Genetical Jargon Demystified

- In typical genetical parlance the hereditary factor that determines the round/wrinkled seed difference as referred to as *"the gene"* for round or wrinkled seeds
- What we mean more precisely is that this *single gene* controls the difference in seed form between the round and wrinkled strains that Mendel worked with

Does Mendel's work suggest that this is the only gene in the pea genome that can affect this particular trait?

If not, then why is round vs. wrinkled refered to as a single-gene trait?

A phenotypic difference in two organisms is said to be a single gene trait if the phenotypic variation is explained by allelic differences in just one gene

The term single gene trait in no way implies that only a single gene product is involved in the development or structure & function of the cells/tissue/organ related to the trait

Other genes are likely to be involved in pea seed morphology but these genes were not functionally different in Mendel's round and wrinkled strains

From Gene to Phenotype

Table 1 Seven characters of *P. sativum* examined by Mendel and a summary of the genes, phenotypes and presumed mutations involved

Trait	Dominant phenotype	Recessive phenotype	Symbol group	Linkage group	Cloned	Gene function	Molecular nature of mutation
Seed shape	Round	Wrinkled	R	V	Yes	Starch branching enzyme 1	0.8-kb insertion
Stem length	Tall	Dwarf	LE	III	Yes	GA 3-oxidase1	G-to-A substitution
Cotyledon color	Yellow	Green	I	I	Yes	Stay-green gene	6-bp insertion
Seed coat/flower color	Purple	White	A	II	Yes	bHLH transcription factor	G-to-A at splice site
Pod color	Green	Yellow	GP	V	No	Chloroplast structure in pod wall	Unknown
Pod form	Inflated	Constricted	V?	III	No	Sclerenchyma formation in pods	Unknown
Position of flowers	Axial	Terminal	FA	IV	No	Meristem function	Unknown

References are given in the text.

From Gene to Phenotype: Yellow vs Green peas

Cross–Species Identification of Mendel's I Locus: The Stay–Green Gene

Science 5 January 2007:   Chlorophyll breakdown during senescence is an integral part of plant development and leads to the accumulation of colorless catabolites. The loss of green pigment is due to an oxygenolytic opening of the porphyrin macrocycle of pheophorbide. Stay**green** (the gene sgr), the indefinite retention of **green**ness in senescing leaves and cotyledons, is a genetic variant that interferes with the normal expression of senescence.   The researchers identified gene homologs in Pisum sativum (**pea**), Arabidopsis thaliana, and Festuca pratensis (meadow fescue), mutations of which partially disable plant senescence. The biochemical properties and map location of this gene in **pea** indicate that it is the same locus that determines yellow (I) and **green** (i) cotyledon color, as originally described by **Mendel** in his seminal paper.
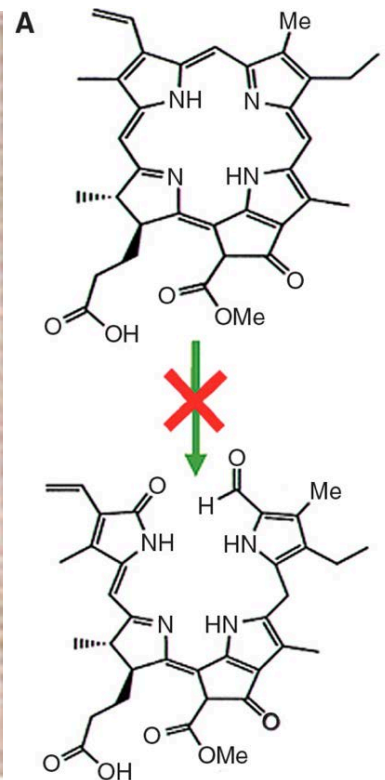
A) In mutant and silenced genotypes, inhibition (X) of the ring–opening step between (top) pheophorbide and the (bottom) red chlorophyll catabolite in the chlorophyll breakdown pathway

This study results in the identification of a gene that plays a fundamental role in chlorophyll catabolism during plant senescence. In addition, it suggests that the cotyledon color trait described by **Mendel** reflects allelic variation in a **pea** gene, homologs of which are responsible for the stay–**green** phenotype in both dicots and monocots.

♦ Mendel proposed that the hereditary determinants for each trait are discrete and do not become blended together in the F1, *but maintain their integrity from generation to generation.*

♦ Mendel also proposed that, for each trait examined, the pea plant contains two copies of the hereditary determinant (gene) controlling the trait, one copy coming from each parent

How did Mendel explain the 3 to 1 (dominant to recessive) F2 ratio in the monohybrid crosses?

