CHAPTER 4

Nettie Stevens & the Problem of Sex Determination

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☐ INTRODUCTION

We may think that we know about the birds and the bees, but animal sexuality takes many unusual forms. Anemone fish, which live on coral reefs, begin life as males but later develop into females. In other coral reef fish, called wrasses, the sequence is reversed. A single male lives with several smaller females. If the male dies, the largest female takes his place. Within days she begins to produce sperm instead of eggs. In sea bass, each individual is both male and female. Although the sea bass has a combined testis and ovary, mating still occurs and an individual almost never fertilizes its own eggs. On the other hand, whiptail lizards, found in the southwestern United States, are always female. These lizards reproduce asexually through the development of unfertilized eggs. Sexual behavior would seem to be useless in this case, but whiptail lizards court and mate much like their sexually reproducing relatives. Before ovulation females behave like females, but after laying eggs they behave like males. Apparently male behavior still serves an important function in whiptail lizards, because isolated females lay fewer eggs than those who engage in pseudosexual intercourse with other females.

Unusual forms of animal sexuality challenge our commonsense notions of what it means to be male or female. These phenomena remind us that sexuality is a complex combination of anatomical, physiological, and behavioral characteristics. They also raise one of the most fundamental questions in biology: What determines sex?

Biologists have always been intrigued by this question, but sex determination generated particular interest around 1900. Perhaps this interest was partly due to the social climate of the times. The close of the Victorian era, "the sexless age," brought a more liberal attitude toward sexual matters. Important scientific advances also made sex determination interesting. The optical quality of microscopes had improved dramatically during the nineteenth century, allowing biologists to observe the nucleus, chromosomes, and other cellular structures with great clarity. By 1900 many biologists were convinced that studying cells—sperm, eggs, and zygotes—was the key to understanding sex. The rediscovery of Gregor Mendel's work on heredity added another new dimension to the study of sex. Was sex inherited according to Mendel's laws? How were patterns of inheritance related to the processes of cell division and fertilization? How did male and female characteristics develop in the embryo?

ALTERNATIVE THEORIES OF SEX DETERMINATION

At the turn of the century, several theories attempted to explain how individuals become male or female. None of these explanations was satisfactory. Supporting data were ambiguous, and biologists disagreed on what specific problems were most important. For example, some biologists were interested in how sex is inherited, while others were more interested in how sexual characteristics develop in the embryo. Not surprisingly, there were also major disagreements concerning research methods, basic assumptions, and philosophical implications of various theories. This situation might appear chaotic, but the very uncertainty surrounding sex determination appealed to ambitious biologists. With perseverance, hard work, and a bit of luck, a scientist might make a discovery of fundamental importance.

Before 1900, most biologists were "externalists." They believed that sex was caused by interactions between a sexually undifferentiated embryo and its external environment. As the embryo developed, it became male or female as a result of these interactions. How did this occur? Experimenting with tiny aquatic animals called rotifers, one influential biologist concluded that temperature was the controlling factor. At high temperatures most rotifers became males, while at lower temperatures nearly all became female. Other biologists claimed that nutrition influenced sex determination. Well-fed caterpillars almost always became females, but malnourished caterpillars usually became males.

Externalist theories were enormously popular around 1900. Not only were they supported by experimental evidence, but they also reflected the popular philosophical position that adult structures develop from scratch in the embryo (**epigenesis**). **Preformationism**, the rival belief that adult structures are already present in miniature form in the egg or sperm, had been widely rejected. It was perhaps natural, therefore, that many biologists believed that sex was undetermined in the zygote and gradually emerged as the developing embryo interacted with its surrounding environment.

Unfortunately for externalists, experimental results are usually open to more than one interpretation. Most of the externalists' experiments were conducted on whole populations rather than individual organisms. Externalists were, therefore, incapable of predicting whether any particular individual would become male or female. Critics quickly pointed out that altered sex ratios in populations could also be explained by selective mortality. The fact that, at lower temperatures, rotifer populations contained mostly females might simply mean that many embryonic males died in the cold. Critics also complained that almost any environmental factor seemed to affect sex ratios. Was there one environmental cause of sex determination, or many?

