

# THEODOR BOVERI: THE LIFE OF A GREAT BIOLOGIST 1862 - 1915

FRITZ BALTZER, 1967

Translated from the German by Dorothea Rudnick.

University of California Press, Berkeley; pp. 85-97.

This excerpt is published by kind permission of the University of California Press.

The illustrations and references have been renumbered.

## The Chromosome Theory of Inheritance, Experiments with Double Fertilized Sea Urchin Eggs (Dispermy Experiments)

If one makes the easy generalization that all great things are simple, this may apply to the idea that chromosomes carry different hereditary factors. But to prove decisively the correctness of this idea was not simple. For this purpose an artful and almost inquisitorial system of experiments was required. The experimenter had to produce egg cells with altered chromosomal arrays and had to be able to follow their development and observe to what abnormalities or peculiarities these alterations led. That appears simple. However, though the chromosome pattern is easy to observe in a fixed and stained cell, this is not true in the living state. How can it be made possible to detect the presence of an altered chromosome set and then to follow the development of the cell in question? Boveri's experiment with double fertilized sea urchin eggs was the first cytological procedure fulfilling these requirements.

His work up to this point had led him to three conclusions: (1) Chromosomes retain their individuality from one cell division to another. (2) The developing egg obtains corresponding chromosome sets from egg and sperm nuclei. (3) Each of these parental sets, separately, is sufficient for normal development of the germ. Given that these three facts identify the chromosomes as carriers of hereditary substance, two opposite possibilities present themselves. Each chromosome may contain the total hereditary substance--this was the view advocated by Weismann--or each chromosome may be the carrier of different portions of the hereditary material. In the latter case, each chromosome would be of different genetic value, an idea that Wilhelm Roux (1) had interjected into the discussion as early as 1883. Boveri decided this question in favor of differential value by the analysis that we shall now discuss.

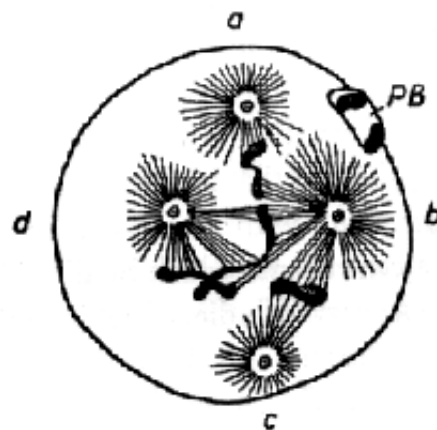
The experiments were carried out in the winter of the 1901-1902 and in the spring of 1905. The final, extremely comprehensive report (2) appeared in 1907. It was preceded by two excellent summaries, a first one in 1902 (3) and a second in 1903, enlarged in 1904 (4, 5). As was often the case with Boveri, an already known developmental anomaly gave him the key to these experiments. In cultures of artificially fertilized sea urchin eggs, one often finds simultaneous fours. In these cases, the egg divides immediately into four cells, without passing through the normal two-celled stage. The division itself proceeds from a four poled astral figure, a tetraaster. Such tetrafoil cleavages appear when the egg is fertilized simultaneously by two sperm. As Boveri found in 1888 (6), in normal fertilization the egg engulfs a centrosome or division organ along with the sperm head. The centrosome divides and forms the two centers of the first cell division spindle. In the double fertilized eggs, two sperm enter the egg. Consequently, four centrosomes are formed, and hence a tetraaster. Boveri felt that this was strong evidence for the correctness of his centrosome theory of fertilization, and that conversely the theory could explain all anomalies of the dispermic eggs. It was for him an example of how the disclosure of a

single new fact [the discovery of the sperm centrosome] suddenly can illuminate a quite distant area, hitherto dark (3).

