

TIMELINE

The chromosome number in humans: a brief history

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Abstract | Following the rediscovery of Mendel's work in 1900, the field of genetics advanced rapidly. Human genetics, however, lagged behind; this was especially noticeable in cytogenetics, which was already a mature discipline in experimental forms in the 1950s. We did not know the correct human chromosome number in 1955, let alone were we able to detect a chromosomal abnormality. In 1956 a discovery was reported that markedly altered human cytogenetics and genetics. The following is an analysis of that discovery.

In 1956 the field of genetics was half a century old, dating from the rediscovery of Mendel's work in 1900 (REFS 1–3). Formal genetics (mutation analysis, mapping and cytogenetics) was already a mature field, as shown by the results of studies in *Drosophila melanogaster* and *Zea mays*, and to a lesser extent in other experimental organisms. The one gene–one enzyme hypothesis of George Beadle and Edward Tatum⁴, which was derived from studies in *Neurospora crassa*, was almost two decades old, and, most importantly, the structure of DNA had been published three years earlier⁵. The golden age of genetics was at hand.

Human genetics was in a different state; overall, the field had been strongly influenced by the earlier biometrical work of Francis Galton⁶, who argued against the applicability of Mendelian rules to human heredity. There were some bright spots, however. One of these was Karl Landsteiner's discovery, in 1901, of the ABO blood group system, a classic example of a human Mendelian character⁷. Later, Landsteiner and his students discovered the Rh (rhesus) factor and other blood group systems, and by the early 1950s nine independent Mendelian human blood group systems had been described. Another important, but largely unrecognized contribution was that of Archibald Garrod⁸, who foresaw the one gene–one enzyme idea through his work on inborn errors of

metabolism in 1909. In 1949 Linus Pauling⁹ and his colleagues separated human haemoglobin molecules by electrophoresis and described **sickle cell anaemia** as a molecular disease. The resultant technology led to molecular studies of other haemoglobinopathies and to the demonstration of malaria as a selective agent in the incidence of sickle cell anaemia¹⁰. In 1955 Oliver Smithies¹¹ described the new technique of starch gel electrophoresis, which led to the discovery of even more human Mendelian variants. Some progress was also made in the formal genetics of humans with the reporting of a small number of autosomal linkage groups¹² and the publication of the LOD (logarithm of the odds)¹³ score method of linkage analysis. But in 1956 not a single chromosomally identified linkage group was known, except for the X chromosome.

This state of affairs was about to change. In 1956 Joe Hin Tjio and Albert Levan¹⁴ reported that the correct human chromosome number was 46, and not 48 as was supposedly established some three decades earlier. The importance of this finding was not the number itself, but rather that now one could confidently distinguish between counts of 46 and 48 chromosomes. An amazing amount of human cytological variation, probably not anticipated by Tjio and Levan, was about to be revealed that would have tremendous effects in more than one area of human genetics.

Human chromosomes: the early period

Chromosomes were first observed in the second half of the nineteenth century, following the incorporation of fixation and staining procedures as standard steps in cytological preparations. The term 'chromosome' derives from the fact that these structures were conspicuously stained ('chroma' means 'coloured' in Greek). Mitotic figures were observed and soon the various stages of mitosis were worked out in plants and animals¹⁵. Human mitotic figures were observed in the late 1800s, but suitable material was not readily available. Early chromosome studies were interested in chromosome movement, not numbers. With the development of the chromosome theory of heredity^{16,17}, however, and the rediscovery of Mendel's work, counting chromosomes became important as it was realized that species have distinct numbers of chromosomes.

