Regeneration

Regeneration is a fascinating phenomenon. The fact that many organisms have the capacity to regenerate lost parts and even remake complete copies of themselves is difficult to fathom; so difficult, in fact, that for a very long time people were reluctant to believe regeneration actually took place. It seemed unbelievable that some organisms could regrow lost limbs, organs, and other body parts. If only we could do the same! Unfortunately, our regenerative capacities are limited to hair, nails, and skin, while the liver and a few other tissues display more restricted regenerative abilities. What if we could grow back lost limbs, or damaged organs? This question has inspired many stories, dating back to Greek mythology, wherein Prometheus was doomed to regenerate his liver after it had been devoured by birds. Regeneration has permeated many imaginations; it has appeared in many literary and religious texts, and has also provoked much interest from the scientific community.

There is a long and rich history of chopping up organisms and subjecting them to a variety of environments designed to test the limits of their regenerative capacities. Furthermore, regenerative phenomena have long acted as a window into otherwise hidden developmental processes. By examining the history of regeneration studies we also learn about the history of studying development and gain interesting insights into how and why development has been studied. This essay provides an introduction to the history of regeneration studies by describing some key characters and exploring their experiments to learn more about our interest in organisms that regenerate.

In the early eighteenth century, observations of regeneration generated wide interest throughout the scholarly community. Results seemed outside the realm of the possible. Abraham Trembley's 1744 report of the remarkable regenerative capacities of a polyp inspired him to name the mysterious creature "Hydra" because of its ability to regenerate multiple heads was reminiscent of the Greek mythological beast. Legend has it Voltaire was so skeptical of the Hydra's extraordinary ability to regenerate that he kept a jar full of them on his mantelpiece with the hopes of observing the process himself.

While examining a sample of pond water, Trembley discovered a curious organism he described as a horn-shaped polyp with many arms. Although this polyp had previously been described by well known microscopist Antony Leeuwenhoek, as well as an anonymous English gentleman in 1704, Trembley was unaware of its identity. He embarked on a series of experiments to determine if the creature was an animal or a plant. To determine what kind of thing it was, Trembley cut it in half, and was surprised when each piece regenerated into a complete polyp. This evidence, however, did not entirely convince him of the organism's identity because during his observations he noticed the polyp moved in a step-by-step fashion, much like an inchworm, ultimately persuading him to think of the polyps as animals rather than plants. Although the scientific community applauded Trembley's results, the preformationists remained skeptical because they believed the embryo developed from preexisting parts.

Preformation stated that all generations of creatures were preexisting either in the maternal egg or the male spermatozoon. Many preformationists thought all embryos were established during creation by God, encased within one another, awaiting future development. This idea of preexistence, or emboîtement (encasement), was first articulated in the 1670s with the work of Nicolas Malebranche, Jan Swammerdam, Claude Perrault and others, and was largely in response to the difficulties raised by epigenetic theories of development. The epigenesists argued that each embryo was newly produced and developed gradually from unorganized material. Convictions about the rightness of preformation versus epigenesis have played an important role in scientific investigations about life, resurfacing throughout history.

Trembley's observations seemed to provide support for the epigenesists, yet he preferred fact collecting to theorizing and avoided adding his own interpretation. A religious man, Trembley only once made a brief comment that favored preformationism. His cousin Charles Bonnet, on the other hand, was a vocal, committed preformationist. Bonnet's work on regeneration convinced him preformed germs existed within organisms, ready to regenerate lost parts.

Bonnet worked with many kinds of freshwater worms, including the annelid Lumbriculus. His 1741 publication demonstrated the concept of polarity, showing a worm cut in two developed a new tail at the posterior end of the anterior piece and a new head at the anterior end of the posterior piece. He cut worms into multiple pieces and observed that the new heads always developed on the anterior end of the new piece, although head growth appeared to have limits. Bonnet discovered that if he cut off a newly regenerated head, another would develop up to twelve consecutive times, after which only a bud-like outgrowth would form. This led Bonnet to conclude that the capacity of a part to regenerate was related to the number of times the organism was likely to be injured in natural conditions. Bonnet also noted that regeneration time was related to temperature, occurring much more slowly in cool weather. In addition, pieces that failed to regenerate after a few days would die.

