Development, Evolution, and Teeth: How We Came to Explain The Morphological Evolution of the Mammalian Dentition

by

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ABSTRACT

This dissertation begins to lay out a small slice of the history of morphological research, and how it has changed, from the late 19th through the close of the 20th century. Investigators using different methods, addressing different questions, holding different assumptions, and coming from different research fields have pursued morphological research programs, i.e. research programs that explore the process of changing form. Subsequently, the way in which investigators have pursued and understood morphology has witnessed significant changes from the 19th century to modern day research. In order to trace this shifting history of morphology, I have selected a particular organ, teeth, and traced a tendril of research on the dentition beginning in the late 19th century and ending at the year 2000. But even focusing on teeth would be impossible; the scope of research on this organ is far too vast. Instead, I narrow this dissertation to investigation of research on a particular problem: explaining mammalian tooth morphology. How researchers have investigated mammalian tooth morphology and what counts as an explanation changed dramatically during this period.

DEDICATION

This dissertation is the result of seven years of research.

Over seven years, I have received the support of an unbelievable number of people. The degree is for me, but this dedication is for them.

With love:

My partner in crime.

My family.

My friends.

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Over the years, I have given presentations of different ideas and pieces of chapters at a number of meetings, including, “The Enamel Knot at 100” in Helsinki, Finland, in December, 2013, the Joint Atlantic Seminar for the History of Biology at Johns Hopkins University in April, 2014, the History of Science Society in Chicago, IL, in November, 2014, the PhilBioMed seminar at the Institute for History and Philosophy of Sciences and Technology in Paris, France, in November, 2014, the Pan American Society for Evolutionary Developmental Biology in Berkeley, CA, in August 2015, &HPS6 at the University of Edinburgh in July, 2016, the Marine Biological Laboratory McDonnell Foundation Workshop in November, 2016, and the Mathematical Biosciences Institute Workshop on “The Biological Challenges in Morphogenesis” in February, 2017.

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CHAPTER 1

INTRODUCTION

"A tooth! A tooth! My kingdom for a tooth!" T.H. Huxley to Charles Gordon, December 8, 1858.

1.0 Morphology Matters

The history of biology in the 20th century has typically been told from the perspective of the gene—of the molecular determinants of heredity and development.[1] With good reason. The advent of molecular biology and genetics has given us incredible insights into our personal histories, as well as our historical connections with the rest of the animal kingdom. The concept of genes, and their interactions, allow us to ask crucial questions about our bodies and our place in nature, like: How do organisms develop one variant of a trait as opposed to another? And, how are organisms related? Genes do not give complete answers to these questions.

During development, genes interact. Their interactions tend to occur in regular ways that can be thought of as networks. These networks can contain modules—subsets of the networks that are fairly self-contained and can be deployed throughout many contexts of the developing body. Gene products, through these modules and networks guide and help build the cells, tissues, and organs that will become an organism.[2] As this process unfolds, cells multiply and divide, changing shapes depending on their location

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[1] There are too many histories of genetics, molecular biology, and the gene to even begin to have a comprehensive list here. For especially popular histories, see: Olby 1974, Keller 2009, Mayr 1982. For insights into the molecularization of developmental biology, see: Crowe et al. (2015), MacCord and Maienschein (in press)

[2] Clearly this is not the process of development for all organisms, but it works for our purposes because it applies to all mammals.

within the emerging body, their neighbors, and the genetic signals that they receive. This process of development is one of shifting forms and genetic modules, the outcome of which is an organism. This outcome, or the phenotype of the organism, is what comes into contact with the world and helps to determine its success. Phenotypes tend to vary within species, and this variation gives natural selection something upon which to act. Development, then, is the source of variation and the raw materials that produce evolutionary diversity. Development is also a process that requires information about how genetic modules direct and regulate morphogenesis, as well as information about how morphogenetic processes produce emerging forms, in order to be explained.

Morphology, a concept introduced by Johann Wolfgang von Goethe at the end of the 18th century, gives us another way to frame the aforementioned questions, and another perspective on what count as answers. As Goethe saw it, morphology was not simply a matter of studying fixed, definite form (*Gestalt*); rather, morphology also necessarily included study of the process of changing form (*Bildung*) (Russell 1916). Morphology, in the sense of *Bildung* can be thought of in two ways: as the processes by which an organism forms during development (morphogenesis), and as the changes in form that occur throughout lineages (morphological diversity). Morphogenesis and morphological diversity thus play out along different timescales—development and evolution, respectively—but are inextricably linked within Goethe's sense of *Bildung* and the concept of morphology.

Nineteenth century researchers interested in exploring the natural history of organisms turned to morphology to guide their investigations, particularly by exploring the relationships between parts.[3] This exploration, especially in the latter part of that century, witnessed a surge of research that took development and evolution to be inextricably linked. The linkage between ontogeny and phylogeny broke as the 20th century dawned; however, the tendency to look at the natural world through the lens of form and its study through morphology did not die.[4] Pursuits of morphological problems, such as descriptions of developing organs and organisms and explanations of phenotypic diversity within the fossil record, have persisted throughout the 20th and 21st centuries. The history of scientists pursuing the study of morphology during this timeframe, though, is largely unwritten.

This dissertation begins to lay out a small slice of that history—of morphological research, and how it has changed from the late 19th through the close of the 20th century. Investigators using different methods, addressing different questions, holding different assumptions, and coming from different fields have pursued morphological research programs—research programs that explore the processes of changing form. At the same time, the way in which investigators have pursued and understood morphology has witnessed significant changes from the 19th century to the modern day. To trace this shifting history of morphology, I have selected a particular organ system—the dentition—and traced a tendril of research on teeth beginning in the late 19th century and ending at the year 2000. Teeth are a useful example here because their morphologies are indicators of dietary adaptations, life history, and evolutionary relationships and have

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[3] The history of morphological research in the 19th century has been covered in great detail. See, for instance, Appel (1987), Churchill (1991, 2007), Coleman (1971, 1976), Laubichler (2000), Lenoir (1981, 1987), Nyhart (1987, 1995), Russell (1916), and many others.

[4] For arguments about the persistence of morphology past the end of the 19th century, see Benson (1981), Maienschein (1981), and Rainger (1981).

been studied across many disciplines. But even focusing on teeth would be impossible—the scope of research on this organ is far too vast. Accordingly, I narrow this dissertation to investigation of research on a particular problem—explaining mammalian tooth morphology. How researchers have investigated mammalian tooth morphology and what counts as an explanation, as we will see, changed dramatically during this period.

In the late 19th century, tooth morphology was a problem for paleontologists and embryologists, and these researchers constructed theories to explain the morphological diversity that they observed in their daily work (as discussed in this dissertation, Chapters 2 and 3). Choices that these researchers made about methods, evidence, and the nature of the relationship between development and evolution were vastly different and led to conflict (Chapter 4). Although these theories have largely been forgotten by modern biologists, their construction and the clash that resulted allow us to reflect on how scientists understand and come to explain the phenomena that they encounter, especially when it comes to explanations of morphology. In this instance, investigators approached morphology in vastly different ways.

Their different approaches, however, converged in that neither side thought it necessary to account for the processes that give rise to form during ontogeny—that is, they did not account for morphogenesis. They also understood the relationship between ontogeny and phylogeny in different ways. As we will see, the investigators' assumptions played a crucial role in shaping their science, and many of the concepts that they drew assumptions about, such as homology and types, as well as the relationship between ontogeny and phylogeny are still in play in the modern field of Evolutionary Developmental Biology (hereafter referred to as "EvoDevo").

At the dawn of the 20th century, evolution was largely set aside by practitioners in the burgeoning field of embryology. Historical research on embryology, particularly focused on the early 20th century, has followed the rise of experimentalism (Maienschein 1991). Morphological research persisted, however, as embryologists continued to provide detailed studies of the processes of tooth development *sans* evolution (Chapter 5). This morphological research did not focus on diversity or accounting for the final forms of the dentition, as had been the case in the 19th century. Rather, investigators were interested in the processes of morphogenesis: *how* these processes build the relationships between the developing parts of the tooth. Morphogenesis had largely been ignored by their 19th century counterparts.[5]

Morphogenetic investigations of teeth also changed over time as researchers brought new conceptual frameworks to bear on interpreting shifting cells and tissues that they witnessed. Within these investigations, researchers yet again drew upon assumptions—this time about how development works, what counts as evidence, and what types of processes are most important to track—to explain the morphogenetic processes that they saw.

By the 1990s, EvoDevo had emerged as a field and researchers again became interested in uniting development and evolution. Just how such a union could be accomplished, and how development could be used to explain evolution, became a persistent problem for the field. Meanwhile, explaining mammalian tooth morphology

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[5] In Chapter 3 we encounter an investigator who tried to explain the morphological diversity of the dentition by looking to developmental processes. However, his way of explaining these processes was to give descriptions of the tissues and structures at different developmental stages. While this type of work was important, and is also seen in the first investigator discussed in Chapter 5, these gross descriptions did not account for *how* the structures came to form.

once again became the problem of explaining their morphological diversity. While the problem and many of the concepts (e.g. homology, type) were the same as researchers in the 19th century had encountered, the methods, evidence, and understanding of how development and evolution can be brought together within a research program were vastly different (Chapter 6). This different perspective is one that united development and evolution by focusing on morphological diversity and morphogenesis, and reconceiving of how to explain both. Development, within this program, became a matter of both morphogenetic processes and the genetic modules that regulate them. Meanwhile, evolution became a matter of surveying morphological diversity without appeals to concepts like homology, and using this survey to shape hypotheses about how such diversity could develop. This combination has become the basis for a highly successful research program within EvoDevo because the research program utilized these reconceptualized versions of morphogenesis and morphological diversity to create an explanation of mammalian tooth morphology. That is, this research program used development to explain evolution.

Accordingly, this dissertation traces teeth, or more specifically, the ways in which researchers have explained mammalian tooth morphology, from the late 19th century to 2000. In tracing this history, we see how morphology changed with regards to the types of questions investigators asked, the methods they used, and the interpretations that they brought to bear on the evidence. We also see that the focus of morphological research changed immensely. Nineteenth century investigators focused on describing and explaining the morphological diversity without appeals to morphogenetic processes, while 20th century embryologists focused on morphogenetic processes without appeals to how they gave rise to diversity. In the late 20th century an EvoDevo researcher who worked on teeth brought these two ways of understanding morphology together and reconceived of how to explain each. Therefore, this is a history of shifting views of morphology and changing assumptions, and a story of how science progresses. In tracing this history, we also see that focusing on morphological diversity and how processes produce it were crucial to the development of a highly successful research program within EvoDevo, and can grant us insights into how to use development to explain evolution.

1.1 Why Teeth?

The dentition is a little-studied anatomical system in the historical literature. While some have written about the advent of dentistry, historical investigations of research on the teeth are notably sparse and populated almost exclusively by scientists (Berkovitz 2012, Glasstone 1965). The absence of historical investigations, however, does not map onto a dearth of scientific inquiry; dentition has a long and storied past within the sciences and natural history.

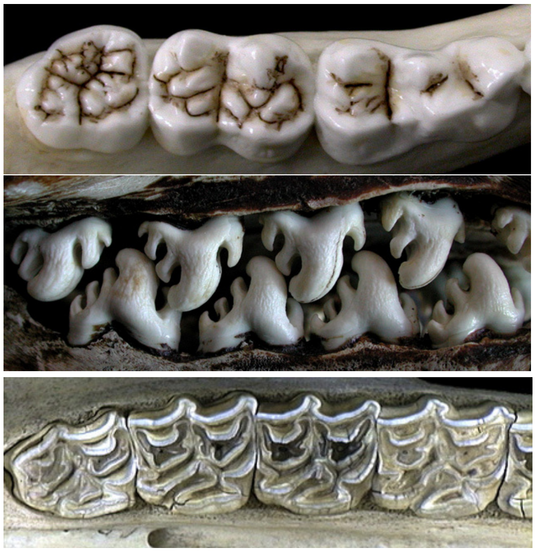

Figure 1. Molar morphological diversity. From the top: molars of the giant panda (<u>Ailuropoda melanoleuca</u>), crabeater seal (<u>Lobodon carcinophaga</u>), and horse (<u>Equus caballus</u>), showing a small sample of mammalian molar diversity. All photos by Phil Myers. (Myers et al. 2017)

Aristotle, for instance, referred to the numbers and appearances of teeth when describing genera in his *The History of Animals*, Book II. Later, in his *Systema Naturae*, Carl Linnaeus overturned the common practice of classifying mammals with respect to their feet, and instead divided the taxon based on the number, appearance, and placement of their teeth. In the 19th century Richard Owen, Thomas Henry Huxley, Oscar Hertwig, and many others recognized the value of the dentition for questions of taxonomy and classification (Hertwig 1874, Huxley 1853, Owen 1845). These are just a few examples, but they are indicative of a broader trend in which teeth have been central to answering questions in natural history and the life sciences.

From issues of species identification (taxonomy), to ordering species (classification), to understanding the evolutionary adaptations of species, scientists have looked to teeth for answers. In particular, they have looked to dental morphology.

Mammalian teeth display a huge range of morphological diversity—far more than in any other vertebrate clade (see figure 1). This diversity manifests in many ways, including the sizes, shapes, numbers and functions of teeth and cusps. One of the most important aspects of morphological diversity of mammalian teeth is the cusp patterns found on molars. Molar cusps vary in terms of size, shape, number, and configuration. These variations give researchers answers about the dietary adaptations, life history, and the phylogenetic relationships of mammals.

1.2 Tooth Development and the Special Characteristics of Teeth

Before we dive into the historical context in which tooth morphology arose as a problem of both ontogenetic and phylogenetic proportions, it is necessary to understand how teeth develop and why they are important for answering questions in the life sciences. Mammalian teeth begin to form from a band of ectodermal epithelium within the oral cavity called the dental lamina (see figure 2). The dental lamina sits on top of mesenchyme (derived from the neural crest). At around the 6th week of human development, or E (embryonic day) 10 in mice (*Mus musculus*), the dental lamina forms localized thickenings, called placodes, that mark the beginning of tooth formation. The timing of the stages of development is slightly different across mammalian species, but the stages are the same and are distinguished by their physical appearance (see table 1).[6] By E11, the epithelial thickening has expanded into the underlying mesenchyme to form the bud stage. The bud stage lasts until around E13.5, and it is during the latter part of this stage that the primary enamel knot appears within the expanding epithelium.[7]

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[6] Because most studies of tooth development are conducted on mice, I use the developmental stages of mice to indicate the progression through the stages of development. It is interesting to note that while researchers often report the stage of development for their findings, they also report the embryonic day of the specimen.

[7] The enamel knot is a cluster of cells within the epithelium of the developing tooth. It is discussed in detail in Chapters 5 and 6.

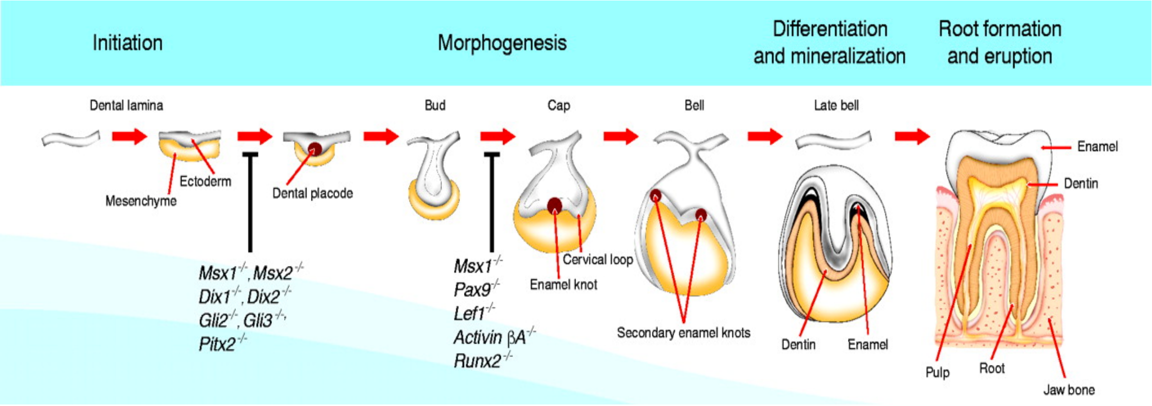

Figure 2. Stages of mammalian tooth development. Adapted from Thesleff (2003)

Following the bud stage is the cap stage. During this stage, the epithelium continues to expand outward and wraps itself around the condensing mesenchyme adjacent to the primary enamel knot. The bell stage follows the cap stage. During the bell stage secondary enamel knots form at the sites of future cusps, and begin to give molars their species-specific occlusal patterns.[8]

Table 1. Stages of Tooth Development

Stage	Mouse[9]	Human	Description
Placode/germ	E 10	6th week	Oral epithelium develops localized thickenings
Bud	E 11-13.5	8th week	Oral epithelium expands into mesenchyme
Cap	E 14-15.5	9th-10th weeks	Epithelium expands and captures mesenchyme, forming enamel organ
Bell	E 16-19	11th-12th weeks	Species-specific cusp patterns emerge

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[8] Note that secondary enamel knots form at the site of future cusps in multicusped teeth.

[9] Note that the embryonic days outlined for mice are approximate in their correspondence to the different stages of development. The timing of these stages has been shown to be different within different strains of mice (Gaete et al. 2004).

Teeth have characteristics that give them a unique status for addressing problems in systematics and dietary behavior. For instance, enamel (see figure 2) is the hardest substance in the body.[10] As the hardest substance, enamel survives post-mortem processes better than any other tissue and makes teeth more prone to fossilization than any other organ system. This means that teeth are represented in the fossil record far better than any other organ system, especially when compared to soft tissues systems like the muscles or alimentary canal. In many cases, teeth are the best or only fossil evidence we have for the existence of a species. This is particularly true for the mammalian clade, which means that the majority of the insights we have about the evolution of mammals have come from teeth.

Teeth also have highly canalized development (Kassai et al. 2005, Polly 2006). This means that while there is variation within species, the basic dental formula and patterns of cusp morphology of any given species is set during development and not prone to large perturbations.[11] In addition, teeth have distinct morphological configurations—the arrangements of cusps, lophs, crests, and tubercles are reliably unique to taxa.[12] And, teeth do not undergo remodeling following their formation, except to wear down with use. This is because the tissues that make up teeth—enamel, dentine,

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[10] Enamel is the hardest substance because it contains the highest percentage of minerals (approximately 95%).

[11] A dental formula is an expression of the numbers and kinds of teeth possessed by a mammal. It is usually written as a fraction, wherein the upper line represents the upper jaw and the lower line represents the lower jaw. For example, the human dental formula is: 2.1.2.3/2.1.2.3 This formula means that humans have 2 incisors, 1 canine, 2 premolars, and 3 molars in each quadrant of the mouth. A quadrant is defined by all teeth on one side of the midline of the mandible or maxilla.

[12] The system of naming dental features can be complex and confusing, and these are only a few anatomical features that can be found. Cusps (also referred to as cones) are elevations on the occlusal surface of the posterior teeth (i.e. premolars, molars). Lophs are ridges on the occlusal surface that connect two cusps. Crests are expanded cusps. Tubercles are nonoccluding prominences on the crown.

cementum—have extremely limited or no regenerative capacity.[13] While this means that dentistry will remain a stable and lucrative trade for the foreseeable future, it also means that the environment has a very limited impact on the overall morphology of an individual's dentition. Canalized development, reliably unique morphological configurations, and lack of remodeling have made teeth highly sought after as taxonomic indicators.

Finally, dental morphology is strongly correlated with diet, such that the size, shape, and arrangement of cusps, crests, lophs, etc. are tied to different dietary habits.[14] This means that although teeth do not remodel throughout life, they are used to understand the life ways of organisms. Understanding the life ways of organisms, and in particular their diets, is bolstered by research showing that information about diet can be inferred by looking at microwear patterns (Scott et al. 2005; Teaford 1988; Unger and Sponheimer 2011, Walker et al. 1978). The relationship between dietary adaptations and teeth is important for understanding teeth as functional units. Cusps on most mammal teeth come into contact during chewing and form an interlocking relationship between upper and lower molars. The ability of teeth to form these interlocking relationships (i.e. the ability of teeth to develop correctly) is what makes them crucial for tearing and shearing foods. Without a functional interrelationship between the cusps on teeth (i.e. the ability to form functional phenotypes), an organism would not be able to eat properly. Natural selection can thus act on these phenotypes because a reduced ability to process

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[13] Scientists have uncovered some evidence of regenerative capacity among odontoblasts (the cells that make dentin), but none among ameloblasts (the cells that make enamel) (Huang 2009). Also note that dental wear has become extremely useful in the fields of paleontology and anthropology, where wear patterns and microwear are indicative of behavior, including diet.

[14] Note that this is a general trend, and not an absolute diagnostic.

food should lead to a lower fitness for an organism. In this way, understanding the both morphogenesis and morphological diversity of cusps and teeth is critical to understanding organismal biology.

1.3 Homologies, Types, and the Relationship between Ontogeny and Phylogeny: Morphology in the 19th Century

In Chapters 2 and 3 I follow the development of two theories that address the problem of morphological diversity in mammalian molars. These theories, as we will see in Chapter 4, came into conflict over the assumptions that each theory's proponents held regarding the value of different types of evidence, the relationship between ontogeny and phylogeny, and about how teeth form. Their assumptions were grounded in the morphological tradition of the late 18th through late 19th centuries.

At the end of the 18th century, Johann Wolfgang von Goethe introduced the term morphology (Russell 1916). This term embodied both the study of form and formation of the organism. As Russell put it, "[Goethe's] interest was not in *Gestalt* or fixed form, but in *Bildung* or form change. He saw that *Gestalt* was but a momentary phase of *Bildung*, and could be considered apart and in itself only by an abstraction fatal to all understanding of the living things" (Russell 1916, 49). This unity of form and formation led to a focus on the relationships between parts both within and among organisms, and became a constant feature of natural history inquiry throughout most of the 19th century.[15]

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[15] Laubichler and Maienschein have referred to this unity of form and formation as a search for the theory of generation (2007).

Morphologists throughout the 19th century, much like their 18th century counterparts, looked at nature and saw patterns—of body plans, of taxa, etc. They attempted to make sense of these patterns and to trace them throughout the animal kingdom in a variety of ways. One of the ways in which investigators sought to trace the relationships between parts and the history of organisms was by defining homologies.[16] The definition of homology was standardized by Richard Owen in 1843, then the Hunterian Professor in the Royal College of Surgeons, London (Owen 1843). In his Hunterian lecture, Owen explained homology as, "the same organ in different animals under every variety of form or function" (Owen 1843, 379). The homology concept, as conceived by Owen, defined organismal structures primarily by their morphological relationships (Rupke 1993).[17]

Owen's definition of homology appealed to a similar concept, *analogue*, defined by his predecessor, Étienne Geoffroy Saint-Hilaire (hereafter, "Geoffroy").[18] Geoffroy was a professor at the Muśeum National d'Histoire Naturelle in Paris during the early 19th century who believed that the structure of an organism's anatomy provided the best insights into relationships among taxa (a view termed "structuralism") (see Appel 1987, Racine 2013). Guided by his structuralist principles, Geoffroy sought to discern common

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[16] The terms "homology" or "homologue" and "analogy" or "analogue" are used here in the sense that Richard Owen defined them. Owen first formally defined analogue and homologue in published notes from his Hunterian lectures in 1843:

"Analogue. A part or organ in one animal which has the same function as another part or organ in a different animal. See Homologue." Pg. 374 "Homologue. (Gr. *Homos*; *logos*, speech.) The same organ in different animals under every variety of form or function." Pg. 379

[17] As opposed to defining structures by their functions. See: Rupke 1993

[18] Analogues, to Geoffroy, referred to similarities in structure regardless of their function.

body plans. His approach, called "transcendental morphology," was defined by a process of abstraction from evidence to infer a unity within animal body plans.[19]

Geoffroy's structuralism and transcendental morphology stood in stark contrast to "functionalism," the prevalent view at the time. The functionalist framework made famous by Georges Cuvier, also a professor at the Muśeum National d'Histoire Naturelle, was highly teleological—the form of organisms and their parts were dictated by their purpose rather than any postulated causes. The friction between Geoffroy and Cuvier, or structuralism and functionalism, famously erupted in 1830 in the halls of l'Académie des Sciences in Paris, as these two prominent anatomists sought to define the course of anatomical research according to their worldviews (Appel 1987, Racine 2013). In the mid-19th century then, "form versus function" was an active and hotly debated subject.

Throughout the first half of his career, Owen followed in Geoffroy's footsteps, looking to the morphological features of the specimens he examined to infer relationships within the animal kingdom (Rupke 1993). He believed that form dictated function. Owen's inferences about vertebrate form led him to propose a unified vertebrate body plan, called the archetype (Owen 1848). This body plan, at least as first conceived by Owen, was an idealized structure from which all known vertebrate body plans were derived.

While Owen was busy defining his archetype, other naturalists and zoologists were also at work defining less encompassing points of unity within animal body plans called "types." "The type concept…emphasized the existence in nature of a limited number of distinct and stable kinds of animal life" (Coleman 1976, 150). Paul Farber has

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[19] Transcendental morphology preceded Geoffroy—see Lenoir (1981)

shown how the type concept had at least three distinct usages (classification, natural history collections, and morphology) during the first half of the 19th century (Farber 1976). For our purposes, the most important usage of type is the morphological, wherein types represented a basic body plan that practitioners believed could be "discerned at various taxonomic levels" (Farber 1976, 100). Despite their vastly different metaphysical and epistemological commitments, Cuvier, Geoffroy, Owen, and Karl Gegenbaur, developed their own understandings of how the animal kingdom could be parsed into separate, autonomous units. The types that these naturalists proposed were unconnected with each other, such that there was no organic connection between the different types. Types were the *Baupläne* from which organisms were molded, and tracing this structural unity became a matter of tracing homologies.

Before the rise of Darwinian evolution, archetypes and types (especially morphological types) were largely idealized forms, not actual physical entities. The homology concept was used to infer "sameness" between structures among organisms in relation to these idealized forms. The application of the homology concept has always been associated with two different problems, "one related to the question of how one could establish 'sameness' between structures and the other related to how one could explain the existence of such 'sameness' between structures of different species" (Laubichler and Maienschein 2003, 195). In other words, the problems of how to identify homologies (a methodological problem) and how to explain them (an epistemological problem) were both crucial to understanding the natural history of organisms (Laubichler and Maienschein 2003).

With the advent of Darwinian evolution in the mid-19th century, the "sameness" that underlay the homology and type concepts could be ascribed to common ancestry rather than an idealized archetype. Now, what had been a desire to trace structural changes throughout a type became an effort to establish linkages within a chain of morphological modification and to trace changes in structural complexity through time.[20] However, as Laubichler and Maienschein point out, ascribing the sameness of homologies to ancestors, "did not solve the problem of how homology between two organs could be detected, nor did it really explain in a mechanistic or verifiable fashion how organs in different species came to be the same" (Laubichler and Maienschein 2003, 195). How, then, were such phenomena traced and explained in the Darwinian era?

To answer this question, let's look to Darwin's *On the Origin of Species*. In his 13th Chapter, Darwin writes,

> Thus, as it seems to me, the leading facts in embryology, which are second in importance to none in natural history, are explained on the principle of slight modifications not appearing, in the many descendants from some one ancient progenitor, at a very early period in the life of each, though perhaps caused at the earliest, and being inherited at a corresponding not early period. Embryology rises greatly in interest, when we thus look at the embryo as a picture, more or less obscured, of the common parent-form of each great class of animals. (Darwin 1859, 450)

In this passage, Darwin has two important messages. First, embryos provide extremely important evidence for the history of organisms. Second, there is a correspondence between the development of an embryo and the evolutionary history of that organism.

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[20] This is not to say that all morphologists accepted Darwinian evolution or the precept of the accumulation of modifications via natural selection. Many did not (Owen, Agassiz, etc.). In spite of objections to the tenets of Darwinian evolution, the acceptance of descent theory (i.e. genealogy) for morphologists like Gegenbaur was "a more potent mode of explanation because it permitted a more complete integration of anatomical facts" (Coleman 1976, 162).

Darwin was by no means the first to point out either of these messages. Martin Rathke, a professor of zoology at the University of Königsberg, was one of the earliest proponents of comparative embryology (Churchill 1991). Rathke turned to careful study of organismal development to answer questions about shared form (Churchill 1991, MacCord et al. 2015). Rathke's contemporaries, such as Christian Pander and Karl Ernst von Baer, utilized embryology towards similar ends (Churchill 1991). Meanwhile, Johann Friedrich Meckel, a professor of anatomy at the University of Halle, and Antoine Etienne Renaud Augustin Serres, a physician in Paris, published works in the first two decades of the 19th century that outlined a recapitulation theory of development. Their recapitulation theory held that the embryonic stages of higher animals recapitulated those of the lower animals on a "great chain of being."

As the Darwinian theory of evolution arose following the publication of *On the Origin of Species*, the recapitulation theory of Meckel and Serres was reborn in a new framework. Instead of organismal development following along a great chain of being, now it followed along the evolutionary history of an organism's species. This theory, termed the biogenetic law and introduced by Ernst Haeckel in his *Generelle Morphologie* (Haeckel 1866), "shifted the explanatory reference frame for homologies towards ontogeny as the observable record of phylogeny" (Laubichler and Maienschein 2003, 196). Under the auspices of the biogenetic law, one could use embryology to discern homologies from analogies. In the wake of Haeckel's biogenetic law, development and evolution became intertwined and spurred research that appealed to both of these processes.

Historians of science have written extensively about the biogenetic law, i.e. that ontogeny recapitulates phylogeny (Churchill 2007; Gould 1977; Guralnick 2002; Maienschein 1978; Ospovat 1976; Rasmussen 1991; Rinard 1981; Russell 1916; among others). The biogenetic law, as formulated in its strong analytical form by Haeckel, entails that phylogeny *causes* ontogeny. Thus, "the fact that a phylogeny contains particular adult forms actually makes it causally necessary that the embryo will assume those forms progressively during its ontogeny" (Maienschein 1978, 132). To Haeckel, phylogeny was both a sufficient cause and a sufficient explanation for an organism's ontogenetic process (Maienschein 1978). Many researchers embraced the causal connection between phylogeny and ontogeny, the consequence of which was that for many no further explanation was sought for the phenomena of development than to appeal to phylogeny. Thus, while many morphologists sought the "unifying generalizations about organic forms and their genesis" (Nyhart 1987, 366), this rarely amounted to an interest in exposing the sources or causes of biological variation. We will see in Chapter 3 how adherence to the biogenetic law drove a research program and shaped explanations of the phenomena that were uncovered while investigating mammalian tooth morphology.

In contrast to this strong form of the biogenetic law, a weak form also existed (Churchill 2007; Guralnick 2002), and was likely the more widely adopted version of recapitulation by late nineteenth century researchers (Churchill 2007). The weak form reflected a "…composite of positions maintained by comparative embryologists as they worked within the framework of the biogenetic law and the program to establish phylogenetic lineages" (Churchill 2007, 59). Within this weak form framework, researchers were less likely to adopt Haeckel's vocabulary of "biogenetic law," "palingenesis," and "cenogenesis," began to question the strict monophyly of major groups, and were less likely to hold Lamarckian inheritance as a foregone conclusion (Churchill 2007). Thus, researchers who accepted a weak form of the biogenetic law understood there to be a special relationship between the unfolding of an embryo during development and its evolutionary history, but did not embrace ontogeny as a strict read-out of phylogeny or necessarily that phylogeny causes ontogeny. For some, such as Edward Drinker Cope and Henry Fairfield Osborn, the flow of information worked in the opposite direction. For this pair, and other proponents of theories like kinetogenesis, the raw materials of evolution were changes in ontogeny that accrued over time and were chiseled into the phylogeny of organisms.[21] In this vein, ontogeny *caused* phylogeny.

From this brief survey of the history of morphology in the 19th century, we can see that there was a rich context for development of theories that explained the morphological diversity of mammalian molars (Chapters 2, 3, and 4). This context arose out of a desire to understand and trace the relationships between parts, which led to investigators embracing an inseparable relationship between development and evolution, as well as type and homology concepts. These factors that were so integral to 19th century morphology also became a crucial component for the establishment of EvoDevo and research, beginning in the 1990s, on explaining mammalian molar diversity.

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[21] The theory of kinetogenesis held that extant structures were modified during development in response to the functional need of the organism. Kinetogenesis is discussed in more detail in Chapter 2.4.

1.4 Morphology in the 20th Century: From Morphogenesis to a New Intersection of Development and Evolution[22]

By the end of the 19th century, the fields that had overlapped in their use of the morphological concepts outlined in Section 1.3 were beginning to diverge as the biological sciences moved towards a vast array of specialties. Paleontology, embryology, and others, while still invested to some extent in morphological concepts, like homology and type, went their separate ways in terms of methods, standards of evidence, and problems that come along with emerging fields.[23] At the turn of the 20th century, experimental embryologists drove the final nail into the coffin of the biogenetic law as a legitimate biological concept, a move that helped sever the relationship between development and evolution that had been so critical to morphologists of the previous century (Maienschein 1978). With the severance of the ties between development and evolution, embryologists turned away from explaining the phenomena that they observed in terms of evolution, and moved towards describing the phenomena of developing embryos in terms of internal factors and processes.

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[22] Note that this section only addresses morphology within embryology/developmental biology during the 20th century. Morphology remained a focus within other fields throughout the 20th century, but the investigation of morphology within these fields is not the focus of this dissertation.

[23] Much has been written about this fracture in the biological sciences and the continuity of morphological principles as the fields went their separate ways. See Coleman (1971) and Allen (1975) for discussions of the “revolt from morphology”, its precipitating factors, and its effects. Meanwhile, see Benson (1981), Maienschein (1981), Rainger (1981) for arguments countering the notion of a revolt. For insights into the split between comparative anatomy and embryology within the German University system, see Nyhart (1987, 1995)

One way in which embryologists focused on the internal workings of developing embryos was by investigating morphogenesis.[24] Morphogenesis is the development of morphological characteristics, which can also be thought of as arranging the spatial distribution of cells. This spatial distribution is accomplished through several processes, including cell proliferation, cell death, and cell movement. Throughout the 20th century, the morphogenetic investigation of developing teeth changed considerably as researchers embraced different assumptions about what counts as evidence, what type of evidence is most valuable, how development works, and what counts as an adequate explanation of development.

In Chapter 5 I show how investigators at the start of the 20th century utilized the same methods and gave the same types of explanations of tooth development as their predecessors, with one significant difference—they consciously set aside evolution from their explanatory frameworks. Here, descriptions of the emerging forms of the developing tooth—descriptions of the changing relationships of the different tissues and structures—were seen as sufficient accounts of tooth morphology (see Section 5.1). With evolution divorced from their explanatory frameworks, these researchers did not feel the need to account for the enormous range of morphological diversity of mammalian teeth, as those in the 19th century had.

As the century wore on, researchers maintained many of the same methods as their 19th century counterparts, but they focused increasingly on giving accounts of development that addressed *how* morphogenetic processes shaped the tooth (see Sections

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[24] For more on the history of morphogenesis within developmental biology, see: Gilbert (2003), Hamburger (1996), MacCord and Maienschein (in press), Sunderland (2011), Trinkaus (2003). Morphogenesis is one of three processes/phenomena of development, as outlined by such embryologists as Bonner (1952), Huxley and DeBeer (1934), Needham (1942), Trinkaus (1984), and Waddington (1956).

5.2 and 5.3). These accounts invariably focused on describing cellular phenomena, such as proliferation and migration. And, in the latter half of the century, these investigators also began to adopt many of the experimental techniques, such as interfering with normal development, that had defined other areas of embryology since the turn of the century (see Sections 5.3 and 5.4).

