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Method paper

Boveri's research at the Zoological Station Naples: Rediscovery of his original microscope slides at the University of Würzburg

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A B S T R A C T

Eric Davidson once wrote about Theodor Boveri: "From his own researches, and perhaps most important, his generalized interpretations, derive the paradigms that underlie modern inquiries into the genomic basis of embryogenesis" (Davidson, 1985). As luck would have it, the "primary data" of Boveri's experimental work, namely the microscope slides prepared by him and his wife Marcella during several stays at the Zoological Station in Naples (1901/02, 1911/12 and 1914), have survived at the University of Würzburg. More than 600 slides exist and despite their age they are in a surprisingly good condition. The slides are labelled and dated in Boveri's handwriting and thus can be assigned to his published experimental work on sea urchin development. The results allowed Boveri to unravel the role of the cell nucleus and its chromosomes in development and inheritance. Here, I present an overview of the slides in the context of Boveri's work along with photographic images of selected specimens taken from the original slides. It is planned to examine the slides in more detail, take high-resolution focal image series of significant specimens and make them online available.

1. The Naples slide collection: an overview

A few years ago I detected hundreds of old microscopic slides in the cellar of our Biocenter which were originally kept in the old Zoological Institute of the University of Würzburg. Among them were slides that had been prepared by Theodor Boveri in the course of his studies on egg maturation, formation of polar bodies, fertilization and early cleavage events. In addition, I found slides containing paraffin sections of dividing *Ascaris* and sea urchin eggs with brilliantly stained centrosomes which most likely had been used by Boveri for his seminal work on centrosomes (Scheer, 2014). Between the stacks of brown cardboard folders of a type still being used today, four elegant book-shape storage boxes with the imprinted inscription *Mikroskopische Präparate* (microscopical slides) stood out (Fig. 1a). The handwritten text on the white labels reads as follows (Fig. 1a, from left to right):

Neapel 1901/02. Versuche an Echiniden-Eiern (experiments on Echinoid-eggs).
Neapel 1911–1912, I.
Neapel 1911–1912, II.
Neapel 1914, II (Box I is missing).

Each box accommodates 100 numbered slots that correspond to a numbered index on the inside of the hinged lid (Fig. 1b). Often two slides are placed back to back in a single slot. The content list is written in Boveri's characteristic, clearly legible handwriting (Fig. 1c). Unfortunately, a number of the listed slides are missing.

The number of slides per box is as follows:

Box Neapel 1901/02: 101 slides.
Box Neapel 1911–1912, I: 175 slides.
Box Neapel 1911–1912, II: 46 slides.
Box Neapel 1914, II: 133 slides.

A further 174 slides dated from 1902 to 1914 are kept in 12 separate folders. Thus altogether 629 Naples slides are still in existence.

The vast majority of the slides are whole mounts of sea urchin embryos stained with borax carmine, but a few paraffin sections are also existent. Two slides taken from the 1901/02 box are shown at higher magnification in Fig. 1d. Depending on the experiment, a slide contains only one or a varying number of sea urchin larvae. In order to provide the necessary distance between slide and coverslip, two hairs serve as spacers. Canada balsam is used as mounting medium. Although it turned yellow over the years, the slides are generally in a surprisingly good condition despite their age. The specimens are well preserved except for their larval skeleton in the form of calcium carbonate spicules which are barely recognizable. It is known that Canada balsam can be acidic which could have caused erosion of the fine skeletal structures.

Each slide is dated by Boveri and provided with essential

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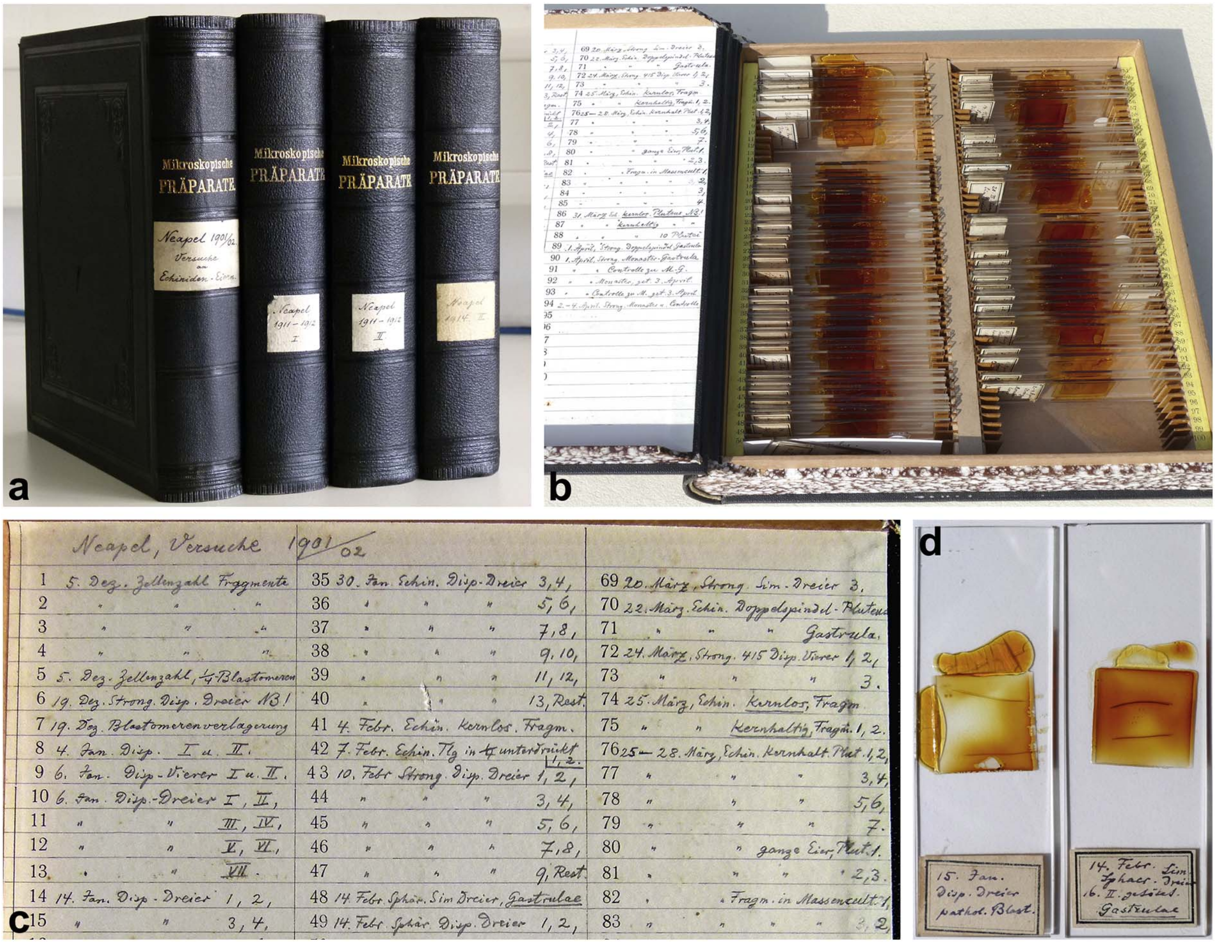

