Eye Color Inheritance: 3P's Learning without Computers

by Cynthia Wynne and Barbara Smentek

"How is Eye Color Inherited?", found at the end of this article, was written by students in our genetics class, and is a product of a six-week summer program that was designed to allow minority students to pose, solve and present a research problem in a scientific field, and thus to increase their beliefs that they can become scientists. In the following paragraphs, we discuss the program in general, how we designed the class to enable students to engage in scientific inquiry, and the events that led to the choosing of this project.

Our genetics class was part of the Summer Science Institute (SSI) at UW-Madison, developed and directed by Jose Rios. In this program, students had the option of studying genetics, exercise physiology or animal behavior. Eight high school minority students, with prior genetics exposure that ranged from a brief discussion of the subject in a high school biology class to an entire semester course in microbiology, chose to pursue genetics within the SSI program. In addition to genetics, the students studied language arts, data analysis and computers. These other classes were designed to help the students develop tools for research, and to compliment the ongoing project in the genetics course.

The first two weeks of the course were designed to introduce students to basic concepts in genetics, with an emphasis on Mendelian and population genetics, and to give them experience posing a feasible scientific problem. We tried to select open-ended activities, so students would have the opportunity to ask their own questions and develop a strategy for how they might solve those questions. For example, in one typical activity we gave the students three taste papers: PTC paper, thiourea paper, and sodium benzoate paper (the ability to taste at least one, and possibly all, is genetic.) Each student tasted all three papers, and recorded their observations. The students were then encouraged to pose a problem, and to develop a method for solving this problem using the data generated by the class. The class brainstormed and came up with several problems; such as "If the person can taste PTC paper, can they taste thiourea paper?" Once they steeled on a problem in our class. they analyzed the data in their data analysis class, write a mini-research paper in their language arts class, word processed the paper in their computer class, and presented the results.

During the beginning of the third week, students became very excited about the activity we did in class. We began this activity by asking students to recall eye colors that they see in their friends and families. We explained the purpose of this activity was to design genetic models that could account for the inheritance patterns of different eye colors. (For example, the simple dominance model is used to account for the inheritance of height in pea plants, while a multiple allele model is used to account for the inheritance of blood types in humans.) Students, eager to share their prior observations, began constructing pedigrees to display what they had observed in the population. The models had to explain these pedigrees. For example, if a student in our class had brown eyes, and her parents had green eyes, the models had to account for this. Students quickly determined that they could not explain the inheritance of eye color with merely one gene, given the varieties of eye colors in the general population. The students, working in groups of two, developed four initial models that they believe accounted for the inheritance of eye color. Three of these models were polygenic inheritance, although the students had no previous experience with such models.

When students were given the opportunity to select a research project, they decided to revise their initial eye color inheritance models in order to produce, as a class, a "final" model that could explain the inheritance of eye colors in the general population. They did this by conducting two pilot studies, and by using the results of each pilot study to further revise their initial models.

We thought that this process of continued model revision was the most exciting aspect of the project, as our students were experiencing the changing nature of science. The following paper, "How is Eye Color Inherited?" includes the students' initial models, results of both pilot studies, the final eye color model that the students generated based on the results of both pilot studies, and the research they carried out to test and analyze their final model.

How is Eye Color Inherited?

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This version has been slightly edited for length

Abstract
The purpose of our study was to test a model we created regarding the heredity of eye color in the population and the number of genes involved in the process of inheritance. Our model included seven colors, with dark brown as the most dominant and light blue/blue gray as the most recessive. Due to the number of shades we observed, we hypothesized that eye color is a polygenic trait, controlled by three genes. We collected data at three locations: Henry Vilas Zoo, Farmer's Market on Capitol Square and Kronshage Hall (U.W. Madison), all in Madison, Wisconsin. First, the 110 participants were given flyers summarizing the purpose of the research. Then we asked them questions about their eye colors and their biological family members' eye colors. We categorized each of the participants and family members as having one of the seven eye color shades. Using our model, we predicted the percentages of different eye color shades that children would have, based on the eye colors of their parents. We compared these expected values to the values we collected in the population. Our model was able to explain the results in 62% of the families we surveyed. However, our model was not able to explain 38% of the cases we studied. One of the major problems with this study was identifying the eye color shades of the participants. If we could repeat this study, we would use a larger sample size, we would try to use observed (rather than reported) individuals, and we would perfect our eye chart.

Introduction
Have you ever looked into someone's eyes and wondered how much melanin was deposited in the superficial layers of their iris? Through this study, we hope to explain the inheritance of eye color and the number of genes (units of DNA that contain the code for a trait) involved.

Researchers in the past have developed several models that describe the number of genes involved in the inheritance of eye color. Some of the models (Davenport, 1907), although no longer accepted, stated that one gene accounted for eye color. In such a case, brown eye color would be dominant, meaning that only one dominant allele (a form of a gene) is necessary for the trait to be expressed. Blue eyes would be recessive, requiring two recessive alleles for the trait to be expressed.