Concerning the fate of these tetrafoil cleavages, it was known according to the work of Driesch (7) that they at first develop normally, then unexpectedly die during gastrulation. Driesch did not discuss the cause of this. Boveri however realized that in tetrasters the chromosomes must almost always be distributed unequally to the four cells. The decisive point is that the chromosomes are bilateral, and can be related to only two centrospheres. In the bipolar spindle this guarantees equal distribution to the two poles. However in dispermic eggs, four centers compete for each chromosome. Chromosome distribution must thus occur at random and for the most part unequally. An irregular set, once established, would be repeated in subsequent divisions. Fixed tetrasters, with the chromosomes stained, later reinforced this view (8a,b).

The implications for the developmental potency of the embryo and its four separate blastomeres are clear: If each chromosome contains all hereditary factors necessary for the development of the different organs, then an unequal chromosome distribution should cause no defect in development. If, however, the chromosomes as carriers of heredity are qualitatively different, then with unequal chromosome distribution there will be germ regions lacking certain hereditary factors. This would express itself in pathological development, either in premature death of the whole embryo or in developmental defects in one of more quadrants. In other words, the study of the tetrafoil cleavages should show whether the whole hereditary substance is contained in each chromosome or whether it is distributed to different chromosomes.

We have previously indicated that the basis for this reasoning can be found as early as 1888 in Boveri's second Cell Study (6). At that time, he found occasional cases of *Ascaris* eggs with tetrasters. An especially good example is shown in figure 1, and it can immediately be seen that the chromosomes will be unequally distributed to the four poles. Boveri closes the commentary on this case with the statement that the nuclei of the resulting daughter cells must receive different qualities, "in case we are to ascribe different qualities to the individual chromatic elements" (6). This idea remained with him. He returned (9) in another article on fertilization in 1892. But it was the special advantages of the dispermic sea urchin eggs that allowed him to proceed with experimentation.



Ê

**FIGURE 1. Tetrapolar spindle in an egg of *Ascaris megalocephala bivalens*. Chromosomes arranged on several spindles. Of the four poles, two (a,c) will receive only one chromosome, b will receive three or four; d, two. PB, polar body. From Boveri (6).**

From the first tetraster observations a second possibility, the production of triasters, quickly emerged. Morgan (10) had already found in 1895 that instead of tetrafoils one often obtained trefoils if the eggs

were shaken shortly after insemination. Obviously the shaking interfered with the division of one of the sperm centrosomes, and thus three instead of four spheres were produced. In a tripolar figure, the possibilities of a more equal distribution of chromosomes naturally are greater than in a tetraaster. If Boveri's basic assumption were correct, he should get better development from such trefoils from the fours. As we shall see, this assumption was fulfilled beyond the most optimistic expectation.

Before we compare these two experimental groups, we must turn our thoughts in another direction. The cardinal point of the discussion up to now has been the unequal distribution of the chromosomes. But since the egg cell possesses, in addition to the chromosomes, an extensive cytoplasmic body, another question must be posed. Does differential division of the cytoplasm possibly play a part in the pathological development of the tetrafoils and trefoils? To test this question, Boveri separated from one another the blastomeres of normal four-celled eggs. This can be done very safely by placing the eggs in calcium-free sea water according to the method of Herbst (11). He then raised these isolated quarter embryos to test their developmental potency. When he started with normal four-celled stages, well developed plutei, that is, artificial quadruplets, resulted. The first four blastomeres in normal development thus are equivalent in cytoplasm and in chromosome array. They also possess, as will be detailed later, equivalent portions of the various cytoplasmic layers. This last is also true for the tetrafoils (and trefoils). Here also, the cytoplasm is separated by the first division into morphologically equivalent cleavage cells. Nevertheless, in isolation, most of the tetrafoil blastomeres die as blastulae; others, less numerous, as gastrulae; and almost none even attempt to form a pluteus. For the isolated blastomeres of the trefoils the same is true, although in this case normal development is more frequent. It was clear, then, from the isolation experiments, that cytoplasmic differences were not responsible for the altered fate of the isolated cells of the tetrafoils and trefoils.

The successive phases of this experiment, carried out by Boveri and his wife in the winter of 1901-1902 in Naples, are graphically depicted in letters to Spemann. The Boveris obtained decisive results with enviable rapidity. After two months the outcome was clear. The idea of using dispermic eggs to determine the qualitative unlikeness of the chromosomes proved exceedingly fruitful.

