Biol 213 Genetics: Wed/Fri, Oct 11 and 13, 2000
Extensions to Mendelian Genetics

The exam. I'm trying, really. It's just that things like notes and problem sets, things that you need RIGHT NOW (and similar things that others need right now), take precedence over grading exams. Anyway, if you want to stick a pin in someone, stick it in me. Brad finished his questions over the weekend.

But let's suppose it comes back on Wednesday (which is certainly my intention). You may be pleased with the results of the exam. Or you may just be relieved that you survived. Or perhaps you didn't survive. That was then. This is now. For those who are satisfied, be aware of the Wily Coyote phenomenon. You may recall Coyote somehow gets himself chained to a bomb with a fuse set to go off in seconds and then goes through superhuman (supercoyote?) efforts to get to a lake miles away. He reaches it at the last moment and jumps in to douse the fuse. Then he climbs out of the lake, soaked but relieved, perhaps even pleased with himself, unaware that the fuse has spontaneously reignited. KABOOM!

It's often the SECOND time out that you run into trouble. This is understandable. The first time, you're extra careful, looking out for every possible pitfall. The second time, however, you know you can do it, your guard drops,... and there you are in the pit. Don't let it happen. Don't let knowing you've done it prevent you from doing it again.

If you did not survive… well, you actually did. You're still here. Take a look at the syllabus. One crummy exam doesn't amount to all that much in the scheme of things. If a rotten score inspires you to look deeply at how you're approaching the course, it may be well worth it. Come in and let's talk.

Outline

(Note that it covers two days. We'll probably get mostly through I.F. by the end of Wednesday)

I. EXTENSIONS TO MENDELIAN GENETICS (Part I)
- A. Overview (pp.76-78)
- B. Sex-linkage and sex-limitation (pp.66-70, 88-90)
- C. Dominance (pp.77-80, 82-83)
- D. Penetrance and expressivity (pp.87-88)
- E. Multiple alleles (pp.81-83)
- F. Interactions between two genes affecting the same trait (pp.90-94)
- G. Interactions between multiple genes affecting the same trait

Table 1: Mendel's Interpretations Disemboweled

Mendel's Interpretation	Our Disinterpretation
Traits do not blend but are determined by unchangeable units	Can't there be traits that are only partially determined by genes? Predisposition to disease, for example. Forget "unchangeable:" genes mutate.
Each trait is determined by two units	OK if one gene is enough for every trait. But what about complex pathways?
The two units may or may not be identical	If an allele is just a variant base sequence of a gene, there should be a huge number of possible alleles.
One character form is recessive to or dominant over another	If dominance is (most often) the presence of an active enzyme vs its absence, who says that 50% of the protein is as good as 100%?
The two character forms carried by a heterozygote are passed to progeny with equal likelihood	What if an allele makes a defective protein that is sickening?
Different traits assort independently	Independent assortment of traits is based on independent assortment of chromosomes. One would think that it would work only if there's one trait per chromosome.

I. EXTENSIONS TO MENDELIAN GENETICS

A. Overview

So far, Mendel has had everything his own way. Yet, if you look at his conclusions, they look awfully suspicious (Table 1). The more we look at the molecular underpinnings of Mendelian genetics, the more we see that the assumptions are OK, sort of, but there's a great deal of room for exceptions to the rules. Let's look at some of these exceptions.

I.A. Sex-linkage and sex-limitation (pp.65-70, 88-90)

Let's start with traits that don't assort independently. That's a strange way of describing sex-linkage, but isn't it the truth? If you consider gender to be a trait, then X-linked traits are those that do not assort independently of gender. Follow Morgan's experiment described on p.67. The symbols used are somewhat confusing, since we've

generally used letters to represent genes or alleles. Here, **X** and **Y** represent chromosomes, with the *w* or w^+ alleles sitting on the former.[1]

SQ1. Use the genotypes of the parents (female X^{w+} X^{w+} and male X^{w+} Y) to construct Punnett squares to predict the genotypes and phenotypes of the F_1 progeny. From Morgan's actual results, which is the dominant phenotype?

SQ2. Use the genotypes of the F_1 generation you just found to construct Punnett squares to predict the genotypes and phenotypes of the F_2 progeny.

