# Cells, Development, and Evolution: Teeth Studies at the Intersection of Fields


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**Abstract** Early in the twentieth century, biology was seen as grounded in the dual foundations of cells and evolution. Cells provided the most basic living unit, and evolution provided a way for cells to become established in different organisms. However, as the twentieth century progressed, cells and cellular level phenomena became embedded in different research traditions within developmental biology with varying connections to an evolutionary framework. While researchers focusing on differentiation could continue to link their research to evolution through heredity, those focused on morphogenesis largely gave up any evolutionary perspective. Morphogenetic research programs continued, without evolution, until late into the twentieth century, when fruitful new insights brought development back into the process of evolution. This chapter takes teeth as an exemplary case study for these changes with special focus on the enamel knot, now thought of as the morphogenetic control center of the developing tooth. Once development, and especially cellular level phenomena, was seen in the light of evolution, the enamel knot became the central component of a new paradigm in evolutionary developmental biology—one that, to this day, continues to provide a means of understanding the development and evolution of teeth. The intersection of cells and "the Darwinian tradition" is a complex relationship. This chapter offers an alternative history of the ways in which development, evolution, and cells were brought together throughout the twentieth century and challenges the common conception that genes are the sole locus of explanation for research at the intersection of development and evolution.

**Keywords** Development • Evolution • Enamel knot • Teeth • Morphogenesis

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## 1 Nineteenth Century Evolution and Development

Charles Darwin dominates discussion of evolution in the nineteenth century, of course, because of his theory by which species evolve through natural selection. Yet Darwin was not a cell biologist, said only a little about embryology—mostly that it provided important evidence in favor of evolution—and had a very limited understanding of heredity. Ernst Haeckel said much more about the ways that development at the individual and species levels intersect, but he also remained focused on theory and did less with mechanistic details of processes in cells, development, and heredity. Neither Darwin nor Haeckel looked closely at causal explanations of morphogenesis, and though Haeckel's contemporary Wilhelm His did, His did not draw on evolution in his accounts (see Richards 2008; Hopwood 2002, 2015).

August Weismann is surely the figure who most energetically sought to bring together these different and mostly divergent approaches to understanding life. Frederick Churchill's masterful study of *August Weismann. Development, Heredity, and Evolution* (2015) provides an excellent starting point for reflecting on the context in which twentieth century researchers began. As Churchill shows persuasively, Weismann had one foot in the natural historical richness of the nineteenth century and another in the experimental search for causal explanations of the twentieth century. Weismann's interpretation of neo-Darwinism provided an excellent foundation for a unified study of life.

Though Weismann lacked detailed knowledge about how heredity works, biochemical and mechanical details of development, and understanding of the role of cells, he nonetheless understood the importance of all those contributions to living organisms. He had a vision of how the pieces could intersect and fit together to explain both organization of an individual and change over time. Germ plasm and chromosomes provided a basis for heredity, development, response to environment, and therefore also evolution through selection. In many ways, it is only now in the twenty-first century as researchers bring together evolution and development into developmental evolutionary biology (devo-evo or evo-devo) that we are able to realize the goals of developmental evolutionary accounts of life that Weismann set out.

As Churchill shows, Weismann was surely one of the most important biologists of any time. Weismann wanted to understand life and address questions such as how an individual comes into existence, grows, acquires the right kind of form, and then gives rise to new generations. Development and heredity both matter. But each individual is part of a species, and therefore evolution also matters. How do species evolve? What counts as evidence? What theoretical interpretations fit the facts and also lead us forward to new observations and interpretations? What do we learn from observing butterflies and so many other organisms that Weismann studied? For Weismann, these were not isolated questions. Biology must address them all, and all at once.

Weismann studied a number of organisms, but he was especially intrigued with butterflies. What causes the differences in structure, behavior, and other details, he

asked. What causes morphogenesis of the different forms? He was convinced that chromosomes are involved and that the environment provides selective pressure so that some of the "determinants" that make up chromosomes win in the struggle for existence, as he put it. He did not know how they do that; he did not have a theory of morphogenesis. Yet he knew that such a theory, which could provide explanations for the differences in organic forms, was central to biology. He did his best, but knew he had a missing developmental piece.

As Churchill put it, "Weismann's contribution was to articulate the controversies, to sketch them in a lineal sequence as an artist might do, breaking new and clearer boundaries for discussion." Weismann, above all, "appeared always to have kept the bigger picture in mind" (Churchill 2015: 572, xii). Evolution, development, cells, heredity, and all the rest of phenomena of living organisms make up biology, and any account of life must embrace them all for Weismann. It took a century for biologists to articulate the questions, develop the methods, and clarify the theoretical framework grounded in developmental genetics and evolution to succeed with Weismann's goals.

In this chapter, we first look briefly at two major movements that sought to explain evolution in the twentieth century: what has come to be known as the Modern Synthesis and at what is called evolutionary developmental (or developmental evolutionary) biology ("evo-devo"). We note how both of these research programs attempted to bring together fields within the life sciences in order to explain evolution, and how these programs understood and utilized genetics in different ways.