I have raised the four blastomeres of simultaneous-tetrafoil eggs in isolation. Most important, as a rule a different thing happens to each cell. One goes to pieces at the blastula stage, one forms mesenchyme and then goes kaputt, another begins gastrulation or even completes it. Once I even got a pluteus--admittedly somewhat rudimentary. Taking everything into consideration, I believe that here we are finally closing in on the nucleus. That the development does not depend on quantity of chromatin but on quality is quite certain [December 1, 1901].

Then two and a half months later, on February 17, 1902: "After I had followed the isolated blastomeres, I returned to the development of the intact dispermic eggs, finding that these do not always die as stereoblastulae, as Driesch stated, but that one gets--rather rarely--gastrulae and even abnormal plutei.

"Fortunately there is another type of dispermy where the relationships are much more favorably displayed. If one shakes the eggs shortly after insemination, many of them form a three-poled figure with simultaneous division into three cells, as Morgan had observed without being able to explain the significance." For these trefoils, as Boveri expected, the chances of correct chromatin distribution are more favorable.

We have raised at least 500 such trefoils in isolation; among them were some twenty absolutely normal larvae, and, from these, all imaginable gradations down to the usual pathological stereoblastulae. These larvae demonstrate clearly that the quantity of chromatin is entirely irrelevant. I have a few larvae in which one third contains tiny little nuclei, the other two thirds much larger ones. The boundaries between the two areas can be demonstrated with extraordinary precision. Nevertheless the morphology

of the larva is not affected by these boundaries. On the other hand, highly pathological monsters are often found showing nuclei of the same size in all areas.

Moreover I beg you," he adds in this letter to Spemann, "not to say anything about the dispermy experiments for the time being. The fact is, if only one thinks of it, the experiment is easy to do. It is in any case simpler," he continues with bitter humor, "than to steal a pocketbook from an inner coat pocket, as recently happened to me in the jostling of a crowded tramcar--unfortunately the pocketbook had 230 chromosomes in it. This affair afforded me the privilege of looking through the album of pickpockets--a handsome gallery--at the central police station. But I was not able to find mine in the array."

Boveri's statement that it is a simple thing "if only one thinks of it, to do the experiment" may occasion a short commentary. Boveri's creative concept of the dispermy experiment is an extremely striking example not only of acute thinking but also of imagination. Its power lies in the intuitive integration of highly disparate data: the death of the dispermic germs, the centrosome theory of fertilization, the distribution by chance of the chromosomes in the multipolar division figures, and the idea of the chromosomes as carriers of diverse hereditary factors. This power of intuition is not necessarily unexpected in so extraordinarily artistic a man as Boveri.

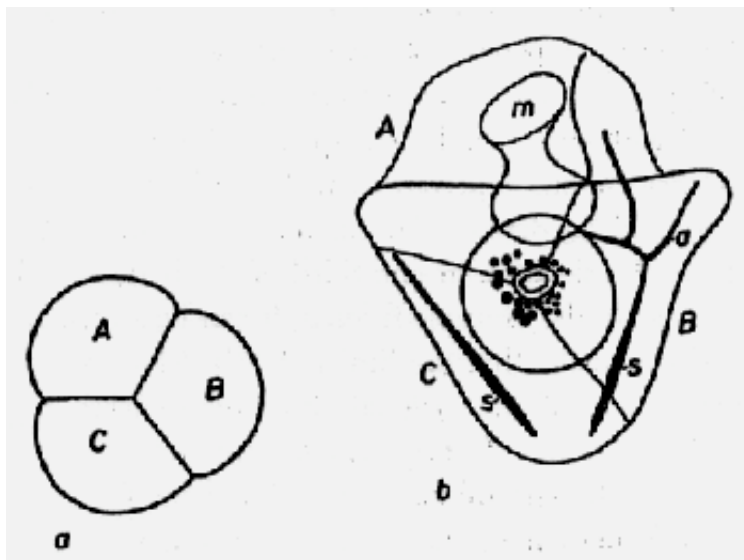
Let us turn now to a more precise calculation of the chances for normal development in dispermic eggs. In other words, how frequently will all four or all three cells receive a complete chromosome set? The eggs of the sea urchin species used contain, in normal fertilization, 36 chromosomes, 18 from the egg nucleus and 18 from the sperm. The two arrays are alike. Boveri's arbitrary assumption was that all 18 chromosomes carry different hereditary factors. Accordingly, the chromosomes may be distinguished as numbers, 1-18. The dispermic eggs contain two sperm nuclei and one egg nucleus, thus three sets of chromosomes 1-18. It was required to calculate with what frequency each cell in the original tetrafoil or trefoil would contain at least one representative of each chromosome type 1-18.

