E. B. Wilson. This discovery coincided closely with the emergence of Mendelism and stimulated research on the possible relationships between Mendel's principles and the meiotic behavior of chromosomes.

KEY POINTS

- Individual chromosomes become visible during cell division; between divisions they form a diffuse network of fibers called chromatin.
- Diploid somatic cells have twice as many chromosomes as haploid gametes.
- The cells of males and females may have different numbers of X and Y sex chromosomes; however, the number of autosomes in these cells is the same.

THE CHROMOSOME THEORY OF HEREDITY

Studies on the inheritance of a sex-linked trait in *Drosophila* provided the first evidence that the meiotic behavior of chromosomes is the basis for Mendel's Principles of Segregation and Independent Assortment.

By 1910 many biologists suspected that genes were situated on chromosomes, but they did not have definitive proof. Researchers needed to find a gene that could be unambiguously linked to a chromosome. This goal required that the gene be defined by a mutant allele and that the chromosome be morphologically distinguishable. Furthermore, the pattern of gene transmission had to reflect the chromosome's behavior during reproduction. All these requirements were fulfilled when the American biologist Thomas H. Morgan discovered a particular eye color mutation in the fruit fly, *Drosophila melanogaster*. Morgan began experimentation with this species of fly about 1909. It was ideally suited for genetics research because it reproduced quickly and prolifically and was inexpensive to rear in the laboratory. In addition, it had only four pairs of chromosomes, one being a pair of sex chromosomes—XX in the female and XY in the male. The X and Y chromosomes were morphologically distinguishable from each other and from each of the autosomes. Through careful experiments, Morgan was able to show that the eye color mutation was inherited along with the X chromosome, suggesting that a gene for eye color was physically situated on that chromosome. Later, one of his students, Calvin B. Bridges, obtained definitive proof for this Chromosome Theory of Heredity.

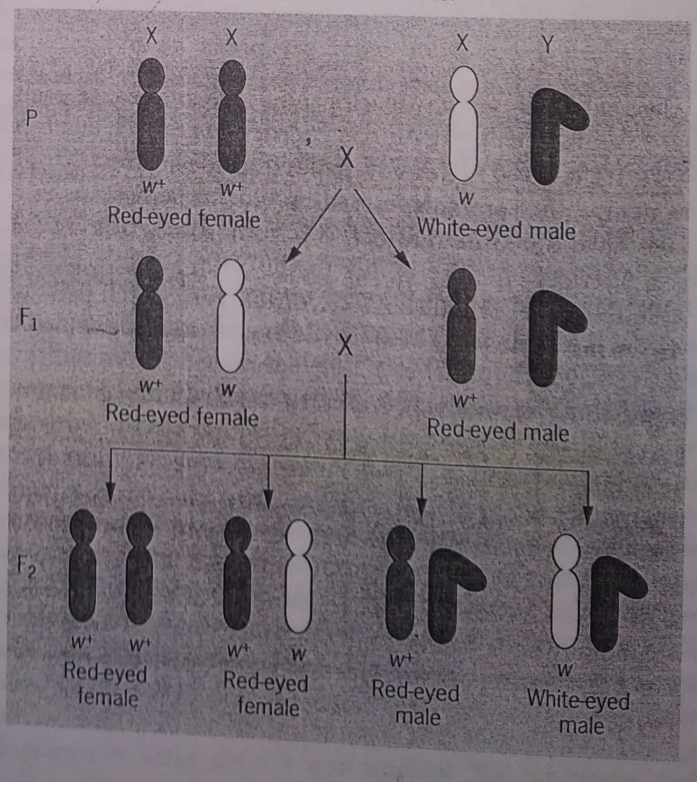
Experimental Evidence Linking the Inheritance of Genes to Chromosomes

