Nettie Stevens Uses Diptera to Describe Two Heterochromosomes

At the same time (1905) that Wilson was working out his hemipteran series of dissimilar idiochromosomes, Nettie M. Stevens was working on Diptera (flies) and other insects, including *Tenebrio*, at Bryn Mawr.[21] Nettie Maria Stevens (1861–1912) (Figure 7) was born in Cavendish, Vermont, where her father was a carpenter. During a summer course in Martha's Vineyard where she was studying to become a teacher, she took an interest in science and went to Stanford University for her B.A. (1899) and M.A. (1900). She became one of T.H. Morgan's students at Bryn Mawr (the best he had had in 15 years of teaching up to that time) and completed her Ph.D. in 1903. She received a fellowship to travel to Europe, studied at the Naples Station, and spent some time with Theodor Boveri at the University of Würzberg. She admired Boveri's work.

In 1905, after she returned, Stevens' first recognition of what she called heterochromosomes was in the mealworm, *Tenebrio*. That same year she studied the housefly, *Musca domestica*. It had five pairs of chromosomes, which all paired as homologs, and one pair, which she designated as h1 and h2, that did not actually pair fully, but which did separate during meiosis I (Figure 8). She later reported a similar finding for *Calliphora vomitoria, Sarcophaga sarracinae*, and *Drosophila ampelophila*.[22] The latter, the fruit fly, was to be renamed *Drosophila melanogaster* and in Morgan's laboratory would become one of the most studied organisms on earth. She had more difficulties with *D. melanogaster* than with any of the other Diptera she studied and used thousands of specimens to isolate a few good cells with details of meiosis. She noted that in the somatic cells, the chromosome number was always a constant eight. In the reproductive cells, the reduction of chromosome number provided, in oogenesis, a constant four chromosomes in all cells. But in the spermatogenesis of the males, she noted what seemed like a smaller chromosome of a pair that she called "heterochromosomes" and gave them the same h1 and h2 designation that she had for *M. domestica*.

Figure 7. *Nettie Stevens in 1904, the year before she discovered what were later named the X and Y chromosomes. (Courtesy of the Carnegie Institution of Washington.)*

Stevens drew some conclusions: "Here, as in similar cases previously described, it is perfectly clear that an egg fertilized by a spermatozoon containing the smaller heterochromosome produces a male, while one fertilized by a spermatozoon containing the larger heterochromosome develops into a female."[23] She also studied the sex ratio of fruit flies bred on grapes and bananas and found no significant differences, the two sexes each fluctuating slightly about the expected 50%, a phenomenon that she also found to hold true for the sex ratio among houseflies. There was no profound effect of