SQ3. Redo the crosses under the presumption that *w* is NOT X-linked but rather sits on some autosomal chromosome (call it A). Which is the first cross that gives different results depending on whether *w* is or is not X-linked?

Just because the trait is **X**-linked, that doesn't mean anything remarkable happens in the F_1 generation. By the way, take a look at the actual data at the top of page 68.

SQ4. What ratio of phenotypes did you expect from your Punnett square (presuming that the trait is X-linked)? How can you explain the discrepancy?

Now redo the same crosses, but this time starting with the female fly white-eyed and the male red-eyed.

SQ5. Now which is the first cross that gives different results depending on whether *w* is or is not X-linked?

It's sometimes possible to tell even with the F_1 progeny whether the trait is **X**-linked. Just think – you may already have the means to discern **X**-linked traits in your own flies!

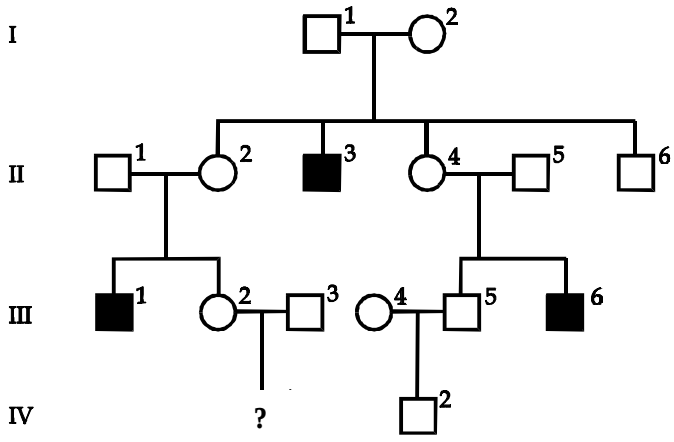
Now let's see how it works in humans.

SQ6. Consider the pedigree to the right of a family with a history of Duchenne muscular dystrophy, a rare condition that causes wasting away and eventual death during childhood. Individual III.2 comes to you worried that her newborn son may someday be afflicted with the disease. Is the trait dominant or recessive?

Both dominant and recessive raises difficulty. If the trait is dominant, then

Fig.1

Family with Duchenne Muscular Dystrophy



[1]The typesetter would have helped us see this more easily if he had given the phenotype of the wild-type female fly as $\mathbf{X}^{w+}$ $\mathbf{X}^{w+}$ rather than $\mathbf{X}^{w+}$ $\mathbf{X}^{w+}$.

why didn't one of the greatgrandparents have the disease? If the trait is recessive, then its appearance in the third generation would indicate that II_1 and II_6 were carriers. It's hard to swallow that two outsiders carry the trait when we're told that the trait is rare. See how things clear up when you consider **X**-linkage.

SQ7. Deduce the manner in which the disease is inherited and write the genotypes of all individuals in the pedigree, to the extent possible.

SQ8. Calculate the probability that IV_1 will get muscular dystrophy.

There is another explanation for the pedigree, however. Suppose that Duchenne muscular dystrophy is just a male thing, for example requiring male hormone for expression. Sex-limited conditions are by no means rare. A predisposition to prostate cancer, for example, might well be an autosomal trait limited in expression to males.

SQ9. Is the pedigree consistent with the hypothesis that the disease is an autosomal dominant trait limited to males?

Sex-linkage and sex-limitation can be distinguished from one another in two ways. First, males affected by sex-linked conditions ($X^- Y$) always pass the trait on to their daughters. They are always carriers. But males affected by autosomal sex-limited conditions (e.g., $C^D C^+$) pass the trait on to their daughters only 50% of the time. Second, rare affected females should arise if the trait is sex-linked traits ($X^D X^D$), and they will pass on the trait to all of their progeny.

I.C. Dominance (pp.77-80, 82-83)

Dominance is perhaps the easiest target because it is a purely human invention. The text describes the case of "incompletely dominant" petal color where the progeny of pure-breeding red plants and pure-breeding white plants have pink petals. It also talks about "codominant" blood groups, where the heterozygote has the immunological properties of both its L^M parent and its L^N parent. How can we understand these terms?

