PARADIGM LOST:
THE HUMAN CHROMOSOME STORY

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The knowledge that humans have a diploid chromosome number of 46 has an interesting history. The original "solid" estimate was either 47 or 48 depending on whether one interpreted the sex determination system in humans as XO or XY. With improved chromosome preparation techniques and common use of cell cultures, the chromosome number was reported to be 46 in 1956. Did this emerging knowledge fit into a paradigm shift of the Kuhnian type? Our argument is: not in the traditional sense.

Keywords: human chromosome number, paradigm shift, sex determination

Thomas S. Kuhn in his highly regarded work, *The Structure of Scientific Revolutions*, argued that science does not progress in an orderly way.

"... when, that is, the profession can no longer evade anomalies that subvert the existing tradition of scientific practice, then begin the extraordinary investigations that lead the profession at last to a new set of commitments, a new basis for the practice of science." (Kuhn, 1970, p.6)

Thus Kuhn suggested that science moves quietly along punctuated by occasional "revolutions" reflecting changes in thought. Accepted scientific thought was defined by Kuhn as a "paradigm." Scientific revolutions occur with a shift in paradigm.

"Its [new thought] assimilation requires the reconstruction of prior theory and the re-evaluation of prior fact, an intrinsically revolutionary process that is seldom completed by a single man and never overnight." (Kuhn, 1970, p.7)

Kuhn primarily uses examples from the history of physics, mathematics, and chemistry to support his ideas of paradigm shifts and scientific revolutions. We sought to find a biological example of a Kuhnian paradigm shift. Our choice for this phenomenon was the establishment of the human diploid chromosome number.

The first report providing evidence for the human diploid chromosome number of 46 appeared on January 26, 1956. (Tjio and Levan, 1956) Joe Hin Tjio and Albert Levan concluded in their research appearing in the journal Hereditas:

"... the almost exclusive occurrence of the chromosome number 46 in one somatic tissue derived from four individual human embryos."

Using lung fibroblasts, Tjio and Levan examined mitotic figures from 256 cells and reported that all but four exhibited 46 chromosomes. They commented:

"We were surprised to find that the chromosome number 46 predominated in the tissue cultures from all four embryos, only single cases deviating from this number."

Had these men, using Kuhn's words, found "anomalies that subvert the existing tradition of scientific practice?" Again using Kuhn's words, did this paper set into motion "the reconstruction of prior theory and the re-evaluation of prior fact?"

Prior to 1956 the accepted value for the number of diploid chromosomes in the human cell was 48. Hans de Winiwarter reached this conclusion for human oogonia in the year 1912. (as cited by Kottler, 1974) T. S. Painter provided evidence for 48 chromosomes in human spermatoagonia in 1923. (Painter, 1923) Both Winiwarter and Painter had impeccable cytology credentials: Winiwarter training with von Beneden and Painter with Boveri. For more than thirty years the accepted diploid chromosome number stood at 48. This number was solidly established in the literature of the 1950's: see Darlington's *The Facts of Life*, 1953, and Storer and Usinger's *General Zoology*, 1957. Even Beatty's 1954 article titled "How many chromosomes in mammalian somatic cells?"
published in the prestigious *International Review of Cytology* concluded there were 48 chromosomes in human cells.

The body of science for nearly a half century had argued for 48 chromosomes; and now, the research team of Tjio and Levan suggest this widely accepted value was incorrect. In Kuhn’s words the stage was set for “an intrinsically revolutionary process that is seldom completed by a single man and never overnight.” This major paradigm shift from 48 to 46 chromosomes did not result in a “Scientific Revolution” in the sense of Kuhn. Confirming evidence was reported within the same year followed by relatively few dissenting words. The battle and war were played by few people and the war was over in less than three years.