Human chromosome numbers were determined in the 1890s, and by 1914 at least 15 investigators had published papers reporting the chromosome number in humans¹⁸. With one exception, the counts were low, with most reporting 24 as the diploid number. The lone exception was Hans de Winiwarter¹⁹, who reported a count of 47 from testes and 48 from fetal ovaries. As we now know, only Winiwarter's counts were close to being correct, apparently because he realized the importance of fresh material and immediate fixation, to avoid the clumping of chromatin. Once the need for fresh specimens and immediate fixation was recognized, reports of low chromosome counts disappeared. But, for a time, some investigators believed there might be natural variation in human chromosome number. Because Winiwarter had worked with Caucasian specimens and some of the 24 counts were from specimens from individuals of African descent, the suggestion was made that blacks and whites might differ by a factor of two in their chromosome number. As preposterous as this notion seems today, no less a geneticist than Thomas H. Morgan considered such variation a possibility and mentioned it briefly in his textbook²⁰. However, by the 1920s, when Theophilus Painter published his counts of 48 in both blacks and whites, this idea was discarded;

the view that the chromosome number in humans was 48 appeared established, remaining so for over three decades.

Theophilus S. Painter (1889–1969)

If anyone must bear the burden for broadcasting the incorrect human chromosome number, it is Painter (FIG. 1). He was a well-trained insect cytologist who specialized in studies of spermatogenesis. After receiving his Ph.D. from Yale University in 1913, he spent a postdoctoral year in Europe in the laboratory of Theodour Boveri, possibly the foremost cytologist of his time. Painter returned to an instructorship at Yale and two years later he moved to a tenure-track position at the University of Texas; he would remain there for the rest of his life, eventually becoming president of the university.

In 1921 he published his first paper on human chromosomes, showing the presence of a Y chromosome in male testicular preparations and a total count of 48 chromosomes²¹. For the next seven years he studied only mammalian chromosomes in organisms as diverse as the opossum, monkey and horse. Then, in 1929, Painter went back to insect cytology; he turned to the cytogenetics of *D. melanogaster*, probably under the influence of Hermann J. Muller, the future Nobel Prize winning geneticist who was in the same department in Texas at the time²². In 1933 Painter discovered that the structures found in the nuclei of the salivary glands of all Diptera were closely paired homologous chromosomes, and with the background of genetic data available on *D. melanogaster*, he carried the cytogenetic analysis of the salivary gland chromosomes to a new level²³. This work brought him a number of honours, and he was soon considered one of the leading cytogeneticists of the time.

When reading the original Painter papers on human chromosomes, one is struck by the fact that Painter is uncertain about the counts. In the first paper he states: “In my own materials the counts range from 45 to 48 apparent chromosomes, although in the clearest plates so far studied only 46 chromosomes have been found.”²¹ He concludes that the true count must be either 46 or 48. In his main paper on chromosome counts in spermatogenesis, he still expresses doubts about the counts, but concludes that the correct count is 48 (REF. 24). When I examined the figures in Painter’s papers, which are *camera lucida* drawings of what one sees in the microscope, I could appreciate the difficulty in making an exact count. Tao-Chih Hsu, a prominent mammalian cytogeneticist



Figure 1 | **Theophilus S. Painter**. Reproduced from REF. 26, courtesy of Centennial Photos, Barker Texas History Centre.

and one of the discoverers of the hypotonic shock method for chromosome spreading, examined a slide from Painter’s collection and remarked that: “it’s amazing that he even came close.”²⁵ (FIG. 2)

What, then, made Painter so positive about 48? In his 1921 paper, the main issue was whether humans had an X0 or XY sex-determining mechanism. In the 1923 paper, the total chromosome number was the main issue. He might have felt that if he was unable to determine the correct chromosome number he would not be able to publish the work. Bentley Glass, in his biographical memoir of Painter, mentions that Painter: “experienced deep chagrin over this error in what had long been regarded as a primary discovery for which he was known and universally cited.”²⁶

Frank Ruddle²⁷ has recently proposed that Painter’s error might have resulted from interpreting chromosome 1 as two chromosomes owing to its large block of heterochromatin adjacent to the centromere. A similar suggestion had been made by Maj Hultén²⁸. This is a possibility, although I believe that the main problem in obtaining accurate counts was the poor quality of preparations at that time: exact counts were not possible. For example, a little known fact, pointed out to me by James Crow, is that Painter also examined testicular material from a **Down syndrome** case supplied to him by Charles B. Davenport²⁹. He reported that there was nothing unusual about the chromosomes. This implies that Painter, or

probably any other mammalian cytologist of the time, could not reliably detect an extra small chromosome.

