

1 BASIC MENDELIAN AND MOLECULAR GENETICS

1.1 Genetic terms you should know and understand

Gene	Autosome	Homologous chromosome
Locus	Sex chromosome	Sister chromatid
Allele	Hemizygous	Tetrad
Dominance	Pleiotropy	Mitosis
Recessiveness	Epistasis	Meiosis
Wild type	Haploid	Independent assortment
Mutation	Diploid	Recombination
Genotype	Nuclear DNA	Crossing over
Phenotype	Mitochondrial DNA	Transcription
Homozygote	Chloroplast DNA	Translation
Heterozygote	Chromosome	

Mendelian genetics skills you should have mastered

Basic Probability (Sum rule, product rule, conditional probability)	Monohybrid (1-locus) crosses Dihybrid (2-locus) crosses Sex Linkage
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1.2 Features of the Genetic Code you should know

A, G (purines),
C, T (pyrimidines)
Genetic Code (based on mRNA sequence)
Degeneracy (wobble)
Replacement mutation (nonsynonymous)
Silent mutation (synonymous)
Frameshift mutations

1.3 Features of Genome Structure you should know

Coding regions code for polypeptides (proteins) or tRNAs, rRNAs

Noncoding regions contain repetitive DNA sequences, e.g., microsatellite loci (Short Tandem Repeats), longer repeats (*Alu* is 300 bp long and copies occur 300,000 times in human genome = 5% of human DNA)

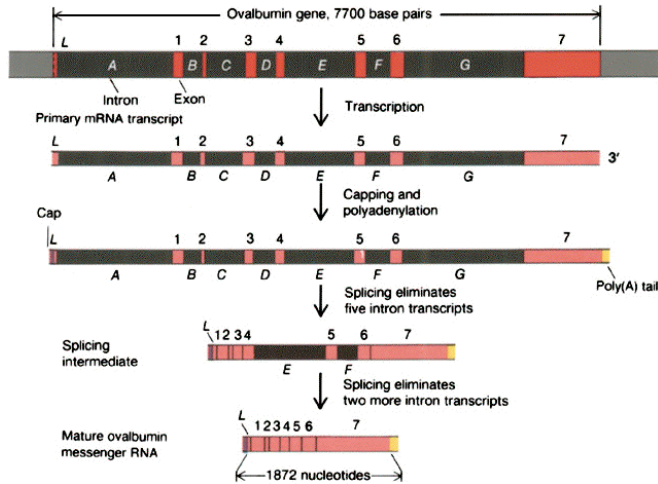
Pseudogenes are the result of duplications that have acquired a mutation producing a stop codon inside the coding region.

		SECOND POSITION					
		U	C	A	G		
FIRST POSITION	U	phenyl-alanine	serine	tyrosine	cysteine	U	THIRD POSITION
		leucine		stop	stop	C	
			stop	tryptophan	A		
					G		
C	leucine	proline	histidine	arginine	U		
			glutamine		C		
				A			
					G		
A	isoleucine	threonine	asparagine	serine	U		
	* methionine		lysine	arginine	C		
				A			
					G		
G	valine	alanine	aspartic acid	glycine	U		
			glutamic acid		C		
				A			
					G		

* and start

Introns (transcribed but not translated portions of a gene)

Exons (transcribed and translated portions of a gene)



2 MENDEL'S POSTULATES

Basis of Mendel's success:

- chose research organism well-suited to his objectives
- experiments carefully designed
- collected large amounts of data
- used mathematical analysis to show that results were consistent with hypotheses

Peas will **self-fertilize** unless they are cross-pollinated by hand. Must clip off anthers to prevent **selfing**. They're available in wide array of distinct **true-breeding types** (produce only progeny like themselves when they self). Grew them for two years to insure true-breeding. Provided **control** for hybridization.

Mendel obtained seeds for plants with distinct characteristics or **phenotypes**. Bred true for flower color, pod shape, seed shape, seed color, etc. True-breeding types used as parents in **hybridization** experiments.

2.1 Mendel's First Postulate:

Genetic characters are controlled by unit factors that exist in pairs in individual organisms.