The first scientific comment of the Tjio and Levan article appeared as an editorial in the British Medical Journal on August 11, 1956. The editorial simply summarized the techniques and findings and went on to say that Dr. Tjio showed preparations of human chromosomes at the Congress on Human Genetics in Copenhagen the week before. (Anon., 1956) The most immediate concern was that the gonadal tissue chromosome number may be different from that of somatic tissue. Winiwarter’s and Painter’s research dealt with sectioned gonadal tissue. Tjio and Levan used somatic cells of tissue cultured embryos. C. E. Ford and J. L. Hamerton addressed this problem in their article published in *Nature* on November 10, 1956. They cautiously reported that the majority of their counts on human spermatogonial tissue resulted in 23 bivalents. Ford and Hamerton reviewed both Painter’s and Tjio & Levan’s work. They raised a new question of why had so many scientists miscounted the chromosomes. Ford and Hamerton cited the personal observation of Dr. Hansen-Melander who kept finding 46 chromosomes in human liver embryo cells. Hansen-Melander’s study was discontinued and never reported because 48 chromosomes could not be found. How many other scientists abandoned research because they could not find 48 chromosomes?

Pivotal to the acceptance of 46 chromosomes as the correct diploid number was the opinion of other notable scientists. Such opinions for change came quickly. T. C. Hsu accompanied by Paul S. Moorhead published the following remarks in the March 1957 *Journal of the National Cancer Institute*:

“the diploid number, whether it is 48, conventionally accepted by man for many years, or more likely 46, recently claimed by Tjio and Levan and by Ford and Hamerton... .” (Hsu and Moorhead, 1957)

Hsu with C. M. Pomerat and P. S. Moorhead a few months later produced counts that “...helped to prove that the stemline number for normal human cells is 46.” (Hsu et al., 1957) An acknowledged leader in chromosome-based research and following a 1952 report where he confirmed 48 chromosomes (Hsu, 1952), Hsu quickly switched to the new number paradigm based on two reports and his own new observations.

In 1958 Tjio and Theodore T. Puck published an article in which they further supported the 46 chromosome paradigm of Tjio and Levan by saying, “the chromosome number in all cultures of normal human cells was always 46.” (Tjio and Puck, 1958a) They presented micrographs of a variety of normal human male and female tissues which demonstrate that the human diploid chromosome number was 46.

Despite the support that the Tjio-Levan paradigm had received, there was still some doubt as to what the actual chromosome number in humans was, as is seen by an October 1958 article written by Frank H. Ruddle, Lawrence Berman, and Cyril S. Stulberg in the journal *Cancer Research*. In this
article Ruddle et al. state, "at present, uncertainty exists on the actual human chromosome number." Ruddle et al. then go on to summarize how Hsu has revised his initial estimate of 48 chromosomes to 46, Kodani's claim of 46, 47, and 48 chromosomes existing in man, and finally Tjio and Levan's report of 46 chromosomes. After two years, not everyone was completely convinced by Tjio and Levan's new paradigm.

In a 1957 article, Masuo Kodani argued for a different paradigm based on a chromosomal study of the testes of Japanese men in which Kodani "found surprisingly that three different diploid numbers exist in man instead of one." (Kodani, 1957) It should be noted that Kodani worked for the Atomic Bomb Casualty Commission and his sources of spermatogonial tissue were taken from the testes of men with epididymitis. (Kodani, 1957) In a 1958 report, Kodani continued to provide evidence for a supernumerary chromosome in human males. These results were once again from non-normal sources: sterile men, some of whom had been exposed to atomic bomb radiation but showed no signs of illness. (Kodani, 1958)

In 1958, a June issue of Nature revealed even more support for the Tjio-Levan paradigm while providing no support for other existing hypotheses, such as Kodani's supernumerary chromosome idea. In this Nature article C. E. Ford et al. restate the findings of the Tjio and Levan paper and go on to cite examples of other scientists who have confirmed Tjio and Levan's chromosome counts. On the other hand Ford et al.'s article stated that in the case of Kodani's paradigm "we are unaware of any other reported instance of 'inert' supernumerary chromosomes." (Ford et al., 1958) Once again the Tjio-Levan paradigm was reinforced.

