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T. H. Morgan as invertebrate embryologist

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Summary

T. H. Morgan is known primarily for his work in genetics and for his "conversion" to the Mendelian-chromosome theory of inheritance in 1910. Standard accounts represent this conversion as evidence of Morgan's having seen the light of truth and progress in science and of his having cast off the shackles of his old embryological and morphological approach. In contrast, this paper suggests that the primary roots of Morgan's interest in heredity and development both lie in his work of the 1890s, and can be seen clearly in the work of 1895 at the Naples Zoological Station, where Morgan intensively studied isolated blastomeres of marine invertebrate eggs.

Keywords: Morgan, development/differentiation, isolated blastomeres, sea urchins

The name Thomas Hunt Morgan for most people means genetics, fruit flies, and rotting bananas. Perhaps it suggests the Nobel Prize in physiology and medicine, which Morgan won in 1933. A few may know also of Morgan's studies of regeneration or frog development. Some may recall Morgan's close friendship with Edmund Beecher Wilson or Edwin Grant Conklin and may envisage them all in Woods Hole for their summer work. But not many will think of Morgan standing in his lab at the Naples Zoological Station or the Marine Biological Laboratory shaking tubes of sea urchin eggs.

Yet embryology of marine invertebrates actually remained Morgan's favorite research subject for most of his life (Mountain, 1987). During the years 1893–1895, he countered the work of Theodor Boveri, discovered the wonders of Naples, and published no fewer than ten papers exploring the development of isolated blastomeres in sea urchins and other marine invertebrates. In many key ways, this early research set the stage for his later conceptualization of the problems of embryology and genetics. The work also played a role in the active debate about development during the 1890's and stands alongside Wilson's and Conklin's more familiar study of cell lineage as a foundation for work on development in the twentieth century.

Morgan's interest in sea urchins and isolated blastomeres arose in 1892–1893, after Wilson returned from his year-long visit in Europe. Wilson had studied with Boveri and then at Naples, in each case learning about the techniques and problems central to European biology. In particular, Wilson (1892) had found promising the shaking of eggs to produce isolated pieces. The Hertwig brothers, Oscar and Richard, had inaugurated the technique, but Boveri and Hans Driesch had demonstrated its usefulness in their researches on fertilization and development.

Boveri (1887, 1888, 1890) had recently completed his "Zellenstudien" when Wilson arrived in Europe in 1891, and he had begun to work with sea urchin egg fragments in order to examine the relative contributions of nucleus and cytoplasm to development (Baltzer, 1967). Boveri took unfertilized eggs and shook them. This causes the coating to give way and the eggs to break up into fragments, many of which continue to survive for a while. He assumed that some of these pieces must contain the egg nucleus while others do not. Since he believed that the nucleus remains intact and the individual chromosomes maintain their individuality, this also meant that some fragments contain the maternal chromosomes while other do not. When he introduced sperm, many of the pieces began to divide. Since not all contained the egg nucleus, this suggested that the presence of egg cytoplasm plus sperm nucleus was sufficient to initiate development. Two sets of chromosomes are not necessary, he concluded.

In 1889, Boveri published the results of a most suggestive set of experiments from which he concluded that "Herewith is demonstrated the law that the nucleus alone is the bearer of hereditary qualities." (Boveri, 1893, p. 232). He had taken egg fragments of *Sphaerechinus* and had fertilized them with *Echinus* sperm. The resulting hybrids had shown characteristics in between the two species in some cases. But in all cases where the fragments had been non-nucleated, Boveri concluded that the larvae had only characteristics of the paternal species. To Boveri, this demonstrated that "the maternal protoplasm, although in this case furnishing a large share of the material for the development of the new organism, is without influence on the form of the organism" (Boveri, 1893, p. 232). This bold conclusion raised obvious questions and possibilities for further work.

Wilson (1893, 1894) saw the possibilities but was not convinced of the efficacy of the nucleus in directing development. He carried out similar experiments with *Amphioxus*, which he had been studying for other reasons, and concluded that there is a great deal more cytoplasmic organization and direction than Boveri would have admitted. But he continued to explore the question and discussed the work at the MBL when he returned in 1892. Morgan was intrigued.

In 1893, Morgan (Boveri, 1893 Introductory notes) translated and published Boveri's earlier paper. He saw the research and the incumbent questions as "of the utmost importance" and as carrying "forward rapidly our understanding of the most vital phenomena of life." Morgan was not persuaded by the evidence for Boveri's conclusion any more than Wilson would have been. But he embarked on an intense series of experiments to discover what really was happening with isolated egg fragments.

