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Ernest Everett Just: Egg and Embryo as Excitable Systems

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ABSTRACT Ernest Everett Just (1883–1941) was an African American embryologist of international standing whose research interests lay in the area of fertilization and early development in marine invertebrates. Perhaps best known for his discovery of the dynamical and structural blocks to polyspermy that sweep over the egg upon fertilization, E. E. Just also was the first to associate cell surface changes with stages of embryonic development. He was deeply familiar with the natural history of the animals whose eggs he studied, and his knowledge of natural settings led him to emphasize the importance of using laboratory conditions that closely match those in nature. Based on more than 30 years of work, he came to believe that it was the cell surface that played the most critical role in development, heredity, and evolution. He promoted a holistic view of cells and organisms in opposition to the gene-centric view that was becoming more prevalent with the rise of genetics, but rejected the vitalism espoused by some biologists of his era, calling instead for "a physics and chemistry in a new dimension …superimposed upon the now known physics and chemistry" to account for biological phenomena. Just's incisive critique of genetic reductionism finds echoes in contemporary multiscale, systems approaches in biology. His speculations on the relationship between developmental and evolutionary mechanisms resonate with today's evolutionary developmental biology. After a brief biographical sketch, this paper outlines and discusses some of Just's scientific contributions, and shows how his ideas remain relevant today. *J. Exp. Zool. (Mol. Dev. Evol.) 322B:191–201, 2014.* © 2014 Wiley Periodicals, Inc.

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For more than 40 years, the work of Ernest Everett Just (1883–1941) (Fig. 1), an African American embryologist known for his pioneering studies of fertilization and early development in marine invertebrates, lay buried in the scientific literature, largely forgotten and invisible to the world of biology. Then, in 1983, Kenneth R. Manning, a historian of science at the Massachusetts Institute of Technology, wrote a biography of Just, *Black Apollo of Science* (Manning, '83), that garnered attention for both author and subject. Stephen Jay Gould wrote a favorable review of Manning's book (Gould, '88 ['83]), and provided some reflections of his own about Just in a column in *Natural History* magazine (Gould, '85). Scott Gilbert, in the inaugural edition of his popular textbook *Developmental Biology*, cited Just (Gilbert, '85); this was likely the first technical citation of Just's work since the early

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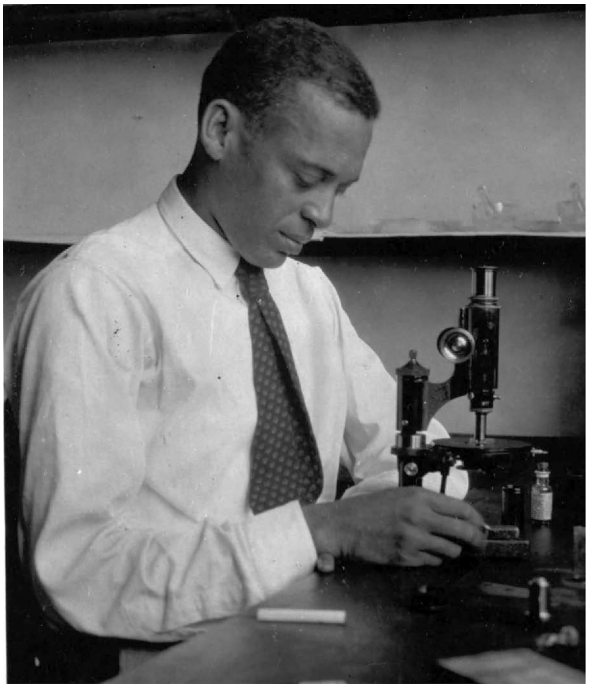

Figure 1. E. E. Just, circa 1925. Courtesy of the Marine Biological Laboratory Archives. Available at: http://hpsrepository.asu.edu/handle/10776/2602.

1940s. Two decades later, in an essay commemorating the 125th anniversary of Just's birth, the renowned geneticist James F. Crow referred to him as "one of the greatest biologists of early 20th century" (Crow, 2008), attesting to the high esteem in which he is now held by many of his latter-day peers.

In the intervening years a number of annual symposia or lecture series were established in Just's honor: at the Medical University of South Carolina in Charleston, the city of his birth, Dartmouth College, his undergraduate institution, and the University of Chicago, where he earned his Ph.D. Since 1994, an award has been given every year in Just's name by the American Society for Cell Biology, and its recipient presents a plenary lecture at the Society's annual meeting. In 1996, a stamp was issued by the U.S. Postal Service in Just's honor and, in 2008, a symposium on the influence of his work in current biological research was held at Howard University, where he taught (with interruptions for research activities) from 1907 until his death. This last event led to the publication of a special issue of the journal *Molecular Reproduction and Development* (see Byrnes, 2009a). Nonetheless, despite these recent and ongoing tributes, the life and work of E. E. Just remain largely unknown to most biologists. Our goal in the present paper is to help rectify this situation by describing Just's contributions and by showing how his ideas were prescient and still remain important today.

BIOGRAPHICAL OVERVIEW[1]

Early Days

E. E. Just was born in Charleston, South Carolina, on August 14, 1883, to Charles Frazier Just and Mary Mathews Just. The oldest of three surviving children—two of his siblings had died earlier of cholera and diphtheria—Ernest and his family moved to James Island, one of the sea islands off the South Carolina coast near Charleston, after the death of his father when he was 4 years old.