Finally in December 1958, Tjio and Puck published an article that cited other scientists who confirmed the 1956 results of Tjio and Levan. They also performed further studies of their own which provided evidence for 46 human diploid chromosomes. By this time the combined data set from all reported sources comprised a population of 74 human individuals who had a chromosome number of 46. (Tjio and Puck, 1958b)

Clearly the shift to the 46 chromosome paradigm was swift and without much dissension. The struggle predicted by Kuhn associated with such a major change in thinking did not occur. However, Kuhn's prediction that once a new paradigm is adopted, it is done so completely, did occur. Although the chromosome number paradigm shift is a poor model for a Kuhnian scientific revolution, an appreciation of the chromosome number history is a good lesson in the process of science.

Why Did T. S. Painter Get the Diploid Human Chromosome Number Wrong?
The answer to this question is surprising and it points to a scientific revolution. A brief review of the history of the chromosome is in order to provide a backdrop for Painter's time.

The following review is based on Gardner's History of Biology. (Gardner, 1972) W. Waldayer gave chromosomes their present name in 1888, after Flemming had already enunciated their role in mitosis in 1879. In 1883 van Beneden described reduction division in Ascaris and Boveri in 1888 implicated chromosomes as hereditary factors. E. B. Wilson and N. M. Stevens reported in 1905 two sex determination mechanisms known as XO and XY. This report built from H. Henking's 1891 publication about the "X" body found in some insect sperm. W. S. Sutton proposed the Chromosome Theory of Heredity in 1902 and C. B. Bridges provided evidence for phenotype and gene correspondence on Drosophila chromosomes in 1916. Painter gathered his scientific heritage from a time where the focus was on what a chromosome did, rather than how many chromosomes there were or what they looked like.

No doubt Painter was the pivotal person in the scientific investigation of the human chromosome number. Painter had an excellent educational pedigree including Yale University; he was a member of the National Academy of Sciences; and for six years he was the President of the University of Texas at Austin. Painter's data collection and interpretation were affected by at least three factors. The three factors were technique, sex determination, and chromosomal anomaly. The role of technique will be dealt with first.
Technique
In an excellent review, Malcolm Jay Kottler described the role of technique in visualizing chromosomes for counting. (Kottler, 1974) He stressed that the early chromosome work used sectioned material for examination, whereas modern work uses chromosome spreads obtained from tissue culture. Clumping of the chromosomes is a greater likelihood in working with sectioned tissue than with tissue culture material. From the first reports of chromosome numbers in human cells in the 1890's to Painter's review in 1930, reported chromosome numbers varied from as few as 8 to as many as 100. (Painter, 1930, and Kottler, 1974) Kottler summarized it best by saying:

“If one wishes to assess human chromosome counts prior to 1920, the ‘failure’ of cytologists was not so much the incorrectness of their counts as their inability to appreciate the conditions for the lifelike preservation of chromosomes and the resulting disparity between chromosomes in their material and in living cells.” (Kottler, 1974)

Winiwarter and Painter were the only investigators during this period to produce chromosomes of the sort that offered the possibility of accurate counting. The most common “accurate” number for human chromosomes in a cell at that time was 24. In 1912 Winiwarter reported 47 chromosomes in human males and 48 chromosomes in human females. (As cited in Painter, 1930) Painter, of course, reported in 1923 forty-eight chromosomes in human male spermatogonia. Winiwarter used fresh tissue and Flemming’s fixative for his work. Painter likewise used fresh tissue and Allen’s modification of Bouin’s fixative. (Kottler, 1974) It must be appreciated that fresh tissue meant working with surgical castrations at the foot of the patient. In fact, Evans and Swezy (in 1929) obtained fresh tissue from very recently executed criminals. (Kottler, 1974) The technique for chromosome preparation had everything to do with the appearance of the chromosome and the accuracy of the count.

Using his modified Bouin’s preparation technique, Painter examined a variety of mammalian species for their chromosome number and their sex determination mechanism. The list included the following: housebat, hedgehog, rabbit, mouse, rat, and horse. (Kottler, 1974) In all cases Painter found an XY sex system in mammals and what proved to be the correct diploid chromosome number for the listed species.