First of all, I advise you not to worry about memorize the terms (except perhaps the night before MCATs). Myself, I just look them up whenever I need them. It's more important to understand the basis behind the phenomenon they describe. Let's examine various kinds of dominance through our old friend, the genetic disease phenylketonuria (PKU). PKU results when the enzyme phenylalanine hydroxylase is not present (see Fig. 12-2, p.316 in the text), leading to a buildup of phenylalanine and the consequent overflow of breakdown products into the blood. These breakdown products interfere with normal neural development and produce mental retardation.

SQ10. Consider two alleles of the gene encoding phenylalanine hydroxylase: P^+, encoding the wild-type enzyme and P^0, encoding a truncated and completely defective enzyme. Which allele do you think is dominant?

OK,... a trick question, sorry. Strictly speaking, alleles aren't dominant, it's the phenotypes they determine that are dominant or recessive (or neither). We often speak

of a dominant or recessive allele, but only when we have a specific phenotype in mind. This must be the case, because a genotype confers many phenotypes, and an allele may be associated in our minds sometimes with a dominant trait and sometimes with a recessive trait (Table 2).

Table 2: "Dominance" depends on the phenotype considered

Phenotype	Genotype			Relation
	P^+P^+	P^+P^0	P^0P^0	
mental retardation	no	no	yes	P^+ phenotype dominant over P^0
Phenylalanine in urine (challenged)[1]	no	yes	yes	P^0 phenotype dominant over P^+
Phenylalanine hydroxylase activity	100%	50%	0%	Partial dominance
presence of enzyme[2]	wild-type enzyme	wild-type enzyme + mutant enzyme	mutant enzyme	Codominance

[1]Presence or absence of phenylalanine determined after person fed high load of phenylalanine
[2]Enzyme type determined by size on a gel

If we look to the disease state, then the wild-type phenotype is dominant, because even half the amount of enzyme is enough to save the person from mental retardation. However, a common test for PKU, measuring phenylalanine in the urine, tells us that the mutant allele is associated with dominance, because half the amount of enzyme is not enough to metabolize the large amount of phenylalanine given in the test. Other tests, e.g. for enzyme activity, give still different answers regarding dominance.

The reality is that heterozygotes make a different level or perhaps different type of protein than homozygotes. The protein encoded by the two alleles may contribute to a wide variety of different phenotypes, some of which may be very similar to the phenotypes of one of the homozygotes. In such cases, we will call that phenotype dominant.

SQ11. There are a great many cases in which the phenotype of a heterozygote encoding half the normal amount of active enzyme is the same as the homozygous wild-type encoding the full amount of enzyme and differs from the homozygous mutant. In other words, there are many cases in which the wild-type phenotype is dominant over the mutant phenotype. What does this fact imply about the amount of enzyme that is normally made relative to the amount that is generally needed?

Examination of the molecular mechanism underlying a trait will often make clear why it is dominant, recessive, or neither relative to a variant trait. For example, consider an inherited bleeding disorder called PI Pittsburgh that is caused by a mutation in a protein that normally inhibits certain proteases, enzymes that chew up protein (PI:

protease inhibitor). The mutation in PI Pittsburgh alters the protease inhibitor so that it gains a new, inappropriate function, blocking the coagulation protein thrombin.

SQ12. Given the molecular basis of PI Pittsburgh, would you expect its phenotype to be dominant or recessive with respect to wild-type?

I.D. Penetrance and Expressivity (pp.87-88)

Even when you're sure of your ground regarding dominance, there may be additional complexities. Consider the PKU phenotype we're most often concerned with -- mental retardation -- which acts as a recessive trait. Fortunately, the disease is preventable if detected at birth. A carefully controlled diet that has a minimal amount of phenylalanine is sufficient to prevent a buildup of toxic metabolites in the blood, and the baby will develop normally. It is possible, then, for a homozygous phenylketonuric to grow to maturity and marry another homozygous phenylketonuric who has benefited from the same controlled diet. What will be the phenotype of their children? Mendelian genetics suggests that their children, progeny of an *aa* x *aa* cross should all have the disease. Given the same care their parents received, however, they will be phenotypically normal.

