Drosophila eye color trait lab

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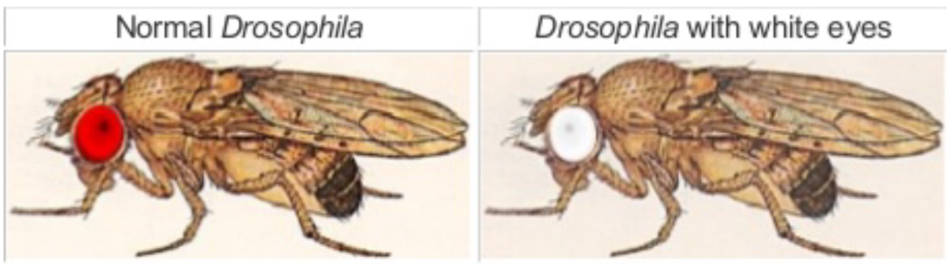
Sometimes, what appears to be a simple trait turns out to be more complex. Consider white-eyed Drosophila. Normally, white-eyed flies are presented in genetics lessons as a good example of a trait that depends on a single gene. Mutations that inactivate the white gene produce flies with white eyes. But there are other genotypes that can also produce white eyes. Let's analyze one of them, and see what we learn.

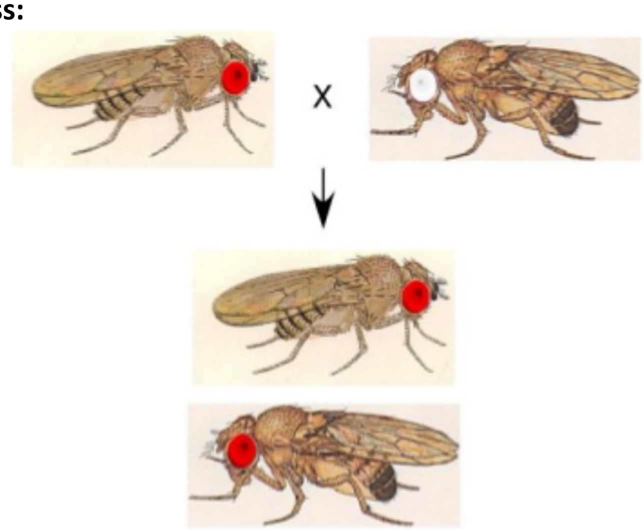
Flies with white eyes



We have a fly with white eyes. It seems likely that the white-eye phenotype could result from a mutation in a gene that is important for eye color. But we don't know unless we do the experiment. As Drosophila geneticist Mel Green used to say, "Ask the flies; *they* know."

There are different methods that scientists use for designing and thinking about experiments. We'll use one here that is common in the Drosophila research community: ask a question. We'll ask, "How is this white-eye phenotype inherited?" We will use the experimental approach of crossing white-eyed flies to wild-type flies to create an F1 hybrid, then we will investigate the F1 by performing a back-cross (test-cross) to the white-eyed parental type, and by performing a cross of the F1 flies among themselves.

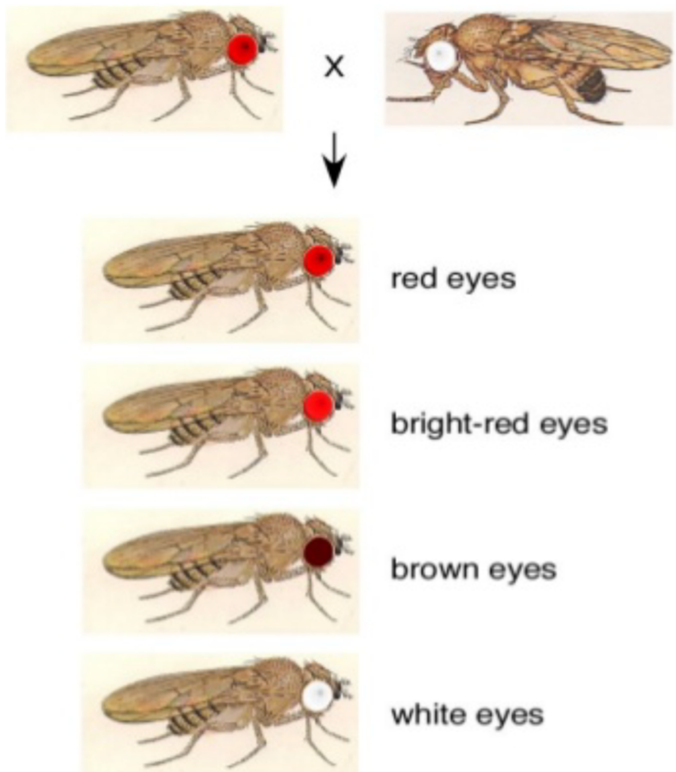
First, the parental cross:



All F1 offspring have red eyes

If all of the F1 flies have red eyes, then we can tentatively conclude that the white-eye phenotype is recessive. In molecular/biochemical terms, we can suggest that the white-eye phenotype may result from failure to produce the colored eye pigment.

