NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Eyes of a Fly: Discovering the Physical Location of Genes

by
Jonatan Alejandro Yepes Giraldo
Universidad de La Salle, Bogotá, Colombia *and*
Colegio Militar Academia Pantano de Vargas, Fusagasugá, Colombia

Introduction

In the early 20th century, biology was at a crucial turning point. Mendel's laws, rediscovered in 1900, revealed that underlying "factors," later known as genes, governed the inheritance of traits. At the time, the molecular nature of heredity remained a mystery; DNA had not yet been identified, and the gene existed only as a theoretical concept, leaving open a crucial question: where are these hereditary factors located?

The hypothesis that hereditary factors resided on chromosomes was proposed by Walter Sutton and Theodor Boveri (1902–1903), following advances in cytology that allowed more detailed observation of chromosome behavior during cell division, mitosis, and meiosis. Their observations paralleled the inheritance mechanisms described by Mendel in 1865 but still lacked direct experimental evidence.

Around the same period, Nettie Maria Stevens, an American cytologist and former student of Thomas Hunt Morgan, made another crucial discovery. While studying the mealworm *Tenebrio molitor,* she found that males and females differed in their chromosome composition. In particular, she noticed that male cells contained one chromosome pair that differed in size and shape, which she referred to as "heterochromosomes." Stevens correctly speculated that these chromosomes determined sex, providing one of the earliest direct links between a physical structure and a biological trait. Her work added weight to the emerging idea that chromosomes could carry hereditary information, a notion that would soon inspire further experiments.

At the same time, Edmund Beecher Wilson reached similar conclusions while studying insects and other organisms. He also identified these unequal chromosomes, but called them "idiochromosomes." In 1905, Wilson introduced the terms X and Y chromosomes, establishing the chromosomal basis of sex determination. This finding provided one of the first cytological explanations for inheritance and paved the way for later research.

It was in this intellectual and experimental context that Thomas Hunt Morgan and his students, Alfred Sturtevant, Calvin Bridges and Hermann Müller, began their work. They revolutionized Mendelian genetics by adding that chromosomes carry hereditary factors, using the fruit fly *(Drosophila melanogaster)* as a model organism. This strategic choice proved decisive; *D. melanogaster* has a short life cycle, is easy to breed, and exhibits multiple visible mutations that facilitate the study of heredity. Their work laid the foundations of modern genetics and, decades later, earned Thomas Hunt Morgan the Nobel Prize.

Part I – An Unexpected Discovery

On the campus of Columbia University, in a small laboratory known as the "fly room," Thomas Hunt Morgan and his collaborators carefully examined a population of *Drosophila melanogaster*, a species increasingly used to study inheritance. The year was 1910.

Rows of glass bottles, arranged in the small room, housed dozens of flies that would soon change the course of biology, and the air of the laboratory, as usual, was filled with the strong smell of fermenting bananas, the flies' food, and with a sense of expectation.

While they carried out their observations, something caught their attention: *D. melanogaster* characteristically has red eyes, the color considered "common" or "wild type." However, they had just found a male fly with eyes of an unusually white color never seen before.

Morgan looked closer. Two more males showed the same unusual trait. In total, three males out of nearly two thousand flies had white eyes instead of red.

He paused. What could have caused this variation? Was it an environmental effect, an injury, or something that could be transmitted from parent to offspring?

Questions

1. If you were Morgan, what would be your next step to determine if the white-eye trait is inherited?

2. Why is the appearance of a new eye color relevant in a genetic investigation?

3. What is the difference between a heritable mutation and a somatic change?

4. What is an allele, and why might it help explain what Morgan observed?

Part II – Mutation or Accident?

Rare deviations such as the appearance of white eyes were often dismissed as developmental accidents or damage from handling. But three independent occurrences of the same anomaly suggested something deeper, perhaps a change that could be inherited.

Because of this, Morgan suspected that the white-eye trait was not an accident but a heritable mutation; a new allele for eye color in *Drosophila melanogaster*.