Technical changes in cytogenetics

At the same time as Painter and others obtained incorrect human chromosome numbers, these same investigators obtained correct counts on several other mammalian species, despite using similar materials and techniques. One of the reasons for this, I suspect, was the limited availability of human specimens. Spermatogonial cells are one of the few sources of rapidly dividing human cells; in humans, such samples in the 1920s and 1930s were difficult to obtain. Malcolm Kottler³⁰, in his excellent history of the human chromosome counting story, relates that cytologists “literally waited at the foot of the gallows in order to fix the testis of an executed criminal immediately after death before the chromosomes could clump.” Such specimens are essentially unlimited in experimental animals. Only a very small percentage of dividing cells under the conditions being used at the time could be accurately counted. In non-human organisms the investigator had the luxury of being able to search for that rare perfect cell; not so with human material.

At the technical level, an equally important drawback was the paraffin sectioning method used by Painter and other cytologists at the time. The paraffin sectioning method involves impregnating a fixed piece of tissue with paraffin and then cutting the paraffin block into very thin slices. Individual sections are attached to a slide, the paraffin dissolved away and the tissue slice stained for observation. This technique did not allow separation of chromosomes from one another, and sectioning could cut through cells, therefore splitting chromosomes and making it difficult to follow the continuity of a chromosome.

Ironically, the same year in which Painter published his first paper on human chromosomes, John Belling³¹ described his squash technique for counting chromosomes in pollen mother cells. This technique involves squashing cells between the slide and a cover slip, which tends to spread the chromosomes. It became the standard for cytological work and was used by Painter for his studies on the salivary gland chromosomes of *D. melanogaster*. The squash method alone probably would not have solved the difficulties in human chromosome counting, at least for testicular material. In fact, Ursula Mittwoch³², who studied testicular material from a Down syndrome case in 1952, used

the squash method and still came up with a count of 48 for both spermatogonial and spermatocytic preparations.

Possibly the most important modification for chromosome studies was the use of the hypotonic shock method to spread the nuclear contents. This technique was introduced by Eleanor Slifer³³ for insect cytology, but was overlooked and later independently rediscovered by three mammalian groups^{34–36}. If the target tissue has a sufficient number of dividing cells, a hypotonic shock might be all that is necessary to obtain good chromosome spreads. This is how Charles Ford and John Hamerton³⁷, using testicular samples, quickly confirmed the 46 count of Tjio and Levan¹⁴.

Another important technical addition to human cytology was the use of colchicine, which arrests dividing cells in metaphase and therefore increases the number of cells suitable for analysis. One of the discoverers of the colchicine effect was Levan³⁸, of Tjio and Levan fame. Colchicine was of limited effectiveness in early human and animal cytological work until it was combined with cell culture. Colchicine not only increased the proportion of cells in metaphase, but also

enhanced the condensation of chromosomes, thereby minimizing overlaps. The combination of hypotonic shock (spreading) and colchicine (increased condensed metaphases) led to the picture-perfect preparations obtained by Tjio and Levan.

Albert Levan (1905–1998)

In the 1950s all the necessary technology was available to obtain high-quality human metaphase preparations, but papers still appeared supporting Painter's reported number of 48 chromosomes. Possibly the most striking of these papers is one by Levan³⁹, which was published in the same year as his classic Tjio and Levan paper¹⁴. Levan (FIG. 3) spent a year at the Sloan Kettering Institute in New York studying chromosomes in human cancer cell lines and perfecting his use of colchicine and hypotonic shock for improved chromosome preparations. The paper showed that several human cancer cell lines and some control cells from fibroblast cultures had heteroploid chromosomes (that is, not a whole-number multiple of the haploid number of chromosomes for that species). Levan states that: "The fibroblast culture gave the expected

normal human chromosome number."³⁹ So months before Levan co-authored with Tjio the classic paper in which they showed that the correct chromosome number in humans was 46, not 48, he was still under the pervasive influence of Painter's count of 48 chromosomes.

