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What Did Sutton See?: Thirty Years of Confusion Over the Chromosomal Basis of Mendelism

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IN December 1902, 2 years after the rediscovery of Mendel's 1865 article, America's leading cytologist, Edmund Beecher Wilson, announced to the readers of Science that a graduate student of his at Columbia University had discovered the physical basis of the "Mendelian principle," by which Wilson meant the segregation of Mendelian factors (Wilson 1902). In an article published the following year, which became a classic of genetics, this student, Walter Stanborough Sutton, explained how the behavior of chromosomes during meiosis—as he interpreted it in his observations of spermatogenesis of the grasshopper *Brachystola magna*—could explain not only Mendelian segregation but also Mendelian assortment. Sutton had done much of the cytological work as a student of Clarence Erwin McClung at the University of Kansas, but his interpretation of his results in light of Mendelism was done at Columbia. Supposing that Mendel's factors were located on chromosomes, he realized that the "association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division [of meiosis]" could explain Mendelian segregation (Sutton 1902, p. 39) and that Mendelian factors on different chromosomes would assort independently if, at the division in which paternal and maternal chromosomes separate "any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the positions of other pairs" (Sutton 1903, p. 234). Although Sutton's conclusions gained general acceptance only gradually (Morgan 1910; Bateson 1922), his brilliant insight brought cytology and genetics together, initiating the long path of discovery leading to the present understanding of the chromosomal basis of inheritance.

Although correct in its essentials, Sutton's analysis contained a critical flaw. As did others at the time, Sutton identified the wrong division of meiosis as the reducing division, the division in which paternal and maternal chromosomes separate. Sutton thought that the separation of paternal and maternal chromosomes and their independent assortment take place during the second meiotic division, while actually they (or, more precisely, their centromeres[2]) separate and independently assort at the first division.

Estella Eleanor Carothers, who in 1913 presented the first clear cytological evidence for the independent assortment of chromosomes, nevertheless perpetuated Sutton's misconception. Carothers followed Sutton as a student of McClung at the University of Kansas and, like Sutton, studied spermatogenesis in Brachystola. Examining slides that Sutton had left behind as well as those she and McClung prepared, Carothers correctly described the segregation and independent assortment of certain recognizable chromosomes in the first division of meiosis. Nevertheless, she supposed that segregation and assortment take place in the second division for all other chromosomes. Confusion over which of the two meiotic divisions was reductive would linger for another 20 years. Despite histories of genetics crediting Sutton for explaining the chromosomal basis of Mendelism, no single work marks the clarification of the understanding of the meiotic divisions. Instead, clarification came from the gradual accumulation and integration of cytological

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[2] Because of crossing over, unknown to Sutton, it is more accurate to say that centromeres, rather than whole chromosomes, segregate at the first division of meiosis.

and genetic observations over more than a quarter of a century.

SUTTON'S THEORETICAL INSIGHTS AND CYTOLOGICAL CONFUSION

To understand the significance of Sutton's theoretical advances, as well as the cytological misunderstandings under which he labored, it is important to understand the state of biology at the turn of the century. Gregor Mendel's principles of heredity, so clearly put forward in his 1865 article, were ignored for 35 years. Their rediscovery in 1900 marked the birth of genetics. Nevertheless, the end of the 19th century saw a flourishing of cytological observations and theoretical advances that rendered biologists receptive to Mendel's analysis and conclusions. The second edition of E. B. Wilson's *The Cell in Development and Inheritance*, written without any knowledge of Mendelism, appeared in 1900 and provides an authoritative window on turn-of-the-century cytology. Many things were already known about chromosome behavior by 1900, including that the somatic chromosome number is preserved through successive mitoses and halved during meiosis. Writing that the reduction in chromosome number that accompanies meiosis is not only "very obviously a provision to hold constant the number of chromosomes characteristic of the species," Wilson went on to point out that, if chromatin is the physical carrier of inheritance, "an infinite complexity of the chromatin would soon arise did not a periodic reduction occur" (Wilson 1900, pp. 243 and 245). It was this consideration, upon which August Weismann (1887) based his famous prediction, that meiosis must entail a reduction division. In this sense, reduction refers to a halving of genetic complexity, not merely to a reduction in chromosome number.