Following the advice of his friend Wien, the physicist in Würzburg, he devised a simple chance-calculating apparatus to determine these frequencies. The three chromosome series 1-18 were represented by 54 numbered balls, three balls marked with each of the numbers. The balls were mixed in a cup and poured at random onto a circular plate with a frame superposed, dividing it into three or four compartments as the case required. For the tetraster calculation a rectangular cross was used, for the triaster count a triradiate frame. The number of balls in the individual sectors was counted. A total of 200 throws was made for each of the two types. The results were that in the tetraster distribution, 64 percent of the cases showed all four quadrants as "defective," that is, in no quadrant were all 18 chromosome types present. In 34 percent one quadrant, and in 2 percent two quadrants, contained the complete assortment. Cases with four normal quadrants for the tetrafoils did not occur in the 200 throws used.

In the triaster tests, however, the result was different. Here, in 11 percent all three thirds were complete, in 42 percent, two and in 36 percent only one. Entirely defective cases with three incomplete chromosome assortments occurred in only 11 percent. In biological terms these figures mean, assuming that in the sea urchin egg all 18 chromosomes of the haploid set carry different genetic factors and that all are required for a normal pluteus, that normal larvae may be expected practically not at all from tetrasters; but from triasters about 11 percent of the eggs should develop normally. This expectation was strikingly confirmed in the experiment. Of 1500 tetrafoils individually cultured, a single one developed into a healthy pluteus (0.07 percent); from 719 trefoils, 58 perfect plutei or 8 percent resulted (2). If one includes defective plutei, the number rises to 79, or 11 percent.

In addition to the cases where normal plutei developed, the partly inhibited embryos were of interest. The defects and anomalies they showed were almost always limited to circumscribed sectors of the body

which corresponded to the three or four original cells. We shall consider only the trefoils in detail. According to the statistical prediction, one of the trefoil cells would contain a complete set of 36 percent of cases, and two cells in 42 percent. Thus embryos normal in one or two thirds of the body are to be expected with corresponding frequency. Morphological examination confirmed this expectation, with special impressiveness in the case of skeletal defects. An example is shown in figure 2. Here the skeleton in one third of the body is lacking. In many cases, as in the example figured, it can be ascertained from the size of the nuclei that the limits of this defect coincide with the limits of one of the original three blastomeres (fig. 2b). Translated into terms of chromosomes, this means that in such cases the particular chromosomes containing the factors requisite for normal formation of the skeleton must have been missing from that particular body region. Which of the 18 chromosomes are involved, the experiment does not say. Here the sea urchin experiment had reached its limit, a limit that the work on *Drosophila* was the first to surmount.



**FIGURE 2. Development of pluteus with skeletal defect from a "simultaneous three." (a). Diagram of the trefoil with cells A, B, and C. (b). Pluteus in same orientation as (a). Each sector has uniform nuclei, shown only in the anal region. Note especially small size of nuclei in sector B and absence of skeleton in sector A (m, mouth; s, apical rod; a, anal rod). From Boveri (2).**

A final fact that we anticipated in connection with the hybrid merogons should be mentioned. Most of the dispermic eggs develop abnormally. The pathological phase, however, begins only in the blastula, or at the beginning of gastrulation. Cleavage is normal. On these grounds, Boveri distinguished two developmental phases. Not until the onset of gastrulation do chromosomes exert their specific individual influences, for which specifically adjusted cytoplasm is requisite. In the dispermic eggs, development becomes pathological because of the absence of certain chromosomes; in the case of the hybrid merogons, because the paternal chromosomes belong to a foreign species.

