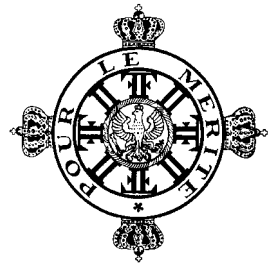
POUR LE MERITE

ERIC WIESCHAUS

# THEODOR BOVERI AND THE CHROMOSOMAL THEORY OF DEVELOPMENT

From the middle to the end of the nineteenth century, the German university system underwent a period of significant growth and was admired across Europe. New buildings and institutes were established, particularly in the biological sciences where the discovery of biologically useful dyes and the development of high-resolution optical microscopes by Ernst Abbe at Zeiss-Jena allowed visualizing cellular components in unprecedented detail. In the period between 1880 and 1900, the structure of the cell and the features of cellular organelles like mitochondria and centrosomes were described, as was the existence of the nucleus, the fact that it contains DNA, the behavior of chromosomes during cell division, the process of fertilization and the development of the embryo. In the history of science, the productivity between 1880 and 1900 can only be compared with the Watson-Crick period of the 1950s where the molecular genetic code was elucidated, and the 1990s when the use of recombinant DNA allowed genes and proteins to be understood molecularly. What is special about the period between 1880 and 1900 is that, with very rare exceptions, almost all this work was done at German universities by German scientists.

Today I am going to talk about the work of Theodor Boveri, who during this period established the connection between genetics and cell biology. His work was transformative and has affected science in ways that can still be felt today. I believe he can be regarded as the single most important figure in German experimental biology at the beginning of the 20th century. Boveri died at the age of 53, on October 12, 1915, almost exactly one hundred years from today and so it makes sense that we should discuss his work at a meeting of the Orden this year.

Boveri was born in Bamberg on Oct 15, 1862, one of three sons in an upper middle class family. His younger brother Walther immigrated to Switzerland where he and Charles Brown established the Brown Boveri manufacturing company. Boveri himself entered the university of Munich with the intention of studying philosophy, but within a year, apparently enamored by the new emerging science of biology, had switched to the study of cell structure and function. (Figure 1) He finished his dissertation in 1885 and remained in Munich as a Lamont Stipend until assuming an ordinarius professorship the University of Würzburg at the age of 31. He became director of the Zoologische Institut in the same year and he remained in Würzburg until his death. In 1913, he was recruited to head the new Kaiser Wilhelm Institute für Biologie in Berlin Dahlem. Although he played a central role in planning the Institute, he ultimately had to decline the nomination to head the institution because of health reasons.

During today's lecture, I will present Boveri's most important experiments linking chromosomal activity and early embryonic development. But before going into those experiments, it is useful to discuss two personal features of his life that put his scientific productivity into perspective, as well as providing insight into how science was done at the time. The first is Boveri's constant battle with illness. His first major episode began as influenza during his first period of peak productivity in 1889, but spiraled into a year and a half of depression. His letters to his brother and sister-in-law in Switzerland indicate how debilitating his condition was. »… Ich kann mich noch immer nicht erholen, besonders das Gehirn ist wie eingefroren«. »Das Ge-

Figure 1: Theodor Boveri 1889 Munich

hen strengt mir die Beine an, das Lesen die Augen, das Schreiben das Gehirn, kurz, jede Tätigkeit strengt mich an, und so sitze oder liege ich irgendwo, suche mir in Gedanken möglichst alle unangenehmen Dingen hervor und male dieselben recht schön schwarz aus«. Eventually, his depression required admission into a sanatorium in Konstanz. It is sign of the strength of his scientific reputation and the extraordinary impact of his experiments that a year after his recovery, he was offered the professorship and directorship at Würzburg. Boveri continued to struggle with health issues throughout his career. These are variously described as influenza, depression, neurasthenie, rheumatism and infections by the nematode worms he worked with. Throughout his research career, repetition of crucial experiments had to be postponed and some of the associated scientific disputes effectively lasted for 15 years because of those health interruptions.