Morgan first tackled the question whether the non-nucleated egg pieces really ever segment. Perhaps it was the case that only pieces with some maternal nuclear material have the capability and that Boveri's assumption otherwise was wrong. In 1894–1895, Morgan had the chance to take a year off from his teaching at Bryn Mawr College and to visit the Stazione at Naples (Allen, 1978). There he examined the question more closely. In fact, he found no evidence that non-nucleated pieces ever segment. Spermatozoa do enter the egg pieces, but nothing further happens (Morgan, 1894). One problem was that Boveri had looked at a large number of pieces at the same time and could not follow the exact situation for each one. Also, he could not follow each living individual throughout the entire process. Morgan, by following in detail what happens to a single piece which he knew by continual visual inspection had no nucleus, could discover Boveri's error. He could supplement the observation of living material with prepared specimens to determine that there was, in fact, no nucleus.

Egg pieces, Morgan showed, may appear to have no nucleus when they really do and could thereby have misled Boveri. Because the egg membrane may break down during the shaking process, if the egg is at an appropriate stage, then the nuclear and chromatin material will be scattered. Small numbers of chromatin granules may enter into pieces that appear to be non-nucleated. By focusing on the chromatin granules rather than on the chromosomes as persistent wholes, Morgan concluded that actually some combination of nuclear and cytoplasmic substance could have (and indeed must have) been present in each case where development of a larval form occurred. Therefore, Boveri's conclusion about the greater importance of the nucleus was not confirmed. As an alternative, Morgan pursued his cautious approach in presenting only "suggestive" or "working" hypotheses, concluding that "A simple mechanical explanation is probably at the root of the matter, but I do not feel warranted in suggesting one" (Morgan, 1894, p. 145).

From related work, Morgan concluded that by the two celled stage and perhaps even earlier, sea urchin eggs are already cytoplasmically differentiated (Morgan 1894, p. 142). Thus, it cannot be the case that the two celled stage remains cytoplasmically isotropic. Yet this is precisely what Driesch had suggested in the conclusion to his famous paper challenging Wilhelm Roux's suggestions about the qualitative mosaic division of cells. Roux had maintained on the basis of his study of frogs' eggs that each cell division parcels out qualitatively differentiated parts of the hereditary nuclear material into each different blastomere. He had stuck one of the first two blastomeres with a hot needle to kill it, then observed the resulting development of a half embryo (Roux 1888). The result is a developmental mosaic, he said, constructed by the acting out of inherited instructions. Roux went on to generate a full-scale mosaic theory of development and to argue for the necessity of experimental manipulations in embryological research (Roux, 1895).

Driesch (1891–1892) initially agreed with Roux and set out to extend Roux's conclusions to sea urchins. He shook fertilized sea urchin eggs apart so that he obtained two isolated blastomeres. Each of these should, following Roux's results, have produced a half embryo. Instead he got two smaller than normal sea urchin larvae. At the two celled stage, Driesch concluded, the blastomeres remain cytoplasmically totipotent and capable of compensating for the missing material (Churchill, 1969). To explore this apparent totipotency further, Driesch (1893) then subjected the eggs to pressure by compressing them between glass plates and rotating the plates 90 degrees through their gravitational field. Still he obtained normal larvae, which had compensated for the changes. Morgan performed the experiments and focused on what happens to the micromeres. On Driesch's account, they should form at the ends of the eggs under pressure. Instead, they formed in the normal position, at the side. The egg had somehow accommodated; it seemed quite capable of acting as an organized whole to interchange and accelerate stages and locations of development, all in order to effect the "proper" outcome (Morgan, 1894, p. 147). Questions about what directs development remained open.

Another set of experiments followed a suggestion by Jacques Loeb (Loeb, 1892) who had been Morgan's colleague at Bryn Mawr for one year and remained his friend and colleague at the MBL (Pauly, 1987). Loeb had discovered that putting sea urchin eggs into seawater, to which an additional two percent of sodium chloride had been added, caused egg segmentation upon the eggs; return to normal seawater. This, even without fertilization. Therefore, the fertilization process could not have been essential for development, and apparently some physiological response to changing physico-chemical conditions stimulated development instead. Morgan liked this suggestion. For Morgan remained an epigenesist who disliked any account of developmental phenomena in terms of inherited, fixed, predeterministic information. Instead, he sought to explain development in terms of internal reactions and reorganizations in response to conditions both inside and outside the egg. As he repeatedly made clear in his writings and lectures, Morgan disliked anything that smacked of preformationism. Because of what he saw as its metaphysical appeal to invisible hypothetical hereditary units, he held such an account as necessarily incapable of providing a properly scientific explanation.

