Historical roots of centrosome research: discovery of Boveri’s microscope slides in Würzburg

Ulrich Scheer

Cell and Developmental Biology, Theodor-Boveri-Institute, Biocentre of the University of Würzburg, Würzburg, Germany

Boveri’s visionary monograph ‘Ueber die Natur der Centrosomen’ (On the nature of centrosomes) in 1900 was founded primarily on microscopic observations of cleaving eggs of sea urchins and the roundworm parasite Ascaris. As Boveri wrote in the introductory paragraph, his interests were less about morphological aspects of centrosomes, but rather aimed at an understanding of their physiological role during cell division. The remarkable transition from observations of tiny dot-like structures in fixed and sectioned material to a unified theory of centrosome function (which in essence still holds true today) cannot be fully appreciated without examining Boveri’s starting material, the histological specimens. It was generally assumed that the microscope slides were lost during the bombing of the Zoological Institute in Würzburg at the end of WWII. Here, I describe the discovery of a number of Boveri’s original microscope slides with serial sections of early sea urchin and Ascaris embryos, stained by Heidenhain’s iron haematoxylin method. Some slides bear handwritten notes and sketches by Boveri. Evidence is presented that the newly discovered slides are part of the original material used by Boveri for his seminal centrosome monograph.

1. The beginning of Boveri’s centrosome research

In 1893, Theodor Boveri was appointed as Professor of Zoology and director of the Zoological Institute at the University of Würzburg. Despite his young age of just 30 years, Boveri had already made a number of seminal and famous observations and established several basic facts about centrosomes, a term introduced by him in 1887 [1]. In my view, the year 1887 marks the naissance of centrosome research. Two scientists, the well-known Edouard van Beneden from Liége in Belgium [2] and Theodor Boveri, then a young (25 years old) postdoctoral fellow at the Zoological Institute in Munich in Germany, almost simultaneously and independently, published their observations on the karyokinetic (mitotic) divisions of fertilized eggs of the nematode Ascaris megalocephala (now Parascaris equorum) [1,3]. Most importantly, both authors realized that the polar bodies of mitotic spindles in cleaving eggs, which have already been described by several authors, such as W. Flemming, O. Hertwig, H. Fol and van Beneden (see [4,5]), were more than just transient spindle pole-associated structures. Rather they observed that these bodies, termed corpuscule central by van Beneden & Neyt [3] and centrosome by Boveri [1] were independent and permanent cell organelles that, by self-replication, were passed on from the mother cell to the daughter cells and appeared to act as organizing centres of cell division.

In his short communication of 1887, Boveri wrote that ‘the centrosome represents the dynamic centre of the cell; its division creates the centres of the forming daughter cells, around which all other cellular components arrange themselves symmetrically. . . . The centrosome is the true division organ of the cell, it mediates the nuclear and cellular division’ (‘Das Centrosoma repräsentiert das dynamische Centrum der Zelle, durch seine Theilung werden die Centren der zu bildenden Tochterzellen geschaffen, um die sich nun alle übrigen Zellbestandtheile symmetrisch gruppiren. . . . Das Centrosoma ist das eigentliche Theilungsorgan . . . .’).
der Zelle, es vermittelt die Kern- und Zelltheilung', [1, pp. 153 and 154]). Not bad for a young postdoctoral fellow, who had just entered the field of chromosome research and, after only 2 years of research, recognized that centrosomes are definite permanent structures throughout the cell cycle, that they duplicate and separate, that they initiate and organize the mitotic spindle, that they determine the plane of cell division and that supernumerary centrosomes cause multipolar mitotic figures. In a subsequent study published 1 year later, Boveri recognized a minute, denser granule in the centre of the centrosome [6]. Twelve years later, he described the cyclical behaviour of this granule in detail and named it centriole [7].

During a visit to the Zoological Station in Naples from January to April 1888, Boveri became interested in sea urchin development. In the same year, he described in a short communication that upon fertilization of sea urchin eggs an aster (‘Strahlsonne’) develops around the midpiece of the incorporated sperm cell, divides and eventually forms the dicentric spindle apparatus. Notably, he made his observations on living sea urchin eggs. Although Boveri was unable to visualize distinct bodies at the spindle poles in vivo, he was convinced that centrosomes must be present and that they divide and are passed on to the blastomeres (‘Wir müssen solche Centralkörperchen, die sich durch Theilung von einer Zelle auf die Tochterzellen vererben, ohne Zweifel auch für die Echinodermeneier annehmen, wenn es hier auch noch durch kein Reagens hat gelingen wollen, dieselben als scharf abgegrenzte Gebilde im Centrum einer Strahlung nachzuweisen’. [8, p. 68]).

