

AN EXAMINATION OF VARIABILITY IN DENTAL DEVELOPMENT ACROSS NORTH
CAROLINA

By

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ABSTRACT

Contemporary research suggests that dental development is biologically stable across human populations. However, research examining trends in dental developmental variation tends to focus on broad population trends and often lacks nuanced information about small-scale relationships at the city and community level. As a result, these studies may gloss over localized genetic and/or environmental trends that may influence variation in dental development. Addressing this gap, tooth mineralization scores were collected from dental radiographs associated with subadult individuals (6–16 years of age) from Community Service Learning Centers (CSLC) affiliated with the East Carolina University School of Dental Medicine (SoDM) with a total sample size of $n = 976$. The CSLC locations are geographically and demographically diverse and include populations across North Carolina, such as Ahoskie, Lumberton (Robeson County), Ross Hall (Greenville, NC), Spruce Pine, and Sylva. Results were compared against previously published research associated with the Subadult Virtual Anthropology Database (SVAD) and contextualized within demographic factors such as geographic location, biological sex, self-described race, and ethnicity. The results demonstrate that the timing of tooth mineralization is similar across North Carolina regardless of geography or demographic

composition. CSLC Sylva shows a high degree of delay in mineralization timing for age with most teeth scoring below their counterparts across North Carolina and within the SVAD sample. This delay is most severe during root development and may be a product of local environmental factors in western North Carolina (CSLC Sylva). Early forming teeth notably exhibit more extreme degrees of delay compared to their later forming counterparts. The results do corroborate the stability shown in previous research, but also capture the variation that may be missed when pooling samples by broad geographic or environmental categories.

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CAROLINA

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Chapter 1: Introduction

The study of human growth and development provides insight into the health of an individual, and the broader health implications of a population. Specifically, the critical periods associated with growth and development are the most vulnerable to external environmental influences that could impact long-term health outcomes (Mousikou et al., 2023). Environmental factors such as disease, infection, nutrition, psychological distress, and other components may compromise an individual's quality of life, and developing individuals are especially prone to potentially irreversible effects of such factors during these critical periods in their development (Mousikou et al., 2023; Reitsema & McIlvaine, 2014).

Despite widespread acknowledgment of these effects on growth and development, studies have often focused on skeletal development. In existing literature, the relationship between environmental factors and dental development is underexplored, with little evidence documenting the changes in tooth mineralization. This has led to the long-standing assumption that dental development is less plastic, more genetically driven, and biologically stable across diverse human populations (Elamin & Liversidge, 2013; Liversidge, 2003). These assumptions suggest that the trajectory and pace of dental development are largely uniform across global populations, regardless of environmental contexts. However, recent studies have begun to challenge this view, suggesting a more complex environmental response in dental ontogeny (Cardoso et al., 2010; Conceição & Cardoso, 2011; Dharmo et al., 2018; Nelson et al., 2021). This study challenges the assumption that dental development is stable across human populations and explores the relationship between local environments and tooth formation.

Early studies often relied on convenience samples drawn from homogenized, centralized populations and prioritized research questions unrelated to environmental influences on dental

development (AlQahtani et al., 2010; Elamin & Liversidge, 2013; Haavikko, 1970; Liversidge, 2009). These samples lacked the diversity to examine subtle patterns of variation, especially those present between local communities or across smaller geographic scales. Consequently, variation in dental development has been attributed to broad genetic or population-based differences, rather than the influence of environmental factors that may differ between communities.

Comparatively, skeletal growth and development studies acknowledge present variation among individuals due to inter-individual variation in developmental timing (IVDT) (Medendorp Rautman & Edgar, 2020). Research suggests that IVDT is influenced by developmental stressors or plasticity that can alter the rate of growth and maturation in response to environmental conditions. For example, the differences present in the development of biological males and females has been a well-documented instance of IVDT, and additional studies have shown population-level differences associated with ancestry, nutrition, and historical events such as slavery or famine (Medendorp Rautman & Edgar, 2020).