By the last decade of the 20th century the evolutionary framing that embryologists had dropped at the turn of the century re-entered developmental inquiry as the field of EvoDevo emerged. Morphogenetic research also changed as new methods, like *in situ* hybridization, allowed researchers to track gene expression patterns in the developing cells and tissues. In this new setting, morphological investigations shifted yet again as research on the morphogenetic processes of the teeth reabsorbed many of the concepts that had guided morphological research in the 19th century.

Jukka Jernvall, a graduate student at the University of Helsinki, began his dissertation work during this time (Chapter 6). Within his dissertation and subsequent research, Jernvall unified and re-conceived of both the 19th century focus on morphological diversity of the dentition with the 20th century focus on how the processes of morphogenesis shape the developing tooth. This combination allowed Jernvall to track the developmental mechanisms of diversity, and begin to give explanations of the morphological evolution of mammalian teeth unlike anything that his predecessors could have imagined. Jernvall's research on morphological evolution, and the enamel knot theory that he created to explain mammalian tooth development and evolution, has been hailed by the editors of *Nature* as one of, "15 examples published by *Nature* over the past decade or so to illustrate the breadth, depth and power of evolutionary thinking" (Gee et al. 2009, 1).

1.5 Thesis

From the mid-19th century to present day, explaining tooth morphology has been an ever-present problem for scientists. Morphology, as originally envisioned by Goethe, embodied the study of formation (*Bildung*). If *Bildung* is the goal of morphological inquiry, then to explain morphology, one should account for both a diversity of final forms (morphological diversity) and how organisms arrive at these final forms (morphogenesis). And yet, throughout this time, researchers have addressed this problem from different perspectives and come to different, and often conflicting, conclusions about understanding and explaining tooth morphology on both developmental and evolutionary timescales.

From 19th century studies of morphological diversity to 20th century studies of tooth morphogenesis, researchers accounted for different aspects of how tooth morphology arises, but did not develop explanations that effectively brought together the processes of development with the diversity of form generated by evolution until Jukka Jernvall's research program emerged in the 1990s. The way in which Jernvall re-envisioned both morphological diversity and morphogenesis was key to his ability to unite these two research programs. To Jernvall, morphological diversity of cusp patterning was divorced from the notion that cusps were homologous entities, which we will see was key to one of the 19th century theories we meet in Chapter 2.

Morphogenesis, on the other hand was best explained, for Jernvall, by appeals to both the movement and proliferation of cells (a 20th century tradition, seen in Chapter 5) as well as tying these cellular phenomena to developmental modules that could regulate them. This combination allowed him to develop the enamel knot theory of cusp development. The enamel knot theory allowed Jernvall to explain both the morphogenesis of tooth development, as well as the morphological diversity of cusp patterns within the mammalian fossil record, creating an explanation for both the development and evolution of mammalian teeth. Jernvall's enamel knot theory and way of reconceiving of how to explain both morphological diversity and morphogenesis can serve as an exemplar for the field of EvoDevo. EvoDevo has struggled since its inception to use development to explain evolution—something that the enamel knot theory does well. Therefore, the thesis of this dissertation is as follows:

The research program of Jukka Jernvall brought these two historical foci on morphology together by reconceiving of what it means to explain morphological diversity of teeth and what it means to explain morphogenetic processes in teeth, the combination of which allowed Jernvall to develop a theory of tooth development and evolution that serves as an exemplar for developing explanations that unite development and evolution for modern EvoDevo.

CHAPTER 2

EDWARD DRINKER COPE, HENRY FAIRFIELD OSBORN, AND THE TRITUBERCULAR THEORY

> Of the many problems in Comparative Odontology, one of the most interesting morphologically and most important phylogenetically is that dealing with the origin of the complex crowns of the mammalian cheek-teeth, and their evolution from a primitive haplodont type. Tims 1903, 131

2.0 Introduction

In Chapter 1 we saw how morphologists in the late 19th century were entrenched within a framework of type and homology concepts and sought explanations for the phenomena they encountered from the perspectives of both development and evolution. Throughout the last quarter of the 19th century, researchers became increasingly interested in describing and understanding the ways in which cusps arise on both developmental and evolutionary timescales—i.e. they were interested in explaining the morphological diversity of cusp morphologies. Numerous theories emerged from morphologists within paleontology and embryology to account for the development and evolution of cusps. Although these researchers were invested in developing theories that could explain both the development and evolution of cusp patterns, their theories did not account for morphogenesis. These theories, by and large, were given as simple statements of general patterns or processes. These simple statements often were not formal statements of a theory, and only gained that title after the fact. This is certainly the case for the two theories described in Chapters 2 and 3—the tritubercular and concrescence theories.

Despite often being such simple descriptive statements, they were called theories by their proponents (and opponents) and often carried an enormous amount of assumptions that made them far more complex and explanatory than they seem upon initial inspection. Background assumptions about the nature of species and types, the relationships between development and evolution, what counts as evidence and what type of evidence is most valuable, and even how evolution proceeds, were often bound to these theories by their proponents. These background assumptions led to conflict between researchers and theories, even in cases when the theories should not *prima facie* be in conflict.

In the late 19th century, two prominent theories were proposed to account for the diversity of mammalian molar cusps and patterns—the tritubercular theory and the concrescence theory. The tritubercular theory was first formulated by the paleontologist Edward Drinker Cope in 1883, and later expanded by Henry Fairfield Osborn beginning in 1888. The tritubercular theory is a description about the generalized pattern through which mammalian molars changed over an evolutionary timescale and can be stated thusly: "The theory consists of two distinct parts: (1) the tritubercular origin of the molar patterns of Tertiary mammals, and (2) the origin of this tritubercular pattern from the reptilian cone" (Butler 1941, 422). That is, the tritubercular molar (see figure 3) is the molar type

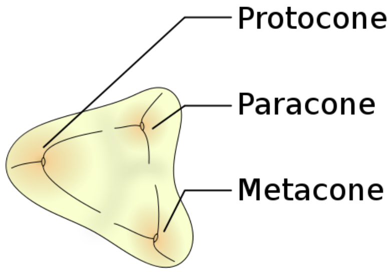

Figure 3. Tritubercular (upper) molar. Protocone is lingual, paracone and metacone are buccal. Image courtesy of Totodu74 (Wikimedia).

from which all subsequent mammalian molar diversity arose (beginning in the Tertiary) and it evolved from simple, cone-like teeth.

The concrescence theory, on the other hand, was formulated by the embryologist and doctor of dental medicine, Carl Röse, in 1892 (Röse 1892). The concrescence theory attempts to explain the process by which molar patterns form during development, and can be stated as follows: teeth are formed by the concrescence (coalescence) during development of initially independent cusps. Although the concrescence theory describes a developmental process, it should not be thought of as appealing to morphogenesis. Röse's theory, as we will see, does not explain how the cusps coalesce during development (i.e. does not address the morphogenetic processes involved), it simply tells us that they do.

Prima facie, it does not seem like these two theories are in conflict—although they both attempt to resolve the same problem of how mammalian molar diversity arises, the tritubercular theory describes an evolutionary pattern, while the concrescence theory explains a developmental process (see Chapter 4). That is, these theories address very different questions. And yet, the proponents of each (Cope and Osborn in the case of the tritubercular theory, and Röse for the concrescence theory) took issue with the other's theory. These conflicts often arose not around the formal statements of the theories, but around the assumptions held by each of these researchers (Chapter 4). Within the construction of these theories and the ensuing debate, evolution and development were brought together and understood in different ways. The history presented in this and the following two chapters thus highlight tensions within research that attempted to explain morphological diversity through appeals to development and evolution.

To understand the debate that arose between the proponents of the tritubercular and concrescence theories, we need to understand the context within which they arose and the assumptions that were tied to each. This chapter tracks the development of the tritubercular theory, beginning with clarifying how Cope understood and explained morphological diversity.[25] His vision of morphological diversity arose from his work with extant organisms and fossils and his desire to sort out the patterns that he saw as latent within these materials. Underlying Cope's work, as well as Osborn's, was a complex view of how development generated morphological diversity present in the fossil record; that is, how ontogeny drove phylogeny.

2.1 Cope, Osborn, and Assumptions

Edward Drinker Cope and Henry Fairfield Osborn sought to decipher the morphological diversity they observed in the fossil record and to explain this diversity by elaborating theories and laws. Throughout their respective careers, both Cope and Osborn shifted their assumptions, picking up some only to abandon or modify them as time wore on (Bowler 1977; Rainger 1981, 1985). Despite their shifting intellectual pathways, some motifs remained throughout their respective discussions of the tritubercular theory. It is these motifs that are the focus of this chapter.

Cope and Osborn both recognized within nature the existence of definite units (morphological types) that represented points of unity within evolution and served as

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[25] My goal here is to track the inception of the tritubercular theory for each of these authors, not how their understanding of the theory changed over time.

evidence for progress in evolution. Each held a concept of homology, although it varied when applied to the mammalian dentition (whole cusp configurations, in the case of Cope, and individual cusps for Osborn). Both believed in the process of change over time within nature, although they each took issue with aspects of Darwinian evolution.

To these men, evolution was a gradual and directed process, wherein extant structures were modified by localized changes during development in response to the functional needs of the organism (a process that Cope referred to as "kinetogenesis"). These changes were wrought by shifts in the growth energy of the developing organism. The adoption by Cope and Osborn of kinetogenesis as the factor underlying modification during development, and ultimately evolution, meant that to these two, not only was ontogeny tied to phylogeny, it *caused* phylogeny. This relationship between ontogeny and phylogeny falls within the bounds of the weak form of the biogenetic law, as discussed in Chapter 1.3. Finally, both men, while recognizing the importance of evidence from embryology and modern comparative anatomy, held fossil evidence as the primary indicator of evolution.

This litany of the metaphysical and epistemological assumptions of Cope and Osborn formed the foundation of their research on the evolution of morphological diversity within the mammalian dentition. While these assumptions are critical to understanding their formulation and defenses of the tritubercular theory, they are not an explicit part of the theory (Chapter 4). Although largely implicit, these assumptions became entrenched within the tritubercular theory, and made it subject to attack.

2.2 Cope's Understanding of Evolution and Mammalian Molar Complexity

Cope's understanding of evolution and the emergence of morphological diversity was formed long before his excavations of Eocene beds in New Mexico and Wyoming from 1872-1874 led him to the fossilized mammal remains from which he would formulate the tritubercular theory. His complex views developed out of a long career spent investigating the comparative anatomy and phylogeny of modern reptiles, fishes, and mammals, as well as their fossilized forms (Gill 1897).

On December 15, 1871 Edward Drinker Cope addressed the American Philosophical Society on, "The Method of Creation of Organic Forms" (Cope 1871). Within this speech, Cope outlined further and with modification the theory of evolution that he had explicated in his 1868 paper "On the Origin of Genera." [26] His 1871 paper addressed what Cope saw as a deficiency in Darwin's evolutionary theory, namely the inability of natural selection to account for the origin of forms: "I propose then to seek for the originative laws by which these subjects are furnished—in other words, for the causes of the origin of the fittest" (Cope 1871, 230).[27]

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[26] Bowler (1977) has shown that between Cope's 1868 and 1871 papers, his evolutionary theory underwent an important shift from that of a theistic evolutionist concerned with rational patterns in nature towards a Neo-Lamarckian whose evolutionary theory was underpinned by utilitarian principles, and that, while Cope always maintained a recapitulationist element to his theory, his appeal to the law of acceleration dwindled as he shifted to a utilitarian, adaptationist view of evolution.

[27] Rainger (1981) has shown how 1871 marked a turning point in Cope's career, wherein he began to work increasingly with paleontological evidence.

Cope's search for an evolutionary theory that addressed the origin of form led him to explicate and expand several laws.[28] Primary among these was the "law of acceleration and retardation," which underpins his entire theory of evolution by tying the appearance of variation to a shift in the tempo of developmental trajectories—that is, it couples phylogenetic and ontogenetic processes, with the later as the causal agent.[29]

Cope envisioned an exact parallel between the ontogenetic and phylogenetic processes, whereby the organism would recapitulate during ontogeny the phylogenetic stages of its evolutionary history. These stages, according to Cope, were equivalent to genera, and were laid out as branching hierarchies that moved from more general (and ancient) to more specialized (and modern). Therefore, Cope was a strong proponent of a weak form of the biogenetic law.

The law of acceleration and retardation granted the embryo the ability to proceed through the normal developmental stages of its parents with the caveat that it could move through some stages faster, thus altering the final form of the organism and producing an evolutionary change. As Bowler (1977) points out, there is one important distinction between ontogeny and phylogeny for Cope; ontogeny is a continuous process, whereas phylogeny is a step-wise process.

Cope's second law, the "law of repetitive addition," was a corollary to the law of acceleration and accounted for the ways in which organisms increase in complexity

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[28] Rainger (1981) has shown that Cope's understanding of morphology was initially informed by his studies of embryology and comparative anatomy, not paleontology. These interests remained with Cope as he became embedded within paleontological research, and helped him establish a morphological tradition in American paleontology. Rainger's characterization of Cope contrasts with that of Bowler (1977), who characterizes Cope primarily as a paleontologist and taxonomist, even in his early years.

[29] Gould (1977) and Bowler (1977) have both addressed in detail Cope's theory of evolution in detail, and, in particular, the relationship between ontogeny and phylogeny. In the early years of his theorizing, Cope maintained a creator as the ultimate causation for evolution; this shifted over the years.

during growth, and as a consequence, throughout evolution. Cope identified two kinds of increase: “the addition of identical segments and the addition of identical cells” (Cope 1871, 235). His goal in formulating a law of repetitive addition was to show “that every addition to structure which has resulted in the complexity of the higher animals, was originally a repetition of a preexistent structure” (Cope 1871, 236). Thus, Cope understood form to evolve through the gradual accretion of modifications, not the appearance of structures *de novo*.

Within the evidence that Cope presented for his law of repetitive addition, we find the first indication of the tritubercular theory. That is, we see Cope laying out an approximate structural sequence of molar patterns roughly mapped to a phylogeny, and then applying his laws and principles of evolution to the sequence.[30] Cope’s understanding of mammalian dental evolution is built on the understanding that a link exists between the morphological diversity and evolutionary history of teeth.

The link between morphological diversity and phylogeny, Cope believed, was accounted for by what he called the “growth force,” or, later, “Bathmism.” The growth force is an energy that builds the embryo by directing the movement of matter in definite directions.[31] The modification of such energy, Cope believed, constituted evolution. Thus, when Cope states “The low mammal *Ornithorhyncus*, possesses but a single tooth in each jaw; the simple teeth of armadillos and cetaceans, increasing as they have done from a single commencement as in the monotreme cited, present examples of repetitive

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[30] Rainger tells us that Cope “developed phylogenies that represented the patterns of evolution” (Rainger 1992, 16), my reading of Cope agrees with Rainger in this respect.

[31] Cope (1894) tells us that “The phenomena of growth are also evidently exhibitions of energy. The term energy is used to express the motion of matter, and the building of an embryo to maturity is evidently accomplished by the movement of matter in certain definite directions (206).”

acceleration of growth force" (Cope 1871, 238), he means that there has been a progressive and directional alteration of teeth within these lineages towards a greater diversity and complexity of form. Complexity, in terms of cusp number and pattern, is seen as a hallmark of progress:

> Complication of a single element of repetition is accomplished apparently by a double repetition. This is best understood by the consideration of the transition from simple to complex teeth. In the cetaceans this occurs in the Squalodonts; the cylindric incisors are followed by flattened ones, then by others grooved on the fang, and then by two rooted, but never double-crowned teeth. This is the result of antero-posterior repetitive acceleration of the simple cylindric dental type of the ordinary toothed cetacean. (Cope 1871, 238)

Cope intends his reader to understand that the law of repetitive addition encapsulates an increase in the growth energy of an organism that directs parts of the organism towards increased structural complexity through the duplication and addition of extant parts. This gradual and directed build-up of structural complexity and thus diversity is at the heart of the tritubercular theory.

Cope further elaborated his rough structural sequence of molar forms:

> A good example of repetitive addition in both linear and transverse directions, may be found in the successive complication of tooth structure seen in Mammalia. In the dolphin, the dental series may be represented thus:—Fig. 7; in the squalodon thus:—Fig. 8; in the cat:—Fig. 9; in the dog:—Fig. 10; in man:—Fig. 11; in some insectivora:—Fig. 12. The circles represented here, are each a simple cusp. (Cope 1871, 242)

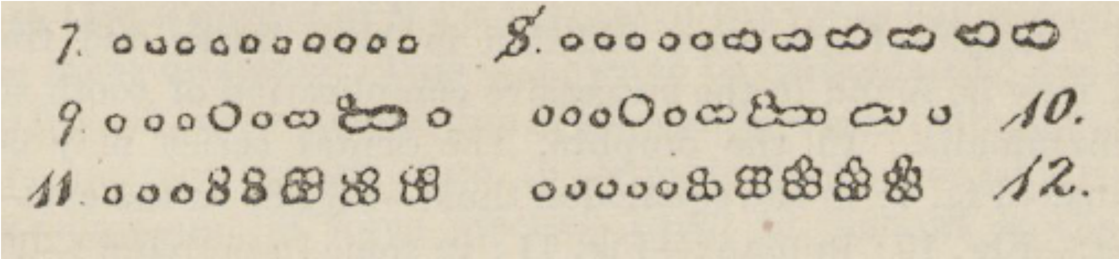

Figure 3. Cope's structural sequence of molars. From Cope 1871, 241. 7=dolphin, 8=squalodon, 9=cat, 10=dog, 11=human, 12=some insectivore

The sequence that Cope proposed is indicative of his commitment to a structural progression occurring throughout mammalian dental evolution (figure 4). One other thing to note here is that the phylogenetic relationships of the clades that Cope represents are highly separated, and his choices include both extant and extinct mammals.[32] William King Gregory (1934) claimed that Cope's priority was not to determine exact phylogenetic relationships between mammals with different tooth types, but to recognize a sequence of increasing structural complexity. It was not Cope's style to give extensive evidence for any single claim; it was, rather, to give an abundance of claims that amounted to a proof.[33]

In his 1873 work "On the homologies and origin of the types of molar teeth of *Mammalia educabilia*," Cope attempted to trace ungulate molars to "a parent quadritubercular type" (Cope 1873, 326), and began to direct his argument of structural progression toward more phylogenetic, and thus evolutionary, ideas. [34] In this work, Cope

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[32] Gill (1897) has noted that, "The great merit of Cope's work on mammals is that he always considered the old and new—the extinct and recent—forms together." (Gill 1897, 236)

[33] Rainger (1992) has shown that Cope was not a particularly careful or accurate worker.

[34] It should be noted that, while Cope was addressing structural evolution within a specific clade, his argument is still very much one of structural progression, and is not very tightly tied to evolution in the phylogenetic sense.

reiterated his argument that "the transition from simple to complex teeth is accomplished by repetition of the type of the former in different directions" (Cope 1873, 71). To this notion of structural progression, he added an important evolutionary component—homology:

> The proper homologizing of the various forms of dental structure of the Ungulates with each other, and with the primitive types of tubercular teeth, is entirely essential to their intelligent classification, and therefore comprehension of their mode of origin.[35] In order to lay a foundation for this work, I define the four types as follows, giving the subdivisions of the first two in brief, and discussing those of the third and fourth more fully afterward. (Cope 1873, 72)

It is difficult to grasp fully and in what sense Cope understood homology to be at work. While the tritubercular theory, as later elaborated by Osborn, would include the assumption of a strict interpretation of cusp homologies, in this treatise, Cope seems to picture homology as a similarity in overall molar structure—homology is a condition of the structural arrangement of cusps, but not of individual cusps. [36] Cope's understanding of homology could be read as reminiscent of Owen's original usage of the term, "the same organ in different animals under every variety of form or function" (Owen 1843, 379) because he sees homology as occurring at the level of the organ, not the individual features of the tooth.[37]

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[35] It is not clear when Cope uses the term "origin" whether he is using it in the sense of *ursprung* (primitive source) or *entstehung* (process of origination).

[36] See Section 2.7.2

[37] See Chapter 1.3 for more information about the homology concept during the 19th century.

2.3 Cope's Tritubercular Theory

Through his paleontological fieldwork in the Eocene beds of New Mexico and Wyoming from 1872-1874, Cope discovered a variety of fossil mammals, including numerous carnivores and insectivores. These discoveries led to his formulation of the tritubercular theory in 1883 (Gregory 1934). Beginning in 1875, Cope turned his attention to outlining the structural evolution of the teeth he discovered in those Eocene beds.

In an announcement titled "Note on the Trituberculate Type of Superior Molar and the Origin of the Quadrituberculate," published in *The American Naturalist* in April of 1883, Cope outlined his view of trituberculy in mammals. Here is the opening:

> It is now apparent that the type of superior molar tooth which predominated during the Puerco epoch was triangular or tritubercular; that is, with two external and one internal tubercles…This fact is important as indicating the mode of development of the various types of superior molar teeth, on which we have not heretofore had clear light. (Cope 1883a, 407)

The announcement is extremely brief—covering only three paragraphs—but in it Cope articulated the idea that, within the mammalian clade, the tritubercular molar, with two cusps on the buccal side of the crown and one on the lingual side, is a type through which nearly all mammalian upper molars passed during evolution. Furthermore, he said, the quadritubercular type is derived from this tritubercular type.

Cope's discussion of molar-crown types and their evolutionary sequence was not new to him in 1883. A decade earlier he had traced the modifications of the upper teeth in ungulates back to an ancestral quadritubercular type and laid out a series of crown types

(Cope 1873). The new point for Cope in 1883 was the breadth of the generalization. While he had discussed ancestral types within different mammalian clades, his fossil evidence from the Eocene allowed him to postulate an ancestral condition for the entire mammalian clade. And, while the announcement is brief and does not have a formal statement of a tritubercular theory, it is often referenced as the first statement of the theory (Gregory 1934; Rainger 1981).

Later that year, on December 7, Cope expanded upon his theory of the tritubercular molar in a paper read before the American Philosophical Society on December 7. This paper, titled "On the Trituberculate Type of Molar Tooth in Mammalia," extended the theory to include lower molars: "The tritubercular or triangular superior molar is associated with a corresponding form of the anterior part of the inferior molar" (Cope 1883b, 325).

Cope's inclusion of lower molars within the tritubercular theory is significant for two reasons. First, the structural sequence of the lower molars during mammalian evolutionary history, and especially the homological nature of their cusps, would become the strongest evidence in support of the tritubercular theory. Second, with the addition of the lower molars, Cope could address the correspondence in form between the upper and lower molars, and speak to the importance of mechanical action in the origin of molar form.

2.4 Cope and the Mechanical Causes of Evolution

Bowler has noted that over the course of his career, Cope committed himself to a neo-Lamarckian vision of evolution—one that hinged on utility and use of morphology during an organism's lifetime as causal in the origin of form (Bowler 1977). This is particularly clear in his treatment of the development and evolution of the "hard parts" of mammals, especially the teeth. Over the course of three decades, Cope published an incredible number of notes and treatises highlighting the mechanical causes of evolution, particularly in teeth.[38]

Cope's mechanical causes of evolution are intimately tied to his perception of a growth energy.[39] Growth energy, and a concomitant motion of parts, was the major factor in determining growth, and especially the individual differences in growth that Cope saw as the basis of organic evolution. According to Cope, "…the course of growth (ontogeny) is determined by motion from sources external to the germ cell. It is this which modifies ontogeny and produces those changes of structure which constitute Evolution. To this aspect of growth I have given the name kinetogenesis" (Cope 1894, 212). Cope's theory of kinetogenesis tied evolutionary change to utilitarian, Lamarckian principles, and allowed him to give an account of evolution that was both mechanical and had causes.[40]

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[38] Note that Cope was an incredibly prolific writer and researcher. Rainger (1992) has noted that Henry Fairfield Osborn, Cope's student and biographer, claimed that Cope identified 1,282 new species and genera of fossils over the course of his career, which is incredible given Cope's specialty was vertebrate paleontology.

[39] According to Rainger (1981), Cope's growth energy was directed by the conscious choice of the organism (in the higher animals, at least)

[40] This is some squishy, metaphysical malarkey.

It also meant that in Cope's framework, evolution and morphological diversity were caused by ontogeny.

2.5 Recap: Cope, Morphological Complexity, and the Origin of the Tritubercular Theory

Edward Drinker Cope held complex and nuanced views of evolution and how morphological diversity arises. His research on his fossil finds from New Mexico and Wyoming reflected his desire to classify and order nature, and his tritubercular theory arose as part of this program. These views were built-up over decades of research comparing modern and extinct species, and included a sense of directed evolution, a desire to parse his fossil finds into morphological types, and the belief in a causal connection between ontogeny and phylogeny (wherein the changes built up during development in response to the functional needs of the organism were the raw material of evolutionary change). All of these assumptions became tied to his tritubercular theory and shaped the way the theory was perceived and discussed by his contemporaries and successors.

2.6 Osborn, Mesozoic Mammals, and the Tritubercular Molar

At the time of his death in 1935, Henry Fairfield Osborn had amassed an astounding 940 publications, ranging from brief communications to full-length monographs. The most important of his scientific works centered on vertebrate paleontology, and recurrent themes throughout his corpus include evolutionary theory and principles, human evolution, eugenics, and educational methods.[41] Osborn's academic training began at Princeton University, where, among other things, he studied psychology and zoology. His first taste of paleontological fieldwork came in 1877 when he joined the Princeton Scientific Expedition to the Bridger and Washakie Basins in Wyoming. Upon graduating from Princeton in 1879, Osborn entered Cambridge University for graduate study in zoology. There he learned embryology from the brilliant Francis Maitland Balfour. While in England, Osborn also attended Thomas Huxley's lectures on comparative anatomy at the Royal College of Science in London.

Osborn's academic training brought him into contact with some of the brightest evolutionary theorists, embryologists, and comparative anatomists of the day, and much like Cope, his training in comparative anatomy and embryology shaped his understanding of vertebrate evolution (Rainger 1985). These views of evolution aligned largely with Cope's—he understood evolution to occur in a directed fashion, sought to group organisms into morphological types, and held the belief that phenotypic changes during development (in response to the functional needs of the organism) led to gradual evolutionary change. He carried these beliefs with him throughout his career, and although he modified them over time, they served as foundational assumptions within his articulations and defenses of the tritubercular theory.

Osborn's commitment to the tritubercular theory arose out of his work with collections of Mesozoic mammals from North America and England.[42,43,44] In 1888,

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[41] Rainger (1985) has argued that Osborn's evolutionary theorizing, while prevalent throughout his career, was not widely accepted by his contemporaries.

[42] Note that Osborn worked with Mesozoic (mostly Jurassic) fossils, whereas Cope had built the theory upon an Eocene sample. Osborn's sample thus pushed the timeline of trituberculy back nearly 100 million years.

Osborn published his monograph "On the Structure and Classification of Mesozoic Mammalia," in which he set out to review and update the descriptions and general classification of Mesozoic mammals and "to discuss the dentition of these genera in its bearing upon the origin and succession of mammalian tooth forms" (Osborn 1888a, 190). In this early text, Osborn declared his commitment to the tritubercular theory, and so it behooves us to understand what Osborn was up to within this text. Osborn's classification, based entirely upon study of the dentition seeks to answer pertinent questions of the day, including: Should Mesozoic mammals be classified as marsupials, insectivores, or both?[45] And, have all modern insectivores passed through a marsupial evolutionary stage?

Osborn made close study of all of the Mesozoic fossils of North America and England to which he could gain access.[46] Like most paleontologists of his day, he spent the majority of his text creating detailed descriptions of specimens in order to construct classifications (Rainger 1985). His classifications aimed to order nature, and due to the

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[43] Owen's collection at the Geological Department of the British Museum; *Plagiaulax* from the Rheims (Lemoine); Puerco (Cope); Mesozoic mammals (Marsh); North American Triassic specimens from Williams College and the Philadelphia Academy of Science; *Ampitherium*, *Amphitylus*, and *Amphilestes* (Lydekker). See: Osborn, 1888a, page 190, for a breakdown of where he got his specimens.

[44] Osborn reflected that, "It was while studying the rich collection in the British Museum that I became convinced of the force and universal application of the tritubercular theory proposed by Cope" (Osborn 1892, 747)

[45] Osborn (1888a) notes that his classification of Mesozoic mammals is based solely upon the dentition because of the limitations of the fossil materials, i.e. limb bones were rare and difficult to assess, and many of the genera present were represented only by their mandibular dentitions. Comparing the usefulness of the dentition for understanding phylogeny and evolution, versus that of the limbs for these topics, was a frequent occurrence for Osborn. This may be related to the prevalence of research on the evolution of the tetrapod limb, as well as its frequent usage for establishing phylogenies.

[46] Rainger (1985) has pointed out that even by the turn of the 19th century, the total number of fossil species and genera known was incredibly small. For example, American collections did not exceed 5000 fossilized species and genera. This means that, while Osborn collected a large sample for his time, he was working with very limited materials, both in terms of specimens representing a species/genus and in terms of generic/specific diversity.

materials at hand, this ordering was based on dental morphology. As Rainger has argued, Osborn's "Structure and Classification," as well as later works, "emphasized the existence of orderly patterns of change" (Rainger 1985, 285), rather than constructing detailed evolutionary lineages. In most instances, this meant creating descriptions solely from type specimens or very small samples.[47] In several cases (genera of the lower Purbeck, *Stereognathus*, and some of the molars of the Rhaetic *Microlestes* and *Hypsiprymnopsis*), Osborn was forced to rely on the figures and descriptions produced by Richard Owen (Owen 1871).

Osborn began "Structure and Classification" with detailed descriptions of 16 genera of British Mesozoic mammals, for which he furnished illustrations of the type specimens in accompanying plates. His discussion of structure in these genera is followed by an attempt at classification, wherein he notes that materials are limited. This classification begins with the separation of his samples into two groups: Multituberculata and everything else.[48,49] He continued his classification with succinct descriptions of a handful of genera in each of these groups. In addition to his succinct morphological descriptions of these specimens, Osborn used illustrations throughout the text to highlight comparative structural details.

The range of Mesozoic fossils available for Osborn's inspection at the time, while not diverse or numerous by modern standards, included enough morphological and

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[47] Type specimens corresponds to what Farber (1976) has called the "natural history collections" understanding of the type concept in the 19th century. Type specimens are objects designated as the name bearer of a taxon and are used to help classify (and reclassify) other specimens of unknown taxonomy.

[48] The classification and phylogenetic affinities of the multituberculates are *still* contested.

[49] While this may seem odd, multituberculates are just really weird. Seriously.

taxonomic diversity for him to be concerned about how best to classify members of his second group (all mammalian fossils, except multituberculates):

> …the mammals of this group are so distinct from the Multituberculata that their zoölogical position must be considered separately, and, unlike this order they conform so little to a common type that when the approximate systematic position of one genus or family has been determined, it by no means settles the question in regard to the remainder. Do they belong to a distinct order? Are they exclusively Marsupials or Insectivores, or do they stand in ancestral lines leading to each of these orders? These are the three forms of the problem, which are conditioned by the wider question whether the Placentalia have ever passed through the marsupial stage, with a peculiar yolk-sac placenta and restricted milk dentition. (Osborn 1888a, 256)

While Owen had preliminarily found within the Mesozoic mammalian fossils evidence for transition to both modern marsupials and insectivores, the prevailing opinion among paleontologists of the time was that Jurassic mammals should all be classified with the marsupials (Osborn 1888a). Osborn weighed in on this issue, noting that,

> The grouping of all these genera in one distinct order is, however, impracticable; first, because the members of at least one family present distinctively marsupial characters; second, it is impossible, with our present knowledge to adding a single character of ordinal value which is universal; third, as to the minor question of systematic arrangement, there is no precedent for including in one order, such types as Kurtodon, Stylacodon and Triconodon, in which the teeth are as diverse as in the recent Rodentia, Insectivora and Carnivora. (Osborn 1888a, 257)

and,

> The supposition that *all* these mammals can be placed in the Marsupialia is equally untenable, or, at least, it may be said to rest upon no foundation whatever. It has been the fate of numerous primitive mammals, at the period of their discovery, to be placed without much reason or question in this order. (Osborn 1888a, 257 [emphasis from author])

Osborn's interest in placing the evolutionary relationships between the Mesozoic mammals and more modern mammalian taxa led him to inspect further their classification with respect to their placement with marsupials and insectivores. He found evidence that *Triconodontidae* should be placed within the marsupial line, while *Amphitheriidae* and *Kurtodontidae* were possibly ancestral to marsupials as well, but their morphological characteristics were too mixed to be definitive. *Stylacodontidae*, on the other hand, displays characteristics that grouped them as primitive insectivores. These characters, the dental formula, and, more notably for our purposes, the presence of tritubercular molars, distinguished them from the other Jurassic groups. Thus, for Osborn, there was an evolutionary branching of mammals distinct already within the Mesozoic fauna, with the hallmark of passage towards placental mammals being the presence of a tritubercular molar form.

Following his examination of these Mesozoic mammals, Osborn endorsed the tritubercular theory:

> ...the derivation of the mammalian molar from the single reptilian cone can be demonstrated by the comparison of a series of transitional stages between the single cone and the three-cone type, and from the latter to the central tritubercular type...The remarkable part played by the tritubercular molar has been unfolded by the discoveries and writings of Cope. It is undoubtedly the ancestral molar type of the Primates, the Carnivora, the Ungulata, the Cheiroptera, the Insectivora, and of several, if not all, of the Marsupialia. (Osborn 1888a, 242)

Much like Cope, Osborn's endorsement of trituberculy did not amount to a formalized statement of theory. In fact, at first, Osborn did not refer to Cope's tritubercular theory as such; he simply called it "trituberculy" or referenced the "tritubercular type," later it was a "law," and finally, by 1897, it was a "theory" (Osborn 1888b, 1888c, 1897). Regardless of what it was called, in 1888 Osborn embraced the notion of a tritubercular type as a bottleneck for morphological diversity for subsequent mammalian dental evolution, that is, he recognized the tritubercular molar as a morphological type. And, within this early adoption of the tritubercular theory, Osborn had already begun to lay out his views on such things as types, homologies, progress in evolution, and kinetogenesis, which he would doggedly carry as assumptions throughout his later defenses of the theory.

2.7 Osborn's Molar Types

Osborn sought to understand the evolution of the dentition within Mesozoic mammals leading to insectivores and placental mammals. He noticed, like many before him, that mammalian molars could be grouped according to the number and arrangement of cusps on the tooth crown. These groups constituted morphological types that could be sorted into an orderly, hierarchical pattern. While many previous authors had discussed the existence of types, Osborn noted, that,

> The dentition in the recent Mammalia is so diverse that the most sanguine evolutionist of fifteen years ago could not have anticipated the discovery of a common type of molar, in both jaws, as universal among the Mammalia of an early period as the pentadactyle foot, and as central in its capacity for development into the widely specialized recent types. (Osborn 1888b, 1067)

In establishing the tritubercular theory, Cope had noted the existence of types, much in line with what Osborn determined (Cope 1883a). However, unlike Cope, who recognized mammalian molar types as constituting homologous entities (at the level of the entire crown), Osborn developed a more nuanced understanding of the relationship between the mammalian molars that was dependent upon two factors: progress and cusp homologies.

FIG. 2.

Figure 4. Osborn's sequence of molar types. From Osborn 1888b, Plate XXV, Figure 2. "Molars of opposite jaws in normal mutual relation." (1) Haplodont; (2) Protodont, (3) Triconodont, (4) Tritubercular, etc.