Boveri’s studies soon brought him international recognition. In 1891, Edmund B. Wilson, 6 years older than Boveri, spent a sabbatical year in Naples and Munich, where he worked with Boveri. Figure 1 shows a photograph of Boveri from this time period.

2. Boveri’s centrosome research in Würzburg

In 1893, Boveri became professor in Würzburg and in the summer of the following year he began to reexamine the fertilization process and early cleavages of sea urchin eggs by using more sophisticated techniques. For his new studies, he now used paraffin sections of fixed material and stained them with Heidenhain’s iron haematoxylin [10]. Heidenhain, at that time prosector at the Anatomical Institute in Würzburg (just across the street from the Zoological Institute), had developed a staining method for the visualization of centrosomes in sectioned mammalian cells, and it is easy to understand that Boveri was eager to try out this technique on his own material (unfortunately, we do not know whether Heidenhain and Boveri maintained personal contact).

Boveri did not collect the material in Naples by himself, but obtained it from his friend W. M. Wheeler, then instructor of embryology at the University of Chicago. In 1893, Wheeler visited the Zoological Institute in Würzburg before he went on a trip to Naples, where he stayed during the winter 1893/1894. Here, Wheeler fixed fertilized eggs of *Echinus microtuberculatus* with picric–acetic acid and made the conserved material available to Boveri. Further processing (paraffin embedding, serial sectioning and staining) was done in Würzburg, and Boveri published a preliminary account of the results in 1895 [11]. The new technique produced interesting results and was therefore quickly applied to *Ascaris* material by Boveri’s doctoral student, Eduard Fürst. He published his doctoral thesis in 1898 under the title ‘On centrosomes of *Ascaris megaloecephala*’ [4]. The haematoxylin-stained paraffin sections of cleaving sea urchin and *Ascaris* eggs provided the basis for Boveri’s comprehensive monograph ‘On the nature of centrosomes’, comprising 220 pages and a total of 111 hand-drawn figures of exceptional artistic quality, which appeared in 1900 [7]. Wilson, in a review of this monograph, wrote in 1901 that it ‘deals with the nature and function of the centrosome, which has become one of the most difficult and perplexing problems of cytology. Students of cellular biology have eagerly awaited a critical discussion by Boveri of the later and in many respects conflicting aspects of this subject, in which he was one of the ablest pioneers’ [12, p. 264].

It is important to mention that in addition to the sea urchin material provided by W. M. Wheeler, Boveri examined another series of *Echinus microtuberculatus* eggs, which he obtained from ‘Herrn Kollegen Sobotta’ [7, p. 42]. Johannes Sobotta (still today widely known by medical students for his *Atlas of Human Anatomy*) has spent several months in 1895 in Naples, where he studied the maturation processes of *Amphioxus* eggs. Thereafter, he was appointed prosector...
at the Institute of Anatomy, Histology and Embryology at the University of Würzburg.

There can be no doubt that in preparation for his centrosome monograph Boveri examined hundreds of microscope slides with haematoxylin-stained sections of cleaving sea urchin and Ascaris eggs. At least one slide is known from the literature, which originally was in the possession of Boveri’s widow, Marcella O’Grady [13]. Joe Gall took photographs and published a spectacular image of the first cleavage division of Ascaris at the metaphase stage with prominent centrosomes in both spindle poles [5,13]. Could it be that more slides were still in existence, hidden somewhere in the cellar of the Zoological Institute in Würzburg?