To test this hypothesis, he conducted a series of experimental crosses. First, Morgan crossed a white-eyed male (mutant type) with a red-eyed female (wild type). He found that all offspring in the first filial generation (F_1) had red eyes, a result that was expected by Mendel's laws if the trait were transmitted as a recessive factor (see Figure 1 below).

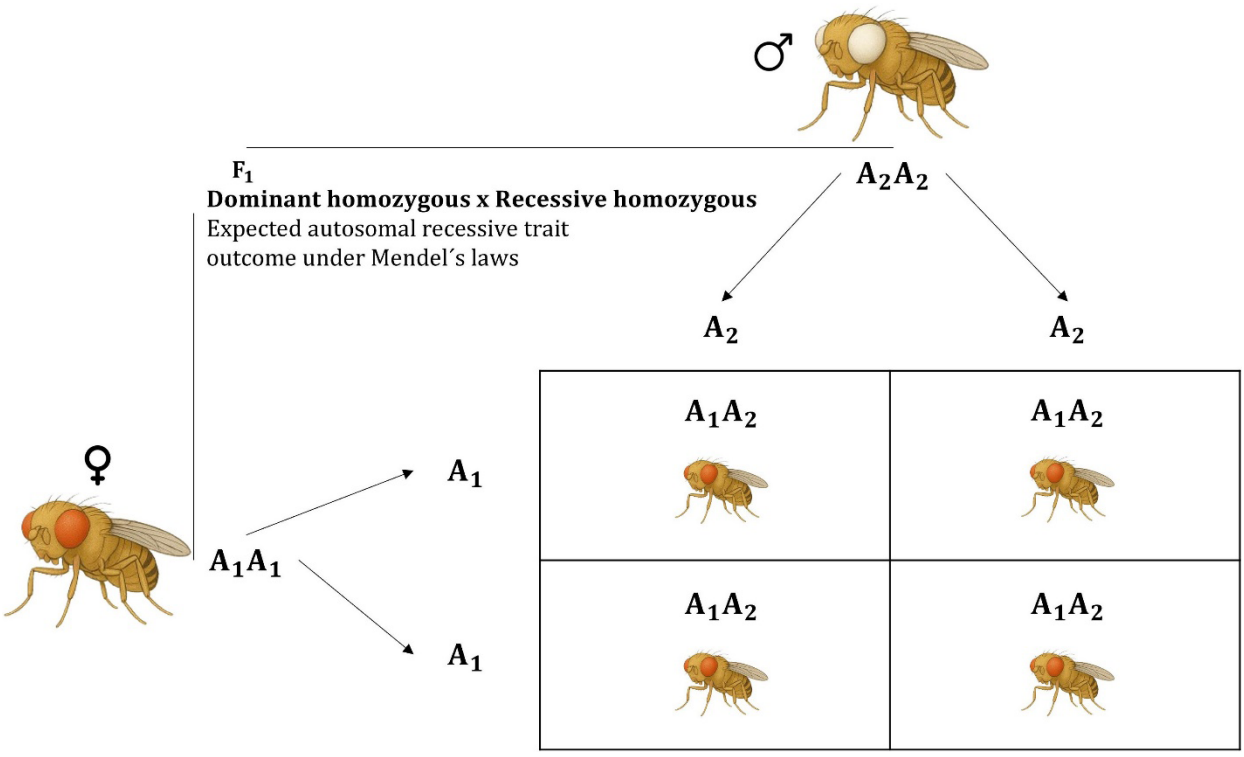

Figure 1. Monohybrid cross between a female with wild-type alleles (A_1) for red eyes and a male with mutant alleles (A_2) for white eyes. All offspring are heterozygous (A_1A_2) with red eyes because A_1 is the dominant allele. *Note:* This model is used for comparison and does not represent the actual chromosome arrangement in male flies, which are hemizygous for X-linked traits.