Bonnet seized upon the work of Lazzaro Spallanzani to discredit spontaneous generation. In a letter, Bonnet challenged Spallanzani to begin investigations into regenerative phenomena, hoping he would further Bonnet's own work with flatworms. Spallanzani rose to the challenge. He returned Bonnet's letter with a description of his many investigations of regeneration involving a wide variety of organisms in various conditions. Unfortunately, Spallanzani's work on regeneration was only published as a short preliminary account in his 1768 Prodromo. Perhaps this was because regeneration was only one of his many scientific interests.

Spallanzani performed hundreds of salamander tail amputations and closely examined the interface between the stump and the regenerated tail. He had trouble believing an organized tail could result from a simple outgrowth, and continued to look for evidence in regenerating tadpole and salamander tails to support his preformationist inclinations. Spallanzani openly reported his observations, even those questioning preformationism, once suggesting tail regenerates in tadpoles appeared to be the result of an elongation, a comment surely disturbing to Bonnet but nevertheless failing to persuade him to seriously consider epigenesis.

Spallanzani subjected regenerating organisms to a variety of environmental conditions and measured their effects on the rate of regeneration. He observed that withholding food from tadpoles would prevent them from growing larger, but this deprivation had no effect on their ability to regenerate. If he withheld food from a salamander for two months after amputating one of its legs, he observed the new leg regenerating at the same pace as a salamander fed regularly. He also measured how the size of an amputation affected the regeneration rate, noting that larger pieces regenerated more quickly than smaller ones. For example, he experimented on salamanders by simultaneously amputating the fingers from one leg and the entire leg from the opposite side. Surprisingly, the full leg and the fingers took the same amount of time to regenerate. He noticed an extra toe would occasionally regenerate, and also a toe would sometimes fail to grow back; amputated parts, however, most often regenerated what had been removed. Spallanzani began experiments on snails and slugs and observed that they were able to regenerate an entire head, albeit with greater difficulty than other parts.

The work of these researchers was widely celebrated. At the turn of the twentieth century scientists were optimistic that new methodologies and tools would enable some of these early results to be revisited and better elucidated. However, before jumping ahead to the twentieth century it is valuable to examine the nineteenth century German context, where the conceptual framing and institutionalization of scientific research permeated scientific enterprise on an international scale.

Although Trembley, Bonnet, and Spallanzani were all communicating and publishing work possibly recognized as "experimental embryology," it was not until much later that this process of investigation was christened Entwicklungsmechanik (developmental mechanics). Wilhelm Roux (1850-1924) is often identified as the founder of this new experimental approach to physiology since his journal

Wilhelm Roux's Archiv für Entwicklungsmechanik der Organismen helped advance the Entwicklungsmechanik approach by publishing only experimental work. Roux convinced many that this new approach would revolutionize the study of development and of biology more generally. Roux is also remembered for promoting the mosaic theory of development, according to which each cell contains unique information determining its fate. He supported the theory with his famous study of frog eggs at the two- and four-celled cleavage stages. He discovered that if he injured one of the first-formed blastomeres, the surviving one would develop into a half-embryo, which he interpreted as evidence that each cell developed independently, and that total development was therefore a summation of multiple mosaic developments.

Hans Driesch was inspired by Roux to conduct similar experiments with early cleavage blastomeres, but used a different technique and a different organism: the sea urchin. Whereas Roux had destroyed one early blastomere by pricking it with a hot needle, leaving it attached to the remaining, developing blastomere, Driesch separated the early blastomeres by placing them in calcium-free sea water, a method developed by his friend Curt Herbst. Much to his surprise the separated blastomeres did not develop into half-embryos, but rather developed into normal, albeit smaller whole embryos.