After discussion of these two movements, we focus on a case study that does not fit the standard narrative of evolution in the twentieth century: that of tooth development. Teeth are extremely important to evolutionary biology, paleontology, forensics, anthropology, and any time we look for concrete evidence of past structural variation (morphological differences within a species) and diversity (morphological differences between species). Our story about teeth tells a history of shifting views of how to investigate and explain morphogenesis during the twentieth century. Morphogenesis has been largely neglected by historical narratives about evolution throughout the twentieth century. Yet it is the process that gives shapes to developing organs and organisms and as such is extremely important at the intersection of development and evolution. Therefore, understanding the changing approaches to studying tooth morphogenesis gives us insight into the central biological questions that Weismann raised and sheds light on how the biological synthesis that Weismann called for can be achieved in modern research.

## 2 Purported Modern Syntheses

The most well-known effort at suggesting a synthesis is surely the one proposed by Julian Huxley in *Evolution: The Modern Synthesis* (1943). Dedicated to Thomas Hunt Morgan, "many-sided leader in biology's advance," this volume opened with Huxley's reflection that:

> Evolution may lay claim to be considered the most central and most important of the problems of biology. For an attack upon it we need facts and methods from every branch of the science—ecology, genetics, paleontology, geographical distribution, embryology, systematics, comparative anatomy—not to mention reinforcements from other disciplines such as geology, geography, and mathematics. Biology at the present time is embarking upon a phase of synthesis after a period in which new disciplines were taken up in turn and worked out in comparative isolation. Nowhere is this movement towards unification more likely to be valuable than in this many-sided topic of evolution; and already we are seeing the first-fruits in the re-animation of Darwinism" (Huxley 1943: 13).

Many biologists and historians have commented on Huxley's synthesis, noting the extent to which it actually synthesized or failed to do so (see Smocovitis 1996; Cain and Ruse 2009; Delisle 2009, 2011, 2017). Joe Cain argued in 2009 that historians should give up the concept "evolutionary synthesis" in favor of a more robust understanding of themes in evolutionary biology (Cain 2009). That same year, Richard Delisle argued that epistemic and metaphysical pluralism within the modern synthesis was so rampant that at least three epistemic frameworks could be identified (Delisle 2009). Cain and Delisle are surely right that there is more to evolutionary biology than the synthesis. Yet the fact that Huxley invoked the idea of a synthesis and so many others took up that idea is worth noting.

Huxley's thinking is clear in his 1927 popular volume of lectures, *The Stream of Life*. In a series of lectures, he explained that all of life is essentially a stream. Evolution connects all the different kinds of organisms back to a beginning point, and development connects one generation to the previous and subsequent generations. Reproduction of individuals involves a "stream" of hereditary material interacting with the environment, one after another through time. Heredity, development, cells, and physiology all operate within the context of evolution. Scientists ask what, how, and also why the world is the way it is. What do we see? Many different instances of life. Why is there so much diversity of forms and functions? Because of evolution. How does each individual arise? Through heredity and development. Understanding evolution also allows us to improve, Huxley urged. And, thus: "Let us not forget that we men are the trustees of evolution, and that to refuse to face this problem is to betray the trust put into our hands by the powers of the universe" (Huxley 1927: 63).

In *The Modern Synthesis* in 1943, Huxley presented a much more detailed discussion of the different approaches to understanding life. Betty Smocovitis has discussed Huxley's motivations and the impact of his rallying call for synthesis (Smocovitis 1996: 138–153) Clearly, he sought both to summon and motivate the energy and attention of biologists to work on evolutionary studies and also to attract external attention for biology generally and evolution in particular. Huxley was

always both a scientist and a publicist. As Smocovitis notes, Huxley was accused of having left out important areas of biology, development among them. The impact of Huxley's call to arms was to give evolutionary biologists a manifesto to which they could point. Over time, it also served as a lightning rod for dissent. Critics within genetics and developmental biology especially saw Huxley's view as limited and as leaving out their favorite fields. They saw him as providing less a real synthesis than a summary of the field of evolutionary biology alone.

Yet some leading evolutionary biologists, including Ernst Mayr, saw Huxley as less important than Theodosius Dobzhansky, whose population genetics grounded evolutionary explanation. For Mayr, the evolutionary synthesis had two central features: evolution as a gradual process based on small genetic changes, and the effects of population genetics and reproductive isolation in changing environments (Mayr 1982: 567). Focusing in this way on genetics as causing variation had the effect of largely ignoring development. Mayr's influence also had the effect of directing generations of researchers and resources towards the study of population genetics as a means to explain evolution. As Depew (2017) points out, "tracking gene frequencies is useful, even indispensable, in bringing evolutionary biology's *explanada* into view, but it cannot identify evolutionary causes. . .." Thus, while the population genetics movement pushed so forcefully by Mayr could track the progress of traits (e.g., genes and alleles) throughout populations (i.e. track evolution), it lacked the ability to explain how those traits got there in the first place. Mayr's account failed to incorporate the importance of embryological development and the processes of morphogenesis. In effect, development seems in Mayr's type of evolutionary account almost just to happen when the genes are in place.

## 3 Evo-Devo and the Return of Development?

By the late twentieth century, it had become clear that development does a lot more work than just transcribing and expressing genes. As seen in *From Embryology to Evo-Devo. A History of Developmental Evolution*, embryologists had always had much to say about both development and evolution throughout the twentieth century (Laubichler and Maienschein 2007).