2.7.1 *Types and Progress*

A major component of delineating orderly patterns, for Osborn, was the determination of the stages, or types, within a mammalian hierarchy of dental complexity:

> As genera fall into smaller groups we at once observe that these groups show a more or less clearly marked specialization for a certain kind of diet, which is usually manifested most clearly in one genus, which we may therefore speak of as typical of the group. The allied genera diverge more or less widely from this type, which, it must be understood, is not selected as the most primitive or the most central but as showing the most clearly defined functional adaptation.[50] While some genera are thus functionally *typical* others are *transitional*, that is, they show a divergence from the central type towards different kind of adaptation. Still other genera are *isolated*; they do not approach other known types but stand apart by themselves, either because few specimens have been found and we are less familiar with their structure, or their molar pattern does not conform with that of any other known genus, or represent a distinct type. (Osborn 1888a, 224 [emphasis from author])

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[50] It is interesting to note that, unlike Cope, Osborn believed that mammalian molar types (and even the tritubercular type) evolved independently in different mammalian groups from a hypothetical ancestor. See: Rainger, 1991 for more information.

Osborn thus developed an understanding of the mammalian dentition leading to placental mammals as a structural progression of types, and types were representative of the functional adaptation of a group of genera. These types were ordered according to the complexity of their molar crowns, in terms of number and arrangement of cusps (figure 5).

The notion of progress in nature, or directed evolution, was a key factor influencing Cope's development of the tritubercular theory. Like Cope, Osborn also adopted both a Neo-Lamarckian and a directed theory of evolution predicated upon the assumption of progress in nature (Rainger 1985).[51] That is, evolution proceeded along certain definite lines. This is apparent even in this early work on mammals, and would become a critical assumption for his defense of the tritubercular theory, his evolutionary theory, and underpin his subsequent research. Osborn envisioned functional adaptation, or the functional need of the organism to adapt to a certain diet, as the driver of form change on both developmental and evolutionary timescales.

In recognizing a functional connection between dental form and diet, Osborn was following in a long tradition. As had many naturalists before him, Owen noted that,

> The dental system thus presents many and peculiar attractions to the anatomist and naturalist, for independently of the variety, beauty and even occasional singularity of the form and structure of the teeth themselves, *they are so intimately related to the food and habits of the animal as to become important if not essential aids to the classification of existing species*. (Owen 1840, Introduction [emphasis added])

In fact, the link between diet and dental structure was a common theme for naturalists from the mid-18th through the 19th centuries, and remains so today. For

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[51] As Rainger (1981, 1985, 1991) has noted, later in his career, Osborn shifted away from his Neo-Lamarckian predilections.

instance, in 1749 Comte de Buffon noted in the first volume of his *Histoire Naturelle* that: “The teeth are also fitted to the nature of their food. In those which live upon flesh, they are sharp, and fitted for holding and dividing; in those which subsist on vegetable diet, they are calculated for pounding or grinding their aliment.” (Buffon and Wright 1831, 216)

Nearly a half-century later, the relationship between dental form and diet provided Georges Cuvier with the example for his principle of the conditions of existence—that is, the parts of an animal must be in harmony with the animal’s environment and mode of life: [52]

> If an animal's teeth are such as they must be, in order for it to nourish itself with flesh, we can be sure without further examination that the whole system of its digestive organs is appropriate for that kind of food, and that its whole skeleton and locomotive organs, and even its sense organs, are arranged in such a way as to make it skillful at pursuing and catching its prey. For these relations are the necessary conditions of existence of the animal; if things were not so, it would not be able to subsist. (Cuvier 1798; translation from Rudwick 2008, 50)

In his second paper on the tritubercular theory in 1888, Osborn clarified his position on functional adaptation (Osborn 1888b). He saw two hypotheses that could account for the change in dental form: first, Darwinian evolution, that new forms have, “been acquired by the selection of accidental variations in the production of new cusps and modelling of old ones” (Osborn 1888b, 1074). The second hypothesis was Cope’s theory of kinetogenesis, that “the interaction of the upper and lower molars in the movements of the jaws has resulted in local increase of growth at certain points, resulting

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[52] Cuvier saw the principle of the conditions of existence as a tandem to his principle of the correlation of parts—i.e. that there must exist a functional relationship between the parts of the organism.

first in new cusps, then in a change of position and of form in the cusps" (Osborn 1888b, 1074).

Based on available evidence, Osborn came to the conclusion that, while the two hypotheses are not mutually exclusive, the "balance of evidence in tritubercular evolution seems to favor the second or kinetogenesis theory" (Osborn 1888b, 1074). Thus, in accordance with Cope, Osborn saw movement in response to dietary needs as driving localized changes in form during development, which resulted in gradual changes on an evolutionary timescale.[53] These localized increases in growth occurred "not at random, but according to a certain definite order" that led phyla through a series of stages from the pre-mammalian haplodont through triconodont and tritubercular stages and on within the mammalian clade to give rise to the diversity of mammalian molar patterns that exist today (Osborn 1888b, 1067).

2.7.2 *Types and Cusp Homologies*

Osborn's adoption of kinetogenesis meant that there was a definite and directed modification of molar structures throughout mammalian evolutionary history that was built upon the gradual modification of extant structures. This principle became central to both his understanding of the structural progression of types, as well as the homological concept of tooth cusps that he built on top of this understanding.

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[53] The theory of kinetogenesis still gives no indication about how localized movements are driven to change.

According to Osborn, the structural progression of types was evidence for there being homological relationships between cusps (see figure 6):[54]

> A nomenclature may be suggested for these cones, with reference to their order of development and primitive position, to keep clearly before the mind their homologies during secondary changes of form and position. The primitive cone may be called the protocone; upon the anterior and posterior slopes of which appear, respectively, the paracone and metacone. (Osborn 1888a, 242)

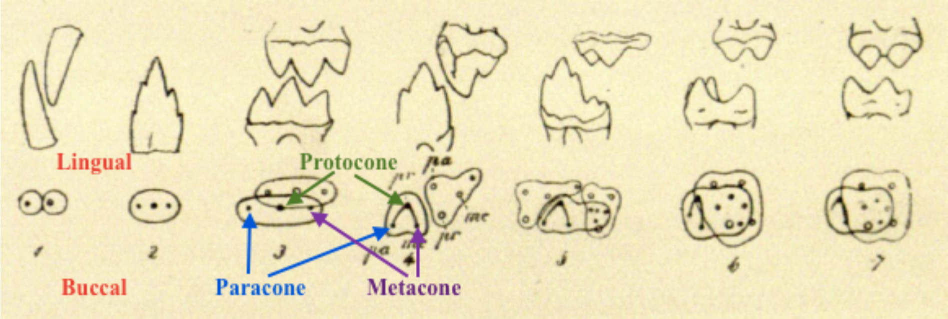

FIG. 2.

Figure 5. Osborn's sequence of molar types with cusp labels. Modified from Osborn 1888b, Plate XXV, Figure 2. Labeled to show homologous cusps across molar types (triconodont [left] and tritubercular [right])

Initially, Osborn's homology scheme was built around the three main cusps of the tritubercular tooth: the protocone (antero-lingual), the paracone (antero-buccal), and the metacone (postero-buccal), with the addition of the hypocone (postero-lingual) to denote the 'heel' of the crown that forms the tubersectorial molar (Osborn 1888a).[55] Osborn quickly expanded upon this early nomenclature to include the protoconule (antero-intermediate) and metaconule (postero-intermediate) (Osborn 1888c).

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[54] Osborn did not elaborate on his concept of homology.

[55] Note that these terms are for the upper dentition; the lower dentition is distinguished in its nomenclature by the addition of the suffix –id, such that the protocone becomes the protoconid, the paracone becomes the paraconid, and the metaconid becomes the metaconid.

This nomenclature was meant to both codify a disparate system of naming cusps according to their relative position on the tooth crown, as well as to indicate the homological relationships between cusps across the mammalian dentition: “The terms for the three main cusps are selected to indicate, as far as possible, the *primitive position* and the *order of evolution*" (Osborn 1888c, 927 [emphasis added]). By primitive position and order of evolution, Osborn meant that, “the separate history of each cone can certainly be traced throughout the series in its various degrees of modification, development, and degeneration” (Osborn 1888a, 242).

Thus, in Osborn’s homological system, the protocone represents the primitive cone—it is homologous to the reptilian haplodont cone that preceded the explosion in dental diversity within Mesozoic mammals. Through gradual modification over developmental and thus evolutionary timescales, additional cusps were built up around this initial cusp, giving rise to other homologous cusps (the paracone and the metacone) and, subsequently, a range of dental types.

Osborn’s application of homology to the dental cusps both spurred the creation of a nomenclature that is in use to this day, and conjoined developmental and evolutionary processes. Osborn’s homology concept for the cusps necessitated that the developmental sequence of cusp formation follow the order of evolution, such that the protocone appeared first during ontogeny. In this way, Osborn united ontogeny and phylogeny, with the former causing the later. Although Osborn’s homology concept led to the creation of a nomenclature that is still the standard of dental morphologists to this day, it was also a point of great contention for the theory when embryologists discovered that the protocone is not the first cusp to appear during development (Röse 1892; Taeker 1892).

2.8 Osborn and the Tritubercular Theory

Henry Fairfield Osborn held a complex and nuanced understanding of the relationship between ontogeny and phylogeny, and the process of evolution. His foray into sorting out the evolution of morphological diversity within mammalian molars was one of his earliest research projects and showcased his desire to classify, order, and understand nature as the result of a progressive and directed evolutionary process. Within this early work on the fossils of the British Mesozoic, Osborn found support for Cope's tritubercular theory and elaborated on it with his own insights into types and homology. These concepts were built upon his understanding that evolution was driven by the gradual and progressive alteration of parts during development—particularly the cusps. Cusps, to Osborn, held a special place for investigating the evolution of morphological diversity because their development also reflected their phylogenetic origins. Thus, for Osborn, ontogeny caused phylogeny and phylogeny was engraved within the ontogeny of the organism. These assumptions that Osborn built into his interpretation of the tritubercular theory and future research became tied to the theory and acted as points with which subsequent researchers would particularly contend.

Osborn continued to endorse and recapitulate the tritubercular theory for the next several decades, defending it against objections from paleontologists whose fossil evidence seemed to run contrary to his own as well as against evidence wrought from embryos (Chapter 4.4). This embryological evidence, uncovered by Carl Röse and Julius Taeker, challenged Osborn's notion of homology and led him to retaliate against the works of these embryologists, as we will see in Chapter 4 (Röse 1892; Taeker 1892).

2.9 The Tritubercular Theory, by the End of the 19th Century

By the end of the 19th century, the tritubercular theory had become the most widely known theory of mammalian dental evolution, to the point where it was discussed across paleontology manuals (Major 1893, Tims 1903). Its ubiquity provoked one dental researcher to proclaim that, "Trituberculism, or, as we rather ought to call it, the reptilian-cone theory, is more than a theory, but has become a dogma" (Major 1893, 198). It was widely accepted among American morphologists, such as William Berryman Scott, Harrison Allen, and Charles Earle. It also met with approval from paleontologists on the European continent, such as the German paleontologist Max Schlosser, who grounded his mammalian phylogenies on the principle of trituberculy.[56]

The popularity of this theory is largely due to the great number of publications that Osborn produced on the topic—it was a frequent source of discussion for him throughout the 1890s and 1900s. However, while the tritubercular theory met with broad acceptance it also met with a host of objections.

These objections came from practitioners in multiple disciplines. Some, like J.L. Wortman, the curator of vertebrate paleontology at the Carnegie Museum and a field paleontologist for Cope, took issue with the inability of the theory to accommodate multituberculates, as well as problems with Osborn's adherence to cusp homology and its

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[56] See: Schlosser 1888, 1889, 1890

concomitant nomenclature (Wortman 1903).[57, 58] This issue with the cusp nomenclature would become a recurrent source of debate for the tritubercular theory, drawing attention from researchers across traditions, like Albert Fleischmann, a zoologist at the University of Erlangen, and Carl Röse, a dentist and embryologist (Fleischmann 1891; Röse 1892).[59] The latter figure would become the leading proponent of the concrescence theory—a theory that dealt with the problem of how mammalian morphological diversity arises, but from an embryological perspective.

2.10 Assumptions of the Tritubercular Theory

The tritubercular theory of Cope and Osborn was meant as a generalization about a pattern observed within the fossil record of mammals. It addresses the problem of how mammalian molar morphological diversity arose and attempts to account for patterns of molar groupings within the fossil record. And yet, its two main proponents understood this pattern and the theory in light of a host of assumptions. Assumptions such as the gradual and directed nature of evolution, the primacy of fossil evidence, the unidirectional causality from ontogeny to phylogeny, and the nature of types and homologies. And, while Cope and Osborn recognized an inextricable link between development and evolution, their theories and research programs did not seek to explain the processes by which different cusp patterns develop—i.e. they did not seek to explain

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[57] Recall that in establishing his classification of Mesozoic mammals, Osborn separated out multituberculates from other mammals (Osborn, 1888a). His subsequent development of the tritubercular theory notably left out multituberculates.

[58] For more about objections to Osborn's homology concept and the nomenclature for the cusps that he derived from it, see Chapter 4.3 and 4.4.

[59] Note that many researchers weighed in on the tritubercular theory. This is just a small sample to show the range of disciplines and traditions that found the theory wanting.

morphogenesis. Development, to both of these men, was black-boxed within Cope’s theory of kinetogenesis, and this served as an adequate way of explaining the changes during development that they saw as driving morphological diversity. These assumptions, while never part of the formal apparatus of the tritubercular theory, led to conflict from the 1890s on. In Chapter 4 we see how these assumptions were connected to the tritubercular theory and how the debate that arose around them played out.

CHAPTER 3

CARL RÖSE AND THE CONCRESCENCE THEORY

3.0 Introduction

In the previous chapter, we saw how Edward Drinker Cope and Henry Fairfield Osborn constructed the tritubercular theory as a result of noticing general patterns of molar form while working with fossilized dentitions. Their goal was to explain the morphological diversity they observed in the fossil record, and their theory arose as an extension of their observations of fossils. While the tritubercular theory was often stated as a simple description of the pattern of molar forms through which mammals passed, it was infused with a number of assumptions about what counts as evidence, how evolution works, and about the relationship between ontogeny and phylogeny. These assumptions, including a type and homology concept as well as the understanding that changes during development (in response to the functional need of the organism, e.g. kinetogenesis) drove the gradual change in phenotypic diversity seen in the fossil record, amounted to a scientific framework for their tritubercular theory in which ontogeny caused phylogeny (and an embrace of a weak form of the biogenetic law). These assumptions were foundational to the tritubercular theory, but were never formally integrated into it (Chapter 4).

In this chapter, we meet another theory that explains the morphological diversity within mammalian molars—the concrescence theory—and its main proponent—Carl Röse. Unlike Cope and Osborn, whose goals were to explain what they perceived of as natural groupings of molar patterns within the fossil record, Röse's goal was to explain how to get complex molar forms from originally simple developmental structures. Although Röse developed his theory to explain a developmental process, he did not take morphogenesis into account—that is, his theory does not explain how the cells and tissues form the cusps during development or how they coalesce, it simply explains how different cusp patterns arise through development. In this respect, Röse's theory is akin to the tritubercular theory of Cope and Osborn, both of whom neglected morphogenesis and explanations of how, exactly, morphological diversity arises on a developmental timescale. Röse, unlike Cope and Osborn, did not begin from observations of a pattern so much as from the assumption of the primacy of the biogenetic law. This assumption of the strong form of the biogenetic law drove his interpretation of the evidence that he accumulated for dental development and led to his creation of the concrescence theory—a theory that explains a process within embryos.

At first glance, the tritubercular theory and the concrescence theory are not incompatible—that is, one describes a pattern within the fossil record, while the other describes the process of how molar patterns form during development. However, the assumptions that the proponents of each theory held for their respective theories led to conflict between the two that brought different ways of tying development and evolution together into tension at the end of the 19th century.

This chapter tracks the development of the concrescence theory, beginning with a brief history of predecessors to Röse's theory, followed by Röse's dissertation work on the development of the vertebrate heart which led to his acceptance of a strong form of the biogenetic law. Röse's adamant acceptance of the biogenetic law became foundational for his future work on tooth development and colored the way in which he understood and explained dental complexity. As Röse formulated the concrescence theory and applied it widely throughout the vertebrate clade as part of his research program on dental development, the biogenetic law served as a means through which his work connected phylogeny with ontogeny, with the former as the causal agent.

3.1 The Context of Concrescence: Dental Development in the 19th Century

While the concrescence theory of dental development and evolution is largely forgotten today, it was proposed independently several times and became a topic of heated discussion within certain circles during the last quarter of the 19th century.[60,61,62] The concrescence theory, broadly conceived, states that the complex dental forms that we see in toothed organisms, particularly mammals, are formed by the coalescence or concrescence during development of initially independent cusps. The individuals who formulated this theory (or string of theories) sought to describe and explain the complicated forms of molars that they witnessed across genera of mammals (and some

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[60] In addition to the concrescence theory of dental development, around the same time arose the concrescence theory of the vertebrate embryo, which states that, "…the vertebrate primitive streak is formed by the growing together in the axial line of the future embryo of the two halves of the ectental line." (Minot 1890a, 514). While Minot (1890a) traces the origins of this theory to observations made by Rathke in 1862, it did not gain popularity until the 1890s. Minot (1890a, 1890b) provides a nice overview of the history of and evidence for the concrescence theory of the vertebrate embryo.

[61] There is at least one current group of collaborators that adheres to a concrescence theory of mammalian molars—the laboratories of Miroslav Peterka (Laboratory of Embryogenesis) and Renata Peterkova (Laboratory of Odontogenesis) that make up the Department of Teratology at the Institute of Experimental Medicine (an EU Centre of Excellence) in the Czech Republic, and the laboratories in France of Laurent Virio and H. Lesot. See: Peterkova et al. (2000) and Peterkova et al. (2002).

[62] Interestingly, these circles appear to break down into American paleontologists and German embryologists.

other scattered clades). The earliest proponents of this theory were paleontologists, broadly scattered around the globe, followed subsequently by embryologists. These investigators appear to have developed the idea of dental concrescence independently, and in the early years, the theory garnered little recognition or interest from any research community.

In 1878, Jean Albert Gaudry, the chair of paleontology at the Museum of Natural History in Paris, noted that,

> If we compare the complicated form of ruminant molars, canines, and incisors with most animals, or with the anterior premolars of land mammals, or to the rear molars of dolphins and some fossil mammals of the Mesozoic period (*Stylodon pusillus* Owen), we must get the idea, that they are composed of several individual teeth, which have moved close to each other and intimately fused, similar to what occurs in other skeletal parts. (Gaudry 1878, translation quoted in Röse 1892e, 625)[63]

In 1880, Florentino Ameghino, the Argentine naturalist and paleontologist, wrote,

> …it is evident that all mammals which possess compound teeth have in past periods possessed a very much larger number of teeth, but of quite simple conical form, like those of the modern dolphin. The most primitive mammals must also have had a number of very elevated teeth, but it is difficult at the present time to determine how large this number was. Nevertheless, if we take as an example a mammal in which the dentition is complete, as in the *Macrauchenia* or in the horse, and if we reduce ancestors of these forms must have possessed more than one hundred and fifty teeth. This number is certainly not exaggerated, because *Priodon*, the giant tatusia, a mammal in an already quite advanced stage of evolution, possesses nearly one hundred simple teeth, and in the dolphin this number rises from one hundred and fifty to one hundred and seventy. (Ameghino (1880) translation quoted in Osborn 1895, 5)

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[63] "Wenn wir die komplizierte Form der Wiederkäuermolaren vergleichen mit den Kaninen und Incisiven der meistern Tiere oder mit den vorderen Prämolaren der Landsäugetiere oder mit den hinteren Molaren der Delphine und einiger fossiler Säuger der Sekundärzeit (*Stylodon pusillus* Owen), so müssen wir auf den Gedanken kommen, dass sie aus mehreren Einzelzähnen zusammengesetzt sind, welche einander nahe gerückt und innig verschmolzen sind, ähnlich wie dies häufig bei anderen Skeletteilen der Fall ist."

Neither Gaudry nor Ameghino, both paleontologists, attempted to gather embryological evidence in support of their claims, and their contributions to a concrescence theory were not recognized until the mid-1890s (Osborn 1895). In 1883, Emile Magitot, a forerunner in experimental dentistry, and coincidentally one of the first people to perform *in vivo* tissue recombination experiments on dental tissues (Glasstone 1936), stated that, “One is pushed to the conviction that the so very diverse tooth shapes all arise from a common archetype, as we find it in fish. This archetype is the cone-shaped tooth. The projections and tubercles of the individual mammal teeth correspond to the individual cone-shaped teeth” (Magitot translation quoted in Röse 1892e, 626).[64,65] While Magitot sought embryological evidence to confirm his hypothesis, his work in this area went largely unrecognized.

The concrescence theory only gained traction in the early 1890s after two German embryologists independently proposed it within a short time span. The first to propose the concrescence theory from embryological evidence was Willy Kükenthal, who was at the time a professor of phylogeny at the University of Jena. Kükenthal introduced his version of the concrescence theory in a brief communication in 1891 (Kükenthal 1891).

After examining the jaws of 30 baleen whale fetuses both as whole mount specimens and as serial sections, Kükenthal noticed that there were several instances in which the hind teeth of the fetuses appeared to have fused into double teeth, begging the

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[64] “Man wird zur Ueberzeugung gedrängt, dass die so sehr vershiedenartigen Zahnformen alle aus einem gemeinsamen Urtypus entstehehn, wie wir ihn bei Fischen finden. Dieser Urtypus ist der Kegelzahn.Die Vorsprünge und Tubercula der einzelnen Säugetierzähne entsprechen einzelnen Kegelzähnen.”

[65] Shirley Glasstone is a fascinating character. In 1936, she became the first person to culture teeth *in vitro* (Glasstone 1936).

question, “Are these double teeth secondary unions or primitive conditions?” (Kükenthal 1891, 366) [66,67]

The answer, Kükenthal believed, lay in *Entwickelungsgeschichte*. By looking to specimens both younger and older than the ones in which he noticed double-teeth, Kükenthal came to the conclusion that the double-teeth were the original condition, representing what might be thought of as a molar, and that the conical teeth in that location in older specimens was the result of division of those developmentally earlier molars. Kükenthal also observed the existence of a correlation between jaw length and tooth crown complexity; namely, that reptiles, which tend to have simple, conical teeth, have long jaws, whereas mammals, which have molars of varying cusp complexity, have shortened jaws.

Based on the correlation between cusp complexity and jaw length, as well as the degradation of molars in baleen whales from complex to simple, Kükenthal reasoned that cusp simplification in whales was due to the lengthening of the jaws, and the converse, that jaw foreshortening during development (and evolution) led to cusp concrescence. Additionally, Kükenthal took his observations of molar degeneration during whale development as evidence for the origin of homodont dentition from heterodont dentition.[68]

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[66] Kükenthal (1891) looked at the following species: *Megaptera boops*, *Balaenoptera rostrata*, *Balaenoptera sibbaldii*, *Balaenoptera musculus*

[67] “Sind diese Doppelzähne secundäre Verschmelzungen oder primitive Zustände?”

[68] With regards to the concrescence theory, and especially Kükenthal’s version of it derived from whale tooth development, Marett Tims (1903) notes that, “Little or no weight attaches to the evidence derived from the disintegration process of the cetacean molars.”

Despite his being the first to push for a concrescence theory from embryological evidence, Kükenthal's is not the name most commonly associated with the theory, nor is he the researcher who drew the most attention from Cope and Osborn, whose tritubercular theory stood in conflict with this way of interpreting mammalian dental evolution. Carl Röse, a dentist trained in the laboratories of Carl Gegenbaur, and Richard and Oscar Hertwig, and who early on was a researcher invested in understanding the development and evolution of mammalian dentition, is most famously associated with the concrescence theory and drew the attention of Henry Fairfield Osborn during the last decade of the 19th century. What follows is a history of Röse's concrescence theory and an attempt to understand the conflict that arose over explaining the origins of morphological diversity within mammalian teeth.

3.2 Röse's Early Work and the Biogenetic Law

Röse began his dissertation research on the anatomy of the human fetal heart at the Munich University Frauenklinik in 1888. After he had completed his analyses of the histological structures of the human fetal heart, he applied to work with Carl Gegenbaur in Heidelberg. Gegenbaur pushed Röse to investigate earlier, embryonic stages of development, for which Röse found difficulty acquiring specimens (Nickol 1992). Because human materials proved unavailable, Röse worked with rabbit embryos, relying on the practices of comparative anatomy.

Early in 1888, Röse moved from Gegenbaur's Anatomical Institute in Heidelberg to the Zoological Institute in Munich where Richard Hertwig offered him more time and specimens to pursue his investigations.[69] In Munich, Röse began his research on the comparative anatomy and development of the vertebrate heart with the explicit desire to demonstrate the extent to which the individual stages of development of the mammalian heart could be attributed to the states of the heart in the lower vertebrates (Röse 1889, 1890). Röse, it appears, sought explicit evidence for the biogenetic law.[70] In Munich, Röse was able to amass a collection of specimens that included embryonic to adult developmental stages, as well as multiple species across the fish, amphibian, reptilian, avian, and mammalian clades.[71] Utilizing the anatomical techniques that he had learned in the laboratory of Gegenbaur, Röse compared several primary components of the heart's structure across the vertebrates: the sinus venosus, the pulmonary veins, the atrial septum, the ventricular septum, and the atrioventricular valves.

It appears that many of the specimens that Röse utilized for this study came to him already preserved. In the instances in which he was able to collect a live specimen, Röse injected it with either chromic acid or alcohol and performed gross dissections. For the few embryological specimens that Röse was able to obtain, he dissected them either under a magnifying glass or created and examined serial sections (Röse 1890). It is interesting to note that most of the embryonic specimens that Röse examined were mammals, whereas the vast majority of the other clades were represented with adult specimens.

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[69] According to Röse (1889) these investigations arose in part as a response to Gustav Born's (1888) work on the heart.

[70] It is unclear why Röse wanted evidence for the biogenetic law. However, it may stem from his exposure to Haeckel's zoological lectures in 1885/1886, and his attendance of Haeckel's zoological praktikum. See Nickol (1992) for further discussion of Röse's personal history and motivations for research.

[71] See: Röse, 1890; 29-30 for list of species

Two years after his move to Munich, Röse published his findings on the development and evolution of the vertebrate heart in Gegenbaur's influential *Morphologisches Jahrbuch*. This 75-page tome, which included 23 illustrations of vertebrate heart dissections, stands as a testament to Gegenbaur's school of comparative anatomy as it wove together evidence from adult and embryological specimens, pinned together by a phylogenetic hypothesis.[72] However, unlike the mentor who had discarded the biogenetic law, Röse's ardent commitment to it is abundantly clear throughout the text. Röse's affirmation of the controversial law is particularly salient at the conclusion of the article:

> It could be perhaps that many minor points in the ontogenetic and phylogenetic development of vertebrates heart are arranged in parallel; I am satisfied, however, to have demonstrated in the main points discussed the causal relationship. There, in the development of the heart most beautifully the great biogenetic law holds true, therefore, as I would like to emphasize in conclusion once again: "Ontogenetic development is a brief outline of the phylogenetic! (Röse, 1890; 90-91)[73]

After this report, Röse never returned his attention to the evolution and development of the vertebrate heart. Despite this abandonment, his work on the heart, and the evidence that he found for the biogenetic law within these investigations greatly influenced his future research. His unmitigated acceptance of the biogenetic law, as applied to the development and evolution of teeth, in many ways determined his

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[72] See Laubichler (2003) for more information on Gegenbaur's evolutionary morphology and conception of transformation (as opposed to *de novo* innovation)

[73] "Es ließen sich vielleicht noch viele untergeordnete Punkte in der ontogenetischen und phylogenetischen Entwicklung des Vertebratenherzens in Parallele stellen; mir genügt indess in den besprochenen Hauptpunkten den causalen Zusammenhang nachgewiesen zu haben. Es bewahrheitet sich demnach, wie ich zum Schlusse nochmals hervorheben möchte, auch in der Entwicklung des Herzens aufs schönste das große biogenetische Grundgesetz: "Die ontogenetische Entwicklung ist ein kurzer Abriss der phylogenetischen!"

understanding of how mammalian dental evolution occurs.[74] To Röse ontogeny was a strict readout of phylogeny, thus any account he gave of the process of development was necessarily also an account of evolution. And, because he embraced a strong form of the biogenetic law, which dictates that phylogeny *causes* ontogeny, his concrescence theory, which was formulated as a simple description of a developmental process, was not just a description, but also an explanation of both the development and evolution of molar patterns.

3.3 Röse, Teeth, and the Concrescence Theory

Following from his heart research, Röse adopted a strong form of the biogenetic law as the basis for his research on the dentition. Beginning in 1891, Röse released a landslide of research on tooth development. While the choice of investigating the vertebrate heart is a bit murky, his reasons for focusing on teeth are clear—Röse was in training to become a doctor of dental medicine, and understanding the development of tooth form across vertebrates became his preoccupation for several subsequent years.[75] These publications began with his move to the anatomical institute in Berlin, and his

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[74] Gregory (1934) has noted that the adherence of embryologists to the biogenetic law was detrimental to acceptance of their theories of dental evolution.

[75] Nickol (1992) notes that despite his training to become a dentist, Röse was not particularly interested in dental practice, at least not at first.

work with Oscar Hertwig, the older brother of Röse's previous mentor, Richard Hertwig.[76]

Upon deciding to work on human dental development, Röse ran into a wall—human fetuses were hard to come by. Despite requests submitted by Oscar Hertwig to the Universität-Frauenklinik, Berlin, on his behalf, the institution was reluctant to make specimens available (Röse 1891a). Thus, Röse had to rely on a network of colleagues to supply his research specimens. Embryonic and fetal materials were gathered from across Berlin, supplied by Drs. Hellner, Müller, Schreiber, and Nagel, as well as from Dr. Cirincione in Naples, and Dr. Born in Breslau. Post-natal materials were made available from Waldeyer's collection at his anatomical institute in Berlin.[77] Due to the breadth of his circle of contributors, Röse was able to amass a sample of eighteen embryonic and fetal specimens, and nine post-natal specimens.[78]

For fresh materials, Röse fixed the specimens using acetic acid (*Chromessigsäure*) or nitric acid (*Picrinsalpetersäure*) to decalcify the materials, or simply hardened the specimens in alcohol. Following decalcification via the application of the acids, the specimens were stained with borax carmine (a red dye that stains nuclei and cytoplasm pink) and sectioned using the paraffin method. The sections were then processed with Blue de Lyon, which discretely highlighted the bone and connective

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[76] Oscar Hertwig had quite a bit of experience with dental research, having published a stand-alone supplement to the *Archiv für Anatomie mikroskopische* in 1874 titled "Über das Zahnsystem der Amphibi und seine Bedeutung für die Genese des Skelets der Mundhöhle: eine vergleichende Anatomisch, entwicklungsgeschichtliche Untersuchung". As a side note, Hertwig dedicated this 208 page supplement to both of his dissertation advisors—Carl Gegenbaur and Ernst Haeckel.

[77] Röse (1891a) provides a brief description of where he obtained his specimens. See: Röse 1891a, 29.

[78] Ahrens (1913) noted that Röse was responsible for developing the "the modern technique of studying dental development", and a part of this was acquiring a sample that was big enough to represent nearly a complete sequence of human ontogeny. (Ahrens 1913, 170)

tissues in the jaws surrounding the developing teeth. The serial sections were cut at about 1/50 Millimeter thick, and the youngest specimens had their whole heads sectioned, either in the sagittal or horizontal plane.

Hertwig requested that Röse make some of the series into wax models, and so he created six wax models using Born's technique. Following their creation (and his studies), he colored the models to make some of the structures, like the early stage tooth germs, more easily identifiable (Mummery 1893, Röse 1891a). Röse partially sectioned the post-natal specimens and examined them using a magnifying glass. The specimens were aged according to His's normal tables (His 1880, 1882, 1885, Röse 1891a).[79] Following from his training in comparative anatomy, Röse used a variety of mammalian and other vertebrate specimens as comparisons for human dental development.

From his serial sections, and especially the wax models of the earliest stages of tooth development, Röse became the first to describe the ontogenetic origins and early developmental history of the dental lamina (*zahnleiste*).[80] The dental lamina is an in-growth of oral ectoderm that represents the earliest evidence of tooth development, and it is from this structure that all dental papillae (the precursors of teeth) emerge and develop (see 1.2 for more information on tooth development). Previous authors had missed recording the earliest stages of this structure; as Röse noted, it was difficult to obtain specimens young enough and well preserved enough in order to observe it (Röse 1891a).

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[79] Normal tables are image tables and plates that define and show the standard divisions of development. In the 1880s, Wilhelm His, an anatomist at the University of Leipzig, began to publish a series of volumes for normal tables of human embryonic and fetal development. For a history of His's and other 19th century normal tables, see: Hopwood (2007).

[80] Röse's wax modelling technique became one of the things for which other dental researchers lauded his work (See: Mummery 1893; James 1909; Ahrens 1913). Röse also called on his opponents to look at his wax models if they took issue with his characterization of development (See: Röse 1892e, 631).

According to Röse, during the early stages of tooth development, the dental lamina gives rise to the dental papillae, and also tethers them together (figure 7). Any successional teeth (i.e. permanent teeth in humans) also develop from the dental lamina. Röse's theory, that all teeth arise from the dental lamina, opposed the leading theories of tooth development of the day, and would also become the basis for his concrescence theory of the origins of mammalian dental morphological diversity.[81]

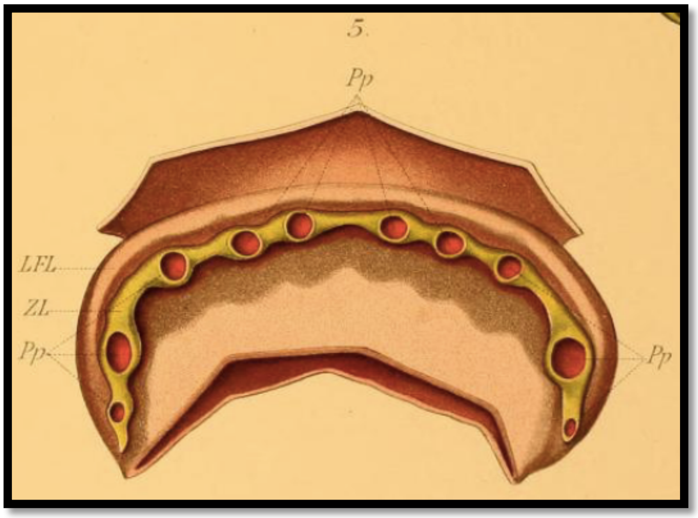

Figure 6. Color diagram of a wax model showing early dental papillae arising from the dental lamina. Human embryo, 4cm long. Pp-papilla; ZL-dental lamina; LFL-vestibular lamina. Figure 5 from Röse (1891a).

In his 1891 introduction to human tooth development, Röse gives an indication of his burgeoning application of the biogenetic law to the dentition:

> The possibility admitted that the multi-rowed dentition of lower vertebrates in the earlier intermediate forms between reptiles and mammals could have completely regressed (zurückgebildet) to a single row, as it occurs in edentulates and

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[81] Mummery (1893) outlines the two other predominant views of tooth development taught in England at the time.

> marsupials, then must also the enamel organ in the immediate formation of this rise a row of teeth. (Röse 1891a, 473)[82]

And, "The primary series of teeth (milk teeth) corresponds to the first rows of teeth of lower vertebrates crowded together. The series of the permanent teeth is caused by gradual reduction of all the following rows of teeth into a single series (Röse 1891a, 474)."[83] These passages promise an approach to dental development steeped in the types of evolutionary transformations that Haeckel had made famous—a promise that Röse made good on beginning the following year.