This is a good example of how the environment may interact with the genotype to produce a phenotype that is not in accord with Mendelian genetics. You might object that the parents and their children still have PKU, it simply isn't expressed, and we can consider the treatment as layered onto the disease state. Unfortunately, that sort of verbal subterfuge doesn't get us out of the woods. Consider the time before the basis of PKU was known. Children were born who lacked phenylalanine hydroxylase enzyme activity, but some, just by luck (or more likely by poverty) were given a diet deficient in phenylalanine and thrived. Others with the same genetic endowment consumed phenylalanine and suffered mental retardation. The outside observer would have no way of knowing the different environmental conditions of the different children and might conclude simply that the disease had variable penetrance. Or, some children, because of their diet, might have milder than usual symptoms of the disease. The same observer might conclude then that the disease had variable expressivity. These are the terms we often use when we don't know what is going on.

True, we can unravel some of the complexities of PKU, but there are a large number of inherited diseases that remain a mystery. The bewildering pattern of phenotypes associated with these diseases are often a result interactions with the environment in ways we cannot fathom.

SQ13. A fraction of the U.S. population is genetically predisposed to atherosclerosis, one of the leading causes of death in our society. The allele responsible for this predisposition is also present in third world cultures where atherosclerosis is virtually unknown. Explain.

Nor is environment the only joker in the deck. The penetrance and expressivity of a trait may be greatly influenced by the action of other genes, a notion we'll return to shortly.

II.E. Multiple alleles (pp.81-83)

Mendel worked with only two alleles per gene. However, considering that an allele is simply a specific base sequence of a gene, any one of which may be mutated, it should come as no surprise that genes may have a large number of alleles. Since many mutations are silent or conservative, many alleles produce phenotypes no different from the wild-type allele. Confining our attention for the moment to alleles that are observed clinically, most have the same effect: to reduce the activity of the encoded protein. There are cases, however, where different mutations may lead to mutant proteins with very different activities. For example, the ABO blood type of an individual is determined by an enzyme that modifies the surface of the red blood cell. The alleles I^A and I^B encode slightly different versions of the same enzyme that place different sugars on a membrane protein, leading (when homozygous) to type A or B blood. The *i* allele, on the other hand, produces a protein that lacks enzyme activity altogether and places no sugar on the protein, leading (when homozygous) to type O.

SQ14. Draw a picture that can help you see the relationships between the *I* gene, the I^A and I^B alleles, and the protein they encode.

How do we know that I^A and I^B are two alleles of the same gene rather than belonging to different genes? Multiple alleles can be distinguished from multiple genes by the ratio of phenotypes seen in the progeny of crosses. If a trait is determined by multiple alleles of the same gene, then a given cross can yield no more than four phenotypes, representing the four possible combinations of 2 alleles per parent. There may be fewer phenotypes, of course, some variation on 1:1:1:1 (e.g. 2:2 = 1:1 or 3:1). For example, an *ab* x *cc* cross might yield phenotypes in the ratio of 1:1 (=2:2) if the phenotypes determined by *a* is dominant over that determined by *c*. Or an *ab* x *bc* cross might yield phenotypes in the ratio of 3:1 if the phenotype determined by *b* is dominant over the others. We'll see below that traits determined by multiple genes behave quite differently.

SQ15. Use the four alleles, *C*, c^{ch}, and c^h, and *c* and the dominance relationships described on p.81 for rabbit coat color to make up as complicated a cross as you can imagine. What do you predict to be the ratio of the phenotypes?

II.F. Several genes affecting the same trait (pp.90-94)

Are all traits controlled by a single gene as implied by Mendel's simplification? We already know that's not true. Recall the multistep pathways leading to the ultimate products. Mutation of any one of those steps would block the pathway. For example, the synthesis of the amino acid tryptophan proceeds in several steps, starting with chorismic acid:

A B C D E

chorismic acid ====> ====> ====> ====> ====> tryptophan

SQ16. Beadle and Tatum isolated many mutants of the fungus *Neurospora*. Suppose they found two independent mutants, M and N, that required tryptophan for growth. The two mutants are crossed, and the F_1 progeny are all wild-type. A cross between two F_1 progeny give F_2 progeny in the ratio of wild-type:mutant = 9:7. What can you conclude?