First he proposed the Principle of Segregation

Mendel's principle of segregation

- Mendel proposed that during gamete formation in the F1, the paired Rr alleles would separate into different gamete cells and that about 1/2 of the gametes would carry the dominant *R* allele and 1/2 would carry the recessive *r* allele.
- This would be true for both the male and female gametes,
- Mendel assumed that the male and female gamete cell would combine at random.
- So, a given R or r male gamete would have an equal chance of fertilizing an *R*-bearing or an *r*-bearing ovule.

Two elements of chance are indicated here:
- the chance that a gamete is R vs. r
- the random (with respect to genotype) combination of male gametes with female gametes

Because of the elements of chance in these processes, *Mendel used his knowledge of the basic rules of probability to make predictions about the genotypic and phenotypic composition of the F2 progeny*

A *probability* is a measure of the likelihood or chance, that an event will have a particular outcome.

A probability is usually expressed as a fraction between 0 and 1.

1 - the event is certain to occur

0 - the event is certain not to happen

In all other cases the chance that a particular event will occur increases as the probability approaches 1.

The <mark>product (both-and)</mark> rule of probability:
the probability of two independent events both occurring is the product of each of their respective probabilities

To use the product rule, the events must be independent: *the occurrence of one event cannot affect in any way the probability of the other event occurring*

This rule can be applied *to multiple independent events*

What is the outcome of such chance events?
Mendel recognized that the basic rules of probability could be used to predict the likelihood of each genotype (*RR*, *Rr and rr*) for a given F1 progeny.

- ***What is the chance that an F2 progeny shows the recessive phenotype?*** According to the <mark>product (*both-and*)</mark> rule of probability, the probability that a given zygote received an *r* allele from *both* the male *and* females gametes is 1/2 X 1/2 or 1/4.

The sum (either-or) rule of probability

The probability of either one of two *mutually exclusive* events occurring is the sum of their respective probabilities

In other words we are determining the probability of one event or another event (either-or rule)

This rule applies to multiple events

What is the chance that an F2 progeny shows the dominant phenotype?

- Here we have to use the ==*product (both-and)*== and the ==*sum (either-or)*== rules of probability.
- To show a dominant phenotype the genotype of the F2 can be *either RR or Rr*.
- The overall probability of a dominant phenotype =

$\downarrow$ **product** $\downarrow$**sum** $\downarrow$ **product & sum**

(1/2)(1/2)   +   2(1/2)(1/2)   = 3/4

[*either* RR]   [*or* Rr]   [overall p]

If a large number of independent fertilization events are examined these phenotype probabilities translate into the fraction of progeny that should be of each phenotype:

- 3/4 of the F2 progeny should show the dominant phenotype and 1/4 the recessive.
- Here then is Mendel's *3 to 1* ratio

Mendel's analysis predicted two classes of F2 round progeny

How to confirm that the round F2s consists of two different genotypes in the proper ratio?

- To confirm these predictions Mendel planted the F2 seeds and allowed the F2 plants to self-pollinate.
- He then examined the phenotypes of the F3 seeds.
- As predicted, F2 plants showing the recessive phenotype always bred true.
- There were two classes of F2 progeny with the dominant phenotype.
- How did the self-progeny of these two classes differ?

Fraction of F2	Phenotype of F2	Phenotype of F3 progeny	Genotype of the F2 plant
1/4	dominant	all dominant	
1/2	dominant	3/4 dominant 1/4 recessive	
1/4	recessive	all recessive	

1. For example, Mendel selfed 519 round plants from the F2 generation of the crosses described above.
2. **He observed that 193 bred true (producing only round progeny) and 372 plants produced both round and wrinkled seeds, giving a ratio of homozygotes to heterozygotes of 1.93 to 1 (~2 to 1).**

Consider these questions:
- *What is the probability that an F2 plant is heterozygous?*
- *What is the probability that an F2 with the dominant phenotype is heterzygous?*

This will be an important distinction when we look at pedigrees

MENDEL EXTENDED HIS EXPERIMENTS BY EXAMINING TRAITS TWO AT A TIME

◆ Mendel also was interested in knowing whether his principle of segregation held true when the parental lines differed with respect to two or more traits.

◆ He crossed a parental line that had round, green seeds with a parental line with wrinkled, yellow seeds.

◆ As expected, all F1 seeds showed the dominant yellow and round phenotypes.

◆ Mendel then allowed the F1 plants to self-pollinate and examined the phenotype of the F2 seeds.

◆ Mendel observed four phenotypic categories in the F2 generation.

Phenotype of F2	Number of F2 (556 total)	Fraction of total progeny
yellow, round	315	9/16
yellow, wrinkled	101	3/16
green, round	108	3/16
green, wrinkled	32	1/16

How to analyze this data so it makes sense in the context of the previous experiments

He analyzed in the following way:

♦ First he looked at the data for seed color only and noted that the ratio of yellow (315 + 101 F2 plants) to green (108 + 32 F2 plants) was *416 to 140* or *3 to 1*.