This observation of partial eggs forming whole embryos led Driesch to interpret the egg as a harmonious and equipotential system. Driesch emphasized epigenetic, external factors of development, as opposed to the internal focus of Roux. Experiments on isolated blastomeres were subsequently conducted with amphioxus, jelly-fish, teleost, ascidian, and triton, among other marine organisms, all of which supported the interpretation that early divisions were quantitative, resulting in equivalent, or totipotent blastomeres. These early experiments by Roux and Driesch are often identified as foundational since they inspired great bodies of experimental work. Interestingly, both experiments can be classified as "regeneration" experiments, although Roux referred to the phenomena as "post-generation" since it tested an organism's ability to regenerate damaged or missing parts.

These experiments, along with many others, were published in Wilhelm Roux's Archiv für Entwicklungsmechanik der Organismen. Embryologists were well primed to respond positively to Roux's sentiments about the role of experimentation in science and began sending their best work for publication in his journal. But embryologists were primed for a reason: institutional change had been brewing in the academic community throughout the nineteenth century. This supposedly new "experimental approach" was not actually new at all, beginning over a hundred years earlier when Trembley published his experiments in 1744.

If we consider biology at the beginning of the twentieth century as building on a large body of work in scientific zoology rather than as a distinct revolt from morphology, our perceptions of what counts as developmental biology are broadened. In addition to the experimental biology of Roux and Driesch, it is important to consider numerous other lines of research, such as work in protozoology, animal behavior, biogeography and classification. The rise of experimental approaches in embryology occurred within the rich context of scientific zoology, as well as a long history of studies of regenerative phenomena.

The decades around the turn of the twentieth century have been identified as pivotal for biology. The early twentieth century saw a new interest in genetics and theories of heredity based in physical, structural units. This shift in focus was a conceptual departure from many of the earlier studies in experimental embryology, where the search for tangible, heritable units was not explicit. Genetics seemed to ignore development, particularly the role of the cytoplasm. Embryologists had demonstrated that unlike the nucleus, the cytoplasm changed during development. Many experiments were conducted to investigate the relative roles of the nucleus, the cytoplasm, and the environment during generation, development, and heredity. Studying regenerative phenomena seemed like a good way to begin elucidating some of these causal factors.

Many leading scientists contributed to the growing body of work on regenerative phenomena. They studied a broad range of organisms known for their regenerative capacities at different developmental stages and levels of organization including eggs, blastomeres, gastrulas, protozoa, and algae, as well as plants and various animal phyla. What phenomena did these researchers consider regeneration? There was some confusion and overlap about definitions of generation, post-generation, regeneration and normal development.

Different organisms subjected to similar tests yielded different results. Different species within the same genus responded uniquely. Response further depended on the specific technique employed as well as the developmental stage of the organism. However, rather than these complications causing a crisis, they inspired a sense of forward momentum and excitement. Many thought regenerative phenomena would provide a means to further investigate emerging theories of development.

To better understand the origins and intricacies of regeneration theories at the turn of the twentieth century, it is best to return to the work of Charles Bonnet. Famous for both his theorizing and his experimental work, Bonnet proposed a preformationist explanation of regeneration in 1745. He predicted the existence of pre-formed germs that were not whole germs, like those contained in the reproductive organs, but rather incomplete germs representing the parts of the body beyond the location of the germ. Rather than appearing as miniature copies of their future part, these germs had the capacity to take on their proper form as they absorbed nourishment. Bonnet assigned a purpose to these germs, namely to replace the damaged part.

Bonnet adjusted his theory to account for his observations of regeneration in Lumbriculus. If he cut the worm in two, a new tail and a new head would develop almost anywhere, regardless of where the cut was made, which demanded an explanation of why the head always developed at the anterior end of the piece and the tail at the posterior end. To account for this phenomenon, Bonnet proposed a further assumption—the existence of nourishing fluids in the body that carried the proper formative substances to the head region. He hypothesized that when the worm was cut in two, the nourishing substance accumulated at the anterior edge and then acted on the head germs, causing the development of a new head. Conversely, the tail's nourishing substances would flow toward the posterior edge of the cut surface to activate tail development. Bonnet did not propose an explanation of where or how these nourishing fluids originated, and did not suggest how or why they were able to flow. This interpretation laid the foundation for the botanist Julian Sachs's theory of formative stuffs, which said there was "stuff" in the cytoplasm that was responsible for regenerative phenomena.