3. A treasure trove of historical microscope slides

When Boveri became professor in Würzburg, he moved into an almost new institute building that had been erected 4 years previously, by the initiative of his predecessor, Karl Gottfried Semper. It is reasonable to assume that after Boveri’s untimely death in 1915, most documents of his scientific work were archived and stored in the institute. In March 1945, the Zoological Institute was largely destroyed during an air raid on Würzburg. I was informed that only a few items could be recovered from the ruins. After the war, the institute was rebuilt on its original site at the corner of Röntgenring and Koelikerstrasse. When I was appointed to the chair of Zoology I (later renamed, Cell and Developmental Biology) in Würzburg in 1986, the institute was housed in this building. In 1992, Zoology and several other institutes moved into the new Biocenter, and it was only then that I became aware of stacks of old microscope folders, many of them unlabelled, together with some historical instruments such as Boveri’s personal microscope (Leitz model Ia, engraved with his name), a camera lucida, dissecting microscopes and microtomes, a complete set of Boveri’s cell studies (vol. 1–6, the first two volumes with Boveri’s handwritten dedication to his predecessor Semper), original drawings by Boveri and four book-shaped boxes filled with slides prepared by Boveri and his wife Marcella during their research stays at the Zoological Station in Naples in winters 1901/1902, 1911/1912 and in spring 1914. Most of the materials are now on display in a permanent exhibition in the foyer of the Biocenter except for the folders that were stored in the cellar and forgotten.

Here is not the place to describe extensively the approximately 700 Naples slides with whole mounts of sea urchin eggs and embryos, stored in the book-shaped folders. Suffice it to say that they are all labelled and dated, and can be assigned to experiments devoted to the role of the nucleus and its chromosomes in development and inheritance. Boveri used two different experimental strategies known as the ‘merogone experiments’ [14] and the ‘dispermy experiments’ [15]. Thus, these slides bear testimony to Boveri’s important discoveries that chromosomes are the carriers of heredity, that they are genetically different and that proper embryonic development depends on a correct complement of chromosomes.

In October 2012, the Theodor-Boveri-Institute of the Biocenter of the University of Würzburg celebrated Boveri’s 150th anniversary with a commemorative symposium. In preparation of my presentation, entitled ‘Boveri’s legacy in Würzburg: letters, microscope slides and other documents’, I browsed through more than a hundred largely unsorted microscope folders stored in the cellar and, much to my delight, retrieved the following folders with slides relevant to Boveri’s centrosome studies (figure 2i):

(i) Three folders, numbered I–III, with Boveri’s handwritten note ‘St. u. Tlg. d. C.’, most likely meaning ‘Stadien und Teilung der Centrosomen’ (stages and division of centrosomes),
(ii) two folders with the writing ‘Sobotta II’ and
(iii) one folder marked ‘Wheeler’.

This was an exciting discovery since, as mentioned above, the material studied by Boveri in his preliminary report on sea urchin centrosomes [11] and the subsequent monograph ‘On the nature of the centrosomes’ [7] was provided by his colleagues W. M. Wheeler and J. Sobotta (in the following termed Wheeler and Sobotta series). All slides in the Sobotta and Wheeler folders contain iron haematoxylin-stained serial sections of cleaving sea urchin eggs. Remarkably, groups of eggs are encased by a very peculiar epithelial sheet, often in multiple layers, of obviously non-echinoderm origin (figure 3b). The solution to this histological riddle is found in Boveri’s preliminary centrosome paper [11, p. 4]. Since it would be desirable, he wrote, to cut through an as-large-as-possible number of eggs, he developed a method that could also be used for other similar purposes. By wrapping a large mass of eggs in shed amphibian skin, the little parcels could be processed like a piece of tissue through all steps of deydration and paraffin embedding. The shed epidermis was obtained from two living species of Cryptobranchus japonicus, the Japanese giant salamander, then held at the Zoological Institute.