Now, let’s do the test-cross, crossing the F1 offspring to flies of the original, parental, white-eyed genotype



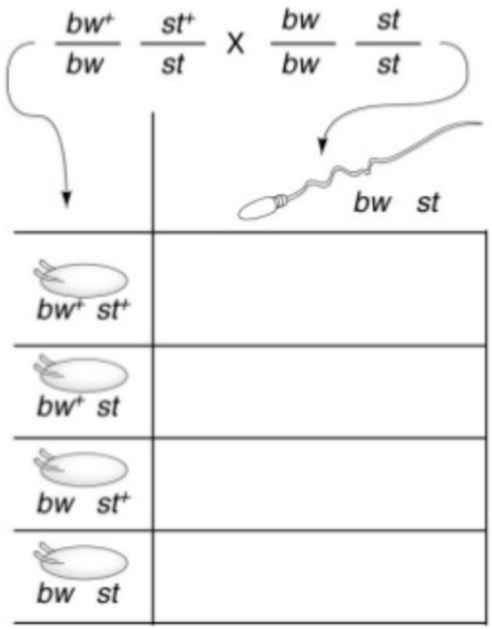
4 different phenotypes, in roughly equal numbers

The data - even as simple as the number of different phenotypes - indicate that we are not following the inheritance of a single gene. A single gene would give us two classes of phenotypes, red eyes and white eyes. Here, we have four. This argues that there must be at least two genes involved here.

The two "extra" phenotypes seem somewhat surprising at first - brown eyes, and bright-red eyes (which we will call scarlet). We'll need to refer to these genes and the alleles in our discussions, so, following the conventions of Drosophila genetics, we'll call the gene that produces brown eyes brown, and we'll call the gene that produces scarlet eyes scarlet. For simplicity, let's refer to the different alleles of these genes this way:

Gene	wild-type allele	mutant allele
brown	*bw+*	*bw*
scarlet	*st+*	*st*

The alleles with the + signs are the wild-type alleles (normal eye color). The *bw* allele gives brown eyes. The *st* allele gives scarlet eyes. If we've thought this through correctly, then the white-eye phenotype must be produced by the combination of the *bw* and *st* alleles. Let's check our reasoning by drawing out a Punnet Square for this cross:



Fill in the table, and see what you get.

¼ of the offspring should carry both a *bw+* allele and a *st+* allele. These should have normal red eyes.

¼ of the offspring should carry a *bw+* allele, but should be homozygous for *st*. These should have scarlet eyes.

¼ should be homozygous for *bw*, and should carry a *st+* allele. These should have brown eyes.

Lastly, ¼ should be homozygous for both *bw* and *st*. These must be the white-eyed flies.
NOW … if our interpretation is correct, we should be able to predict the outcome for the cross of F1 flies among themselves. Let's see if we can.

Crossing the F1 Flies among themselves
We'll draw out a Punnet Square for this. With two genes, and two alleles of each, it's just too many things to keep in mind all at once. Punnet's handy table will help us keep things straight. Both males and females are heterozygous for both genes; therefore, they should each produce 4 types of gametes (the same ones drawn out for the female in the Punnet Square above). This time, however, let's try filling in the table only for homozygous-mutant alleles. Maybe this will make it easier to see what the table tells us.

		Male Gametes			
		$bw^+\ st^+$	$bw^+\ st$	$bw\ st^+$	$bw\ st$
Female Gametes	$bw^+\ st^+$				
	$bw^+\ st$		*st* *st*		*st* *st*
	$bw\ st^+$			*bw* *bw*	*bw* *bw*
	$bw\ st$		*st* *st*	*bw* *bw*	*bw st* *bw st*

Of the 16 squares in the table, 9 (which are blank here) should have at least one + allele for each gene. These genotypes will all produce normal-eyed flies.