PROBLEM

Design a simple experiment deciding whether selective mortality is responsible for altered sex ratios in the populations of rotifers grown at two different temperatures.

The rediscovery of Gregor Mendel's work in 1900 stimulated considerable interest in studying patterns of inheritance. Some biologists, "Mendelian internalists," enthusiastically argued that sex was inherited according to Mendel's principles. Some Mendelians claimed that every gamete carried a sex-determining factor (what we would now call a **gene**). Each egg or sperm carried either a male or a female factor. The combination of factors in the zygote determined the sex of the offspring. Critics immediately pointed out that if this were true, then sex ought to be inherited according to Mendel's 3:1 phenotypic ratio. Mendelians responded by hypothesizing selective fertilization; certain combinations of gametes were more likely than others.

PROBLEM

Suppose that sex is a simple Mendelian characteristic determined by two hereditary factors, one for maleness and one for femaleness. Based on this assumption, is it possible to account for the 1:1 sex ratio found in most species of animals? Can the hypothesis of selective fertilization help explain this sex ratio?

Mendelian theory appealed to many biologists, but it had great difficulty explaining how sex could be inherited. An obvious weakness was accounting for the 1:1 sex ratio found in most species. If selective fertilization occurred, it ought to be observable, but sperm and eggs seemed to combine randomly. More serious, perhaps, the Mendelians could not adequately explain what the sex-determining factors were, where they were found in the cell, or exactly how they caused an individual to become male or female. Critics insisted that Mendelian factors must have a physical existence, but in 1900 this could not be demonstrated. The very idea of sex-determining "factors" smacked of preformationism, because it suggested that the sex of an egg, sperm, or zygote was set even before development began. This struck critics as a retreat to discredited theories of the past.

Some biologists ("non-Mendelian internalists") tried to find a middle ground between the externalists and the Mendelians. Like the externalists, they believed that sex determination must be understood as a gradual, developmental process. Also like the externalists, they were highly suspicious of the invisible Mendelian "factors." Unlike the externalists, however, these biologists looked for the causes of sex determination inside the embryo. Primarily trained as embryologists, they believed that complex physiological changes in the nucleus or cytoplasm caused embryos to become male or female.

Non-Mendelian internalism was particularly popular in the United States. It attracted prominent biologists such as Thomas Hunt Morgan and Edward Beecher Wilson. Both of these men later became champions of the theory of sex chromosomes, but in 1900 they were leading critics of Mendelism. They changed their minds about sex chromosomes partly as a result of important discoveries made by their student and colleague: Nettie Maria Stevens.

THE MAKING OF A CELL BIOLOGIST

The dawning of the twentieth century brought many new opportunities to aspiring professional women in the United States. Although they would not be guaranteed the right to vote for another two decades, women in 1900 were beginning to enter professions that had previously been closed to them. Talented women, routinely denied educational opportunities during the nineteenth century, could now pursue graduate training at some of the best universities in the nation. This opened new possibilities for careers in science, where jobs were becoming increasingly specialized. Women could pursue both research and teaching, although they continued to face many barriers to promotion and professional advancement.

The opportunities and constraints facing women in science during the early twentieth century are illustrated by the career of Nettie Stevens (Figure 4.1). As a young woman, Stevens taught high school and was a librarian, traditional occupations for unmarried women during the late nineteenth century. Her teaching duties included courses in physiology and zoology, as well as mathematics, Latin, and English. Her interest in zoology may have been encouraged by summer field biology courses that she took at the seashore near Martha's Vineyard during the early 1890s. In 1896, at the age of 35, Stevens entered college at Stanford, one of several new universities that admitted women. After receiving her B.A. in 1899 and her M.A. in 1900, Stevens left California to become a doctoral student at Bryn Mawr College.