Fig. 1. Boveri's microscope slides prepared at the Zoological Station in Naples. (a) Four book-shaped storage boxes with handwritten labels (see text). (b) Open box to reveal the slides. Note the inventory list on the inside of the lid. (c) Higher magnification of the numbered and dated inventory list. The heading reads *Neapel, Versuche 1901/01* (Naples, experiments 1901/02). The abbreviations *Disp.-Dreier* and *Disp.-Vierer* stand for "dispermic three" and "dispermic four", i.e., triaster or tetraaster embryos. *Kernlose Fragm.* are nuclear-free egg fragments and *kernhaltige Frag.* are egg fragments containing a nucleus. (d) Higher magnification of two slides from 1902. Note the two hairs each supporting the coverslip in order to prevent flattening of the specimen. Despite yellowing of the mounting medium Canada balsam the optical properties of the slides are generally good. The slide on the left (*15. Jan*) contains three pathological blastulae derived from triaster eggs. The slide on the right (*14. Febr*) contains about 25 triaster *Sphaerechinus* embryos, all arrested at the early gastrula stage (*Gastrulae 16.II. getötet*, i.e. killed on Feb. 16).

information such as the type of experiment and a brief specification of the content. Some slides bear additional handwritten notes on a second label (Fig. 3a). The meaning of the acronym *NB!* on several slides is not entirely clear (Fig. 3a). Most likely it stands for *Nota Bene* and was used by Boveri to highlight particularly important specimens including those he has drawn for his publications. However, it is quite difficult to identify on a microscope slide those objects displayed in Boveri's publications. This is because Boveri often drew the objects when they were mounted temporarily in glycerol. Slight movements of the coverslip allowed him to roll the object in any direction and thus to integrate all relevant aspects into a single composite drawing. Only after completion of the drawing the object was permanently mounted in Canada balsam.

The slides document Boveri's seminal experiments with sea urchin eggs and embryos, known as the "dispermy" and "merogone" experiments (see below), which provided evidence for the nuclear control of development or, as Boveri put it: "all essential traits of the individual and the species are determined by the chromatin of the egg nucleus and of the sperm nucleus" (Boveri, 1904, p. 113).

Interestingly, the majority of the slides (354 out of 455) are devoted to the merogone experiments (for a comprehensive review see Laubichler and Davidson, 2008). Over many years the Boveris repeated these experiments again and again in order to meet the criticisms by others and to improve the experimental setup. In his last publication which appeared posthumously three years after his untimely death, Boveri wrote: "On no other experiment have I spent so much time as on the breeding of merogonic hybrids from *Sphaerechinus* eggs" (Boveri, 1918, p. 435). This may explain that the merogone slides outnumber by far the dispermy slides.

2. The dispermy experiments: creating blastomeres with different chromosome sets

Edmund B. Wilson once wrote that Boveri's experiments with double-fertilized eggs "form his crowning achievement, whether in respect to excellence of method or importance of results" (Wilson, 1918, p.74; for reviews see Baltzer, 1962; Sander, 1993; Moritz and Sauer, 1996; Maderspacher, 2008). Boveri's brilliant idea was to produce blastomeres with different chromosome sets and to analyze the effects on embryonic development. In his own words: "We give the cell a nucleus with some parts lacking and follow the effect of this defect" (Boveri, 1902, p. 81).

Sea urchin eggs can be easily fertilized in vitro. When sperm is added in excess, often two sperms enter an egg. These dispermic eggs develop tetrapolar mitotic figures and divide immediately into four rather than two cells. The four spindles compete for the chromosomes with the result that the four daughter cells inherit abnormal and different chromosome combinations. When eggs are shaken shortly after fertilization, tripolar spindles may also form (mechanical strain