Pollen from round-seeded plant used to pollinate wrinkled-seeded plant, & vice versa.

<u><--Reciprocal Monohybrid Crosses--></u>							
	Maternal parent		Paternal parent		Maternal parent		Paternal parent
P:	wrinkled	x	round		round	x	wrinkled
F1:	all round				all round		
	(selfed)				(selfed)		
F2:	2737 round		923 wrinkled		2750 round		920 wrink
	F2 Ratio	2.99:1				3: 1	

Prevailing notion of heredity at the time was that the traits of parents **blended** together to produce a hybrid with an intermediate phenotype. But blending of characteristics was not observed in Mendel's experiments—he saw **particulate** inheritance.

Based on his experiments, Gregor Mendel concluded that certain "factors" (which we now call genes) are *passed from parents to their offspring (Darwin's first postulate)*. According to Mendel, each organism has a pair of these factors that control the development of a specific trait. When two organisms produce offspring, each parent gives the offspring one of the factors from each pair. In the offspring, the two genes - one from each parent - act together.

2.2 Mendel's Second Postulate: Dominance/Recessiveness:

When two unlike for a single character are present in a single individual, one unit factor is dominant to the other, which is said to be recessive.

In the above monohybrid cross, a recessive factor is covered up by a dominant factor in the F1 of a cross, but it reappears in the F2 in a predictable proportion (1/4). The F1 plants all look like one of the parents (round), but retain the potential to produce wrinkle-seeded offspring.

Thus, F1 plants are no longer true-breeding: they are **hybrids**. Mendel invented terms **dominant** and **recessive** to describe this phenomenon. **The trait that is masked in the F1 is recessive.** The other is dominant. According to the principle of dominance, one gene in each pair may be "stronger" and prevent the other from being seen. The recessive gene can, however, reappear in the F2 generation.

2.3 Mendel's Third Postulate: Equal Segregation

During the formation of gametes, the paired unit factors segregate randomly so that each gamete receives one or the other with equal likelihood.

Demonstrate using a Punnet Square:

Using modern notation (capital letters denote dominant allele, lower-case letters denote recessive allele, and the letter is often derived from the abnormal (mutant) form, rather than from the normal (wild-type) form:

		F1 Female Eggs		Equal Segregation of genes into gametes. Random union of gametes. All these combinations <u>equally likely</u> .
		1/2 W	1/2 w	
F1 Male Sperm	1/2 W	1/4 WW	1/4 Ww	
	1/2 w	1/4 Ww	1/4 ww	
1 WW : 2 Ww : 1 ww 3 with dominant phenotype : 1 recessive				

2.4 Putting it all together

Parental phenotype	round		wrinkled	
Parental genotype	WW	x	ww	diploid--two copies of gene
Gametes	W		w	haploid--single copy of gene
F1			Ww	diploid--W dominant to w
F1 gametes			___ W and ___ w	
F2 (from selfed F1)			___ WW: ___ Ww: ___ ww	

2.5 Test Cross:

Since the **WW** and **Ww** individuals all have the same phenotype, how would you determine if you actually had the above genotypic ratio?

(That is, If an individual has the recessive phenotype (**wrinkled**), we know what it's genotype is (**ww**). But if it has the dominant phenotype (**round**), we don't know what the genotype is. Individuals with a dominant phenotype can be either **homozygous** (WW) or **heterozygous** (Ww) in genotype.

To determine genotype, can cross an individual with the dominant phenotype to a plant with the recessive phenotype (and recessive genotype).

	WW	x	ww		Ww	x	ww
		⊥				⊥	
Genotype			Ww		1/2 Ww		1/2 ww
Phenotype			all round		1/2 round		1/2 wrink.

2.6 The Dihybrid Cross and Mendel's Fourth Postulate: Independent Segregation

During gamete formation, the segregation of one gene pair is independent of other gene pairs

What if plants differ in 2 traits? Mendel used the dihybrid cross in which 2 traits are examined simultaneously: seed color (G,g) and seed shape (W,w)

P:	yellow, round		green, wrinkled
	GG WW	x	gg ww
gametes:	G W		g w
F1:			Gg Ww yellow, round

Q: What kinds of gametes are produced by F1 individuals, and in what proportions?

