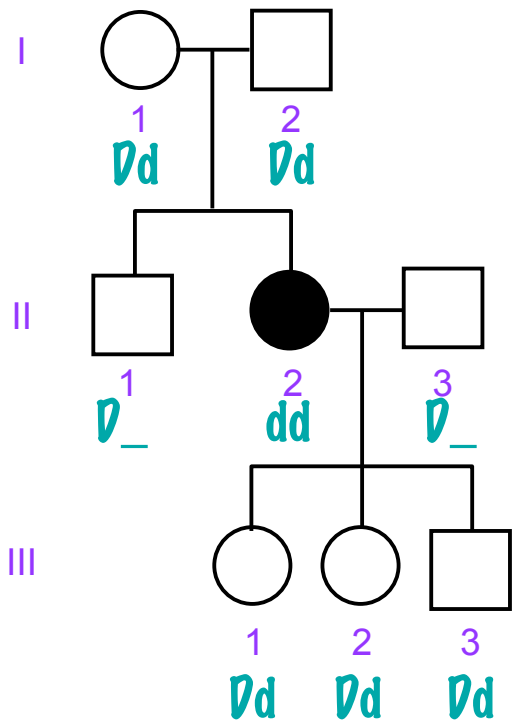

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Let...

D = unaffected (no disease)

d = affected (disease phenotype)

Rare traits: people marrying into the family are assumed to be homozygous normal

Probability

Need:

- to be able to predict genotype/phenotype ratios
- measures how well our observed data fit predictions

Why bother?

- genetic counseling — risk assessment
- testing hypotheses — need to predict outcomes, then assess how well the predictions match the real results
- mapping disease genes — depends on being able to calculate probabilities of specific outcomes

Product Rule

The probability of two or more **independent events** all occurring (event #1 and event #2 and…)

= the product of the individual probabilities

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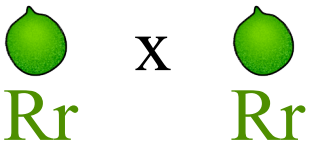

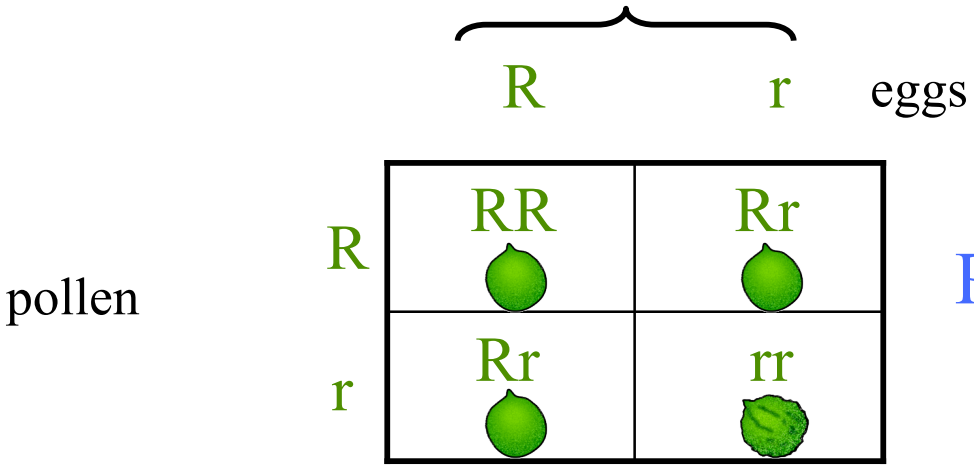

Probability of wrinkled pea?

Product Rule

The probability of two or more **independent events** all occurring (event #1 and event #2 and…)

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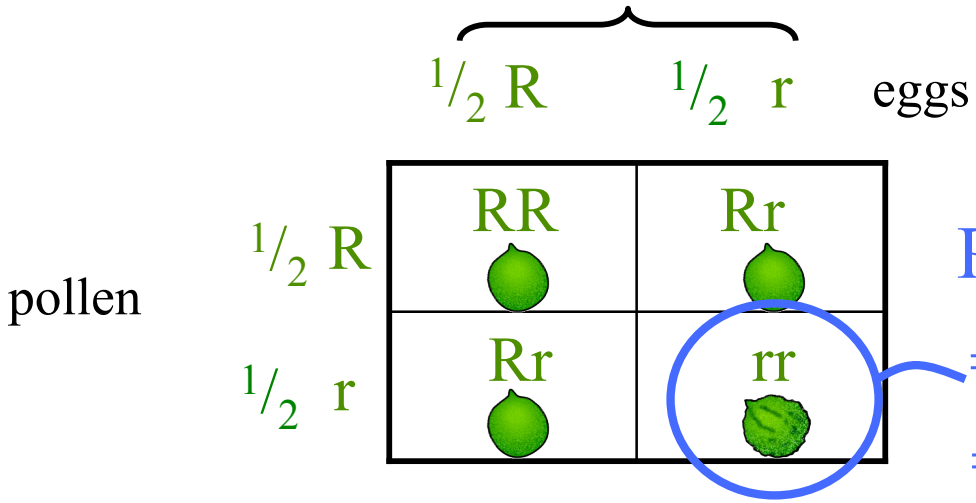