Figure 2: Marcella O'Grady Boveri

The second personal feature is his marriage in 1897 to an American scientist, Marcella O'Grady (Figure 2). O'Grady was herself a remarkable woman. In 1888, she was the first female to graduate in biology at MIT, and was a founding member and professor of biology at Vassar College where she taught from 1891 to 1896. In 1896, she took a year paid sabbatical leave to do research in cell biology in Boveri's Institute at Würzburg. They were married a year later in 1897. Although she published her thesis work in1903, she never published another paper, choosing instead to work closely with her husband on his own research program. Over the past fifteen years, as the original note books and slides from Würzburg have been recovered and examined, it has become increasingly evident how active her participation was in all the experiments carried out between 1897 and Boveri's death in 1915. She traveled with him to the Naples

biological station every year and even after his death continued to prepare his manuscripts for publication. Nowadays it is not uncommon to regard the final set of Boveri's experiments as joint products by Theodor and Marcella Boveri. The couple had one child, Margret Boveri, born in 1900. After Boveri's death and when her daughter had completed her education and begun her career as a journalist, Marcella O'Grady, then 64 years old, returned to the United States. She then took a position as head of the biology department at Albertus Magnus College in New Haven, Connecticut. She held that position until she was 79. She died in 1950 at the age of 87.

Boveri's main contribution was to move biology from the powerful descriptive technologies that had just become available when he was a student at the University of Munich to a more mechanistic understanding based on experimental manipulation. To give a flavor of Boveri's science I have selected two of his most important experiments to present for you today. As a practicing scientist in the 21st century, it is impossible not to be impressed by the logical brilliance with which Boveri distilled complicated biological questions, and simultaneously by the incredible crudeness of the actual experimental approaches he was forced to use. It is this dialectic between logical cleanness and messy experiments that make Boveri's work so interesting.

Although Boveri worked on a variety of organisms during his career, the two experiments I would like to talk about were done on embryos from sea urchins and were carried out at the Naples Marine Station founded by Anton Dohrn. Marine organisms like sea urchins are advantageous objects for research because they are transparent and easy to obtain. The fundamental question that Boveri chose to address was the role of genes and genetic information in controlling the pattern of differentiation of the embryo.

By the time Boveri did his experiment the basic features of fertilization were known; that the egg and sperm both contain nuclei that after fertilization fuse to form the nucleus of the embryo, and that the cytoplasm of the embryo is derived from the maternal cytoplasm of the egg. Boveri observed that even before any development in the

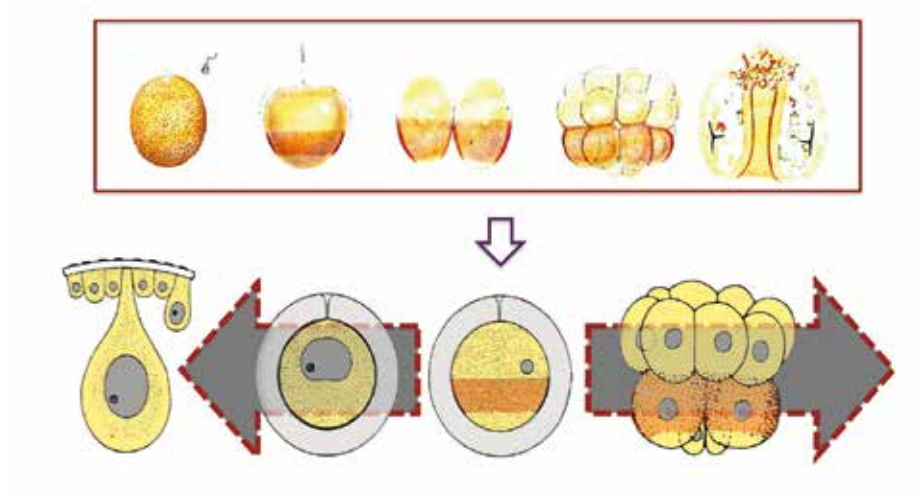

Figure 3: Cytoplasmic Localization before Fertilization Predicts the Fate of Cells formed in that region. The existence of a band of red cytoplasm in the sea urchin egg allowed Boveri to trace the development of cells derived from certain regions of the egg cytoplasm, and to relate those cell fates to the organization of the egg cell in the ovary of the female sea urchin that produced that egg. Figures modified from Boveri's original manuscripts (Boveri, T. [1901]. Die Polarität von Oocyte, Ei und Larve des Strongylocentrotus lividus. Zool. Jb. Anat. Ontog. 1: p. 630-653)