The three folders mentioned above ‘i’ contain some additional slides with sections of cleaving sea urchin eggs, but mostly slides with serial sections through the oviduct of Ascaris, filled with early embryos at about the first cleavage stage. Unfortunately, none of the slides is dated, but some bear Boveri’s handwritten notes, little sketches and Indian ink dots marking specific sections (figure 2b–d). Several lines of evidence demonstrate that the sections are stained with Heidenhain’s iron haematoxylin. Firstly, some Ascaris slides are labelled ‘Alk. Ess. (H)’, meaning fixation with alcohol–acetic acid and staining with H, which almost certainly stands for Heidenhain’s haematoxylin. Secondly, the staining method produces a characteristic pattern at the surface of densely packed Ascaris eggs as described by Boveri (figure 3c). Boveri had used a regressive staining method, i.e. the sections were first overstained and then differentiated by removing excess dye in a ferric alum solution. He noted that the dye remained bound at the contact sites of juxtaposed eggs, which he explained by a mechanical exclusion or limited accessibility of the differentiation solution [7, p. 12 ff]. In fact, all Ascaris sections display this diagnostic surface-staining pattern (figure 3b). It is also seen in the photographs taken by Joe Gall, thus confirming his assumption that the slide was stained using Heidenhain’s haematoxylin method [5,13]. And thirdly, only Heidenhain’s haematoxylin produces the distinct centrosome labelling as seen in the slides. Taken together, all the data support the conclusion that the slides with Ascaris and sea urchin serial sections had been used by Boveri for his centrosome papers [7,11] and hence date back to the period between 1894 and about 1898.
4. Taking a closer look at some of the slides

(a) The sea urchin slides

Although, as Boveri wrote, the Wheeler and Sobotta series of cleaving sea urchin eggs were prepared from the same species (Echinus microtuberculatus) and processed in the same way (fixation in picric–acetic acid and staining of the paraffin sections with iron haematoxylin), the results differed to some extent, underlining the ‘capricious behaviour’ of the haematoxylin stain. In the Wheeler series, entire centrosomes were stained in contrast to the Sobotta material, which often displayed a preferential centriole staining. Boveri emphasized the need to combine both datasets in order to understand the ‘cycle of centrosome metamorphosis’ from the first zygotic division to the next division of the blastomeres, during which one spindle pole makes two poles. Altogether, 55

Figure 2. Folders with microscope slides used by Boveri for his centrosome studies. The slides can be dated to the period from 1894 to about 1898. Each slide contains several rows of serial sections of sea urchin and Ascaris embryos at the first cleavage stages, stained with Heidenhain’s haematoxylin. Boveri’s handwritten notes are on the outside of the folders (a) and on several slides with sea urchin (b) or Ascaris material (c,d); the roundish objects on the slides are oviduct cross sections). In addition, specific sections are marked by Indian ink dots or their position is indicated (d; II.7 – 9 stands for second row, sections 7 – 9).

Figure 3. (a) In order to facilitate histological processing of sea urchin eggs, batches of fixed eggs were wrapped by Boveri in shed pieces of salamander skin (an epidermal cell nucleus is denoted by the arrow) and processed like a piece of tissue. (b) Even after prolonged destaining (differentiation) of iron haematoxylin-stained sections of Ascaris oviduct, the contact sites of the tightly packed eggs retain the stain. The resulting staining pattern is diagnostic for the haematoxylin method. (c) The same situation depicted by Boveri (adapted from [7]). Scale bars, 50 μm.
slides are still in existence revealing all stages of the centrosome cycle, as described and depicted by Boveri in ch. 3 of his centrosome monograph [7].

At metaphase/early anaphase, Boveri described the centrosomes as clearly delineated entities of considerable size (‘als wohlbegrenzte kugelige Gebilde von beachtlicher Größe’; p. 31) from where the astral rays appear to radiate out from the centrosomal surface. Occasionally, a tiny dot is recognized in the centre of a destained centrosome, most probably a centriole (insert in b, arrow). (c) This micrograph of overstained, yet clearly delineated centrosomes was taken from a slide labelled ‘Sobatta II. s. stark’ (Sobotta II, very strong). Boveri wrote [7, p. 33] that he intentionally left some slides for 8 days in the staining solution leading to totally black centrosomes (c’). (d) Two tiny dots, apparently connected by fine filaments (‘Verbindungsgurte’), are seen inside an anaphase centrosome (arrows). A comparable situation is depicted in the drawing (d’). Scale bars, (a–d) 20 μm and (insert in b) 10 μm.

Figure 4. Images of centrosomes and centrioles during the first cleavage division of *Echinus microtuberculatus* eggs. Comparison between photographs taken from original slides (a–d) and Boveri’s drawings (a’–d’, adapted from [7]). Centrosomes were described by Boveri as moderately and uniformly stained spheres (a,a’) or as spheres interspersed with a filamentous scaffold (b,b’). Astral rays appear to radiate out from the centrosomal surface. Occasionally, a tiny dot is recognized in the centre of a destained centrosome, most probably a centriole (insert in b, arrow). (c) This micrograph of overstained, yet clearly delineated centrosomes was taken from a slide labelled ‘Sobatta II. s. stark’ (Sobotta II, very strong). Boveri wrote [7, p. 33] that he intentionally left some slides for 8 days in the staining solution leading to totally black centrosomes (c’). (d) Two tiny dots, apparently connected by fine filaments (‘Verbindungsgurte’), are seen inside an anaphase centrosome (arrows). A comparable situation is depicted in the drawing (d’). Scale bars, (a–d) 20 μm and (insert in b) 10 μm.