In the 1880s Edward Pflüger observed that the early cleavage planes of the developing frog egg were always oriented with respect to gravity regardless of how the egg was turned before the division began. The first two planes were vertical, the third was horizontal, and later cleavages formed the smaller cells in the upper hemisphere. Pflüger theorized that the embryo must be isotropous (equivalent) around the primary meridian axis. However, as Thomas Hunt Morgan pointed out in his 1901 publication Regeneration, studies of regenerating pieces of adult animals such as the planarian suggested there could not be one specific organized plane of symmetry in the organism. If a piece of an adult planarian was cut from one side of the median, a new plane of symmetry would develop, which was also true in the egg. Morgan interpreted this as evidence that the meridian of any egg, or regenerating adult piece, had the possibility of becoming the median and suggested adopting the word "totipotent" rather than isotropous to describe this developmental property. Introducing the term totipotent broadened the discussion to include the possibility of variable cellular potentials, since Morgan defined totipotent cells to be those having the potential to do the same thing, despite physical differences, whereas the word isotropous was synonymous with equivalent.

Pflüger further theorized regeneration always replaced exactly what was lost. He suggested some sort of nutritive material organized at the wounded surface and a molecular force organized the new cells into appropriate tissue layers until the entire missing part was replaced. He further predicted the importance of cell-cell communication and suggested that it was polarization in the protoplasm (cytoplasm) ensuring, for example, that heads developed anteriorly and tails posteriorly. There were some obvious objections to Pflüger's theory. For example, experimental evidence showed regeneration did not always replace exactly what had been lost.

August Weismann, one of the most influential theorists, spurred opposition and set the stage for a great body of experimental work with his publication The Germ Plasm: A Theory of Heredity (1893). His theory generated excitement and controversy since it raised grand, overarching questions and made predictions about what was likely occurring at the microscopic level, thus inspiring vast amounts of experimental work.

Weismann said vital units were present in the germ plasm collectively representing all parts of the individual. These units within the germ plasm were transmitted to offspring and distributed qualitatively at each cell division. Weismann saw Roux's famous developmental mechanics experiment as support for his theory. Roux's experiments on frog eggs led him to theorize that material was separated between the right and left side of the embryo during the first division and the second division then separated the anterior and posterior halves of the embryo. Although Roux's conclusions were limited to the first two divisions of the frog's egg, Weismann extended his hypothesis of qualitative divisions to include all organisms and most divisions. Known as the Roux-Weismann hypothesis of qualitative cell division, the theory posited that the unequal distribution of cellular material during each cell division caused physical differences in the developmental capacities of each cell.

To account for the complexities of development Weismann was aware he needed to explain the processes of differentiation, morphogenesis, and regeneration. He theorized that the germ plasm was arranged to contain serial units with special properties. He called each of these organizational units "biophores," which grouped together formed higher units or "determinants" that acted to specify cell type. Determinants were then grouped to form larger units, the "id." Each id contained a complete set of determinants. A complete set contained all characteristics, or cell types, of the adult organism. Ids were thought to be arranged in a linear series on the chromosome, or "idant." Development coincided with the disintegration of the id into increasingly smaller units until only one kind of determinant remained to determine the fate of the particular cell. Weismann's theory was testable. Regenerative phenomena suggested many testable hypotheses.

Throughout the 1890s Weismann's theory was rejected as too simplistic and at odds with a growing body of experimental results. Driesch's observations that sea urchins separated at the two-cell stage developed into two complete, albeit smaller, larvae, rather than incomplete, half-versions, challenged the Weismann-Roux hypothesis. These results inspired Driesch to propose his theory of the organism as a harmonious equipotential system wherein the fate of a cell was determined by its relation to the organism as a whole.

Despite the growing evidence challenging the Roux-Weismann mosaic theory of development, neither was willing to concede. Roux invoked auxiliary hypotheses to account for the new results. He proposed the existence of "reserve germ plasm," a concept particularly important in the explanation of regenerative phenomena. Some organisms preserved a backup set of determinants not stored in the germ cells but spread throughout the body, particularly in those areas liable to injury and capable of regenerating lost parts.