It might seem odd that an aspiring scientist should choose a small women's college for graduate training, but Bryn Mawr was an excellent choice for Stevens. The biology department had developed a national reputation under the leadership of Edmund Beecher Wilson, perhaps the leading cell biologist in the United States. Although Wilson had moved on to Columbia University, he maintained close ties with his former department. Wilson's place at Bryn Mawr was taken by his friend, Thomas Hunt Morgan, who was already a prominent biologist, well-known for his studies on animal heredity and development (see Chapter 5).

Working with Morgan, who was five years younger than she was, Stevens enjoyed opportunities that women before her did not have. Although she collaborated with Morgan on some work, Stevens also conducted independent research. Through Morgan's influence, Stevens was able to spend two summers in Europe working in the

FIGURE 4.1 Nettie Maria Stevens. *Source:* The Carnegie Institution of Washington.

laboratory of Theodor Boveri at the prestigious Naples Zoological Station. At the time, Boveri was one of the world's leading experts on chromosomes. Stevens also went to work at the Marine Biological Laboratory at Woods Hole on Cape Cod, where Morgan, Wilson, and other leading American biologists spent their summers doing research.

By the time she completed her Ph.D. in 1903, Stevens was a seasoned scientist. She had already written several scientific papers for leading biological journals. The instructorship at her alma mater, which she took immediately after graduation, involved heavy teaching responsibilities, but it provided a laboratory for her research. Fortunately, Stevens won research grants from one of the most prestigious scientific foundations in the United States: The Carnegie Institution of Washington. This financial support allowed her some free time to pursue research directed at one of the great biological questions of the day: how are chromosomes involved in sex determination?

CHROMOSOMES, ACCESSORY CHROMOSOMES, AND SEX CHROMOSOMES

Today we take for granted that chromosomes carry the units of heredity (genes), but establishing this fact was not a straightforward process of discovery. The term **chromosome** was first used in 1888 to describe the tiny, threadlike structures in the nucleus that many cell biologists had studied during the preceding decade. The movements of chromosomes during cell division and their significance for both sexual and asexual reproduction became major scientific problems. By 1900, the processes of mitosis, meiosis, and fertilization had been accurately described in both plants and animals. There was considerable disagreement about the function of chromosomes, however. If you reflect upon our current understanding of how chromosomes work, you will realize that very little of this knowledge comes from direct observation. Thus the development of a satisfactory theory of chromosomes involved considerable speculation, as well as the piecing together of fragmentary evidence. Two controversial hypotheses were particularly important for guiding Nettie Stevens's research.

Biologists knew that chromosomes usually come in pairs, the members of which have the same size, shape, and placement of centromeres. During the late 1890s, cell biologists discovered that unpaired chromosomes are also sometimes found in cells. What we now call the X chromosome was referred to as the "accessory chromosome," and in 1902 one of Wilson's students, Clarence E. McClung, implicated it in sex determination. Thinking that it was found only in some sperm (and never in eggs), McClung speculated that if a zygote received the accessory chromosome, it became male; if not, it became female (Figure 4.2). This, of course, turned out to be incorrect, but it was an important hypothesis for two reasons. First, it stimulated considerable interest in studying the relationship between chromosomes and sex. Second, in suggesting that there are two types of sperm, McClung was on the right track.

A broader speculation was made by another of Wilson's students, Walter Sutton, and independently by the German biologist Theodor Boveri. According to the Sutton-Boveri hypothesis, chromosomes maintain their individuality and physical integrity even when they are not visible. This was a controversial claim because many biologists believed that chromosomes formed more or less randomly before each cell division. Sutton also emphasized the striking parallel between Mendel's law of segregation, which applied to hereditary factors, and the separation of chromo-

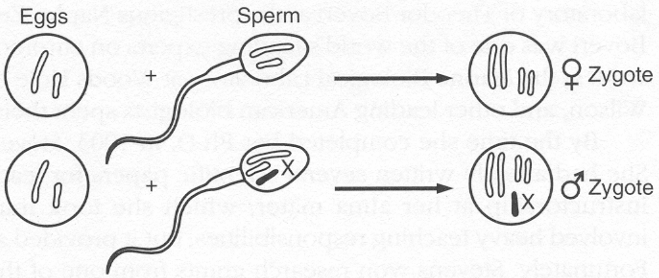

FIGURE 4.2 Clarence E. McClung's hypothesis of sex determination by accessory chromosomes. McClung claimed zygotes with an accessory (X) chromosome became males; those without the accessory (X) chromosome became females. Later studies refuted McClung's hypothesis.

some pairs during meiosis. Did chromosomes carry Mendelian factors? If so, did the accessory chromosome carry a sex-determining factor?