Another telling incident with regards to bias comes from Hultén, who was an undergraduate student in the same department as Tjio and Levan when they were carrying out their classic work. She remembers being told by the director of the Institute, Arne Müntzing, that: "earlier that year Doctors Eva and Yngve Melander working on normal human fetal cells had problems with their chromosome preparation as they could only find cells with incomplete chromosome plates, the maximum number being 46." (REF. 28) In fact, according to a recent report by Peter Harper⁴⁰, the Melanders showed their results to Levan before he left for his work at the Sloan Kettering Institute in 1955. Clearly, he was not prepared for the possibility that 48 was not the correct human chromosome count. The evidence was so overwhelming that no one appeared to question Painter's 48 count. Levan, who was a well-established professor at Lund, had already made an important contribution with his work on colchicine in plant material. He had switched most of his laboratory over to work on animal cancer lines and was interested in improving chromosome analysis so that he might be able to detect significant cytological changes in cancer.

Joe Hin Tjio (1919–2001)

Tjio, of Chinese ancestry, was born in Java in 1919 (FIG. 4). Java was then part of the Dutch East Indies. Tjio learned photography from his father, several languages in Dutch colonial schools, and agronomy in college. At the start of the Second World War he held a position in Java as an agronomist, working on potato breeding, and had become involved in plant cytogenetics. The Japanese invaded Java and in 1942 Tjio was arrested by the Japanese military and subjected to torture as a possible source of military information. He was a prisoner of war for three years until the Second World War ended. When the war ended he was considered a displaced person and was taken to Holland, where the Dutch government provided him a fellowship for study in Europe. He spent some time at the Royal Danish Academy in Copenhagen and also established a relationship with Levan at the University of Lund in Sweden.



Figure 2 | **Camera lucida drawing of a human spermatogonial metaphase made by Theophilus S. Painter.** The drawing presumably shows 48 chromosomes. The cytological methods used were state of the art for the time, but it is still difficult, if not impossible, to make an exact count. Reproduced with permission from REF. 25 © (1979) Springer Verlag.

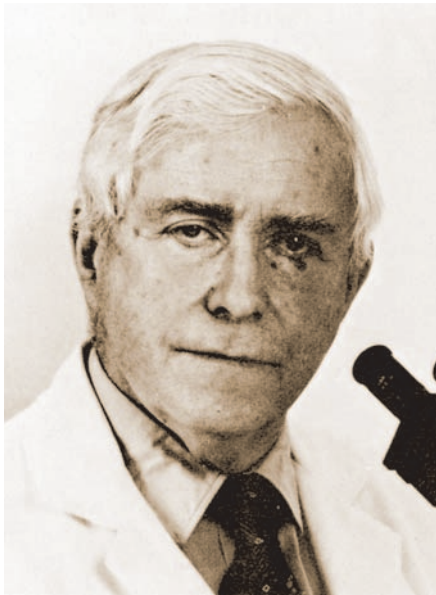


Figure 3 | **Albert Levan**. Reproduced with permission from REF. 25 © (1979) Springer Verlag.

In 1948 Tjio obtained a position in Zaragosa (Spain), directing plant cytogenetic research, but took summers off to continue working with Levan. He became a regular visitor to Lund and concentrated his efforts on chromosomes in cancer. He wanted to know whether there were consistent chromosomal changes associated with malignant growth, but was hampered by a lack of good data on normal human chromosomes. The virus laboratory in Lund had access to fetal specimens, and late in 1955 he was told that there were several fetal cell cultures on which he could study normal chromosomes. He came back to Lund in December and in a short time had the results that were the basis of the classic 1956 paper.

Tjio's unusual background and life experiences might have made him more likely than others to question authority regarding '48'. He also must have been aware of the Melanders's results. In their 1956 paper, in fact, Tjio and Levan credit the Melanders and another member of their group, Stig Kullander, with having made early observations of 46 chromosomes in human cell cultures. In a 1978 article describing the discovery, Tjio remarks that the early observations of sectioned testis material were difficult to interpret and that the observations from smear preparations in the early 1950s were of poor quality and wishful interpretations of the configurations⁴¹. I believe, however, that the overriding factor in challenging '48' was the quality of his preparations. They were simply the best human metaphase spreads that had been made. There was no question about the count!