Just was home-schooled by his mother until age 13, when he left to go to the Colored Normal Industrial Agricultural and Mechanical College, a teacher-training school in Orangeburg, South Carolina. Three years later he graduated with a license to teach, but he and his mother had grander plans for his future. Seeing an advertisement in a magazine, Mary decided to send him, in 1900, to Kimball Union Academy (KUA), a boarding school in Meriden, New Hampshire.

In 1903, Just graduated from KUA and entered Dartmouth College in Hanover, New Hampshire. He had intended to major in the classics at Dartmouth, but in his sophomore year he switched to biology, concentrating on zoology. He took on an independent research project under William Patten, a biology professor known for his book on the embryology of the limpet, who was at the time interested in the anatomy and evolution of frogs and other vertebrates. So pleased was Patten with Just's work that he acknowledged him in a footnote in his book The Evolution of Vertebrates and their Kin (Patten, '12). Just graduated from Dartmouth in 1907 as an academically elite Rufus Choate scholar. He held the distinction of being the only one to graduate *magna cum laude* that year (there were no *summa cum laude* graduates).

Howard University and Woods Hole

After leaving Dartmouth, Just straightaway went to Howard University in Washington, DC, the premier institution of higher education for African Americans, where he accepted a faculty position in English. In 1909 he moved to the Biology Department. Quickly climbing the academic ladder, in 1912 he was appointed both Professor of Biology in the College of Arts and Sciences and Professor of Physiology in the College of Medicine. With support from the Rosenwald Fund, he established a graduate program in zoology, and served as the first chairman of the Zoology Department.

Given the interest Just had shown in research as a student at Dartmouth, it is not surprising that, soon after he joined the Biology Department at Howard, he began to look for new opportunities to conduct research. Through a contact established by Patten, in the summer of 1909 Just began working at the Marine Biological Laboratory (MBL) at Woods Hole, Massachusetts, under the supervision of embryologist Frank R. Lillie, who

[1]All of the information in this overview comes from Manning's biography of Just, *Black Apollo of Science: The Life of Ernest Everett Just* (Manning, '83).

was both the director of the MBL and the head of the Department of Zoology at the University of Chicago. At first, Just worked mainly as an apprentice under Lillie. Despite his junior status, he succeeded in producing an important paper that was widely cited and which would influence the whole course of his scientific career. Titled "The relation of the first cleavage plane to the entrance point of the sperm" (Just, '12), the paper reported the discovery that the plane of first cleavage of the egg of the marine worm *Nereis limbata* is dependent on where on the surface of the egg the sperm enters. One conclusion from the paper was that the whole *Nereis* egg surface prior to fertilization has equal potential for accepting a fertilizing spermatozoon. As Gould would write in his essay on Just, this discovery helped to shape Just's later explicit holism, his epigenetic view that the embryo's developmental trajectory is not pre-set from the beginning—as the preformationists believed—but is contingent on external factors (Gould, '85). In addition to this work, Just also published, in 1913 and 1914, two papers on the breeding habits and egg-laying behaviors of the marine worms *N. limbata* and *Platynereis megalops* (Lillie and Just, '13; Just, '14). In 1915, he enrolled in the Ph.D. program in zoology at the University of Chicago, with Lillie as his major advisor. The research he had done for the 1913 and 1914 papers formed the basis of his dissertation; all he had to do was meet coursework and residency requirements, which he did in Chicago during the 1915–1916 academic year, receiving the Ph.D. degree in 1916.

By the late 1920s, Just was viewed as "a first rate experimentalist" and was broadly respected (Manning, '83, p. 110). He published many of his experimental methods for the handling eggs and embryos in articles in the MBL journal *The Collecting Net*. Eventually these were brought together in the form of a book titled *Basic Methods for Experiments on Eggs of Marine Animals* (Just, '39b), which is available online in somewhat modified form (Cohen, '99).

Europe

Ironically, it was Just's success as an experimentalist at Woods Hole that catalyzed his departure. By 1928, he was growing weary of the many requests for help and advice engendered by his knowledge of marine eggs and embryos and his expertise in handling them. But this changed beginning in 1929 when he made his first transatlantic voyage. It was to Naples, to the *Stazione Zoologica*, where he pursued research on marine animals. The experiences he had in Europe, and the acceptance he received, gave Just a new level of confidence and led him to broaden his scientific perspective. Altogether, Just made ten trips abroad: several were to Naples, several were to Berlin, one was to Switzerland, two to Paris, and the last was to a marine laboratory in a remote fishing village, Roscoff, on the rugged coast of Brittany in France.

Just's first excursion to Germany, which took place on the heels of the one to Naples, was to the *Kaiser-Wilhelm-Institut für Biologie* (KWI) in Berlin-Dahlem. The invitation, by Max Hartmann, a famous protozoologist, was to work for six months, from January to June 1930, at the KWI. The Germans were impressed with his work, and they were eager to see if his ideas about the active role of the egg cell cortex could be extended to the unicellular freshwater protozoan *Amoeba proteus*. Just returned to Germany to continue this line of work in the summer of 1931, and again in 1932.