embryo had occurred, pattern could be observed in the egg – a band of orange-pigmented cytoplasm extending around the lower half of the egg (Figure 3). By following further development, as the embryo divides into two cells and then four, Boveri showed that only certain cells incorporated this pigmented cytoplasm, and that ultimately those cells gave rise to the intestine and other internal organs. It was also possible for Boveri to trace back that cytoplasmic organization (or »polarity«) through earlier stages, back to the arrangement of cells in the ovary of the female that gave rise to that egg. The possibility that one could trace the pattern at any stage forward and backwards argued that the pattern in the embryo could be explained

by a previously existing pattern in the egg, driving home the idea that in biology, spatial patterns build on previously existing patterns. But how much of the final complicated pattern of an organism was already prefigured in the egg? It was only possible to see a single graded red pigment band, but was there more information, more molecules? The first of the famous Boveri experiments addresses this question. What Boveri did was identify two different species of sea urchin in the Bay of Naples (*Sphaerechinus* and *Echinus*) that were distant enough that the embryos developed distinct morphologies, but close enough that sperm from one species could be used to fertilize the eggs from the other species.

When two species were crossed, any morphological features that depended on the pattern of the egg cytoplasm should assume the species-specific character of the mother. If, instead, the feature depended on information from the nucleus, the hybrid might show either the character of the father or the mother, or a mixture of both. It was this latter result Boveri saw when *Sphaerechinus* females were crossed with Echinus males. The embryos showed an intermediate morphology, with the skeletal spicules show the thickness of one species, but the branching pattern of the other. This argued that nuclear information from the father of the different species contributed to the final pattern, but did not say how much it contributed. The ideal experiment would be to engineer a situation where the egg cytoplasm was from one species and the nucleus only from the other.

Boveri's next step is an example of his originality, and also of the primitive experimental procedures possible at the time. If you violently shake a collection of eggs before they are fertilized, some of the eggs are fragmented such that they would lose their own nuclei. When these eggs were fertilized by sperm from the same species, they developed into embryos that were characteristic of the species, but were smaller and had smaller nuclei that were derived only from the sperm. The interesting variant of this experiment was to generate enucleate fragments from one species and fertilize them with sperm of the other. In this way Boveri could make embryos that had egg cytoplasm exclusively from one species but nuclear material