From the mid 1930's to the early 1950’s cytologists did not return to counting chromosomes. Virtually no new evidence was offered during this period. (Kottler, 1974) However, by the early 1950’s, the techniques that could led to improved chromosome visualization had been developed. Kottler summarized the developments as follows:

“The Tjio and Levan discovery was the direct and inevitable result of the combined use of four new preparative techniques in mammalian cytology. Each of the new techniques – tissue culture, hypotonic pretreatment, colchicine pretreatment, and squashes – contributed to the dispersal of chromosomes and the reduction of chromosome overlap.” (Kottler, 1974)

Tjio and Levan simply had a much better chance of counting chromosomes correctly when compared to the cytologists working in the first quarter of the twentieth century. Painter found 48 chromosomes because that is what the methodology at that time would allow.

Sex Determination
The second factor influencing Painter's judgment was sex determination. In Painter’s Stadler lecture, he gave insight as to his research approach in the early 1920’s:

“By 1920 because of the work of McClung, and E. B. Wilson, and many others we knew that sex determining chromosomes occurred in insects and other invertebrates... But very little was known about the chromosomes of vertebrates. ... From my study of the opossum I knew what to look for in primary spermatocyes of man and there I found an X-Y complex quite similar in morphology to the X-Y of the opossum. ... The identification of the X-Y complex in the male has stood the test of time, and this was the main point of interest to me in making this study.” (Painter, 1971)
Painter's interest in chromosomes was fixed on the sex determination process. At the beginning of the century insects were found to have an "XO" sex system: females had 2n chromosomes and males had 2n-1 chromosomes. The sex chromosome had been isolated and identified; however, the status of the smaller Y sex chromosome was unclear. Winiwarter's superior preparation techniques led him to report that humans likewise had an "XO" sex determination system. Working with armadillo, Painter found the Y chromosome in mammals and contradicted Winiwarter's findings. Painter reasoned that if, in fact, mammals were "XY" rather than "XO" then the spermatogonial cells should have an even number of chromosomes and not the odd number reported by Winiwarter.

Painter turned next to human testis as an example of mammalian tissue. He published a preliminary report in 1921 where he stated the following about human chromosomes:

"In my own material the counts range from 45 to 48 apparent chromosomes, although in the clearest equatorial plates so far studied only 46 chromosomes have been found."

(Painter, 1921)

Winiwarter and his colleagues continued to argue for 47 chromosomes in human males based on an "XO" hypothesis. Painter and others overwhelmed Winiwarter and by the mid 1930's the XY system for humans was accepted as well as an even chromosome number which happened to be calculated at 48 chromosomes.

If one seeks to find a scientific revolution in the sense of Kuhn then the real war was fought between 1910 and 1930. The topic was the sex determination system in mammals and whether spermatogonia had an even or odd number of chromosomes. Kottler (1974) argued that Painter may have accepted 48 as the number because he wanted to establish the sex determination mechanism in the literature more than the number of chromosomes. Forty-eight was an even number, one which Winiwarter obtained for oogonia and rather than argue along three fronts (XY, even number, and 46) Painter opted for just two fronts: XY and even number. Painter's 1921 counts could support either 46 or 48 as the diploid number.

Source of Material
The third factor that may have influenced Painter was the source of the human testicular material. Theophilus S. Painter in his 1923 report, "Studies in Mammalian Spermatogenesis," established that the human diploid chromosome number was 48 by saying, "48 is, in all probability, the correct diploid or somatic chromosome number for both the male and female of the white and Negro races."

(Painter, 1923) He arrived at this number by studying the testicular tissue of one white and two black inmates of the Texas State Insane Asylum in Austin. He further reported in detail the "cause for the removal of the testes was excessive self-abuse coupled with certain phases of insanity which made the removal of the sex glands desirable." (Painter, 1923)

From research results obtained in the 1960's, an abnormal chromosome number is not uncommon in violent male mental patients. (DeRobertis et al., 1970) Painter unknowingly may have recorded the first chromosomal anomaly associated with mental illness rather than what he thought was the normal human diploid chromosome number. As appealing as this idea may seem, Kottler argued against it by stating:

"Since one or both of the extra chromosomes would undoubtedly be a sex chromosome, a trivalent or quadrivalent of the sex chromosomes would form and spermatogenesis would eventually break down. But Painter described just normal spermatogenesis with a normal XY bivalent." (Kottler, 1974)

Conclusion
Our original goal with this report was to demonstrate that the 46 chromosome paradigm would illustrate a biological scientific revolution
in the manner of Kuhn. However, the 48 chromosome paradigm was abandoned too easily and too quickly for it to be considered a Kuhnian revolution. From our vantage point of being 40 years from the chromosome paradigm shift, it is lost that the original issue was not the diploid chromosome number but rather how sex determination takes place in humans. Prior to the 1950’s the technique for chromosome visualization was quite arduous. Advances in cancer research during the 1950’s made the knowledge of the human diploid chromosome number essential. Tjio and Levan were able to augment the Winiwarter and Painter research because at least four advances in chromosome preparation came together in 1955. Appreciating this history has caused us to reevaluate Kuhn.

The reviews from the book jacket of The Structure of Scientific Revolutions states the following about Kuhn’s work, “... is a landmark in intellectual history... he erects from ground up a structure in which science is seen to be heavily influenced by nonrational procedures.” With tidiness we offer these observations about a “landmark in intellectual history.” Kuhn states on page 11, “The study of paradigms, including many that are far more specialized than those named illustratively above, ... .” (Kuhn, 1970) Those illustrations named above include ‘Ptolemaic astronomy’, ‘Aristotelian dynamics’, and ‘corpuscular optics’. It is not clear whether Kuhn would consider the chromosome number shift to be large enough intellectual game for one of his paradigms. Perhaps he would consider the game to be the role of chromosomes in heredity.

Kuhn acknowledges the role of equipment and technique towards progress:

“Again and again complex special apparatus has been designed for such purposes, and the invention, construction, and deployment of that apparatus have demanded first-rate talent, much time, and considerable financial backing. ... From Tycho Brahe to E. O. Lawrence, some scientists have acquired great reputations, not from any novelty of their discoveries, but from the precision, reliability, and scope of the methods, they developed for the redetermination of a previously known sort of fact. ... The existence of the

paradigm sets the problem to be solved; often the paradigm theory is implicated directly in the design of the apparatus able to solve the problem.” (Kuhn, 1970, pages 25-27)

It is clear that Winiwarter and Painter were experts in the methodology of the visualization of fixed tissue chromosomes; however, they did not invent the techniques but rather applied them. In a similar fashion Tjio and Levan pulled together techniques to better visualize chromosomes. The techniques combined were not expressly invented to be applied to the chromosome number controversy. It is this habit of scientists to repurpose technology, techniques, and equipment that Kuhn seems to underappreciate in what he calls “the nature of normal science.” (See chapter 3 title in Kuhn, 1970, page 23.)

Kuhn considers only the big questions in science. He does not dwell on the nuances of mixing buffers and developing tools to mince freshly castrated tissue. Kuhn looks for revolutions in thought and not to the day-to-day practice of “normal science.” For Kuhn the Eureka experience has to do with thinking about planetary motion in a bath tub rather than being in a deer blind thinking about how to soften up Drosophila salivary glands as Painter was purported to have done. (John Bieseke, personal communication) Kuhn is an observer rather than a player in the business of science.

With apologies to Milton, was the 48 chromosomes story a Paradigm Lost? Knowledge of the human diploid chromosome number became important after it was discovered. Turner’s and Klinefelter’s syndromes can be appreciated only if one has the knowledge that humans normally have 46 chromosomes in each somatic cell. The cytologists in this report backed into their discovery (number of chromosomes) while their focus was on something else (sex determination). Perhaps Kuhn has the perfect vision of hindsight for many scientists do not know that they are in the midst of a scientific revolution until it is over. Did Gregor Mendel know he was a part of a scientific revolution? Tjio and Levan brought the scientific community to a new embarking point with their refinement of the human chromosome number. Perhaps science is
more a series of journeys with some trips without
destination rather than a series of constructed
scientific revolutions.

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