Yet study of development had followed its own course, separate from the specialization of evolutionary biology. Thus came the call by the 1980s for integration of evolution and development, known as evo-devo. Some called for evolutionary development and others for developmental evolution, with somewhat different emphases. A symposium in 2000 took place at the Society for Integrative and Comparative Biology (formerly American Society of Zoologists). The session formally introduced the new Division of Evolutionary Developmental Biology, chaired by Rudolf Raff. In his introduction to the session, philosopher of science Richard Burian pointed to research a century earlier, when study of cell division, embryology, evolution, heredity, and so on were connected. "These problems were generally held to be intimately interconnected, so much so that many biologists

thought of them as inseparable, forming a single nexus" (Burian discussed in Maienschein and Laubichler 2014: 157). After a century of specialization and divergence, it was time to reconnect and reunify biological thinking—but how was this unification to be accomplished?

Pigliucci (2017) and Depew (2017) illuminate the conceptual framework of evo-devo. As an addendum to these eloquent contributions, we would like to add a further point: that evo-devo is a theoretically and epistemically diverse field. Evo-devo researchers utilize many methods, embrace many ways of explaining developmental phenomena, and often enter the field after being trained in disciplines such as molecular biology, genomics, paleontology, and developmental biology. While the goal of evo-devo has always been to unite development and evolution, and specifically to use development to explain evolution, it has struggled since its inception to achieve this goal—a point that we will return to in the conclusion.

If the hallmark of the Modern Synthesis was tracking gene frequency changes within and between populations, then the hallmark of evo-devo has been tracing the roles of genes during development. In effect, evo-devo traded in population genetics for developmental genetics. At first, this meant identifying genes and recognizing their temporal–spatial distributions throughout embryos. Early on, however, researchers recognized that genes are not just expressed; they are also controlled. From this recognition came the search for regulation, and uncovering gene regulatory networks that underlie developmental phenomena has become a mainstay of the field.

## 4 Genes, Cells, and Unifying Development and Evolution

So far we have seen how August Weismann laid out a unified vision of biology that brought development, heredity, and evolution together. Weismann, as we have noted, was unable to bring this vision to fruition because he had no theory of morphogenesis. That is, Weismann lacked the ability to move from the genetic determinants in the germ plasm to the level of cells in order to explain how the germ plasm can account for development. We have also seen how the Modern Synthesis of the mid twentieth century, as dictated by Ernst Mayr, broke Weismann's unified vision of biology by discounting development as relevant to its goals. Investigators working within the Modern Synthesis framework turned to population genetics and tracking changes in gene and allele frequencies through time. The field of evo-devo, which emerged in the last quarter of the twentieth century, brought development back into questions about evolution, and did so by shifting from population genetics to developmental genetics, and more recently, by searching for gene regulatory networks that underpin development.

In this history leading from Weismann to the Modern Synthesis to evo-devo, genes have remained central. They have been depicted as the locus of explanatory value for evolutionary biologists from the mid-twentieth century to the present.

Within the history of science, this depiction is common. Historians who have dealt with evolutionary biology, developmental biology, and evo-devo have tended to privilege the molecular aspects of these fields (MacCord and Maienschein 2017). While genes have had an undeniable impact on these movements, a great deal of research has taken an alternative path: following the cellular processes that build the embryo, e.g., morphogenesis.

We mentioned in the previous section that evo-devo has struggled since its inception to find a way to achieve the goal of using development to explain evolution. This, we argue, has in part been due to an overemphasis on the role of genes as the locus of explanation for both development and evolution. Cells and cellular processes build morphology and traits within the developing embryo. Cells are also not under the sole control of genes. Thus, a gene-centric perspective of development, morphology, and evolution cannot completely account for the development and evolution of morphological traits. In order to do so, the cellular processes of development (morphogenesis) need to be taken into account.

In the following section, we turn to our particular case study that allows us to follow twentieth century research programs that traced morphogenesis, leading to a modern example of merging explanations that include genes and cells, and development and evolution. By following a series of research programs from 1913 to the year 2000 that revolved around individual tooth development, we show how researchers interested in tooth development initially made a conscious decision to ignore evolution and the theoretical musings inherent to Darwinian evolution at the turn of the twentieth century. Tooth development research continued throughout the century, largely untouched by the Darwinian paradigm or by molecularization. However, in the 1990s, when researchers invested in understanding the morphogenetic development of teeth and how they achieve their morphological diversity embraced a new developmental and evolutionary biology, they reintegrated the Darwinian paradigm and genetics back into their research. This case study ends with a modern research program in evo-devo that has succeeded in using development to explain evolution by building a theory of development that takes both genetic and morphogenetic processes into account. Thus, this case both traces a history of morphogenetic research as it changed throughout the twentieth century and also indicates how morphogenesis now intersects with the Darwinian tradition.

## 5 A Case Study: The Enamel Knot

### *5.1 Why Teeth?*

Teeth play an oversized role in our understanding of mammalian evolution, in part because they are the bits of the body that fossilize the best and in part because their morphology varies so widely and distinctively across species. The morphological

diversity witnessed in teeth has long been used to identify species and construct phylogenies of both extinct and extant organisms. But why are they so distinctive?

For answers, we need to look closely at development and specifically at morphogenesis. How does each tooth emerge and gain its distinctive morphological features? It took the full twentieth century to work out fundamentals of tooth morphogenesis, which required accumulation of different kinds of evidence and different approaches to interpreting that evidence. We tell that story below, in brief, drawing on Kate MacCord's extensive research on this topic. A small cluster of cells within the developing tooth, called the enamel knot, plays a central role, and tracing its history shows us how morphogenetic research changed throughout the twentieth century (MacCord 2017).