♦ Likewise, the ratio of round (315 + 108) to
wrinkled (101 + 32) seeds was *423 to 133* or *3.18 to 1*.

♦ *For each trait, the F2 seeds exhibited the 3:1 (dominant: recessive) ratio that he had observed previously when analyzing the traits individually.*

How to explain the 9:3:3:1 distribution of progeny?

If the traits are combining at random, then the *product rule of probability* can be used to calculate the expected frequency of round, yellow plants (both dominant phenotypes):

3/4 (round) X 3/4 (for yellow) = 9/16 (both round and yellow).

This is exactly what Mendel observed. The frequency of the other phenotypic categories can be explained in a similar fashion

♦ Mendel examined other combinations of traits (two at a time) and obtained similar results for each pair of traits:
♦ the phenotypes were combined at random in the F2 progeny to give a 9:3:3:1 ratio of progeny.

Recall that for the product rule to apply the different traits must be combining at random – that is they must be independent events

MENDEL'S PRINCIPLE OF INDEPENDENT ASSORTMENT
To explain the random combination of phenotypes in the F2 progeny Mendel proposed that during gamete formation, *the segregation of one gene pair occurs independently of the segregation of other gene pairs*.

This *principle of independent assortment* makes very specific predictions about the *composition of gametes produced by an F1 plant*.

- The genotype of the F1 round, yellow plants can be symbolized as *RrYy* to indicate that the plant is heterozygous for the dominant and recessive alleles for two different traits.
- Mendel's *principle of segregation* predicts that 1/2 of the gametes produced by such a plant should carry the dominant *R* allele and 1/2 should carry the recessive *r* allele; likewise 1/2 of the gametes should bear the *Y* and 1/2 the *y* allele.
- Mendel's *principle of independent assortment* means that whether a gamete receives the dominant *R* allele or the recessive *r* allele has no bearing on whether the same gamete receives the dominant *Y* or recessive *y* allele (see figure below)
- Mendel predicted then that a *RrYy* plant would produce four different types of gametes in equal numbers:

50% of the meiotic divisions:

50% of the meiotic divisions:

ab   a   a   A   A   AB   aB   a   a   A   A   Ab

b   b   B   B   B   B   b   b

metaphase/anaphase of first division
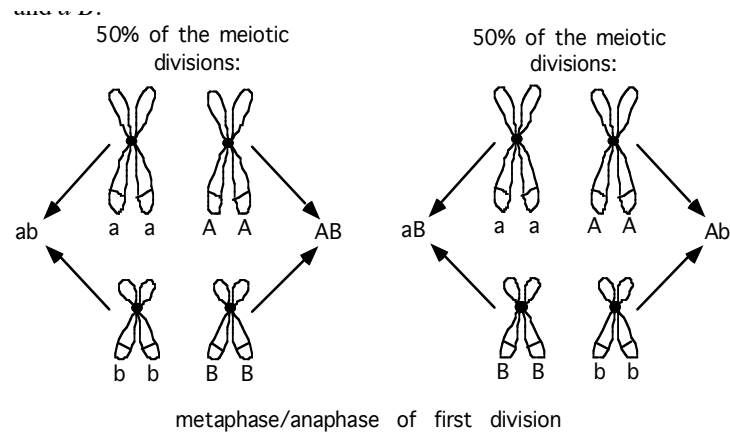
1/4 *YR* = 1/2 chance of *Y* X 1/2 chance of *R*
1/4 *Yr* = 1/2 *Y* X 1/2 *r*
1/4 *yR* = 1/2 *y* X 1/2 *R*
1/4 *yr* = 1/2 *y* X 1/2 *r*

MENDEL USES A TESTCROSS TO CONFIRM HIS PREDICTIONS

To provide additional evidence for his principle of independent assortment, Mendel need to demonstrate that an F1 plant which is heterozygous for two different gene pairs produces four different types of gametes in a 1:1:1:1 ratio. He did this by performing a **testcross** between the F1 plant and a plant that had the recessive forms of the traits under examination:

F1 round, yellow plant   X   wrinkled, green plant
   RrYy   rryy

This punnet square shows the expected progeny phenotypes and ratios if the F1 round, yellow parent produces the four types of gametes in equal proportions:

<center>Gametes from F1 parent</center>

	1/4 RY	1/4 Ry	1/4 rY	1/4 ry
Gamete from tester ry **strain**	**1/4 RrYy** round, yellow	**1/4 Rryy** round, green	**1/4 rrYy** wrinkled, yellow	**1/4 rryy** wrinkled, green

A testcross is informative about gamete ratios because the tester strain contributes only recessive alleles to the progeny.