By 1903, when Sutton advanced his interpretation of meiosis in terms of Mendelian segregation and assortment, there was substantial reason to believe that specific chromosomes carried specific hereditary factors. Thomas Harrison Montgomery (1901), studying spermatogenesis in Hemiptera, had noted the continuity and morphological individuality of chromosomes, and Theodor Boveri (1902), in his studies of double-fertilized sea urchin eggs, provided evidence that chromosomes differ qualitatively in their effects on development. Also, it had been concluded by Montgomery (1901) that at the synapsis stage, maternal chromosomes unite with paternal chromosomes rather than maternal with maternal and paternal with paternal.

Sutton wrote three articles (Sutton 1900, 1902, 1903) on the morphology and behavior of chromosomes in Brachystola. The first, "The spermatogonial divisions of *Brachystola magna*," written while Sutton was a masters degree candidate at Kansas, describes the chromosome group of male Brachystola and presents camera lucida drawings of its appearance in the meiotic divisions of the testis. The second article, written when Sutton was a doctoral candidate at Columbia, "On the morphology of the chromosome group in *Brachystola magna*" (Sutton 1902, p. 26), argues that the "ordinary chromosomes" (*i.e.*, autosomes) may be arranged in pairs according to their prophase lengths and volumes and that "the same number and size-relations of chromosomes" are seen through consecutive cell divisions. From this and his observations of synapsis, Sutton drew four essential conclusions: (1) chromosomes have morphological individuality; (2) chromosomes come in homologous pairs according to their size, with one from each pair inherited from the mother and one from the father; (3) chromosomes of each pair synapse before the meiotic divisions; and (4) synapsed chromosomes separate during meiosis.[3]

While these conclusions are correct, Sutton cited no specific observation that would have allowed him to distinguish the maternal and paternal members of a homologous pair of autosomes and therefore had no way of identifying the meiotic division in which they separate and independently assort. His unsupported claim that these events occur in the second division is, in fact, wrong.

What might have led Sutton to misidentify the division in which parental chromosomes separate? Sutton believed, correctly, that the first of the two meiotic divisions is longitudinal,[4] but was wrong in thinking that it is "essentially like that occurring in ordinary mitosis" (Wilson 1900, p. 286) and therefore equational. In Sutton's words:

> When the ordinary chromosomes [autosomes] divide in the first mitosis of the spermatocytes, the separation takes place along the line of the longitudinal split and therefore, except that the chromosomes are joined together by pairs, differs in no respect from an ordinary spermatogonial division.
>
> Sutton (1902, p. 32)

Sutton's misidentification of the first division as equational was apparently influenced by the mistaken belief, then held also by McClung, Montgomery, and Wilson, that maternal and paternal chromosomes join end to end during synapsis and that the second meiotic division is transverse,[5,6] separating paternal and maternal chromosomes:

[3]Sutton acknowledged in his 1902 article that Montgomery (1901) had drawn essentially the same conclusions.

[4]Longitudinal separation meant the separation of chromosomes aligned side by side.

[5]Transverse separation meant the separation of chromosomes joined end to end.

[6]Commenting on the synapsed chromosomes in Brachystola, Sutton wrote that ". . . the eleven double chromosomes are made up each of two limbs of equal size and we find it difficult to believe that these limbs do not represent the members of the pairs, joined together at their polar ends" (Sutton 1902, p. 28).

