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SERIES ON HISTORICAL PROFILES — TIMELINE

Theodor and Marcella Boveri: chromosomes and cytoplasm in heredity and development

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Abstract | The chromosome theory of heredity, developed in 1902–1904, became one of the foundation stones of twentieth-century genetics. It is usually referred to as the Sutton–Boveri theory after Walter Sutton and Theodor Boveri. However, the contributions of Theodor Boveri and his co-worker, Marcella O'Grady Boveri (also his wife), to the understanding of heredity and development go beyond the localization of the Mendelian hereditary factors onto the chromosomes. They investigated the interaction of cytoplasm and chromosomes, and demonstrated its relevance in heredity and development.

In appreciation of his visionary ideas for genetics, which were formulated in the early years of the twentieth century, Theodor Boveri has been called "the first genetic engineer"[1]. He is primarily remembered for his identification of chromosomes as the site for Mendelian factors, later called genes[2,3]. However, molecular and developmental biologists are finding his results on fertilization and early embryonic development, and his hypothesis on the genetic causes of cancer (BOX 1), to be of new relevance today[4–9]. Theodor Boveri's wife, Marcella O'Grady Boveri, was important as his collaborator for over two decades. She has also recently been rediscovered[9,10]. The finding of another 'creative couple in the sciences' illustrates the situation at the turn of the last century, when women in Germany and the United States fought for access to scientific careers[11–14]. This article traces the lives of the Boveris and investigates the main stages of their attempts to understand inheritance and early embryonic development at a time before and during the establishment of genetics and embryology. Their work was based on cytology combined with experimental manipulation of chromosomes and cytoplasm during the processes of fertilization and ontogenesis. From the beginnings of his career, Theodor Boveri belonged to the group of scientists who, following Carl Naegeli, saw the cell nucleus as containing the substances responsible for the determination and inheritance of the characters of the cell[15]. However, the Boveris also investigated the contribution of the cytoplasm to heredity. In their view, heredity included both the transmission of traits to the next generation of individuals and the process of embryonic development from fertilized egg to the differentiated somatic cells of the embryo.

Biographies

Theodor Boveri: becoming a zoologist. Theodor Boveri was born in the northern Bavarian town of Bamberg, Germany, in 1862. His father was a physician with a passion for botany and music. The young Boveri, as passionate as his parents about arts and music, was destined to become an engineer or architect, to which end he attended the Realgymnasium — a school focusing on sciences and mathematics. In 1881 he enrolled at the University of Munich, Germany, beginning with courses in history, philosophy and classical languages. However, after one term he changed to anatomy, became an assistant to the anatomist Carl von Kupffer and eventually finished his doctoral dissertation on nerve fibres under Kupffer's supervision in 1885. A 7-year scholarship then gave him the freedom to do what he wanted, so he moved to the Zoological Institute in Munich under the directorship of Richard Hertwig. Hertwig and his brother Oscar were famous for their description in 1875 of fertilization as a cellular process involving the fusion of an egg cell and a spermatozoan and the fusion of their nuclei. Theodor Boveri concentrated on cytology and the investigation of fertilization, cell division and early embryonic development using microscopy, sophisticated staining techniques and his highly developed drawing skills. As early as 1887 he obtained his 'habilitation', which qualified him as a university lecturer in zoology and comparative anatomy. A short illness then interrupted his career and left him susceptible to rheumatism and neurasthenia.

After his scholarship expired, he worked for a short time as an assistant in Richard Hertwig's institute, where he met the American cytologist Edward Beecher Wilson and developed a lifelong friendship. In 1893, at 30 years old, Boveri was appointed professor

Box 1 | **A genetic cause for cancer, 1914**

Towards the end of Theodor Boveri's life, he and his wife, Marcella O'Grady Boveri, started a new experimental system using the lens and cornea epithelium of the rabbit to investigate the cellular causes of cancer. Based on his understanding of the processes of cell division and the relevance of each individual chromosome for normal development, in 1914 Theodor Boveri proposed the hypothesis that cancer cells derive from cells with an irreparable defect within the chromosomes. In this hypothesis, each malignant tumour would derive from a particularly injured primordial cell and have a changed metabolism. Owing to the defect in the chromosomes, the cell would no longer react to the conditions of its surroundings and would start multiplying again. Endless multiplication was thought to be the natural tendency of single cells, something that was inhibited in the context of differentiated tissue[50]. This hypothesis of a chromosomal or genetic cause of cancer was only reconsidered in recent decades in the light of new findings on genomic rearrangements and cancer genetics[7–9,51].

of zoology and comparative anatomy at the University of Würzburg, Germany, when the prosperous Würzburg was a centre of medical and scientific research. Boveri's impressively large institute had been erected just 4 years previously, close to the institute for physics where Wilhelm Conrad Röntgen discovered X-rays in 1895 (REFS 7,16,17).

Marcella O'Grady: a pioneering woman in science. In 1896, the academic world of Würzburg was confronted with another novel development. A woman, Marcella O'Grady, trained in biology and head of the department of Biology at Vassar Women's College, New York State, USA, came to study with Boveri and to work for her doctoral dissertation; Wilson had provided the contact. At that time, women had no regular access to German universities, and for the first time the old and internationally renowned Würzburg Society for Physics and Medicine had to admit a woman to its sessions. Boveri was then its president, and Röntgen an esteemed member. A year later Marcella O'Grady and Theodor Boveri married in the United States. Their only child, Margret, was born in Würzburg in 1900. The Röntgens, who had supported Marcella since her arrival, became lifelong friends of the Boveris, sharing holidays and conversations on recent scientific developments[9,10,17].