Associated research explicitly addressing the impact of the environment on the dentition is less straightforward. Most studies that do explore the possibility of variation in other contexts (i.e., ethnicity), rely upon homogenized samples from a single population (Thevissen et al., 2024). As new research begins to explore new perspectives (Cardoso et al., 2010; Conceição & Cardoso, 2011; Dhamo et al., 2018; Nelson et al., 2021), it reveals a significant gap in the literature that addresses the degree to which development is influenced by the environment. Even so, recent research still often lacks the ability to use sampling strategies that explore ontogenetic variation at this smaller scale. Such large-scale studies often gloss over community level

variation that may impact how a clinician defines an individual's maturity (Cardoso et al., 2010) or may impact how teeth are used in settings such as forensic anthropology (Stull et al., 2021).

This thesis explicitly addresses the relationship between individual environments and the developing dentition by examining tooth formation at the community level throughout the state of North Carolina. As a result, this work provides a better understanding of the relationship between local [environmental] context and dental developmental variation. Such work informs broader studies in bioarchaeology and paleoanthropology (Dean & Liversidge, 2015; Halcrow et al., 2007). Given the lack of research on this small scale, this study does not begin with a specific prediction, as the absence of prior literature makes it difficult to anticipate the presence of variation between communities. Instead, the goal is to explore the variation present in each community to determine if similarities or differences exist across regions in North Carolina. Understanding these subtle patterns of variation has important implications for clinical assessments of maturity and forensic applications and may lead to a better understanding of environmental impacts on growth, health, and development across small-scale populations.

Chapter 2: Background

2.1 – Historical Background

According to Bogin (2020), research on the growth and development of the human skeleton has undergone massive change over the last three thousand years. Such research dates to Sumerian Mesopotamia, where depictions of higher-status individuals were shown to be significantly larger in size than their lower-status counterparts, with texts suggesting a relationship between human growth and development, and how biosocial conditions may influence these biological processes. Continuing into the eighteenth and nineteenth centuries, longitudinal methods and statistical approaches became common practice in observing the relationship between skeletal growth and biosocial conditions, with many other fields of research (evolutionary ecology, behavioral development, etc.) observing the same trends in human growth and development (Bogin, 2020; Bateson et al., 2004). This recurrent trend has been observed, with childhood growth and development providing information associated with the health of a population and their relationship to environmental and/or social stress. “Health” is typically compromised by any number of factors, eventually leading to negative consequences that affect an individual’s quality of life. “Stress” is one of these factors, typically associated with a physiological and/or social change caused by strain on the individual due to environmental pressures (Reitsema & McIlvaine, 2014).

The relationship between health, stress, growth, and development can be subsumed under the broader process associated with developmental plasticity. Developmental plasticity is defined as the ability for a single genotype to alter its developmental processes and phenotypic outcomes due to variation in environmental conditions (Moczek et al., 2011). In biological anthropology, the use of developmental plasticity has a long history that dates to Franz Boas’ study of cranial

variation in immigrant children (Boas, 1912). The understanding of the plasticity of the human skeleton is well documented. Hoppa (2005), Scheuer & Black (2004), and Bogin (2020), conducted research on historical and contemporary environmental conditions such as malnutrition, physiological stress, and infectious disease and their role in development. Compared to the extensive research done on the developmental plasticity of the human skeleton, the mineralization of the dentition is understudied. In the context of growth and development, growth is defined as a quantifiable increase in size or mass over time, while development is established by a progression of stages that eventually lead to an organized mature state (Bogin, 2020).

2.2 – Dental Development

A myriad of research has been done on the development of dentition through studies that focus on measuring the tooth from crown to root (Liversidge et al., 1998), tooth eruption (when the tooth advances from the alveolar crypt to a functional occlusal position) (Hurme, 1948; Liversidge & Molleson, 2004; Scheuer & Black, 2004), and tooth mineralization (the formation of teeth from crown to root) (AlQahtani et al., 2010; Demirjian et al., 1973; Scheuer & Black, 2004; Stull & Corron, 2021). Measurement of the crown and/or root components is a less reliable metric of development, requiring information about the population and the associated demographics (Nelson et al., 2021). Dental eruption is highly variable across human populations, with variation resulting from environmental influences such as missing teeth, malocclusion, space in the growing jaw, etc. (Scheuer & Black, 2004). Due to these limitations, tooth mineralization is the most reliable method to examine the development of the dentition (Lopes et al., 2018). For this reason, tooth mineralization is used to describe development in the proposed study.