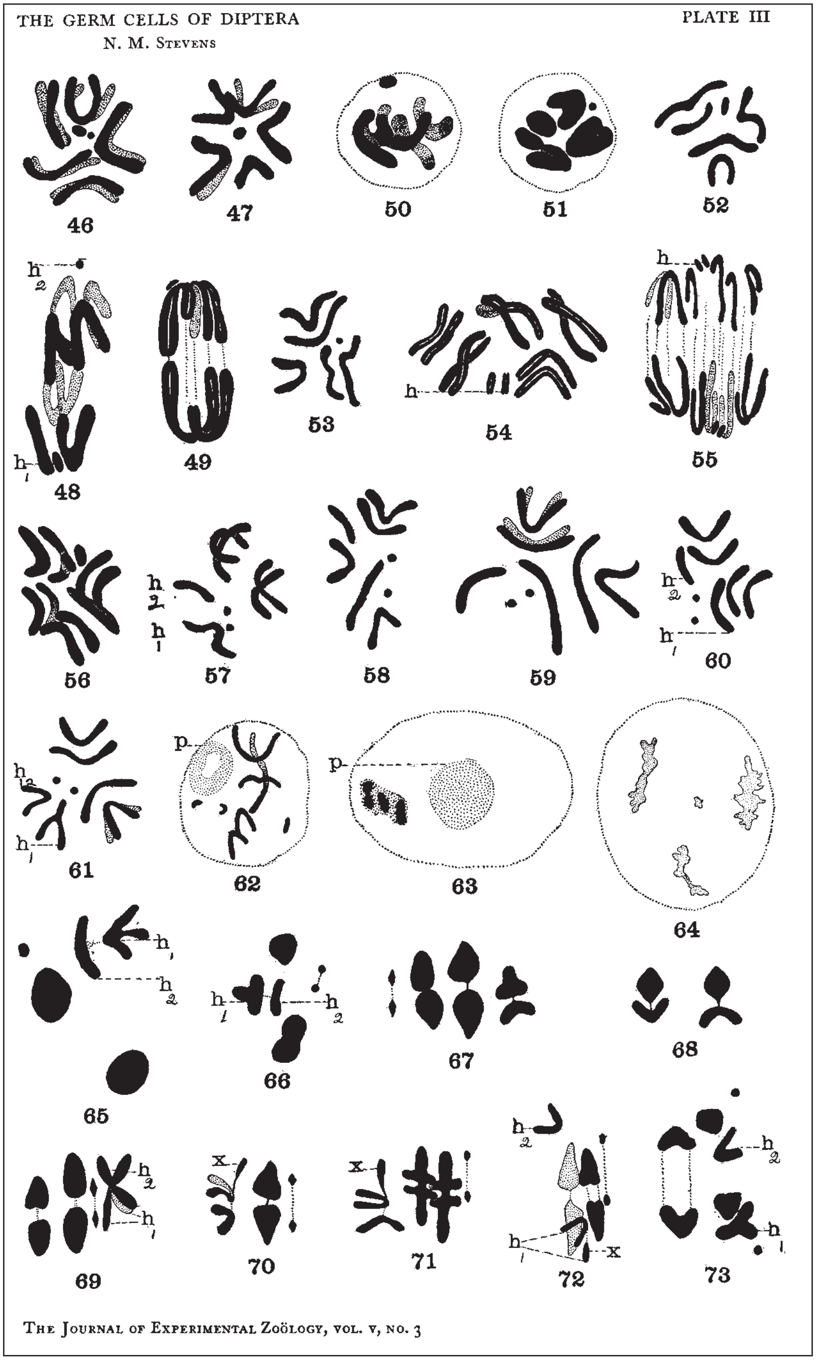

Figure 8. *Nettie Stevens had great difficulty obtaining good chromosomes of* Drosophila ampelophila *(later,* D. melanogaster*). From her successful specimens, she believed that two chromosomes (she called them heterochromosomes), which she designated as h1 and h2, corresponded to what Wilson later called the X and the Y in males* (items 57 and 58 are D. melanogaster *males*). *(Reprinted from Stevens N.M. 1908. A study of the germ cells of certain Diptera with reference to the heterochromosomes and the phenomena of synapsis.* Journal of Experimental Zoology **5**: *359–374.)*

food on sex determination and the sex-determining mechanism seemed tied to the heterochromosomes. Stevens added a final note in her paper that was misinterpreted by Morgan and his students in the early days of their study of *D. ampelophila*. She stated, "A preliminary statement in regard to the chromosomes of *Drosophila* was made at the International Congress of Zoology in Boston, August 21, 1907. The question as to whether an odd chromosome or an unequal pair of heterochromosomes was present in the cells was then unsettled."[24] Morgan thought he would play it conservatively and stayed with the "odd chromosome" interpretation. Until 1916, *D. melanogaster* was **XO** for males and **XX** for females. Note that McClung's idea of an accessory chromosome was now dead. Most species that were examined did not have an extra solitary chromosome involved in male determination. Instead of **XO** (= male) and **OO** (= female) for McClung's symbolism, the typical situation was **XO** (= male) and **XX** (= female) when one sex had a single sex chromosome.[25]