Therefore the gamete composition of the F1 parent can be directly inferred from phenotypic composition of the progeny.

Figure 3-4 of your text illustrates how a Punnet Square can be used to explain the 9:3:3:1 F2 progeny ratios starting with the gamete composition of the F1 parents predicted by Mendel's principle of independent assortment.

Note that genotype frequencies can be determined from this chart.
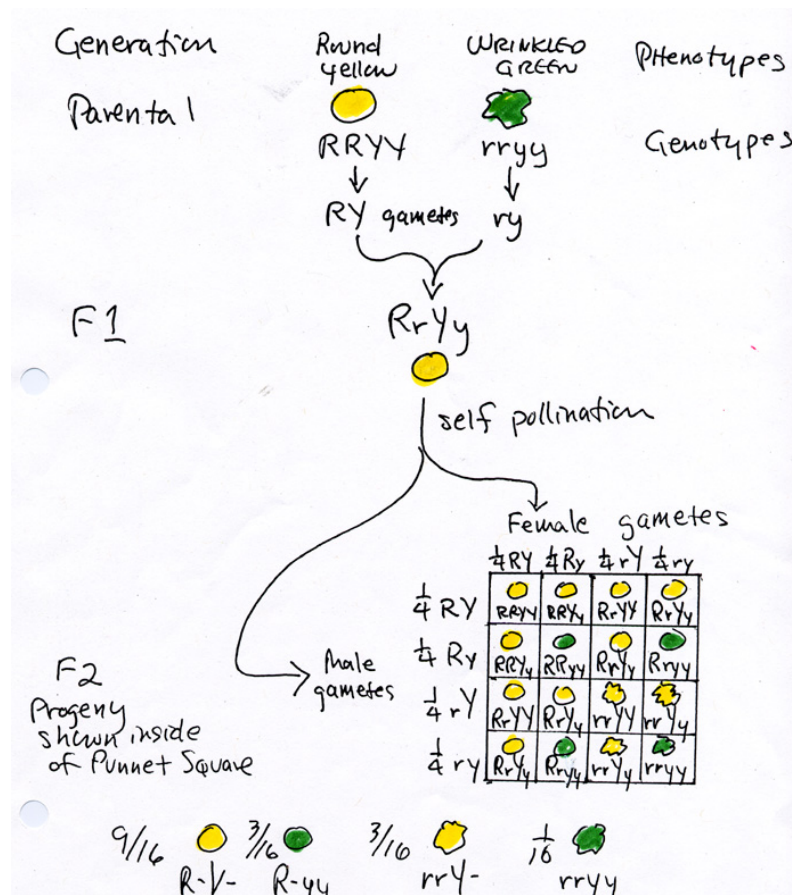
What is the probability of this genotype: RRYy

- could draw up the Punnett square, fill in all the square and count up the number of squares with this genotype

Courtesy of the Wellcome Institute Library, London. Noncommercial, educational use only.

Group portrait of the Cambridge University Natural Science Club. R. C. Punnett is in the second row, far right. Ernest Rutherford, the physicist, is in the top row, far left.
From the web site *DNA from the Beginning* http://vector.cshl.org/dnaftb/

The disadvantages of using a Punnett Square

- it time-consuming and an inefficient way to do this calculation (that is, determining the probability of this genotype: RRYy)
- easier to consider that since the genes are assorting independently, you can use the product rule of probability chance RR (1/2 X 1/2) X Yy [2 (1/2)(1/2)]

Sample probability question to illustrate previous point

- different chromosomes = independent assortment
- independent assortment = independent rule = product rule of probability

A friend of yours is trying to generate a strain of fruitflies that is homozygous for four autosomal mutations (aabbccdd). Each gene is on a different chromosome. He generates male and female AaBbCcDd heterozygotes. Next he mates a male and a female fly:

AaBbCcDd X AaBbCcDd
⬇

He collects 200 progeny flies and carefully examines their phenotypes. He is disappointed to discover that none of the 200 progeny exhibits all four recessive phenotypes although he does find a couple of flies with 3 of the recessive phenotypes. Assuming that he scored the progeny phenotypes correctly, where did your friend go wrong? You must illustrate your answer with the appropriate calculation.

Mendel's work provided a powerful conceptual framework for thinking about heredity. He was the first individual to describe the basic rules of **transmission genetics**, that is, the mechanisms involved in the passage of genes from one generation to the next. All of the ratios of progeny that Mendel observed in his F1 and F2 generations could be explained as the consequence of three simple processes:

- Segregation of discrete, paired alleles into separate gamete cells
- Independent assortment (segregation) of the paired alleles controlling different traits
- Random fertilization of gametes