In 1933 and 1934, Just returned to Europe, mainly to Naples, to work on the manuscript for his book *The Biology of the Cell Surface*, going back to Europe once again, in 1935, to Switzerland this time, to continue his writing. In 1937, he travelled to Paris with the intention of finding some arrangement that would allow him to stay in Europe and continue his research, but was unable to secure funding or obtain a position. Finally, in the spring of 1938, he returned to Paris, where he worked at the Sorbonne, putting the finishing touches on his cell surface book. Later that year, he returned again to France, finally settling at *Station Biologique de Roscoff* in Brittany.

Final Days

Just's intention was to stay at Roscoff indefinitely, despite the rustic conditions, in order to continue his marine research. He had been unsuccessful in securing research funding from foundations in the United States, and likewise had been unable to obtain retirement pay from Howard University. Penniless, he was nonetheless determined to go on. In late 1939, the Nazis invaded France, and by the summer of 1940 they had reached Paris and its surrounds, including Roscoff. Just was captured and briefly interned at a prisoners' camp. Only with the help of the father of his new second wife, Hedwig Schnetzler, a German citizen, as well as the U.S. State Department, was he released and allowed to travel, with Hedwig, to the United States. Hedwig settled in East Orange, New Jersey, with relatives. But Just, needing to provide for his new family (Hedwig was pregnant), returned to teach and pursue research at Howard. Finally, in the fall of 1941, just as the semester was swinging into full gear, he became violently ill; it turned out that he had pancreatic cancer. On October 27th of that year, Just died. He was 58.

HISTORICAL CONTEXT

According to developmental biologist and science historian Scott Gilbert, American biology in the early 1900s was "a house divided" (Gilbert, '88). On one side were the classical embryologists; on the other were the newly-minted geneticists. The embryologists had an "egalitarian" view of the cell; they believed that all of the parts of the cell work together as one harmonious whole, with no part dominant over any other. The geneticists, on the other hand, believed that genes in the nucleus controlled what happened in the cell. A leader among these early geneticists was Thomas Hunt Morgan, the future Nobel laureate and author of the gene theory who, based on genetic experiments using the fruit fly *Drosophila* *melanogaster*, proposed that genes were arrayed in linear fashion on chromosomes in the nucleus.

Significantly, before 1910, Morgan was an embryologist in the classical mold. Like other embryologists, he believed that the whole structure of the egg cell, including (especially) the cytoplasm, was important for development and inheritance. But then he switched and began advocating for nuclear dominance over cytoplasm. By doing this, Gilbert argues, Morgan "drove a wedge" into embryology, splitting it in two. By the time Morgan had written his highly influential book, *The Theory of the Gene*, he was arguing for a complete separation of the two fields. Genetics would be concerned with the inheritance of traits, embryology with the expression of traits during development. Morgan maintained this position when he wrote his 1934 book *Genetics and Embryology.* He believed that genetics and embryology intersected at only a single point: the study of the *expression* of genes in the cytoplasm.

Gilbert describes what transpired at the hands of Morgan as a kind of "supersessionism" akin to what happens when a religious sect "claims superiority to the original sect from which it emerged" (Gilbert, '98). He argues that Morgan and the geneticists "... employed a rhetorical strategy ... to distance themselves from embryology, proclaim their science to be superior ... and redefine embryology in terms of the new genetic discipline" (Gilbert, '98). Garland Allen describes the situation similarly. He writes:

> A consequence of this sort of analytical dissection [of the organism, which was seen by the geneticists as the sum of its parts] was the creation of a new dialectic at the disciplinary level: the dissociation of the holistic process of reproduction into two separate processes: transmission (genetics) and development (embryology). That this split occurred at the professional as well as the conceptual level highlights the pervasive way in which the mechanistic materialist approach dominated the organization of the "new biology" (Allen, 2007, p. 150).

Both Gilbert and Allen emphasize that the rift between genetics and embryology that was created has only begun to close with the rise of evolutionary developmental biology (Evo-Devo) in the late twentieth-to-early twenty-first centuries (Gilbert, 2001; Allen, 2007).

The separation that Morgan helped to orchestrate was acceptable to the embryologists for a while. As long as the geneticists stayed on their own turf—inside the nucleus—things were tolerable. Besides, embryologists could argue that the geneticists still had not explained the critical problem of differentiation: how an unchanging set of genes could direct the very different sets of events occurring in different types of cells and at different stages of development. But the "uneasy truce" was broken when geneticists, now interested in looking at gene expression, began to venture outside of the nuclear envelope into *embryologist* territory—the cytoplasm. With this move, the geneticists essentially "laid claim to embryology" (Gilbert, '88). In response, some in the embryologist camp sought to reconcile the two disciplines in such a way that the importance of the cytoplasm would be preserved.

THE ROLES OF NUCLEUS AND CYTOPLASM IN DEVELOPMENT

One of the people who attempted to close the widening gap between embryology and the newly-emerging field of genetics was E. E. Just. At the 1935 meeting of the American Society of Zoologists in Princeton, New Jersey, Just challenged Morgan's nucleocentric view. He also offered his own explanation for how an embryo's cells can undergo differentiation despite their having identical sets of chromosomes during development. It was an explanation that was explicitly cytoplasm-centered. Calling it the theory of "genetic restriction" or "potency sequestration," Just proposed that the role of the nucleus during embryonic cleavage and differentiation was to sequester a specific set of factors from the cytoplasm, leaving the others free to determine the characteristics of one or another type of cell (Just, '39a).[2] Thus, in Just's model the nucleus and its chromosomes play a role that is distinctly secondary to that of factors in the cytoplasm. Yet Just also emphasized that the interaction was not one-way: "The cell is a unit: the nucleus influences the plasma, the plasma the nucleus. The cell reacts as a whole. Sharply to divorce these two constituents of the protoplast is to make them abstractions" (Just, '32).