Multiple Sex Chromosomes Are Found by Wilson and Payne

Complicating the story was the work of Fernandus Payne, who greatly extended Wilson's story by discovering multiple sex chromosomes in a variety of bugs. Payne worked with both Wilson and Morgan at Columbia University. For his doctoral work, he studied reduviid bugs. These were unusual because they had more than two sex chromosomes. In 1910, Payne studied *Acholla multispinosa*.[26] In the somatic tissue of males, the chromosome number was 26 for males and 30 for females. What we would today call autosomes numbered 20 in both sexes. In the female, ten sex chromosomes were present: four medium and six small. In the male, in addition to the 20 autosomes, there were six sex chromosomes: one large, two medium, and three small. Payne argued that the eggs uniformly had ten autosomes and five sex chromosomes (three small and two medium). In addition, the males produced two classes of sperm; those that were female determining had three small and two medium along with the ten autosomes, like the eggs, but the male-determining sperm had only one large sex chromosome in addition to their ten autosomes. Payne realized that in living things, diversity, not uniformity, was a common occurrence, and he doubted the efforts of looking for one determining mechanism of sexuality (Figure 9): "Those who believe that the odd chromosome is merely a delusion in the minds of a few investigators still cling to the universality of van Beneden's law. However, the law is no longer of universal application. Not only the odd chromosome but a number of other irregularities have been recently described, the present case of *Acholla* giving the greatest variation in number."[27] Despite Payne's criticism, van Beneden's law was essentially correct—the somatic or diploid chromosome number is usually even and the gametic or haploid chromosome number is half that of the somatic number and can be odd or even.

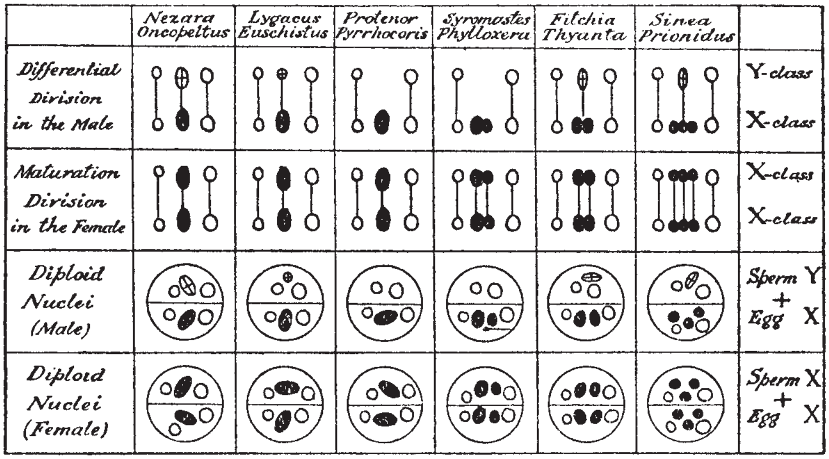

Figure 9. *Multiple sex chromosomes. Payne extended Wilson's observation that some bugs (the three columns on the right) had multiple sex chromosomes. Their existence made both Payne and Morgan skeptical of universal models of sex determination. (Reprinted from Wilson E.B. 1909. The chromosomes in relation to the determination of sex.* Science Progress **4:** *90–104.)*

Wilson's Solution: Sex Chromosomes Are Represented by X and Y

Wilson's clarification came to him in a series of brilliant papers that he published between 1905 and 1911. In 1905, he had independently found chromosomes of unequal size (idiochromosomes) present in the males of some insects he studied, just as Stevens had found what she called heterochromosomes present in the males of her insects. Wilson was not sure of the full significance of the function of these chromosomes, but he was certain that they established evidence for the continuity of the chromosomes because the two forms, larger and smaller, could be followed into the germ cells and among the subsequent progeny. They also separated during the first meiotic division, as did the solitary accessory chromosome. Wilson thus stressed the meiotic implications in his first paper. Although he was sympathetic to a sex-determining role for them, he argued "that as far as the Hemiptera are concerned, neither the suggestion I have made, nor the hypothesis of McClung has at present any support in observed fact."[28] Wilson added a footnote citing Stevens' work on the beetle *Tenebrio* that had just come to his attention (Figure 10). "She was able to determine, further, the significant fact that the small chromosome is present in the somatic cells of the male only, while in the female it is represented by a larger chromosome."[29]

Wilson, using Stevens' phrasing, but assigning the sex chromosome distinction to somatic cells instead of the spermatogonial, was almost there. He