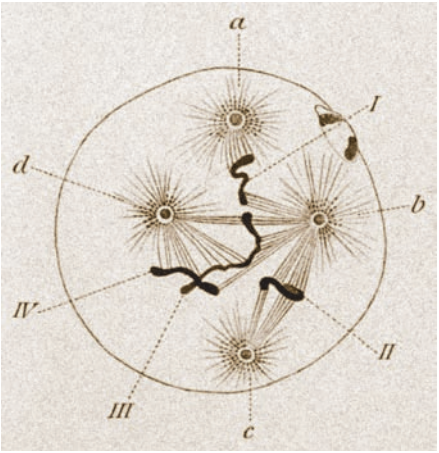


Figure 1 | **Mechanisms of cell division, 1888.** The figure illustrates a method used by Theodor Boveri: the use of 'nature's experiments' plus staining techniques, microscopic observation and drawing. The abnormal presence of four centrosomes (a, b, c, d) in a fertilized egg leads to an unequal distribution of chromosomes (I–IV) in the daughter cells. It allows for the interpretation of the interplay of centrosomes, spindle and chromosomes during cell division. Reproduced from REF. 36, courtesy of the Bildarchiv Preussischer Kulturbesitz, Berlin, Germany.

Marcella had already lived a professional life as a biologist before she came to work at Theodor Boveri's institute. Born in 1865 in Boston, USA, the daughter of an architect, she was the first woman to graduate in biology from the Massachusetts Institute of Technology. She subsequently taught science at Bryn Mawr School, Pennsylvania, USA, where Edmund Wilson became her mentor. She then continued her studies at Bryn Mawr College for Women in the years 1887 to 1889, supported by a fellowship and specializing in comparative zoology and embryology. In 1889 she became head of the department of biology at Vassar Women's College, visiting the Marine Biological Laboratory at Woods Hole, Massachusetts, to do embryological research on several occasions. She is described by her daughter as a typical Vassar woman, dedicated to civic virtue, her life devoted to modern science[9,10,17].

As Theodor Boveri's wife she published only one paper, in 1903, supposed to be the basis for her doctorate, which she never completed[16]. Instead, she saw her duty and best service to science in the support of her frail husband, and he acknowledged her contributions and collaboration in his publications and letters to colleagues. However, she never acted as the visible co-author of her husband.

Marcella's contributions cannot be overestimated. Of course, she did the work a spouse was supposed to do: caring for the child and the home; supervising servants, as was common for members of the academic middle class; and providing an informal space at home, where Theodor's colleagues and their wives could meet. But, being highly educated, she could also discuss scientific ideas and problems with her husband; a highly skilled colleague without any ambitions for herself, loyally supporting him. She worked with Theodor in experiments during his regular research leave at the Stazione Zoologica in Naples, Italy[17]. Her professional contacts with the United States, Bryn Mawr and Vassar college, enabled several female students of zoology to come to Boveri's institute to do research in embryology and cytology, and she also provided the contact that enabled the American physicist Edna Carter to do her Ph.D. with Röntgen's successor in Würzburg in 1906 (REF.9). Thus, Marcella acted as an important supporter of American women in science who came to Germany for further scientific training in the years before the First World War. In doing so, she also strongly supported women in Germany in their struggle to get access to university.

Theodor Boveri: the professor. Theodor Boveri supervised the work of doctoral students, both male and female. The German women either married or became teachers, the American women went back to their colleges. Only men continued to have university careers in Germany. Nonetheless, women had scientific contributions to make. Although the most prominent student of Theodor Boveri was Hans Spemann, who developed his own branch of experimental embryology for which he was awarded the Nobel prize in 1935, the crucial experimental work on the organizer was made by Spemann's doctoral student, Hilde Mangold[17,18]. Among the visiting scholars from the United States, Nettie M. Stevens is probably the most important for the history of genetics. She stayed in Würzburg twice (in 1901–02 and 1908–09) and she immediately saw the applicability of Boveri's chromosome theory of heredity to the problem of sex determination[19–21]. Using her highly developed skills as a cytologist, and techniques she most probably learned at Boveri's institute, she also investigated the number and size of the chromosomes of *Drosophila melanogaster*, thus laying the ground for the chromosome maps that were developed in the group of Thomas Hunt Morgan[22,23].

Theodor Boveri became highly esteemed within contemporary zoology and cytology in the first decade of the twentieth century. When the newly founded and prestigious Kaiser Wilhelm Society for the Advancement of Science decided to establish a well-funded research institute for biology in Berlin-Dahlem in 1912, Theodor Boveri was asked to develop the concept and to become its first director[22]. Boveri chose the institute's first heads of department, and the institute itself became a principal centre of German genetics in the 1920s and 1930s[23]. The influential geneticist Richard Goldschmidt worked here until he had to emigrate in 1936 (REF. 24), and two of the scientists that Boveri brought to the institute were awarded Nobel prizes later in their careers: Otto Warburg and Hans Spemann.