For one thing, you can conclude that these are not two alleles of the same gene, since the ratio cannot be derived from 1:1:1:1. Furthermore, it is no normal Mendelian dihybrid cross, where you would of course expect phenotypes in the ratio of 9:3:3:1. But let's stop with the numerology and figure out what phenotypes you WOULD you expect? Suppose the M and N mutants have different defective proteins in the pathway leading to tryptophan, so neither can grow without the amino acid. . . but if they have the same phenotype, how can we distinguish them? In this experiment, we can't, so let's combine the phenotypes:

Table 3: Interaction between two genes encoding enzymes in the same pathway
Cross: *MmNn* x *MmNn*

	MN	***Mn***	***mN***	***mn***
MN	*MMNN*	*MMNn*	*MmNN*	*MmNn*
Mn	*MMNn*	*MMnn*	*MmNn*	*Mmnn*
mN	*MmNN*	*MmNn*	*mmNN*	*mmNn*
mn	*MmNn*	*Mmnn*	*mmNn*	*mmnn*

Phenotypic ratio: Trp^+ : Trp^- = *M-N-* : (*mmN-* or *M-nn* or *mmnn*) = 9 : 7

And there's the 9:7 ratio.

Or recall another old favorite: eye color in *Drosophila* (Fig. 2).

Pathway to eye color in *Drosophila*

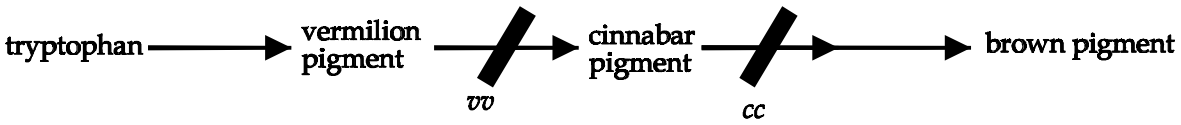


Fig. 2: Interaction of multiple genes in the pathway leading to eye color

SQ17. You cross a vermilion-eyed fly with a cinnibar-eyed fly to obtain F_1 progeny, then you cross these progeny to get F_2 progeny. What phenotypic ratio do you expect?

Once more a dihybrid cross with a twist. What phenotype would you expect of each of the four types: *V-C-*, *V-cc*, *vvC-*, and *vvcc*? Again, a Punnett square might help.

Table 4: Interaction between two genes encoding enzymes in the same pathway
Cross: *VVCc* x *VvCc*

	VC	***Vc***	***vC***	***vc***
VC	*VVCC*	*VVCc*	*VvCC*	*VvCc*
Vc	*VVCc*	*VVcc*	*VvCc*	*Vvcc*
vC	*VvCC*	*VvCc*	*vvCC*	*vvCc*
vc	*VvCc*	*Vvcc*	*vvCc*	*vvcc*

Phenotypic ratio: brown : vermilion : cinnibar = *C-V-* : *--vv* : *ccV-* = 9 : 4 : 3

SQ18. Why does vermilion appear at a higher frequency than cinnibar?

These modified phenotypic ratios were clearly taken from the basic 9:3:3:1 ratio, combining the appropriate elements. Here's another:

SQ19. Draw a pathway where mutations in two enzymes might lead to a 15:1 phenotypic ratio?

In SQ17, you knew the pathway underlying the observed phenotypes and deduced the phenotypic ratios implied by the pathway. More often the situation is reversed. You are given the phenotypic ratios and have to deduce the gene interaction that causes it. Here is an example.

SQ20. You, a genetically inclined farmer, are poking around a farmers' market, scoping out the competition, when you run across a display of strange corn. The ears of corn have some kernels that are the usual yellow color, but some look bright golden and others sickly green. You figure if you can breed a strain that is pure gold, you'll corner the market. Accordingly, you buy a few ears, and when you get home, you count the kernels to get a handle on the genotypes of the kernels you have and the ideal strain you'd like to develop. From the count below, suggest what are the genotypes of the different colored kernels.

Yellow kernels	**247**
Golden kernels	**154**
Sickly green kernels	**23**

SQ21. Use a c^2 analysis to assess the reasonableness of your hypothesis.

G. Interactions between multiple genes affecting the same trait

Mendel chose characters that had two discrete states: wrinkled vs round, green vs yellow, etc. Choosing such traits is one major reason he obtained comprehensible results. Contemporaries, who generally subscribed to theories of inheritance based on the blending of parental traits, would probably have chosen characters that can be described on a continuous scale, and they would have missed Mendel's discovery of the particulate nature of inheritance.