There are 3 squares that have two copies of *st* and at least one copy of *bw+*. These genotypes should produce scarlet-eyed flies.

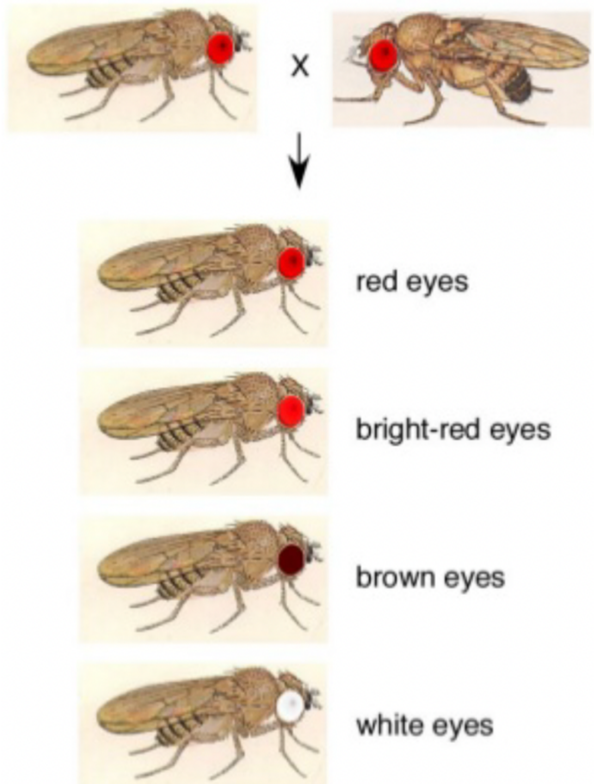
Similarly, there are 3 squares with two copies of *bw* and at least one copy of *st+*. These genotypes should produce brown-eyed flies.

Lastly, there is 1 square for *bw st* double-homozygotes. This indicates that only 1 out of 16 offspring should have white eyes.

Is this what we see when we do the cross?

[Note that this time, we have enough information about the genotypes of the flies that we can write down what we think their genotypes might be. We can formally state that we have a hypothesis: our current, tentative understanding of what's going on. According to the hypothesis that the F1's are heterozygous for *bw* and for *st*, we expect to find a 9:3:3:1 ratio of phenotypes among the offspring.]

Let’s do the cross:



4 different phenotypes, in very unequal numbers

When we actually do this cross, we recover a great many wild-type, red-eyed flies. We recover many fewer scarlet flies, and somewhat fewer brown-eyed flies. We recover very few white-eyed flies. These numbers are "in the general direction" of the 9:3:3:1 ratio, but there are many more wild-type flies and fewer white-eyed flies than predicted. In fact, many people who do this particular cross recover no white-eyed flies at all.

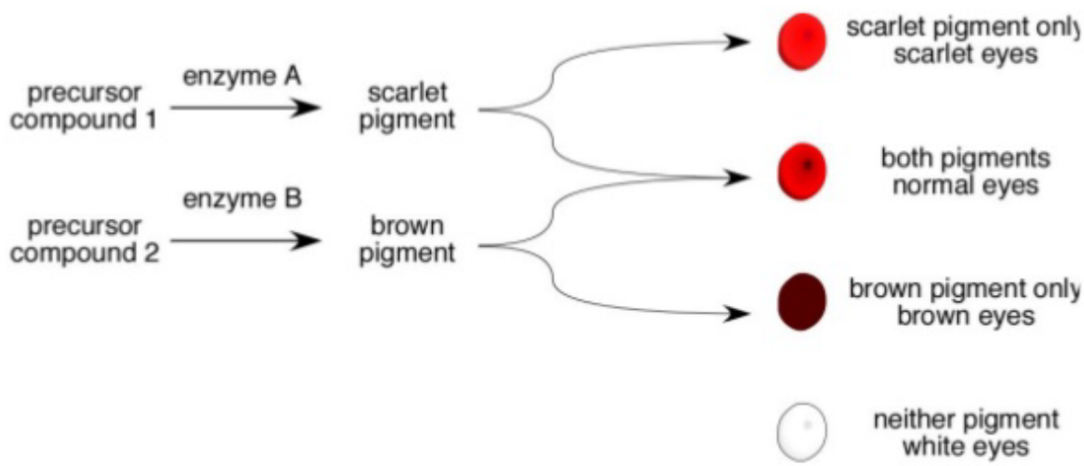
When we perform a statistical test with the numbers (a chi-square test), we find that our numbers just don’t match. In the phrasing of the chi-square terminology, "we must reject the null hypothesis." Translated into conversational English, this means "there's more going on here than we predicted." We discuss this below.