Although Just's theory was ultimately (though not in his lifetime) experimentally disconfirmed, he was nonetheless right about some things: his proposed mechanism required active organizing processes at early embryonic stages in order to work, and he was correct in his identification of some of these. They were mesoscale physical effects that had no explanation in terms of the physics of the day. These processes, which Just had observed and measured in the egg's cortical cytoplasm, are now widely

[2]In order to set the stage for the unveiling of his theory of "genetic restriction" or "potency sequestration," Just first has to present an argument against the viability of a rival theory, that of "embryonic segregation," which had been put forth by several early twentieth century embryologists, including E. G. Conklin and Just's own mentor F. R. Lillie (Just, '36). These embryologists, in an attempt to explain the problem of differentiation, that is, how the cells of the embryo can undergo regional differentiation despite having an identical set of chromosomes, had resorted to the notion that a selective distribution of ooplasmic determinants during cleavage ("segregation") was involved. Just begins by chiding these avowed epigeneticists for adopting a back-door preformationism, and then proceeds to undermine their argument with (empirically informed) logic reminiscent of the best medieval Scholastics. Most decisively, the segregation theory was inconsistent with the maintenance of the totipotency of blastomeres in many species throughout multiple cleavage events. Just's fearless integrity is exemplified by his willingness to take on, intellectually, even one of his most avid supporters (see Manning, '83, p. 277).

recognized and studied: contraction (e.g., cortical flows and related viscoelastic effects) and conduction (calcium ion oscillations and transients) (reviewed in Newman, 2009). Consideration of such phenomena, which lay outside the frameworks of both the embryologists and the geneticists among Just's contemporaries, allowed him to see past his peers to discern the outlines of the next century's biology.

Throughout the 1940s and into the early 1960s, that is, between the time that differential gene action was recognized as the means by which inherited chromosomal determinants contributed to the organism's phenotype and the time of the deciphering of the genetic code and the mechanism of protein synthesis, there were several speculative models advanced concerning the genotype–cellular phenotype relationship. Lacking our present knowledge, some of these models placed more emphasis on the purported agency of the genes; others placed more emphasis on the presumed active role of the cytoplasm (Sapp, '87). The notion of the cell as a hierarchy, with the implied analogies of the cell to the human social order, was not lost on some investigators of the era. For example, Joshua Lederberg—a pioneer in the study of extrachromosomal genetic elements in bacteria—anticipated how the study of such elements could eventually extend beyond cell and individual organism into the human realm when he wrote: "At each level of interaction pathological deviations can be found, ranging from sick plastids and malignant tumors ... to human serfdom" (Lederberg quoted in Sapp, '87, p. 120).

Given the increasing recognition of the multileveled nature of development and physiological regulation (Forgacs and Newman, 2005; Gorelick and Laubichler, 2008; Bonduriansky, 2012; Holland and Rakyan, 2013), we can see that Just's egalitarian view of the reciprocal interaction between nuclear and cytoplasmic factors was arguably more biologically valid than the authoritarian notion popular during the rise of molecular genetics, encapsulated in the apothegm of the physicist Erwin Schrödinger: "The chromosome structures are at the same time instrumental in bringing about the development they foreshadow. They are law-code and executive power or,—to use another simile, they are architect's plan and builder's craft—in one" (Schrödinger, '44, p. 22).

SCIENTIFIC CONTRIBUTIONS: OVERVIEW

E. E. Just was a prolific researcher and author. He wrote over seventy papers and two books (*The Biology of the Cell Surface* and his methods manual) over the course of his 32 year scientific career, from 1909 to 1941. He published in reputable journals, including some German ones (e.g., *Protoplasma*, *Naturwissenschaften*). His contributions were in a number of areas.

First, Just observed and sought to replicate developmental processes under natural conditions. He carefully observed the breeding habits of the animals whose eggs he studied. In many ways he was a naturalist. Indeed, some of his earliest work at Woods Hole was on the breeding behavior of the marine worms *N. limbata* and *P. megalops*. Because of his deep knowledge of the natural settings in which fertilization occurs, he was able to devise laboratory experiments whose conditions closely matched those in nature. As a result, he was highly successful in getting eggs to fertilize and embryos to develop normally. In fact, he was famous at Woods Hole and beyond for his uncanny ability to do this. He developed what he called "indices of normal development," mainly based on the quality and timing of fertilization envelope separation. These allowed him to predict with a high degree of certainty whether or not a given egg would develop normally. Moreover, Just's insistence on the normality of the egg under study, combined with his deep knowledge of natural settings and his philosophical gravitation toward what is known as *organicism* (or materialistic holism, which emphasizes the holistic integrity of living systems (Gilbert and Sarkar, 2000)), place him squarely in the company of today's ecological developmental (Eco-Devo) biologists (Byrnes and Eckberg, 2006), who focus on development as it occurs in nature (see Gilbert, 2001; Gilbert and Epel, 2009).