Overstained specimens that were intentionally left for 8 days in the staining solution, centrosomes did not grow further in size, but rather appeared as sharply delineated black spheres (figure 4c). Boveri noted, with a sense of irony, that those researchers who wanted a jet black staining of centrosomes should be satisfied by this result (p. 33). After sufficient extraction of the haematoxylin stain, a minute dot (or two, depending on the stage of the centrosome cycle), which he named *centriole*, emerged inside the decolourized centrosomes (figure 4b, insert). Notably, Boveri found (at metaphase/anaphase) two juxtaposed centrioles within each centrosome, most probably representing the disengaged centrioles prior to their replication (figure 4d). At later stages of mitosis, he described remarkable structural and topological changes of the centrosomes. The initially spherical centrosomes flatten into thin disc-like structures and attach to the polar surface of the daughter nuclei.
In Boveri’s words, the nuclei reveal a tendency to cling to the centrosomal disc as closely as possible—a process that, as we now know, is mediated by the LINC-complex proteins of the nuclear envelope [16]. Next the centrosomal disc adopts a dumbbell-shaped configuration, divides, and the two halves condense and segregate to opposite sides of the nuclei where they establish the bipolar mitotic apparatus for the next cleavage (figure 5c). Boveri’s description of the centrosome/centriole cycle in cleaving sea urchin eggs was later fully confirmed and extended by electron and immunofluorescence microscopy studies [17–19].

Figure 5. Flattening, expansion and division of centrosomes in preparation for the second cleavage of Echinus microtuberculatus eggs. Comparison between photographs taken from original slides (a–c, all from the Wheeler series) and Boveri’s drawings (a’–c’, adapted from [7]). (a,a’) At telophase of the first division (shown is the karyomere stage when individual chromosomes become surrounded by a nuclear envelope), centrosomes no longer appear as compact spheres but are spread out in the form of disc-like structures. (b,b’) Upon fusion of the karyomeres the centrosomal discs attach to the poleward faces of the daughter nuclei. (c,c’) Eventually each elongated centrosome divides, the two halves condense into compact structures and migrate to opposite positions of the daughter nuclei to form the new spindle poles. Scale bars, 20 μm.

Figure 6. (a) Microscope folder with Boveri’s handwritten note ‘Eier von A. meg., nicht aufgeklebte Schnitte. Färbg. nach Delafield’ (Eggs of Ascaris meg., sections not affixed. Staining according to Delafield). The paraffin sections were applied to glass slides without using adhesives such as gelatin or egg albumen which might have interfered with the staining procedure. (b) Photograph of a section through a dividing Ascaris egg, stained with Delafield’s haemalum. Note the absence of centrosome staining. Astral rays terminate at ill-defined focal bodies within each spindle pole (arrows). The reddish tinge results from en bloc staining of the eggs with borax Carmine prior to sectioning. The arrowhead denotes a polar body. Scale bar, 20 μm.

(b) The Ascaris slides
Clearly, the histological staining method introduced by Heidenhain [10] marked a major breakthrough in centrosome research. This is best illustrated when we consider the outcome of earlier histological staining protocols applied by Boveri, in particular haemalum staining according to Delafield. In the microscope folder depicted in figure 6a, I found eight slides with sections of cleaving Ascaris eggs stained with Delafield haemalum. Under such conditions, the astral rays of the bipolar spindle apparatus are seen to converge towards central elements which, however, are not specifically stained and therefore
remain structurally ill defined (figure 6); to the best of my knowledge, drawings of such specimens have never been published by Boveri). In striking contrast, the same focal structures are intensely stained with Heidenhain’s iron haematoxylin method and, fortunately enough, 14 of these slides are still in existence (figure 2c,d). Depending on the duration of the differentiation process, either the whole centrosomal body (figure 7a) or only the internal centriole (figure 7b) is displayed, allowing Boveri to describe all stages of both the centrosome and the centriole cycle during the first two cleavages of Ascaris eggs (ch. 4 of his centrosome monograph [7]). After the first cleavage of the Ascaris egg, the single centrosome in each of the two blastomeres has to double in preparation for the next division. Boveri distinguished between the processes of centrosome duplication and separation. An early phase of centrosome separation with the two sister centrosomes still close-by is shown in figure 7c. The next stages, migration of the two sister centrosomes to opposite poles of the nucleus (the blastomere on the right-hand side is only partially included in the section). (a–c) Scale bars, 20 μm and (insert in a) 10 μm.