A: 1/4 G W 1/4 G w 1/4 g W 1/4 g w

If a gamete gets the the allele *G*, rather than *g*, **this has no influence on whether it receives *W*, rather than *w*, as long as the loci bearing these genes are unlinked.** This is because in peas, *G/g* and *W/w* are on separate pairs of homologous chromosomes. If two traits are controlled by genes that are on the same pair of homologous chromosomes, they might be linked. We will discuss linkage in detail later in the semester.

Example: Punnet Square Method:

		G W	Female G w	gametes g W	g w	16 possible combinations
Male Gametes	G W	GG WW	GG Ww	Gg WW	Gg Ww	
	G w	GG Ww	GG ww	Gg Ww	Gg ww	
	g W	Gg WW	Gg Ww	gg WW	gg Ww	
	g w	Gg Ww	Gg ww	gg Ww	gg ww	

Collect together the offspring with the same phenotype:

	Genotype	Generalized Genotype	Phenotype
1/16	GG WW	9/16 G- W-	yellow, round
2/16	GG Ww		
2/16	Gg WW		
4/16	Gg Ww		
1/16	GG ww	3/16 G- ww	yellow, wrinkled
2/16	Gg ww		
1/16	gg WW	3/16 gg W-	green, round
2/16	gg Ww		
1/16	gg ww	1/16 gg ww	green, wrinkled

9:3:3:1 ratio

Example: Another method of solving a dihybrid problem: _

Branching Diagram Approach. Much faster and easier once you are used to it! Can use it to enumerate all possible outcomes of a cross, or to quickly calculate just the fractions that interest you. In the first column under “F2”, write the F2 fractions (probabilities) for the G locus. In the second column, write the F2 fractions for the W locus. The expected fraction of each F2 two-locus genotype (in column 3 below) is simply calculated by multiplying the fraction in column 1 by the fraction in column 2. The fourth column below show the phenotype associated with each F2 genotype, and the fifth column shows the phenotypic ratio

P	GG WW	x	gg ww	
F1	Gg Ww	x	Gg Ww	
F2				
Fractions for g locus	Fractions for w locus	Two-locus genotypes	Phenotypes	Phenotype ratios
1/4 GG	1/4 WW	1/16 GG WW	yellow, round	9/16 yel. rd
	1/2 Ww	2/16 GG Ww	yellow, round	
	1/4 ww	1/16 GG ww	yellow, wrinkled	
1/2 Gg	1/4 WW	2/16 Gg WW	yellow, round	3/16 yel wr
	1/2 Ww	4/16 Gg Ww	yellow, round	
	1/4 ww	2/16 Gg ww	yellow, wrinkled	
1/4 gg	1/4 WW	1/16 gg WW	green, round	3/16 gr rd
	1/2 Ww	2/16 gg Ww	green, round	
	1/4 ww	1/16 gg ww	green, wrinkled	

2.7 *Two Character Test Cross for an individual having the dominant phenotype for two different traits.*

P: Gg Ww x gg ww

		Male gametes		Phenotype
		G/ W/	g/ w/	
Female gametes	G/ W/	Gg Ww	1/4 yellow, round	
	g/ W/	gg Ww	1/4 green, round	
	G/ w/	Gg ww	1/4 yellow, wrinkled	
	g/ w/	gg ww	1/4 green, wrinkled	

Reminder: In a **test cross** the relative frequencies of the different gametes produced by the heterozygous parent can be observed directly in the progeny, because the recessive parent contributes only recessive alleles.

In a **backcross**, hybrid individuals are crossed with one of the parental genotypes. So the above cross is both a testcross and a backcross.

3 SOME SIMPLE RULES OF PROBABILITY

Random events are extremely important in Mendelian genetics. The union of gametes in fertilization is random with respect to the genotype of the gamete, and the proportions of offspring of different types in a genetic cross are the cumulative result of many random events. Understanding some simple laws of probability will allow you to predict the types and frequencies of progeny from complex crosses. It will also allow you to conduct tests of genetic hypotheses.