Weismann's theory of regeneration shared some common ideas with Bonnet's. Weismann proposed that latent cells resided throughout the body, particularly in animals with regenerative capacities and even more so in those parts liable to injury. Weismannn provided a sophisticated account of the location of these pre-formed germs, locating them on chromosomes in the nucleus, but these not the germs responsible for development. Weismann identified regenerative processes as distinctly different from early developmental processes occurring in the egg, an assumption that distinguished his theoretical framework from many, such as that of Morgan or Driesch, who considered studying regenerative phenomena an excellent way to learn about development. Weismann predicted regenerative germ cells, or determinants, would be unique depending on their location in different body layers, each locally endowed with the capacity to regenerate the corresponding distal region, a claim Morgan spent much time refuting.

Weismann further predicted that because a newly regenerated part had the capacity to regenerate again, new latent germ cells were deposited during regeneration throughout the new part. Both Weismann and Bonnet's theories suffered from the same problem: the untestable assumption of invisible pre-formed germs responsible for regeneration. Many found aspects of Weismann's theory problematic, but it nevertheless provided researchers something to which they could respond.

For example, Thomas Hunt Morgan was determined to refute Weismann. He was convinced his work on regenerative phenomena provided clear evidence showing Weismann could not be right. Although best known for his work in genetics with the fruit fly, Drosophila, Morgan's first passion was development. Questions about development inspired him at the beginning of his career and led him to perform extensive experiments on regenerative phenomena. These studies produced over thirty papers, a series of lectures, and a synthetic book titled Regeneration based on these lectures and published in 1901.

Why was Morgan interested in regeneration? Methodologically, he was concerned that many of the experiments designed to investigate development involved highly invasive techniques that were ultimately disruptive to the normal course of development. He questioned the reliability of these results and was therefore skeptical of many accounts of development. He was convinced that studying regeneration provided an opportunity to generate testable hypotheses about basic developmental processes, as regeneration seemed to mimic the natural process of development.

Morgan and Weismann illustrate two distinct, dominant positions guiding and motivating early-twentieth-century studies of regeneration: those like Weismann who thought regeneration was a special case and those like Morgan who saw it as representative of early development and therefore a fundamental property of organisms. Both positions contained many embedded assumptions, and members from each camp spent much time arguing why the other position was implausible and lacked proper experimental justification.

At the turn of the twentieth century many hoped regenerative phenomena would solve the mysteries of development and were ready to follow Morgan's lead. Morgan's scientific path, however, soon moved in other directions. No one blamed Morgan for shifting his focus away from regeneration. In fact, some applauded him. Developmental biologist John Tyler Bonner suggested "he did so because he was unable to devise a micro-theory, and just this is what he did so successfully in the gene theory of heredity" (Bonner, 1952, 4–5). In 1952, more than fifty years after the publication of Regeneration, Bonner highlighted the mysterious nature of regenerative phenomena: "More than anything else, this sensing of the whole, this making of a perfect whole from a small bit of a previous whole, is what seems marvelous to us, so much so that we become, I think, psychologically affected and troubled, and cannot believe that a solution to such a problem would be anything but difficult, if not impossible" (Bonner, 1952, 241).

Unanswered questions in early development eventually enticed Morgan to return to experimental embryology and regenerative phenomena at the end of his career. Morgan spent his final years of research investigating fertilization and early development in ascidians. In Embryology and Genetics Morgan struggled with experimental findings that evaded genetic explanation and devoted an entire chapter to regeneration, as many regenerative phenomena did not fit within a genetic framework. Morgan introduced new terms, such as "compensatory regeneration" and "physiological balance" to help explain processes that genetics could not. As gene theory developed, regenerative phenomena continued to pose challenges and Morgan continued to recognize these challenges as opportunities.

Although the story of regeneration is ongoing, this account finishes in the early twentieth century, after which time many studies of regeneration faded into the background or became engulfed or masked by studies of heredity. By introducing key characters, places, experiments, and concepts, this essay explores the interplay between studies of development and embryology, and studies of regenerative phenomena. Exploring how others investigated regeneration will perhaps offer insight into how we might approach regeneration and development today, for although context changes, many questions remain unchanged.

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