Unfortunately, Boveri’s term centroplasm did not survive and has been replaced by the acronym PCM (pericentriolar material). Boveri ascribed to the centroplasm, and not the centrioles, the capability of organizing and inserting the astral arrays, based on his observation that they end at the peripheral region of the centroplasm, without contacting the internal centrioles (‘...daß die Spha¨renstrahlen in allen von mir untersuchten Objekten nicht bis an das Centriol herangehen’ [7, p. 117]). He was entirely correct, as shown decades later by electron microscopy studies [21]. Most remarkably, the recently discovered concentric arrangement of proteins around the centriole [22] had been anticipated by Boveri's student E. Fürst in 1898, based upon the concentric destaining of centrosomes during the differentiation step of the iron haematoxylin staining (‘Die Art, in der sich bei der Entfa¨rbung die Verklei-

derung der schwarz gefa¨rbten Stelle vollzieht, wu¨rde viel eher fu¨ r die Annahme einer concentrischen Structur der

5. Concluding remarks

Boveri’s visionary concepts, laid down in his masterly monograph ‘On the nature of centrosomes’ [7], set the stage for our current understanding of the structure and function of centrosomes. It is truly fascinating to observe the same microscopic images of centrosomes and centrioles that Boveri had studied more than a century ago. This allows us to follow his path of
visual perception, interpretations, conclusions and theories about the significance of these intriguing cellular structures, to which he ascribed an important regulatory role for cell division (‘Denn die Bedeutung der Centrosomen für die Zellteilung ist viel weniger eine direkt mechanische als eine regulatorische.’ p. 155)—a truly modern view of the role of centrosomes, which is now supported by a growing body of evidence. Moreover, and this is probably less known, he considered centrosomes not as indispensable elements (‘nichts überhaupt Unerläßliches’, p. 155), but rather as the best way for establishing bipolarity of the mitotic apparatus and for coordinating nuclear and cellular divisions. ‘Ich möchte sagen: die Teilung mit Centrosomen ist die eleganteste Lösung einer Aufgabe, die auch auf andere und wohl mehrfache andere Weise gelöst werden kann.’ (‘I want to say: division involving centrosomes is the most elegant solution to a problem that can also be solved in other and probably multiple other ways.’ p. 155).

The microscope slides hold all the information on which Boveri built his conception of centrosomes as the ‘true division organ’ of the cell. He conceived that cell division is regulated and determined by the division of the centrosome (‘... daß die Zweiteilung der Zelle durch die Zweiteilung des Centrosoms bedingt wird’, p. 132), which in turn is initiated by the preceding division of the centriole into two daughter centrioles (p. 97). He argued that the bipolar organization of the spindle apparatus is a consequence of the precise doubling (‘Zweiteilung’) of centrosomes, and that an abnormal increase in the number of centrosomes would lead to multipolar spindles, with the inevitable consequence of an unequal distribution of the chromosomes to the daughter cells (figure 8). In later years, Boveri could experimentally support this concept by his famous dispermy experiments on sea urchin eggs, carried out in Naples (as mentioned above, the slides of these experiments do exist, but need to be evaluated). He observed that abnormal chromosome contents (now termed aneuploidy) produced all sorts of cellular malfunctions in the developing sea urchin embryos, such as cell death, loss of cell–cell adhesion and unlimited growth of specific cells [23,24], which ultimately let him to postulate a link between supernumerary centrosomes, multipolar spindles, aneuploidy and cancer [25,26]. No doubt, Boveri’s microscope slides represent a valuable treasure trove for students of the history of science.

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References