Next, Morgan crossed the F_1 offspring with each other. The results in the second generation (F_2) were surprising:

Phenotype	*Observed Number*
Red-eyed females	2,459
Red-eyed males	1,011
White-eyed males	782
White-eyed females	0

Only the males exhibited white eyes! The results did not align with Mendel's predictions (see Figure 2, next page). A linkage between a non-sexual trait such as eye color and sex had never been observed. Morgan wondered why this particular trait appeared only in males.

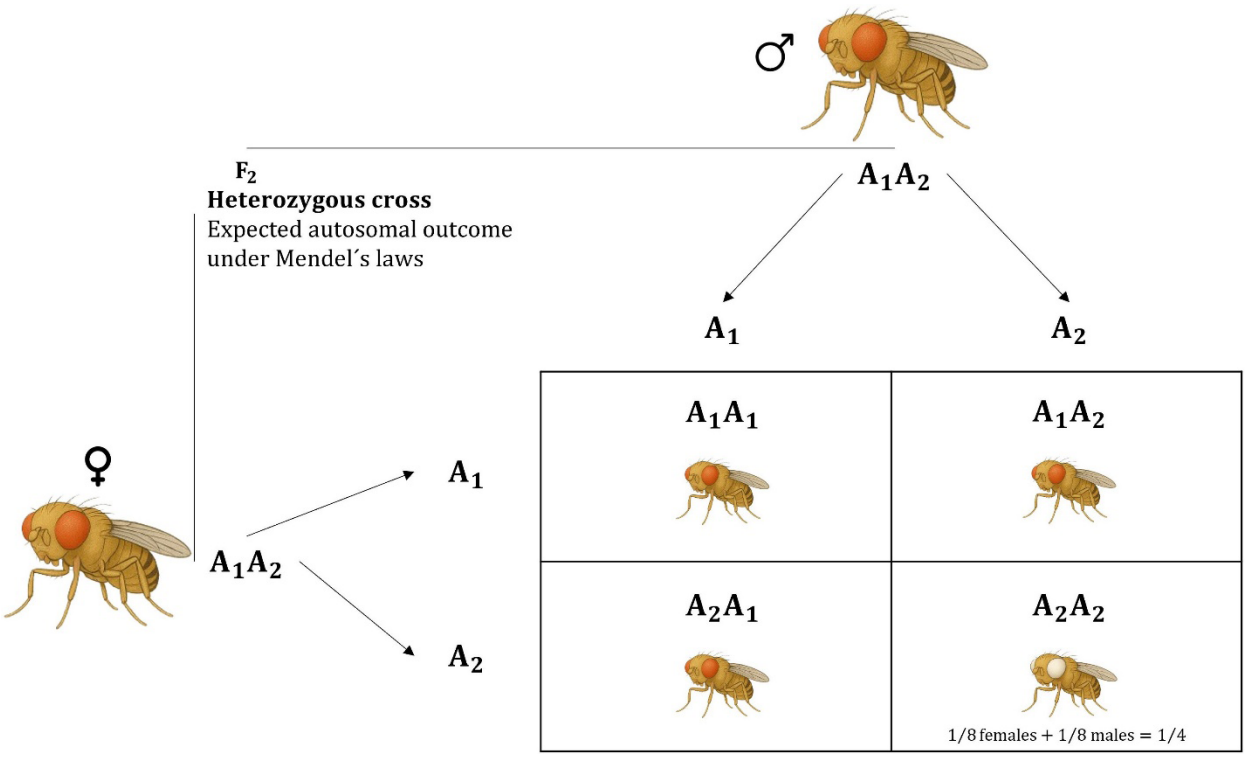

Figure 2. Expected genotypes of offspring from two heterozygous individuals (A_1A_2) of the F_1 generation based on Mendelian genetics. Note that for traits found on autosomes, Mendelian inheritance predicts approximately equal numbers of males and females, such as shown in homozygous recessives.