Second, using only a light microscope Just was able to study the structure of the egg cell cortex, or ectoplasm, and closely observe how it changed during the process of fertilization. He discovered that a "wave of negativity" sweeps over the egg during fertilization; it is a wave of ectoplasmic structural change that blocks additional sperm from binding to the egg surface, and it is associated with what is known as the fast block to polyspermy. He distinguished this fast wave from the different, slower wave of fertilization membrane separation. He noted that the rapid wave of negativity preceded the slower one defined by membrane separation.

Third, Just was able to show that Frank Lillie's theory of fertilization known as the "fertilizin" theory (Lillie, '13, '14)—which proposed that fertilization resulted from an interaction between sperm and egg via cell surface macromolecules and in this way extended Emil Fischer's principle of stereocomplementarity from the molecular realm to the cellular one (see Gilbert and Greenberg, '84)—held true for a range of marine invertebrates, including *Nereis*, *Arbacia*, *Echinarachnius* and others. Lillie had found that a diffusible substance given off by eggs that are in the fertilizable state, which he called fertilizin, caused agglutination of sperm and was necessary for the fertilization process. Lillie proposed that fertilizin was the middle piece in a three-part interaction involving sperm, fertilizin, and egg (Lillie, '13, '14). In 1930, Just wrote a long defense of the theory (Just, '30), which was being criticized for borrowing terminology from Paul Ehrlich's side chain theory of immunity (Ehrlich, 1897), which had fallen into disfavor at the time.

Fourth, he studied the effects of various factors on the artificial parthenogenetic activation of eggs. His results here conflicted with those of Jacques Loeb, whom Just felt (and others acknowledged) had used poor experimental technique. Just also took issue with Loeb's overt reductionism, his belief that the goal of biology was to engineer life to suit human purposes. He challenged Loeb both in his publications and in his presentations at scientific conferences.

Fifth and finally, Just made important contributions in the area of embryo morphogenesis. He was the first to show that the adhesiveness of the cells in the developing embryo—their ability to stick together—is exquisitely dependent on developmental stage, so that if one cell is out of synchrony with the others, it can no longer adhere to them and development cannot proceed normally (Just, '31). These findings of Just's informed Johannes Holtfreter's concept of *tissue affinity* (Holtfreter, '39; see Grunwald, 2013, for a discussion of Just's contributions to the field of cell adhesion research). There is also evidence that Just's work contributed to Holtfreter's concept of *autoneuralization*, or autoinduction (Holtfreter, '47, '48, '91), in which a variety of nonspecific agents can induce the formation of neural tissue in the amphibian gastrula ectoderm.

The discussion below will focus in more detail on Just's contributions in three areas: fertilization and experimental parthenogenesis; the role of the cell surface in development; and the evolutionary dimensions of development. His contributions in the areas of Eco-Devo and embryo morphogenesis have been discussed elsewhere (Byrnes and Eckberg, 2006; Byrnes, 2009b), and will not be elaborated upon further here.

FERTILIZATION: THE FAST AND SLOW BLOCKS TO POLYSPERMY

In *The Biology of the Cell Surface*, Just wrote that: "Before the actual separation of the vitelline membrane, an ectoplasmic change beginning at the point of sperm-entry sweeps over the egg which immunizes it to other spermatozoa ..." (Just, '39a, p. 107). He described the site of sperm entry as "negative" to the entry of additional spermatozoa, and all other points along the surface of the egg as initially "positive." He noted that the "negativity" moved around the egg in a wavelike manner "at a rate which varies with that at which the sperm head disappears within the ectoplasm" (p. 107). He observed that the wave of negativity precedes the actual beginning of fertilization envelope elevation, and that "before the membrane begins to separate at the site of sperm-entry, other spermatozoa can no longer enter at any point on the egg" (p. 107).

Using dilute seawater, Just was able to correlate the wave of instability that swept around the egg with structural changes that occurred at the egg cell surface. In a particularly elegant and innovative experiment (Just, '21), using a light microscope, he was able to expose *Echinarachnius parma* eggs to dilute seawater at precisely timed intervals after insemination and measure the position of fertilization membrane (envelope) separation at that time (Fig. 2). He was able to observe an "outflow of cytoplasm" and a movement of droplets from the cytoplasm into the perivitelline space at the point on the surface where the membrane was just beginning to separate (the "point of susceptibility"). He noticed that prior to membrane liftoff, the egg was resistant to the cytolytic effect of dilute sea water, and that immediately after liftoff, when the fertilization envelope was fully formed all around the egg, it was once again resistant. Thus, there was a momentary period of "susceptibility" that was bracketed in time by periods of "resistance" to the effect of the dilute sea water. From these experiments, Just was able to show that the fertilization envelope forms as a result of a wave of structural change that moves around the egg cell surface, from the point of sperm entry to the other side. We now know that this wave was in fact the wave of cortical granule exocytosis.

Here, and in similar studies, Just was inferring and documenting what is known as the *fast block to polyspermy*, a phenomenon that later was shown to be due to a shift in egg cell membrane potential (Jaffe, '76). However, it is important to note that Just was not directly observing a change in membrane potential, which is almost instantaneous over the egg's entire surface. Rather, he was likely observing the cortical structural changes that accompany the release of calcium that precedes fertilization envelope liftoff. The salient point is that Just was aware that a rapid block existed, and that it was different from the slower block, a mechanical one due to fertilization envelope separation, which had been discovered by Fol several decades earlier (Fol, 1879).