The classic 1956 paper

The Tjio and Levan paper was the first in the 1956 volume of *Hereditas* and was also presented as a demonstration at the First International Congress of Human Genetics (Copenhagen, 1956) (FIG. 5). Muller was attending the Congress and was so impressed with the work that he encouraged Tjio to come to America and work with Theodoure T. Puck, a pioneer in the new field of human somatic cell genetics. Tjio resisted because he was upset with the McCarthy hearings that were embroiling in the United States at the time. Muller apparently sent him clippings from the New York Times that were critical of McCarthy; Tjio relented and joined Puck's laboratory in 1957. He and Puck published a confirmatory paper on the human chromosome number⁴², and Tjio earned the Ph.D. degree that he had not previously acquired but richly deserved. In 1959 Tjio was invited to the US National Institutes of Health as a visiting scientist where he remained for the rest of his career.

One of the unfortunate aspects of this bit of history is that Tjio and Levan apparently had a dispute over authorship of the paper. Who should be the first author? According to a brief account by Rich McManus⁴³, which was derived from an interview with Tjio, Levan had claimed first authorship on the basis of being head of the laboratory. This seems unlikely, however, as the two men had published at least five joint papers previously and Tjio was always the first author. It seems more likely that an argument arose over who was the prime force behind the work. There is no doubt that Tjio did the laboratory work and made the original counts. As Levan had missed the correct chromosome number in his work at Sloan Kettering in 1955 (REF. 39), it seems proper that Tjio should have been the first author. In this day of mega-authorships, such an argument seems strange when only two authors are involved, especially as there was enough credit to go around.

48 or 46: what does it mean?

I first heard about the Tjio and Levan work at the First International Congress of Human Genetics in Copenhagen in 1956. Tjio and Levan did not present a paper, but they had a poster demonstration that attracted much attention. I was a postdoctoral fellow and remember at least one senior person saying 'very interesting, but what difference will it make?' Formal genetic analysis and a good deal of molecular studies are independent of chromosome number. For example, the field of yeast genetics grew by leaps and bounds in the 1960s and 1970s, but the correct

chromosome number in yeast was not known until 1985 (REF. 44). Clearly, the absence of a chromosome count did not inhibit the growth of yeast genetics. As mentioned in the introduction, human genetics in 1956 was in a different state, and the Tjio and Levan report would have an important effect on the field.

Surprisingly, it did not happen immediately. But in 1959 reports appeared identifying numerical chromosome abnormalities in Down⁴⁵, Turner⁴⁶, and Klinefelter syndromes⁴⁷. Reports of XXX females⁴⁸ and XYY males⁴⁹ were soon published; the field of medical cytogenetics was born. With the development of amniocentesis in the 1960s the field developed an important clinical application. In 1956 no one could be identified as a medical cytogeneticist. Today there must be at least a thousand professional medical cytogeneticists worldwide and ten times that number in supporting positions. At the least, the Tjio and Levan paper created a minor industry. Tjio and Levan took no part in this cytogenetic work, apparently having gone back to their studies of chromosomes in cancer.

In 1960 Peter Nowell⁵⁰ discovered that phytohaemagglutinin stimulated white blood cells to divide, a tremendous stimulus to human cytogenetics. That same year he and David Hungerford discovered the Philadelphia chromosome, a supposed deletion of chromosome 22 in patients with **chronic myelogenous leukaemia**⁵¹. This was the first specific chromosomal change associated with a human cancer, and Tjio



Figure 4 | **Joe Hin Tjio**. Reproduced with permission from REF. 25 © (1979) Springer Verlag.