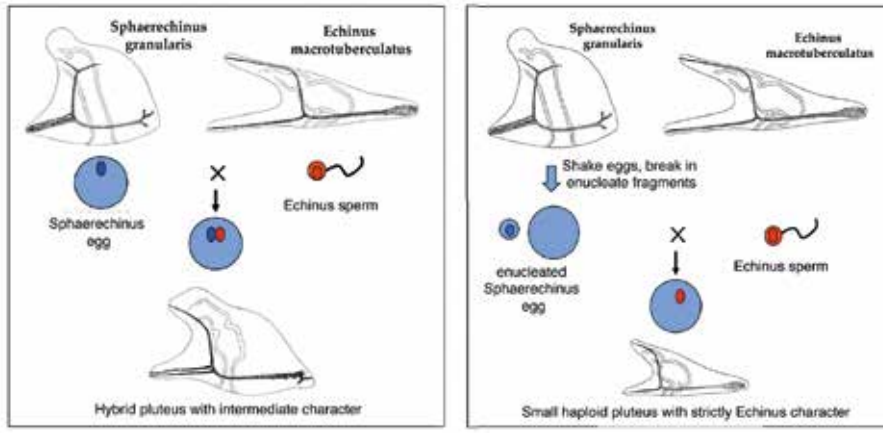


Figure 4 : Boveri's 1889 experiment demonstrating the role of the nucleus in determining embryonic morphology. Embryos formed by crossing sea urchins of two different species show morphological features intermediate between the two species, even though the egg cytoplasm is derived entirely from the maternal species. When the female pronucleus is removed before fertilization, the resulting embryo is still derived entirely from maternal cytoplasm but shows a completely paternal morphology, indicating that the nucleus rather than the cytoplasm controls the final form of the embryo. Images modified from Boveri 1889 and from the translation by Thomas Hunt Morgan published in 1893.

exclusively from the other. In Boveri's hands the results were very clear. The resultant embryos looked exactly like the species from which the sperm had been derived and argued that the character of the embryo depended primarily on information in the nucleus.

It is useful to compare Boveri's experiments with those of Gregor Mendel, whose famous hybridization experiments using pea plants established the basic outlines of heritability. Both Boveri's and Mendel's experiments were essentially genetic approaches, analysis of hybrids. Using data from his crosses, Mendel developed a conceptual framework for genetics. He defined rules for the behavior of genetic factors that allows predicting the outcomes of crossing experiments,

but his conclusions were abstract and did not have a mechanistic biological basis. Mendel's experiments were published in 1866 and were totally forgotten. In the twenty years between Mendel and when Boveri did his experiment in 1889, microscopes had been invented, dyes were discovered and the structure and behavior of the nucleus described. Boveri therefore conceptualized the problem of heredity in terms of the cellular structures and organelles that had just been visualized during the period when he was studying at the University of Munich. He thought of the problem in terms of nuclei and cytoplasm – the driving goal of most of his experiments was to distinguish the various roles of those structures.

The sea urchin hybridization experiment argued that the nucleus conveyed most of the information that specified the final form of the embryo, but it did not define where that information was, or exactly how future morphology could be encoded in the structure. At the time, the most intriguing thing that was known about the nucleus was its behavior during cell division. Before the cell itself divided, the nucleus was observed to condense and to partition into dark staining bodies called chromosomes. From Walter Flemming's work and others in the 1880s, it was known that in a given species the number of chromosomes was constant, and that the small nuclei of eggs and sperm had half the number of chromosomes present in other cells on the body. What was not known was whether chromosomes were different from each other and whether the entire information content of the nucleus was present in each chromosome, or whether individual chromosomes contained distinct parts of that information. To address this question, one would like to remove a single chromosome and determine the consequences on development.

It was that question that is addressed in the final set of Boveri experiments I would like to present today. This experiment was based on a peculiar and unpromising initial observation in 1889, namely that eggs fertilized by more than one sperm invariably developed abnormally. By following the development of such embryos. Boveri realized that the defects could be traced back to the earliest stages and was related to an abnormal behavior of chromosomes. In contrast

to the orderly division of the embryo into two cells and then four cells, with a correspondingly orderly replication of chromosomes and their equal distribution to two daughters, Boveri saw that when an egg had been fertilized by more than one sperm (Figure 5a), the resultant embryo divided immediately into four cells and that the chromosomes were distributed randomly between the four daughter cells.

This random distribution means that although the average amount of chromosomal material in each cell was high enough to support normal development, many of the cells would lack specific chromosomes (Figure 5b). This would be a random event but would occur with a predictable frequency. If the missing chromosome contained necessary information that could not be supplied by other chromosomes, then the cell and its progeny would develop abnormally. Other cells that were missing other chromosomes would give rise to regions of the embryo that also showed defects, but the defects would differ depending on the specific role of that chromosome in development.