## 5.2 *Background*

The part of teeth that we see is called the crown. The surfaces of crowns that meet (or occlude) are covered in bumps called cusps. Mammals have an enormous range of sizes, shapes, and numbers of cusp configurations on their teeth—far more than any other clade. In the nineteenth century, paleontologists and embryologists came up with a number of theories to account for this phenotypic diversity by relying on discussions of both development and evolution.

Paleontologists Edward Drinker Cope and Henry Fairfield Osborn developed the tritubercular theory, which held that the tritubercular molar (a three-cusped structure where the cusps are arranged in a triangle) is the common type from which all subsequent mammalian molar diversity arose. As an alternative, the embryologist Carl Röse elaborated the concrescence theory—a late nineteenth century theory that held that teeth are formed by the fusion during development of initially independent cusps. Both the tritubercular theory of Cope and Osborn and the concrescence theory of Röse were evolutionary at their roots, building on interpretations of how evolution and development work to shape the morphological diversity of mammalian teeth (MacCord 2017).

In the twentieth century, however, embryologists interested in teeth shifted their gaze from dental diversity to individual tooth development, and in the process evolution faded into the background. This shift stemmed in part from a growing distaste for appeals to evolution for the explanatory framework of development (Ahrens 1913). To provide what they considered an adequate account of tooth development, embryologists sought to explain the processes by which teeth go from being a small clump of cells to a fully grown organ with characteristic form. This is the problem of morphogenesis.

Morphogenesis within the research programs of these embryologists changed over time in terms of how it was investigated and explained. From the early twentieth century, where morphogenesis was understood as the dynamic shifts in the relationships between tissues and structures during development, to the mid-twentieth century focus on the movement and proliferation of cells, researchers

understood morphogenesis in different ways. This shifting history of morphogenesis becomes particularly interesting in light of the history of interpretation of what came to be known as the enamel knot. The enamel knot is a cluster of cells within early stages of developing teeth. The cells of the enamel knot do not divide, and yet they signal for cells in the surrounding tissues to proliferate. This combination of an inert cluster of cells surrounded by rapidly proliferating cells shapes the developing tooth. Today the enamel knot is at the core of explanations of how teeth develop their characteristic forms. And yet, over the course of the 100+ years since its discovery in 1913, the enamel knot has moved into and out of the explanatory framework of tooth development.

Shifts in the enamel knot's explanatory value are tied to shifting concepts of morphogenesis. This story is, therefore, one of changing scientific choices about methods and concepts, and it shows the ways evolution variously faded into the background or was seen as playing a central causal role. A focus on four research programs throughout the twentieth century shows how the enamel knot emerged and was understood in different ways depending upon how researchers investigated and explained morphogenesis; together they offer a case study of twentieth century research in a Darwinian world, as well as insights into evo-devo's problem of how to use development to explain evolution.

## *5.3 Discovery of the Enamel Knot: Hans Ahrens*

Hans Ahrens's research on tooth development culminated in an article in 1913 (Ahrens 1913). Here, Ahrens does not work with a single question in mind so much as with a suite of questions derived from gaps he saw in the literature. Ahrens wanted a more detailed understanding of the morphogenesis of the developing cells and tissues, which he was convinced required closely observing developing teeth rather than relying on theory (Ahrens 1913: 172). He sought to challenge established theories of dental development—especially those that had relied heavily on appeals to evolution such as Röse's concrescence theory.

Through working with local clinics and hospitals around Munich, Ahrens amassed an astonishing sample of human fetal and postnatal remains. He fixed his materials in formalin, applied a number of contrast dyes, and made sections for every stage of development (Ahrens 1913). Ahrens was primarily concerned with characterizing the fine morphological changes through which the tooth forms. Research into how teeth develop had not yet taken the experimental turn that had characterized many historical depictions of embryology at this time, as Garland Allen (1975, 1979) describes. Nor did evolution play an explanatory role for Ahrens.

Ahrens firmly believed in the necessity of reconstructing structures through serial sections and wax models (Ahrens 1913: 170), and he modified the wax modeling technique developed and made famous by Gustav Born (1883). He was exquisitely careful with his preparations, pressing each section between writing paper saturated with pure formalin and rubbing it with his thumb before running it

through an alcohol and toluene series. Using this method, Ahrens was able to create impeccable serial sections and sequences that afforded him a view into changing relationships between different parts of the oral tissues. He then used serial sections of younger specimens following techniques of earlier researchers to get at development from the earliest stages (Born 1883; discussed by Hopwood 1999 and Radlanski 1995).

As part of his broad study of tooth development, Ahrens discussed development of what he called the enamel organ (Ahrens 1913: 184), which forms the enamel of the tooth crown and gives the tooth its characteristic form. Because the folding of tissues during the early stages of the enamel organ development is complex, Ahrens used his serial sections and wax models to help him visualize the shifting forms of tooth morphogenesis. Through these reconstructions of early stage teeth, he first noticed a cluster of cells that he called the "enamel knot" (Ahrens 1913: 188, 192). Ahrens did more than simply establish that there is such a structure. He also noticed that the enamel knot bulges out of the enamel organ into the surrounding tissue, causing the formation of two grooves. These "enamel grooves," Ahrens believed, ultimately become the cusps. Ahrens thus placed the enamel knot as a main factor in explaining individual tooth development.