Many important traits show a continuum of states. Animal examples include susceptibility to hypertension, obesity, and certain drugs. Traits that differ by small degrees are called quantitative traits. Mendel was right to ignore them. They too can be explained by the action of genes following the same rules Mendel elucidated, but since many genes work together, the situation is much more complicated than wrinkled seed cases.

The length of corncobs, varying nearly continuously from small to large, is an example of a quantitative trait. Figure 3 shows the results between a cross of two strains of corn, one small, the other large. In the case of both parents, there is a distribution of heights, but the distributions of the two do not overlap. The F_1 generation has a distribution different from either parent, intermediate in height. It looks for all the world like blending. If that were so, however, the F_2 generation should be even more homogeneous, if anything, giving a tighter distribution. This is not the case. The F_2 distribution of heights centers around the same median length as the F_1 distribution, but the former is more spread out. So while a simple Mendelian treatment can't account for the length of corn cobs, blending doesn't work either.

SQ22. Suppose that corn length were a Mendelian trait governed by two alleles, one conferring shortness and the other conferring length. What result would you have expected from the experiment shown above?

SQ23 Suppose that the trait has variable expressivity (the degree of expression of the trait varies from one individual to the next). Would that permit the above results to be explained by the action of a single Mendelian gene?

How can we reconcile the results shown in Figure 3 with the action of pairs of alleles? We can't, if there is only one gene. But it is possible if we consider the trait to be determined by more than one gene. Let's consider the relatively simple case in which the action of just two identical genes, $Height_1$ and $Height_2$, each with two alleles, *H* and *h*, can give complex length distributions.

Figure 4 shows the usual Punnett square with a dihybrid cross. The wrinkle this time is that the two genes combine equally to determine the same trait. Suppose that *H* encodes some protein important in growth, never mind whether the allele is at the $Height_1$ locus or the $Height_2$ locus. Then we can distinguish the possible progeny by the number of wild-type alleles they possess. The F_2 cross gives the usual 16 possible combinations of gametes, but grouping the combinations by number of *H* gives a ratio of length phenotypes as 1 : 4 : 6 : 4 : 1. These are binomial coefficients, the coefficients of the expansion:

$$(a + b)^4 = \mathbf{1}a^4 + \mathbf{4}a^3b + \mathbf{6}a^2b^2 + \mathbf{4}ab^3 + \mathbf{1}b^4$$

The coefficients appear in the phenotypic ratios because they represent the number of combinations of four alleles that have a given number of *H* alleles. Note that the results