Loeb's techniques held real possibilities for getting at what happens in the course of epigenetic development, Morgan thought. He therefore adopted the procedure himself. He, too, observed the segmentation which followed the return to normal seawater. "That this segmentation corresponds in any way to the normal stages," however, he "could not verify as the process seemed to me too irregular" (Morgan, 1894, p. 149). Once again, he remained cautious about drawing theoretical conclusions from the empirical data. It remained to be discovered with further studies just what the results meant.

In a further set of experiments, he fertilized eggs of *Asteria Forbesii* with *Arbacia* sperm. While expecting not to obtain any results with such a hybrid, he nonetheless thought it might provide further insight into Boveri's earlier experiments on hybrids. It might illuminate the relative contributions of different parents, for example. In fact, some embryos did develop. They differed from both parental species in both segmentation timing and size and suggested that neither nucleus nor cytoplasm had prevailed. Once again, however, the significance of the results remained to be discovered (Morgan, 1894, p. 152).

All these lines of study Morgan pursued in Naples, following each in various ways during the first half of 1895. He asked a series of questions of his experimental larvae produced from isolated blastomeres. What happens when the isolated blastomeres rejoin after they are shaken apart; how does that affect the result in size and sequence of developmental stages? (Morgan, 1895) How many cells do the half embryos which result from isolated blastomeres have in comparison with the whole, normal cases; is the number the same, as Driesch said, or roughly half, as Wilson maintained? (Morgan, 1895a) Does the abnormal embryo invaginate and proceed otherwise just like normal, or alternately in some adaptive way to respond to the altered circumstances? (Morgan, 1895b) To what extent is the unfertilized egg already organized in some way; does it have organ-forming germ regions, as Wilhelm His had called them, or just vaguely prelocalized areas? (Morgan 1895a, Morgan and Driesch, 1895) What relative roles doe the nucleus and the cytoplasm play in directing development? (Morgan, 1895c)

The pursuit of answers to these questions led Morgan through a series of experiments and several organisms during the mid 1890s. At the same time, he continued to study frog development as well. This work, stimulated by that of Roux and his contemporaries in the 1880s (Eduard Pflüger, Gustav Born, the Hertwigs, and others), culminated in *The Development of the Frog's Egg* in 1897 and summarized the situation to date (Morgan, 1897). The frog studies raised as many questions as they solved, and the key questions about development remained. Yet the study of isolated blastomeres also seemed by 1896 to have reached its limits for Morgan. After a final paper in that year, he turned to other related work, which built on what he had learned.

That final paper, on the "Number of Cells" in *Amphioxus* larvae resulting from 1/2, 1/4 and 1/8 blastomeres (Morgan 1896), reviewed the earlier experimental work and pointed to the varying results obtained. Different organisms, different experimental designs, and different researchers had found different results and had made interpretation difficult. Clearly, however, the experimental cases developed differently from normal cases. Morgan concluded from this fact, in its various manifestations, that cleavage results in some way from the cytoplasmic organization of the egg. And since the experimental cases necessarily have abnormal organization after the disappearance of half the embryonic material, then it makes sense that the development must be different. He was convinced of this on *a priori* grounds, but then confirmed it with careful cell lineage study of the normal and abnormal cases. Invagination and details of cell lineage vary in the experimental cases. Experimentally induced development of isolated blastomeres — which would never occur in normal conditions since the protective coating of the egg keeps the parts together — stimulates a process like regeneration in the remaining material. What is left must act as a new whole and must generate its own responsive and interactive developmental responses to the altered conditions in which it finds itself. It is conditions internal to the embryo itself which direct the coordinated development, Morgan believed.

At the end of his paper, Morgan suggested that something like a wholistic process might be at work in this regenerative activity. As he concluded:

> Despite the physical constraints that have been brought to bear on the developing egg, there remains always a tendency for the egg or part of the egg to reach its prescribed goal despite, even inspite of the modifications impressed on the egg from the outside. This it seems to me is one of the most important results of the experimental work of the last few years...
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> In much the same way, an animal or plant tends in many cases to replace a part of itself that has been lost or injured by external agencies; i.e. we say the whole is regenerated from a part. We can find no chemical or physical explanation for any of these phenomena. It does not make our problem easier to admit the possibility that factors may be present in the ontogeny that are dependent on principles unknown and unrecognized by the chemist and physicist. We call these 'vital' factors and in many of the fundamental problems of Biology, such as development, cell-division, and regeneration these vital processes come to the front. So far as we can see at present the vital factors that control the development do not make use of the many known chemical and physical properties of matter, but it seems to me that it is very rash at present to conclude therefore that the vital processes of living things are necessarily only the complex of known physical and chemical processes (Morgan, 1896, p. 292).