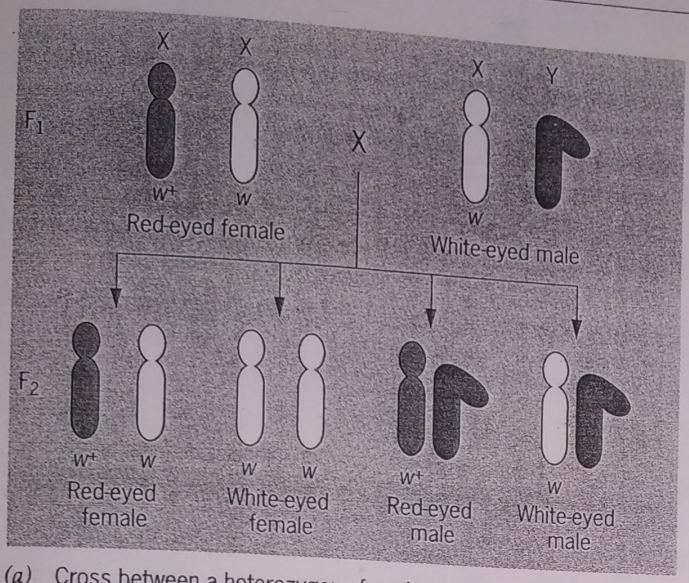
Morgan's experiments commenced with his discovery of a mutant male fly that had white eyes instead of the red eyes of wild-type flies. When this male was crossed to wild-type females, all the progeny had red eyes, indicating that white was recessive to red. When these progeny were intercrossed with each other, Morgan observed a peculiar segregation pattern: all of the daughters, but only half of the sons, had red eyes; the other half of the sons had white eyes. This pattern suggested that the inheritance of eye color was linked to the sex chromosomes. Morgan proposed that a gene for eye color was present on the X chromosome, but not on the Y, and that the white and red phenotypes were due to two different alleles, a mutant allele denoted w and a wild-type allele denoted w^+.

Morgan's hypothesis is diagrammed in Figure 5.3. The wild-type females in the first cross are assumed to be homozygous for the w^+ allele. Their mate is assumed to carry the mutant w allele on its X chromosome and neither of the alleles on its Y chromosome. An organism that has only one copy of a gene is called a **hemizygote.** Among the progeny from the cross, the sons inherit an X chromosome from their mother and a Y chromosome from their father; because the maternally inherited X carries the w^+ allele, these sons have red eyes. The daughters, in contrast, inherit an X chromosome from each parent—an X with w^+ from the mother and an X with w from the father. However, because w^+ is dominant to w, these heterozygous F_1 females also have red eyes.

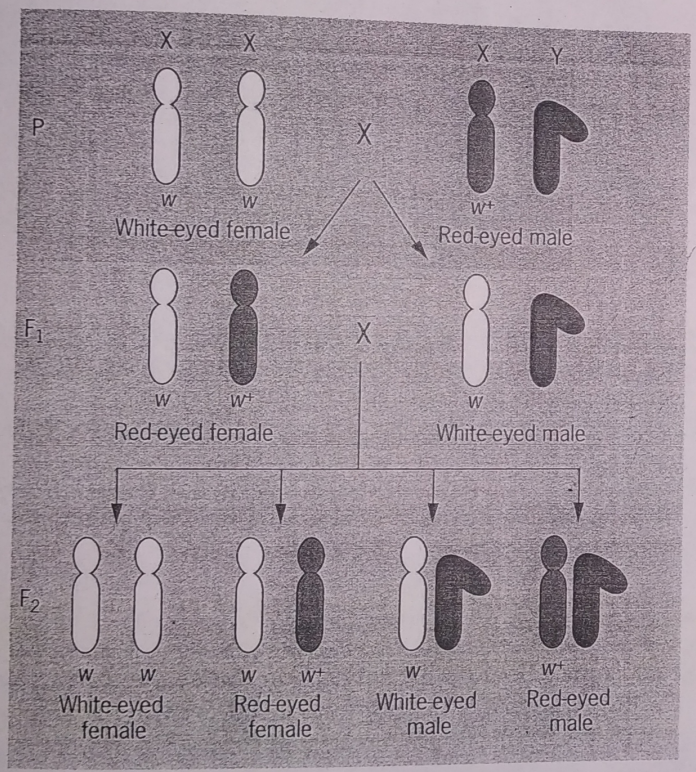
When the F_1 males and females are intercrossed, four genotypic classes of progeny are produced, each representing a different combination of sex chromosomes. The XX flies, which are female, have red eyes because at least one w^+ allele is present.