To test this idea, Boveri followed the development of more than 1500 embryos formed by multiple fertilizations. All of the embryos initially developed normally, but at a precise time in development they became abnormal. The abnormalities were sectored in a manner that suggested that the abnormal cells were derived from one of the original four cells. In embryo XV in the right panel of Figure 5c, for example, a sector of cells has lost the ability to adhere. Boveri concluded that chromosomes were distinct and that each chromosome provided its own unique input into development. He also concluded that the information in chromosomes was first utilized after the initial cell divisions had been completed and the embryo had assumed a ball-like blastula shape. Prior to that stage, chromosomal content was irrelevant – suggesting that up to that point, development relied on information in the egg cytoplasm provided by the mother.

Although the ideas can be traced back to 1889, the first complete description of these experiments was published in 1902. By the time they were published they had assumed a special significance because of unexpected developments. On April 22, 1900 Carl Correns

stantially neglected since their publication in 1866. Correns re-discovery of Mendel, coupled with similar citations by De Vries and Tschermak, opened the scientific community to the simplicity of the Mendelian formulation, and the ideas rapidly took hold of the scientific community. The abstract descriptive nature of the Mendelian laws was puzzling and, even among Mendelian advocates, needed a mechanistic physical explanation. In his 1902 paper, Boveri suggests the possibility chromosomes might contain the hereditary factors proposed by Mendel, an idea he developed further in 1904. That genes were on chromosomes came to be known as the Sutton-Boveri hypothesis, and continued to be referred as such until the 1930s.

The central idea that emerges from Boveri's view of development is that spatial patterns are present in the maternal cytoplasm as molecular distributions from the earliest stages, but that these patterns are simple. It is the subsequent activity of genes and chromosomes that actually build the ultimate functional patterns in the final organism.

His experiments were done at the beginning period of experimental biology, at stage when descriptive technologies had reached a new powerful level but the ability to manipulate biological material was extremely primitive. One hundred years later, we can ask how well his overall viewpoint has been verified with time. In the eighty years that followed his experiments, it was not possible to identify the genes and molecules that controlled development but this became possible in the 1980s with the combination of genetics and molecular biology.

Work on Drosophila has provided the best example where Boveri's views have proven themselves to be applicable. The first and best-characterized maternal cytoplasmic determinant was the Bicoid protein characterized by the laboratory of Ordenskanzlerin Christiane Nüsslein-Volhard (Driever and Nüsslein-Volhard,1988). Even before fertilization, the Bicoid RNA is localized in the anterior end of the egg, in the region that will give rise to the head of the embryo. When the egg is fertilized, the RNA is translated into a protein that diffuses from the source and sets up a gradient along the length of

the egg. This maternal concentration gradient is analogous to the maternal polarity Boveri described in sea urchins eggs.

The Bicoid protein is a transcription factor that binds to specific regions of chromosomes and activates at specific concentrations the expression of distinct genes. The chromosomal genes are analogous to the chromosomal factors Boveri postulated to control embryonic pattern. In my own lab, over the past ten years we have initiated a more quantitative biophysical analysis of these genes. By our counts, there may be 1000 genes controlled by Bcd binding, and they can be grouped with respect to their sensitivity to Bicoid concentration. Much of our current work has been to establish conditions in which we can observe Bicoid's activity in single living embryos, to count the number of molecules, measure their movement and use the system to understand the general principles of DNA binding and gene activation.

One consequence of the early action in Boveri's model is that cells in the embryo are programmed from the earliest stages to the particular types of structures they will eventually form. That this is the case for Drosophila was actually demonstrated by Walter Gehring in one of his early experiments at the time when I first met him at Yale. Gehring showed that isolated cells from the anterior half of the embryo retained that fate, even when they were isolated from their normal neighbors and cultured for long times. This stability of programming is a hallmark of early determinative event and provides a general test for early maternal determinants.