Questions

1. According to Mendelian inheritance, how would a recessive trait be expected to segregate?

2. What genetic hypothesis could explain the absence of white-eyed females?

3. Explain what an autosome is and why this cross cannot be explained by autosomal inheritance.

Part III – The Decisive Cross

As you discovered in Part II, interestingly, when Morgan crossed the red-eyed heterozygous F_1 flies, the traits in the F_2 offspring did not assort independently (they depended on sex) nor did they behave as a simple recessive trait. This clear deviation from Mendelian genetics seemed to follow the pattern of known sex chromosomes: X and Y.

In *D. melanogaster,* females have two X chromosomes (XX) and males have one X and one Y (XY) (Figure 3). Based on the unexpected inheritance patterns he had observed, Morgan hypothesized that the trait was linked to a sex chromosome.

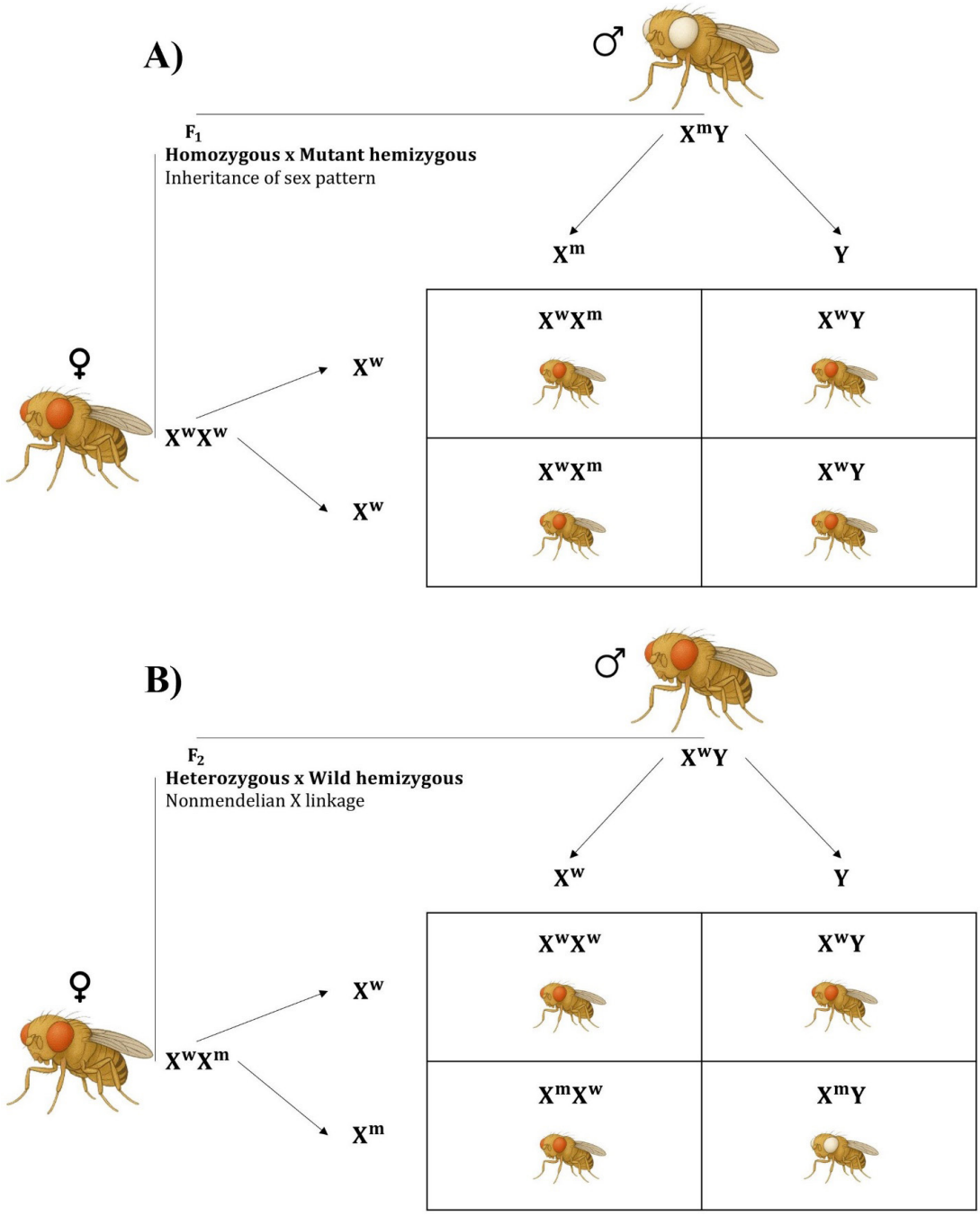

Figure 3. Panel A: Offspring observed by Morgan in the F_1 generation, following the inheritance pattern of sex chromosomes (X and Y) for wild-type (w) and mutant-type (m) alleles. *Panel B:* Offspring in the F_2 generation between a heterozygous dominant female (X^wX^m) and a male with the wild-type allele (X^wY). Hemizygous (hemi = half) describes an organism that has only one copy of a gene instead of the usual two.

To evaluate his new hypothesis, Morgan conducted another test cross. This time he crossed the original white-eyed male with an F_1 red-eyed heterozygous female. Results from this cross (F_2) were:

Phenotype	*Observed Number*
Red-eyed females	129
Red-eyed males	132
White-eyed females	88
White-eyed males	86

White-eyed females now appeared! This confirmed that the trait could be expressed in females, but only under the conditions suggested by Morgan: a female needs to acquire two copies of the white-eye allele on her X chromosomes.

In other words, if the Y chromosome does not carry information about eye color, this explains why males who inherit a single X with the mutant allele directly express the trait, while females need two recessive alleles to show it (Figure 4).