In 1892, Röse unleashed a series of fourteen publications that documented tooth development in everything from humans to marsupials to reptiles.[84] Throughout these papers, Röse documented the gross similarities (or, to him, homologies) that occur throughout dental ontogeny. Among the first of these articles was "Über die Entstehung und Formabänderungen der menschlichen Molaren," wherein Röse articulated the concrescence theory for the first time.

In the opening to his article, Röse discusses his search for clarity on the typical molar forms of humans (Röse 1892a). Textbooks of anatomy and dentistry, according to Röse, were full of diverse and conflicting information regarding what, exactly, the normal human molar looks like. Röse points especially to Zuckerkandl as the person who had most thoroughly addressed the issue, referring to his chapter in Scheff's *Handbuch*

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[82] "Die Möglichkeit zugegeben, dass die vielreihige Bezahnung der niederen Vertebraten bei den früheren Zwischenformen zwischen Reptil und Säuger sich völlig zurückgebildet haben könne bis auf eine Reihe, wie sie bei Edentaten und Beutlern vorkommt, dann müsste doch das Schmelzorgan ganz in der Bildung dieser einen Zahnreihe aufgehen."

[83] "Die Milchzahnreihe entspricht den ersten zusammengedrängsten Zahnreihen der niederen Vertebraten. Die Reihe der bleibenden Zähne ist entstanden durch allmähliche Reduction aller folgenden Zahnreihen in eine einzige."

[84] The following year, he doubled this number to 28 publications!

der Zahnheilkunde, Band I., wherein Zuckerkandl examined 542 skulls and claimed that the typical molar form in the maxilla was four-cusped, while in the mandible it was five-cusped (Zuckerkandl 1890). These typical forms, according to Zuckerkandl, are found in the "inferior" races much more frequently than the Indo-Europeans, where reduction in cusp number is more frequent. Röse aimed to examine this claim, and in so doing, establish a more thorough understanding of human dental form and its diversity. In order to accomplish this, Röse looked at the skull collections in Strasbourg, Basel, and Freiburg, examining in total 1241 maxillae and 828 mandibles (Röse 1892a). Before he addressed the question of normal human molar form, however, Röse deemed it necessary to formulate an evolutionary perspective.[85]

Röse begins by reviewing the theories of Cope, Osborn, and other paleontologists, whose views of dental evolution were underpinned by assumptions about the gradual tempo of evolution and mechanical necessity of different forms. These assumptions hinged on changes of form wherein the functional needs of the organisms wrought changes in cusp formation through modifications of ontogenetic processes. These assumptions, Röse concluded, were not sufficient to explain dental evolution.[86]

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[85] "Bevor ich jedoch auf die Resultate meiner Statistik näher eingehe, dürfte es angebracht sein, zunächst einmal die Frage von der Entstehung und Form der Mahlzähne überhaupt näher zu beleuchten." Röse, 1892a: 392.

[86] "Die mechanische Erklärung von Cope, der z.B. die Entstehung der seitlichen Höcker dadurch erklärt, daß die Blutgefäße der Zahnpulpa an der Basis der ursprünglichen Conus mehr Bildungsstoff ablagern und die Ernährung kleiner Zeitenzacken besser begünstigen sollen (weshalb?), sei durchaus nicht hinreichend." Röse, 1892a: 394.

> Compared with the data of the previous authors mentioned, that the present form of the molars was formed by gradual transformation of an originally simple conical teeth, I have come to quite different results. Through my investigations into the development of the teeth I have been pushed to the idea that *molars as well as premolars originated by fusing several simple conical teeth into today's highly organized dental structures.*[87] (Röse 1892a, 394-395 [emphasis from author])

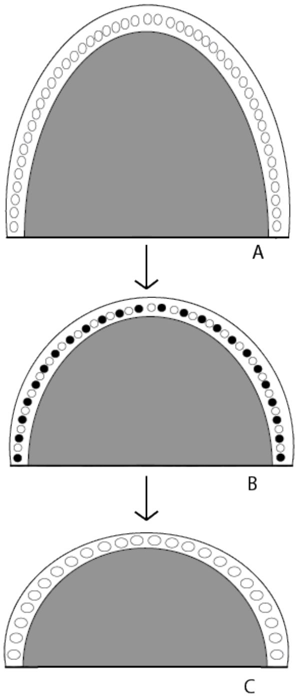

Figure 8. Diagram of Röse's argument against Cope and Osborn. A. Circles represent original number of dental papillae. B. Open circles represent papillae that will survive, small black circles represent papillae that will disappear. C. Small circles represent the papillae that have survived (note the fewer number of papillae).

Röse found these types of gradual perspectives insufficient because within his interpretation of the biogenetic law, all ancestral forms are recapitulated during ontogeny. Within this framework, the dozens of single, conical teeth that are found in the jaws of reptiles, crocodiles for example, would need to be accounted for in order to understand the more complex molar forms of modern mammals. The evolutionary framework of Cope and Osborn, so Röse believed, contained a major fallacy—that is, the problem that some simple, conical progenitor teeth transformed into complex molars and premolars while others simply disappeared: “Why should it be that one inexplicable, highly organized molar should develop from

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[87] *“*Gegenüber den Angaben der genannten Autoren, daß die heutige Form der Molaren entstanden sei durch allmähliche Umwandlung eines ursprünglich einfachen Kegelzahnes, bin ich nun zu durchaus anderen Resultaten gekommen. Bei meinen Untersuchungen über die Entwickelung der Zähne drängte sich mir fast als selbstverständlich der Gedanke auf, daß **die heutigen Molaren und ebenso die Prämolaren enstanden sind durch Verschmelzung mehrer einfacher Kegelzähne** zu den heutigen hoch organisierten Zahngebilden*.”*

two originally identical teeth, while the equal neighbors would completely perish?"[88] (Röse 1892a, 399)

Röse's concrescence theory corrected Cope's and Osborn's supposed fallacy by accounting for the persistence of all ancestral teeth throughout the ontogenetic process (see figure 8). The concrescence theory, as envisioned by Röse can thus be articulated along the following lines: complex teeth are formed by the concrescence during development of initially independent cusps. In other words, early on in dental ontogeny, dental papillae (tooth precursors) arise from the dental lamina. These papillae far exceed the number of teeth that will ultimately reside in the fully-formed dental arcade. The number of papillae that initially form corresponds to a phylogenetically deep ancestral state (most likely akin to the crocodilian jaw). Throughout the developmental process, these papillae join together to give rise to more complex dental forms.

There are two points to note here about Röse's concrescence theory. First, this theory, while usually given as a simple statement about the process of molar formation, sometimes included an auxiliary hypothesis within Röse's work. Namely that as ontogeny proceeds, the maxilla and mandible of the organism foreshorten, bringing the numerous dental papillae into proximity, causing them to concresce. This statement of causality can be found peppered throughout Röse's many texts dealing with the concrescence theory, however it doesn't appear to be a central tenet of the theory and is rarely mentioned.

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[88] "Weshalb soll denn aber von zwei ursprünglich gleichartig entwickelten Zähnen der eine auf eine bisher unerklärliche Weise zum hochorganisierten Molaren sich ausbilden, während die gleichberechtigten Nachbarn ganz zu Grunde gehen müßten?"

Second, it is important to note that Röse never explicitly states that the concrescence of the papillae follows the phylogenetic history of the organism. However, his figures (figure 9) and references to things like calling the Crocodilian tooth an "embryonic type" make this a reasonable inference.[89]

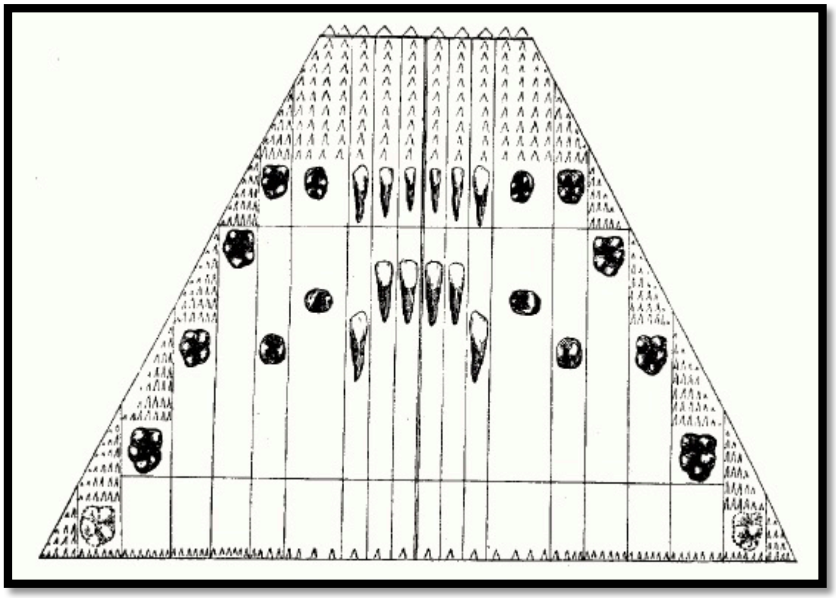

Figure 7. Röse's diagram of the concrescence theory. From Röse 1892a, 404. Figure caption reads: "Schema der Entwickelung des menschlichen Gebisses aus einem reptilienähnlichen Gebisse." Caption translates to: "Diagram of the development of human dentition from a reptilian dentition."

In order better to comprehend Röse's vision of the concrescence theory of dental development and evolution, it is necessary to return to the dental lamina and Röse's understanding of what that structure does. Within the context of Röse's acceptance of the biogenetic law, the dental lamina took on an evolutionary meaning—it became the structure that not only connected and gave rise to teeth during ontogeny, but also that which tethered mammalian molar forms to their evolutionary roots. Through this

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[89] See Röse 1892g, 133 for his reference to the crocodile tooth as an embryonic type.

structure, Röse believed, the reptilian cone-like teeth of our evolutionary ancestors were preserved and arose as primordia. These primordia crowded together throughout the ontogenetic process (which was a recapitulation of evolutionary history) of higher vertebrates like mammals, possibly due to a foreshortening of the jaws.

Thus, the many-toothed dental series seen in reptiles, especially in crocodiles, was foreshortened within the mammalian jaw, crowding the primordia into close proximity, and ultimately forcing their coalescence into what we see as the multi-cusped mammalian molars (Röse 1892a).

In addition to elaborating the way in which tooth crowns are formed, Röse also uncovered evidence for the order of cusp formation in humans and marsupials (Röse 1892a; Röse 1892c).[90] Through his investigations of human molar development, Röse noticed that in maxillary molars, the mesial buccal cusp (paracone) develops and calcifies first, followed by the mesial lingual and distal buccal cusps (protocone and metacone, respectively) (figure 10).

The timing and order of cusp development that Röse uncovered for the maxillary dentition stood in opposition to the order proposed by Osborn's homology concept—namely, that the protocone is always the first cusp to develop, regardless of placement within the upper or lower

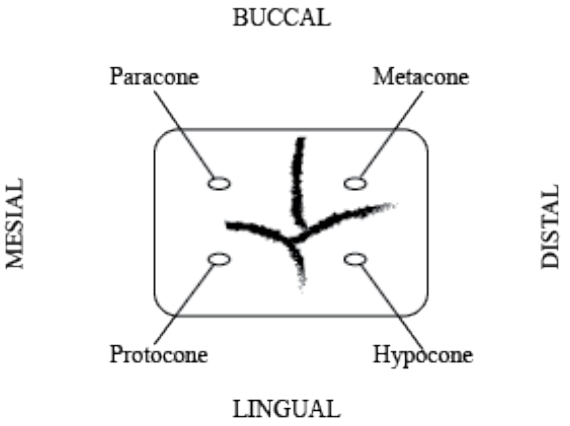

Figure 10. Diagram of typical upper quadritubercular molar.

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[90] Röse went on to note the order of cusp formation across many other taxa. Around the same time, Taeker (1892) also uncovered evidence for the order of cusp formation. For a brief overview of this history, see: Tims (1903)

dentition (Osborn 1888a). This piece of embryological evidence proved to be the main point of contention between Röse and Osborn and undermined one of Osborn's core assumptions for the tritubercular theory.

Following from his investigations of development of the human dentition, Röse extended his taxonomic scope to include the development of teeth across the vertebrate clades. From elephants (Röse 1893a) to marsupials (Röse 1892c), to crocodiles (Röse 1892f) and edentates (Röse 1892b), Röse sought to understand the process of tooth development and how it potentially differed across taxa, leading him to apply his concrescence theory across many toothed organisms.[91, 92]

3.4 Reception of Röse's Work and the Concrescence Theory

In the years following Röse's development of the concrescence theory, his research and the theory met with mixed reviews. At first, his research and his human tooth development models were lauded by dental researchers worldwide. In 1893, John Howard Mummery, the President of the British Dental Association, presented a series of Röse's models of human dental development to the Odontological Society of Great Britain (Mummery 1893). The models that Mummery brought to the society meeting were copies, created by Alfred Ziegler, of Röse's wax models that were made for his 1891 paper, "Ueber die Entwickelung der Zähne des Menschen." Mummery's

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[91] Röse examined tooth development in many more taxa, including: humans (1891, 1892a), lungfish (1892d), reptiles (1892g), garmans (1894a), fish (1894b), and cows (1896).

[92] It should be noted that establishing the concresence theory across toothed organisms does not appear to have been Röse's main motivation for his broad developmental work. His application of the concresence theory in many papers amounts to only a brief mention.

presentation of these models and Röse's research on human tooth development was called by the Chairman of the society, "the paper of the evening" (Mummery 1893, 194).[93] In 1909, W. Warwick James, the noted dental surgeon and dental researcher again highlighted Röse's adaptation of Born's modeling technique to visualize the early structures and processes of dental ontogeny. Both Mummery and James admired Röse's theory of tooth formation from the dental lamina, in addition to his wax models.

Furthermore, in 1913, Hans Ahrens, a researcher at the Dental Institute of the University of Munich whose own work would challenge some of Röse's findings, noted that,

> It is to Röse's undisputed credit that the modern technique of studying dental development developed. He had almost complete material--he examined newborns in 19 stages--which he sectioned in series and in various planes. He was in a position to present his findings physically, through reconstruction. For this purpose, he made wax models of 6 stages. In this way, he could see the first primordia (Anlage) of the human teeth, which it is unclear if people had previously observed. He also showed that the development of the permanent teeth is independent of the enamel organ of the deciduous tooth, and comes from the dental lamina, just like the deciduous teeth. (Ahrens 1913, 170-171)[94]

At the turn of the 20th century then, Röse's work on describing the transformations involved in dental development, particularly human dental development, were highly valued within the dental research community. But what of the concrescence theory?

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[93] Röse's models and research on human dental development continued to be acknowledged as important throughout the dental community. See: James (1909)

[94] "Es ist das unbestrittene Verdienst Röses, die modern Technik der Zahnentwickelung erschlossen zu habben. Er verfügte über ein fast lückenloses Material—er untersuchte bis zum Neugeborenen 19 Stadien—welches er in Serien geschnitten hat und zwar in verschiedenen Schnittrichtungen. So war er in den Stand gesetzt, seine Befunde mittelst des Rekonstruktionsverfahrens körperlich darzustellen. Zu diesem Zwescke fertigte er von 6 hauptsächlich in Betracht kommenden Stadien Wachsmodelle an. Auf diese Weise konnte er die erste Anlage der Zähne deim Menschen, die bisher noch nicht sicher beobachtet war, feststellen. Ferner konnte er zeigen, dass die Entstehung der bleibenden Zähne unabhängig von dem Schmelzorgan des Milchzahnes aus der Zahnleiste genau so wie die Entstehung der Milchzähne erfolgt."

While Röse’s work on human dental development was lauded by many dental researchers, his concrescence theory (which was an extension of this work on development) met with more mixed reviews. American paleontologists largely panned Röse’s concrescence theory. However, the theory received support from the German embryological community. Schwalbe weighed Röse’s evidence and found the theory credible, as did H.W. Marett Tims (Schwalbe 1894; Tims 1903).

CHAPTER 4

A CONFLICT OF ASSUMPTIONS: TRITUBERCULAR THEORY VERSUS CONCRESCENCE THEORY

4.0 Introduction

Prima facie, there is nothing logically incompatible between the formal statements of the tritubercular theory and the concrescence theory. The tritubercular theory describes a morphological pattern within the fossil record, while the concrescence theory describes the process of how individual teeth acquire their characteristic forms. And yet, conflict arose between the proponents of these two theories. In the words of Henry Fairfield Osborn, “the difference between the palaeontological and embryological theories is radical…There is no middle ground between them” (Osborn 1907, 7). Despite Osborn’s sentiments, I contend that the conflict that arose was not over the formal contents of each theory, but over the assumptions that each of these researchers held. This chapter shows how and why Cope, Osborn, and Röse tied the assumptions raised in Chapters 2 and 3 to their theories, why these assumptions were in conflict, and what the relationship was between these assumptions and the tritubercular and concrescence theories.

4.1 The Relationship Between Assumptions and Theories Within the Research of Cope, Osborn, and Röse

At its core, Röse's concrescence theory is a description about how molar patterns form during development—that is, it is a theory about a developmental process. Röse's concrescence theory was developed under the assumption that the biogenetic law was true. Within his dissertation research on the development of the vertebrate heart, Röse found what he believed to be proof of the causal relationship between phylogeny and ontogeny. His confirmation of the strong form of the biogenetic law in these early studies led to his application of it in his further research.[95] In his research on dental development he uncovered empirical evidence for a broad uniformity of dental development across vertebrate taxa, which led him to account for a problem, namely that teeth arise from developmentally simple papillae tethered to the dental lamina.[96] For Röse, the question became: How do complex molar forms arise from these originally simple structures?

Because Röse's earliest research had confirmed in his mind the truth and primacy of the biogenetic law, he was apt to apply it to his solutions to future problems, such as how complex molars arise from simple structures. While Röse provided no direct observations of the concrescence of the dental papillae, it seemed plausible that these simple papillae did, in fact, represent ancestral and haplodont teeth (because of their early appearance during ontogeny and thus deep placement within phylogeny). It was also

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[95] For more about the strong versus weak forms of the biogenetic law, see Chapter 1.3

[96] Recall that at the time in which Röse was working, the earliest stages of dental development were debated and his finding of teeth arising from a dental lamina that tethered together all of the dental papillae was novel.

plausible, within this framework (wherein dental papillae represent an ancestral haplodont condition) that one had to account for all of these ancestral haplodont teeth when creating an account of tooth development. If the ancestral haplodont teeth had to survive, and, if they had to (in some instances) develop into more complex forms, then they could do so by coalescing during development. Thus, the assumption that the biogenetic law was true meant to Röse that the concrescence theory did not simply aim to describe a process, but was meant as an explanation of both the development and evolution of molar patterns. Röse's acceptance of the biogenetic law also meant that for him phylogeny caused ontogeny.

Cope's and Osborn's tritubercular theory, on the other hand, was a general description about the pattern through which mammalian molars changed on an evolutionary timescale. The tritubercular theory, as understood by Cope and Osborn, was grounded in a series of assumptions, namely that evolution occurs in a directed fashion dictated by the functional needs of the organism, that molars can be grouped into a hierarchy of types, and that homologies exist between molar cusps "with reference to their order of development and primitive position" (Osborn 1888a, 242).

We have seen that there is a rich historical context for the assumptions held by Cope, Osborn, and Röse (Chapter 1). We have also seen how these researchers framed their theories in the context of these different assumptions. What, then, is the relationship between their assumptions and the theories that they proposed? Put another way, are these assumptions part of the theories or are these assumptions held by the researchers but not part of the formal apparatus of the theories? In the first case, providing evidence to counter the assumptions would undermine the respective theories, whereas in the latter case, the theories would hold regardless of countering the assumptions.

The relationship between assumptions and theory here is interesting because of the messiness inherent in the works of these three authors. Cope, Osborn, and Röse, at points throughout their texts all make their assumptions explicit—whether it is Osborn stating his preference for kinetogenesis over Darwinian evolution or Röse proclaiming that, “Ontogenetic development is a brief outline of the phylogenetic!” (Osborn 1888b, Röse 1890, 91). Their statements about how development and evolution work, the homologous nature of cusps, and so on make it difficult to sort out the exact impact of these assumptions for the theories that they have proposed. It is this difficulty of separating assumptions held by researchers and assumptions integrated into theories that led to conflict between the tritubercular theory and the concrescence theory at the end of the 19th century.

4.2 Statements of the Tritubercular and Concrescence Theory and Assumptions

To clarify the relationship between the researchers’ assumptions and theories, it is necessary first to explicate both. Here is a formal statement of the tritubercular theory, along with some of the assumptions that Cope and Osborn held:

Tritubercular theory (TT): “The theory consists of two distinct parts: (1) the tritubercular origin of the molar patterns of Tertiary mammals, and (2) the origin of this tritubercular pattern from the reptilian cone” (Butler 1941, 422).

TT Assumption (TTA) 1: Tempo of evolution is slow (Cope, Osborn)

TT Assumption (TTA) 2: Cusps are homologous (Osborn)

TT Assumption (TTA) 3: Ontogeny causes phylogeny—Kinetogenesis gives rise to changes during ontogeny that become the raw materials of evolution (Cope, Osborn)

TT Assumption (TTA) 4: Evolution is directional (Cope, Osborn)

Here is a formal statement of the concrescence theory, along with the assumptions that Röse held:

<u>Concrescence theory (CT)</u>*:* Teeth are formed by the concrescence during development of initially independent cusps.

CT Assumption (CTA) 1: The tempo of evolution is fast.

CT Assumption (CTA) 2: Phylogeny causes ontogeny.

CT Corollary Assumption: Enough tooth primordia are present in the dental lamina to 1) represent the haplodont condition (untestable—unknown how many primordia would be necessary), 2) represent a haplodont condition for each cusp in the mouth (testable).

4.3 The Controversy that Arose Between Assumptions

Here we have a case in which morphologists from different fields (paleontology and embryology) used different types of evidence (fossils and embryos, respectively) to address the same problem—the morphological diversity seen in mammalian molars. And yet, while these morphologists addressed the same problem, their approaches and the questions driving their research programs were very different: how to account for natural patterns of molar groupings within the fossil record (Cope and Osborn), and how to derive complex molar forms from originally simple developmental structures (Röse). On its face, the formal statements of these two theories are not incompatible, and yet conflict arose.

The conflict between the proponents of the two theories emerged in two areas. For Röse, the tritubercular theory contained a major fallacy—that is, the problem that some simple, conical progenitor teeth transformed into complex molars and premolars while others simply disappeared (Röse 1892a). In other words, Röse took issue with the differential survival of tooth primordia required by his interpretation of the tritubercular theory. He thought that the tritubercular theory would entail differential survival of tooth primordia because within the tritubercular theory, changes in cusps are built up gradually over evolutionary time (via kinetogenesis). Röse did not see how the gradual tempo of evolution (*TTA 1*) that allowed for small changes to be built up over time due to kinetogenesis (*TTA 2*) assumed by Cope and Osborn could accommodate the survival of all the tooth primordia that had to represent the ancestral condition, as assumed by the biogenetic law (*CTA 2 and corollary*). Röse's commitment to the concrescence theory required that all tooth primordia be accounted for and survive throughout ontogeny (*CTA 2 corollary*). Despite this objection, Röse supported the possibility that the tritubercular theory was correct in terms of the pattern that it dictated for the fossil record (Röse 1892c).

Osborn's objection to Röse's work lay not in his focus on embryological evidence (which Osborn thought to be an important line of inquiry). Rather, Osborn objected to the fact that Röse's embryological evidence for the order of cusp formation conflicted with the assumption of cusp homology that Osborn had developed (*TTA 2*) (Röse 1892a; Röse 1892b).[97] Recall that Röse uncovered evidence that in maxillary molars, the paracone (mesial buccal cusp) develops and calcifies first, followed by the protocone and metacone (mesial lingual and distal buccal cusps, respectively). Recall also that Osborn's homology concept of teeth required that the protocone (or protoconid) was always the first cusp to develop (Osborn 1888). Thus, if Röse's embryology was correct, then Osborn's order of cusp formation could not accurately reflect the phylogenetic history of the mammals.

If the assumption of homology was a formal part of Osborn's theory, then Röse's evidence about the order of cusp formation would pose a serious challenge to the entire tritubercular theory. From Osborn's perspective, the homologous nature of cusps was a crucial component to the tribubercular theory, and Röse's findings led him to defend the theory against this embryological evidence (Osborn 1895).

If it was the case that the assumptions held by these researchers were also necessary for their theories, then providing evidence to counter the assumptions should have undermined their theories. Conversely, if the assumptions were not part of their theories, then the theories should have held up in the face of evidence that challenged the assumptions. We can look to the historical record of how other scientists reacted to the tritubercular and concrescence theories and the various assumptions of Cope, Osborn, and Röse to determine the relationship between their assumptions and theories.

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[97] I could not find evidence of Cope defending the tritubercular theory.

4.4 Challenges to the Tritubercular and Concrescence Theories

In the late 19th and early 20th centuries, the tritubercular and concrescence theories were widely discussed by morphologists interested in dental morphological complexity. Many challenges arose to the theories, based on the assumptions tied to them as well as the formal statements of the theories. What follows is a small sample of this historical debate.

English zoologist Edwin Stephen Goodrich found the tritubercular theory to be seriously flawed in two main respects (Goodrich 1894). Goodrich objected first to the multiple independent acquisitions of the tritubercular type within mammalian evolution—a point that Osborn often underscored in his research (Osborn 1907). Goodrich also objected to the application of a type concept, especially in light of the polyphyletic nature of the tritubercular tooth. “The words ‘common’ and ‘central,’ as applied to a type of structure, have no significance nowadays unless equivalent to ancestral. It seems hardly necessary to point out that such mythical types hovering over organs, and compelling them to assume a certain form, have no place in modern biology” (Goodrich 1894, 6). Despite the objections he raised against the assumptions that Osborn had tied to the tritubercular theory, he still believed that the pattern that Cope and Osborn delineated within the fossil record had merit:

> There is much evidence for the view that the upper molars of the Pro-mammalian ancestor were of the tritubercular, and the lower molars of the tubercular-sectorial types; in fact, I think, we cannot do better than accept Prof. Cope’s generalisation, if not as a definitely established theory, at all events as an excellent working hypothesis, 'that the superior molars of both ungulate and unguiculate mammal have been derived from a tritubercular type; and that the inferior true molars of both have been derived from a tubercular-sectorial type. (Goodrich 1894, 6-7)

While Goodrich found merit in trituberculy, at least as a working hypothesis, some other paleontologists found it harder to accept. Charles Immanuel Forsyth Major, a physician and avid paleontologist in his free time, found multituberculates to be a major stumbling block for the initially simple teeth that the tritubercular theory proposed for mammalian evolution (Forsyth Major 1894).[98] Forsyth Major believed that the primitive mammalian molar crown initially bore many cusps, not few, and so the pattern of evolution that the tritubercular theory outlined could not hold. A few years later, J. L. Wortman, a former field assistant to Cope, concluded that Osborn's nomenclature and homology system were inaccurate because the homologies and order of development and evolution that he assigned to the molar cusps did not match the fossil evidence for premolars evolution (Wortman 1902).

The following year, H.W. Marett Tims, a zoologist and embryologist at Cambridge University, took issue with the tritubercular theory based on evidence that the protocone is not the first cusp to develop and on the deficiency of paleontological evidence to support the theory (Tims 1903). Tims found support within his own research on tooth development for a version of the concrescence theory (Tims 1903).

In 1916, William King Gregory, a zoologist and paleontologist trained under Osborn, reviewed the evidence for the tritubercular theory, and found support for the pattern of molar evolution described in the theory, but not for all of the assumptions that Cope and Osborn had held (Gregory 1916). Specifically, Gregory objected to Osborn's

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[98] Multituberculates are a taxon of extinct rodent-like mammals. The name "multituberculate" derives from the fact that their molar teeth have several rows of small cusps (or tubercles).

homology concept that saw the protocone as the most ancestral cusp, and thus homologous to the reptilian haplodont molar:

> In conclusion, I continue to endorse the Cope-Osborn theory, insofar as it derives all the more complex molar patterns of typical placental mammals respectively from the ‘tritubercular’ and ‘tuberculosectorial’ types of upper and lower molars. But I am forced to reject that theory both in its explanation of the origin of the tritubercular type and in its identification of the origin of the tritubercular type and its identification of the protocone as the summit of the haplodont reptilian molar. (Gregory 1916, 239)

Despite Gregory’s objections to the assumptions, he continued to find the pattern of evolution described by the tritubercular theory useful. In a review of the tritubercular theory and the debate that had arisen around it for the preceding 50 years, Gregory noted that, “In the judgment of palaeontologists the theory of trituberculy has proved to be of great value in deciphering the evolutionary history of the dentition in many families of mammals, including man” (Gregory 1934, 169).

As the 20th century wore on, the assumptions that surrounded the tritubercular theory were taken less seriously than in the decades directly following Osborn’s first defense of it. In 1927, anthropologist Wilton Marion Krogman reviewed the theories that explained the origins of mammalian molars (Krogman 1927). In this review, Krogman took issue with many of the assumptions attached to the tritubercular theory, but held that the pattern that it laid out was accurate (Krogman 1927). Regarding the pattern of evolution determined by the tritubercular theory, Krogman concluded that, “no matter how much the Cope-Osborn theory may be attacked, it still stands as a monument to the genius that conceived it” (Krogman 1927, 50).

The renowned paleontologist George Gaylord Simpson found that the pattern laid out by the tritubercular theory had stood the test of time: “…the primary problem, that of finding a central type from which all higher mammalian molar patterns were derived and to which they could be referred…was solved in its essentials by Cope…" (Simpson 1936, 792). Despite his endorsement of the pattern laid out by the tritubercular theory, Simpson found flaws in some of the assumptions that Osborn held. The continued controversy surrounding the theory, Simpson suggested, was not due to paleontological evidence (that supported the pattern determined by Cope and Osborn), but rather to researchers taking issue with the assumptions that were carried along with it.

In a personal communication with Krogman, published as part of Krogman's "Anthropological Aspects of the Human Teeth and Dentition," Simpson noted that, "Difference of opinion as to how this pattern originated does not mean opposition to the Cope-Osborn theory. On the contrary, if a more firmly established theory of derivation can be proposed than the rather widely disallowed one advocated by Osborn up to 1907 the theory will be very strongly supported thereby in its main contention" (Krogman 1927, 53). In this passage, Simpson meant that the contention surrounding the tritubercular theory was with Osborn's assumption of cusp homology—that the protocone must be the most ancestral cusp. Simpson clarified further, "The protocone is not the oldest cusp of the mammalian molar. The original reptilian cusp has given rise by fission or unequal budding to the paracone and metacone" (Krogman 1927, 53).

Thus, it seems that as the 20th century wore on and new researchers joined the search for the origins of mammalian molars, the focus of debate over the tritubercular theory resided not with the assumptions of Cope and Osborn, but with the formal statement of the theory, as laid out in section 4.2. What, then, happened to the concrescence theory? What objections did it meet with, and on what grounds did investigators object to this theory?

Paleontologists who engaged with Röse's concrescence theory largely objected to the assumptions that he attached to it, particularly the biogenetic law. An excellent example of this can be found in William King Gregory's "A Half Century of Trituberculy: The Cope-Osborn theory of Dental Evolution with a Revised Summary of Molar Evolution from Fish to Man" (Gregory 1934). After laying out a brief history of Röse's work, Gregory objected to the theory on the grounds that the biogenetic law, particularly its strong form, had been discredited as an explanatory framework[99]:

> In the period in which the theory of trituberculy was developed it was widely taken for granted that ontogeny repeats phylogeny, so that as soon as it had been determined by Röse, M.F. Woodward, and others, that the so-called protocone of the molars was not the first cusp to be formed in the embryonic molars of man and other mammals, it was felt that the cardinal assumption of the theory of trituberculy, namely, that the protocone was on the inner side of the upper molars crowns, had been refuted. (Gregory 1934, 189)

While paleontologists objected to Röse's assumptions, embryologists in the early 20th century took issue with the concrescence theory on different grounds. Hans Ahrens, whose work on tooth development is examined in Chapter 5, was among the first to challenge the empirical basis of the concrescence theory (Ahrens 1913). Working with early stage human embryos, Ahrens could find no evidence for the concrescence of the tooth germs, and concluded that his work eliminated "the only reasonable viable support for the concrescence theory" (Ahrens 1913, 253).[100] That same year, dental embryologist

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[99] Note that here I state that Gregory believed that the biogenetic law had long been discredited when he wrote his review of the tritubercular theory in 1934. Gregory did not distinguish between a strong and weak form of the law.

[100] "Damit entfällt die einzige einigermassen brauchbare Stütze der Concrescenztheorie."

P. Adloff, also investigated the earliest stages of human dental development and found no evidence that the individual tooth germs concresce during development (Adloff 1913).

4.5 Theories, Assumptions, and the Fates of the Tritubercular and Concrescence Theories

In the last section we saw how morphologists challenged the tritubercular and concrescence theories in the decades after they were proposed. What, then, was the fate of each of these theories? And, what light can the historical record shed on the nature of the relationships between assumptions and theories for the tritubercular and concrescence theories?

As the 20th century wore on and new researchers joined the search for the origins of mammalian molars, the focus of debate over the tritubercular theory resided not with the assumptions of Cope and Osborn, but with the formal statement of the theory, as laid out in section 4.2. And, the tritubercular theory did not fail on the basis of the assumptions that Cope and Osborn attached to their theory. In fact, it could be argued that the theory did not fail at all. The tritubercular molar is still recognized as having arisen in the Tertiary, and as a key innovation in the evolution of therian mammals. Researchers continually investigate the pattern that gave rise to the tritubercular molar from a reptilian and proto-mammalian ancestor, as well as the way in which form has changed from this common molar pattern (Jernvall 1995, Luo et al. 2001, Luo et al. 2007).[101]

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[101] In modern literature, the tritubercular molar is referred to as a tribosphenic molar. For the purposes of continuity, and to avoid confusion, I retain the usage of the term tritubercular throughout, even when referring to literature that uses the term tribosphenic.

While researchers have continued to embrace the tritubercular molar as critical to early therian evolution, they have since shed the usage of types, imbued with a notion of progress, that Cope and Osborn embraced. They have also discarded the theory of kinetogenesis and directionality in evolution. Thus, while most of the assumptions that Cope and Osborn tied to the tritubercular theory were dismissed, the central role of the tritubercular molar for Tertiary mammals (premise 1 of the theory), as well as the evolution of the tritubercular molar from a more simple reptilian and proto-mammalian ancestor (premise 2 of the theory) are intact.

What, then, happened to the concrescence theory? Following the works of Ahrens and Adloff, the concresence theory was largely abandoned (Adloff 1913, Ahrens 1913).[102] It is not surprising, then, that evolutionary biologist Percy Butler declared in 1941 that, "The concresence theory in its original form is now completely discredited…" (Butler 1941, 422). Even before Ahrens and Adloff provided empirical evidence that opposed the concrescence theory, the main assumption that Röse held, the biogenetic law, had been soundly discredited by experimental embryologists at the turn of the 20th century (Maienschein 1978).

The historical record shows that the relationship between theory and assumptions in the case of the tritubercular and concrescence theories is complicated. Objections to the assumptions outlined in section 4.2 were directed at the tritubercular and concrescence theories both from within and outside of the fields in which they were developed. This fact points to the assumptions being held as necessary parts of the

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[102] The premise of dental germ fusion giving rise to molars was reincarnated by the Dutch anatomist, Louis Bolk, in 1921; however, his "Tooth Germ Fusion Theory" did not seem to gather wide acceptance (Bolk 1921).

theories. However, neither the tritubercular nor the concrescence theory fell on the basis of researchers' objections to the assumptions. While the assumptions that underlay the tritubercular theory have been discarded, the formal content of the theory remains. And, the concrescence theory was overturned on the basis of empirical evidence that did not align with the formal content of the theory. Thus, it appears that while the assumptions were foundational to the formation of both the tritubercular and concrescence theories, and were often the foci of conflicts between these two theories, they were not formally integrated into either theory.