The findings in Drosophila have been extended to many of the organisms used for research in laboratories around the world. Maternal RNAs deposited in the egg provide cues that activate chromosomal activity in defined regions of many different kinds of embryos. One important exception, however, has emerged. Maternally localized RNAs capable of setting up spatial patterns have not been found in mammalian embryos. This is perhaps not surprising given that most of the cells that are formed in early mammalian embryos are dedicated to forming the placenta and extra-embryonic membranes. In human embryos, for example, the cells that will give rise to the

embryo are set aside late and only become visibly patterned 14 days after the egg is fertilized. Consistent with that view, the cell behaviors of early mammalian embryos are extremely flexible and unlike the mixing experiment of Walter Gehring on fly embryos, similar mixing experiment with mammalian embryos suggest a total flexibility of the cells with respect to embryonic fate (Tarkovski, 1963). It is the unprogrammed nature of mammalian embryos that allows the derivation of embryonic stem cells from mammals. It has been impossible to establish embryonic stem cells from fly embryos, presumably because the early cytoplasmic differences restrict any individual cell's potential.

These observations suggest that the Boveri formulation may not apply to mammalian embryos, or that the maternal information provided in the egg is of an extremely general or fluid nature. If this is the case, we have to consider the possibility that pattern does not always have to be built on previous patterns, that somehow chromosomal activity in the early mammalian embryo is capable of generating reproducible pattern with no previous input. In a way, this would be an extreme version of Boveri's experiment with hybrid sea urchin embryos, where interactions between genes within the nucleus would not only define the final form but also the underlying spatial pattern. Understanding how this happens represents, I believe, one of the major challenges to be addressed by modern molecular biology.

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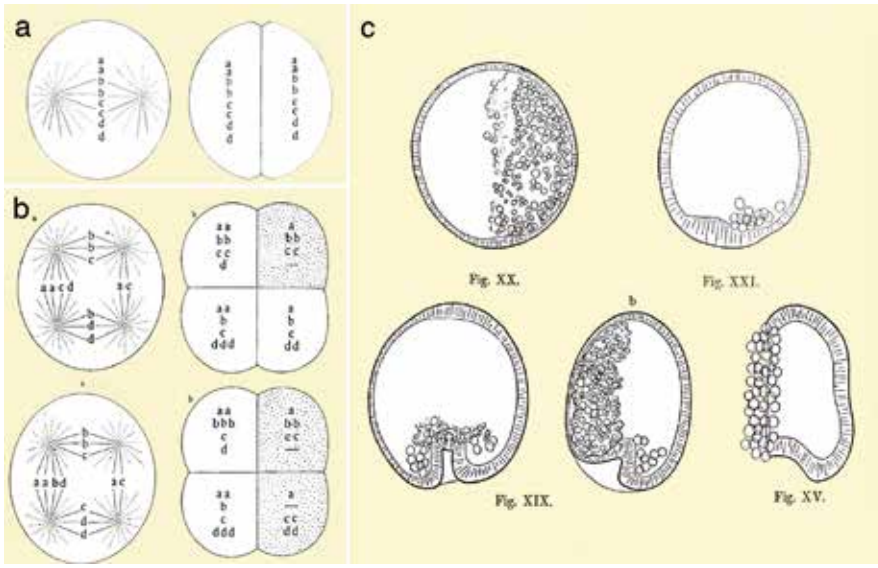


Figure 5: Boveri's use of polyspermy to demonstrate the unique developmental potential of Individual Chromosomes. a) In normal development, sea urchin eggs are fertilized by a single sperm and the resultant embryo contains a maternal and a paternal copy of each chromosome. When the cell divides, chromosomes align on a single spindle and after cell division, each daughter cells receives a single copy of each chromosome. b) When sea urchin eggs are fertilized by two sperm, the first division divides the embryo immediately into four cells rather than two. The three sets of chromosomes are distributed between those four cells, resulting in some cells that are missing specific chromosomes. c) The regions of the embryo formed by such cells will become abnormal when the embryo reaches a stage when the genes on that chromosome become essential for development. The resultant embryos are »mosaic« mixtures of normal and abnormal cells. Figure modified from the original drawings in Boveri's 1907 description of the experiments.

who was at the University of Tübingen submitted a manuscript to *Berichte der deutschen botanischen Gesellschaft* in which he described the results of Mendel's genetic experiments, which had been sub-

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