However, after exhausting negotiations, during which he fell ill, Theodor Boveri declined the offer of the directorship in Berlin, preferring to stay in Würzburg. The position in Würzburg promised better financial support for his wife and daughter in the event of his death, which occurred much earlier than expected. After a final research leave in Naples in 1913–14, Theodor Boveri's health deteriorated rapidly and he died in 1915 (REFS 16,17). The most convincing hypothesis as to the cause of death is a

retrospective diagnosis, which suggests that Boveri tragically died of an infection with one of the model organisms of his research, the parasite *Ascaris lumbricoides*.

The widow. Marcella Boveri continued to supervise her husband's doctoral students, and published his last unfinished paper in 1918. After the war, and with inflation creating difficult financial circumstances, she raised their daughter to follow a career of her own. When Margret, after having begun to study biology, decided to work as a secretary at the Stazione Zoologica, Marcella Boveri felt free to go back to the United States. In 1927, at the age of 64, she accepted an offer from the Catholic Albertus Magnus College for Women in New Haven, Connecticut, USA to establish a new department of zoology. Here she worked as a professor until her retirement in 1943. She published her late husband's work on cancer in English in 1929, helped German colleagues to visit the United States and transmitted the latest developments in the German biological sciences to her students. Again she provided facilities for young women to study science, something that they would otherwise have been unable to do. However, as far as is known today, Marcella Boveri did no research of her own during her time in the United States[9,10]. Her daughter Margret discontinued her scientific career and became a well-known political journalist in West Germany[17].