Other scientists (Galton, 1889; Galloway, 1912; Hughes, 1944) concluded that more than one gene is involved in the inheritance of eye color based on the complexity and variation observed in the population. Their models, some of which are currently used, suggest that eye color is inherited in a polygenic fashion, which means that many genes contribute to the variation of the trait. They hypothesized that the complexity is due to melanin, a pigment responsible for the brown color in hair, skin, and eyes. Depending on the amount and placement of melanin in the superficial layers of the iris, the shade can vary. If no eye pigment is visible (every eye, with the exception of albinos, contains pigment), an optical effect similar to the sky appearing blue on a clear day occurs and the eye appears blue. If a small amount of melanin is massed together in small particles, the iris appears to be a darker shade of blue. If the same amount of pigment is thinly dispersed over the iris, a yellow film forms over the blue optical effect, creating a green iris. As the amount of melanin in the iris increases, the brown film over the blue effect darkens, creating shades from hazel to dark brown (Winchester, 1966). Sometimes, if a substantial amount of melanin is massed into groups, the eyes may appear to be different colors in different lights. Masses of melanin can be found in several patterns which affect the overall shade of the iris: irregular placement, radial arrangement around the pupil like the spokes of a wheel, or concentric circles around the pupil (Moody, 1967). All these observations indicate that eye color is a complex matter and is controlled by many genes.

With no knowledge of these studies, we generated several models and developed pilot studies to test them. Through the pilot studies we wanted to see how well the models represented the population. Based on our pilot study, we hoped to develop one highly accurate model that accounts for variation in eye colors in the population. We hypothesized that eye color must be controlled by more than one gene, as one gene can only result in three shades, and we believe there are more than three shades. As a group, we decided that eye color is controlled by three genes which code for seven shades.

Methods

On July 13, 1993, we performed a class activity in which we created a model that described the number of genes involved in eye color and the patterns of inheritance. As a group we brainstormed four models. The first model described nine genes controlling eye color (see Fig. 1, Model 1). This model worked on a system of hierarchy, where dark brown eyes had dominance over brown and brown eyes had dominance over blue. With this model, an eye color was expressed only when the gene for that particular color was homozygous dominant (only dominant alleles were present), regardless of what other heterozygous combinations (one dominant allele and one recessive allele) were present. If more than two homozygous dominant genes were present, the one highest up on the hierarchy would be expressed. The second model suggested that eye color involved five genes working in a codominant fashion, meaning that every combination of alleles yields a different variation (see Fig 1, Model 2). Brown was the most dominant eye color, dark blue was the most recessive eye color, and seven eye colors were listed in between. The third model described eye color as being inherited through three genes in a codominant fashion (see Fig. 1, Model 3). The eye colors were listed similar to the second model. The fourth model described seven basic eye colors coded by three genes (see Fig. 1, Model 4). The color was determined by the number of dominant alleles present. The colors were arranged from darkest to lightest, with brown having six dominant alleles and blue having no dominant alleles.

Figure 1: Four Hypothetical Models

Model 1: This nine-gene model works on a system of hierarchy where colors that are ranked high have dominance over those that are ranked lower. For example, a person with the genetic code Aa Bb cc Dd EE ff GG Hh I I would have green eyes because it is the homozygous dominant gene highest up on the hierarchy. A problem with this model is that there are 19,683 possible combinations of genes, making this model extremely difficult to test.

Dark brown	AA		
Brown	BB		
Hazel	CC		
Green-hazel	DD	Green	EE
Blue-green	FF		
Blue	GG		
Gray-blue	HH		
Gray	I I		

Model 2: This model describes five genes that codominantly code for nine eye colors. The problem with this model was that brown-eyed parents could not have a blue-eyed child, a combination we observed in our first pilot study.

Dark brown	AA BB CC DD EE
Brown	AA BB CC DD Ee
Light brown	AA BB CC DD ee
Hazel	AA BB CC dd ee
Green	AA BB Cc dd ee
Yellow	AA BB cc dd ee
Light blue	AA Bb cc dd ee
Blue	AA bb cc dd ee
Dark blue	aa bb cc dd ee

Model 3: This model describes eye color as being inherited by three genes working in a codominant fashion with eight colors arranged from darkest to lightest. A problem with this model is that, once again, two brown-eyed parents can't have a blue-eyed child.

Dark brown	EE FF GG
Brown	Ee FF GG
Light brown	Ee Ff GG
Hazel	Ee Ff Gg
Green	ee ff GG
Light green	ee ff Gg
Blue	ee ff Gg
Light blue/Blue gray	ee ff gg

Model 4: This three-gene model determines eye color by the number of dominant alleles present. With this model, two light brown-eyed parents can have a blue-eyed child. This model was selected to use for the study after a second pilot study had been conducted and this model had been further revised.