Figure 5.3 ■ Morgan's experiment studying the inheritance of white eyes in *Drosophila*. The transmission of the mutant condition in association with sex suggested that the gene for eye color was present on the X chromosome but not on the Y chromosome.





(a) Cross between a heterozygous female and a hemizygous mutant male.



(b) Cross between a homozygous mutant female and a hemizygous wild-type male.

Figure 5.4 ■ Experimental tests of Morgan's hypothesis that the gene for eye color in *Drosophila* is X-linked. In each experiment, eye color is inherited along with the X chromosome. Thus, the results of these crosses supported Morgan's hypothesis that the gene for eye color is X-linked.

The XY flies, which are male, have either red or white eyes, depending on which X chromosome is inherited from the heterozygous F_1 females. Segregation of the *w* and w^+ alleles in these females is therefore the reason half the F_2 males have white eyes.

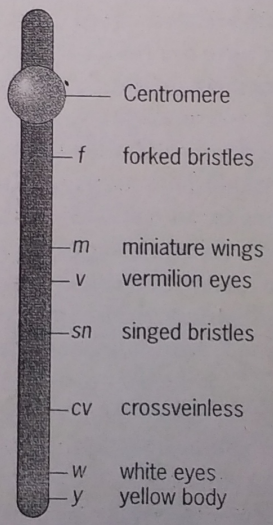
Morgan carried out additional experiments to confirm the elements of his hypothesis. In one (Figure 5.4*a*), he crossed F_1 females assumed to be heterozygous for the eye color gene to mutant white males. As he expected, half the progeny of each sex had white eyes, and the other half had red eyes. In another experiment (Figure 5.4*b*), he crossed white-eyed females to red-eyed males. This time, all the daughters had red eyes, and all the sons had white eyes. When he intercrossed these progeny, Morgan observed the expected segregation: half the progeny of each sex had white eyes, and the other half had red eyes. Thus, Morgan's hypothesis that the gene for eye color was linked to the X chromosome withstood additional experimental testing.

Chromosomes as Arrays of Genes

Morgan and his students soon identified other X-linked genes in *Drosophila*. In each case, simple breeding experiments demonstrated that recessive mutations of these genes were transmitted along with the X chromosome. As the evidence accumulated, it became clear that many genes were located on the X chromosome. However, Morgan's research group also identified genes that were not on the X chromosome. These genes followed the Mendelian Principle of Segregation, but they did not segregate with sex, as the gene for eye color did. Morgan correctly concluded that such genes were located on one of the three autosomes in the *Drosophila* genome. Thus, each *Drosophila* chromosome appeared to contain a different set of genes.

Morgan's laboratory then attempted to determine the relationships among the genes on a particular chromosome. They proceeded on the assumption that the genes were arranged in a linear array—an idea inspired by cytological evidence that the chromosome was a long, thin thread. In just a few years, Morgan's students were able to show that genes were indeed situated at different sites, or loci (from the Latin word for "place"; singular; locus), on a linear structure. This analysis, which we will discuss in Chapter 7, produced the world's first genetic maps—diagrams showing the positions of genes and the relative distances between them (Figure 5.5). Morgan's laboratory pioneered the methods for genetic mapmaking and

Figure 5.5 ■ A map of genes on the X chromosome of *Drosophila*.



laid the foundation for subsequent research on the physical structure of chromosomes. Eventually, the linearity of chromosomes was connected to the linear structure of DNA (see Chapter 9).

These early studies with *Drosophila*—primarily the work of Morgan and his students (see A Milestone in Genetics: Morgan's Fly Room at the end of this chapter)—greatly strengthened the view that all genes were located on chromosomes and that Mendel's principles could be explained by the transmissional properties of chromosomes during reproduction. This idea, called the **Chromosome Theory of Heredity**, stands as one of the most important achievements in biology. Since its formulation in the early part of the twentieth century, the Chromosome Theory of Heredity has provided a unifying framework for all studies of inheritance.