CHAPTER 5

THE ENAMEL KNOT

5.0 Introduction

In Chapters 2 and 3 we saw that mammals have a large range of morphological diversity in their teeth, in terms of the sizes, shapes, numbers, and configurations of their cusps, and that 19th century paleontologists and embryologists came up with a number of theories to explain this morphological diversity. Foundational to their different explanations of the morphological diversity was an understanding of development of individual teeth—either as implicit assumptions (kinetogenesis, in the case of Cope and Osborn) or as an explicit component of their theory (cusp concrescence, in the case of Röse). These theories were also largely evolutionary at their roots.

In the 20th century, embryologists shifted their gaze from explaining dental diversity to explaining individual tooth development, shedding evolutionary explanations in favor of strictly developmental ones. This shift stemmed, in part, from a dissatisfaction with earlier depictions of dental development, and therefore calls for new explanations, as well as a growing general distaste for appeals to evolution as part of the explanatory framework of development (Ahrens 1913.).[103] In grappling with the problem of tooth development, embryologists sought to give accounts of the processes by which teeth go

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[103] It is interesting to note that whereas Röse had sought unifying principles of dental development by appealing to phylogeny, this new group generally assumed that the processes that they were describing were consistent across species. Structures, such as the enamel knot, were identified and confirmed across species, but an understanding of processes, such as cell proliferation during tooth development, were gleaned from a variety of mammalian species and discussed as though they were generalizable.

from a small clump of cells within the dental lamina to a fully grown organ—that is, they sought to give explanations of morphogenesis. An important way in which researchers did this was to focus on the question of how a tooth develops its characteristic form.

In addressing tooth development and morphogenesis, embryologists of the 20th century built research programs that were infused with a number of assumptions about what counts as evidence, what type of evidence is most valuable, how development works, and what counts as an adequate explanation of development. These assumptions become 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 stage developing teeth. The cells of the enamel knot do not divide or proliferate, 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 the leading theory of how teeth develop their characteristic forms—the enamel knot theory (discussed in Chapter 6). 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. These shifts in the enamel knot's explanatory value are tied to the assumptions underlying the research programs of the scientists who investigated it. These assumptions guided researchers in terms of how they set up their experiments, how they interpreted what they saw, and how they explained tooth development and morphogenesis—thus shaping the history of the enamel knot.

This chapter tracks the history of the enamel knot by focusing on a series of four research programs throughout the 20th century. Throughout these four research programs, we see the enamel knot emerge and be understood in different ways—its role in shaping the tooth interpreted according to the different ways in which researchers understood and explained development and morphogenesis.

5.1 Discovery of the Enamel Knot: Hans Ahrens, 1913

By the end of the 19th century, Carl Röse had produced a landslide of literature that established the fundamentals of human tooth development (Röse 1891a, 1891b, 1892c, 1892d, 1892e).[104] Röse's research in this area went largely unchallenged for nearly 20 years, mostly because his work represented a huge advance in the field—his sample sizes and modelling techniques afforded him insights into the complicated structures of developing teeth that no one had previously achieved (Ahrens 1913, Bolk 1907). In 1913, Hans Ahrens, an assistant at the Dental Institute of the University of Munich, was prompted by his mentor, Johannes Rückert, the first conservator of the Anatomical Institute of Munich, to study the development of human teeth. Rumblings in the field had raised doubt about the completeness of Röse's depiction of human tooth development (Bolk 1907), and, "thus a review of Röse's work seems highly desirable" (Ahrens 1913, 171).[105]

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[104] Note that the citations for Röse on human tooth development are just a small sample of the research that he produced on the topic. His writings on human tooth development (to say nothing of tooth development in other organisms) is far more extensive than cited here. Also note that other dental researchers, such as Wilhelm Leche, contributed to the topic of human tooth development, but none so extensively or comprehensively as Röse.

[105] "So scheint eine Nachprüfung der Röseschen Arbeiten dringend wünschenswert."

Ahrens's research on tooth development culminated in his 1913 text, "Die Entwickelung der menschlichen Zähne" (Ahrens 1913).[106] Here, Ahrens does not work with a single question in mind, so much as with a suite of questions derived from gaps he saw within the literature, such as, what is the relationship between the dental lamina and the dental papillae? And what is the process by which the epithelium transforms into the tooth crown? Ahrens thus desired to explain tooth development by looking closely at developing teeth through the applications of different staining techniques, serial sections, and wax models, and by comparing his own observations with those found in the literature.

Ahrens was primarily concerned with characterizing the fine morphological changes through which the tooth forms—he achieved this through his histological methods. Research into how teeth develop had not yet taken the experimental turn that has characterized many historical depictions of embryology at this time.[107] And, unlike Röse, Ahrens sought an understanding of tooth development that began with evidence, not theory: "It is really time that the scientific treatment of tooth development is redirected back into exact channels, and is limited at first to the determination of bare facts, which later can be summarized into theory" (Ahrens 1913, 172).[108] In this way,

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[106] In German (Ahrens publications are all in German), the enamel knot is called the "schmelzknoten." Ahrens term for the enamel knot is derived from its placement within the enamel organ—the structure that will become the tooth crown. In some respect, though, the term enamel knot is a misnomer—the enamel knot appears in the inner enamel epithelium during day 13 (late bud stage/early cap stage) of dental development, well before mineralization occurs, and so it is not directly associated with the enamel of the tooth.

[107] See Allen (1975) and Maienschein (1991) for excellent overviews of the experimental turn in embryology. Although, as Schickore (in print) points out, there is a great deal of experimentation inherent in microscopy and the preparation of tissues.

[108] "Es ist wirklich an der Zeit, dass die wissenschaftliche Bearbeitung der Zahnentwickelung wieder in exakte Bahnen einlenkt und sich zunächst auf die Feststellung nackter Tatsachen beschränkt, die später einmal von einem Berufenen zu einer Theorie zusammengefasst werden können."

Ahrens sought to challenge the established theories of dental development, especially the concrescence theory.

Through working with local clinics and hospitals around Munich, Ahrens amassed an unparalleled sample of human fetal and postnatal dental remains. He fixed his materials in formalin and applied a number of contrast dyes (Borax carmine, Eosin, etc.). He made frontal, horizontal, and sagittal sections for every stage of development and was able to tailor his sectioning thickness to meet the demands of the specimen. Ahrens embedded the smallest specimens in paraffin—creating serial sections of the entire head. While most specimens were sectioned at a thickness of 20µm, younger individuals were sectioned at 10µm, granting Ahrens a finer resolution for the youngest, and thus smallest, stages of tooth development (Ahrens 1913).[109]

Ahrens firmly believed in the necessity of reconstructing structures through serial sections and wax models: "It is impossible to make a clear and complete picture of dentition without a reconstruction method that is designed for complicated structures like the developing teeth" (Ahrens 1913, 170).[110,111] He was exquisitely careful with the preparation of his sections, creating his own technique for collecting and preserving them, which included 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

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[109] 20µm is the thickness employed by Röse in his study of dental development of humans (Röse 1892)

[110] "Es ist unmoglich, ohne das Rekonstruktionsverfahren sich von den raumlich so komplizierten Gebilden, wie sie die sich entwickelnden Zahne darstellen, ein nur annähernd klares und vollstandiges Bild zu machen."

[111] For a discussion of the use of three-dimensional models for understanding and evaluating anatomy, see Hopwood (1999) and Radlanski (1995)

sequences that afforded him a view into the changing relationships between oral tissues. The serial sections of the younger specimens were then used to create three-dimensional wax models based on the methodology of Röse and Gustav Born (Born 1883).[112]

Ahrens's extensive samples and meticulous collection techniques allowed him to investigate a variety of claims about dental ontogeny. For instance, Ahrens found that the dental lamina was present in embryos much younger than Röse had thought (11mm crown-rump length vs. 15mm crown-rump length). The dental lamina is the structure from which the dental papillae develop. Recall from Chapter 3, that through this structure, Röse believed, the reptilian cone-like teeth of our evolutionary ancestors were preserved and arose as primordia (e.g. papillae). These primordia crowded together throughout ontogeny, and concresced into the cusps on tooth crowns. This crowding together during development constituted the core of Röse's concrescence theory.

Through his reconstruction techniques and inclusion of very young specimens, Ahrens found evidence to discredit Röse's concrescence theory on two grounds. First, the number of dental papillae necessary to concresce into the multi-cusped molars and premolars of humans were not present within the dental lamina.[113] Second, he could find no evidence for the concrescence of the papillae through his observations. Thus, to

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[112] This technique was first applied to dental development by Röse (1892b). It is interesting to note that while wax three-dimensional modeling fell out of favor with many embryologists and developmental biologists by 1900, it was used pervasively in the study of tooth development throughout the 20th century, and computer-assisted three-dimensional modeling was critical for the development of the current paradigm surrounding the role of the enamel knot in tooth development. See Hopwood (1999) for a discussion of the history of His' embryological modeling techniques, and Gaunt (1955) for examples of wax modeling within the field of dental development.

[113] "Die von Röse angeblich beobachtete Umwachsung mehrer Papillen durch die Zahnleist beruht auf Täuschung." Ahrens 1913, 253

Translation: The alleged observation by Röse of the concrescence of several papillae via the dental lamina is deceptive.

Ahrens, his work eliminated, “the only reasonable viable support for the concrescence theory” (Ahrens 1913, 253).[114]

In addition to evaluating the evidence underlying the concrescence theory, Ahrens sought in his work to establish accurately the fundamentals of tooth development, especially the changing spatial relationships of the epithelium and mesenchyme. His goal was not to create his own overarching theory of dental development, but to point to observations that accounted for the changes that he witnessed in developing teeth and which potentially contributed to the final form of the tooth.[115] These observations were typically about structures and the changing dimensions of tissues. In setting up his research, he wanted to use his data to investigate the claims of Röse, and he was also in a unique position to observe and document the changing forms of the teeth as they developed and the spatial relationships of their components. His serial sections and wax models were especially useful in this regard.

5.1.1 *Ahrens and the Enamel Knot*

Ahrens’s publication is structured to retrace dental ontogeny, beginning with the dental lamina and proceeding on to the structure of the permanent molars, after which he addresses the concrescence theory (Ahrens 1913). Following his observations of the dental lamina, Ahrens moves on to “The Development of the Enamel Organ” (Ahrens 1913, 184). The enamel organ is an epithelial structure in the developing tooth that will

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[114] “Damit entfällt die einzige einigermassen brauchbare Stütze der Concrescenztheorie.”

[115] Unlike the researchers that we met in previous chapters (Cope, Osborn, and Röse), Ahrens did not call his observations or generalizations about development “theories”.

form the enamel of the tooth crown and give the tooth its characteristic form. His motivation in tracing the development of the enamel organ was that the available literature describing the process through which it develops was quite poor:

> The only question which has been dealt with by previous authors, and which has been much debated, is whether the mesoderm or the epithelium plays the active role in the initiation [of tooth development]. Though the majority of authors have distinguished themselves in the latter sense, they have not taken pains to actually trace the process of invagination of the epithelium, and thus to prove the assertion. (Ahrens 1913, 185)[116]

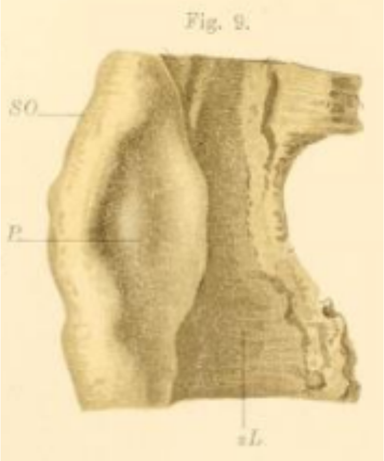

Figure 11. Rendering of a wax model of the dental epithelium of a mandibular tooth from a 32mm long fetus. SO= schmelzorgan (enamel organ), P= enamel knot, zL=zahnleiste (dental lamina). Figure 9 from Ahrens 1913, Tafel 18.

Because the folding of tissues during the early stages of the enamel organ is complex, Ahrens turned to his serial sections and wax models to help him visualize the shifting forms. It was through these reconstructions of early stage teeth that he first noticed a cluster of cells that he called the “epithelial knot.”

Ahrens first noticed the epithelial knot within a wax model of a 32mm long embryo (figure 11). The structure appeared “as a thickening between two grooves in the epithelium of the enamel organ” (Ahrens 1913, 188).[117] He described the bump as an “epithelial knot”

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[116] “Die einzige Frage, die wir von den Autoren behandeltfinden und uber die viel gestritten wurde, ist, ob das Mesoderm oder das Epithel die aktive Rolle bei der Einstülpung spielt. Wiewohl die Mehrzahl der Autoren sich im letzteren Sinne entsehieden haben, hat sieh doch keiner der Muhe unterzogen, den Vorgang der sogenannten Einstülpung am Epithel auch wirklich zu verfolgen und damit erst den Beweis der Behauptung zu erbringen.”

[117] “Ganz besonders aber auffalend ist eine Verdichtung in der Mitte derselben an der Vereinigungsstelle der beiden Falten...” Ahrens, 1913; 188

Translation: It is especially striking that a condensation is in the middle of the latter [enamel grooves] at the junction of the two folds…

due to its placement within the epithelium of the enamel organ, but later changed its name to “enamel knot” (Ahrens 1913, 188).[118] After finding the structure within a wax model, he checked his serial sections (figure 12), where he noted the presence of an oval-shaped arrangement of cells within the epithelium surrounded by “randomly arranged and intensely colored cells” (Ahrens 1913, 188). Ahrens continued to trace the structure through his wax models and serial sections, noting changes in its form, along with the concomitant changing form of the enamel organ, throughout ontogeny.

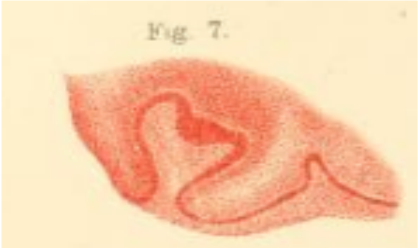

Figure 12. Section of a maxillary molar from a 32mm long fetus. The red cluster in the center is the enamel knot. Figure 7 from Ahrens 1913,

The enamel knot became of interest for Ahrens because of its placement within the developing enamel organ—the structure that gives rise to the tooth crown. The epithelium of the enamel organ was particularly important because it both gives rise to the final form of the tooth, and is also “the only element which is the formative element,” of the tooth (Ahrens 1913, 191).[119] In addressing the enamel organ as the “formative element,” Ahrens was answering the question of which tissue—the epithelium or the

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[118] Ahrens would go on to refer to this “epithelial knot” as the “enamel knot” on page 192. Enamel knot, in German, is “schmelzknoten.” The term is derived from the fact that the enamel knot develops within the enamel organ—the epithelial precursor of the tooth crown. The name is a bit of a misnomer—the enamel organ does not create enamel until well after the enamel knot has disappeared.

Only one researcher had previously identified the structure that came to be called the enamel knot within the literature—Axel Ohlin, in his 1896 “Om tandutvecklingen hos Hyperoodon,” a Swedish publication that has been cited four times (three of those times are after 1995). Unlike Ahrens, Ohlin’s finding of this structure did not lead to its uptake within the community, thus, although Ohlin has chronological primacy, it would be problematic to say that his work had any impact on the history of the enamel knot throughout the 20th century.

[119] “Das Epithel des Schmelzorgans ist einzig und allein das formgebende Element.”

mesenchyme—directs dental development.[120] Ahrens believed that the epithelium, in particular the enamel organ, played this role, and his interest in the enamel knot was tied to the formative role of the epithelium in tooth development.

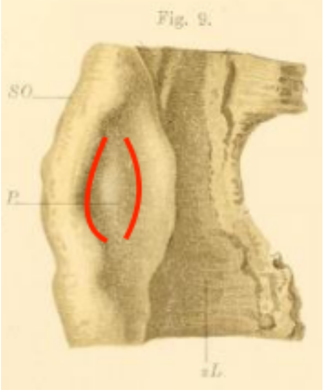

Figure 13. Labelled rendering of wax model. Red lines indicate enamel grooves. Modified from Figure 9 from Ahrens 1913, Tafel 18.

Ahrens did more than simply establish the fact that there is a structure within the developing enamel organ and name it the enamel knot. Through his histological interrogation of the fine morphological changes of developing teeth through serial sections and wax models, Ahrens noticed that the enamel knot bulges out of the enamel organ into the surrounding mesenchyme, causing the formation of two grooves in the epithelium (figure 13). Ahrens named these grooves the "enamel grooves" (Ahrens 1913, 192). The enamel grooves, according to Ahrens, deepen throughout ontogeny to form "the mold of the labial and lingual cusps or cusp rows of molars" (Ahrens 1913, 252).[121] Thus, the enamel knot, positioned within the active material of the epithelium (the enamel organ) directs the overall form of the tooth by causing the formation of the enamel grooves, which ultimately become the cusps. By establishing the enamel knot's position within the active enamel organ, and its role in causing the formation of the enamel grooves, Ahrens placed the enamel knot as a main factor in explaining individual tooth development.

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[120] The question of whether the epithelium or the mesenchyme was the 'formative' element was prominent throughout the later part of the 19th and well into the 20th century.

[121] "Diese 'Schmelzrinnen' bilden, indem sie sich vertiefen, die Gussform der labialen und lingualen Höcker bzw. Höckerreihen der Backenzähne."

5.1.2 *Summary and Assumptions*

Ahrens's techniques and research constituted a step forward in understanding tooth development. Previous authors had covered a huge range of topics within tooth development, but had often published these findings piecemeal—leaving the field scattered and schematized. Ahrens's work attempted to change that by meticulously tracing development from the earliest appearance of tooth germs through to their final forms and placing all of these observations within a single publication.

For Ahrens, much like for Röse, an adequate explanation of tooth development was a description of the fine morphological changes through which teeth form. However, unlike Röse, Ahrens developed his explanation of tooth development without appeals to evolution. He created his descriptions of morphological changes by using the wax models he created and his serial sections stained to highlight different tissues to trace changes in structures throughout ontogeny. Ahrens inferred relationships between structures from these static representations of different stages of development. This inference is exemplified in his explanations of the relationship between the enamel knot, the enamel grooves, and the final cusps of the tooth. Ahrens witnessed in his wax models and serial sections the structure that he called the enamel knot. As he monitored the enamel knot throughout his specimens, he noticed a set of grooves surrounding the enamel knot, which he named the enamel grooves. Due to their location at the base of the enamel organ, which ultimately becomes the tooth crown, and to his histological techniques that "showed" him the continuance of these grooves, Ahrens inferred that 1) they formed due to the enamel knot, and 2) that they were the basis of cusp formation. At no point did

Ahrens collect evidence for these claims from direct observations of living tissues—this was not necessary within his research framework or his understanding of what counted as an adequate explanation of development.

Ahrens also understood that development was accomplished by changes in cellular-level phenomena, such as gross changes in cell morphology and structure, but he made no appeal to cellular-level processes, such as mitosis, cell death, or cell migration—none of which was necessary for his explanation of development.

5.2 Erwin Reichenbach, 1926/1928

Ahrens's histological work on tooth development built on the research of previous authors, like Röse, to give a more accurate depiction of the processes of tooth development, especially as they applied to the changing form of the enamel organ throughout ontogeny. And, Ahrens's work, unlike many who preceded him, created an understanding of tooth development *sans* evolutionary explanations.[122] Evolution, to Ahrens, pointed to the unbridled theorizing that had led Röse to develop the concrescence theory—a theory that he had found no evidence to support. Due to the connection between evolution and theory, Ahrens chose to start from the evidence in front of him and build his accounts of development strictly from the phenomena he witnessed. Despite the advances that Ahrens made in describing tooth development, there was still much to

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[122] Evolutionary explanations continued after Ahrens's 1913 publication, see for instance Bolk (1920, 1921, 1922), but it was far less popular in the dental literature to appeal to evolution as part of the explanatory framework or use dental development to test evolutionary hypotheses.

learn about how teeth develop from an initial clump of cells within the dental lamina into a fully formed organ.

> The development of the teeth and their histological structure has always been the object of extensive research. 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[123]

The proliferation of histological studies, in particular, became a point of contention because while these works did much to characterize the fine morphological changes through which the tooth forms, the literature was scattered, schematized, and often came to contradictory results:

> The enamel pulp and the enamel organ, as regards their finer morphological structure in the individual stages of development, have not yet been subjected to any thorough systematic investigation and summary treatment. It is true that information and publications are found scattered here and there. But even these are inaccurate and contradict themselves to a great extent… Reichenbach 1926, 524[124]

In the mid 1920s, Erwin Reichenbach, an assistant at the Dental Institute of the University of Munich, attempted to give an explanation of tooth development that built on and went beyond Ahrens's studies. Like Ahrens, Reichenbach set aside evolutionary considerations. Histological research, by Reichenbach's estimation, was important, but had not sufficiently addressed the mechanical transformations that teeth undergo. In order

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[123] "Die Entwicklung der Zähne und ihr histologischer Aufbau war schon von jeher der Gegenstand ausgedehnter Forschungen. Während auf dem Gebiete der formalen Zahnentwicklung die Forschungen hauptsächlich zuletzt durch die Arbeiten von Ahrens zu einem gewissen Abschluß gekommen sind, haben die Autoren die vornehmlich die Zahnhistogenese bearbeiteten, bisjetzt nicht genügend Klarheit in alle Probleme dieses schwierigen Kapitels bringen könne."

[124] "Die Schmelzpulpa und die Schmelzepithelien nämlich sind, was ihren feineren morphologischen Aufbau in den einzelnen Entwicklungsstadien betrifft, noch keiner eingehenden systematischen Untersuchung und zusammenfassenden Bearbeitung unterzogen worden. Wohl finden sich da und dort, zerstreut liegend, Angaben und Veröffentlichungen. Aber selbst diese sind ungenau und widersprechen sich in weitgehendem Maße..."

to do this, researchers had to look to the localized changes within the tissues—to characterize the transformations of cells within the tissues of the developing tooth and the pressures that shape and move them throughout ontogeny. That is, they had to explain tooth development in terms of morphogenesis.

> 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.[125] Reichenbach 1928, 53. [emphasis mine]

Reichenbach thus shifted the discussion of tooth development from the characterization of fine morphological detail of tissues to the characterization of the cells and the forces that shape them. That is, he sought to give a biomechanical account of tooth development and morphogenesis (figure 14).

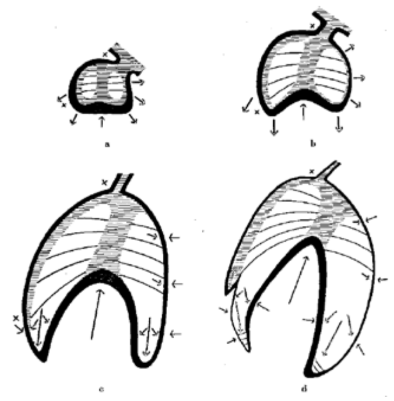

Figure 14. The ways in which biomechanical forces shape the developing tooth as imagined by Reichenbach. Figure 26 from Reichenbach 1928, 504.

In his two-part Habilitationsschrift for the University of Munich, published in 1926 and 1928, Erwin Reichenbach investigated the transformations of the enamel pulp and enamel epithelium during pig dental development. The enamel pulp is the mesenchymal structure that lies adjacent to the enamel epithelium, also known as the enamel organ. These two

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[125] “Abgesehen von den rein biologischen Faktoren, deren Analyse heute noch kaum zugänglich ist, kann auch mechanischen Kräften ein normativer Einfluß bei der Gestaltung der Zahnkrone zuerkannt werden. Durch die auf bestimmte Stellen lokalisierte Proliferationimäußeren Schmelzepithel, die ihrerseits wieder zur Dehnung anderer Abschnitte desselben führt, verbunden mit der Flüssigkeitszunahme im Innern des Schmelzorgans und der ungleichen Differenzierung der Schmelzpulpa, werden.”

structures, Reichenbach believed, were underrepresented in the dental literature and the physical properties of their transformations were poorly understood.

Understanding the biomechanics of the enamel pulp and enamel epithelium was important to Reichenbach because he understood there to be limitations on what traditional morphological explanations could account for. For instance, the tooth germs arise within the dental lamina at regular intervals: "With the question of why these develop only at certain intervals of the dental lamina, and not in its entire extent, we touch upon a fundamental question of developmental history, for whose complete explanation the morphology does not provide us with any kind of handle" (Reichenbach 1928, 491).[126] Morphological inquiry had not given a sufficient explanation for why the tooth germs form at regular intervals, nor had it given an explanation of what happens to the surface of the dental lamina between the tooth germs (Reichenbach 1928, 494).

Reichenbach amassed and processed his own collection of pig teeth, gathering 96 specimens that ranged from 1.5 to 19.5 cm long. He created frontal and sagittal sections of 5 to 10μm thicknesses for his specimens, and applied several types of contrast dyes.[127] Using these techniques, Reichenbach refocused the histological study of tooth development from purely morphological descriptions to descriptions that focused on the forces that shape the tooth by looking at cell proliferation, and cellular and intercellular

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[126] "Mit der Frage, warum diese nur in bestimmten Abständen der Zahnleiste sich entwickeln und nicht in deren ganzer Ausdehnung, berühren wir eine Grundfrage der Entwicklungsgeschichte, zu deren restloser Aufklärung die Morphologie uns jedenfalls keine Handhabe bietet."

[127] Note that Reichenbach's section thicknesses were even finer than Ahrens in some instances. Whereas Ahrens's thinnest sections were 10μm, Reichenbach chose to also section at 5μm—especially within the youngest, and thus smallest specimens. This finer sectioning thickness gave him an even finer grained resolution for investigating the morphological changes within the tissues, especially when it came to tracking cell proliferation and changing cell morphology.

fluid movements.[128] Within his search for a biomechanical explanation of morphogenesis, Reichenbach took special interest in the active elements of the developing tooth—the movements and mitoses of cells and fluids and the pressures that shaped them. He assumed that these active elements were what shaped the developing tooth. Inactive components of the tissues were viewed as impediments to growth, and thus useful morphologically, but not ultimately causal for transformations.

5.2.1 *Reichenbach and the Enamel Knot*

Reichenbach's 1926 and 1928 publications, "The Transformation of the Enamel Pulp and Enamel Epithelium during Tooth Development," reads as a direct response to many of the claims that Ahrens made regarding the formation of the enamel pulp and enamel epithelium, and particularly tests the formation and role of the enamel knot in shaping the enamel epithelium. Just like Ahrens, Reichenbach saw that an understanding of how these two tissues developed was crucial to explaining how teeth acquire their characteristic forms. And so, Reichenbach used his observations to assess many of Ahrens's claims about the enamel knot, the enamel grooves, and how the enamel pulp and enamel epithelium develop.

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

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[128] In many ways, Reichenbach's view of morphogenesis harkens back to D'arcy Thompson's view in *On Growth and Form*: "The form, then, of any portion of matter, whether it be living or dead, and the changes of form which are apparent in its movements and in its growth, may in all cases alike be described as due to the action of force. In short, the form of an object is a 'diagram of forces', in this sense, at least, that from it we can judge of or deduce the forces that are acting or have acted upon it…" 11 (Quote taken from the 1961 abridged edition, edited by J.T. Bonner). Reichenbach's focus on kinematics at the cellular level speaks to a strongly physical view of morphogenesis and the development of form, as advocated by Thompson.

enamel knot to the surrounding tissues? And, what happens to the enamel grooves that Ahrens deemed the precursors of the cusp rows? In order to investigate these questions, Reichenbach turned to his serial sections and a variety of staining techniques.

Based upon his observations of stained serial sections, Reichenbach came to the conclusion that the enamel knot was not so distinguishable a structure as Ahrens had thought. Ahrens believed that the enamel knot formed within and was surrounded by cells of the enamel organ. In contrast, Reichenbach found it to lie directly adjacent to the enamel pulp, and thus be in contact with the mesenchyme underlying the enamel organ. He also had trouble clearly distinguishing it, especially in later stages, from the underlying mesenchyme.

Reichenbach also had difficulty determining how the enamel knot formed. If it was through passive properties, i.e. not increased mitosis or cell movement, then, he reasoned, there should be evidence of a localized increase in individual cell bodies (Reichenbach 1928, 494). He found little evidence for this. If the enamel knot formed through active properties, such as mitosis or cell movement, then there should be evidence for this phenomenon—Reichenbach noted that there was no local increase in mitosis.[129] In fact, it appeared to him that the level of mitosis within the enamel knot was roughly the same as that of the surrounding tissues (Reichenbach 1928, 495). Thus, Reichenbach, came to the conclusion that the formation of the enamel knot was, "due most likely to passive aggregation of existing cell material" (Reichenbach 1928, 535).

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[129] Reichenbach's observations of mitosis were inferred through a combination of staining and observations of changes in cell size and shape, as well as relative positions of the cells' nuclei.

Throughout his investigation of the transformation of the enamel knot, Reichenbach paid attention to the cells and tissues surrounding the structure, and was particularly interested in the observing the enamel grooves.

> The "enamel grooves" of Ahrens, which are of essential importance for the later formation of the tooth, are nothing more than shallow wells filled with mesoderm, which appear only as a result of the formation of the "enamel knot," and disappear again in the course of the further growth of the enamel. (Reichenbach 1928, 535)[130]

Recall that for Ahrens, the formation of the enamel knot creates the surrounding enamel grooves within the epithelium; these enamel grooves become the basis for the cusps of the tooth crown. In this way, the enamel knot shapes the tooth and is a main factor for explaining how teeth develop their characteristic forms. Reichenbach's findings removed the enamel grooves from the discussion of what shapes tooth development, thus opening the question of what, if anything, was the role of the enamel knot in tooth development.

With regard to the role of the enamel knot in tooth development, Reichenbach made three main observations. First, that the enamel knot forms through passive processes (Reichenbach 1928, 535). Second, that while the surrounding epithelium and mesenchyme change shape, the enamel knot remains a static structure (Reichenbach 1928, 498). Third, that the enamel knot disappears as the tissues surrounding it grow (Reichenbach 1928, 535). These observations led Reichenbach to the conclusion that the enamel knot played no active role in shaping the developing tooth, but maybe served as a temporary reservoir of cells (Butler 1956).

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[130] Reichenbach noted that, "Die von Ahrens als für die spätere Formbildung der Zahnkrone von wesentlicher Bedeutung angenommen "Schmelzrinnen" sind nichts weiter als flache, von Mesoderm erfüllte Mulden, die nur infolge der Ausbildung des "Scmelzknotens" in Erscheinung treten und im Verlaufe des weiteren Wachstums des Schmelzorgans wieder verschwinden."

5.2.2 *Summary and Assumptions*

To Reichenbach, an adequate explanation of morphogenesis had to account for the biomechanical activities that shape the tooth—i.e. differential mitosis, and cell and intercellular fluid movements. He found value in the works of researchers like Ahrens, who traced the fine morphological changes in structures, but did not believe that such accounts were adequate explanations of tooth development. In order to gather evidence to give a biomechanical explanation of development, Reichenbach used techniques very similar to Ahrens—serial sections stained to allow observations of the differences between tissues. However, Reichenbach observed these serial sections somewhat differently from Ahrens. Whereas Ahrens had used his serial sections and wax models to infer the relationships between structures, Reichenbach used his materials to infer mitosis and movement. Reichenbach did not have direct access to evidence for these cellular processes—he used only fixed samples. His inferences were drawn from observing the appearances of cells; by noting the changes, like cell size and distance between nuclei, in individual cells across serial sections representing different stages of development, Reichenbach believed that he had evidence for mitosis and movement.

When it came to the enamel knot, Reichenbach found that the enamel grooves did not enlarge to become the tooth cusps, removing the explanatory role that Ahrens had assigned to the structure. Furthermore, Reichenbach found that levels of mitosis within the enamel knot were the same as the surrounding tissues. Reichenbach's biomechanical understanding of morphogenesis put the emphasis on active properties of development like mitosis and movement, and the physically passive properties of tissues were deemed as impediments to growth that only indirectly affected overall form. Given Reichenbach's understanding of what development is and how it should be characterized, 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 that were necessary to explain development.

5.3 Nozue and colleagues, 1971-1973

Following the work of Ahrens, many other researchers investigated the enamel knot—while it was rarely the main focus of study, it became a steady component of inquiry for those interested in tooth development. Researchers like Isaac Schour, Erwin Reichenbach, P. Santone, Erich Blechschmidt, Percy Butler, and William Gaunt and Albert Miles prodded at questions of its morphological significance (Blechschmidt 1953, Butler 1956, Gaunt and Miles 1967, Reichenbach 1926, 1926, Santonè 1935, Schour 1929). These questions were embedded within a larger framework of understanding how the tooth develops its characteristic form and appealed to morphogenesis as the best way to explain this development. These studies utilized histological techniques, much in the ways that Ahrens and Reichenbach had, to trace the enamel knot as part of larger studies about tooth development in different organisms.

While Ahrens did not look at mitoses within the developing tooth, Reichenbach and many others who followed did. The results of these studies frequently came to conflicting conclusions about the enamel knot, especially regarding mitosis within the structure. Santonè maintained that there was an increase in mitosis within the enamel knot, while Reichenbach and Schour found no difference between levels of mitosis within the enamel knot and the surrounding tissues (Reichenbach 1926, 1928, Santone 1935, Schour 1929). Meanwhile, Blechschmidt reported that mitosis ceased within the enamel knot early on (Blechschmit 1953). Thus, while the enamel knot had been subjected to investigation by a number of researchers, the level of mitosis within its cells was unknown, and relatively little was known about the distribution of mitosis overall within the early stages of tooth development (Nozue 1971a, 4).

As we saw with Reichenbach, tracing mitosis within the early stages of tooth development became an important problem for dental embryologists because differential cell proliferation was considered a main factor for shaping tooth development. Thus, knowing which cells were dividing and where was an important problem. In light of conflicts within 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 35 human fetuses, ranging from 50 to 125mm crown-rump length, and fixed his specimens in formalin. He created serial sections of lower lateral incisors at 15-20μm thicknesses and stained his sections with hematoxylin and eosin. Using these methods, Nozue observed that “mitoses, although present in the surrounding part of the enamel knot, are not found in the enamel knot itself,” and that “mitoses were observed all over the tooth bud…mainly in the cells adjacent to the enamel knot” (Nozue 1971a, 2). Thus, while cells within the enamel knot did not divide and proliferate, the cells in the mesenchyme and epithelium immediately adjacent to the enamel knot experienced heightened mitosis. Nozue did not draw out this point further, but differences in mitosis throughout the structure led him to conclude that the "distribution of mitoses in the enamel organ requires further quantitative study" (Nozue 1971a, 4).[131]

After investigating the levels of mitosis, Nozue turned his investigation of the enamel knot toward something he had noticed during his mitosis study. In the course of his first study of the enamel knot, Nozue observed the presence of "globular substances of brownish black or yellowish brown color in the enamel knot" (Nozue 1971b, 139). These structures had not previously been reported in association with the enamel knot, and so Nozue undertook a histological examination of developing teeth to investigate what these globular substances could be.