Nonetheless, we do recover 4 classes of phenotypes, just as we did with the test-cross. The numbers of individual flies differ, but there are still the same 4 phenotypes. These data force us to conclude that there are two genes, and that *bw st* double-homozgotes have white eyes.

The biochemistry revealed by the genetics

We'd like a satisfying explanation for these phenotypes, and how the genotypes produce them, so how can we interpret these observations?

We are dealing with pigments here. To produce pigments, cells require enzymes. Enzymes are encoded by genes, which are subject to mutation. Perhaps the brown-eye phenotype results from failure to produce a scarlet pigment, and the scarlet-eye phenotype results from failure to produce a brown pigment. Without either pigment, the eyes would be white. We can diagram this as follows:



This type of model has been tested by isolating the eye pigments. It turns out to be pretty much the way it works. The primary difference from what we show here is that there are two additional genes that interfere with production of the brown pigment, and that produce scarlet-colored eyes. These genes are named vermillion and cinnabar.

We have presented this example to illustrate the following important point:
Genetics is not just about figuring out how inheritance works. It is most powerful as a tool for figuring out how biology itself works. When we find a mutant allele that alters a phenotype, we learn about how the gene product functions. Here, just by following eye color phenotypes in a couple of crosses, we learned enough to infer a fair amount about the biochemistry of pigment production in Drosophila.

Some comments on statistical analysis
Statistical analysis is extremely powerful. In some instances, one of which is described above, the statistical analysis reveals that there is more going on than we thought. This itself is important. It is also cautionary, however.

When we are forced to reject our hypothesis, we need to think carefully about just what our hypothesis really is. In the situation described here, we wrote our hypothesis as "our F1 flies are heterozygous at both *bw* and *st*." We based our predictions on this statement, and expected a 9:3:3:1 ratio of phenotypes among the offspring.

Even when we perform this cross with proven alleles of *bw* and *st*, so that we are absolutely certain of our genetics, the numbers of flies just don’t match the predictions. We still find that the statistical test requires that we reject our hypothesis. At first, this doesn’t seem to make sense. How can we reject a hypothesis that we already know is true?

The answer is that *we didn't write out the entire hypothesis*. That is, our hypothesis included assumptions that we did not carefully articulate. One of those assumptions was that all genotypes are equally viable. We should, perhaps, have written this into the hypothesis: "our F1 flies are heterozygous at both *bw* and *st*, genes for which no alleles make the flies unhealthy." When we reject the hypothesis, we do so on, the basis of this second part - the assumption that all the flies will be equally viable.

Drosophila are typically grown in half-pint milk bottles, or even smaller vials, containing a cornmeal-like food sprinkled with yeast. In this small, contained ecosystem, there is competition for food. Flies compete with each other, and bacteria and molds compete with the yeast. Drosophila larvae that grow fastest out-compete the others, which may suffer developmental delay, or even death. It turns out that *bw st* homozygotes do not fare well in this competition.

We mentioned above that some people who perform this cross recover no white-eyed flies. In general, this is because the competition was too intense-there were too many larvae in the bottle relative to the amount of food. This occurs commonly in laboratory classes, when flies are put into the bottles and allowed to lay eggs for an entire week. It can be avoided, or at least minimized, by allowing the flies to lay eggs for only 24 hours before transferring them to new food. With less competition, the numbers of flies approach the 9:3:3:1 ratio we predict from the Punnet Square.

It has been our experience that nearly all crosses with Drosophila encounter some kind of problem such as this, so that we are required to "reject the hypothesis," even though we already know precisely what the alleles are. Take care, and always examine your assumptions.

	bw+/st+	bw+/st	bw/st+	bw/st
bw+/st+	bw+bw+/ st+st+	bw+bw+/ st+st	bw+bw/ st+st+	bw+bw/ st+st
bw+/st	bw+bw+/ st+st	bw+bw+/ stst	bw+bw+/ st+st	bw+bw/ stst
bw/st+	bw+bw/ st+st+	bw+bw/ st+st	bwbw/ st+st+	bwbw/ st+st
bw/st	bw+bw/ st+st	bw+bw/ stst	bwbw/ st+st	bwbw/ stst