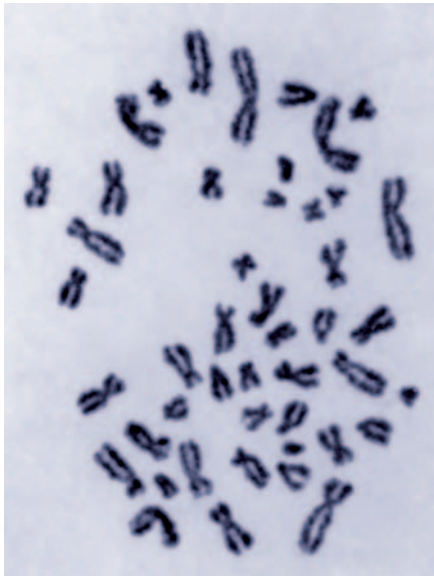


Figure 5 | A human metaphase plate, from the original Tjio and Levan paper, showing 46 chromosomes. Reproduced with permission from REF. 14 © (1956) Blackwell Publishing.

devoted a good deal of his later career to the study of the Philadelphia chromosome. In the late 1960s another major Swedish contribution to human cytogenetics took place with the introduction of chromosome banding by Torbjorn Caspersson's group⁵². This cytological technique allowed essentially perfect discrimination of one chromosome from another, analysis of meiotic pairing⁵³ and, most importantly, accurate analyses of chromosome rearrangements. Banding was the tool that Janet Rowley⁵⁴ used to show that the Philadelphia chromosome was in fact a translocation between chromosomes 9 and 22, and not a simple deletion of chromosome 22. This finding had profound implications for understanding the origin of cancer, as well as cancer cytogenetics.

As mentioned in the introduction, in 1956 not a single human gene had been assigned to a specific autosomal chromosome. This was still true 10 years later, although by 1966 pedigree analyses had confirmed about 600 autosomal and 70 X-chromosome linked genes. In the 1960s Henry Harris's group⁵⁵ and Boris Ephrussi and Mary Weiss⁵⁶ produced mouse-human somatic cell hybrids, which preferentially lost human chromosomes. The pattern of selective human chromosome loss from these hybrids raised the possibility of human gene mapping by correlating the chromosomal and gene expression patterns in these hybrids. The first example of mapping a human gene to a specific autosome was carried out in this way 2 years later⁵⁷.

Later, when gene isolation became possible, labelled probes were hybridized to human chromosome spreads⁵⁸ and even more mapping information was obtained.

Therefore, the Tjio and Levan work not only led to contributions directly related to human cytogenetics, but was also a major building block for early progress in formal human genetics.

Conclusions

What would human genetics history be without Tjio and Levan? There is no evidence that anyone was planning a cytological challenge to the prevailing idea of 48 human chromosomes. It might have been several more years before human cytogenetics could begin and before such important discoveries as the Philadelphia chromosome would be made. If the time span were long enough, formal human genetics probably would have developed independently through DNA polymorphism and pedigree studies. In fact, it is possible that the **Human Genome Project** could have gone ahead and the correct human chromosome number discovered in a completely molecular fashion.

It is interesting to speculate as to what would human genetics history be had Painter concluded in his 1923 paper that the correct count was 46. Tjio and Levan would still have done their work showing that unambiguous and beautiful human chromosome preparations were possible. Their work would not have attracted as much attention, but it would have been noted by enough workers and applied, although perhaps not as quickly, to the discovery of human aneuploids. And Painter's reputation would have remained unblemished.

Most writers on this interesting period of human genetics have ascribed the problem of the continuing incorrect chromosome count following Painter to 'preconception'³⁰. The number was supposed to be 48 so subsequent investigators did everything possible to make their counts 48. The surprise is why there were apparently no criticisms of the drawings published by Painter. It would be interesting to know whether any of the reviewers of Painter's publications on human chromosomes said anything about the quality of his preparations. I would agree that preconception was an important impediment to the delayed discovery of the correct human chromosome number, but I think that the surprising lack of criticism of the quality of the original data might have been equally important.