Let us conclude. By the assumption of a differential genetic significance of the chromosomes, a large number of facts observed by Boveri in his dispermy experiment can be brilliantly explained. This assumption forms a keystone in the construction of the chromosome theory of inheritance, the first foundation of which had been laid by Rabl and Boveri 15 years previously with the theory and evidence for the individuality of the chromosomes. One understands the judgment of Wilson (12), quoted here with some omissions:

One who, like the writer, had puzzled in vain over the riddle presented by the double-fertilized eggs of sea urchins could not read Boveri's complete and beautiful solution without a thrill. This result, wholly new, . . . is fundamental to our entire view of the cytological basis of heredity. As is often the case with

discoveries of the first rank it gives at first sight little suggestion of its far-reaching importance or the difficulties that had to be surmounted in its attainment. It brings forward the long-sought crucial evidence of the direct influence of the nucleus [chromosomes] in determination and development; all attempts to shake the force of that evidence have proved unavailing.

And one also understands the reproach that Goldschmidt makes to contemporary biology (13), that it is scarcely conceivable that this classical experiment is completely passed over in many genetics textbooks of the present day.

Boveri's theory was rapidly supported by conclusive facts. It was already known that single elements of a chromosome set may have different form and different size, and that these differences are repeated regularly through subsequent divisions. Nevertheless, as Boveri, speaking as a cell physiologist, correctly put it, "The more insight grows, the more we realize that in these questions the morphological is only the foundation for what we ultimately want to know: Namely, what physiological properties are attributable to these chromatin elements which undergo so dramatic a history" (4,5). Some physiological facts were already at hand. Various investigators, chiefly the Americans--proceeded however by Henking (14)--had found in insects a single, morphologically identifiable chromosome, obviously concerned with determination of sex. In these cases the male forms two sorts of sperm cells, different in their chromosome content. If the egg is fertilized by one sort, a male develops; fertilization with the other type yields a female.

To these findings on insects, similar ones on various nematode species were later added by Boveri, his students, and other workers (15). Parallel situations were later found in humans, butterflies, *Drosophila*, and many other forms. With the discovery of such "sex chromosomes" and the information that they carry numerous genetic factors, a simple explanation was at hand for sex-linked inheritance, as for example, color blindness in man.

On the other hand, there are species in various animal groups in which a bisexual generation, with separate males and females, regularly alternates with an hermaphrodite generation. The chromosome cycle of these forms, which include the threadworm *Rhabditis nigrovenosa*, interested Boveri particularly. "The problem," he says, "that is set by this developmental cycle, is clear. If there are two different sorts of spermatozoa, distinguishable by their chromosome number, one determining the female, and the other the male sex, why then do fertilized eggs [of the generation with separate sexes] develop into individuals of only one type [the hermaphrodite generation]? And how can such an individual form both sperm and eggs? And how, among these sperm can two different types reappear, distinguishable by their chromosome content?" (16). The problem formulated here by Boveri was independently and simultaneously solved by Schleip (17). In *Rhabditis*, this alternation of sexual generations is achieved by specialized chromosome divisions in sperm maturation, deviating from the normal type.

The same line of thought is found in one of Boveri's last works, concerned with the special reproductive pattern in bees. As is well known, the workers and the queen develop from fertilized eggs, the drones from unfertilized ones. In addition to these three types there are, in exceptional cases, hermaphrodite bees (Zwitterbienen) in which both male and female characters are combined. For an explanation, Boveri assumed that the eggs in this case, as in the females, were fertilized, but that the sperm nucleus, retarded, finally united with one of the daughters of the egg nucleus. In 1888 he had described such an anomaly in sea urchins as "partial fertilization" (18). Only 26 years later did he have the opportunity to test his hypothesis in bees. The apiarist Eugster in Constance had supplied a famous collection of hermaphrodite bees to the zoological collection in Munich. Boveri investigated the mosaic of male and female characters in Eugster's bees. "The view," he concluded, "that the hermaphrodite bees originate by so-called partial fertilization has a probability closely approximating certainty" (19).