In this study, Nozue gathered both fetal human and mouse specimens. The human fetuses were fixed in formalin, sectioned at 10-20μm, and stained with hematoxylin and eosin. The mouse embryos were fixed with a number of materials, sectioned at thicknesses ranging from 4-15μm, and then stained with one of five different histological stains: hematoxylin and eosin, Masson Goldner, Methyl Green-Pyronin, Feulgen, and Mallory-Heidenhain. These different fixatives and stains allowed Nozue to begin to differentiate what the globular substances he had noticed in the enamel knot could be.[132] Using these methods, Nozue concluded,

> Although cell death in the dental epithelium has not been reported, the globular substances might be the products of cell death or degeneration. The fact that the substances, presumably products of the cell death, were observed conspicuously

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[131] In addition to recognizing a lack of mitosis within the enamel knot, Nozue also observed that the adjacent mesenchyme became more compact with the appearance of the enamel knot, and lost this compactness when the enamel knot disappears.

[132] The differences between and applications of these different stains is an important project, but for the sake of time and space, are not part of the discussion here.

> in the enamel knot is suggestive of the significance of the enamel knot. (Nozue 1971b, 142)

The presence of cell death within the enamel knot was an important finding because cell death was considered a main process that shapes development through morphogenesis (Saunders 1966).

Nozue continued his investigations of the enamel knot by teaming up with two other dental researchers based in Tokyo—Tadao Kirino and Motohiko Inoue. Together, the trio began a new course of investigation into the role the enamel knot plays in tooth development. While a number of researchers had previously investigated the enamel knot in the course of broader studies, none had used experimental techniques. That is, none had tried to intervene in the course of normal development to determine what the influence of the enamel knot could be on shaping surrounding tissues.

By the early 1970s, using experimental techniques to look at tooth development was a well-established practice. While the first *in vivo* experiment using teeth was conducted in 1874, it was not until 1936 that researchers implemented *in vitro* techniques to observe development in living teeth (Legros and Magitot 1874, Glasstone 1936).[133] In 1952, Shirley Glasstone, a researcher at the Strangeways Research Laboratory in Cambridge, England, became the first person to manipulate experimentally developing teeth—she cut tooth germs in half and watched them grow *in vitro*.[134] Finally, in 1969, Edward Kollar and Grace Baird conducted the first tissue recombination experiments on

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[133] Legros and Magitot (1874) subcutaneously grafted pieces of tooth germs into guinea pigs and dogs, while Shirley Glasstone (1936) used the hanging drop method to monitor the development of whole tooth germs and excised dental papillae *in vitro*.

[134] "Tooth germs" to Glasstone in her 1952 paper correspond to the epithelium and mesenchyme that make up the developing tooth. She took these germs from rabbit embryos at days 20, 21, and 22. In rabbits, Glasstone notes, the cusps form during the 21st day.

teeth *in vitro*—recombining epithelium and mesenchyme to investigate the inductive phenomenon that existed between these two tissues (Kollar and Baird 1969; MacCord 2013).[135]

Thus, by the time that Kirino and colleagues sought to use experimental techniques on the enamel knot, their application to tooth development in general, and the interactions between epithelium and mesenchyme in particular, had already been widely implemented. Although investigating the interactions between epithelium and mesenchyme were popular in embryology, and experimental techniques were utilized throughout a wide array of dental development research programs, no researcher had looked at the enamel knot experimentally or as a part of epithelial-mesenchymal interactions. "A considerable amount of research has been reported on the epithelial-mesenchymal inductive phenomenon in the tooth morphogenesis. However, little is known about the relation between the enamel knot and mesenchyme with reference to morphogenesis" (Kirino et al. 1973, 117).

Throughout the course of his previous studies, Nozue had recognized a possible relationship between the enamel knot and the underlying mesenchyme by noting the changes in the dental papilla throughout the lifespan of the enamel knot (Nozue 1971a, Kirino 1973). Nozue and his colleagues thought that this relationship might have to do with the epithelial-mesenchymal interactions that shape the tooth, and sought to determine the enamel knot's role in such interactions.

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[135] See: Kollar and Baird (1969, 1970a, 1970b). Note that Kollar and Baird, in pursuing epithelial-mesenchymal tissue recombination studies drew heavily from the works of John Saunders and Mary Rawles. See: Cairns and Saunders (1954) and Rawles (1963) Tissue recombination experiments were not new, and their deployment within embryology helped to solidify the search for the chemical determinants of development. See: Jane Oppenheimer (1991) for an overview of Herbst's work and Spemann's later contribution to studies of tissue interactions.

In addressing the relationship between the enamel knot and the underlying mesenchyme, the trio drew a conceptual tie between the enamel knot and a well-known epithelial-mesenchymal interaction—that which exists between the apical ectodermal ridge (AER), an epithelial structure, and the underlying mesenchyme (Kirino et al. 1973, 117). The interaction between the AER and the mesenchyme shapes the outgrowth of the tetrapod limb, with the AER acting as a signaling center, inducing growth of the surrounding tissues. Thus, Kirino, Nozue, and Inoue sought to understand the role of the enamel knot in the inductive interaction between the epithelium and mesenchyme of the developing tooth (Kirino 1973, 117).

In order to investigate this role experimentally, Kirino and colleagues drew on that conceptual connection between the enamel knot and the AER. In earlier studies, researchers had found that they could alter the development of the limbs in fetal mice by injecting pregnant mice with a potent DNA crosslinker called Mitomycin C (Tanimura 1968).[136] This alteration of development was thought to result from interrupting the inductive stimulus between the AER and the underlying mesenchyme. Drawing on their analogy between the enamel knot and the AER, Kirino and colleagues reasoned that this type of intervention could be used to investigate the relationship between the enamel knot (an epithelial structure) and the underlying mesenchyme. By injecting Mitomycin C into pregnant mice, Kirino et al. hoped to interrupt what they thought could be an inductive stimulus between the enamel knot and the underlying mesenchyme in order to understand

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[136] Mytomycin C is a potent DNA crosslinker, thus it arrests the subsequent replication of DNA and can ultimately result in cell death.

"the inductive phenomenon between the enamel knot and the mesenchyme" (Kirino et al. 1973, 117).

In order to test their hypothesis, namely that the enamel knot is involved in the interaction between the epithelium and mesenchyme, the group injected cohorts of 100 pregnant female mice with mitomycin C on the 11th, 12th, 13th, and 14th days of pregnancy. The fetuses from these samples, along with 25 control mice were harvested, fixed, sectioned, and stained with hematoxylin and eosin. Frontal sections of the molars were used for comparison.

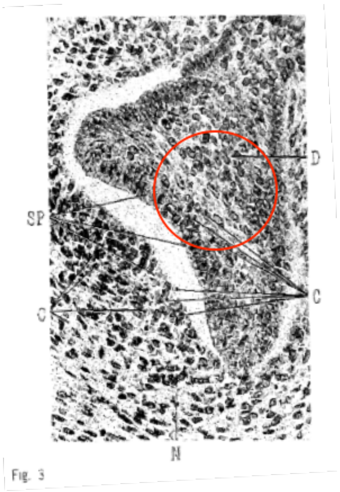

Figure 15. Section of a developing tooth that lacks the enamel knot (approx. day 14). Red circle indicates area in epithelium where enamel knot would normally form. C= cell death, D= dental epithelium, M= Mesenchyme. Figure 3 from Kirino et al. 1973, Plate 2.

Following these preparations, Kirino and colleagues found that "in the tooth germ lacking the enamel knot, mitosis was hardly seen and cell modifications were recognized, and the globular cells decreased in number, especially in the mesenchyme" (Kirino et al 1973, 123). Additionally, they noticed that, "in the tooth germ which was deficient in the enamel knot, subsequent development was interrupted" (figure 15) (Kirino et al. 1973, 122). These observations indicated to the group that the enamel knot was in some way causally necessary for inducing proper mitosis and cell death within both the epithelium and adjacent mesenchyme and thus played a central role in shaping the developing tooth.

5.3.1 *Summary and Assumptions*

Nozue and colleagues represent an interesting 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 observations of these phenomena 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 the different tissues—epithelium and mesenchyme—in directing morphogenesis. By turning to experimentation in this framework, the group sought to define the enamel knot in terms of its signaling capacity, i.e. whether or not it could direct surrounding tissues in their morphogenesis. Thus, to Nozue and colleagues, development was still considered in terms of moving and dividing cells, but they also understood that tissue interactions, i.e. the signaling between tissues, could direct tissue growth and cell proliferation.

While Nozue and colleagues were interested in the interactions between epithelium and mesenchyme, their experimental methods granted them only indirect access to evidence about the enamel knot's role in these interactions. And, through their Mitomycin C experiment, they could not determine what caused the "inductive phenomenon" between the enamel knot and the mesenchyme—that is, they could not identify what signals were producing the effects they witnessed or how these signals were operating. While they did not provide direct evidence for signaling from the enamel knot, their work implied the possibility that it had this property.[137] Thus, the enamel knot gained explanatory value for the problem of individual tooth development.

5.4 The Enamel Knot's Finnish Renaissance: Jukka Jernvall, 1994

The vast majority of the research that took the enamel knot into account during the mid-20th century utilized strictly histological techniques—tracing the fine shifts in morphological details and looking at things like the movements of cells and fluids in order to explain development in terms of morphogenesis (Blechschmidt 1953, Butler 1956, Gaunt and Miles 1967, Santone 1935). In the broader scope of dental research, most investigators turned towards experimental techniques but did look at the enamel knot or prod at its explanatory value (for example, see: Glasstone 1952, 1954, 1958, 1964, 1967a, 1967b, 1971; Kollar and Baird 1969, 1970a, 1970b). While the research of Nozue and colleagues represents an important shift in the way in which investigators understood the enamel knot and used it to explain development, their work went almost completely unnoticed.[138] By the time that Nozue and colleagues took up their investigations on the enamel knot, it was largely relegated to typological obscurity—

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[137] Note that Kirino et al (1973) did not refer to the enamel knot as a signaling center or directly state that it had a signaling property. This conclusion is implicit within the discussion of the enamel knot as being involved in an "inductive phenomenon" with the mesenchyme and with the groups comparison of the enamel knot to the AER, which had already been touted as a signaling center. While Kirino and colleagues never directly mention signaling in their article, it is implicit that the enamel knot works in the same way as the AER—by signaling to surrounding mesenchyme—and in this way directs the morphogenesis of the tooth. Thus, a new property for the enamel knot was tentatively uncovered—signaling.

[138] A google scholar search of Nozue's, Kirino's, and Inoue's publications conducted on February 21, 2017 revealed that each of the publications has less than 10 citations.

existing almost entirely within the confines of oral histology texts. Unfortunately, their work did little to change this. And yet, the seeds were planted.

The fate of the enamel knot began to change 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 of tooth development at a period in which 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.[139] Developmental biologists, using this technique, were in search of spatial information regarding gene activity in the developing embryo in order to get clues about the functions of newly cloned genes.[140] The possibility of locating genes as they were expressed in cells and tissues *in situ* had profound implications for developmental biology—now, 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 and ecology. His graduate field work at a Miocene site in Peshawar, Pakistan, with Lawrence Martin of SUNY Stony Brook and Mikael Fortelius of the University of Helsinki gave Jernvall

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[139] According to Levsky and Singer (2003), the earliest *in situ* hybridizations were performed in the 1960s using probes labeled with radioisotopes. The first methodological report of flourescent *in situ* hybridization is from Bauman et al (1980).

[140] For a history of the uses of in situ hybridization, see Koopman. 2001

insights into the study of teeth as biological and species indicators. This work, Jernvall acknowledges, gave him an appreciation of form and pushed him to explore in his initial dissertation experiments the morphogenetic potentials of cell populations within the developing tooth in order to better understand how teeth gain their characteristic forms.[141]

5.4.1 *Jernvall and the Enamel Knot*

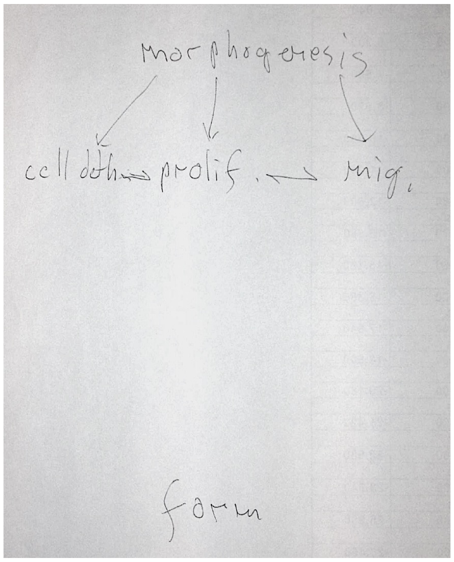

Figure 16. Note written by Jernvall on the back of an experiment sheet sometime around 1991. Jernvall sees form as a matter of morphogenesis, which is accomplished through cell death, proliferation, and migration.

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 within research programs for decades and relegated mostly to oral histology texts.[142] Jernvall was interested in the problem of how teeth develop their characteristic forms. To Jernvall, this problem of development was also a problem of morphogenesis (figure 16); 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

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[141] Personal communication. Interview: 18.September.2012.

[142] Private Communication. Interview 18.September.2012.

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

In order to track mitosis within developing tooth germs, Jernvall, like Reichenbach and Nozue, turned to histology, but with an important difference. Whereas Reichenbach and Nozue had sought to infer mitosis through serial sections that had been stained post-mortem with different types of contrast dyes, Jernvall utilized a technique called BrdU labelling. In this method, the BrdU label is injected into pregnant mice and is incorporated into the S-phase cells of both the mother and the fetuses.[143,144] BrdU labelling allows investigators to mark cells that are actively going through the cell cycle and then observe them post-mortem through serial sections.

Jernvall was searching for areas where proliferation was heightened and tracking the movements of the cells in those areas. Using BrdU labeling, Jernvall found areas of enhanced proliferation surrounding a ball of cells that showed no mitotic activity (figure 17)—a finding reminiscent of Nozue (Nozue 1971). At this point in the study, Jernvall was concerned that his findings indicated a flawed methodology—how else could one account for a static

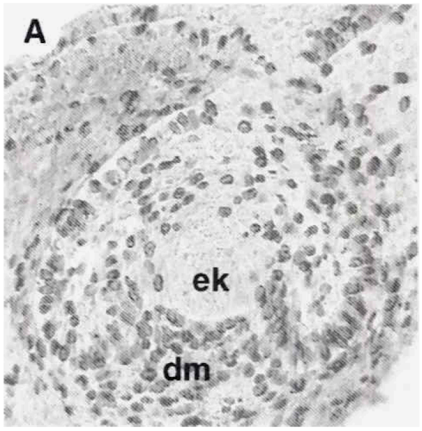

Figure 17. Transverse section of a day 14 tooth. Darkened areas indicate BrdU labelled cells. ek=enamel knot, dm=dental mesenchyme. Figure 2A from Jernvall et al. 1994, 465.

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[143] S-phase is the DNA replication portion of interphase. Cells go through S-phase prior to beginning their division.

[144] Jernvall used a Cell Proliferation Kit produced by Amersham (Code RPN.20). According to the methods section in Jernvall (1994), the "tissues were labeled by BrdU for 1 h, fixed 5 min in MeOH and overnight in 4% paraformaldehyde." There was no chase for this experiment.

area within a rapidly proliferating tissue?

In the parlance of Reichenbach, such a structure could be viewed as an impediment to growth. But Jernvall's understanding of development appealed to more than the just physical forces that Reichenbach and Blechschmidt sought—he also understood that development could be characterized by revealing the signals (i.e. understanding the interactions between the cells/tissues within the developing tooth) that cause the movements and mitosis that Reichenbach had understood development to be. To Jernvall, an understanding of development required both of these perspectives.

While considering the possible methodological issues that had led him to find an inert cluster of cells within a rapidly proliferating tissue, Jernvall came across an article by Lee Niswander and Gail Martin that looked very broadly at FGF-4 gene expression throughout the developing mouse embryo (Niswander and Martin 1992). In this article, Niswander and Martin 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 went on to replicate Niswander and Martin's search for FGF-4 expression. He collected molars of mice ranging from day 13 to day 16. This sample was fixed, embedded in paraffin, sectioned at 7µm, and treated with an Fgf-4 cDNA probe for *in situ* hybridization (Jernvall 1994). Using this method, Jernvall found that FGF-4, a potent mitogen, is expressed within the static cells of the enamel knot.[145]

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[145] It is somewhat surprising, if not paradoxical, that the enamel knot cells express such a potent mitogen because these cells do not divide themselves, yet induce rapid proliferation in surrounding cells.

Jernvall next created computer-assisted 3D reconstructions of the serial sections that he had used to look at BrdU labeling and FGF-4 expression (figure 18).[146] By combining both the data of cell proliferation from BrdU labeling and FGF-4 expression within a single three-dimensional 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 relationship between the enamel knot, FGF-4 expression, and cell proliferation in surrounding tissues (figure 18):

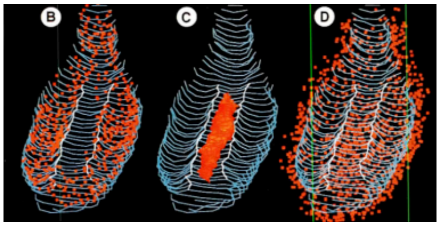

Figure 18. 3D reconstructions of day 14 tooth germs showing BrdU labeled cells and Fgf-4 expression. (B) Epithelium. Red=BrdU cells. (C) Red=Fgf-4 expression (restricted to ek area). (D) Mesenchyme. Red= BrdU cells. Figure 3B-D from Jernvall et al. 1994, 466.

> Our results indicate that, in addition to stimulating mesenchymal cell division, FGF-4, diffusing from the enamel knot, may also enhance proliferation of adjacent epithelial cells. This could provide a mechanism whereby the enamel knot controls the formation of tooth cusps: it may simultaneously stimulate cusp growth (via FGF-4 synthesis) and direct folding of the inner enamel epithelial-mesenchymal interface (by not proliferating itself). (Jernvall et al. 1994, 466)

Jernvall's work on the enamel knot demonstrates the emergence of a way of understanding tooth development wherein both cellular processes (e.g. mitosis and cell movement) and signaling (e.g. gene expression) were necessary. He recognized that the FGF-4 expressed by the enamel knot could be inducing the rapid proliferation of the surrounding tissues, thus shaping the tooth.

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[146] Surprisingly little has changed in the process of creating three-dimensional models between Ahrens and Jernvall. The process for each requires creating serial sections, outlining the tissues/structures to be modeled, and then retrofitting the outlined serial sections back together to create the 3-D form. While the process is similar, Jernvall replaced wax with computer-assisted animation, and thus forewent the tactile properties of wax modeling. Computer-assisted modeling, while non-tactile, allows for the integration of several types of data well beyond what is possible with wax modeling. For instance, Jernvall was able to combine his gene expression data, along with his cell proliferation data, and overlay all of this onto a single 3D rendering. While this is possible with wax models, the process would be far more difficult and require more artistic interpretation in order to render the other data onto the models.

5.4.2 *Summary and Assumptions*

Whereas a number of researchers had investigated the enamel knot as part of larger research programs in the first half of the 20th century, and interest in it dissipated in the second half, in the wake of Jernvall's work, the enamel knot became a central focus for those seeking to explain tooth development (Thesleff and Nieminen 1996, Thesleff and Sharpe 1997). Much like the research of Nozue and Kirino, Jernvall understood that development had to be explained by understanding both the morphogenetic processes, such as mitosis, as well as the signaling mechanisms, such as gene expression, that drove these processes. However, Jernvall made some significant adjustments to the research program developed by Nozue and colleagues. His ability to track mitosis was not limited to inferring the process post hoc from stained serial sections—he was able to label cells within the developing teeth that were going through the cell cycle and then detect those cells post mortem via serial sectioning.

BrdU labeling is not without problems. The method requires the assumption that cells in S phase will proceed into the mitosis phase of the cell cycle. Also, the harsh preparation procedures required for BrdU labeling can affect the staining quality of the sample (Rothaeusler and Baumgarth 2006), and the BrdU signal also dissipates over time, leading to an underestimation of cell proliferation and an overestimation of cell death (Sauerzweig et al. 2009). The method also requires the samples to be fixed and sectioned, forcing the investigator to observe the vestiges of mitosis within dead tissues. So, while Jernvall may have gained more direct access to tracking the process of cell proliferation, he still was not able to observe directly the morphogenetic processes that he wanted to track.[147]

In addition to tracking cell proliferation, Jernvall sought to uncover the molecular underpinnings of the paradoxical relationship between the non-proliferating enamel knot cells and the rapidly proliferating epithelium and mesenchyme surrounding the enamel knot. Kirino and colleagues had pointed to the enamel knot's role in this phenomenon, but could not through their methods determine the actual signal (or gene) that caused it. Jernvall's adoption of *in situ* hybridization techniques allowed him to determine that FGF-4 is expressed by the cells within the enamel knot. This method gave Jernvall insight into the genetic causes of the morphogenetic processes that he was tracking.

Like BrdU labeling, *in situ* hybridization techniques and looking at gene expression patterns require the investigator to accept a number of assumptions. First and foremost among these assumptions is the inference that an expression pattern, correlated with a phenomenon (such as cell proliferation), indicates a causal relationship between the gene that is expressed and the phenomenon. Jernvall assuaged this assumption by testing the capacity of FGF-4 to induce cellular proliferation *in vitro* (Jernvall 1994). In this experiment, Jernvall introduced FGF-4 releasing beads into *in vitro* cultures of mesenchyme and epithelium and tracked cell proliferation of these cultures versus control cultures. He found increased mitosis in the cultures with the FGF-4 soaked beads. Second, genes affect development as part of complex regulatory systems of gene interaction. By tracking a single gene, the researcher can gain insight into the causal

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[147] Note that it is only within the last ten years that investigators have acquired the ability to directly observe mitosis within living tissue. These observations are made using mice from the Fucci mouse line, and, in the case of teeth, are monitored *in vitro* (see: Sakaue-Sawano et al. 2008).

process that shapes development, but cannot give a definitive answer about *how* this gene acts. In a move that was somewhat prescient for the time, Jernvall recognized this limitation within his work:

> The expression of a number of transcription factors, growth factors and structural molecules have been analyzed in developing teeth, but so far the extremely restricted patter of *Fgf-4* expression is unique…It is possible that *Msx-2* is involved in regulating transcription of *Fgf-4* and/or the gene(s) responsible for the cessation of proliferation in the enamel knot. (Jernvall et al. 1994, 467)

5.5 The Enamel Knot Throughout the 20th Century

Beginning in the early 20th century, researchers invested in explaining the development of teeth moved away from integrating evolution into their explanatory frameworks. As they did so, they also largely abandoned the search to explain morphological diversity, and chose instead to focus on morphogenesis of the developing tooth. Throughout this chapter we have seen how this line of morphogenetic inquiry shaped our understanding of the enamel knot. Over the course of the 20th century, the way in which investigators understood the enamel knot changed substantially within research programs. This change in the explanatory value of the enamel knot stemmed from assumptions about how to explain development and morphogenesis inherent in the research programs in which it was investigated.

To Ahrens, an adequate explanation of tooth development was a description of the fine morphological changes through which teeth form. He developed this description by using his wax models and serial sections to trace changes in structures throughout ontogeny. Ahrens inferred relationships between structures from these static representations of different stages of development. In the work of Ahrens, the enamel knot was central and causal for explaining tooth development.

In contrast to Ahrens, Reichenbach understood and sought to explain development in biomechanical terms—as the active movement and proliferation of cells and intercellular fluids. Once Reichenbach ruled out the relationship between the enamel grooves and the tooth cusps, he left open the question of what, if anything, was the enamel knot's role in tooth development. Through his research, Reichenbach found that the enamel knot forms through passive processes, doesn't change shape or move while the surrounding epithelium and mesenchyme change, and disappears as the tissues surrounding it grow. These properties were not in alignment with the active factors shaping development that Reichenbach sought to characterize and which he believed were necessary for an explanation of development. Thus, the enamel knot did not appear to be a significant factor in development. If anything, the enamel knot was involved in shaping the tooth in a passive role—as an impediment to the growth of the surrounding tissues.

Following the work of Reichenbach the enamel knot lost all explanatory value for tooth development. It popped up in the publications of many investigators over the next two decades, but ultimately receded into obscurity by the mid 20th century. Nozue and colleagues' revival of prodding the value of the enamel knot for explaining development did little to change this, despite introducing a new way to interpret the object. Their work sought to explain development by appeals to both morphogenetic processes (e.g. mitosis) and the signaling capacity of the enamel knot; these two perspectives were necessary for understanding development. The group opened the door to using experimental methods to discern the role of the enamel knot in development and their work hinted at the potential role of the enamel knot for shaping the developing tooth, but their methods could not discern how exactly the enamel knot influenced development.

Finally, in the early 1990s, Jernvall became interested in the problem of tooth development. He understood development as morphogenetic processes, such as cell proliferation, cell death, and cell migration. He also understood that genes helped to direct and regulate these processes. Both of these components were necessary for Jernvall's explanation of development, and by building his explanation on both morphogenetic processes and the genes that help to regulate them, he reconceived of how to explain morphogenesis. His research took into account the biomechanical concerns of Reichenbach (i.e. tracking differential mitosis throughout development), the fine morphological structures of Ahrens (i.e. using modeling techniques to interpret the changing structures of the developing tooth), and the signaling concerns of Kirino and colleagues (i.e. discerning the genetic underpinning of the enamel knot's ability to shape the tooth). Through this combination, Jernvall moved the enamel knot back into a central role for explaining the growth and development of the emerging tooth. In the next chapter, we will see how Jernvall built this morphogenetic understanding of tooth development and the enamel knot into a theory that united morphological diversity and morphogenesis in a way that reconceived of how to explain each.

CHAPTER 6

JUKKA JERNVALL, MAMMALIAN MOLAR DIVERSITY, AND BRINGING DEVELOPMENT AND EVOLUTION TOGETHER

6.0 Introduction

In Chapters 2 and 3 we saw how 19th century paleontologists and embryologists dealt with morphological diversity. Through their research, their theories, and the assumptions they held, Cope, Osborn, and Röse brought together development and evolution in the service of explaining how mammalian molar diversity arises. To these researchers, ontogeny and phylogeny were intertwined, albeit in different ways for each of them (Chapter 4). These 19th century morphologists also gave explanations of the morphological diversity that they witnessed without appealing to the processes that give rise to form during development—that is, they did not incorporate morphogenesis into their explanations. In the 20th century, the study of development and evolution diverged as investigators sought to explain tooth formation. In Chapter 5 we saw how some 20th century embryologists shifted their gaze from explaining dental diversity to individual tooth development. This shift entailed a divestment of evolutionary explanations and thinking from embryological research concerning teeth, as well as a shift towards focusing solely on the processes of how teeth develop without attempting to explain the diversity of forms to which these processes lead.

At the end of Chapter 5 we saw how Jukka Jernvall reinterpreted the explanatory value of the enamel knot (Jernvall 1994). This new interpretation brought together the standard methods of investigating development at the time (i.e. patterns of gene expression) with a less mainstream focus on monitoring the processes of morphogenesis (i.e. cell proliferation). In this work, as with his later studies, Jernvall's methods were developed in the service of a single problem—understanding how developmental mechanisms can shape morphological diversity. In his focus on this problem, Jernvall reinterpreted the frameworks laid out in the accounts of the 19th century morphologists (Chapters 2 through 4) as well as those of the 20th century embryologists (Chapter 5) in order to give an explanation of the development and evolution of mammalian dentition.

By reinterpreting these earlier frameworks, Jernvall developed the enamel knot theory within his dissertation, which provided a new way of explaining how morphogenetic processes generate morphological diversity.[148] The enamel knot theory can be stated along the following lines: the development and patterning of individual tooth cusps are driven by signaling centers called enamel knots.[149] While Jernvall developed the theory within his dissertation, he expanded and tested it over subsequent years, making it both stronger and more generalizable. Jernvall's enamel knot theory provides a more complete explanation of mammalian tooth morphology than any of the work in either of the previous frameworks that we have encountered within this dissertation. It explains morphology on both developmental and evolutionary timescales and serves as an exemplar for EvoDevo for how to develop explanations that unite development and evolution.

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[148] Note that Jernvall does not refer to his conclusions about the way in which the enamel knot shapes the developing tooth as a "theory." However, his collaborator and former advisor, Irma Thesleff, has referred to it as a theory in multiple publications (Thesleff and Nieminen 1996, Thesleff and Sharpe 1997).

[149] This is my phrasing for the enamel knot theory. As noted in the previous footnote, the enamel knot theory was never standardized by Jernvall.

6.1 The Structure of This Chapter

In order to dissect how Jernvall built a research program that reinterpreted the frameworks encountered in chapters 2 through 5 and built his enamel knot theory, I begin with Jernvall's dissertation research (including his 1994 paper) and see it as the starting point for his enamel knot theory as well as the basis for his research from 1994 through 2000. I track how the questions and methods that Jernvall developed in his dissertation were explored through further research in his laboratory. The materials and ideas presented in his dissertation drove Jernvall's laboratory's focus for many years and resulted in both a number of publications and a number of related research questions that would be investigated in separate experiments and build up evidence for the enamel knot theory that became the core of his research program (Hunter and Jernvall 1995, Jernvall et al. 1994, Jernvall et al. 1996, Jernvall et al. 1998, Jernvall and Selänne 1999, Keränen et al. 1998, Vaahtokari et al. 1996a).

Although Jernvall's research is ongoing, I stop at the year 2000 because that is when his publication record matches the goal set up in his dissertation—to bring together developmental and evolutionary perspectives in the service of understanding mammalian molar diversity. This approach leads to a somewhat idiosyncratic timeline, but it is meant to track the conceptual pathway required for Jernvall to integrate development and evolution in order to understand dental morphology.

Jernvall began his undergraduate career at the University of Helsinki, training in microbiology and population biology. When University of Helsinki professors Mikael Fortelius, a paleontologist, and Irma Thesleff, a developmental biologist, put out a joint call for a PhD student to examine dental development, Jernvall was one of the few to answer.[150] The pair sought a candidate to train across their disparate disciplines in the hope of crafting a scientist who could bring the discoveries on tooth development from both fields together.[151,152] Jernvall took up the PhD position and began working on a dissertation "to address the general diversity of mammalian molar tooth shapes in the context of both evolution and development" (Jernvall 1995, 3).

Jernvall's 1994 study, discussed in Chapter 5, was just a small part of his dissertation. Jernvall defended and published his dissertation in 1995. At 61 pages, it is brief, yet its content is extremely rich. Within his dissertation, Jernvall covers many aspects of the development and evolution of mammalian molars, employing thought experiments, analyses of paleontological collections, and experiments in developmental biology.

Jernvall's dissertation is divided into four sections: an introduction, a section on "Patterns of tooth shape diversity," a section on the "Development of tooth shape," and a section called, "Thesis: Developmental mechanisms of tooth shape." "Patterns of tooth shape diversity" and "Development of tooth shape" correspond to paleontological and developmental studies, respectively. While Jernvall recognized that the distinction between the two subjects was somewhat arbitrary, he kept them separate on historical

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[150] Private communications with Mikael Fortelius (28. March. 2013) and Irma Thesleff (7. March.2013).

[151] Björn Kurtén was known for applying the quantitative methods based on the population ecology of the Modern Synthesis to fossil materials.

[152] Private communications with Mikael Fortelius (28. March. 2013) and Irma Thesleff (7. March.2013). Note here that the term development is interpreted very differently by paleontologists/evolutionary biologists and developmental biologists. According to Jernvall, "…'tooth development' might make a paleontologist think of the formation of tooth cusps, while a developmental biologist would be thinking of the molecular machinery of cell differentiation" (Jernvall 1995, 4)

grounds.[153] Within the paleontology-oriented section, Jernvall focuses on morphological diversity, while the developmental section focuses on morphogenenetic processes.[154] Even though work on these two elements (morphological diversity and morphogenesis) is presented in separate sections, his presentation of the materials and the conclusions that he draws from his analyses are highly iterative between the two. While the dissertation marks the start of his career, we find him laying out a problem that would drive the research from his laboratory for many years to come, namely tying developmental mechanisms to morphological diversity.

The following sections of this chapter parallel the structure of Jernvall's dissertation, leading us through Jernvall's dissertation research and publications until 2000 in order to highlight how he built a research program that combined and reconceived of morphological diversity and morphogenesis, and how he built and extended the enamel knot theory. In section 6.2 I track the main experiments contained within the "Patterns of tooth shape diversity" section of Jernvall's dissertation. The main purpose of this section of Jernvall's dissertation was not to, "make evolutionary scenarios of how each tooth shape evolved, but to find the minimum requirement for developmental mechanisms of tooth shape" (Jernvall 1995, 47). Here we see Jernvall investigating the evolution of a single cusp—the hypocone—in order to draw out principles about cusp development and evolution in general (6.2.1), as well as Jernvall

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[153] Jernvall reasoned that, "the disciplines of evolutionary and developmental biology have gone through several transformations, creating their own questions and terminology" (Jernvall 1995, 4).

[154] Morphology here is meant in the way that Goethe intended. As Russell put it, "[Goethe's] interest was not in *Gestalt* or fixed form, but in *Bildung* or form change. He saw that *Gestalt* was but a momentary phase of *Bildung*, and could be considered apart and in itself only by an abstraction fatal to all understanding of the living things" (Russell 1916, 49)

developing new ways to survey the morphological landscape of mammalian dentition in order to offer hypotheses about how such diversity arose (6.2.2).

This section details the paleontological portion of Jernvall's dissertation work and several publications that arose from his dissertation research. In it we see Jernvall's reconceptualization of things like molar types and the nature of homologies, as well as his ability to move between analyses of fossils and explanations about their diversity that appeal to development.

In section 6.3 I begin with an overview of prevalent 20th century theories of tooth development that were meant to explain morphological diversity. In this discussion, I lay out how Jernvall's enamel knot theory was distinct from these prior theories—he shifted the focus from explaining tooth identity (i.e. whether a tooth is a molar, premolar, canine, or incisor) to explaining how individual cusps develop and form into patterns on the tooth crown. This move allowed Jernvall to draw hypotheses about how changes in cusp development and patterning could shift to create the morphological diversity he witnessed in his paleontological research. The enamel knot theory, as we see in section 6.3.1 was built from work that took cusps to be the unit of morphological significance and attempted to explain how cusp development can lead to morphological diversity.

When Jernvall first developed the enamel knot theory—within his dissertation research (which includes his 1994 paper)—it applied only to mice and was based on a limited understanding of the genes expressed by the enamel knots and how the enamel knots are controlled.[155] Thus, while Jernvall's enamel knot theory was groundbreaking, it left open many questions, like whether it could explain development in mammals other than mice (his experimental organism), what genes were expressed by the enamel knot besides *Fgf-4*, and how individual cusps are patterned during development. In this section I trace the early development of the enamel knot theory. The work discussed in this section is primarily drawn from Jernvall's dissertation, but his 1994 paper is also discussed in order to show how his dissertation expanded on this previous publication.

In section 6.4 I discuss work that Jernvall undertook following his dissertation defense. Beginning with his research as a postdoc with his mentor, Irma Thesleff, and continuing through his promotion to junior group leader, Jernvall both conducted and directed research aimed towards addressing questions that were left open within his dissertation. These included particularly questions that would provide further evidence for the enamel knot theory and allow him to use it to explain morphological diversity. In order to address these questions, Jernvall turned away from the expansive research program that united morphological diversity and developmental mechanisms laid out in his dissertation in order to focus on developmental mechanisms alone. While many of his publications between 1995 and 2000 are in developmental biology, I show how the different questions that he and the colleagues he worked with (including Thesleff, and students and postdocs in his lab) built towards realizing the research program that Jernvall laid out in his dissertation. This work following his dissertation made the enamel knot theory both stronger and more generalizable.

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[155] The initial iteration of the enamel knot theory applied only to mice because they were the organism on which experiments for Fgf-4 expression and BrdU labelling were performed. The generalizability of the theory was only hypothetical until Jernvall could show that the enamel knots performed in the same way, using the same genes, in other mammals.