Probability of wrinkled pea?

= r from mom and r from dad

= 1/2 x 1/2

= **1/4**

Sum Rule

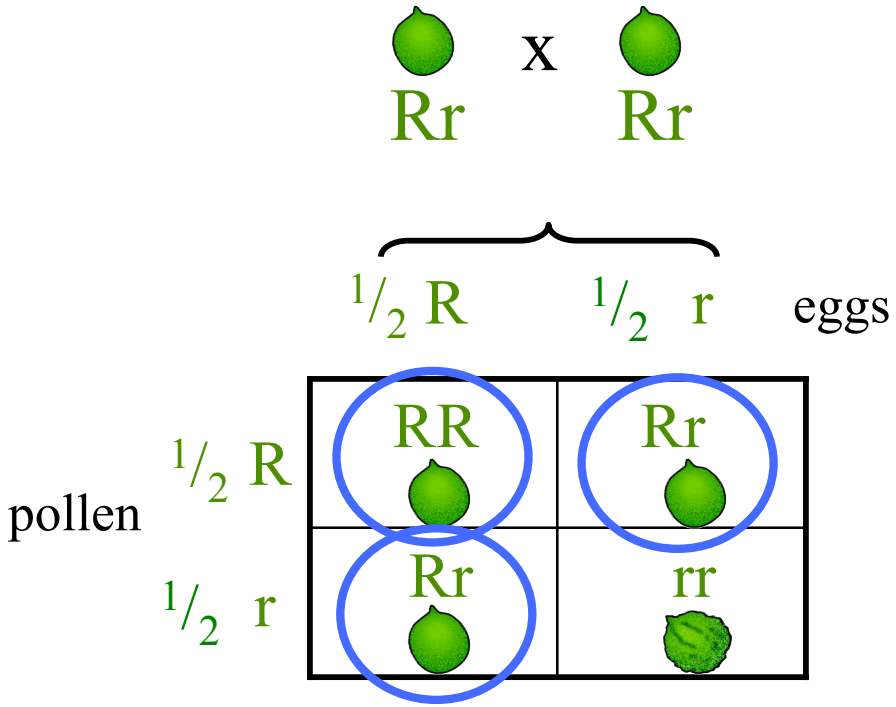
The probability of two or **more mutually exclusive** outcomes (outcome #1 or outcome #2 or…)

= sum of the individual probabilities

Sum Rule

The probability of any one of 2 or more mutually exclusive outcomes (outcome #1 or outcome #2 or…)

= sum of the individual probabilities



Probability of round pea?

= RR or Rr or Rr

= 1/4 + 1/4 + 1/4

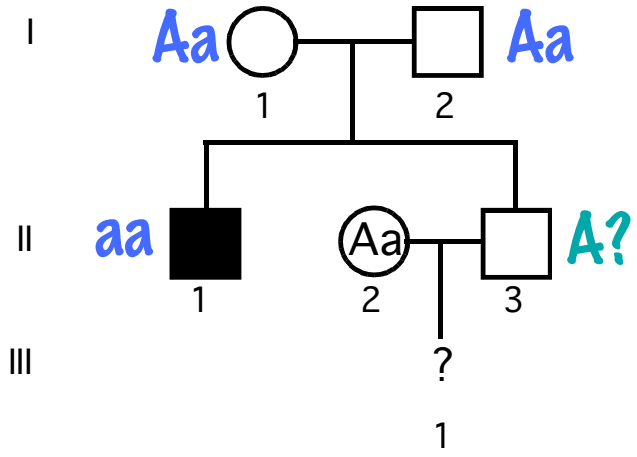
= **3/4**

Practice Question: Product Rule in Pedigrees

Example: Albinism…

a = no pigment

What is the probability that III-1 will show the trait?



P(III-1 is aa) = 2/3 x 1/4 = 1/6