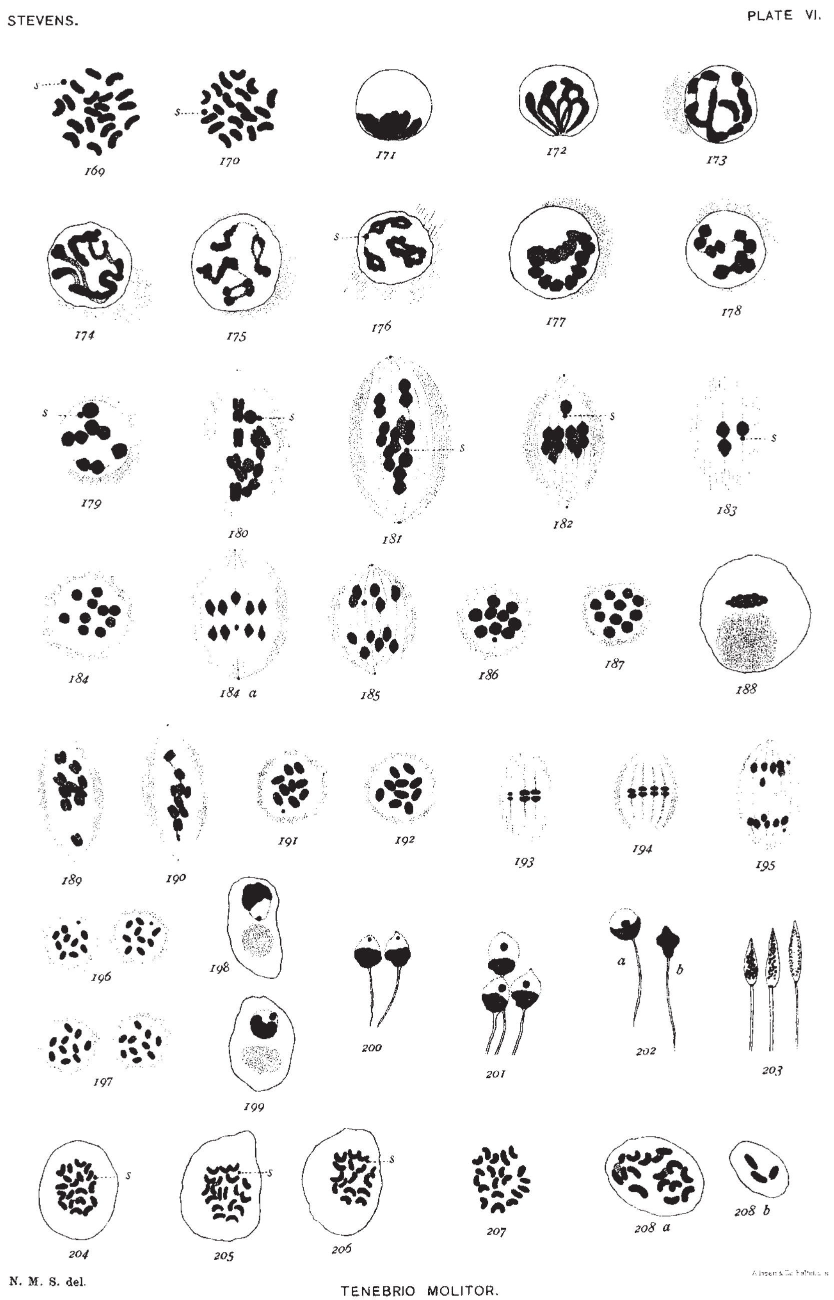

Figure 10. *Nettie Stevens discovered two different sex chromosomes in* Tenebrio. *Stevens' 1905 illustrations of chromosomes in* Tenebrio molitor *implied the existence of sex chromosomes. Note that 184 shows the female having ten chromosomes of about the same size. In 184a, there are nine such chromosomes and a small dot. Stevens inferred that two of the ten chromosomes in the female, and one of the nine chromosomes of uniform size in the male, were what Wilson later called sex chromosomes. (Reprinted from Stevens N.M. 1906. Studies in spermatogenesis. Carnegie Institution of Washington.)*

did not immediately see in his mind *two* larger chromosomes in the female and, until he and Stevens both saw this, the proof of sex determination was still tentative. If he did see this relationship, his phrasing was misleading. I believe that Wilson in that same paper does make that inference when he states that in *Anasa*, "Each spermatid-nucleus thus receives seven chromosomes, one half the spermatogonial number, and an accessory chromosome, in the usual sense of the word, is present; but the spermatids nevertheless consist of two groups, equal in number, one of which contains the smaller and the other the larger of the idiosomes."[30] The next year, both extended their observations and stressed that association. Stevens did so with her dipteran studies and Wilson with further studies on his bugs.