Regarding this *slow block*, Just wrote: "As the membrane lifts off, it carries away any supernumerary sperm whose activity is in contrast to the immobilized sperm previously engulfed by the egg" (Just, '19). He also observed that "membrane" elevation at a given point on the surface prevented sperm entry not only at that point, but "at any point on the egg surface" (Just, '19). Just is thus credited with inferring the existence of the fast block and further elucidating the slow block to polyspermy.

PARTHENOGENESIS AND THE CONFLICT WITH JACQUES LOEB

Jacques Loeb was a prominent biologist at the Rockefeller Institute for Medical Research in New York City who pursued research at Woods Hole in the summer and who, though considerably older, was a contemporary of Just's. Just and Loeb knew each other quite well, and at first they were on good terms. Loeb was active as a political liberal in helping to promote "racial uplift" for blacks; he wrote a number of articles criticizing "scientific racism," the belief that blacks are inherently (biologically) inferior to whites. Furthermore, it was largely through Loeb's recommendation that Just was awarded the first Spingarn medal from the National Association for the Advancement of Colored People (NAACP), in 1915. But their relationship took a sharp turn for the worse when Just began criticizing Loeb's work.

Parthenogenesis is defined as the activation of egg development in the absence of sperm. It occurs naturally in some organisms, such as species of aphids, water fleas, honey bees, and lizards, to name a few. Parthenogenetic development is initiated by a nonspecific trigger that acts at the egg surface (its cortex, or ectoplasm). Loeb had shown in the late 1800s that he could induce

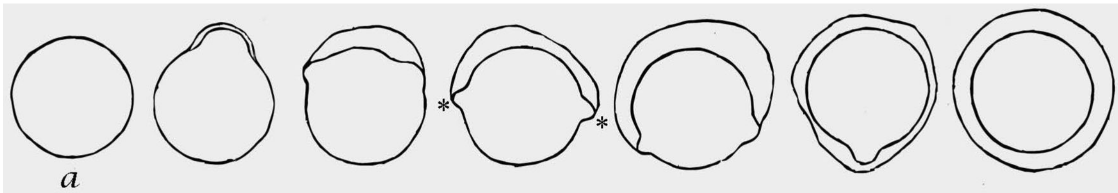

Figure 2. Movement of the "Point of Susceptibility" around the *Echinarachnius* Egg Cell Surface. This experiment demonstrated that the fertilization envelope forms as a result of a wave of structural change that moves around the egg surface. a: Fertilized egg in which membrane-separation has not yet begun. ***Asterisks*** added to indicate the positions of the "points of susceptibility" at one of the time points used in the study (Just, '39a, p. 110).

sea urchin eggs to develop parthenogenetically by treating them with sea water that had a higher-than-normal concentration of salt (hypertonic sea water). However, Loeb noticed that irregularities arose during the development of the embryos, and only a fraction of them developed to the larval stage. After a number of failed attempts—some notoriously involving sloppy experimental technique—Loeb hit upon a two-step method that seemed to work much better than the original (osmotic) one. Called the "superficial cytolysis-corrective factor" method, it involved treating the eggs first with butyric acid and then with hypertonic sea water. Just wryly explained the method in this manner: "the fatty acid causes 'superficial cytolysis' and the hypertonic sea water treatment following 'saves' the egg from its impending death" (Just, '39a, p. 229).

Around this same time, working with Lillie, Just also began to study parthenogenesis. Using a variety of marine invertebrate eggs (*Nereis*, *Arbacia*, *Echinarachnius*), he found that different experimental conditions—hypertonic sea water, hypotonic sea water, UV light, higher temperatures—could all cause parthenogenetic activation, albeit with varying degrees of effectiveness. He also carefully took apart Loeb's two-step method, and obtained results which did not agree with Loeb's. He found that (i) the order of the treatment was completely inconsequential, (ii) only one of the factors—either the fatty acid or the hypertonic seawater—was sufficient to induce parthenogenesis, and (iii) the cytolytic effect of the butyric acid was due to the fact that the eggs were being overexposed to it. "Thus, the sequence in the treatment so strongly demanded by ... the theory," he wrote, "not only is not supported by fact but is contradicted by it" (Just, '39a, pp. 229–230). Just presented his results at the joint meeting of the American Association for the Advancement of Science (AAAS) and the American Society of Zoologists held in Chicago in 1920, which Loeb also attended.

Much to Just's chagrin, Loeb's parthenogenetic achievements had been hailed in the popular press as a major milestone for science. They were being considered as the creation of life, a kind of "immaculate conception" (Loeb cited in Pauly, '87). Indeed, Loeb had become a celebrity. The press followed his research eagerly. In 1902, he was profiled in McClure's magazine (see Pauly, '87). In the magazine article, Loeb stated that his purpose in doing the work on parthenogenesis was to "go to the bottom of things. I wanted to take life in my hands and play with it. ...I wanted to handle it in my laboratory as I would any other chemical reaction—to start it, stop it, study it under every condition, to direct it at my will!" (Loeb quoted in Pauly, '87). Pauly ('87) writes that "Loeb discovered artificial parthenogenesis because he was seeking to control life on its most basic level; it was a natural consequence of his conviction that biology was and should be an engineering science concerned with transforming the natural order." Here Loeb appears to share traits in common with some contemporary bio-entrepreneurs, such as J. Craig Venter, who recently stated that "[n]ot too many things excite my imagination as trying to design organisms—[or] even people, for long term space flight, and perhaps colonization of other worlds" (Venter, 2011).