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- Correns, C. G. Mendel's Regel über das Verhalten der Nachkommenschaft der Rassenbastarde. *Deutsch. Bot. Gesell.* **18**, 158–168 (1900) (in German).
- Tschermak, E. Über künstliche Kreuzung bei *Pisum sativum*. *Zeit. Landw. Versuch. Oest.* **3**, 465–555 (1900) (in German).
- de Vries, H. Sur la loi de disjonction des hybrides. *C. R. Acad. Sci. Paris* **130**, 845–847 (1900) (in French).
- Beadle, G. W. & Tatum, E. L. Genetic control of biochemical reactions in *Neurospora*. *Proc. Natl Acad. Sci. USA* **27**, 499–506 (1941).
- Watson, J. D. & Crick, F. H. C. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature* **171**, 737–738 (1953).
- Galton, F. *Natural Inheritance* (Macmillan, London, 1889).
- Landsteiner, K. Über Agglutinationseigenschaften normaler menschlicher Blute. *Wien. Klin. Wochenschr.* **14**, 1132–1134 (1901) (in German).
- Garrod, A. E. *Inborn Errors of Metabolism* (Oxford Univ. Press, Oxford, 1909).
- Pauling, L., Itano, H. A., Singer, S. J. & Wells, I. C. Sickle cell anemia, a molecular disease. *Science* **110**, 543–548 (1949).
- Allison, A. C. Protection afforded by sickle-cell trait against subtertian malarial infection. *BMJ* **1**, 290–294 (1954).
- Smithies, O. Zone electrophoresis in starch gels; group variations in the serum proteins of normal adult human subjects. *Biochem. J.* **61**, 629–641 (1955).
- Jameson, R. J., Lawler, S. D. & Renwick, J. H. Nail-patella syndrome: clinical and linkage data on family G. *Ann. Hum. Genet.* **20**, 348–360 (1956).
- Morton, N. E. Sequential tests for the detection of linkage. *Am. J. Hum. Genet.* **7**, 277–318 (1955).
- Tjio, J. H. & Levan, A. The chromosome number of man. *Hereditas* **42**, 1–6 (1956).
- Wilson, E. B. *The Cell in Development and Inheritance* (Macmillan, London, 1896).
- Boveri, T. Über mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. *Verh. Phys.-Med. Ges.* **35**, 67–90 (1902) (in German).
- Sutton, W. S. The chromosomes in heredity. *Biol. Bull.* **4**, 231–251 (1903).
- Oguma, K. & Makino, S. A revised check-list of the chromosome number in vertebrates. *J. Genet.* **26**, 239–254 (1932).
- de Winiwarter, H. La formule chromosomale dans l'espèce humaine. *C. R. Seances Soc. Biol. Fil.* **85**, 266–267 (1921) (in French).
- Morgan, T. H. *Heredity and Sex* (Columbia Univ. Press, New York, 1914).
- Painter, T. S. The Y-chromosome in mammals. *Science* **53**, 503–504 (1921).
- Carlson, E. A. *Genes, Radiation, and Society. The Life and Work of H. J. Muller* (Cornell Univ. Press, Ithaca, New York, 1981).
- Painter, T. S. The morphology of the X-chromosome in salivary glands of *Drosophila melanogaster* and a new type of chromosome map for this element. *Genetics* **19**, 448–469 (1934).
- Painter, T. S. Studies in mammalian spermatogenesis. II. The spermatogenesis of man. *J. Exp. Zool.* **37**, 291–336 (1923).
- Hsu, T. C. *Human and Mammalian Cytogenetics: An Historical Perspective* (Springer, New York, 1979).
- Glass, B. Theophilus Shickel Painter: August 22, 1889–October 5, 1969. *Biogr. Mem. Natl Acad. Sci.* **59**, 309–337 (1990).
- Ruddle, F. H. Theophilus Painter: first steps toward an understanding of the human genome. *J. Exp. Zool.* **301A**, 375–377 (2004).
- Hultén, M. A. Numbers, bands and recombination of human chromosomes: historical anecdotes from a Swedish student. *Cytogenet. Genome Res.* **96**, 14–19 (2002).
- Davenport, C. B. Mendelism in man. *Proc. 6th Int. Congr. Genet.* **6**, 135–140 (1932).
- Kottler, M. J. From 48 to 46: cytological technique, preconception and the counting of the human chromosomes. *Bull. Hist. Med.* **48**, 465–502 (1974).
- Belling, J. On counting chromosomes in pollen mother cells. *Am. Nat.* **55**, 573–574 (1921).