Ahrens's techniques and research helped organize previously diffuse studies of teeth. He meticulously traced development from the earliest appearance of tooth germs through to their final forms and placed all these observations within a single publication. For Ahrens, an adequate explanation was a description of the fine morphological changes through which teeth form. He relied on his sections and at no point did he feel it essential to observe living tissues directly. He also understood that development occurs through cellular-level processes, but he made no appeal to those processes such as mitosis, cell death, or cell migration.

Ahrens's histological work on tooth development built on the research of previous authors to give a more accurate depiction of the processes of tooth development. His work, unlike that of many of his predecessors like Cope, Osborn, and Röse, created an understanding of tooth development without evolutionary explanations. Evolutionary explanations did continue after Ahrens's 1913 publication, but researchers concerned with dental development did not often appeal to evolution as explanatory nor use dental development to test evolutionary hypotheses. Explanation came from the local details of morphological change, not through some distant evolutionary past. The era was post-Darwinian, but the biology ignored evolution because it did not seem to add anything to explanation.

## *5.4 Erwin Reichenbach, 1926/1928*

Despite Ahrens's advances, there was still much to learn about how teeth develop from an initial clump of cells. As Erwin Reichenbach noted, "While in the field of tooth development, the research has mainly, through the work of Ahrens, come to a certain conclusion, the researchers have chiefly worked on dental histology, but

cannot sufficiently clarify all the problems of this difficult issue" (Reichenbach 1926: 524).

Other researchers added histological studies, but these accumulating reports became a point of contention because even though they helped characterize fine morphological changes through which the tooth forms, they remained scattered, schematized, and often had contradictory results. In the mid 1920s, Reichenbach, an assistant at the Dental Institute of the University of Munich, attempted to give an account of tooth development built on and extending beyond Ahrens's studies. Like Ahrens, Reichenbach set aside evolutionary considerations as unimportant in his focus on details of dental development.

In line with the research program outlined by D'Arcy Thompson in his famous *On Growth and Form* (1917), Reichenbach believed that tooth development had to be explained by describing transformations of cells and especially the pressures that shape and move them throughout ontogeny. Reichenbach called for mechanical accounts in particular: "Apart from the purely biological factors whose analysis today is hardly accessible. . . mechanical forces can also have a formative influence on shaping the tooth crown. The change in liquids inside of the enamel organ along with the unequal differentiation of the enamel pulp result in specific points of localized proliferation within the enamel epithelium, which in turn stretch out other sections" (Reichenbach 1928: 53). Reichenbach thus shifted discussion of tooth development from characterization of fine morphological detail of tissues to characterization of cells and forces that shape them. In doing so, he also shifted the way in which morphogenesis was to be investigated and explained within developing teeth.

In his two-part Habilitationsschrift for the University of Munich, published in 1926 and 1928, Reichenbach investigated morphogenesis during development of pig teeth, seeking to give a biomechanical account for how the tooth goes from a small cluster of cells (known as a germ) into a fully formed organ (Reichenbach 1928: 494). Reichenbach amassed and processed his own collection of pig teeth, gathering specimens, creating sections, and applying several types of contrast dyes. Within his search for a biomechanical explanation of development, Reichenbach took special interest in active elements of the developing tooth—movements and mitoses of cells and fluids and the pressures that shape them. Reichenbach's publications read as a direct response to many of Ahrens's claims, particularly about the formation and role of the enamel knot.

Reichenbach was interested in questions like where do the cells of the enamel knot come from and how do they coalesce into a cluster? What is the relationship of the enamel knot to surrounding tissues? And, what happens to the enamel grooves that Ahrens deemed the precursors to cusps? From observations of his serial sections, Reichenbach concluded that the enamel knot was not so distinct a structure as Ahrens had thought. He had trouble clearly distinguishing it, especially in later stages, from the underlying tissue.

Reichenbach also had difficulty determining how the enamel knot formed. If it was through passive properties rather than increased mitosis or cell movement, then he reasoned that there should be evidence of a localized increase in individual cell

bodies (Reichenbach 1928: 494). He found little evidence for this. Nor did he find evidence of active properties such as mitosis or cell movement. Thus, Reichenbach concluded that formation of the enamel knot was "due most likely to passive aggregation of existing cell material" (Reichenbach 1928: 495, 535). His observations led him to conclude that the enamel knot plays no active role in shaping the developing tooth, but might serve as a temporary reservoir of cells (Butler 1956).

To Reichenbach, an adequate explanation of development had to account for the biomechanical activities that shape the tooth—i.e., morphogenesis was conceived of as differential mitosis, and cell and intercellular fluid movements. He found value in work like Ahrens's that traced fine morphological changes in structures, but he saw such accounts as inadequate to explain tooth development and morphogenesis. Reichenbach used techniques very similar to those of Ahrens but saw them differently. Whereas Ahrens had used his serial sections and wax models to infer dynamic relationships between tissues and structures, Reichenbach used his materials to look for mitosis and movement. Like Ahrens, he did not have direct access to the living processes but drew inferences from observing appearances of cells and changes that he believed provided evidence for mitosis and movement.