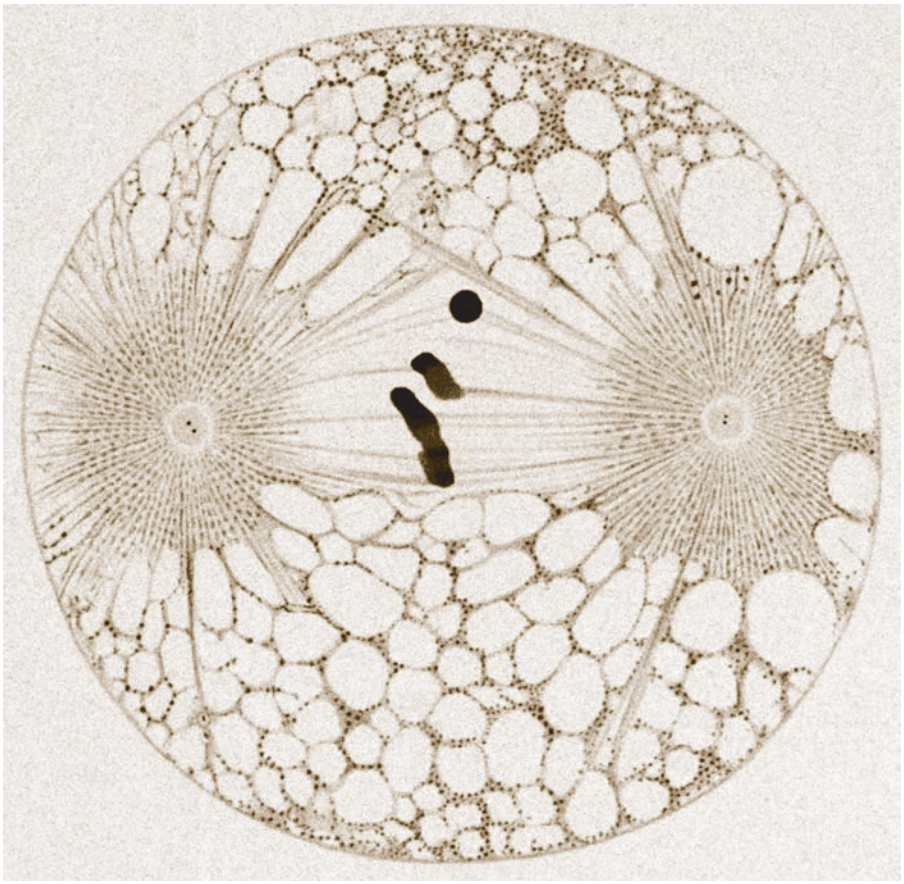


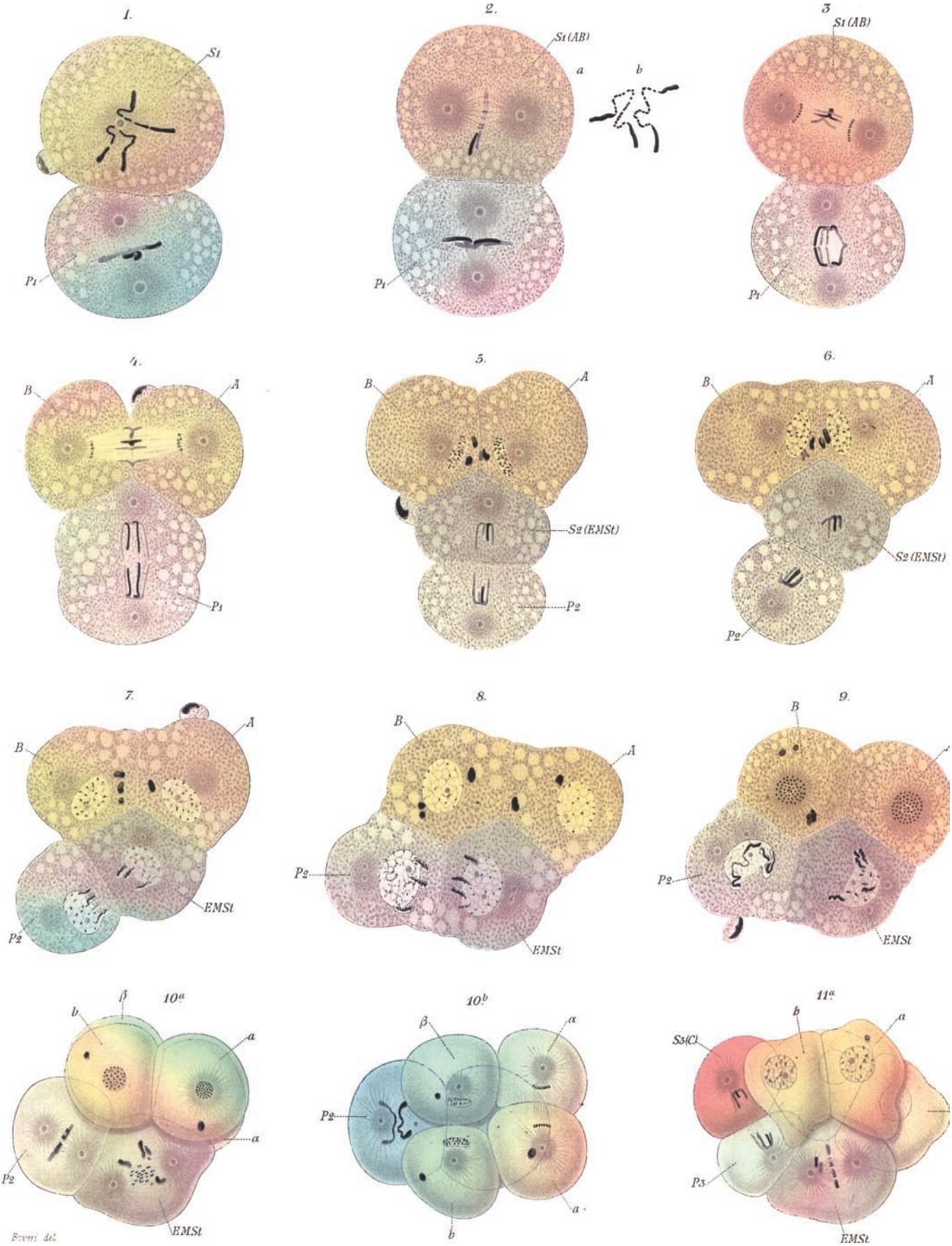
Figure 2 | **Cell division in *Ascaris megalocephala* bivalens, in high optical enlargement, 1901.** This lithograph illustrates the high quality of the visualization techniques used in 1901 for documenting the cellular structures involved in cell division. Cellular details are shown in a quality which cannot be surpassed in light microscopy. The figure shows chromosomes in the middle of the dividing cell, the spindle and two centrosomes, two centrioles in each centrosome are also visible. Note that the cytoplasm is perceived as structured. Reproduced from REF. 54, courtesy of the Bildarchiv Preussischer Kulturbesitz, Berlin, Germany.

The work

The scientific work published under the name Theodor Boveri covers the three decades from 1885, and from 1896 it was supported by Marcella O'Grady Boveri. The rediscovery of the Mendelian laws in 1900 is usually seen as the important starting point for the discipline of genetics, because it helped to introduce new experimental approaches in the understanding of heredity, which, in the hands of Morgan and his group of young men after 1911, led to the influential theory of the gene and the mapping of genes onto chromosomes[25–27]. Less well known is the importance of cytological work at the end of the nineteenth and the beginning of the twentieth century on chromosomes and their regular behaviour during cell division and meiosis as the carriers of hereditary or Mendelian factors (known then as 'Anlagen'). The Boveris were particularly interested in how the interplay of chromosomes and cytoplasm contributes to fertilization, inheritance and embryological development. To this end, they designed sophisticated experiments to introduce slight deviations from the normal processes of fertilization and cell division and presented the results with superb visualization techniques. They also used naturally occurring differences in these mechanisms to draw conclusions on the relevant factors involved. Some of the experiments with *Ascaris* and sea urchins can be described in modern terms as 'chromosomal engineering' because they were designed to change the number of chromosomes and their combination with cytoplasm, so that their interdependence and relevance for inheritance and embryonic development could be observed.

Most of the work dealt with in this article was based on the use of specimens of the genus *Ascaris* in Munich and Würzburg, or sea urchins in the Mediterranean, particularly in Naples. The parasitic round worms of the horse, *Ascaris megalocephala* univalens and bivalens (known in today's nomenclature as *Parascaris univalens* and *Parascaris equorum*), have the advantage of having one and two pairs of chromosomes, respectively, which could be made clearly visible and discernable during cell division. The disadvantage of *Ascaris* is that the fresh worms, when opened to extract the germ cells, cause severe allergic reactions in the experimenter. The advantage of sea-urchin egg cells is their transparency and their availability in large numbers (thousands to millions) for experimental investigation, meaning that fertilization could be observed under physiological and artificial conditions.