Probability is defined as the proportion of times a particular event is expected to occur in numerous repeated trials. Ranges from 0 to 1. For example, if one tosses a coin 10 times and gets 6 heads and 4 tails:

Expected probability if coin is fair : 0.5H, 0.5T

Observed proportion is 0.6H, 0.4T

A genetic example:

F2 of a monohybrid cross between round and wrinkled seeds: Mendel observed 5474 round seeds, and 1850 wrinkled seeds:

Q: What was the observed proportion of wrinkled seeds:

A: $1850/[1850+5474]=0.253$.

Q: What was the expected probability of wrinkled seeds in this case?

A:

3.1 Addition (sum) rule

Heads and Tails are **mutually exclusive** events. The combined probability of two or more mutually exclusive events occurring is the **sum** of their individual probabilities. Combined probabilities are usually denoted by using an “OR”

$$P[\text{head or tail}] = P[\text{head}] + P[\text{tail}] = 1/2 + 1/2 = 1.$$

Q: What is probability of rolling a six-sided die and getting an even number (a 2 OR a 4 OR a 6)?

$$A: P[2] + P[4] + P[6] = 1/6 + 1/6 + 1/6$$

3.2 Product rule

The joint probability of **both of two independent events** occurring is the **product** of their individual probabilities. Joint probabilities are usually denoted by using an “AND”

Q: If you flip two coins (coins are independent), what is the probability of getting a head on the first AND a head on the second?

$$A: P[\text{head on first and head on second}] = P[\text{head}] * P[\text{head}] = 0.5 * 0.5 = 0.25$$

Q: If a couple want to have two children, what is the probability that the first will be a girl, and the second will be a boy?

$$A: P[\text{girl on first and boy on second}] = P[\text{girl}] * P[\text{boy}] = 0.5 * 0.5 = 0.25$$

Q: If a couple want to have two children, what is the probability that they will have one girl and one boy?

A: This can happen two different ways: they can have a girl, then a boy, as above, or they can have a boy, then a girl. So

$$P[\text{girl then boy or boy then girl}] = P[\text{girl then boy}] + P[\text{boy then girl}] = 0.25 + 0.25 = 0.5.$$

3.3 Conditional probability (probability given some constraint or condition)

Q: You roll a 6-sided die: given that an even number is rolled, what is the probability of it being a 2?

A: $P[2]/P[\text{even}] = P[2 \mid \text{even number}] = 1/6 / 1/2 = 1/3$

4 SIMPLE DEVIATIONS FROM MENDELISM

4.1 Incomplete Dominance (partial dominance):

Cross red-flowered 4-o'clocks (snapdragons) with white-flowered ones:

P:	Red	x	White
F1:	Pink		
F2 ratio:	1 Red	2 Pink	1 White
	R₁R₁	R₁R₂	R₂R₂

All genotypes have different phenotypes with the heterozygote intermediate.

Traits that appear to be determined by systems of **complete dominance at the gross phenotypic level** may be cases of **incomplete dominance at the biochemical level**.

WW homozygote and **Ww** heterozygotes for seed shape in garden peas both yield plants with round seeds. However, these two genotypes produce **different** types of **starch grains**, with the starch in **Ww** intermediate in structure between those of **WW** and **ww**

Must be specific about the level of trait we are describing (gross phenotypic or biochemical for example.

4.2 CODOMINANCE:

Sometimes, traits associated with both alleles are observable in a heterozygote. Heterozygotes for red blood cell antigens (genetically determined chemicals on the membranes of red blood cells) often express properties of both alleles.

