Single gene trait showing Pleiotropy - A problem

This problem is used in my undergraduate genetics class. The problem is introduced once the students have learned about sex-linked and autosomal traits and know about the different kinds of dominance. They are expected to be able to look at the data from F_1 and F_1 reciprocal crosses and determine the mode of inheritance.

In the lecture in which this problem is used - I would have discussed the concept of **pleiotropy**.

> Definition: **Pleiotropy** - one gene affects multiple phenotypes. This may be due to the fact that a single gene affects several different biochemical pathways and a gene may affect phenotypes at different levels of organization. Experimentally, pleiotropy is observed as a correlation between two phenotypes in the F_2 and other segregating generations (e.g., backcross generations).

While students happily memorize the definition, they are generally unable to look at data and extract useful information from those data. The following example from my graduate student days is quite useful in illustrating that genetics is an applied science.

I give this problem to a group of three to four students at the end of class. They need a minimum of 10 to 15 minutes to solve this problem as a group. For a complete answer, I require that students do all appropriate 2 tests.

The Problem:

As a graduate student at the University of Illinois I noticed a number of orange-eyed blow flies (*Phormia regina*) in one of my stocks. Wildtype *Phormia* all have red eyes.

I mated several pairs of *orange-eyed* flies in an attempt to get a pure breeding line.
The cross of *orange* x *orange* produced only *orange* offspring. When these *orange* flies were interbred, they only produced *orange* offspring.

The F_1 cross of wildtype (red-eyed) females to *orange* male*s* and the reciprocal F_1 cross of *orange* males to wildtype (red-eyed) females produced only red-eyed progeny.

I continued with the F_2 crosses. The first flies eclosed from the pupa case 12 days after the first instar larvae were placed on fresh pork liver. Many more flies eclosed from the pupal case on day One. Males and females were combined.

Results of the F2 cross	**F1 cross was wildtype females to *orange* males**	
	Red Eye	**Orange Eye**
Eclosed Day 12	**1274**	**64**
Eclosed Day 13	**485**	**507**

Results of the F2 Reciprocal cross	**F1 reciprocal cross was orange females to *wildtype* males**	
	Red Eye	**Orange Eye**
Eclosed Day 12	**972**	**57**
Eclosed Day 13	**267**	**335**

Using the information on day of eclosion and eye color, how is eye color inherited? Explain the difference in ratios between Day 12 and Day 13.

Background information about the black blow fly *Phormia regina.* This information is not needed to solve the problem but may prove useful.

Adult *Phormia regina* were obtained from the colony maintained in the University of Illinois Department of Entomology and were used to establish an experimental colony in the University of Illinois Department of Psychology. The flies from the Entomology stock had been maintained in the laboratory at least 25 years although the origin of the line is unknown. Adult *Phormia* were housed in 30.5 cm x 30.5 cm x 30.5 cm metal mesh insect cages (American Biological Materials Co.). Larvae were reared on raw pork liver in 20 cm x 15 cm dia glass battery jars.

The larvae, pupae, and adults were maintained at 25$\pm$ 1°c on a 16/8 hr light/dark cycle. Flies were reared in a constant environment room with relative humidity at 50%.

Adult flies were given *ad lib* access to water and sugar cubes and were given fresh pork liver daily for a protein source and as an oviposition site. Eggs were collected every day, moistened with distilled water, and kept overnight on liver in covered plastic petri dishes. The next day, 200 to 300 first instar larvae were placed on one-half to three-quarter pounds of fresh pork liver in a battery jar and covered with sawdust. On the fifth or sixth day, the larvae were separated from the liver and placed into fine sawdust for pupation. Separation was achieved by dumping the liver,

sawdust, and larvae on a large wire mesh placed over a collection box. The larvae are photonegative and will crawl down through the mesh into the box. They were then easily poured into a box of fine sawdust. After two days in the fine sawdust, the pupae were sieved out and placed into pint paper holding cups for three or four days. At the end of this period, batches of approximately 50 pupae were put into glass bottles, placed into the controlled environment room, and allowed to eclose*.

Flies tend to emerge from the pupa case at dawn (lights on) $\pm$ 2 hours. Flies that miss this window generally go into a rest state and do not emerge from the pupa case until the next day. The majority of wildtype flies eclose 12 days after pupation. A few wildtype flies emerge 13 days after pupation.