Brown	AA BB CC	6 dominant alleles
Light brown	Aa BB CC	5 dominant alleles
Blue	Aa Bb CC	4 dominant alleles
Light blue	Aa Bb Cc	3 dominant alleles
Green	aa Bb Cc	2 dominant alleles
Hazel	aa bb Cc	1 dominant allele
Blue gray	aa bb cc	0 dominant alleles

After discussing theoretical problems with the models, we decided to conduct a pilot study in order to begin testing our models. This study took place on July 14, 1993. Based on the results of the pilot study, the models were revised and re-evaluated. We chose the fourth model because it best represented the sample we collected. We then conducted another pilot study on July 19, 1993, in order to revise our model. Then, after the model had been revised and a final model had been generated (see Fig. 2) we prepared an eye color chart, a questionnaire, and flyers to use in our study). We found seven people whose eyes closely matched our model's eye colors and photographed their eyes for the chart. The questionnaire took into account multiple marriages, adopted children, and the fact that, especially in Caucasian children, eye color doesn't fully develop until age four. The flyer was written to explain our study more thoroughly.

Figure 2: Our Working Model

Dark Brown	AA BB CC
Brown	Aa BB CC
Light Brown	Aa Bb CC
Blue	Aa Bb Cc
Hazel	aa Bb Cc
Green	aa bb Cc
Blue Gray/Lgt.Blue	aa bb cc

We conducted our data collection at the following places: the Henry Vilas Zoo on July 23, 1993; Farmer's Market (on Capitol Square) on July 24, 1993; and Kronshage Hall, U. W. Madison on July 25, 1993. First, we paired off and looked for unoccupied families. We introduced ourselves and explained the purpose of our study and asked for their eye color, their spouse's eye color, and their biological family's eye colors. Once we finished filling out our questionnaires/ pedigrees of the subject's family, we moved on to the next family. Sometimes some of the members of the subject's immediate family were not present, in which case the subject had to recall their eye colors.

After collecting our data, we organized the 176 collected pedigrees (618 total people) into a table for further analysis. We looked at certain parent eye color combinations (brown x brown, for example) and tabulated the number of children in each combination according to their eye color. Then we calculated percentages of each eye color and compared them to theoretical percentages derived from our model. The theoretical percentages were calculated by looking at Punnett squares of every possible parent eye color combination.

Results of Pilot

One hundred and six people were involved in our first pilot study. Seventy-five people were involved in our second pilot study. The results of these pilot studies are summarized in Figure 3. (Figure missing)

Results

Six hundred and eighteen people were involved in our study. The results are summarized in Figures 4.1-4.7. (Figures missing)

Discussion

According to our graphs, we were able to draw several conclusions. After comparing the graphs to one another and the expected percentages, we found that 62% of the pedigrees collected fit the model while 38% contradicted it. The observed percentages of the more dominant colors seemed to match the expected percentages more closely than the more recessive colors. This suggests that the order of recessive colors in our model may need revision. We found that in similar parent combinations of different shades of brown (dark brown x green and brown x green, for example), the total observed percentage of all the browns in one graph closely matched the total percentage of all the browns in the other graph. This seems to suggest that there may be no genetic distinction between shades of brown, and that they could be combined. According to our model, brown is more dominant than blue, and when crossed with each other, a greater percentage of brown should appear. However, when dark brown and brown were crossed with blue, we observed a much higher percentage of blue, even when the shades of observed brown were combined.

Several reasons might account for the 38% disagreement with the model. The sample size could have been too small to get an accurate representation of the population. It was sometimes hard to determine a subject's eye color due to poor light. If the subject's family was not present, he or she had to remember them. These recollections might have been inaccurate.

We ran into many problems doing this project. It was very hard finding information on eye color because many of the books and articles dealt with eye color in animals rather than eye color in humans. There are many questions we've asked about eye color, but finding one specific question was very hard. Classifying eye colors was one of our biggest challenges, because some of us disagreed on what certain shades of color were, such as hazel. Some of us thought hazel was green, and others thought hazel was light brown. Many people were rude to us when we asked for their eye color. Some people cut us off before we could tell them what it was were doing, and said " I don't have time," or "Leave me alone!"

Determination of a persons eye color was very hard. For example, some people said their eyes were hazel, but we observed green. While collecting data, we found a man who had one brown eye and one blue eye. This made things difficult because we were only looking for the same color in both eyes. Some people said their eye color changes with their attitude. For example: there was a woman who had light brown eyes said, " My eyes turn gray when I get angry." We haven't looked into this as a part of our project, all though this did make things difficult because we were only looking for permanent eye color.

Finding whole families was a problem because we needed to see what eye colors were inherited from the parents to the children. The information received about family member's eye color that we didn't observe could have been incorrect. Another problem we encountered was people with children from different marriages. We found a woman who had been married 5 times and had at least 12 children. The information received from this woman had to be thrown out because it was too confusing and difficult to understand.

If we were to repeat this project, we would revise our model using the information received from our study. We would do this by rearranging the colors, so that the model would be better able to explain the observed data. Since we experienced some difficulty in distinguishing certain shades of color, we would improve our eye color chart by using only photos of the eyes rather than including the area in between.

We would need time to gather more data so that we could increase our sample size. Also we should look for better locations to find whole families. We think it would be better to see all of the family members so that we can verify the eye color of each family member. We are also interested in looking at the structure and different patterns of color of the iris, because we believe that these factors influence eye color.

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