2 alleles: **L^M** and **L^N** and three genotypes, all recognizable in blood tests:

Genotype	L^ML^M	L^ML^N	L^NL^N
Phenotype	MM	MN	NN

The snapdragons that exhibit incomplete dominance on a gross phenotypic level actually exhibit codominance on a subcellular level--**Pink flowers of heterozygotes have red and white plastids present in cells.**

4.3 An example of dominance, codominance, and multiple alleles

ABO blood groups, like MN blood groups are determined by antigens on the surface of red blood cells:

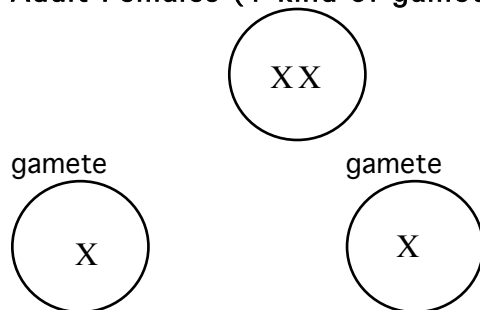
	A	B	AB	O
Phenotype				
Genotypes	A A A O	B B B O	A B	O O
Red cell Antigens	A	B	A&B	neither
Serum antibody	anti-B	anti-A	neither	anti-A anti-B

4.4 Sex-Linked Inheritance

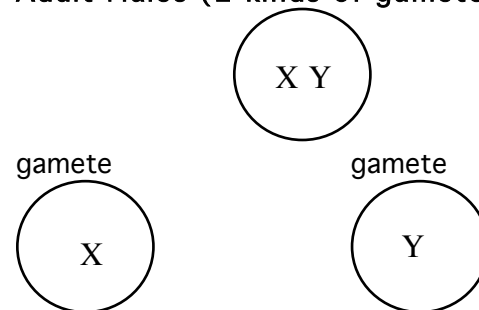
Many human traits are determined by X-linked **recessive alleles**: Color blindness, Hemophilia, Muscular Dystrophy (1 form)

Segregation is not the same as for autosomal genes. **In females**, segregation for X-linked genes is the same as for autosomal genes.

Adult Females (1 kind of gamete)



Adult Males (2 kinds of gametes)



X-Linked Recessive: Color blindness:

Let **C** be wild-type allele, **c** be mutant allele that causes color blindness. Behaves exactly like a simple Mendelian dominant/recessive system.

Females can be	$X^C X^C$ normal	$X^C X^c$ normal	$X^c X^c$ color blind
Y chromosome does not carry this gene, so			
Males are:	$X^C Y$ normal	$X^c Y$ color blind	

Punnet square method: $X^C X^c$ female and $X^c Y$ male. (All combinations equally likely)

		Eggs		Equal Segregation and random union of gametes
		$1/2 X^C$	$1/2 X^c$	
Sperm	$1/2 X^C$	$1/4 X^C Y$	$1/4 X^C X^c$	
	$1/2 Y$	$1/4 X^c Y$	$1/4 X^c X^c$	

Males inherit the Y chromosome from their fathers. Females inherit an X chromosome from their fathers.

All females have normal sight, and half the males are color blind. Half of female progeny are heterozygous carriers and can have color blind sons.

When the allele is RARE, females homozygous for the trait will be very rare, but males that carry the trait will be much more common

5 PEDIGREE ANALYSIS

The analysis of segregation by generating controlled crosses and counting large numbers of progeny is not possible in human beings. But the mode of inheritance of a trait can sometimes be determined by examining **pedigrees**.

A family tree that shows the phenotype of each individual. Important application of probability in genetics is its use in pedigree analysis.

Pedigree diagrams

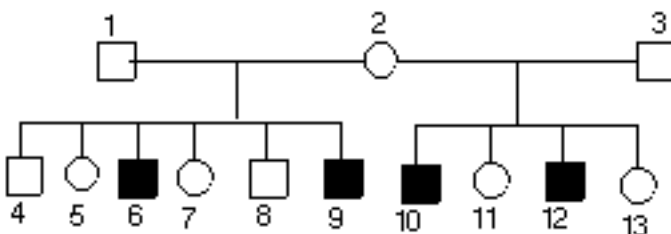
	Unaffected individuals (wild-type phenotype)	Affected individuals (mutant phenotype)	Partially affected individuals (rarely used)
MALE:			
FEMALE:			

Symbols:

circle: female
square: male
diamond: sex unspecified
solid color: affected individual
horizontal line connecting male and female: a mating
two horizontal lines: mating between relatives
vertical line: connects parents and offspring symbol with slash: deceased individuals

Extended example of a pedigree problems

Because of the small number of progeny, pedigrees can often be ambiguous – more than one model may explain the observed pattern of inheritance. For this reason, we often assume that the genetic abnormality is rare in the population. This means that, in a given family, very few (usually one) of the first generation individuals carry the abnormality and very few (usually none) individuals who "marry" into the family carry the abnormality. This assumption may help to rule out certain models.