This hypothesis that some coordinating action might be in play led Morgan into an intensive study of regeneration, which peaked in a series of lectures at Columbia and his *Regeneration* of 1901 (Morgan, 1901).

Throughout, as a result of his frog studies and the related work on isolated blastomeres of marine invertebrates, Morgan gradually articulated the convictions about the nature of science more generally and about the study of development more particularly that characterized his work throughout his life (Manier, 1969). He also represented a position characteristic of his American colleagues, which came to dominate developmental biology through the twentieth century (Maienschein, 1987).

In essence, Morgan's view which emerged and gained reinforcement during this time held that the unfertilized egg experiences some cytoplasmic differentiation from its beginning. It has different regions, characterized by the different materials which make up those regions. In addition, the nucleus contains chromosomes which may have something to do with heredity, but they necessarily work under the direction of the cytoplasm. As cleavage begins, the regions of the egg are divided up into different cells, but these separate blastomeres continue to interact and coordinate their activities. After all, the cytoplasm of all the cells is physiologically interconnected in fundamental ways. External factors may act on the egg or the embryo and cause it to react in various ways. But ultimately it is the largely inherited internal structure, and reactions and reorganizations within it, rather than the external forces which directs development. Throughout, the process is epigenetic.

In addition, science is only justified in offering well founded working hypotheses which emerge out of the empirical evidence and which are testable. This all meant that, in Morgan's view, Roux's qualitative nuclear differentiation could not provide an acceptable explanation of development because it depended on the existence of what he regarded as unobservable and non-testable hypothetical units. Nor was Driesch's idea of isotropy acceptable, since it conflicted with available evidence. Something more complex was required.

Exactly what difference organization makes and how the developmental process works, however, remained unclear. We must pursue productive (meaning testable) lines of research and continue to generate legitimately scientific working hypotheses to guide further work, Morgan urged. Probably some version of a mechanical account would prevail, but that might prove incorrect. And just what sort of mechanical account remained unclear at any rate. In order to make progress and to gather answers to well-defined questions, Morgan resolved to look elsewhere: to problems of regeneration, to questions about how such a distinctly recognizable and bivalent characteristic as sex is determined in individual organisms, and to further explorations of the role of nuclei in development.

Morgan's views as generated in the course of this work on sea urchins and related marine invertebrates clearly made a great difference to his own research program. The work of 1893–1895 reoriented his questions toward regeneration and toward assessing the relative roles of individual cells within the whole organism. It moved him to sharpen his questions and his techniques as he recognized that Roux, Driesch, Boveri and others had not established all they thought they had because of imperfections in their research approaches. One must strive to be convincing in one's results and avoid speculative hypotheses not grounded in empirical fact, Morgan came to insist during this time.

This work of Morgan's did not simply affect his own work and his own intellectual development, however. Because he was addressing mainstream questions that provided a focus of interest for other leading biologists of the day, and because he published in the major journals (notably in Roux's *Archiv für Entwickelungsmechanik der Organismen*), this work brought the professor from Bryn Mawr to center stage in European biology. The reactions, and even criticism, he received from his friend Driesch and others helped him to sharpen his experimental approach and to articulate the next step in his research program. In addition, since Morgan read widely in the available literature and responded to current exciting research, a careful look at his work of this time reveals to later readers a great deal about what was happening in experimental embryology generally. The reader also learns what Driesch, Boveri, Wilson, and others were doing. Thus, it is important to recognize Morgan as a first rate invertebrate embryologist, concerned with key questions of heredity and development. He was not a geneticist before 1910. But he did not undergo a sudden conversion at that time from concern with embryology to concern with heredity. Science generally does not work that way. Rather, Morgan's work of the mid 1890s reveals his ongoing and evolving interest in the related problems of heredity and development. The way that he framed his questions later and the way he did his science grew out of deep commitments developed during this period. The primary roots of Morgan's genetics research program lie right here, in the experimental study of isolated blastomeres in marine invertebrates.

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References and Notes

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