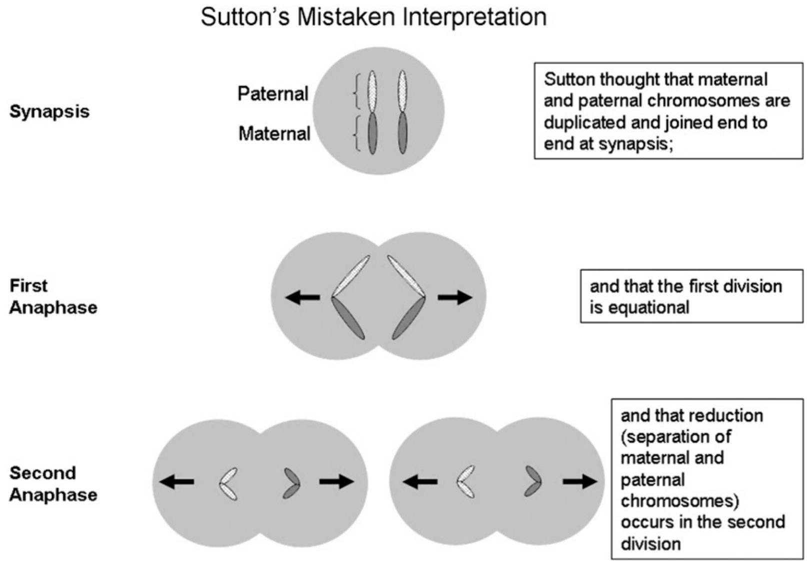

FIGURE 1.—"Unquestionably transverse": Sutton's understanding of meiosis. Sutton believed that homologous maternal and paternal chromosomes joined end to end prior to the first meiotic division. At the first meiotic division, in supposed analogy with mitosis, the duplicated chromatids would separate longitudinally from their sisters, making the first meiotic division equational. At anaphase of the second meiotic division, the maternal and paternal chromosomes, still joined at their ends, separate, making the division transverse and reductional.

> The [second meiotic] division occurs at the point of junction of the two limbs and is unquestionably transverse—*separating the two chromosomes at the point where they fused in synapsis two generations before.*
>
> SUTTON (1902, p. 33)

In Figure 1, chromosomes segregate and assort in the second meiotic division. As we now understand (Figure 2), homologous chromosomes pair side by side during synapsis and both meiotic divisions are longitudinal.[7] The longitudinal division in meiosis I separates maternal and paternal homologs, making the first division reductional and the second equational, the opposite of what Sutton thought.

In "The chromosomes in heredity," his third, final, and deservedly most famous contribution, published in 1903, Sutton proposed a specific cytological basis for Mendelian segregation and independent assortment, thus bringing together cytology and genetics.[8] In the opening paragraph of his article, he denied any influence from Mendelism on the formulation of his cytological conclusions:

> The general conceptions here advanced were evolved purely from cytological data, before the author had any knowledge of the Mendelian principles, and are now presented as the contribution of a cytologist who can make no pretensions to complete familiarity with the results of experimental studies on heredity.
>
> SUTTON (1903, p. 231)

Despite this disclaimer, Sutton's knowledge of genetic principles, if not of Mendelian genetics itself, definitely influenced his interpretation of his cytological observations. Indeed, he wrote that he originally thought, as did Montgomery, that "all the maternal chromosomes must pass to one pole and all the paternal ones to the other," altering this conclusion only because it was "seen to be at variance with many well known facts of breeding" (SUTTON 1903, p. 232).

Seeking cytological evidence to explain how an individual might produce gametes containing chromosomes that are not derived solely from one parent or the other, Sutton undertook a "more careful study" of the meiotic divisions and, finding "no evidence in favor of parental purity of the gametic chromatin," claimed that:

> . . . many points were discovered which strongly indicate that the position of the bivalent chromosomes in the equatorial plate of the reducing division is purely a matter of chance—that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the position of other pairs.
>
> SUTTON (1903, pp. 233–234)[9]

In fact, because he did not notice an unequal pair of autosomes present in his material that would have allowed him to distinguish a maternally derived autosome from its paternal homolog, Sutton could not have distinguished maternal chromosomes from paternal ones.

Sutton's misidentification of the second meiotic division as the reductive division must certainly invalidate his supposed evidence—that the position on the equatorial plate of the chromosomes derived from the synapsis of a maternal and paternal pair is a matter

[7]At the time, although it was incorrectly thought that in insects chromosomes synapse end to end, it was thought, correctly, that they synapse side by side in higher plants and vertebrates (SUTTON 1903, p. 248).

[8]Sutton did not present any new experimental data in his 1903 article but instead recapitulated the observations and conclusions from his 1902 article.

[9]Remarkably, Sutton added a footnote after "strongly indicate," stating that "Absolute proof is impossible in a pure-bred form on account of the impossibility of distinguishing between maternal and paternal members of any synaptic pair" (SUTTON 1903, p. 233). It would be interesting to know when this footnote (that undermines his claimed cytological evidence for independent assortment) was added and whether it was in response to comments from others, perhaps Wilson or an editor of his article.