It is probably hard to imagine, starting from today's textbook knowledge of genetics, how difficult it was in the late nineteenth century to establish an understanding of the role of the chromosomes and their interplay with the cytoplasm for inheritance and embryonic development[15,28,29]. Early geneticists, such as William Bateson or



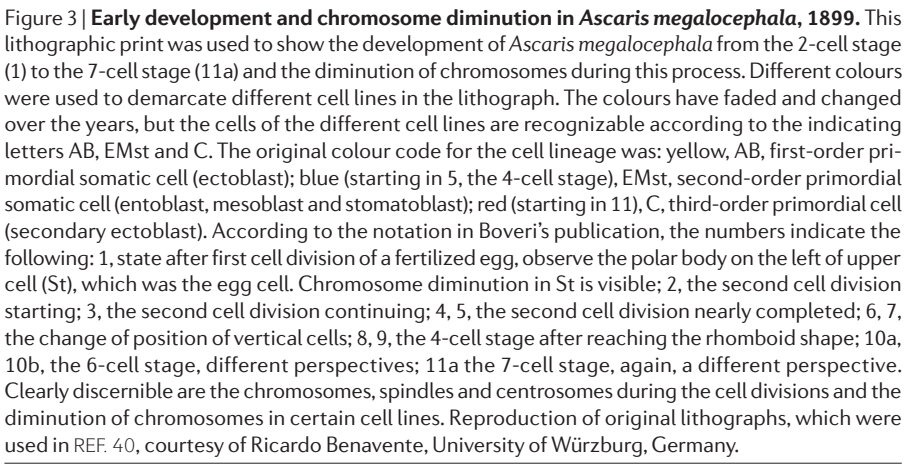


Figure 3 | **Early development and chromosome diminution in *Ascaris megalocephala*, 1899.** This lithographic print was used to show the development of *Ascaris megalocephala* from the 2-cell stage (1) to the 7-cell stage (11a) and the diminution of chromosomes during this process. Different colours were used to demarcate different cell lines in the lithograph. The colours have faded and changed over the years, but the cells of the different cell lines are recognizable according to the indicating letters AB, EMst and C. The original colour code for the cell lineage was: yellow, AB, first-order primordial somatic cell (ectoblast); blue (starting in 5, the 4-cell stage), EMst, second-order primordial somatic cell (entoblast, mesoblast and stomatoblast); red (starting in 11), C, third-order primordial cell (secondary ectoblast). According to the notation in Boveri's publication, the numbers indicate the following: 1, state after first cell division of a fertilized egg, observe the polar body on the left of upper cell (St), which was the egg cell. Chromosome diminution in St is visible; 2, the second cell division starting; 3, the second cell division continuing; 4, 5, the second cell division nearly completed; 6, 7, the change of position of vertical cells; 8, 9, the 4-cell stage after reaching the rhomboid shape; 10a, 10b, the 6-cell stage, different perspectives; 11a the 7-cell stage, again, a different perspective. Clearly discernible are the chromosomes, spindles and centrosomes during the cell divisions and the diminution of chromosomes in certain cell lines. Reproduction of original lithographs, which were used in REF. 40, courtesy of Ricardo Benavente, University of Würzburg, Germany.

Wilhelm Johannsen, disregarded cytology as inexact, and preferred the logic of Mendelian hybridization experiments and counting the offspring[30,31]. The cytologists themselves had their controversies on how to interpret these tiny entities called chromosomes, which only became visible during cell division with the help of dyes[15,30,32].

Theodor Boveri started by investigating fertilization and the formation of the polar bodies in the egg cells of *Ascaris*, building on the work of Eduard van Beneden[15,33,34]. In his studies of cell division he used a high microscopic resolution that allowed the observation of the two centrosomes and the spindle apparatus, which both participate in the distribution of the chromosomes into the two daughter cells. He identified two independent cycles during cell division: the duplication of the centrosomes and the duplication of the chromosomes. These findings are still recognized as being of value today[5,6] (FIGS 1,2).

It was difficult to prove experimentally if, and in what sense, the chromosomes were relevant for inheritance. Efforts to remove the nucleus of frog egg cells with a fine syringe and supplant it with the nucleus of a toad species, to create a viable cell, had failed. Boveri saw such experimental intervention as seriously disruptive: he preferred to transfer a nucleus from another species into an enucleated sea-urchin egg cell, partly using the natural process of fertilization. He enucleated sea-urchin egg cells by shaking them, and fertilized the fragments with the sperm of a different sea-urchin species. It was known that such fragments could be fertilized by sperm of the same species, the only difference to normal development being that, in accordance with the smaller size of the enucleated egg, the size of the resulting larvae was smaller. Boveri chose two species that showed morphological differences in the calcified skeleton of their larvae. He found the expected result: the enucleated eggs developed into larvae showing characteristics of the 'father', whereas the controlled fertilization of complete eggs with sperm of the different species resulted in intermediate forms[35].

This 'merogonic experiment', published in 1889 and extended in 1895 (REF. 36), is seen by many as the paradigmatic experiment proving that the cell nucleus and chromosomes are the sites of hereditary material. However, after a series of experiments carried out over the next 25 years, Boveri had to concede in his last, unfinished paper that he never could be sure that the maternal nuclear material in the enucleated egg had been removed completely[37].

The chromosome theory of heredity

Chromosome diminution in the somatic cell line. Nevertheless, this first experiment of 1889 must have guided Boveri towards his further observation of the chromosomes. One way to approach them was to follow August Weismann's central postulate on heredity, which was the division between somatic cells and the cells of the germ line. According to Weismann, and radically different from today's understanding, only the cells of the germ line contained all the unaltered hereditary Anlagen to be transmitted from one generation to the other. The somatic cells would only receive the Anlagen needed for their specific differentiation. Theodor Boveri used the embryonic development of *Ascaris megalocephala* to show, by careful microscopic observations, that the chromosomes were transmitted unaltered within the cells of the germ line, whereas in the early somatic cells the chromosomes were dissolved. He published his full results in 1899, including elaborate cell-lineage studies and their visual representation[38] (FIGS 3,4). These findings are appreciated by today's biologists as the first demonstration of a cytoplasm-induced rearrangement of chromosomes during ontogenesis[5,6].