Both Mendelians and non-Mendelians studied chromosomes, and in the years following 1900 both groups realized that these nuclear structures were important for understanding sex determination. The two groups, however, tended to interpret the results of their studies differently. Mendelians claimed that chromosomes *caused* heredity, because they carried the hereditary factors postulated by Mendel. Because it carried the Mendelian factor for sex, the "accessory chromosome" was really a "sex chromosome." Non-Mendelian internalists such as Morgan and Wilson were more cautious, admitting only that chromosomes were somehow *correlated* with heredity. Perhaps it was the case that the accessory chromosome simply acted as a "marker." The chromosome indicated the sex of an individual, but it did not actually cause the individual to be male or female. In 1903, when Nettie Stevens earned her Ph.D., the exact nature of chromosomes and their role in sex determination remained open questions.

STEVENS'S STUDIES ON SEX DETERMINATION

The uncertainty over sex determination is borne out by Stevens's early research. Together with Morgan, she did experiments on aphids to test the claim that temperature alters sex ratios in populations. These experiments failed to confirm the externalists' hypothesis. In her early cellular studies of aphids (which she did by herself), Stevens also failed to detect McClung's accessory chromosomes. Thus, at the end of 1904, she concluded that although it seemed likely that sex was somehow determined by the eggs and sperm, exactly how it was determined remained unclear.

During the next two years, Stevens completed a comparative, cellular study of several species of insects, drawn from a diverse group of beetles (Coleoptera), butterflies and moths (Lepidoptera), and true bugs (Hemiptera). This was painstaking work. First she dissected the tiny gonads from the insects. These were fixed in a preservative solution, embedded in paraffin blocks, and sliced into very thin sections. The tissue sections were then mounted on microscope slides and stained with one of several dyes. Careful observations of the tissue sections revealed gametes in various stages of development. If a cell had been just beginning to divide and the tissue had been cut at just the right angle, all of the chromosomes were clearly visible and could be accurately counted.

In the common mealworm, *Tenebrio molitor*, Stevens found that the body cells of females contained 20 large chromosomes, while body cells in males contained 19 large chromosomes and 1 small chromosome (the Y chromosome). She also found that although mealworm eggs always contained 10 large chromosomes, there were two types of sperm: 50 percent contained 10 large chromosomes, 50 percent contained 9 large chromosomes plus the 1 small Y chromosome. This pattern was found in the other species that she studied except that, in a few species, the small (Y) chromosome was completely absent. In these cases, 50 percent of the sperm contained a set of chromosomes identical to that found in eggs, while in the other 50 percent the number of chromosomes was one fewer than that found in eggs. Stevens's results could be generalized as follows: for any given species, all eggs are the same, but there are two distinct types of sperm.

In her conclusion, Stevens pointed out that a male is produced whenever an egg is fertilized by a sperm carrying the small Y chromosome (or lacking this chromosome); a female is produced whenever an egg is fertilized by a sperm carrying the large X chromosome (Figures 4.3(A) and 4.3(B)). Having stated this conclusion, however, she refused to claim that the X and Y chromosomes could accurately be described as "sex chromosomes." These chromosomes seemed to play some hereditary role in sex determination, but how they did so was still unclear. In other words, Stevens had found an important correlation between chromosomes and sex determination, but she couldn't prove causation. She cautiously concluded that further evidence was needed before one could speak confidently of "sex chromosomes."