In section 6.5 I highlight Jernvall's application of Geographic Information Systems (GIS) to the developing teeth. This novel approach to depicting and analyzing form helped Jernvall to visualize the developmental differences in enamel knot gene expression and cusp patterning between species that are responsible for morphological diversity. Finally, in section 6.6 I show how, in 2000, Jernvall used the enamel knot theory to explain morphological diversity between mouse and vole molars.

6.2 Morphological Diversity

As noted above, Jernvall's dissertation is divided between paleontology and developmental biology, or morphological diversity and morphogenesis, respectively. In his section on paleontology, his aim was to survey the morphological landscape of mammalian dental diversity in order "to find the minimum requirement for developmental mechanisms of tooth shape" (Jernvall 1995, 47). In order to survey this morphological landscape, Jernvall selected cusps as the morphological variables on which to focus. He chose cusps because it is the patterns that cusps form on the tooth crown that most researchers interested in the morphological diversity of teeth focus on. Cusps were the main morphological feature of Cope, Osborn, and Röse, and other 19th century researchers who sought to explain mammalian tooth diversity, but had been neglected by the 20th century researchers covered in Chapter 5.[156]

One of the problems that arises when trying to understand and explain morphological diversity is that, while diversity of cusp patterns is recognized in the literature, it had never been described across the entire mammalian clade in a single place (Jernvall 1995). Thus, in order to explain morphological diversity of mammalian teeth and "find the minimum requirement for developmental mechanisms of tooth shape" (Jernvall 1995, 47), Jernvall had to start by surveying the morphological landscape of the dentition.

6.2.1 *Evolution of a Cusp: The Hypocone*

Jernvall's survey of the morphological landscape of the dentition began with looking at the evolution of a single cusp—the hypocone. The hypocone is the cusp that sits in the disto-lingual corner of upper molars (figure 19). This cusp has a high functional significance for the dentition—when appended to the typical three-cusped molar pattern, it increases the occlusal area of the teeth, giving mammals more surface area with which to process foods. Evolutionary biologist Percy Butler has noted that acquiring a hypocone effectively doubles the area devoted to crushing food (Butler

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[156] Cusps continued to be a main focus of paleontologists and evolutionary biologists throughout the 20th century. See, for instance Butler (1939) and Osborn (1978). Embryologists and developmental biologists, as seen in Chapter 5, focused on the overall development of the tooth and/or describing the transformations of certain features of the tooth (e.g. dental lamina, enamel pulp), but rarely gave detailed accounts of individual cusp development. Those who did investigate cusp development, generally gave descriptions of the changes in cells and tissues, but did not structure theories to account for how they form or how they are patterned.

1981). Hypocones have been reported broadly across mammalian taxa, but at the time in which Jernvall began his dissertation research, little work had been done to document the extent to which it was present within mammals (Jernvall 1995). Thus, the hypocone was posited to have both a high functional significance and be broadly present across mammals, but the breadth of the hypocone's presence within mammals was unknown.

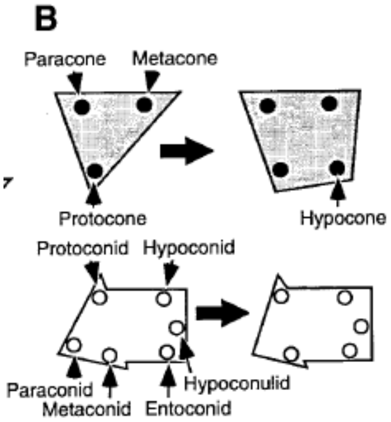

Figure 19. Schematic representation of occlusion for tribosphenic molars (left) and quadritubercular molars (i.e. tribosphenic with a hypocone) (right). Mesial is left, buccal is up. Figure 2B from Jernvall 1995, 7.

Jernvall's interest in the hypocone stemmed from interactions early in his PhD studies with John Hunter, who in the early 1990s was a student of paleontology stationed at the University of Helsinki on a Fulbright Scholarship. Together, the two decided to investigate further the linkage between the functional significance of the hypocone and its broad presence within mammals. In order to do this, they focused on Cenozoic mammals (~65 million years ago to present).[157] This research was published both within Jernvall's dissertation as well as in an article within the *Proceedings of the National Academy of Sciences* that same year (Hunter and Jernvall 1995, Jernvall 1995).

The pair utilized the collections at the American Museum of Natural History in New York, and culled primary and secondary literature for morphological data about extant and extinct mammalian molars. They divided the morphological data into three

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[157] Cope's research on mammalian molars was centered on the Jurassic Period (~206-144 million years ago) and Osborn extended that timeline by incorporating data from the Triassic Period (~248-206 million years ago). In relation to the samples utilized by Cope and Osborn to formulate the tritubercular theory, the samples that Jernvall and Hunter used are much more recent.

categories: no hypocone, hypocone shelf present, and hypocone present.[158] When they mapped these categories onto a phylogenetic tree, it became apparent that the hypocone was widely distributed across taxa, including Metatherians, Eutherians, Artiodactyls, Primates, Chiropterans, Rodents, and Lagomorphs (Hunter and Jernvall 1995, Jernvall 1995). Based on the wide distribution pattern across highly separated taxa, Hunter and Jernvall concluded that the presence of the hypocone in so many taxa was most likely due to multiple convergent evolutions (greater than 20) (Hunter and Jernvall 1995, Jernvall 1995). The pair also realized that there was probably more than one way to evolve a hypocone (figure 20).

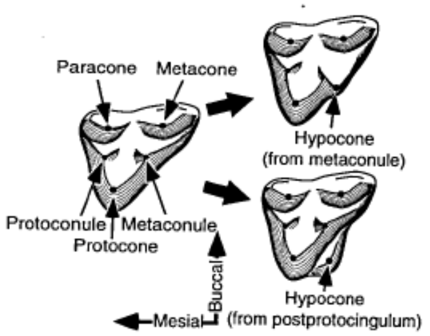

Fig. 3. The two most common paths for the hypocone evolution: The metaconule (top) is displaced lingually and enlarged or the postprotocingulum (bottom) forms a new cusp. Adapted from Hunter & Jernvall (1995).

Figure 20. Possibilities for hypocone evolution. Figure 3 from Jernvall 1995, 8, showing two ways to evolve a hypocone.

The broad presence of the hypocone across mammals required explanation, for which Hunter and Jernvall turned to the functional interrelations of teeth, organismal biology, and ecology. In order to do this, the two paired their morphological data with information from the literature regarding primary diets.[159] They broke diet into three categories: faunivore, generalist, and herbivore. By simply tabulating the numbers of taxa with no hypocone, a hypocone shelf, or a hypocone (i.e. the hypocone states within their

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[158] A hypocone shelf is an expansion of the crown in the disto-ligual corner of a tribosphenic molar. This expansion is not occlusal, but sits in the position where a hypocone would be found.

[159] It is interesting to note that an organism's diet is often inferred from its dental morphology. The data that the pair used to judge the diet of each species is unclear. However, nearly all inferences about diet for fossilized specimens are related to tooth morphology. This presents an interesting piece of circular logic—the pair have been using diet inferred from the dentition in order to track the relationship between the dentition and diet.

data) that fell into each of these categories, Hunter and Jernvall showed that generalists and herbivores had overwhelmingly evolved hypocones or hypocone shelves, whereas faunivores rarely had either of these characters (figure 21). This point in and of itself was interesting, though not unsurprising, and pushed Hunter and Jernvall to take their analysis one step further.

Their next step was to track the trends in hypocone states through time. In doing so, Hunter and Jernvall sought to evaluate how morphological evolution, and its relationship with diet, played out over an evolutionary timescale. The two took their morphological and taxonomic data and plotted it on timelines representing the epochs of the Cenozoic. By putting time on the x axis, number of species on the y axis and plotting the morphological categories (hypocone, hypocone shelf, and no hypocone) through time, they could visualize trends in morphological radiations (figure 22). By pairing these trends with their data about diet, they could begin to infer radiations of different dietary types during the Cenozoic.

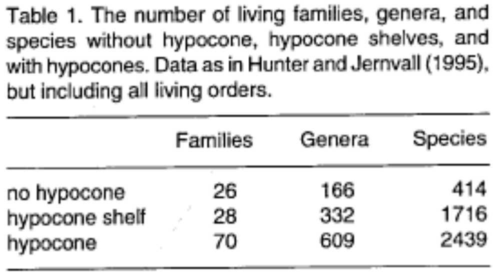

Table 1. The number of living families, genera, and species without hypocone, hypocone shelves, and with hypocones. Data as in Hunter and Jernvall (1995), but including all living orders.

	Families	Genera	Species
no hypocone	26	166	414
hypocone shelf	28	332	1716
hypocone	70	609	2439

Figure 21. Hypocone states. Table 1 from Jernvall 1995, 7

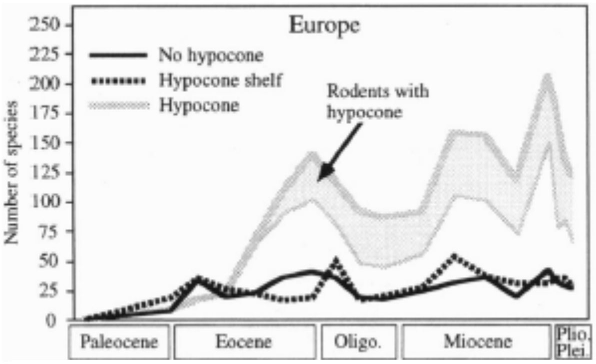

Figure 22. European species diversity through time of fossil mammals with hypocones, with hypocone shelves, and without hypocones. Part of Figure 3 from Hunter and Jernvall 1995, 10720

From their research on the hypocone, Hunter and Jernvall came to several conclusions about the nature of cusps, and thus some of the requirements for developmental mechanisms of tooth shape that Jernvall sought. First, cusps usually evolve gradually. Second, "the multiple convergent evolutions of the hypocone makes it a likely hypothesis, that acquiring a cusp is not difficult per se, and developmental evolution for making new cusps seems to be flexible" (Jernvall 1995, 9). Third, while the evolution of a hypocone appeared to be a relatively simple step, many molar tooth shapes that evolved from the quadritubercular molar (a molar with a hypocone) display extensive increases in phenotypic complexity (Jernvall 1995, 11).

6.2.2 *Tooth Types and Surveying Morphological Diversity*

As noted previously, although many researchers had provided detailed descriptions of tooth morphologies, general descriptions of tooth shape diversity throughout mammals had not been thoroughly investigated prior to Jernvall (Jernvall 1995).[160] Classifying tooth shape diversity, Jernvall realized, is particularly important for two reasons. First, cusp nomenclature and ways of describing tooth shape were adapted for particular mammalian groups. Osborn developed his cusp nomenclature on the basis of the well-defined cusps of the tritubercular mammals of the Mesozoic (Chapter 2.7.2) (Osborn 1888). This system, which assigns cusp names based on their relative positions on the crown, is not easily extended to other types of molar shapes, such as the extreme

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[160] Paleontological texts are littered with fine morphological descriptions of teeth. For an excellent example of a comprehensive text of molar morphology, see Owen (1845). Despite such an interest in dental morphology, the point here is that no one had attempted to give an overview of morphology or morphological principles of the dentition throughout mammals.

lophodont molar of elephants (figure 23).[161] Second, in order to offer likely hypotheses about the morphological evolution of mammalian molars, it is critical to understand the range of shapes that have been achieved through evolution.

Thus, in order to supplement existing knowledge of tooth shape diversity, Jernvall devised a homology-free system of classifying tooth shapes based on their appearance.[162] He did this by classifying molar crown morphologies into types. Types, to Jernvall, were collections of shape variables rather than the collections of homologous cusps that Osborn had envisioned. Recall that for Osborn, cusp homologies were defined by their "primitive position" and "order of evolution" (Osborn 1888, 927), and that this definition meant that cusps had to evolve according to a certain order and create certain patterns (or types). This led Osborn to define a directional evolution through his molar types and got him into trouble with embryological data about the order of cusp development. By uncoupling homology from types, Jernvall did not tie himself to a certain order for cusps to develop or evolve, and thus did not need to construct or explain a particular order of evolution through types.

Figure 23. Molar of an Asian elephant. Available: http://paleopix.com/blog/wp-content/uploads/2012/11/Elephant-Asiatic-tooth-RT25.jpg

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[161] 'Lophodont' refers to teeth that have elongated ridges (called 'lophs') that run between cusps. In the case of elephants and some rodents, it is impossible to identify relative cusps, and thus implement the cusp nomenclature devised by Osborn (1888).

[162] Note that the classification system that Jernvall devised was only for upper molars. However, there is no reason to believe that this system could not be extended to lower molars as well.

Jernvall defined his types on the basis of six variables: cusp type, buccal cusp number, lingual cusp number, number of longitudinal lophs, number of transverse lophs, and crown height.[163] Cusp type was given three possible states: sharp (cusp slopes are straight, i.e. come to a point), round (cusp slopes are convex), and loph. Buccal and lingual cusp number were given four possible entries: 0, 1, 2, and many. Longitudinal and transverse loph number were given the same variable. Finally, crown height could be brachydont (low crowned, i.e. mesio-distal length exceeds the height of the crown), hypsodont (high crowned, i.e. the height of the crown exceeds its mesio-distal length), or hypselodont (reserved for molars that are ever-growing and do not form roots).

> This kind of shape classification does not describe the 'absolute' diversity of molar tooth shape, but rather the variables of quantity (cusps, lophs), location (buccal/lingual cusps), orientation (lophs), and general shape (sharp, round, loph, height), provide a rather simple means of describing basic molar crown types. (Jernvall 1995, 14)

Based on Jernvall's six variables, there are 720 possible crown types. After removing crown height because he recognized that the height of the crown doesn't necessarily affect the topography of cusps and crests, the number is lowered to 270.[164] Of the 270 possible crown types, only 8 out of a possible 30 (26.7%) non lophodont types are realized within mammals, while 15 of a possible 240 (6.3%) lophodont types are realized within mammals.

Jernvall next calculated the Poisson distribution of crown types of living families and orders in order to estimate the randomness of the distribution of crown types—i.e. to

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[163] Lophs are ridges formed by the elongation of cusps.

[164] After tabulating the number of possible patterns based on these six characters, Jernvall removed crown height from further analyses, noting that, "crown height does not necessarily affect the topographical relations of cusps and crests" (Jernvall 1995, 14).

determine expected frequencies of crown type appearance. His results indicated that, “compared to the expected frequency distribution, the living families are clearly clustered into a few evolutionarily realized upper molar crown types” (Jernvall 1995, 15). Following testing of expected versus realized crown types within orders, he showed similar high clustering with a higher number of possible crown types not realized. These results led Jernvall to conclude that, “the upper molar crown types of living mammals are not a random sample, but rather a very selected set of all the theoretically possible tooth morphologies” (Jernvall 1995, 15). Jernvall’s results show that out of all the possible tooth types, only a relatively small number actually occur in nature, and that these realized crown types are not evenly distributed throughout the mammalian clade.

Jernvall hypothesized that the disparity between expected and realized crown types was due to historical, functional, and ecological constraints (Jernvall 1995, 15). Historical constraints referred to evolutionary history. For example, “…having several lingual cusps with one buccal cusp would ‘reverse’ the occlusal relations of the tribosphenic pattern, and the upper molars would occlude lingually to the lower molars…” (Jernvall 1995, 15).[165] Thus, given the evolutionary history of crown types, wherein mammals largely evolved through a tribosphenic (tritubercular) molar type (where there are two buccal cusps and one lingual cusp), developing an upper crown type with two lingual cusps and one buccal cusp would take a lot of reshuffling of cusp patterning.

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[165] ‘Tribosphenic’ refers to the tritubercular pattern of Cope and Osborn, where the protocone is on the lingual side of the crown, and the paracone and metacone are on the buccal side of the crown.

Functional constraints, i.e. the need to maintain a working dental occlusion for mastication, were tied to historical constraints. For example, Jernvall found that the reversal of the tribosphenic pattern, as hypothesized above, "…might be functionally disadvantageous considering food flow during mastication" (Jernvall 1995, 15). Thus, a functional disadvantage could result in malocclusion, leading to an inability for the organism to properly process its food.

Finally, ecological constraints referred to the "limits on structural solutions caused by natural selection alone" (Jernvall 1995, 15). For example,

> …the evolution of very lophed tooth shapes, which are mostly to be found among herbivorous mammals, happened after the evolution of the hypocone and increase in the occlusal area. Even if multiple lophs can be formed without new cusps (as in squirrels), there might be little ecological reason to have them unless the tooth shape is generally adapted to utilize plants. (Jernvall 1995, 15)

Ecological constraints thus mean that the organism's dental morphology must conform to the diet that it takes advantage of.

Interestingly, Jernvall does not see development as a factor constraining the appearance of different molar types. "Because almost all possible cusp combinations can be found when the whole dentition (especially premolars) is taken into account, the missing upper molar crown types can be assumed not to result from any developmental constraints. The same seems to be true for the non-realized lophodont tooth shapes" (Jernvall 1995, 15). The reversal of the tribosphenic upper molar described above is a good example of this point.

> The reverse cusp configuration might not make sense in the context of tribosphenic molars, but in theory the tribosphenic upper molars could have evolved in the lower jaws. This would have reversed the realized cusp patterns for upper molars (the lower molars would have been now uppers), and indeed, lower

> molars of tribosphenic teeth do have several lingual cusps with fewer buccal cusps. (Jernvall 1995, 15)

The year after he published his dissertation, Jernvall collaborated again with John Hunter and his mentor, Mikael Fortelius, to apply his crown types specifically to understanding Cenozoic ungulate radiations (Jernvall et al. 1996).[166] Using the data on ungulates that Jernvall had compiled for his dissertation, the threesome sought to compare morphological diversification (i.e. the radiation of different crown types) to taxonomic diversification (i.e. the radiation of different genera).[167] By comparing crown types to numbers of genera over time (figure 24), Jernvall and colleagues noted that morphological and taxonomic diversification do not share a simple relationship, i.e. that, "ungulate crown type diversity does not appear to be a simple function of taxonomic diversity" (Jernvall et al. 1996, 1490).

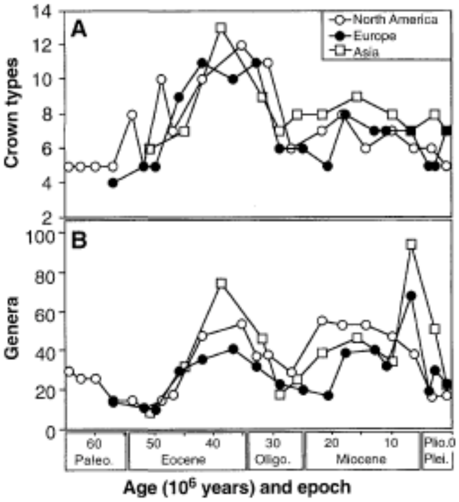

Figure 24. Crown type (A) and generic (B) diversity over time. Figure 2 from Jernvall et al. 1996, 1490

The group turned to ecological factors to understand the disparity between morphological and taxonomic diversity, and found that "the analysis of morphological

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[166] The choice of ungulates for this investigation is twofold: First, ungulates, along with rodents, are the most taxonomically diverse group of herbivorous mammals both today and in the fossil record (Fortelius 1985, Janis and Fortelius 1988). Second, Fortelius' specialty is Cenozoic ungulate paleobiology (see: Fortelius 1985).

[167] Note that a great deal of the data that Jernvall compiled for his dissertation and the Jernvall et al. 1996 article came to him from Fortelius (personal communication with Fortelius 28. March. 2013).

trends describes the ecological aspect of ungulate radiations better than taxonomically based analyses alone" (Jernvall et al. 1996, 1491).

6.3 Development of Tooth Shape: The Enamel Knot Theory

By the time that Jernvall took up his research on dental morphological diversity, the development of cusps had been thoroughly described in terms of the morphological changes of tissues and structures that occur during ontogeny. Embryologists, and later developmental biologists, were well aware of morphological changes that occurred as a tooth moved from being a tooth germ through the bud, cap, bell, etc. stages, and became a fully-formed tooth. And, paleontologists and evolutionary biologists relied on cusp details, including the patterns they form on the crown, to make taxonomic and ecological inferences from fossilized remains. And yet, very few theories of tooth development were focused on explaining how cusps form and why cusps are arranged into the patterns that make them so important.

The majority of 20th century theories that explained dental morphological diversity focused on meristic variation within the dental arcade (the rows of teeth in the upper and lower jaws)—that is, they explained how different types of teeth (molars, premolars, canines, incisors) form within the jaw, but not how mammals had evolved such diverse tooth phenotypes. Meristic variation rested on the idea that there are a number of tooth types (molars, premolars, canines, incisors) present within the dentition, and that these types are developmentally related in some sense. William Bateson, in his famous *Materials for the Study of Variation* amassed an enormous body of data about meristic variation within the dentition, and found that the most variable morphologies in the dentition occurred at the ends of the dental arcade (Bateson 1894). Zoologist and evolutionary biologist Percy Butler in 1939 developed a field theory of tooth formation that held that morphogenetic fields could account for the meristic variation of the dental arcade, "i.e. with each tooth displaying similarities to others nearby because of the influence of a common field but with graded differences because of position" (Townsend et al. 2009, S35). Butler later went on to suggest that teeth evolve as part of a system rather than as individual organs (Butler 2001). Butler's field theory was widely accepted as a means of explaining dental morphology, and was later adapted to human dentition by the oral surgeon Albert Dahlberg (Dahlberg 1945).

In 1978, John Wright Osborn proposed an alternative to the field theory. Osborn's clone theory held that "a single clone of programmed cells led to the development of all teeth within a particular class" (Townsend 2009, S35). Both Butler's and Osborn's theories relied on gradients, but were at odds in terms of whether the formation of teeth was due to environmental factors (in the case of Butler, the interaction of different morphogen gradients) or internal factors (in the case of Osborn, the preprogrammed cells of the clone).[168]

The field and clone theories of tooth formation were the main 20th century theories that attempted to explain dental morphology. These theories were developed by evolutionary biologists and are interesting for our purposes because they take the morphological unit to be the tooth type—i.e. incisors, canines, premolars, molars. These

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[168] For excellent reviews about the similarities and differences between the field and clone theories, see: Osborn 1978 and Townsend et al. 2009.

theories were constructed to account for the variations that exist within and between these tooth types, not for the morphology of a given tooth. That is, they were not meant to account for how cusps appeared and were arranged on the tooth crown. Thus, the focus of 20th century dental theories was explaining the meristic variation of the dental arcade, not the diversity of the mammalian dentition or the cusp patterning that exists on the molar crowns.

While theories of dental development focused on how different teeth (molars, premolars, canines, incisors) form within a single dental arcade, other research on tooth formation focused on describing the morphological changes that take place during the stages of tooth formation (Chapter 5). By the early 1990s, the development of the mammalian tooth crown and the cusps had been thoroughly described in terms of morphological changes, but had yet to be described in terms of what drives the cusps to form and how they are arranged on the crown. Jernvall's enamel knot theory of tooth development addressed exactly this.

6.3.1 *The Development of the Enamel Knot Theory*

Jernvall developed the enamel knot theory of tooth development from his dissertation research, including his 1994 publication. In order to track how he arrived at the enamel knot theory, I begin with Jernvall's dissertation research on tooth development, and show how that work, along with his 1994 publication, led to his enamel knot theory.

Following the section of his dissertation on the morphological diversity of mammalian teeth, Jernvall turned to uncovering the morphogenetic mechanisms that drive cusp formation and patterning in order to, "…identify some of the mechanisms which control the evolutionary changes of the tooth shapes" (Jernvall 1995, 25). In order to do this, Jernvall focused on three sub-problems of the development of tooth shape: 1) defining the general patterns of tooth cusp development, 2) elaborating a new theory for the developmental control of cusps, and 3) understanding how to adjust the initiation and location of each cusp independently during development (Jernvall 1995, 24).

For the sake of space, I will not review Jernvall's coverage of the general patterns of tooth cusp development. It is sufficient here to note that after synthesizing literature on cusp development across mammals, Jernvall arrives at the hypothesis that relative cusp size is determined mainly by the order of cusp development (i.e. the first cusp to form is usually the largest, and so on) (Jernvall 1995, 24). Jernvall's main contribution in this section of the dissertation is laying out the developmental control of making and patterning cusps by exploring the role of the enamel knot and establishing the enamel knot theory.

At the end of Chapter 5, we saw how Jernvall and colleagues uncovered a potential new role for the enamel knot in explaining tooth development. This work, published in 1994, was a small part of Jernvall's dissertation research. The 1994 publication is oriented towards developmental biology; however, his dissertation as a whole takes a different approach. In the beginning of the enamel knot section of his dissertation, titled, "Developmental control for making a cusp: a new model," Jernvall starts with the statement that,

> The inference of the causal mechanisms between the genome and the cusp development is crucial for linking genetic control of development with evolutionary patterns. Therefore, ontogenetic models should also incorporate mechanisms for controlling evolutionary changes in cusp patterns. (Jernvall 1995, 31)

Thus, Jernvall's goal in investigating how teeth, and particularly cusps, develop and are patterned, was to understand the ontogenetic basis of morphological diversity. In this way he sought to link development and evolution.

In Chapter 5, we saw how Jernvall investigated tooth development starting with tracking cell proliferation in order to understand how form emerged. Upon finding the enamel knot in his BrdU studies of mitosis, he looked at the molecular signaling of the enamel knot by tracking the expression of *Fgf-4* (Jernvall 1995, Jernvall et al. 1994). In addition to uncovering the signaling property of the enamel knot, tracking the expression of *Fgf-4* led Jernvall to one further, critical discovery—the realization that secondary enamel knots correspond to the development of the cusps.[169]

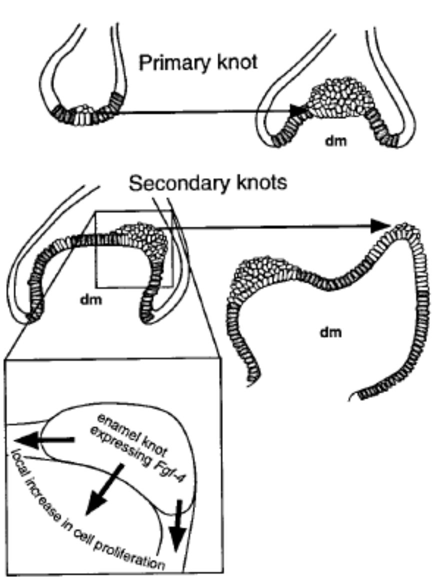

Figure 25. Schematic representation showing the inner enamel epithelium and the enamel knots. Non-dividing enamel knot cells (white) and dividing cells surrounding the enamel knots (grey). Figure 26 from Jernvall 1995, 33.

Secondary enamel knots are transient cell condensations within the enamel epithelium that appear at the tips of the cusps during days 16 and

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[169] In the remainder of this Chapter, the phrase 'primary enamel knot' will be used to indicate what was called in Chapter 5 the 'enamel knot'. The phrase 'secondary enamel knot' will refer to the thickening within the epithelium that directs the formation of an individual cusp, as indicated by Jernvall et al. (1994) and Jernvall (1995). The term 'enamel knot' will be used when both of these sets of structures are indicated.

17 of development (figures 25 and 26).[170] Prior to Jernvall, Erich Blechschmidt and H.W. Marett Tims had noted the presence of these thickenings, but these authors did not connect them with the enamel knot or assign them an explanatory value (Blechschmidt 1953, Jernvall et al. 1994, Tims 1901). Jernvall recognized the structures in the course of examining the expression of *Fgf-4* within the developing tooth, and labelled them as secondary enamel knots because they appear after the (primary) enamel knot and have many of the same properties—they are histologically similar, the cells do not proliferate, they appear and then quickly disappear, and they express *Fgf-4*.[171]

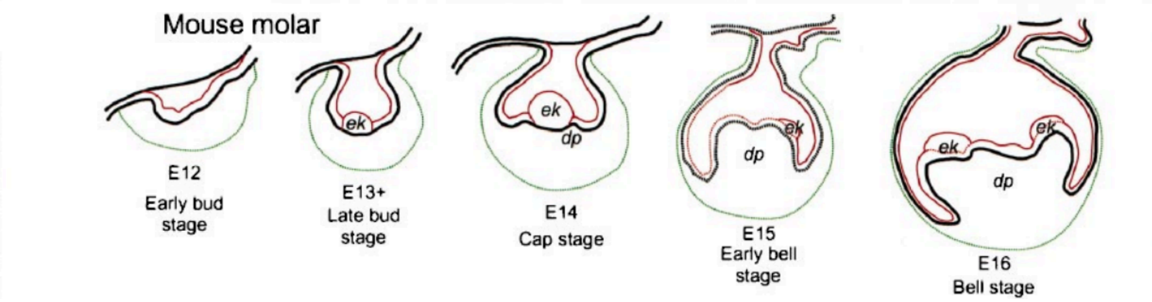

Figure 8. Representation of developmental stages of the mouse molar from days 12 through 16. Figure 2 from Keränen et al. 1998, 478.

In the 1994 paper, Jernvall focused his analysis on the primary enamel knot, noting of the secondary enamel knots that these "structures were observed at the cusp tips and their appearance corresponded to the formation of individual cusp morphology" (Jernvall et al. 1994, 463). He also noted that these structures were non-proliferative areas where "*Fgf-4* transcripts were transiently present in the epithelial cells at the future cusp tip regions" (Jernvall et al. 1994, 465), and that their transient appearance "corresponds to

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[170] Note that these are stages within mice. The standard organism in which to investigate tooth development was mice by the time that Jernvall started his investigations.

[171] The primary enamel knot can be detected in mice at day 3.5, and the time from formation to disappearance lasts about 12 hours (in mice).

the foldings (and number of cusps) of epithelium that create individual cusps…" (Jernvall et al. 1994, 466). Beyond this, there is little mention of the role of the secondary enamel knots in tooth development in Jernvall's 1994 paper.

In his dissertation, however, Jernvall devotes much more space to describing these structures and their possible roles in tooth development—for good reason. The secondary enamel knots are significant because if they act as signaling centers in the way in which the primary enamel knot does—by both directing outgrowth of surrounding tissue and acting as impediments to growth themselves, thus shaping the epithelium—then each cusp on a given tooth can be said to be under individual control. That is, each cusp has its own regulatory module driving its development. A regulatory module here means that a standard set of gene pathways interact in a set way to give rise to a cusp. If each cusp is controlled by its own regulatory module, then instead of developing an explanation of the entire tooth crown, which was often the case for theories that accounted for molar morphology (section 6.4), one could develop an account of the formation of a single cusp (i.e. figure out what gene pathways are involved and how they interact) and then ask questions about how cusp initiation could be changed in temporal and spatial terms in order to account for tooth morphology. Asking questions about how the regulatory modules control cusp formation would thus give insights into how the cusps form patterns on the tooth crown and how those patterns could change throughout evolution. This is exactly what Jernvall set out to do through his enamel knot theory—to explain the morphogenetic development of teeth in such a way that could also explain the morphological diversity of cusp patterns throughout mammals.

Jernvall began his discussion of the roles of the enamel knots in development by elaborating the ways in which they control cusp initiation and could determine molar morphology.

> Given that the change in tooth germ shape is produced by the unequal growth of the dental epithelium, the cessation in cell division of the enamel knot may play a crucial role in this folding. By remaining non-proliferative, the enamel knots could cause the unequal growth of the inner enamel epithelium that establishes the locations of cusp tips and directs the folding of cusp slopes. Moreover, because tooth growth progresses from the tip down, the forming of a new cusp can be considered as a single event. (Jernvall 1995, 33)

and

> Taken together, by creating a non-dividing area of enamel epithelium, and by simultaneously stimulating the surrounding cells to divide, the enamel knots could meet the minimum requirements for the control of cusp formation. That is, they could produce temporally and spatially specific folding of the inner enamel epithelium. (Jernvall 1995, 35)

Thus, using his understanding of the enamel knots, Jernvall created a theory of tooth development wherein cusps were the unit of morphological importance. According to this enamel knot theory of tooth development emerging within Jernvall's dissertation, "developmental control of tooth shape is mediated by genes affecting the spatial and temporal activation of the enamel knot" and by modifying the temporal and spatial activation of cusps, the theory could also explain the morphological diversity of mammalian teeth (Jernvall 1995, 1).

If the function of the secondary enamel knots is to initiate cusp formation, and following this initiation step they disappear, how then may they control the relative sizes of the cusps? Jernvall showed in an earlier part of his dissertation that relative cusp size was determined mainly by the order of cusp development. In order to determine the association of the enamel knots with the control of relative cusp size, Jernvall compared cusp initiation in teeth with different cusp heights. That is, he compared cusp development in mice to that in the gray short-tailed opossum (*Monodelphis domestica*). Jernvall chose *Monodelphis* because the protoconid and metaconid cusps in this species (first and second cusps to form, respectively) are much larger than the surrounding cusps, and the protoconid is larger than the metaconid.

In order to make these comparisons, Jernvall acquired histological sections of post-partum *Monodelphis* joeys from Kathleen Smith at Duke University. He tracked the appearance of the enamel knot and cusp development both in these sections and through digitally rendered three-dimensional reconstructions of the developing first lower molars of both mice (E14 through E17) and *Monodelphis* (post partum days 6 through 13). Using these materials, Jernvall determined that enamel knot formation and action appeared to be similar in mice and *Monodelphis* and that,

> …the initiation of the second *Monodelphis* molar cusp (metaconid) clearly demonstrates the temporal association of the enamel knots with cusp initiation; the metaconid enamel knot is present a day before the metaconid is clearly visible. The metaconid knot forms after the protoconid knot has disappeared indeed, the protoconid tip already has a dentin cap at the time of metaconid initiation. (Jernvall 1995, 37)

Thus, the appearance of secondary enamel knot formation corresponds to the relative size difference between the cusps—in *Monodelphis*, the protoconid is the largest cusp and its secondary enamel knot forms and disappears before the secondary enamel knot of the metaconid forms. Jernvall concluded his discussion of the relationship between secondary enamel knots and relative tooth size:

> …the formation of the enamel knot fits the temporal initiation of cusps, and it can be hypothesized, that all cusps, whether large or small, require similar enamel

> knots. A new secondary enamel knot appearing late in tooth development and its subsequent earlier developmental shift could be a mechanism for evolution of a new large cusp from a cingulum, such as the repeated evolution of the hypocone. (Jernvall 1995, 38)

While Jernvall hinted at the possibility that altering the timing of secondary enamel knot appearance could have evolutionary implications, he did not further investigate this possibility within his dissertation. In order to investigate adequately the question of the relationship between the timing and placement of the enamel knots and the shifts in cusp morphology apparent in the morphological diversity of the mammalian dentition, Jernvall needed a lot more evidence about what the mechanisms of cusp formation were and how they were controlled.

Following the defense of his dissertation, Jernvall became a postdoc in Irma Thesleff's laboratory in the Institute of Dentistry at the University of Helsinki.[172] Jernvall's dissertation left open a number of questions, such as whether enamel knots work the same way in other mammals as they do in mice (the organism he had used for his enamel knot research). What, if anything, is the relationship between the primary and secondary enamel knots, and do they express the same signals? What causes the temporal and spatial activation of the secondary enamel knots? If Jernvall wanted to use the enamel knot theory of development to explain morphological diversity of mammalian teeth, these questions needed to be addressed. Over the next several years, Jernvall worked closely with Thesleff, research associates, and graduate students to expand on the research program started within his dissertation and begin to answer some of these questions from the perspective of developmental biology. By beginning to answer these

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[172] Thesleff's laboratory is now in the Institute of Biotechnology at the University of Helsinki.

questions, Jernvall made the enamel knot theory more generalizable, in the sense that it could be applied to more organisms and that it could provide an explanation of morphological diversity in addition to a description of developmental events.