Definitions:

Eclose: (v) to emerge from the pupal stage

Eclosion: (n) emergence of an adult insect from the pupal stage

Instar: (n) the life stages of an insect larva. Each instar ends with a molting of the cuticle.

Answers and observation on student responses:

- Students are generally able to determine that **orange** eye is an autosomal recessive trait.
- They can generally explain this with the following information: The **orange eye** is recessive because it disappears in the F_1. **An orange eye** is autosomal because the F_1 and F_1 Reciprocal generations are the same.
- Students have a harder time explaining the ratios. There are confident that they should expect a 3:1 ratio. If asked directly a few students are not sure if the ratio should be 3 red: 1 orange or 3 orange:1 red.
- The students must now test the results of the F_2 generation against an expected 3:1 ratio. In my class, this must be done with a 2 test.
- None of the data for the F_2 or F_2 reciprocal cross for Day 12 fit a 3 to 1 ratio. Similarly none of the data for the F_2 or F_2 reciprocal cross for day 13 fit a 3:1 ratio. (see 2 tests on page 5 and 6).
- Some students realize that orange eyed flies might be a bit slower in developing due to pleiotropy. They combine the Day 12 and day 13 data. The combined data shows a good fit to the expected 3:1 ratio (see 2 tests on page 5 and 6).

Skills Utilized in This Problem:

- Using F_1 and F_1R data to determine if a trait is autosomal or sex-linked; dominant or recessive. F_2 data is only need to confirm the observations on F_1 data.

- Developing the expected ratios and the expected numbers in the F_2 generations based on a proposed model.

- Using 2 test for Goodness of Fit.

- Recognition that two phenotypes (eye color and developmental time) can be affected by a single gene.

- *Know your organism*: A homozygous line can show some variation in a phenotype. (My students were often confused that not all wildtype flies emerged on day 12 and not all orange-eyed flies emerged on day 12). This is why I provided all the information on rearing of *Phormia* in my lab.

- Collapsing of data to eliminate the effects of development time to test the inheritance of eye color.

Common Errors:

- Looking for "meaningful" ratios in the F_2 rather than testing against the 3:1 ratio that they should expect based on the F_1 results. For example they get a 20:1 ratio on Day 12 and a 1:1 ratio on day 13.

- Interpreting each piece of data separately. For example, some students have *orange* eyes being lethal on Day 12 but wildtype red eyes being lethal on day 13. They are lost when I ask for data to back up that answer.

Statistical Tests on F_2 data; H_0: 3 Red: 1 orange

Results of the F2 cross Day 12	**F1 cross was wildtype females to *orange* males**		
	Red	**Orange**	
Obs Day 12	1274	64	1338
Exp Day 12	1003.5	334.5	1338
2 =	290.58, 1 df Reject H_0 at .05 level		

Results of the F2 cross Day 13	**F1 cross was wildtype females to *orange* males**		
	Red	**Orange**	
Obs Day 13	**485**	**507**	992
Exp Day 13	744	208	992
2 =	359.26; 1 df Reject H_0 at .05 level		

Results of the F2 cross Combined Days	**F1 cross was wildtype females to *orange* males**		
	Red	**Orange**	
Obs Day 13	**1759**	**571**	2330
Exp Day 13	1747.5	582.5	2330
2 =	0.28; 1 df Accept H_0		

2 tests were calculated using the special case for two phenotypic classes.

$$2 = \quad (|obs\text{-}exp|\text{-}1/2)^2/exp$$

Statistical Tests on Reciprocal F_1 data; H_0: 3 Red: 1 orange

Results of the F_2 cross Day 12	**F_1 cross was orange females to *wildtype* males**		
	Red	**Orange**	
Obs Day 12	1082	69	1151
Exp Day 12	863.25	287.75	1151
2 =	220.72, 1 df Reject H_0 at .05 level		

Results of the F_2 cross Day 13	**F_1 cross was orange females to *wildtype* males**		
	Red	**Orange**	
Obs Day 13	**267**	**335**	602
Exp Day 13	451.5	150.5	602
2 =	299.94; 1 df Reject H_0 at .05 level		

Results of the F_2 cross Combined Days	**F_1 cross was orange females to *wildtype* males**		
	Red	**Orange**	
Obs Combined Day	**1349**	**440**	1753
Exp Combined Day	1314.75	438.25	1753
2 =	3.47; 1 df Accept H_0		

2 tests were calculated using the special case for two phenotypic classes.

$$2 = \quad (|obs\text{-}exp|\text{-}1/2)^2/exp$$