Theodor Boveri interpreted the fragmentation of chromosomes as the visual trace of the partial transmission of the hereditary Anlagen on the chromosomes to the somatic cells. At the time, this interpretation was needed to explain cell differentiation during embryonic development while simultaneously seeing the chromosomes as hereditary material.

The persistence of chromosomes in the germ line. Around the year of 1900, two further problems had to be solved before the establishment of the chromosome theory of heredity. The first question was whether the different chromosomes that are visible during cell division are persistent and stay the same in subsequent cell generations, or if they are transitory and assembled in a new combination before every cell division. The description of chromosomes during fertilization was Boveri's starting point. It had to be demonstrated that the egg cell and spermatozoan each contribute a set of chromosomes to the next generation of cells. In 1902, in his theory of fertilization, Boveri demonstrated that both sets of chromosomes were equal and that the mechanism of chromosomal distribution to the two daughter cells normally worked with such precision that each cell acquired a complete set of chromosomes[39]. Combining this finding with the observations on the reduction division during the development of the germ cells (later called meiosis), Boveri could show that the chromosomes behaved exactly as Weismann had postulated: the germplasm of the parents had to be reduced before the germ cells united during fertilization.

The 'individuality' of chromosomes. The second problem to be solved was whether every single chromosome had a specific relevance for the development of an organism. Using microscopy, Boveri demonstrated that the number, shape and size of chromosomes stayed the same during various cell divisions. However, the Boveris went further and designed sophisticated experiments with fertilized sea-urchin eggs. The idea was to find a mechanism to exclude certain chromosomes from participation in embryogenesis, to see if each chromosome had a specific relevance in normal development. The Boveris used a particular effect occurring in laboratory fertilization, when one egg