The February 1906 paper gave Wilson an opportunity to rethink the way he could describe Stevens' heterochromosomes, his own idiochromosomes, and McClung's accessory chromosome. "They may also be designated (whenever it is desirable to avoid circumlocution) as sex-chromosomes or 'gonochromosomes.'"[31] Fortunately, the former, and not the latter, of these two terms became the preferred phrase for discussion among geneticists and cytologists. Wilson recognized that his designation did not imply that the larger sex chromosome was female determining and the smaller chromosome male determining, because the male had both chromosomes in the somatic cells and in the diploid germ cells as they entered spermatogenesis. He felt uncomfortable with interpreting sex determination as a simple case of dominance and recessiveness (patency and latency), remarking that "...we are still ignorant of the action and reaction of the chromosomes on the cytoplasm and on one another, and have but a vague speculative notion of the relations that determine patency and latency in development."[32]

Wilson Describes Homogametic and Heterogametic Sexes

By April 1910, Wilson was confident in his interpretation: "In many species of insects there are two classes of spermatozoa, equal in number, which in the early stages of their development, differ visibly in respect to the nuclear constitution; while there is but one class of egg, which is of the nuclear type identical to one of the classes of spermatozoa. That is to say, if the two kinds of spermatozoa be designated as the 'X-class' and the 'Y-class,' respectively; the eggs are all of the X-class. The male may, accordingly be designated as the *heterogametic* sex, the female as the *homogametic*."[33] Wilson also now used his symbolism to represent the sexual karyotypes: "The female diploid groups contain accordingly **XX**, the male **XY** (being otherwise identical); and upon reduction each mature egg contains one **X**; while half the spermatozoa contain **X** and half **Y**."[34] Wilson came up with a plausible suggestion. "Could we regard the sexual differentiation as due primarily to factors on a quantitative rather than a qualitative nature, most of these difficulties would disappear; and such a conception would be in harmony both with the cytological facts and with the experimental evidence regarding sex-heredity" (Figure 11).[35] This