Nondisjunction as Proof of the Chromosome Theory

Morgan showed that a gene for eye color was on the X chromosome of *Drosophila* by correlating the inheritance of that gene with the transmission of the X chromosome during reproduction. However, as noted earlier, it was one of his students, C. B. Bridges, who secured proof of the chromosome theory by showing that exceptions to the rules of inheritance could also be explained by chromosome behavior.

Bridges performed one of Morgan's experiments on a larger scale. He crossed white-eyed female *Drosophila* to red-eyed males and examined many F_1 progeny. Although as expected, nearly all the F_1 flies were either red-eyed females or white-eyed males, Bridges found a few exceptional flies—white-eyed females and red-eyed males. He crossed these exceptions to determine how they might have arisen. The exceptional males all proved to be sterile; however, the exceptional females were fertile, and when crossed to normal red-eyed males, they produced many progeny, including large numbers of white-eyed daughters and red-eyed sons. Thus, the exceptional F_1 females, though rare in their own right, were prone to produce many exceptional progeny.

Bridges explained these results by proposing that the exceptional F_1 flies were the result of abnormal X chromosome behavior during meiosis in the females of the P generation. Ordinarily, the X chromosomes in these females should **disjoin,** or separate from each other, during meiosis. Occasionally, however, they might fail to separate, producing an egg with two X chromosomes or an egg with no X chromosome at all. Fertilization of such abnormal eggs by normal sperm would produce zygotes with an abnormal number of sex chromosomes. Figure 5.6 illustrates the possibilities.

If an egg with two X chromosomes (usually called a diplo-X egg; genotype X^wX^w) is fertilized by a Y-bearing sperm, the zygote will be X^wX^wY. Since each of the X chromosomes in this zygote carries a mutant w allele, the resulting fly will have white eyes. If an egg without an X chromosome (usually called a nullo-X egg) is fertilized by an X-bearing sperm (X^+), the zygote will be X^+O. (Once again, "O" denotes the absence of a chromosome.) Because the single X in this zygote carries a w^+ allele, the zygote will develop into a red-eyed fly. Bridges inferred that XXY flies were female and that XO flies were male. The exceptional white-eyed females that he observed were therefore X^wX^wY, and the exceptional red-eyed males were X^+O. Bridges confirmed the chromosome constitutions of these exceptional flies by direct cytological observation. Because the XO animals were male, Bridges concluded that in *Drosophila* the Y chromosome has nothing to do with the determination of the sexual phenotype. However, because the XO males were always sterile, he realized that this chromosome must be important for male sexual function.

Bridges recognized that the fertilization of abnormal eggs by normal sperm could produce two additional kinds of zygotes: $X^wX^wX^+$, arising from the union of a diplo-X egg and an X-bearing sperm, and YO, arising from the union of a nullo-X egg and a Y-bearing sperm. The $X^wX^wX^+$ zygotes develop into females that are red-eyed, but weak and sickly.