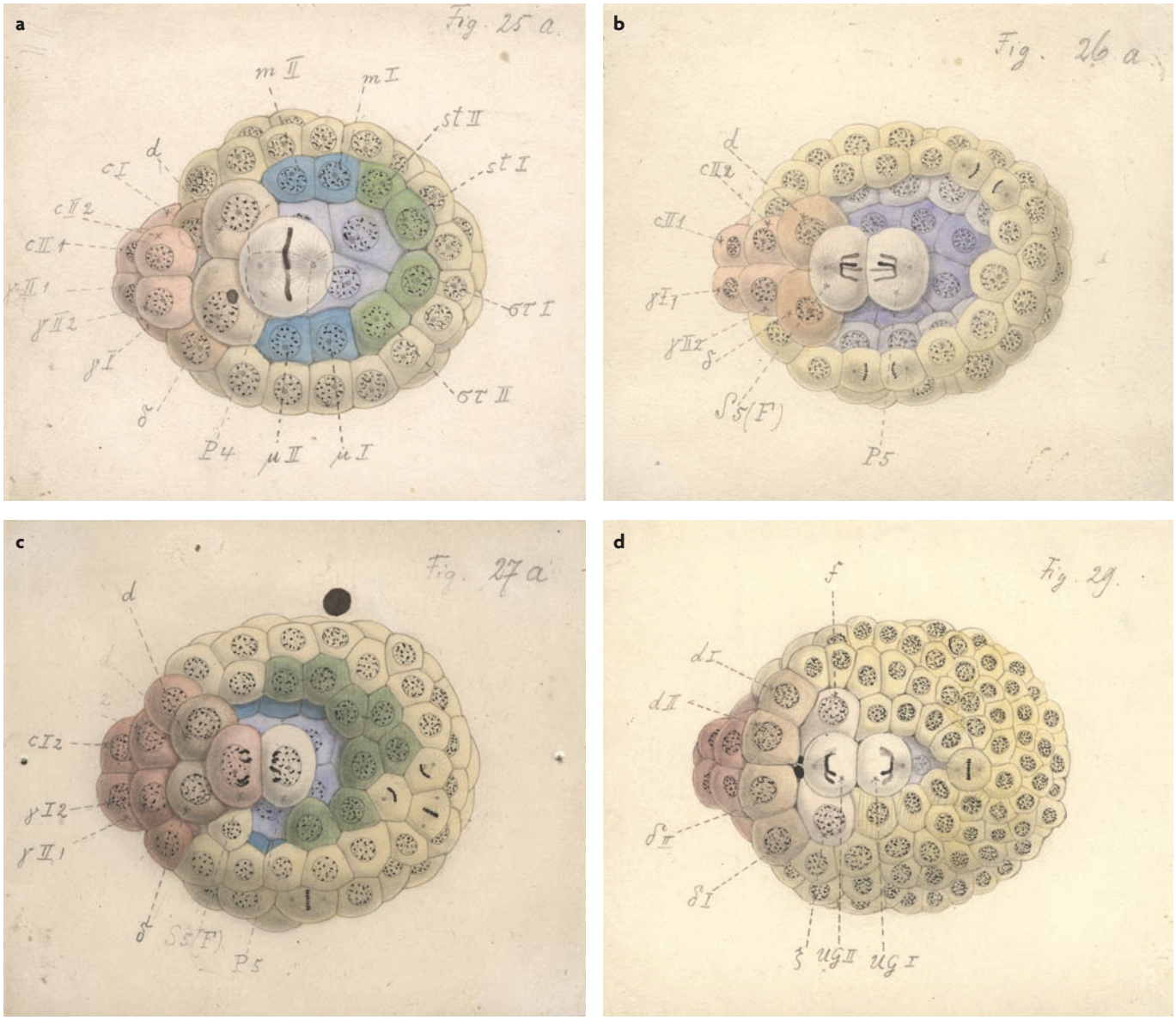


Figure 4 | **Chromosomes in the germ line of *Ascaris megalocephala*, 1899.** The development of *Ascaris megalocephala* at a later stage than is shown in FIG. 3. This figure demonstrates the preservation and continuation of the chromosomes and supports Weismann's hypothesis that only the germ line contains the complete hereditary material. Boveri traced the different cell lines from fertilization to a stage comprising more than 100 cells. In the drawings he chose the ventral view and used colour codes to identify different cell lineages. The four images show consecutive stages of growth and differentiation. **a** | Ventral view, entoblasts and mesoblasts (light blue) go down into the depth. **b** | Ventral view, stomatoblasts from the back start to cover the mesoblast, which goes into the depth. **c** | The process continues (the black spot is an artefact of no scientific significance). **d** | Ventral view, the primordial somatic cell S_5(F), separated into f and ζ. The stem cell P_5 has divided into two primordial germ cells, UGI and UG II. The chromosomes, spindle and centrosomes are visible in the germ line. Reproduction of original lithographs, which were used in REF. 40, courtesy of Ricardo Benavente, University of Würzburg, Germany.

cell is fertilized by two sperm cells. In this case, two centrosomes were introduced into the egg; these doubled into four and created two spindle apparatus instead of one. The chromosomes in the doubly fertilized egg comprised one maternal and two paternal sets of 18 chromosomes each. The combination of chromosomes and spindles resulted in an unequal distribution of chromosomes during the subsequent cell division. This first cell generation could be separated into single cells, each starting embryonic development. In contrast to cells with the normal set of chromosomes, these cells generated various malformations in the sea-urchin larvae.

To interpret these findings and decide if each of the 18 chromosomes had a specific relevance, the Boveris used a probability-calculating apparatus to determine the frequency of all possible combinations of chromosomes in the four cells after the first cleavage. They then compared these ratios with those of viable larvae resulting from their experiment. It is too complicated to describe all the stages of the experiments here but the first results, published in 1902, led to the conclusion that each chromosome contained a specific hereditary property for the organism; it was not the overall quantity of chromatin that counted[40].

This result, combined with the cytological observations of reduction division, led to the conclusion that the chromosomes harboured the Mendelian hereditary factors. The Mendelian law of segregation could be applied to the chromosomes in germ-cell development and fertilization. Boveri published this conclusion, today called the chromosome theory of heredity, in 1904 under the rather modest title "Results on the constitution of the chromatic substance of the cell nucleus"[41]. The following years saw the confirmation of these findings, with the individuality and persistence of each chromosome shown in *Ascaris* in 1909 (REF. 42).

Chromosomal sex determination. Boveri did not immediately recognize one area of study that could be used in support of his chromosome theory of heredity — the correlation between sex difference and the difference in the number or size of chromosomes. It was the visiting scholar Nettie M. Stevens who, after her visit to Würzburg in 1904, immediately saw the applicability of the theory to the problem of sex determination[21–23]. It took Boveri some time to take the new interpretation on board and start research on chromosome sex determination at his institute[16,43].

The cytoplasm

With the support of the visiting women scientists Nettie M. Stevens, Mary J. Hogue and Alice M. Boring, Theodor and Marcella investigated the process of chromosome diminution during the embryonic development of *Ascaris*. They used doubly fertilized eggs to create disturbances in the distribution of chromosomes in various cell lines; they also used the centrifugation of fertilized eggs to alter the distribution of substances in the cytoplasm and the position of the nucleus within the cytoplasm, and they used UV light to specifically destroy the origin cells of particular cell lines[44–48]. In 1910 these combined approaches led to the conclusion that Weismann was wrong in his assumption that the chromosomes engineered their own rearrangement during ontogenesis. The experiments showed that the cytoplasm was responsible for the organization of chromosomes in somatic cells and thus for differentiation. They showed that embryonic development was dependent on the changing position of the spindle apparatus within the embryonic cell, and on the time-and-place-dependent position of substances within the cells[49] (FIG. 5). Developmental biologists now see these findings as the first formulation of an 'epigenetic theory of early embryonic bifurcative cell determination', which for many years was forgotten or misunderstood, but has been recently rediscovered as a result of new methods in embryology[4–6].