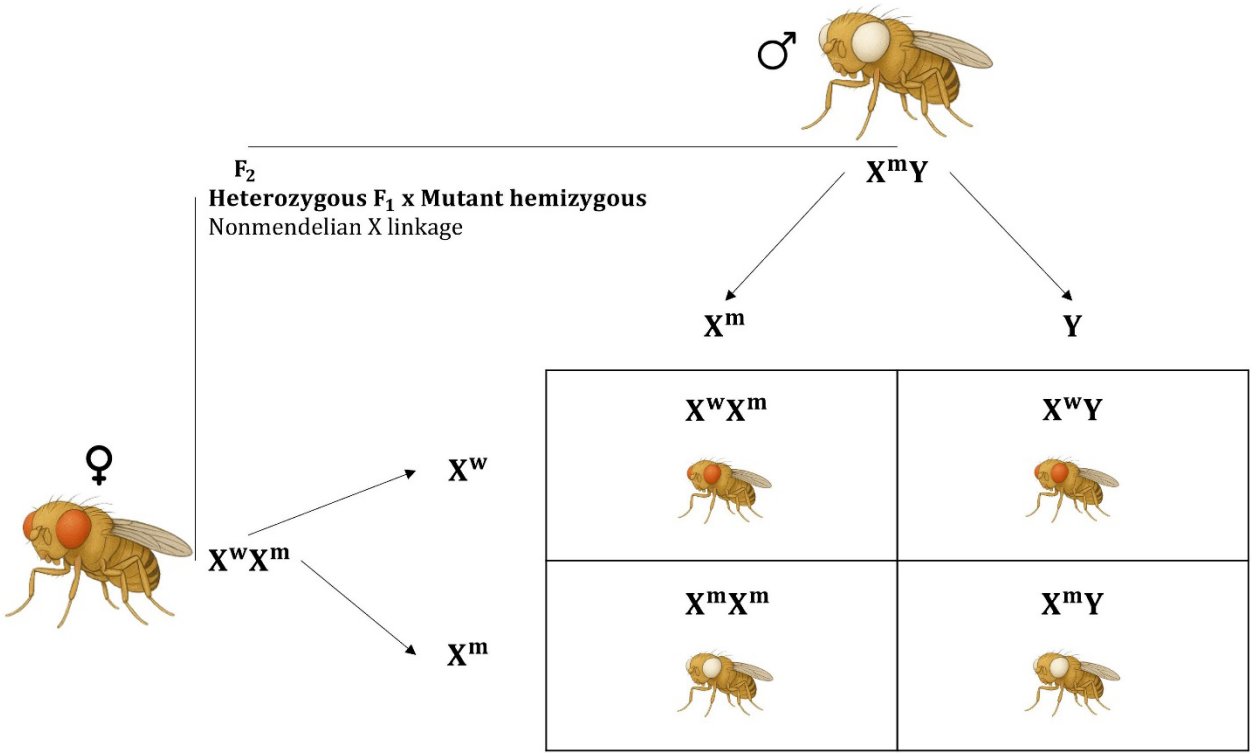

Figure 4. Cross confirming the linkage of the eye color gene to the X chromosome. Although this cross predicts equal numbers of XX females and XY males expressing the mutant allele, it is important to note that males include individuals from the F_1 generation who also showed the condition, making the mutant allele more frequently expressed in males than females, a characteristic of recessive X-linked inheritance.

Questions

1. How do the expected Mendelian F_2 results differ from those obtained by Morgan?

2. Why do females need two recessive alleles to show white eyes, while males need only one?

Part IV – X-Linked Inheritance

The gene responsible for eye color in *D. melanogaster* is linked to the X sex chromosome. This discovery showed that some traits could depend on sex and also led to a bold hypothesis: *genes are physically located on chromosomes and can be inherited together.*

Morgan, along with his students Alfred Sturtevant, Calvin Bridges and Hermann Müller, began to observe that certain traits were inherited together more frequently than Mendel's laws predicted. This led to the concept of genetic linkage, which proposes the following: *when two or more genes are located very close to one another on the same chromosome, they tend to be inherited as a unit rather than independently during meiosis*.

In other words, linked genes share a DNA strand and due to their proximity, have a lower chance of being separated by genetic recombination. The closer they are, the less likely they are to separate. This concept helped clarify inheritance in general, and also the transmission of X-linked genes. Thanks to this, two inheritance X-linkage mechanisms were identified:

- *X-linked Recessive Inheritance*
  - In males (with only one X chromosome), a single defective copy of an X-linked gene is enough to manifest a condition.
  - In females (with two X chromosomes), two defective copies are needed. If only one is defective, they are usually asymptomatic carriers or have mild symptoms.