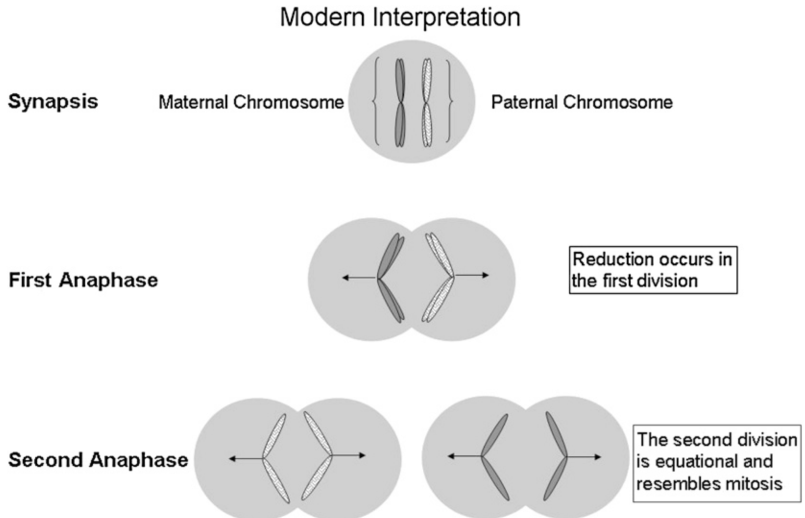

Figure 2.—The present understanding of the two meiotic divisions. Prior to the meiotic divisions, maternal and paternal chromosomes duplicate and synapse side by side. Migrating to opposite poles, maternal and paternal centromeres separate reductionally in the first meiotic division. Sister centromeres separate equationally in the second division.

of chance, which was his cytological explanation for independent assortment. But flawed as it was, Sutton's synthesis of Mendelism and cytogenetics opened the way to many of the fundamental genetic advances that followed.

CAROTHER'S CYTOLOGICAL CONTRIBUTIONS AND CONTINUED CONFUSION REGARDING THE TIME OF CHROMOSOME SEGREGATION

Despite his erroneous belief that homologous chromosomes separate and independently assort during the second division of meiosis, Sutton realized in 1903 that reduction occurs during the first division for one particular chromosome—an observation that would be essential to the work of Eleanor Carothers. The accessory chromosome of Brachystola, which McClung had identified as the determinant of sex,[10] segregated to one pole in the first meiotic division. Sutton believed that the reductive division for sex chromosomes must occur in a division (first division) different from that for other chromosomes (second division). Generalizing, he wrote: "Thus we are confronted with the probability that reduction in the field of one character occurs in one of the maturation divisions and that of all the remaining characters in the other division" (Sutton 1903, p. 245).

In 1913, Carothers showed that the first meiotic division is reductional for at least one pair of autosomes, and she also provided compelling cytological evidence for independent assortment in the same division. The enabling observation for both conclusions was her discovery of an unequal pair of homologous chromosomes in Brachystola and two other species of orthopteran insects. Like the accessory chromosome and contrary to Sutton's supposition regarding autosomes, the members of the unequal pairs segregated in the first division of meiosis. Further, by comparing the segregation of the members of the heteromorphic pair in Brachystola with that of the accessory in individual meioses, Carothers was able to document their independent assortment (Figure 3). Of the 300 first meiotic divisions that she scored, the accessory chromosome migrated to the same pole as the smaller autosome "146 times, or in 48.6 percent of the cases; and the larger one, 154 times, or in 51.3 per cent of the cases" (Carothers 1913, p. 494). She declared this observation the "essential part" of her work: the "segregation of at least part of the paternal and maternal chromatin according to the law of chance" (Carothers 1913, p. 500). Thus, Carothers correctly identified the first division of meiosis as the reduction division for both the accessory and the unequal pair and by comparing them demonstrated their independent assortment.