With the benefit of hindsight, Stevens's conclusion seems curiously conservative, but other prominent biologists were even more hesitant about drawing general conclusions from a relatively small sample of data. Wilson made similar observations of chromosomes and published his results at about the same time Stevens did. Like Stevens, he refused to endorse the idea of sex chromosomes. Although he admitted that chromosomes provided the best "working hypothesis" for explaining sex determination, he remained open to the possibility that they were simply indicators, rather than determiners of sex. Morgan was even more reluctant to accept the idea of sex chromosomes. Some of Stevens's contemporaries, particularly embryologists, never acknowledged that chromosomes might play a role in sex determination.

THE DISCOVERY OF SEX CHROMOSOMES

Looking back at Stevens's contribution to science after her death in 1912, Thomas Hunt Morgan claimed that biologists had been too cautious about sex chromosomes. But Morgan, and to a lesser extent Stevens and Wilson, were among this conservative group. Part of their conservatism stemmed from the need to confirm Stevens's hypothesis with data from other species. This turned out to be a vexing puzzle. In some groups (birds and butterflies), it turned out that eggs were heterogametic and sperm were homogametic (Figure 4.3(C)). The development of unfertilized eggs was

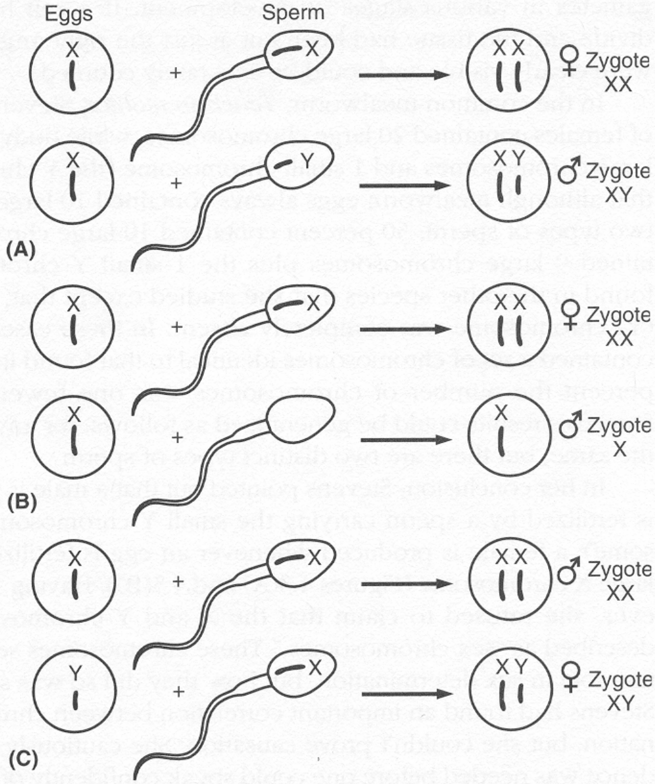

FIGURE 4.3 Sex determination by chromosomes as understood by Nettie Stevens and her contemporaries. (A) The XX, XY system found in mammals and some of the insects studied by Stevens. (B) In some other insects there is no Y chromosome; females have two sex chromosomes (XX), but males have only one (X). (C) Other biologists later discovered that in birds and some insects, the male is XX and the female is XY. Some cell biologists used the symbols WW and WZ to distinguish this from the XX, XY system.

also a major problem to be explained. In some species that reproduced asexually, unfertilized eggs developed into females, but in other species they developed into males. Numerous examples of individuals that had both male and female characteristics, also had to be explained. All of these pieces of the puzzle needed to fit together before many biologists accepted the idea of sex chromosomes.

From a historical perspective we can see that the "discovery" of sex chromosomes was not so much a single event as a period of transition in ideas about sex determination. In 1902 Clarence McClung first claimed that sex chromosomes existed, but the hypothesis that he put forward to explain sex determination turned out to be incorrect. During the next ten years, several biologists studied the accessory chromosomes in many species of animals and tested alternative hypotheses about sex determination. As a result, they gradually changed their views on how sex is determined. This was a major shift, for it meant changing the type of problem studied, the methods used, and some basic philosophical assumptions. Although biologists did not completely abandon the goal of explaining how sexual characteristics develop in the embryo, interest shifted to the problem of how sex is inherited. Stevens, Morgan, Wilson, and other biologists also had to set aside some of their fundamental philosophical commitments—for example, their fear that Mendelism would lead scientists backward to discredited preformationist ideas. As they made this intellectual shift, they also had to embrace a new set of scientific methods, particularly the experimental breeding of fruit flies (*Drosophila*) and other organisms. In 1914 Morgan was finally ready to present a comprehensive theory of sex determination by chromosomes in his popular book, *Heredity and Sex*.