One can begin to understand why Just may have been motivated to criticize Loeb. Recall that Just was the experimentalist who wanted to "here slightly exaggerate, there lightly fret the tones out of which the harmony of the living state arises" (Just, '39a, p. 30). He felt that "[a] cell is never a tool. Nor is it an instrument on which to whet one's physics and chemistry" (Just, '39a, p. 28).[3] On the other hand, Loeb's goal was to use whatever blunt instrument he could find to engineer life. That is why he tried different approaches until he found one that worked, or so he thought. But Just's response to Loeb was to carefully and methodically—using excellent laboratory technique—do his own set of experiments. And when he had finished, Loeb's double treatment method lay in shambles.

Based on his work on parthenogenesis, Just was able to conclude that "...the egg-cell like many other living cell—nerve or

[3]In this attitude Just also has contemporary counterparts. The late evolutionary biologist Carl Woese, for example, wrote that "a society that permits biology to become an engineering discipline, that allows science to slip into the role of changing the living world without trying to understand it, is a danger to itself" (Woese, 2004).

muscle, for example, possesses independent irritability [exemplifying what present-day physicists refer to as "excitable media" (Levine and Ben-Jacob, 2004)]. It has full capacity for development. Neither spermatozoa nor experimental means furnish the egg with one or more substances without which the initiation of development would be impossible" (Just, '39a, p. 237). He concluded that "the egg as a living cell is self-acting, self-regulating and self-realizing—an independently irritable system" (Just, '39a, p. 238). Whereas Loeb was intent on trying to prove that an external agent was the primary cause of development, Just was led by his experimental data to recognize that living matter had the fundamental property of "independent irritability," its ability to respond in a productive fashion to diverse stimuli (Just, '39a, p. 237).

THE CELL SURFACE AND THE REACTIVE EGG

Just's study of the phenomena occurring at the surface of the egg during fertilization and parthenogenesis inexorably led him to believe that it was the cell surface, specifically the ectoplasm, which was the most critical player in development. By virtue of its excitability the cell surface would mediate interactions with the environment and also mediate cell–cell interactions. Indeed, it was this inherent property of the cell surface that most caught Just's attention. He bemoaned the fact that others had overlooked it in their haste to provide a mechanistic explanation for the early events of development. He wrote: "Here lay at the same time the possibilities and the failure of the work on experimental parthenogenesis. Every single investigator who erred in 'proving' an external agent (or agents) to be the cause of development neglected an opportunity to extend our knowledge concerning that fundamental manifestation of living matter, its independent irritability" (Just, '39a, p. 237). Thus, Just's conflict with Loeb was of the most basic kind: he fundamentally disagreed with Loeb regarding what their experiments (to the extent that he could trust those of Loeb) were revealing about the workings of the cell and of nature.

ORIGINS AND EVOLUTION OF DEVELOPMENT

The origin of living organisms and their capacity to undergo developmental change was a constant theme in Just's work. "[H]ow out of non-living matter did life arise?" he asked in a 1933 paper titled "Cortical cytoplasm and evolution" (Just, '33). Although vitalism, the doctrine that living systems are governed by principles different from the physical ones pertaining to non-living systems, retained some respectability among scientists and philosophers in the pre-DNA era (e.g., Sinnott, '50), Just explicitly rejected this notion. Significantly, however, this did not lead him to a naïve mechanistic view. It is worth quoting him at length to appreciate the subtlety of his approach to these questions:

> In biology the term, mechanistic, is used as the antipode of vitalistic. Since practise has legitimatized this usage, there may be little reason to quarrel with it. ... The term, non-mechanistic, by no means implies vitalism. Not every physicist who opposes the mechanistic conception deems it necessary to support a non-physical, super-natural concept. Rather, he holds that the behavior of the ultimate particles of matter is not rigidly determined, perfectly predictable. ... Physico-chemical analysis into ultimate particles and the hypotheses derived from such work establish the fact of the existence of similarities between living and non-living. By virtue of its peculiar organization in space as well as in time, however, the living thing occupies a level in the natural world above that of chemical compounds. From this organization spring those characteristics by which we commonly distinguish a living thing from a non-living. ...Having agreed that there exists no chemistry peculiar to living things and that physical properties are possessed by the living and by the non-living as well, we have remaining the task of evaluating the differences (Just, '39a, p. 14).

Recognizing that "physics has grown beyond 'classical physics"' (Just, '39a, p. 14), he called for "a physics and chemistry in a new dimension ... superimposed upon the now known physics and chemistry" in order to study life, which despite being "a combination of chemical stuffs exhibiting physical properties ... represents in its ... structure and behavior the highest order of complexity in nature" (Just, '39a, p. 3).

Just's focus on the cell surface led him to speculate on its importance in the evolution of the very first cell. In a short chapter titled "Ectoplasm and evolution" in *The Biology of the Cell Surface*, he wrote: "The play of factors in the environment—of temperature, of gases and of electrolytes—upon the living organism must be first on the cytoplasmic surface. Even if we assume that the primordial living thing was a mass of homogeneous protoplasm structurally the same throughout, there must have early arisen a differentiation between surface and interior" (Just, '39a, p. 357).