Carothers clearly recognized that Sutton's claims to have seen cytological evidence that "in the reduction division chromosomes are separated into groups which are neither purely paternal nor purely maternal" to be a hypothesis extrapolated from genetic data and not a cytological observation (Carothers 1913, p. 499). She wrote:

> This last suggestion, I believe, was pure theory advanced to meet the known experimental facts which show that either parent may transmit the characters of its ancestors of the opposite sex. It is strange that so careful an observer should have overlooked the very thing that was present (the unequal tetrad was first found on Sutton's slides) offering definitive chromosomic proof for his theory.
>
> Carothers (1913, p. 499)

Despite her clear demonstration that the accessory and heteromorphic pair segregate reductively in the first division of meiosis, Carothers did not generalize

[10]Owing to an undercount of the number of chromosomes in the female, McClung erroneously concluded that there was no accessory chromosome in the female.

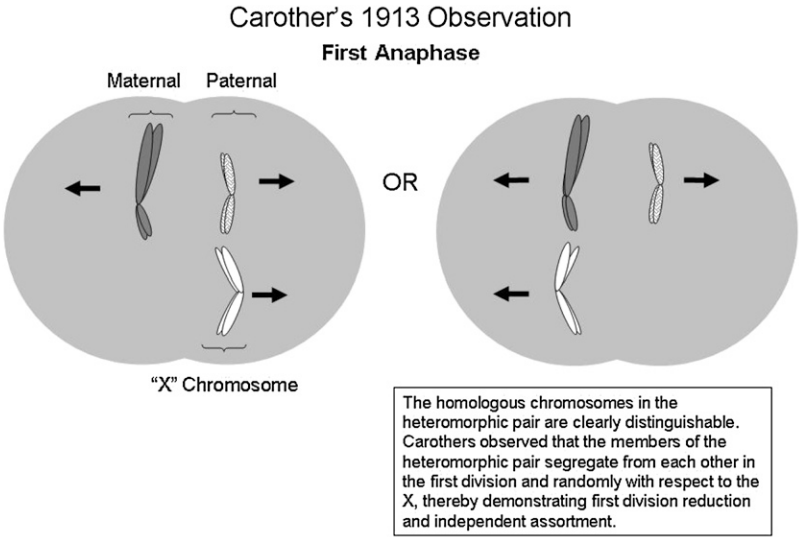

FIGURE 3.—Carothers's observation of reductional segregation and independent assortment in the first division of meiosis. One chromosome of a homologous pair was clearly larger than the other, making it possible for Carothers to see that the maternal and paternal chromosomes of the heteromorphic pair separated in the first meiotic division. She also saw that the homologs segregated randomly with respect to the accessory (X) chromosome; she counted 146 meioses in which the accessory segregated with the short autosome and 154 in which it segregated with the long one. This was the first clear cytological evidence for independent assortment.

from her data. Instead, she speculated that the unequal chromosome pair was an anomaly and that all other chromosome pairs would segregate reductively in the second meiotic division, as Sutton had thought. On the nature of the meiotic divisions, she wrote:

> While all the other tetrads are made of qualitatively equal parts and follow the typical Orthopteran plan of division (longitudinal in the first spermatocyte and transverse in the second) already worked out for Brachystola by Sutton, this tetrad divides transversely in the first spermatocyte, as is evident from an examination of the prophases.
>
> CAROTHERS (1913, p. 493)

Thus, she mistakenly believed that the first-division reduction of the unequal pair is anomalous and that all other pairs divide reductively only in the second meiotic division. Moreover, like Sutton, she believed that chromosomes synapse end to end rather than side by side, thereby requiring the first division of the heteromorphic chromosome pair to be transverse, as she erroneously claims to have been evident. Carothers is nevertheless unequivocal about the reduction being in the first division for the unequal pair: "The unequal size of the two dyads allows no mistake on this score; otherwise, we should have one large and one same chromatid passing to each second spermatocyte" (CAROTHERS 1913, p. 493). Whereas Sutton went too far with his data, making unsupported theoretical leaps, Carothers did not extend her conclusions about the reduction division of the unequal pair to the remaining chromosome pairs.