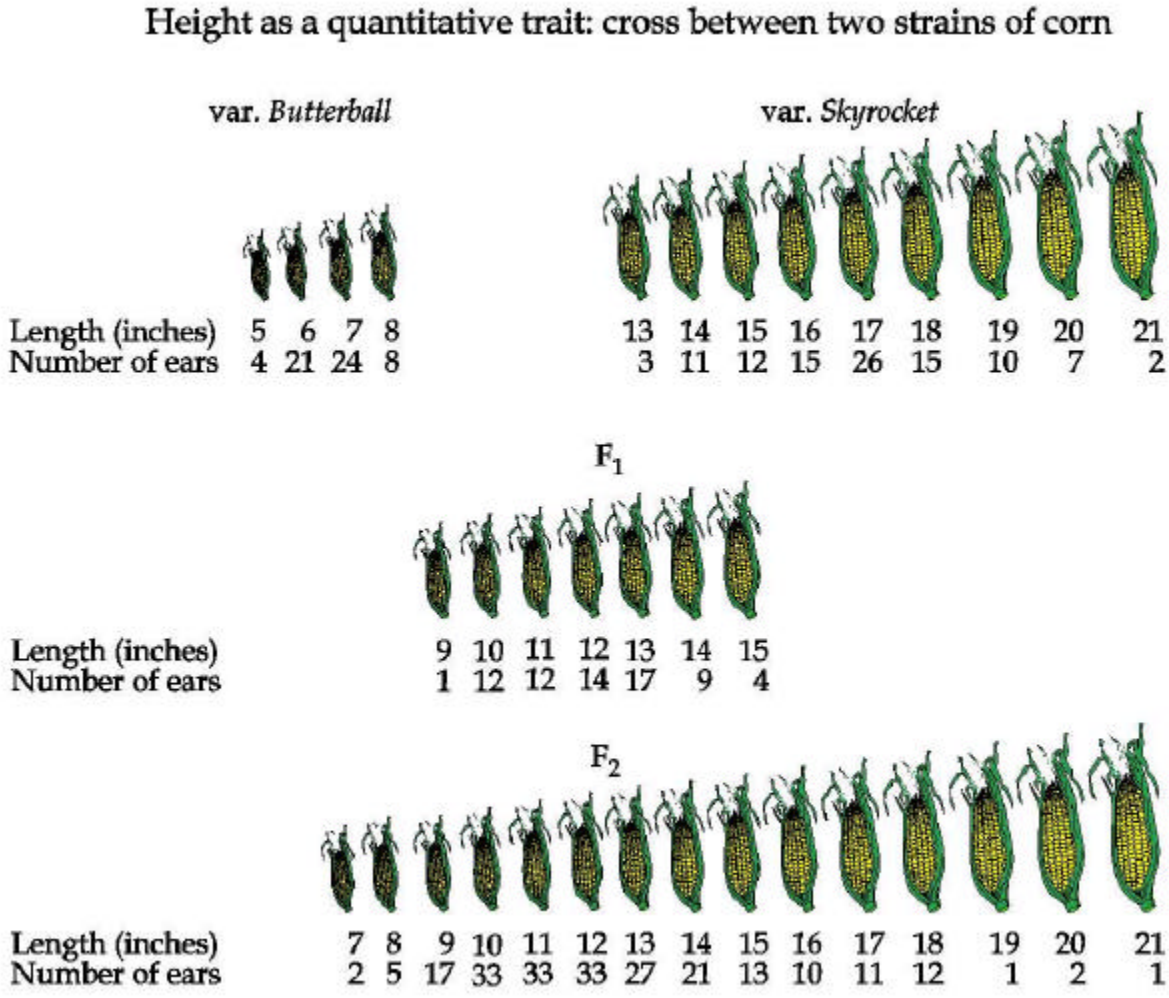


Fig. 3: Cross between two strains differing in a quantitative trait. Two pure breeding strains, *Butterball* and *Skyrocket*, were crossed with each other and the progeny of this cross selfed to yield the F_2 generation. The distribution of ear lengths is shown for each generation. The median values are 7 inches and 17 inches for the two parents, 12 inches for the F_1 generation, and 12 inches for the F_2 generation.

obtained require that the two genes, even though identical, segregate independently from one another. They may, for example, be on different chromosomes.

SQ24. How many discrete lengths would you predict to be possible if there were four identical *Height* genes?

I should stress that what I've described is just <u>one</u> way to get quantitative traits from genes that follow the rules of Mendelian genetics. You'll find another in Problem Set 7.

Fig. 4: Dihybrid Cross: (H_1 h_1 H_2 h_2) x (H_1 h_1 H_2 h_2)

	***H*₁ *H*₂**	***H*₁ *h*₂**	***h*₁ *H*₂**	***h*₁ *h*₂**
***H*₁ *H*₂**	$H_1\ H_1\ H_2\ H_2$	$H_1\ H_1\ H_2\ h_2$	$H_1\ h_1\ H_2\ H_2$	$H_1\ h_1\ H_2\ h_2$
***H*₁ *h*₂**	$H_1\ H_1\ H_2\ h_2$	$H_1\ H_1\ h_2\ h_2$	$H_1\ h_1\ H_2\ h_2$	$H_1\ h_1\ h_2\ h_2$
***h*₁ *H*₂**	$H_1\ h_1\ H_2\ H_2$	$H_1\ h_1\ H_2\ h_2$	$h_1\ h_1\ H_2\ H_2$	$h_1\ h_1\ H_2\ h_2$
***h*₁ *h*₂**	$H_1\ h_1\ H_2\ h_2$	$H_1\ h_1\ h_2\ h_2$	$h_1\ h_1\ H_2\ h_2$	$h_1\ h_1\ h_2\ h_2$

Very Large : Large : Medium : Small : Very small

1 4 6 4 1