Here is a sample human pedigree and one way to analyze it. Use A or a to

represent the abnormality if it is dominant or recessive, respectively.

Parents connected by horizontal lines. Vertical lines lead to their offspring.
Try the possible models one by one.

1. If the abnormality were due to a dominant mutation in a gene on an autosome (autosomal dominant):

A - dominant abnormal allele

a - recessive normal allele

Then either 2, or both 1 and 3 would have to show the abnormality for it to be present in the children. This also does not fit the data.

2. If the abnormality were due to an autosomal recessive mutation:

a - recessive abnormal allele

A - dominant normal allele

In order for the second generation to have affected individuals (aa), both parents of each family must be carriers.

That is: 1, 2, and 3 must be Aa.

You would predict that their children would have a 25% chance of being affected (aa). The observed frequencies are 33% and 50% in the two families which is not statistically significant for this small sample size. Therefore, this model fits the data. However, this model assumes that three non-blood relatives all carry the abnormal gene (1, 2, and 3). If we assume that the abnormality is rare, then the chance that three randomly selected individuals will have the abnormality is very small. Therefore, this model is a possible explanation of the data, but it is not the most likely. You would have to look at more children in this family to determine if the chance meeting you have proposed actually took place. At this point, it is reasonable to try other possible models to see if they fit the data better.

3. If it is sex-linked (X-linked) dominant, 2 would have to be abnormal or both 1 and 3 would have to be abnormal to pass the abnormality to their children. This does not fit the data. (We'll discuss sex-linkage later in the semester.)

4. If it is sex-linked recessive:

1 and 3 must be X^AY in order to be normal

2 must be X^AX^a in order that the abnormality run in the family, someone has to carry it.

We would predict that the resulting daughters would be:

50% X^AX^A unaffected

50% X^AX^a unaffected carriers

No affected daughters are observed, but the sample size is too small to be significant. (Note that statistical arguments are of limited value in pedigree analysis because of the small sample sizes involved.)

We would predict that the resulting sons would be:

50% XY unaffected

50% XY affected

Both affected and unaffected sons are observed. Therefore, the data fit this model as well. This is also a more likely and reasonable model than autosomal recessive since it requires only one individual (2) to have the genetic abnormality. You could distinguish between sex-linked recessive and autosomal recessive by looking at more children in this family to see if any

affected daughters of 1 and 2 or 2 and 3 appear. If so, the abnormality must be autosomal recessive. (Why?)

Hints for solving pedigree problems:

Recessive autosomal traits: Affected individuals have both parents that are unaffected. That is, affected individuals are produced from mating between 2 **carriers**. When recessive allele is **rare**, the relatives of a carrier are more likely to also be carriers than are random individuals selected from the population. Therefore matings between relatives may increase the frequency of homozygosity for deleterious recessive alleles. Another way to say this: When a recessive allele is rare, it is more likely to become homozygous through inheritance from a common ancestor than from parents who are completely unrelated.

Dominant autosomal trait: Does the trait appear in all the offspring of an affected individual (both sons and daughters). This pattern suggests autosomal dominance.

X-linked recessive traits: Does the trait appear exclusively in males, but not in all generations? If so, this suggests an X-linked dominant trait. In small pedigrees, may not be possible to distinguish X-linked from autosomal recessive traits since affected individuals could all be males due to chance.

X-linked dominant: Does a trait appear in a single male and in all his daughters, but none of his sons? This is the pattern expected from an X-linked dominant.

Y-linked trait: Does the trait appear exclusively in males, and do all the male offspring of an affected male also have the trait. This is the pattern expected with an X-linked dominant.