Reichenbach's biomechanical understanding of tooth development put the emphasis on active properties of development, and physically passive properties of tissues were deemed impediments to growth that only indirectly affected overall form. Given Reichenbach's understanding of how to investigate and explain morphogenesis, it is unsurprising that the enamel knot played no explanatory role for him—his observations of the structure rendered it devoid of the active properties necessary to explain development.

## *5.5 Nozue and Colleagues*

Following Reichenbach, tracing cell proliferation within early stages of tooth development became an important problem for dental embryologists because they considered differential mitosis a main factor for shaping tooth development. Thus, knowing which cells were dividing and where was important. In light of conflicts in the literature, Tetuo Nozue, a member of the Faculty of Medicine in the Anatomy Department of the University of Tokyo, decided to investigate the enamel knot more closely and discern "whether or not mitoses are found in this structure" (Nozue 1971a: 1).

Nozue gathered human fetal remains, and fixed, sectioned, and stained his sample. Using these materials, he found that while cells within the enamel knot did not divide and proliferate, the cells immediately adjacent to the enamel knot experienced increased mitosis. (Nozue 1971a: 4).

In his next study, Nozue gathered both fetal human and mouse specimens (Nozue 1971b). Both the human and mouse specimens were fixed, sectioned, and stained, but for mice, Nozue used a wider array of stains that would allow him to examine different properties of the cells and tissues. Nozue concluded that cell

death accounted for some of the observations, which was an important finding because cell death, along with cell proliferation and migration, were considered the main processes of morphogenesis that work together to shape development.

Nozue continued his investigations by teaming up with two other dental researchers in Tokyo—Tadao Kirino and Motohiko Inoue. The team used experimental methods in ways that previous researchers had not, allowing them to intervene in the course of normal development to determine the influence of the enamel knot on shaping surrounding tissues. By the early 1970s, using experimental techniques to look at tooth development was a well-established practice. Yet no researcher had looked at the enamel knot experimentally and little was known about the role that the enamel knot played in the morphogenetic processes that shape the developing tooth (Kirino et al. 1973).

The group devised an experiment in which they injected a chemical, called Mitomycin C, into pregnant mice. Mitomycin C was known from previous work to interrupt the communication between tissue layers that are adjacent during development, called epithelium and mesenchyme (Tanimura 1968). Teeth, like many other organs, develop through epithelial–mesenchymal interactions, and the enamel knot (an epithelial structure) was likely to be affected by this chemical if it had a role in these epithelial–mesenchymal interactions. The results of this experiment indicated in two ways the crucial role that the enamel knot plays in tooth morphogenesis. First, the group noticed that in cases where the chemical had prevented the enamel knot from forming, the subsequent development of the tooth was interrupted. That is, without an enamel knot, tooth formation stalled. Second, mitosis was extremely low and the cells were irregularly arranged in these specimens without enamel knots, indicating that the enamel knot played a role in cell proliferation and cell arrangement.

Nozue and colleagues represent an important change in the history of research on the enamel knot. While they recognized the importance of tracking morphological processes at the cellular level, like mitosis and cell death, and incorporated these observations into their explanatory framework, they also were the first to utilize experimental methods to test the role of the enamel knot in tooth development. This testing grew out of the increased interest and activity in dental research surrounding the roles of epithelium and mesenchyme in directing morphogenesis. In turning to experimentation, the group sought to define the enamel knot in terms of its signaling capacity, i.e., whether or not it could direct morphogenesis in surrounding tissues. Thus, Nozue and colleagues still considered morphogenesis in terms of moving and dividing cells, but they also understood that tissue interactions, i.e., signaling between tissues, could direct tissue growth and cell proliferation.

Despite their advances, their experimental methods granted them only indirect access to evidence about the enamel knot's role in tooth development. They could not determine what caused the inductive phenomenon between the enamel knot and adjacent tissue—that is, they could not identify what signals were producing the effects they witnessed or how these signals were operating. Yet their work

nonetheless implied the possibility that it had this property. Thus, the enamel knot gained new value for explaining individual tooth development.

## 5.6 The Enamel Knot's Finnish Renaissance: Jukka Jernvall

Investigators of tooth development utilized experimental techniques but did not look extensively at the enamel knot or explore its explanatory value. While the research of Nozue and colleagues represents an important shift in the way investigators understood the enamel knot and used it to explain development and tooth morphogenesis, their work went almost completely unnoticed, possibly because of their publication within obscure Japanese journals. Even at the time, the enamel knot was largely relegated to typological obscurity—existing almost entirely within the confines of oral histology texts.

The enamel knot's fate began to change only in the early 1990s when Jukka Jernvall, a doctoral candidate at the University of Helsinki, took an interest in understanding tooth development. Jernvall began his investigations at a time when developmental biology was undergoing massive changes. The first fluorescent in situ hybridization was conducted in 1980, and by the end of the decade its application had become widespread within the developmental community. Developmental biologists using this technique sought spatial information regarding gene activity in the developing embryo in order to get clues about the functions of newly cloned genes (see Koopman 2001). The possibility of locating genes in situ had profound implications for developmental biology—after a century of searching for the formative signals of development, the presence of differentiating signals (e.g., gene expression) could be localized and recorded in temporal-spatial parameters according to the development of the organism.