Tragically, Nettie Stevens died of breast cancer in 1912, just when the idea of sex chromosomes was becoming well established. In the years that followed, her contribution to the discovery was often viewed as that of a data collector, whose careful observations were used by others (Morgan and Wilson) to create the chromosome theory of sex determination. This interpretation has now been rejected by most historians, who have pointed out her important theoretical contributions to this discovery. Several years before Morgan and probably several weeks before Wilson, she cautiously proposed an explanation for sex determination by chromosomes. For the rest of her short career, she continued to gather evidence to support this theory. Together with her two male colleagues, Stevens played a critical role in the discovery of sex chromosomes.

☐ EPILOGUE

This case study might be interpreted as the victory of Mendelism over two rival theories. It is well to remember, however, that throughout his life Morgan held out hope that a single theory would explain both the inheritance and development of sex. With the rise of molecular biology, studies of heredity and development have finally converged much as Morgan had hoped that they would. For example, scientists have recently discovered a male sex-determining region (SRY) on the Y chromosomes. The specific genes making up this SRY region, the proteins for which they code, and their developmental functions are important problems in current research.

We are also increasingly aware that being female or male involves more than simply the possession of XX or XY chromosomes (or the genes that they carry). Pieces of chromosome are sometimes lost or translocated to other chromosomes. As a result, we now realize that there are some XX males and XY females. Sexuality, it now appears, is a complex phenomenon involving sex organs, secondary sex characteristics, and behaviors that can only partly be explained in terms of genes and chromosomes. The physical, biological, and social environment also plays a crucial role.

If the environment is important, what about the discredited externalist explanations of sex determination? Early theories of environmental sex determination were based upon poorly controlled experiments and were rightly rejected by biologists. Perhaps there was more than a grain of truth in these incorrect ideas, however. Textbooks often omit this fact, but not all animals have sex chromosomes. Other genetic systems exist, and in some of these cases the environment plays an important sex-determining role. For example, in many species of reptiles environmental factors such as temperature act as developmental "switches." When eggs are incubated at some temperatures, males are produced; at other temperatures, females are produced. Temperature seems to influence the production of important enzymes and hormones in the developing embryo. Discovering exactly how this happens will require a better understanding of heredity, development, and the environment.

QUESTIONS AND ACTIVITIES

1. What does this case show about the following aspects of doing biology?
   — uses and limitations of indirect evidence
   — resolution of scientific controversies
   — revision of scientific theories
   — positive role of incorrect hypotheses
   — gradual versus sudden discoveries
2. Why is it difficult to identify a single scientist as the discoverer of sex chromosomes? How did each of the following scientists contribute to this discovery: McClung, Sutton, Stevens, Wilson, Morgan?
3. How was McClung's hypothesis about accessory chromosomes incorrect? Can you think of a plausible explanation for his mistake? Why are incorrect hypotheses, like McClung's, sometimes very important in science?
4. The Sutton-Boveri hypothesis cannot be confirmed solely on the basis of microscopic observation. Were biologists in 1903 justified in accepting the hypothesis even without conclusive proof? Why was the hypothesis necessary for the theory of sex chromosomes?
5. In her cellular studies, Nettie Stevens concluded that the X and Y chromosomes were correlated with sex but did not necessarily cause sexual differentiation. What is the difference between correlation and causation? What other forms of evidence might have been used to demonstrate a causal relationship between chromosomes and sex?
6. Consider the sea bass, which is both male and female, or reptiles whose sex is partly determined by temperature. If biologists had known about these unusual examples in 1905, how might this information have influenced the acceptance or rejection of the sex chromosome theory?

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