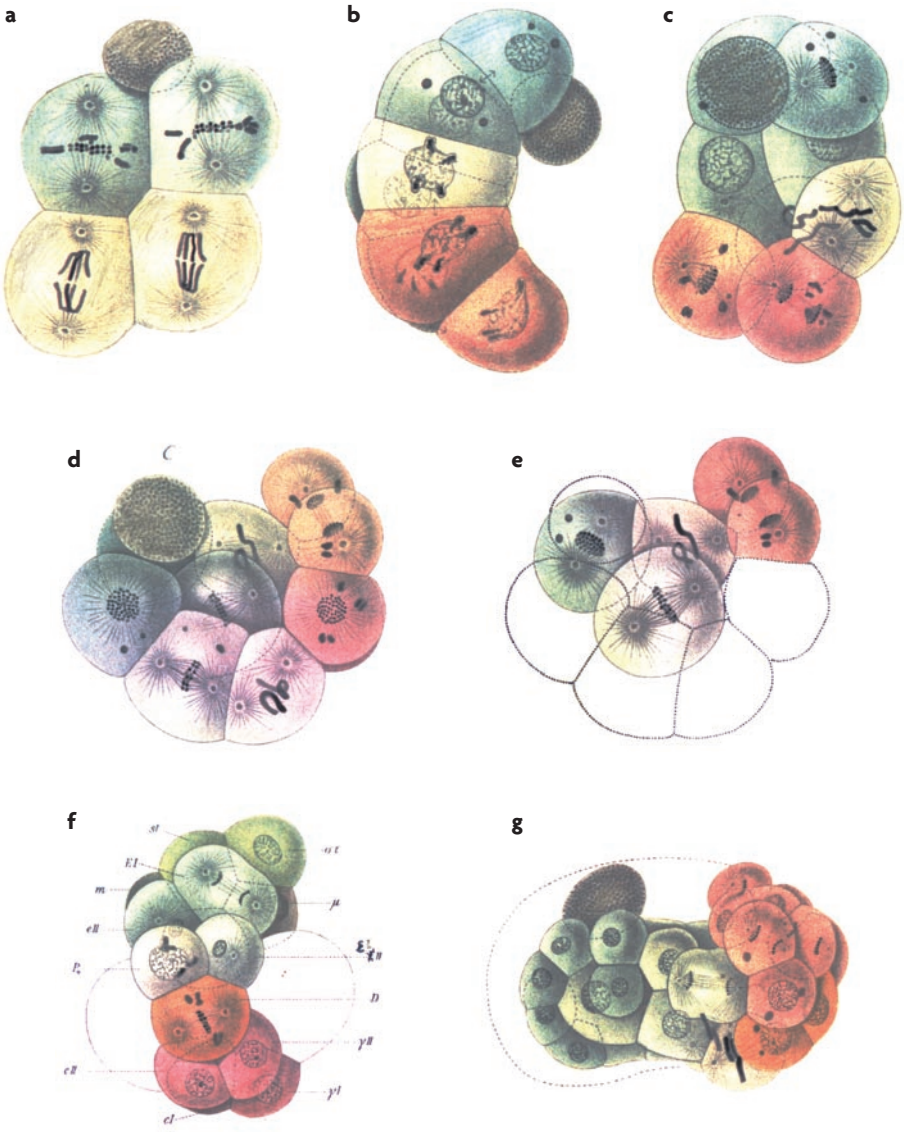


Figure 5 | **Embryonic development of *Ascaris megalocephala* univalens, after experimental intervention in early phases of development, 1910.** The distorted embryonic formations, called Ballkeim, are the result of fertilized eggs, which had been exposed to strong centrifugation to change the spatial relationship of cytoplasm and chromosomes. The experiment was designed to find out if factors in the cytoplasm were responsible for the diminution of chromosomes in certain cell lines. The first cell divisions of the fertilized egg after centrifugation result in a granulated cell and other cells, which divided and differentiated, showing chromosomes during cell division and chromosome diminution in certain cell lines. **a** | Transition from 4-cell to 8-cell Ballkeim, including chromosome diminution. **b, c** | 8-cell Ballkeim, seen from different perspectives. **d, e** | Transition to 16-cell stage. **f** | Only half of the Ballkeim developed after the respective primordial cell was destroyed by UV light. **g** | Older Ballkeim, seen from a different side. The different colours indicate different cell lines. Reproduction of original lithographs, which were used in REF. 40, courtesy of Ricardo Benavente, University of Würzburg, Germany.

In the final experiments involving the fertilization of enucleated sea-urchin eggs in 1913–14, the interaction between the cytoplasm and chromosomes during ontogenesis proved more relevant than previously expected[39]. Years previously, Boveri had used these experiments to establish the chromosomes as the hereditary material[37,38].

Now he considered the hypothesis that the cell nucleus did not carry the Anlagen, which geneticists of the time already called genes, but that the nucleus carried factors that only enabled the development of organs in cooperation with cytoplasmic factors. The experiments using fertilization of egg cells with sperm from different sea-urchin species allowed the conclusion that in early embryonic development, before gastrulation, the chromosomes were not relevant at all, as they did not show any particular influence[39]. In this early phase, everything was determined by the cytoplasm; after that it was the combined interaction of cytoplasm and chromosomes that determined the development of an organism. For Boveri, the investigation of this interaction was a task for the science of heredity. However, it took nearly a century until this interaction made its way back into genetics, for example in the work of Christiane Nüsslein-Volhard.

Conclusion

The Boveris' chromosome theory of heredity was hugely influential in determining the immediate course of genetics and its emphasis on the transmission of Mendelian factors from one generation to the next. However, the cytoplasm, the relevance of which was so impressively shown by Theodor and Marcella, has been revisited more recently by scientists and historians of science[4,6,25]. Why did it take so long to re-include early findings and what does this tell us about the dynamics of genetics and the transitory nature of its paradigms? We need to understand how particular experimental approaches go through use and disuse, and how scientists determine what is considered a relevant question in genetics and what is not.

The history of the Boveris also illustrates the problem of women in science; their contributions have been silenced for a long time. Setting the record straight in this respect is another important task for the history of genetics.

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FURTHER INFORMATION

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