Jernvall's work on tooth development grew out of this period of in situ hybridizations and the search for gene expression patterns. Importantly, though, his investigations were also influenced by his training in paleontology. His graduate fieldwork at a Miocene site in Peshawar, Pakistan, gave Jernvall insights into teeth as biological and species indicators. This work, Jernvall acknowledged in personal communication, gave him an appreciation of form and pushed him to explore in his dissertation experiments the morphogenetic potentials of cell populations within the developing tooth in order to understand better how teeth gain their characteristic forms.

Jernvall's move towards utilizing the enamel knot to explain tooth development began with an accidental finding. He began his research program with no idea of what an enamel knot was, which is not surprising given that it had been marginalized for decades. Jernvall was interested in the problem of how teeth develop their characteristic forms. To him, this was a question of morphogenesis, a phenomenon composed of the processes of cell death, cell proliferation, and cell migration, all of which had genetic underpinnings. Jernvall began his research on tooth development

and morphogenesis by asking, as Reichenbach and Nozue had, where is mitosis happening within the developing tooth?

In order to track mitosis, Jernvall devised an experiment to label cells in developing mouse embryos that were actively undergoing mitosis. When the labeled specimens were harvested and sectioned, Jernvall was able to observe where cells were proliferating at the different stages of development. Using this technique, Jernvall found areas of enhanced cell proliferation surrounding a ball of cells that showed no mitotic activity—a finding reminiscent of Nozue (1971a, b). This finding indicated to Jernvall a flawed methodology—he was not yet familiar with the structure called the enamel knot and thought that the presence of a static area within a rapidly proliferating tissue was a possible artifact of his labeling technique.

While puzzling over what he had found, Jernvall came across an article by Lee Niswander and Gail R. Martin that looked broadly at the expression of the gene FGF-4 throughout the developing mouse embryo (Niswander and Martin 1992). They had found FGF-4 expression in the location where Jernvall had discovered the inert cluster of cells—a structure that they labeled the enamel knot.

Jernvall's understanding of how to investigate and explain morphogenesis appealed to more than just the physical forces that Reichenbach had sought; he also understood that development could be characterized by revealing the genes that could cause the movements and mitosis that Reichenbach had understood development to be. To Jernvall, an understanding of development required both of these perspectives. Because of his commitment to approaching morphogenesis from both of these perspectives, Jernvall decided to replicate Niswander and Martin's experiment within the teeth and looked for gene expression at different stages of tooth development. He found that FGF-4, which is a gene that greatly enhances cell proliferation, was expressed by the non-proliferating cells of the enamel knot. This led Jernvall to consider the possibility that the enamel knot, by both not dividing itself and by expressing genes like FGF-4 that cause heightened cell proliferation in the surrounding tissue, could be shaping the developing tooth.

In order to track the possible connection between FGF-4, cell proliferation, and the enamel knot more closely, Jernvall made computer-assisted 3-D reconstructions of his serial sections that incorporated his data on cell proliferation and gene expression into the models. By combining this data within a single 3-D model of each of the stages of mouse molar development in which the enamel knot was present, Jernvall was able to recognize the tight spatial and temporal relationship between the enamel knot, FGF-4 gene expression, cell proliferation in surrounding tissues, and the emergence of the tooth cusps. Thus, the enamel knot gained a central role in explaining tooth development, and it did so because Jernvall brought together morphogenesis, cellular phenomena, and genetics.

Jernvall's work demonstrates the emergence of a way of understanding tooth development and morphogenesis wherein both cellular processes and gene expression are necessary. In his 1994 paper, Jernvall referred to the enamel knot as a potential control center rather than a signaling center. In doing so, Jernvall sought to clarify that the enamel knot did not merely act in terms of a signaling capacity.

Rather, the enamel knot both directs surrounding cells to proliferate and through its own static properties shapes the outgrowth and transformation of the tissues surrounding it. Thus, tooth morphogenesis to Jernvall was the result of the physical forces that come from cells rapidly proliferating around a static object, like the enamel knot, as well as the genes that cause the rapid proliferation. This research became the basis of Jernvall's enamel knot theory, which holds that the development and cusp patterning of mammalian molars are driven by morphogenetic control centers called enamel knots.

Over the next six years, Jernvall and his lab worked to expand knowledge of the enamel knot in terms of its role in shaping teeth (Jernvall 1995, 2000; Jernvall et al. 1998, 2000; Keränen et al. 1998, 1999; Pispa et al. 1999; Vaahtokari et al. 1996). They sought information about what signals the enamel knot expresses throughout its life cycle as well as what roles these signals have on the cellular processes shaping the surrounding tissues.

In directing this line of research, Jernvall's goal was not simply to understand how the enamel knot shapes a mouse tooth; rather his goal was to understand how the enamel knot can underlie the enormous diversity of molar forms that had provoked Cope, Osborn, and Röse in the nineteenth century to devise their theories of tooth development and evolution. Thus, Jernvall's group built their theory of the enamel knot's role initially by looking at mice (the traditional model organism of tooth development), but also took a comparative approach by checking whether the same processes were at play in the development of vole teeth (a close relative of mice) and asking how the morphological differences between the two species could be achieved by altering the temporal and spatial arrangement of enamel knots (Jernvall 1995; Keränen et al. 1998).

This comparative work came to fruition in 2000, when Jernvall's lab tied their detailed analyses of the processes that produce dental morphology to evolution (Jernvall et al. 2000). By comparing the relationship between enamel knot gene expression patterns and emerging morphology in developing teeth across mice and voles, the group turned the enamel knot theory of tooth development into a theory of both development and evolution. Through research that took into account cellular processes and gene regulation, Jernvall was able to develop a theory of tooth development that could be used to explain tooth evolution, thus achieving the longstanding goal of evo-devo.