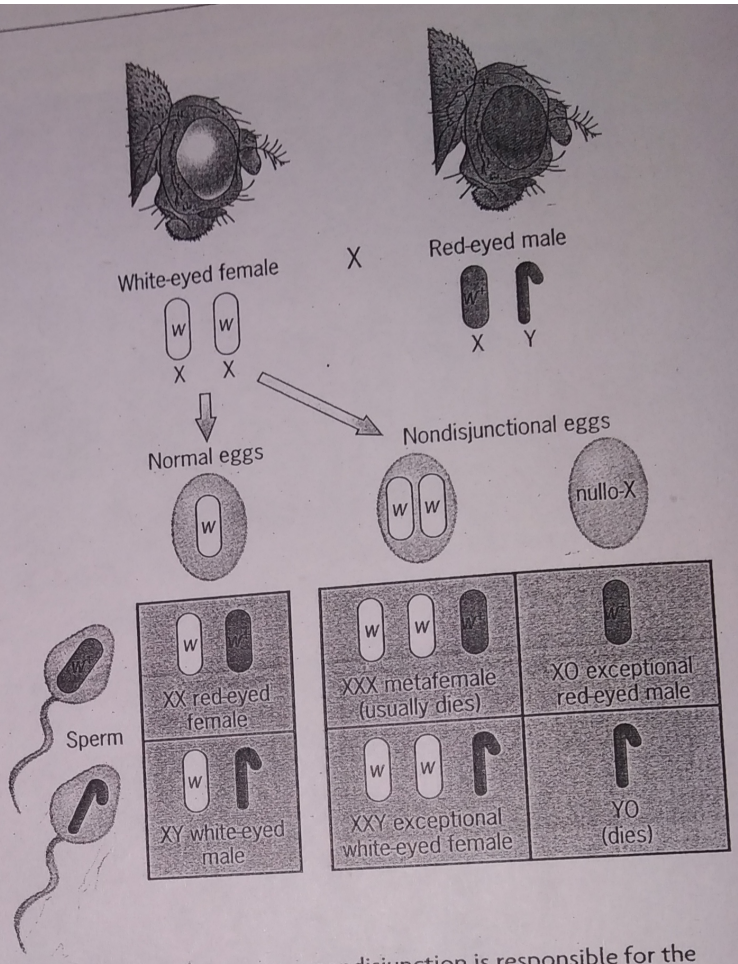


Figure 5.6 ■ X chromosome nondisjunction is responsible for the exceptional progeny that appeared in Bridges' experiment. Nondisjunctional eggs that contain either two X chromosomes or no X chromosome unite with normal sperm that contain either an X chromosome or a Y chromosome to produce four types of zygotes. The XXY zygotes develop into white-eyed females, the XO zygotes develop into red-eyed, sterile males, and the XXX and YO zygotes die.

These "metafemales" can be distinguished from XX females by a syndrome of anatomical abnormalities, including ragged wings and etched abdomens. Generations of geneticists have inappropriately called them "superfemales"—a term coined by Bridges—even though there is nothing super about them. The *YO* zygotes turn out to be completely inviable; that is, they die. In *Drosophila*, as in most other organisms with sex chromosomes, at least one X chromosome is needed for viability.

Bridges' ability to explain the exceptional progeny that came from these crosses showed the power of the chromosome theory. Each of the exceptions was due to anomalous chromosome behavior during meiosis. Bridges called the anomaly **nondisjunction** because it involved a failure of the chromosomes to disjoin during one of the meiotic divisions. This failure could result from faulty chromosome movement, imprecise or incomplete pairing, or centromere malfunction. From Bridges' data, it is impossible to specify the exact cause. However, Bridges did note that the exceptional XXY females go on to produce a high frequency of exceptional progeny, presumably because their sex chromosomes can disjoin in different ways: the X chromosomes can disjoin from each other, or either X can disjoin from the Y. In the latter case, a diplo- or nullo-X egg is produced because the X that does not disjoin from the Y is free to move to either pole during the first meiotic division. When fertilized by normal sperm, these abnormal eggs will produce exceptional zygotes.

Bridges observed the effects of chromosome nondisjunction that had occurred during meiosis in females. We should note, however, that with appropriate experiments the effects of nondisjunction during meiosis in males can also be studied.

The Chromosomal Basis of Mendel's Principles of Segregation and Independent Assortment

Mendel established two principles of genetic transmission: (1) the alleles of a gene segregate from each other, and (2) the alleles of different genes assort independently. The finding that genes are located on chromosomes made it possible to explain these principles (as well as exceptions to them) in terms of the meiotic behavior of chromosomes.

The Principle of Segregation

(Figure 5.7). During the first meiotic division, homologous chromosomes pair. One of the homologues comes from the mother, the other from the father. If the mother was homozygous for an allele, *A*, of a gene on this chromosome, and the father was homozygous for a different allele, *a*, of the same gene, the offspring must be heterozygous, that is, *Aa*. In the anaphase of the first meiotic division, the paired chromosomes separate and move to opposite poles of the cell. One carries allele *A* and the other allele *a*. This physical separation of the two