- *X-linked Dominant Inheritance*
  - A single mutated gene copy on the X chromosome is enough to manifest the condition in both sexes.
  - A female inheriting the mutated gene from one parent will express it and can pass it on to offspring.
  - A male with the mutated gene will pass it on to all his daughters but none of his sons (since sons inherit the Y chromosome).

Questions

1. How would you experimentally differentiate dominant from recessive X-linked inheritance?

2. What would happen if the allele were X-linked dominant instead of recessive?

Part V – Confirming the Chromosome Theory and Conclusion

The evidence was clear: the gene for eye color in *Drosophila melanogaster* was linked to the X chromosome, the same chromosome that determines female characteristics. This was the first documented case of sex-linked inheritance in an experimental organism and provided concrete evidence supporting the chromosome theory of inheritance.

Until then, Morgan had been skeptical of the idea that genes were physically located on chromosomes. But his results with *D. melanogaster* led him to reconsider. The direct association between a specific gene (eye color) and a specific chromosome (X) offered strong empirical support for the hypothesis proposed by Sutton and Boveri a decade earlier.

Morgan, based on his own data, concluded that the only possible interpretation was that the eye color gene is located on the X chromosome. Therefore, genes must have a specific physical location on chromosomes.

This discovery marked a turning point in genetics. It not only validated the chromosome theory but also opened the door to later studies on genetic linkage and gene mapping, which his team would pursue in subsequent years, with student Alfred Sturtevant playing a key role.

Questions

1. How do these results support Sutton and Boveri's hypothesis?

2. Why was this discovery so important for the development of genetics?

3. Investigate the following: what practical applications did this model have in subsequent research?