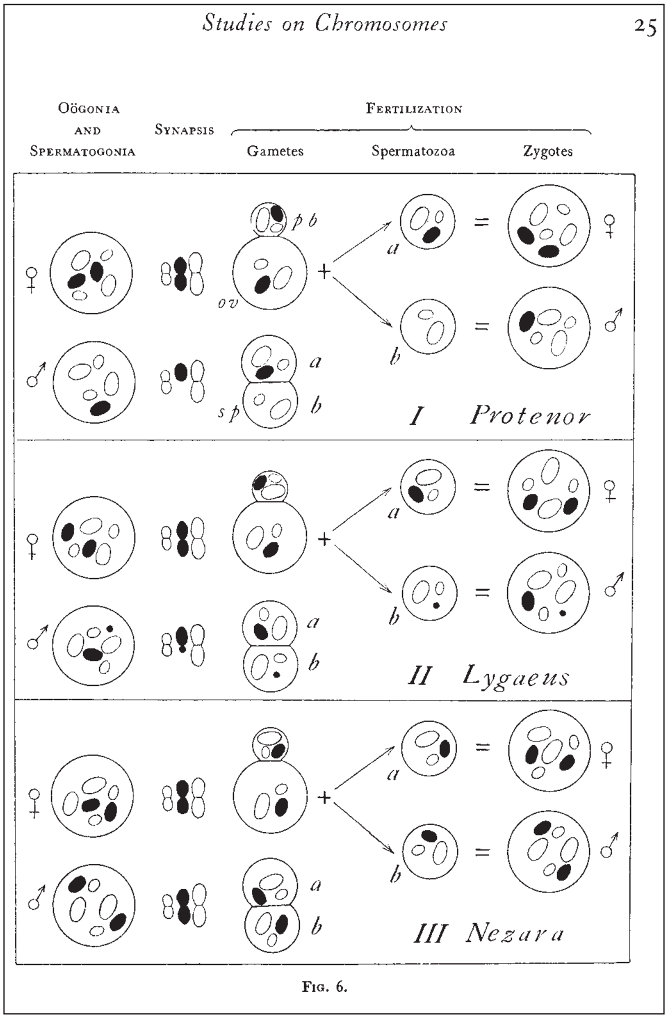

Figure 11. *Wilson identified three ways in which sex chromosomes (his idiochromosomes) may appear. In* Protenor, *the male is XO and the female is XX; in* Lygaeus, *the male is XY and the female is XX; in* Nezara, *the male XY idiochromosomes are equal in size and the XX female cannot be cytologically distinguished from the male. The* Protenor *form (XO) suggests a quantitative basis for sex determination. (Reprinted from Wilson E.B. 1906. Studies on chromosomes. III. The sexual differences of the chromosome groups in Hemiptera, with some considerations on the determination and inheritance of sex.* Journal of Experimental Zoology 3: *1–40.)*

quantitative model, indeed, was absorbed by Morgan and his students in their interpretation of sex determination in *Drosophila*. (For a summary of the contending interpretations of sex chromosomes, see Table 1.)

Just as there is some confusion regarding who should have priority for working out Mendelism—Mendel, de Vries, Correns, or Tschermak von Seysenegg—with each having some flaw of omission or interpretation, so too does the Wilson–Stevens discovery present the same problem. Stevens had stated her confusion on the "odd chromosome" or "heterochromosomes" in her fruit fly work; Wilson is flawed in initially seeing the female represented by

Table 1. *Contending interpretations of the sex chromosomes.*

Researcher	Genus	Comment
Henking	*Pyrrhocoris*	X element as a nucleolus or chromosome.
McClung	*Xiphidium*	Accessory chromosome as a male-determining chromosome; female = OO male = XO.
Paulmier	*Anaxas*	Short chromosome from a degenerating chromatin.
Montgomery	*Euschistus*	Chromatin nucleolus.
Sutton	*Brachystola*	Accessory is a true unpaired chromosome.
Wilson	*Anaxas*	The short chromosome is an accessory chromosome.
Stevens	Diptera and *Tenebrio*	Heterochromosomes (h1 and h2) in male but females have two h1. Ambiguity in fruit flies (odd versus heterochromosomes is unsettled).
Wilson	*Lygaeus*	Two chromosomes of unequal size are male; the larger represented twice is female; Wilson calls these idiosomes.
Wilson	Many bugs (Hemiptera)	A range of idiosomes: some with equal size in both sexes, some with unequal size, and some with idiosomes as two of same size in females and one of same size as in females but solitary in male.
Payne	Reduviids	Multiple heterochromosomes.
Wilson	Many bugs and flies	XX = female; XY or XO = male.

Henking had no idea what the X element was and speculated on its possible role as a nucleolus. McClung renamed the X element as an accessory chromosome and assigned it a male-determining role. Montgomery sided with Henking and saw it as a nucleolus. Paulmier noted that it was a shorter chromosome present in the males (thus, male determining, as McClung claimed). Sutton saw the accessory as a true chromosome and not a nucleolus. Wilson recognized the existence of idiosomes (which Stevens independently called heterochromosomes), and both claimed that the male had one of each and the female had two of the larger of the two idiochromosomes or heterochromosomes. Wilson finally called them sex chromosomes and assigned XX to females and XY to males. Payne worked out numerous cases of multiple sex chromosomes in bugs. Most researchers of macroscopic animal organisms use the XY and XX system, but whether the heterogametic sex is male or female varies widely. At the time of this cytological study (1890–1910), all of the heterogametic bugs and Diptera were male. Genetic but not cytological studies of moths and poultry in Great Britain in the early 1900s suggested that this was not universal.

"a larger chromosome" instead of two larger chromosomes. Making the transition from an accessory chromosome to a sex chromosome took some additional time and work with more organisms for both Stevens and Wilson to arrive at identical results: the XX female and the XY male as we see them today. The working out of a clean story minus its flaws requires a process of comparison and repetition. It is less likely that the complete story emerges in a single observation or experiment.