## 6 A New Synthesis of Development and Evolution: Bringing Cells Back

Weismann's vision was to unite development, heredity, and evolution. He was committed to a comprehensive biological research program and believed that these three areas necessarily intersect and could and should be addressed together. The frustration for Weismann's program was that he did not have the ability to move

from his germ plasm theory of inheritance up to the level of cells or morphogenetic processes in order to provide an adequate account of how the germ plasm could give rise to form. He did not understand enough about development or genetics and did not have ways to tie those processes to evolution sufficiently.

The history of biology in the twentieth century, as we have noted, has often been told from the perspective of the gene—of the molecular determinants of inheritance and development. Looking at genes allows us to ask crucial questions about our bodies and our place in nature but does not give complete answers.

Other chapters in this volume have referred to the reintegration of development into evolutionary theory in the last third of the twentieth century, in the movement that came to be called evolutionary developmental biology ("evo-devo") (Depew, Pigliucci). Pigliucci points out that at the core of evo-devo is an emphasis on linking genes to morphology through development and that, "... one of its major contributions so far has been a marked shift of emphasis in the study of morphology and development, from the sort of classical population genetic studies focused on structural genes to an emphasis on regulatory genes and their potential to help us build a credible theory of the origin of evolutionary novelties." Pigluicci points us towards gene regulation as a way of surmounting the divide between genes, development, morphology, and evolution.

During development, genes interact. Through these interactions, genes guide and help build the cells into tissues and organs that will become an organism. As this process unfolds, cells multiply and divide, changing shapes and identities depending on their location within the emerging body, their neighbors, the physical forces that they encounter, and the genetic signals they receive. As Lewis Wolpert put it, "genes control development by controlling cell behavior" (Wolpert 1994). But, are these complex and multivariate shapes that we see throughout organisms, as well as the processes that give rise to them, solely the output of gene regulation?

Stuart Newman and colleagues in 2006 pointed out that while cellular activities may be largely governed by gene regulation, the results (e.g., compressive forces, cellular asymmetry, etc.) may produce mechanical responses within the cells and tissues that are not governed by genes, but affect organismal form (Newman et al. 2006). Cells, then, and the forces that surround and shape them into tissues and traits are likely not completely subservient to gene regulation. One excellent recent example of this is research revealing that mechanical forces (e.g., stretching) control cell division in epithelia (Gudipaty et al. 2017). Research such as this shows us that more than networks of gene regulation are necessary in order to connect genes, development, morphology, and evolution.

The process of development is one of shifting cells, changing forms, and genetic regulation, all of which interact to produce an organism. This outcome, or the phenotype of the organism, is what comes into contact with the world and is subjected to evolutionary pressures. Phenotypes, guided by developmental processes, vary within species, and this variation gives natural selection something upon which to act. Development, then, is both the source of variation and the source of evolutionary diversity. Development is also a process that requires information about how genes direct and regulate morphogenesis, as well as information about

how morphogenetic processes produce emerging forms, in order to be explained. This later component—the tracking of morphogenetic processes—has been much neglected by historians of science, but not by scientists.

In this chapter, we have seen how investigators tracked the morphogenetic processes that give rise to teeth throughout the twentieth century. They did so, at first, by breaking Weismann's unified vision of biology. In 1913, Ahrens made a conscious decision to depart from the nineteenth century trend of explaining individual tooth development by appeals to evolution as part of his explanatory framework. Even as researchers such as Reichenbach in the late 1920s focused on morphogenesis and the mechanics of cellular forces as the key to explaining how teeth form, evolution lay by the wayside. This trend continued within studies of tooth development—whether from the perspective of morphogenesis or otherwise.

Jernvall, working in the tradition of closely studying the behavior of cells and their morphogenetic properties, provided the bridge that Weismann lacked between the mechanisms of inheritance (i.e., the genes) and the phenotype. The research framework that Jernvall built for tooth development—focused on form and understanding the mechanisms (both cellular and genetic) that produce it—extended easily to incorporate evolutionary thinking. As Jernvall wrote in his dissertation, "Mammalian teeth are morphologically diverse structures whose shapes reflect developmental and ecological processes. By using a comparative approach combining new morphological, embryological and molecular evidence, this paper addresses molar tooth shape diversity, and how changes in molecular mechanisms can produce observed diversity patterns" (Jernvall 1995: 1). In other words, understanding the ways in which form arises throughout development, and the mechanisms (both cellular and genetic) that produce this form, can be extended through comparisons across species to give us insights into how we get so many different patterns of cusps throughout mammalian teeth.

Study of the enamel knot and Jernvall's developmental evolutionary approach gives us a new synthesis that brings Weismann's vision to reality. It also shows different ways that a Darwinian paradigm has affected research—from the nineteenth century desire to elaborate explanations that were both developmental and evolutionary in nature, through the twentieth century setting aside of evolutionary concerns, up to Jernvall's reinvestment in developmental evolution. Jernvall's approach also shows us that when it comes to bridging the divide between genes, morphology, development, and evolution, one should look both to genes and to cells. Cells, after all, are what build morphological characters. Only with Jernvall were the pieces put back together again for teeth. Weismann would have been pleased.

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