**REFERENCES:**

1. Roux, W. 1883. †ber die Bedeutung der Kernteilungsfiguren. Leipzig.
2. Boveri, Th. 1907. Zellenstudien VI: Die Entwicklung dispermer Seeigelier. Ein Beitrag zur Befruchtungslehre und zur Theorie des Kernes. Jena Zeit. Naturw. 43: 1-292.
3. Boveri, Th. 1902. †ber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh. d. phys.-med. Ges. WYrzburg N.F. 35: 67 - 90. Reprinted on this CD-ROM.
4. Boveri, Th. 1903. †ber die Konstitution der chromatischen Kernsubstanz. Verh. D. Zool. Ges. 13. WYrzburg.
5. Boveri, Th. 1904. Ergebnisse Yber die Konstitution der chromatischen Substanz des Zellkerns. Jena. G. Fischer.
6. Boveri, Th. 1888. Zellenstudien II. Die Befruchtung und Teilung des Eies von *Ascaris megalocephala*. Jena Zeit. Naturw. 22: 685 - 882.
7. Driesch, H. 1893. Entwicklungsmechanische Studies V, VI. Zeit. wiss. Zool. 55.
- 8a. Baltzer, F. 1909. Die Chromosomen von *Strongylocentrotus lividus* und *Echinus microtuberculatus*. Arch. f. Zellf. 2.
- 8b. Baltzer, F. 1911. Zur Kenntnis der Mechanik der Kernteilungsfiguren. Arch. Entw.-Mech. 32.
9. Boveri, Th. 1892. Befruchtung. Erg. d. Anat. u. Entw.-Gesch. 1: 386-485.
10. Morgan, T. H. 1895. A study of variation of cleavage. Arch. Entw.-Mech. 2.
11. Herbst, C. 1900. †ber das Ausinandergehen von Furchungs- und Gewebs-zellen in kalkfreiem Medium. Arch. Entw.-Mech. 9.
12. Wilson, E. B. 1918. In Ršntgen, W. C. (ed.) Erinnerungen an Theodor Boveri. TYbingen, J. C. B. Mohr.
13. Goldschmidt, R. B. 1954. Erlebnisse und Begegnungen. P. Parey.
14. Henking, H. 1891. †ber Spermatogenese und deren Beziehung zur Eientwicklung bei *Pyrrhocoris apterus* L. Zeit. wiss. Zool. 51.
15. Boveri, Th. 1909. †ber ŐGeschlechtschromosomenŒ bei Nematoden. Arch. f. Zellf. 4: 132 - 141.
16. Boveri, Th. 1911. †ber das Verhalten der Geschlechtschromosomen bei Hermaproditismus: Beobachtung an *Rhabditis nigrovenosa*. Verh. d. phys.-med. Ges. WYrzburg. 41: 83 - 97.
17. Schliep, W. 1911. †ber die ChromatinverhŠltnisse bei *Angiostomum (Rhabdomena) nigrovenosum*. Ber. naturf. Ges. Freiberg i. Br. 19.
18. Boveri, Th. 1888. †ber partielle Befruchtung. Sitz.-Ber d. Ges. f. Morph. u. Physiol. MYNchen. 4.
19. Boveri, Th. 1915. †ber die Entstehung der Eugsterschen Zwitterbienen. Arch. f. Entw.-Mech. 41: 264 - 311.

---

[Home](#) [Welcome](#) [Info Link](#) [Other Sites](#) [What's New](#) [Instructions](#)

[Return to Chapter TOC](#)

---

*Last Updated 17 January 97*

Ê