32. Mittwoch, U. The chromosome complement in a Mongolian imbecile. *Ann. Eugenics* **17**, 37 (1952).
33. Slifer, E. H. Insect development: VI. The behavior of grasshopper embryos in anisotonic, balanced salt solution. *J. Exp. Zool.* **67**, 137–157 (1934).
34. Hsu, T. C. Mammalian chromosomes *in vitro*. I. The karyotype of man. *J. Hered.* **43**, 167–172 (1952).
35. Hughes, A. Some effects of abnormal tonicity on dividing cells in chick tissue cultures. *Quart. J. Microscopical Sci.* **93**, 207–220 (1952).
36. Makino, S. & Nishimura, I. Water-pretreatment squash technique: a new and simple practical method for the chromosome study of animals. *Stain Technol.* **27**, 1–7 (1952).
37. Ford, C. E. & Hamerton, J. L. The chromosomes of man. *Nature* **178**, 1020–1023 (1956).
38. Levan, A. The effect of colchicine on root mitosis in *Allium*. *Heredity* **24**, 471–486 (1938).
39. Levan, A. Chromosome studies on some human tumors and tissues of normal origin, grown *in vivo* and *in vitro* at the Sloan–Kettering Institute. *Cancer* **9**, 648–663 (1956).
40. Harper, P. S. The discovery of the human chromosome number in Lund, 1955–1956. *Hum. Genet.* **119**, 226–232 (2006).
41. Tjio, J. H. The chromosome number of man. *Am. J. Obstet. Gynecol.* **130**, 725–724 (1978).
42. Tjio, J. H. & Puck, T. T. Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. *J. Exp. Med.* **108**, 259–268 (1958).
43. McManus, R. NIDDK's Tjio ends distinguished scientific career. *NIH Record* [online], < http://www.nih.gov/news/NIH-Record/02_11_97/story01.htm > (2 Feb 1997).
44. Carle, G. F. & Olson, M. V. An electrophoretic karyotype for yeast. *Proc. Natl Acad. Sci. USA* **82**, 3756–3760 (1985).
45. Lejeune, J., Gautier, M. & Turpin, R. Etude des chromosomes somatiques de neuf enfant mongoliens. *Compt. Rend.* **248**, 1721–1722 (1959) (in French).
46. Ford, C. E., Jones, K. W., Polani, P. E., De Almeida, J. C. & Briggs, J. H. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* **I**, 711–713 (1959).
47. Jacobs, P. A. & Strong, J. A. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* **183**, 302–303 (1959).
48. Jacobs, P. A., Baikie, A. G., MacGregor, T. N. & Harndon, D. G. Evidence for the existence of the human 'super female'. *Lancet* **II**, 423–425 (1959).
49. Sandberg, A. A., Koepf, G. F., Isihara, T. & Hauschka, T. S. An XYY human male. *Lancet* **II**, 488–489 (1961).
50. Nowell, P. C. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leucocytes. *Cancer Res.* **20**, 462–466 (1960).
51. Nowell, P. C. & Hungerford, D. A. A minute chromosome in human chronic granulocytic leukemia. *Science* **132**, 1497 (1960).
52. Caspersson, T., Zech, L. & Johansson, J. Differential banding of alkylating fluorochromes in human chromosomes. *Exp. Cell Res.* **60**, 315–319 (1970).
53. Hultén, M. Chiasma distribution at diakinesis in the normal human male. *Hereditas* **76**, 55–78 (1974).
54. Rowley, J. D. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243**, 290–293 (1973).
55. Harris, H. & Watkins, J. F. Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species. *Nature* **205**, 640–646 (1965).
56. Ephrussi, B. & Weiss, M. C. Interspecific hybridization of somatic cells. *Proc. Natl Acad. Sci. USA* **53**, 1040–1042 (1965).
57. Weiss, M. C. & Green, H. Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proc. Natl Acad. Sci. USA* **58**, 1104–1111 (1967).
58. Morton, C. C., Kirsch, I. R., Taub, R., Orkin, S. H. & Brown, J. A. Localization of the β -globin gene by chromosomal *in situ* hybridization. *Am. J. Hum. Genet.* **36**, 576–585 (1984).

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Competing interests statement

The author declares no competing financial interests.

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