LINGERING CONFUSION AND ULTIMATE RESOLUTION OF MEIOTIC REDUCTION

The misunderstandings over synapsis and reduction in Carothers's 1913 article were not anomalous and confusion would persist for two more decades. CAROTHERS (1917, 1921) presented two additional articles on the behavior of heteromorphic chromosome pairs in which she described several heteromorphic chromosome pairs, all of which segregated in the first division of meiosis: "When heteromorphic, the members of these pairs segregate during the first maturation division" (CAROTHERS 1921, p. 459). Qualifying her observation with the phrase "When heteromorphic," Carothers still thought it likely that all heteromorphic pairs were anomalous and that normal chromosomes separated reductively in the second meiotic division. Other investigators were similarly confused. In the third and final edition of *The Cell*, E. B. Wilson wrote:

> In the foregoing pages no attempt has been made to discuss the order of succession of the reduction- and the equation-divisions. An answer to this long-disputed question must obviously rest upon our means of identification of the reductive division and, as will be evident from the foregoing, *such identification can only be made with complete certainty in cases where the synaptic mates are visibly distinguishable* by differences of form size, structure, or mode of attachment. A sufficient number of such cases are now known to demonstrate that *the two divisions do not in all cases follow the same order, and that even in the same division the bivalents may differ individually in this respect.*
>
> WILSON (1925, pp. 572–573)

To be fair, Wilson's explanation of this statement makes it clear that he was aware of "strong" arguments and evidence that indicated that meiosis I was the "prevailing and perhaps the universal order of division" (WILSON 1925, pp. 573–574). Although uncertainty remained, Wilson acknowledged that most biologists had come to accept the first division of meiosis as the reduction division.

In 1926 and 1931, Carothers wrote her final articles on the cytology of heteromorphic homologs, revealing her persistent confusion about reduction.[11] After

[11]In the 1930s, Carothers continued her study of grasshoppers but not on heteromorphic homologs, reporting on interspecific hybridization and the effect of X-rays on embryos. She died in 1957.

observing unequal homologs in various species segregating in both the first and second meiotic divisions, she concluded that, "The members of a given pair may possess a tendency to segregate at one division in preference to the other but this is seldom a fixed condition"[12] (Carothers 1926, p. 434). When homologs segregate in the first division, she presumed, some mechanical peculiarity is involved:

> In heteromorphic pairs . . . where the difference is associated with the spindle fiber insertion, segregation has been found to occur only at the first maturation division. Probably the reason for this is to be found in the mechanical conditions involved. Certainly, we should not assume from this that when the tetrads are composed of homomorphic dyads they also segregate at the first division.
>
> Carothers (1931, p. 347)

Carothers's ultimate conclusion was that reduction occurs in either meiotic division, with specific chromosome pairs showing a preference to segregate in one division or the other. Although she was aware of crossing over and of Janssens's 1909 chiasmatype hypothesis (Carothers 1926, p. 426), she failed to interpret her data in terms of crossing over. Remarkably, Cyril Darlington used Carothers's 1931 observations in his 1932 text, *Recent Advances in Cytology*, as evidence for "When and Where Crossing-over occurs" (Darlington 1932, p. 264). Darlington explained Carothers's carefully gathered data about heteromorphic chromosome pairs as follows:

> i. Where no chiasma [crossover] is formed in the segment between the spindle attachment [centromere] and the inequality, the first division must be reductional.
>
> ii. Where one chiasma is formed between them, the first division must be equational.
>
> iii. Where more than one chiasma is formed between them, either result may obtain.
>
> Darlington (1932, p. 275)

Darlington therefore understood reduction as we understand it today: the first division is reductive for centromeres and for the region of a chromatid proximal to a single chiasma and equational for the region distal to that chiasma; while the converse is true for the second division.

CONCLUSION

Sturtevant (1965) wrote in his history of genetics that Sutton's 1903 work and Carothers's 1913 cytological demonstration marked the end of a phase in biology. Following Sturtevant, most geneticists and historians have assumed that Sutton correctly interpreted Mendel's observations in terms of the behavior of choromosomes. In reality, confusion and controversy clouded the relationship between chromosome behavior and genetics for three decades following Sutton's major conceptual advance.[13]

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[12]Nevertheless, referring to the heteromorphic autosomal pairs described in her 1913 article, Carothers (1926, p. 429) states that a "somewhat hasty review of the slides on which my work was based failed to reveal any case of post-reduction (second division segregation) of the members of the unequal pair."

[13]Sutton left Columbia in 1903 without completing his doctoral dissertation, returning in 1905 to attend medical school. After a distinguished career as a surgeon, he died 2 days after an appendectomy in 1916 at the age of 39. For more about the work and life of Sutton, see Crow and Crow (2002) and McKusick (1960).