Anticipating the concept of mutual adaptation between organism and environment that Lewontin describes in *The Triple Helix: Gene, Organism and Environment* (Lewontin, 2000), Just wrote: "In a certain sense we should not speak of the fitness of the environment or the fitness of the organism: rather, we should regard organism and environment as one reacting system" (Just, '33). Just's view here is similar to that of today's epigeneticists in the sense that both he and they see the organism or cell as a system that responds (reacts) to its environment. Indeed, epigenetics according to the pioneering geneticist Conrad H. Waddington is the branch of biology that looks at the causal interactions between genes and their products, which bring the phenotype into being (Van Speybroeck, 2002).

Just regarded three life-processes as fundamental: *respiration*, *contraction*, and *conduction*. Of these, respiration, a sine-qua-non of vital activity, was primary. This is a phenomenon we would now identify with the non-equlibrium thermodynamics of dissipative, open systems (Prigogine and Stengers, '84; Eu, 2002). Contraction and conduction, both of which Just observed during egg activation, were for him the mechanistic bases of morphological development (Just, '33). In modern parlance, "contraction" relates to viscoelasticity and "conduction" to the activity of excitable media. The physical bases of these phenomena were only dimly understood in Just's time, with theoretical understanding of mesoscopic materials, nonliving and living, only coming into its own in the second half of the twentieth century. They indeed represent "physics and chemistry in a new dimension," and the recent developmental biological literature attests to how central these processes have become to the analysis of embryogenesis (Forgacs and Newman, 2005; Purnell, 2012).

But Just, deeming merely mechanistic accounts of living processes unsatisfactory owing to their apparent goal-directedness, also postulated an intrinsic relationship between development and evolution, one which was reflected in nature's implementation of the fundamental morphogenetic processes: "Animal evolution advanced rapidly or slowly, to a higher or lower stage, depending on the degree of ectoplasmic behavior exhibited as contraction and conduction. Animals to-day differ largely because of differences in these two manifestations of life" (Just, '33, p. 26). Inheritance for him was not just propagation of genes, but propagation of *process*: "What I reject is the proposition affirmed by the embryologists and tacitly admitted by the geneticists—namely that the genesis of form and function and the genesis of particular characters in minute areas of the form are distinct and opposed" (Just, '36, p. 271). Here Just anticipated the rise of "physico-genetic" perspectives in evo-devo, that is, the notion that gene action in the origination and development of biological pattern and form is mediated by the mobilization of mesoscale physical processes (Newman and Bhat, 2009; Jaeger et al., 2012; Newman, 2012).

CONCLUSION

E. E. Just's focus on the cell surface and its changes during the early development of marine invertebrates led him to see egg and embryo as entities responsive to environmental factors; his knowledge of natural history gave him a special appreciation of the importance of natural settings in development. His vision of the intrinsic relationship between evolution and development, and his rejection of abstract notions of the gene's agency anticipated the current century's rise of systems perspectives in evolutionary developmental biology. As biology has turned in a less gene-centric direction in recent years, with a greater emphasis on the important role that nonnuclear determinants play in gene expression, and a greater appreciation of the importance of physical processes in development, the work and ideas of Just seem increasingly relevant and prescient.

In a recent essay, Just's biographer Kenneth Manning wrote that one of the things that first drew his attention to Just was a footnote in Gunnar Myrdal's 1944 book *An American Dilemma: The Negro Problem and Modern Democracy*, in which Myrdal cites Just as "the quintessential example of an American dilemma" (Manning, 2009). Just performed at the highest level; his work was of the highest quality; and for a time (until the early 1940s) his papers were extensively cited. But because he gravitated toward Europe and the ideas of European biologists, and because he did not refrain from publicly challenging prominent American scientists with whom he disagreed, he was sidelined by his American counterparts, despite the fact that his ideas were no less valid, and in many ways were more prescient, than theirs. The fact that Just was black made it easy for them to do this. Denied funding for research, he could not find a position at a major university. He was treated as an outsider. His papers were ignored. As Gilbert explains, his work was met with "polite neglect" (Gilbert, '88).

Most histories of evolutionary developmental biology focus on those scientists—such as Ivan Schmalhausen, Richard Goldschmidt and Conrad Waddington—who believed that changes in gene expression during development, and the responsiveness of the phenotype to environmental factors, were important for evolution (Amundson, 2005; Laubichler and Maienschein, 2007). In Just, we find a scientist who not only criticized the gene theory as being too nucleocentric, but who also favored a view of development and evolution that we would now consider to be one of self-organization and emergence.

Just was also part of an *avant-garde* of scientists who, dissatisfied with the mechanical materialism they had inherited from the 19th century, were searching for a language to describe a systems approach to biology while also being careful to steer clear of a metaphysical vitalism. In addition to those mentioned above, Paul Weiss and Ludwig van Bertalanffy were among the few others working in this vein. Just was unique in his conviction that a new physics of complex, organized materials and dynamical systems would be the key to taking the next steps.

Largely because the mathematical concepts and experimental techniques of the period had not advanced far enough (Crow, 2008), no coherent counter-theory to the rising genecentic one emerged from the collective activity of these figures, even when lines of communication were open among them (Newman and Linde-Medina, 2013). In Just's case, the social barriers of racism, along with the novelty of his ideas and the assertiveness with which he expressed them, conspired to keep him out of this circle of kindred spirits. Notwithstanding his social marginalization, however, we can now recognize that in a scientific sense, E. E. Just was both of, and ahead of, his time. A consideration of his observational, experimental, and theoretical work together reveals him to be an inspired predecessor in evolutionary developmental biology.

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