6.4 The Developmental Mechanisms of the Enamel Knots: Translating Jernvall's Dissertation into Developmental Biology and Expanding the Enamel Knot Theory

Jernvall's dissertation was notable for its divergence from mainstream developmental biology. While he performed developmental experiments using mainstream methods, such as BrdU labeling, *in situ* hybridization, etc., Jernvall saw the resulting data as evidence for both developmental and evolutionary processes, and so, his discussions and analyses of the data were laden with hypotheses about how his data for morphogenesis connected to morphological diversity. Drawing hypotheses from developmental experiments to explain evolutionary patterns was well outside the mainstream of developmental biology at the time.[173] Thesleff, on the other hand, was a straightforward and mainstream developmental biologist. Respected in her field for her work on tooth and other embryonic organ development, her research represented an excellent application of the field standards. The open questions from Jernvall's dissertation required developmental biology in order to answer them, and so he focused his research efforts into developmental experiments instead of approaching tooth

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[173] At this time, developmental biologists were largely concerned with finding the molecular bases of development. Using *in situ* hybridization techniques, many sought to establish the expression patterns of different genes within developing tissues. At this time in the 1990s, the discovery of the genes expressed within the developing tooth was the main problem that concerned Thesleff's laboratory. Personal communications with Jernvall (5.October.2012) and Thesleff (7.March.2013).

morphology from multiple perspectives. Jernvall has noted that he "went mainstream" in his work with Thesleff over the next several years.[174] To Jernvall, going "mainstream" meant that he used the methods and ways of framing questions that were popular within the field of developmental biology. This meant that Jernvall focused on finding gene expression patterns, through methods like *in situ* hybridization, and did not follow up on differential cell proliferation and the morphogenetic aspects of tooth development.

In this section, I show how Jernvall worked with Thesleff, research associates, and graduate students to answer many of the open questions from his dissertation. These questions evolved as new experiments provoked Jernvall and the group to answer new questions about the morphogenetic mechanisms involved in cusp development and patterning. Throughout this time period, Jernvall worked with this group to expand their understanding of the developmental mechanisms of the enamel knots and the enamel knot theory.

In his dissertation, Jernvall expanded beyond the *Fgf-4* expression patterns that he had published the previous year. At the time, Thesleff, along with a team of her graduate students at the University of Helsinki, was looking at the expression patterns of different genes in the enamel knot. Thesleff's student, Anne Vaahtokari, took the lead on a set of experiments, published in 1996, that investigated whether some of the molecular mechanisms involved in the outgrowth of the apical ectodermal ridge (AER) were also

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[174] Personal communication with Jernvall (5.October.2012). Jernvall noted in personal communication that he had to use a lot of the standard methods and ways of framing his questions used in the field of developmental biology at this time. Despite going "mainstream," Jernvall's collaboration with Thesleff in developmental biology was not his only pursuit during this time. He continued to work on paleontological problems, and even collaborated with anthropologists. In spite of this "mainstream" approach to questions about the developmental mechanisms of the enamel knot, Jernvall still managed to bring his own unique perspective into the research and publications in a number of ways.

present in the enamel knots (Vaahtokari et al. 1996a). Jernvall worked closely with Vaahtokari to shape this research and write the resulting publication. This work was published a year after Jernvall's dissertation, but some of the preliminary results were included within the dissertation.

Vaahtokari and colleagues used *in situ* hybridization of developing mouse molars to look at the expression patterns of *Sonic Hedgehog* (*Shh*) and six different *BMPs* (*Bmp-2* to *Bmp-7*) within the primary enamel knot. They then compared these expression patterns with that of *Fgf-4* and cell proliferation from Jernvall's earlier study. Their findings indicated a number of things. First, while the expression of *Fgf-4* has a tight spatial relationship with the primary enamel knot, *Shh*, *Bmp-2*, and *Bmp-7* had nested expression domains that overlap with the primary enamel knot, but did not correspond exactly to its morphology. Second, they found that *Shh*, *Bmp-2*, and *Bmp-7* expression appeared starting on day 13 (before the formation of the primary enamel knot) and continued throughout the lifespan of the primary enamel knot, whereas *Fgf-4* expression begins on day 14 (the day that the primary enamel knot appears).

Taken together, their findings indicated that some of the same molecular signals that are utilized by other signaling centers, such as the AER, are involved in the formation and action of the primary enamel knot. They also indicated that the genetic network controlling primary enamel knot formation and action was complicated and required further study of different genes.

Notably absent from this study, as well as Jernvall's dissertation is information about the expression of these molecules within the secondary enamel knots. Thus, at this point, Jernvall and his surrounding group left the questions of what genes the secondary enamel knots expressed and how they operate open for further research.

> An obvious question in the context of tooth shape is the molecular similarities between the primary and secondary enamel knots. Only *Fgf-4* is currently known to be similarly expressed in different enamel knots (Jernvall et al. 1994). The expression patterns of the other genes remains to be shown but there are indications that the expression of *Bmp-2* and *Bmp-4* is not restricted to the secondary enamel knots. (Jernvall 1995, 39-40)

Two years later, Jernvall and Thesleff's group expanded their knowledge of the repertoire of gene expression within the primary enamel knot. After finding a paper in *Science* that demonstrated the role of *p21* in terminating the cell cycle and inducing differentiation, and which also showed that *p21* was expressed in the oral cavity of a 14-day old mouse embryo, Jernvall was inspired to look more closely at the role of *p21* in initiating and terminating the primary enamel knot (Personal communication, Jernvall et al. 1998, Parker et al. 1995). His question became: what causes the primary enamel knot to form and then to disappear?

P21 was a good candidate for monitoring the life history of the primary enamel knot because it had previously been shown to be involved in the cessation of cell proliferation in muscle cells (Parker et al. 1995). As seen in Chapter 5, one of the hallmarks of the enamel knot is that the cells are non-mitotic, i.e. they are terminally differentiated. The group decided also to look at the expression of *Bmp-4* because it had previously been shown to be involved in apoptosis in rhombomeres and limbs, and was known to participate in the epithelial-mesenchymal induction of tooth development (Graham et al. 1994, Vainio et al. 1993, Zou and Niswander 1996).

The group performed *in situ* hybridizations for *Bmp-4* and *p21* along with Tunel-staining (an assay that detects apoptotic cells) on day 13 through day 15 mouse embryos. Their findings indicated that the *Bmp-4* expressed in the dental mesenchyme at the onset of primary enamel knot formation induced expression of *p21*, which resulted in the cessation of cell proliferation in the primary enamel knot cells. After approximately 24 hours, the cells of the primary enamel knot begin to die via apoptosis (Vaahtokari et al 1996b). Jernvall and colleagues compared the expression pattern of *Bmp-4* with the pattern of apoptosis in the primary enamel knot (determined from Tunel staining) from days 14 to 15 and found a close correspondence between the expression of *Bmp-4* and apoptosis.

These results led Jernvall and colleagues to conclude that the formation of the enamel knot is induced by *Bmp-4* signaling in the mesenchyme. *Bmp-4* induces the expression of *p21*, which causes the cells to differentiate, rendering them non-mitotic. After about a day of expressing a variety of genes, the primary enamel knot cells undergo apoptosis, most likely induced by *Bmp-4* (Vaahtokari 1996a).

Jernvall and colleagues thus had some insight into the mechanisms that caused the enamel knot to form and disappear. However, they still lacked an understanding of whether these mechanisms were specific to mice, or could account for mammalian dental development more broadly. They also lacked insights into the developmental mechanisms of the secondary enamel knots—i.e. the mechanisms that give rise to the individual cusps.

That same year, Soile Keränen, a graduate student with Jernvall and Thesleff, took the lead on a publication that looked at the expression patterns of eight genes known to be associated with regulating tooth development: *Bmp-2*, *Bmp-4*, *Fgf-4*, *Shh*, *Lef-1*, *Msx-1*, *Msx-2*, and *p21*.[175] Keränen and the group sought to compare the expression of these genes across both the primary and secondary enamel knots. For this set of experiments, Jernvall pushed for a comparative approach, and so the group looked at the expression of these genes in both mice and the sibling vole (*Microtus rossiaemeridionalis*). They chose to study mice and sibling voles because, "whilst their molar morphologies are very dissimilar, they are relatively close phylogenetically and thus their gene sequences should be quite similar" (Keränen et al. 1998, 484).

By comparing the expression of these genes via *in situ* hybridization across mice and voles between days 12 and 16, the group drew a number of important conclusions. First, they showed that antisense mRNA probes made from mouse cDNA were able to cross-hybridize with vole tissue—this allowed them to compare gene expression across mouse and voles. Second, "both the mouse and vole secondary enamel knots express almost the same set of molecules as the primary enamel knot, *Bmp-4*, *Fgf-4*, *Lef-1*, *Msx-2*, *p21* and *Shh* (*Bmp-2* was downregulated), which all correlated similarly with morphology in both mouse and voles" (Keränen et al. 1998, 483).

Within the article, the authors briefly described the relationships between the expression of the different genes and the emerging morphology of the tooth. Despite these references to the relationship between gene expression and morphology, they had no way of tracking, except through stained serial sections, the correspondence between the two. As Jernvall had noted previously, "the molecular and morphological changes in

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[175] "The genes Bmp-2, Bmp-4, Fgf-4 and Shh encode signal molecules, Lef-1, Msx-1, and Msx-2, are transcription factors and p21 participates in the regulation of cell cycle" (Keränen et al. 1998, 477).

developing teeth are very fast and the temporal and spatial order of events are not easily depicted from serial sections" (Jernvall et al. 1998, 162). Although tracking morphological and molecular changes in the secondary enamel knots was lacking from this paper, Jernvall was already working on developing a technique to track and represent these two lines of information more effectively than using computer-generated 3-D reconstructions of serial sections (see Section 6.6).

6.5 Representing and Analyzing Tooth Shape

In 1996, Jernvall applied for a grant from the Academy of Finland in order to establish, as a junior group leader, a research unit within the developmental biology program.[176] This unit would focus on the "ecological and development determinants of tooth shape evolution," and more specifically, work to "identify the genes and genetic pathways that are responsible for the evolution of developmental programs and, hence, for the major morphological changes observed in the fossil record" (Jernvall 1996, 1). A major component of this grant was dedicated to further developing a "three-dimensional system of relation molecular activities with developing morphology" using Geographic Information Systems (GIS) (Jernvall 1996, 1). The coupling of GIS to developing morphology was novel when Jernvall proposed it, and the application of GIS would prove critical to Jernvall's ability to connect gene expression data (and thus inferred

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[176] Jernvall went on to earn this grant from the Academy of Finland, with Fortelius and Thesleff serving as co-PIs on the project. Jernvall remained a post doc/research fellow at the University of Helsinki until he was named a group leader (essentially a PI) there in 2000.

mechanisms of development) with the emerging morphology of the developing tooth (Section 6.7).

Jernvall first applied GIS to developing teeth in his 1998 paper in order to analyze shape change in relation to the primary enamel knot in the first lower molar of the mouse (Jernvall et al. 1998). The application of GIS was limited within this paper, showing only the morphological changes that occur in the late bud (day 13) through late cap stage (day 15) of molar growth. Although the application was limited, it served as a proof of concept that digital elevation models could be made from whole mount cultured specimens (i.e. specimens that had not been sectioned) and that GIS could be applied to this data.

In 1999, Jernvall collaborated with Lena Selänne, a Master's student in his laboratory, to introduce a methodology for creating digital elevation models (see figure 27) of both developing teeth and small paleontological specimens and applying GIS to analyze these models (Jernvall and Selänne 1999). Digital elevation models were made by scanning specimens using a confocal microscope. The resulting scan data was run through a series of software packages to create high-resolution images (digital elevation models) of the specimen. Digital elevation models such as these were useful because the greyscale values correspond to height differences, and the height information could be used to create three-dimensional reconstructions of the specimens. These three-dimensional reconstructions with height information allowed for the digital elevation models to be treated like geographic data because the height information could be treated similar to topographic differences on a terrain. With this

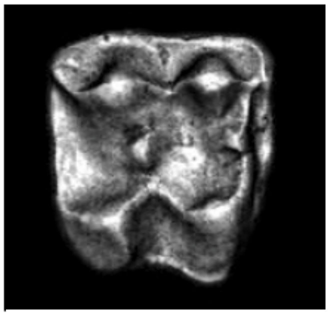
Figure 27. Digital elevation model of a tooth. Figure 5 from Jernvall and Selänne (1999).

three-dimensional information in hand, Jernvall and Selänne applied GIS to the digital elevation models. GIS allowed the pair to quantify and summarize different aspects of the tooth shape, like the surface areas of different parts of the cusp slopes.

Traditional techniques for analyzing shape data are usually based on landmarks (i.e. defined features such as the tip of the paracone), and are not easily applied to very small specimens or developing teeth, where the standard landmarks have not yet mineralized. [177] Therefore, quantification of things like the shapes and slopes of the developing tooth cusps were nearly impossible before GIS. The combination of digital elevation models and GIS thus allowed Jernvall and Selänne to analyze differences in shape between specimens that was previously impossible.

6.6 Evolutionary Modification of Development in Mammalian Teeth

Beginning with his 1994 publication that reinvigorated interest in the enamel knot for explaining tooth development, Jernvall worked with colleagues at the University of Helsinki for a number of years to expand understanding of the enamel knots' roles in development. Through a series of experiments, the group showed that the enamel knots express a number of genes that appeared to coordinate the development of the cusps (Jernvall et al. 1994, Jernvall 1995, Jernvall et al. 1998, Keränen et al. 1998, Vaahtokari 1996a). They showed that the secondary enamel knots gave cusps individual control mechanisms, that primary and secondary enamel knots used the same genetic modules to

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[177] "Standard" landmarks for morphometric analyses of teeth usually include things like cusp tips and the lowest part of foveae (pits) on the crown.

control development, and that these modules were consistent across mammals (or at least across mice and voles) (Keränen et al. 1998). They discovered part of the mechanisms that cause the enamel knots to form and their cells to undergo apoptosis (Jernvall et al. 1998, Vaahtokari 1996b). Finally, Jernvall's group figured out how to create digital elevation models of whole mount developing teeth and apply GIS to these specimens (Jernvall et al. 1998, Jernvall and Selänne 1999).

By the late 1990s Jernvall had enough knowledge about the mechanisms through which the enamel knots shaped the development of teeth, along with techniques to analyze forms without landmarks, to return to the problem laid out in his dissertation—"to address the general diversity of mammalian molar tooth shapes in the context of both evolution and development" (Jernvall 1995, 3). Thus, Jernvall was finally in a position to use the enamel knot theory to explain morphological diversity.

With this in mind, Jernvall began a new set of experiments with his graduate student, Soile Keränen, and his former advisor, Irma Thesleff. These experiments led to a paper published in the *Proceedings of the National Academy of Sciences* in 2000 (Jernvall et al. 2000). The trio, led by Jernvall, sought to bring together the molecular mechanisms of the enamel knot that controlled individual cusp development with the shape analysis tools offered by GIS and an evolutionary perspective. In this way, they sought to use the enamel knot theory in order to explain morphological diversity.

The evolutionary perspective was brought about by comparing the morphological development of the first molars of mice (*Mus musculus*, Murinae) and voles (sibling vole, *Microtus rossiaemeridionalis*, Arvicolinae). Both mice and voles are muroid rodents; "the first members of the Murinae date back to the Middle Miocene, and the first arvicoline date back to the Early Pliocene" (Jernvall et al. 2000, 14444).[178] Mice and voles, although closely related, display different molar morphologies (figure 10). Jernvall et al. offered a description of the contrasting morphologies:

> Mouse lineages have commonly retained the ancestral number of main cusps, but the cusp pattern has evolved from having buccal and lingual cusps diagonal to each other to having them opposite or parallel. In contrast, voles have retained the diagonal cusp pattern, but have evolved several new cusps on their first lower molars…Additionally, voles have retained the longitudinal crest connecting anterior and posterior cusps, whereas this crest has been lost in mice. (Jernvall et al. 2000, 14444)

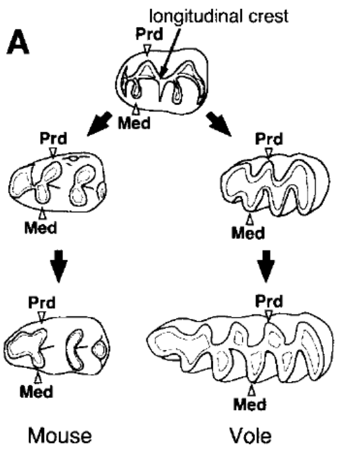

Figure 28. Examples that illustrate a morphological transformation series from the common ancestor of mice and voles to their modern phenotypes. Protoconid (Prd) and metaconid (Med) labeled. Anterior left, buccal top. Figure 1A from Jernvall et al. 2000, 14445.

Based on the morphological differences between these two species and their common ancestor, Jernvall and colleagues had two main morphological features to explain. First, the placement of mouse molar cusps in parallel rows, rather than diagonal rows (as seen in the ancestral state—see figure 28). Second, the elongation of the vole molar that would allow the addition of more cusps. In order to explain these differences in terms of evolutionary morphology, Jernvall and colleagues turned to the developmental mechanisms of the enamel knot.

The group scanned the interface between the epithelium and mesenchyme of developing teeth of mice and voles from days 14 through 17 and created digital elevation models from the scans.[179] Next, they did *in situ* hybridization on whole mounts and serial

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[178] The Miocene extends from ~23 to 5.3 MYA, while the Pliocene extends from ~5.3 to 2.6 MYA.

[179] Recall that days 14 through 17 cover the appearance of the primary and secondary enamel knots. Thus, this timeframe covers from the appearance of the primary enamel knot through to the beginning of cusp formation.

sections to look at the expression patterns of *Fgf4*, *Lef1*, *p21*, and *Shh*.[180] They scanned epithelia of the whole mounts in order to capture the expression patterns of these four genes within the epithelium, and processed and superimposed the scans in order to create images of the co-expression of all four genes. Next, they analyzed the digital elevation models and gene co-expression models using GIS (figure 29).

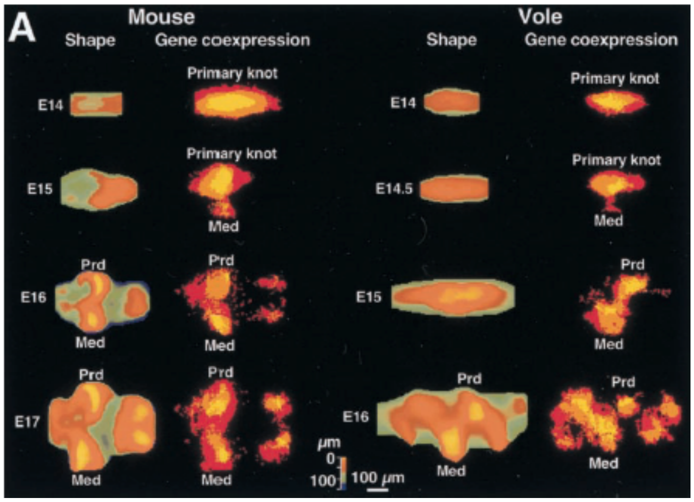

Figure 29. DEMs showing formation of cusps and corresponding coexpression of Fgf4, Shh, Lef1, and p21. Coexpression all 4 genes (yellow), lacking Fgf4 (orange), lacking Fgf4 and Lef1 (red). Figure 3A from Jernvall et al. 2000, 14446.

Using these methods, Jernvall's group was able to infer a number of things about both the developmental and evolutionary processes that shaped the differences between mouse and vole molars. First, they showed that enamel knot gene expression occurs at species-specific locations. This patterning led them to conclude that "the derived parallel cusp configuration of the mouse is not generated by local (e.g., allometric) changes in growth patterns *after* the initiation of cusp development" (Jernvall et al. 14447). Therefore, it is likely that cusp configurations are changed prior to the initiation of cusp

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[180] Recall that these genes had been shown in previous experiments to be expressed by the enamel knots.

development by modification of the regulatory domains that determine cusps—i.e. cusp patterning is determined in advance of cusp development. The trio came to the conclusion that evolutionary modification of cusp development in the mouse is a case of heterotopy.

The analysis of subtle differences in growth between the mouse and vole tooth germs indicated that the vole tooth germ experienced a faster rate of longitudinal growth than the mouse tooth germ (8.5μm/hour in vole versus 3.6μm/hour in mouse) (Jernvall et al 2000, 14447). The trio linked the faster growth of the vole tooth germ with the elongation and alteration of cusp patterning in the vole because it could allow for a more "extensive iteration of the vole cusp pattern longitudinally," meaning that more tooth germ gives more space for new cusps to develop (Jernvall et al 2000, 1447).

The morphological analyses also showed that there was a two-day gap between the initiation of the primary enamel knot in the crown base and appearance of cusp formation. In order to probe what happens during this interval, they examined how the patterns of gene expression correlated with the emerging morphology seen in the digital elevation models. More specifically, they looked at the cross correlation between gene expression at day 15 and the shape topographies at different stages of development. Their results showed that gene expression of day 15 molars correlated more highly to the shape topography of day 16 molars than to any other day (including day 15), meaning that gene expression precedes the onset of morphology. If, in fact, gene expression precedes morphological development (as is indicated by their results), then the evolutionary alteration of the cusps in mice to develop in parallel rows must "affect very early stages in development" because they had already shown that the first two cusps appear at species-specific locations (Jernvall et al. 2000, 14447).

In order to investigate this apparent prepatterning more closely, and to connect it with the morphological evolution of mice and voles, they compared the gene coexpression patterns with the shape topography for each day of development. Their results seamlessly blend development and evolution:

> The patterns show that both vole and mouse molars initiate the second cusp (the metaconid) midway on the side of the primary enamel knot. However, this common pattern of gene expression in mouse and vole is followed by a shift to expression patterns that corresponds to the future species-specific cusp topographies. In vole, the first cusp, the protoconid, forms from the posterior part of the primary knot, whereas in the mouse, the protoconid forms more anteriorly, next to the metaconid. Therefore, the evolutionary shift of the protoconid and metaconid cusp configuration is achieved during development by changing the location within the primary enamel knot that gives rise to the protoconid. (Jernvall et al. 2000, 14447)

In the conclusion of their paper, they again wove together developmental mechanisms and evolutionary morphology:

> In conclusion, we suggest that evolutionary divergence in molars has involved at least two separate hierarchical developmental processes in mouse and vole. First, the anterior shift of buccal cusps in mouse lineages is achieved by changing the spatial regulation of genes operating in signaling networks before the formation of first cusps…Second, increased cusp number in vole lineages is caused by a greater number of iterations of the established lateral topography. (Jernvall et al. 2000, 14448)

Thus, in his article published in 2000, Jernvall brought together developmental mechanisms and evolutionary morphology. By comparing modern morphologies of closely related species with an ancestral state, Jernvall posed questions about the morphological evolution of teeth in mice and voles. In order to answer these questions, he turned to developmental mechanisms, using his enamel knot theory of how enamel knots control individual cusp development in order to posit scenarios that could account for the evolutionary shifts in morphology that gave rise to these two phenotypes.

While this article achieves the goal that Jernvall set out in his dissertation, namely, "to address the general diversity of mammalian molar tooth shapes in the context of both evolution and development," it left open a number of questions about the development and evolution of mammalian teeth (Jernvall 1995, 3). What controls the placement of cusps, and how can cusp placement be altered? What controls the addition of cusps, and how can this process be adjusted to give rise to more (or less) cusps?

As Jernvall wrote in his dissertation,

> The model of the enamel knots as a mechanism for making a single cusp does not explain how development of a multicusped molar is controlled. This is evolutionarily speaking, the "real" task because the diversity of mammalian teeth is largely in the cusp patterns which are made of different combinations of cusps. (Jernvall 1995, 42)

While understanding the developmental mechanisms that control individual cusps is an important step, and one that led to hypotheses about morphological evolution, important questions about how cusps are patterned remained. That is, the big question remaining was: how are the modules that control individual cusp development regulated such that different tooth cusp patterns are generated?

In the intervening years, Jernvall has worked on finding answers to some of these questions, relying on combining developmental mechanisms with morphological evolution, as well as modeling (Jernvall 2002, Kangas et al. 2004, Kassai et al. 2005, Kavanagh et al. 2007). Jernvall's pursuit of an explanation of mammalian tooth morphology that took into account both developmental mechanisms and morphological diversity, has bloomed into a highly successful research program which includes a theory of molar morphology unlike any heretofore proposed. An article written by the senior editors of *Nature* lists works from Jernvall's laboratory as one of their, "15 examples published by *Nature* over the past decade or so to illustrate the breadth, depth and power of evolutionary thinking" (Gee et al. 2009, 1).

6.7 Jernvall's Research: Re-envisioning and Synthesizing 19th Century Research Programs on Morphological Diversity and 20th Century Research Programs on Morphogenetic Processes

The research program that Jernvall developed from his dissertation to 2000 can be seen as a synthesis of the research programs focused on the morphological diversity and morphogenetic processes of teeth of the 19th and 20th centuries, respectively (Chapters 2-5). Jernvall's synthesis of morphological diversity and morphogenesis arose from his ability to draw hypotheses about one from the other. This point is embodied within his enamel knot theory. While the theory explains how individual cusps develop and become patterned on the tooth crown, it can be extended to explain how modification of the development of individual cusps (in terms of timing or placement) gives rise to morphological diversity. In order to fit together research on morphological diversity and morphogenetic properties, and develop the enamel knot theory, Jernvall had to reconceive how research in these areas should be done.

Like Cope and Osborn, Jernvall looked broadly at the morphological diversity of mammalian molars and tried to derive generalizable principles from this survey. Whereas Cope and Osborn tended to take these generalizable principles and declare laws and theories, Jernvall saw them as the starting point for generating hypotheses that could be tested by looking to development. Take for instance Jernvall's study of the hypocone (section 6.2.1). His research led to the conclusion that the broad presence of the hypocone within mammals was the result of multiple convergent evolutions. This conclusion led Jernvall to the hypothesis that "…acquiring a cusp is not difficult per se, and developmental evolution for making new cusps seems to be flexible" (Jernvall 1995, 9). The hypothesis that acquiring new cusps is "not difficult" or "flexible" can be tested with developmental experiments—something that Jernvall's lab has done (Kassai et al. 2005, Kavanaugh et al. 2007).

Jernvall also surveyed morphological diversity very differently than Cope and Osborn—he believed that the diversity he saw could be reduced to a simple matter of shape variables (section 6.2.2). While these shape variables have a history, they are not governed solely by this history, and so cusp homologies did not drive the patterns of crown types for Jernvall in the way that they did for Osborn.

Similarly, Jernvall moved beyond understanding morphogenetic processes as a simple matter of cell proliferation and movement, or development as a simple matter of gene expression, as he strove to combine information on the cellular phenomena of development with gene expression patterns that could drive these phenomena. Jernvall combined this cellular and gene expression information with new ways of analyzing developing shapes (GIS). This new way of conceptualizing morphogenesis resulted in the enamel knot theory of tooth development, which is focused on explaining how individual cusps arise and how they are patterned.

6.8 Bringing Together Development and Evolution, Morphogenesis and Morphology, and a Lesson for EvoDevo

At the conclusion of Chapter 1, I stated that the thesis of this dissertation was the following:

The research program of Jukka Jernvall brought these two historical foci on morphology together by reconceiving of what it means to explain morphological diversity of teeth and what it means to explain morphogenetic processes in teeth, the combination of which allowed Jernvall to develop a theory of tooth development and evolution that serves as an exemplar for developing explanations that unite development and evolution for modern EvoDevo.

This dissertation traces a history of how researchers have investigated and explained the problem of mammalian tooth morphology from the late 19th century to 2000. It shows how these different research programs, while addressing the same problem, focused on different driving questions, were driven by assumptions about methods, evidence, and the relationship between development and evolution, and came to different conclusions about how best to explain morphology.

Morphology, as envisioned by Goethe, embodied the study of formation (*Bildung*). If *Bildung* is the goal of morphological inquiry, then in order to explain morphology, one should account for both a diversity of final forms and how organisms arrive at these final forms. This requires investigating both morphological diversity (evolution) and morphogenesis (development) and building an explanation that can account for both of these. From 19th century studies of morphological diversity to 20th century studies of tooth morphogenesis, researchers explained different aspects of how tooth morphology arises, but did not develop explanations that effectively brought together the processes of development with the diversity of evolution.

EvoDevo is a broad field, encompassing many organisms, techniques, goals, and theoretical and epistemic frameworks. Although broad, EvoDevo is bound together by the tenet that understanding development gives the best insights into understanding organismal evolution. Just how to use development to explain evolution is a problem that has plagued EvoDevo practitioners since the field's inception (Laubichler and Maienschein 2007, 2013).

Throughout this chapter we have seen how Jernvall was able to unify development and evolution within a single explanatory framework by shifting away from the ways in which previous researchers have investigated and explained morphological diversity and morphogenetic processes. These shifts allowed him to develop a research program that unified the two perspectives and worked iteratively between them. Thus, Jernvall's work has brought these two historical foci on morphology together by reconceiving of how to explain morphological diversity (as shape variables, and through hypotheses about development), how to explain morphogenetic processes (through a combination of cellular phenomena, gene expression patterns, and GIS mapping), and how to use morphogenetic processes to explain morphological diversity (the enamel knot theory). The combination allowed him to develop the enamel knot theory, which can explain mammalian tooth development and evolution.

From this history of Jernvall's research program and creation of the enamel knot theory, we can render at least one major lesson for the field of EvoDevo, which has long sought a way to use development to explain evolution. Namely, that development is a process whereby genetic modules regulate morphogenetic processes. Both of these are crucial for explaining how development gives rise to the form, or phenotype, of an organism. The phenotype of the organism is what comes into contact with the world, and phenotypic variation gives selective processes something upon which to act. Therefore, without understanding how phenotypes arise during development, EvoDevo lacks a way of using development to explain evolution. Jernvall would not have been able to develop an understanding of the enamel knots without appeals to both genetic modules and morphogenetic processes. He also would not have been able to construct the enamel knot theory, which relies on the interactions of these two sides of development to explain morphological diversity. Therefore, Jernvall's research program and enamel knot theory serve as examples to the EvoDevo community, showing how investing in understanding both genetic modules and morphogenetic processes, and how these can be altered during development, can lead to a theory whereby development can explain evolution.

6.9 Conclusion

At the outset of this dissertation, I raised the historical point that the history of biology in the 20th century has been told from the perspective of the gene. And yet, this dissertation has traced a history of morphological inquiry that occurred largely irrespective of genetic data. Morphology and genes have been seen in the historical literature, as well as the scientific literature, mostly as distinct entities, and the latter has received the lion's share of attention from both scientists and historians for the past century. This perspective has merit—genes tell us a great deal about our bodies and our place in nature—and yet, throughout this dissertation, I have shown how morphology has also shed light on these topics. While the history of biology has followed the assumption of the gene as the locus of explanatory value for processes like development and evolution, the history that I have presented provokes a reevaluation of that perspective. What is the danger of allowing this assumption of gene primacy to stand, and what can we learn from its reevaluation? In order to answer these questions, we need first to understand more about the assumptions at work.

Assumptions, as we have seen throughout these chapters, are complex. They can be put on display, as in the case of Osborn's endorsement of kinetogenesis, or they can lurk in the background, as in the case of Röse's adoption of a strong form of the biogenetic law. They can apply to standards of evidence, as in Osborn's belief that his own paleontological evidence outweighed the embryological evidence offered by Röse. They can apply to methods, as in Reichenbach's use of stains and sections to follow cellular proliferation. Assumptions enter into every step along the scientific process, and shape the outcomes of science in many ways. More work needs to be done in order to uncover what kinds of assumptions play a role in science, how they do so, and even what assumptions are. But, even without this kind of detailed analyses of assumptions and their roles in science, we can begin to answer the questions laid out here.

The danger of allowing the assumption of gene primacy to stand is that experimental evidence has shown that genes are not the only factors that shape development or, consequently, evolution. During development, cells divide, move around, and die. In going through these morphogenetic processes, cells give rise to morphology, and thus also shape evolutionary diversity that would not even exist with genes alone. Stuart Newman and colleagues pointed out in 2006 ways in which cellular activities may be, in part, shaped by biomechanical influences, which affect organismal form (Newman et al. 2006). Recent work on epithelia shows how mechanical forces (e.g. stretching) can control cell division in this tissue (Gudipaty et al 2017). Thus, research has shown us that more than gene regulatory networks are necessary in order to explain development and evolution. If the goal is to draw on development to explain evolution, then this explanation should account for the processes of morphogenesis as well as gene regulation. This is exactly what Jukka Jernvall has done.

When we look at Jernvall and the enamel knot theory, we see that genes were not the exclusive evidence upon which his enamel knot theory was constructed. Nor were genes the sole evidence that led him to rediscover the enamel knot. Jernvall's work on the enamel knot began by tracing cells in order to learn more about the morphogenesis of tooth development. That is, he wanted to know how cells and cell populations give rise to tooth morphology. Through tracing these cells, Jernvall noticed the enamel knot, which he confirmed by turning to genes. The lesson here is not that genes came to the rescue of morphology, rather that the two were at work in tandem within Jernvall's research. From his homology-free system of dental morphology to studying differential cell proliferation within the developing tooth, morphology was crucial to Jernvall's ability to envision the developmental and evolutionary capacity of the enamel knot.[181]

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[181] Note that the concept of homology has changed considerably since it was first coined by Richard Owen in 1843. Owen's homology concept, and that which was employed by Cope and Osborn, was a morphological concept—i.e. investigators turned to the morphological details of the animal body to trace homologies. Today, the morphological homology concept is still utilized by morphologists across the life sciences, however, it is accompanied by a genetic concept of homology as well. The genetic concept of homology holds that homologies exist at the level of gene sequences, and homological genes can be traced throughout lineages by looking at sequence similarity and base pair differences.

And so, the history presented in this dissertation provides an alternative narrative of developmental research from the late 19th through 20th centuries, and shows us how different types of assumptions have played a role in shaping explanations of tooth morphology. Morphogenesis is a developmental process, which I have shown has a deep, rich history worthy of historical scrutiny. This history has much to tell us about how development works and how we can explain developmental processes. I have also shown that morphogenesis, and a focus on morphology, has a critical role to play in our modern ability to achieve the goals outlined by evo-devo. While more work needs to be done to figure out how to extend Jernvall's framework of uniting morphogenesis and genes, and development and evolution, to research programs that do not focus on teeth, the work that I have presented here shows us how to work among different types of evidence, and different research fields, in order to use development to explain evolution. Thus, the research that I have presented in this dissertation shows a route for further fruitful research, both in the history of biology (where morphogenesis has been woefully neglected) and in evo-devo (where using development to explain evolution has been both a constant goal and constant problem for the field).

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BIOGRAPHICAL SKETCH

MacCord attended the University of Pittsburgh as an undergraduate. At the University of Pittsburgh, she worked under the tutelage of Jeffrey H. Schwartz, and earned a BPhil in Anthropology from the Honors College in 2009. After graduating from the University of Pittsburgh, MacCord attended the University of Cambridge on a Gates Cambridge Fellowship. At Cambridge, she earned an MPhil in Human Evolutionary Studies under the tutelage of Jay Stock. MacCord entered the PhD program in History and Philosophy of Science at Arizona State University in August, 2010. She spent the 2012-2013 school year as a Fulbright Fellow at the University of Helsinki, working in the laboratory of Jukka Jernvall. She has been employed as a Project Coordinator at Arizona State University, in the Center for Biology and Society, since August, 2013. MacCord currently lives in Tempe, Arizona, with her fiancée, Challie Facemire, and three cats: Finn, the Kraken, and Ruh. Following graduation, MacCord will take up a position as a Program Administrator and McDonnell Research Fellow at the Marine Biological Laboratory in Woods Hole, MA.