The survey of the literature from the 1890s through 1910 reveals how the sex chromosome story evolves piecemeal from the many contributions of scientists, sometimes colleagues, sometimes rivals, and each with errors generated by speculations based on incomplete knowledge (Table 1). Yet each successive finding narrows the interpretive range and in the span of 20 years, a coherent story emerged that has essentially remained unchanged since 1910 (Figure 12). Wilson's 1910 description could appear almost unchanged in an introductory biology course today. It is not a story of the victory of one class of scientists over another. It is not the story of a dying out of competitors. It is the "winning of the facts" that triumphs.

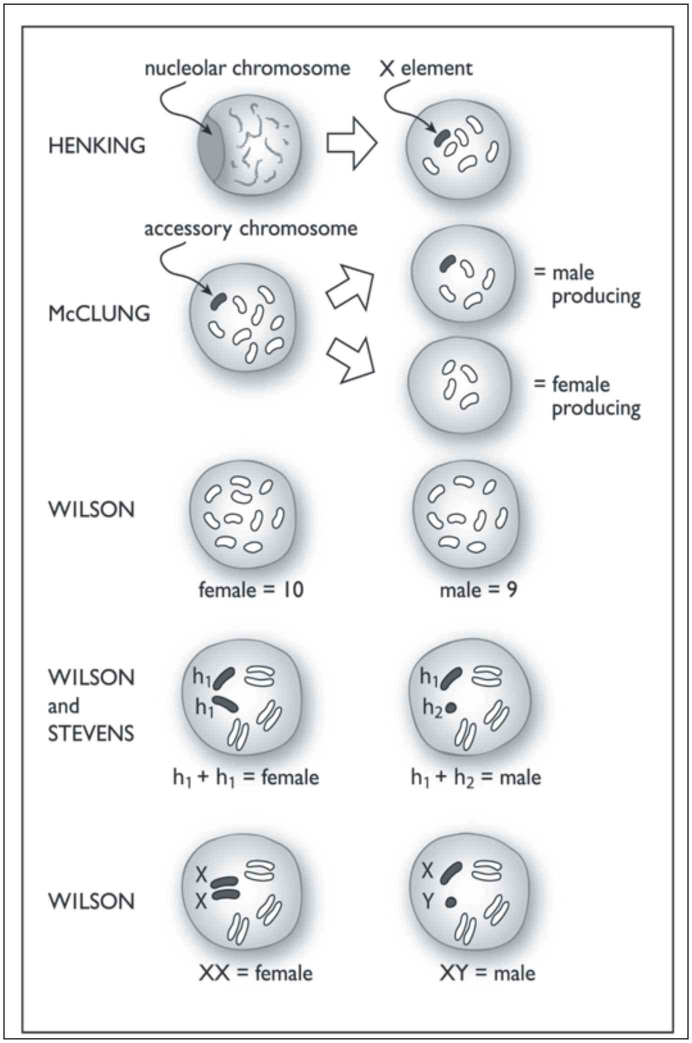

Figure 12. *Clarifying the idea of sex and chromosomes. The working out of the sex chromosomes spanned about 17 years (1893–1910). Henking identified an X element that behaved as a chromosome at one stage of meiosis, but seemed to be a nucleolar-like object near the nuclear envelope at an earlier stage. McClung, using more favorable material, saw a unique chromosome in males that he did not see in females. He called this the accessory chromosome and believed that it was male determining. In his disputes with McClung, Montgomery argued that the accessory chromosome was Henking's X element. Wilson noted that in some beetles, the male chromosome count was one less than the female, but because the chromosomes were about the same size, he could not make an interpretation associating one particular chromosome with sex. By 1907, both Wilson and Stevens had identified organisms with what Stevens called heterochromosomes (h1 and h2), which were morphologically distinct. In these species, the same chromosome number was present in both males and females, unlike Wilson's earlier series. Wilson clarified the story by calling the heterochromosomes sex chromosomes and giving them the modern designation of X and Y. He also argued that neither X nor Y was specific for sex determination. Instead, he believed that a quantitative relation existed, with one sex* XX *(the female in the species studied by Stevens and himself) and the other sex with a single X (either* XO *or* XY*). (Figure drawn by Claudia Carlson.)*