Tooth mineralization is assumed to be biologically stable and genetically driven, with little external influence (Elamin & Liversidge, 2013). Deciduous teeth and early forming teeth begin development before weaning when environmental factors have a lower impact on their formation (Scheuer & Black, 2004). However, according to Dhamo et al. (2018) and Nelson et al. (2021), biocultural factors may play a role in delaying or accelerating the development of later forming teeth such as the premolars, permanent canines, and the second permanent molars. While studies that focus on environmental factors and their association with dental development are limited, there is extensive research that shows how the development of tooth enamel (amelogenesis) is affected by the environment, resulting in developmental defects of the enamel (DDE) (Nota et al., 2020). Based on the disagreement between previous studies and contemporary studies, and the broad amount of research done on other developmental events such as DDE, more research should be completed to explore the relationship that exists between local environmental conditions and the development of the human dentition (Bogin, 2020; Christensen et al., 2019; Liversidge, 2003; Scheuer & Black, 2004).

2.3 – Genetic & Environmental Influence

The development of the dentition is a complex process that is strongly regulated by genes and proteins, though it is also known to be influenced by additional factors such as the environment and epigenetic alteration (Chow et al., 2024). This is due to the intricate process associated with the development of the face, as it must be finely tuned to form bilaterally with the convergence of the bones and tissue that make up the nose and the upper and lower jaws (Hooper et al., 2017). As such, the regulation of these genes is what establishes how the teeth develop, and disruption of this regulation results in developmental and facial anomalies (Chow et al., 2024; Hooper et al., 2017; Smith et al., 2017). According to Chow et al. (2024), every

chromosome has a role in the development of the dentition, and as of April 2024, Bioinformatics for Dentistry has identified and archived 132 genes associated with the development of the dentition. These genes encode a total of 125 proteins and 7 microRNA molecules, with chromosomes 3, 7, and 17 having the most genetic influence (Chow et al., 2024). As such, dental development is genetically driven, though not immune to the effects of the environment.

Environmental factors are known to affect the mineralization of the dentition, causing not only the delay, but also the possible acceleration of tooth mineralization (Dhamo et al., 2018). One such factor is socioeconomic status, as it affects the overall health, stress, diet, and nutrition of the individual (Nelson et al., 2021). Low-income households often struggle with various barriers that limit the improvement of their health status, and unequal opportunities lead to poor dietary habits in childhood and adolescence due to food availability (Rogers et al., 2015). These poor dietary habits can lead to the presence of carious lesions on the dentition. Dental caries, also known as tooth decay or cavities, are a common childhood disease. Caries form through interactions between acid producing bacteria, the fermentation of carbohydrates, and factors including the saliva and teeth of the individual, resulting in the localized destruction of the tooth (Selwitz, et al., 2007). Communities that are the most susceptible to dental caries are often low in socioeconomic status, where minority groups are often overrepresented and health and living conditions are poor (Naidoo & Myburgh, 2007). Despite efforts to prevent dental carries through frequent dental health checks (such as work at each CSLC), high-risk communities end up failing to benefit from these measures and are often excluded from receiving proper care (Gratrix & Holloway, 1994).

Recent research suggests that the development of the dentition is affected by these environmental factors, focusing on the socioeconomic status of the individual and the presence

or absence of dental caries (Dhamo et al., 2018; Nelson et al., 2021). Past studies that show that the environment has little impact on the teeth were often influenced by the selected samples not showing the degree of sample diversity necessary to properly explore the environmental impacts on the developing dentition (Elamin & Liversidge, 2013). Due to the selection of these samples, it is currently unknown whether developmental stability is a universal trait of human dentition, or if this assumption of stability is due to coarse sampling techniques that lack a nuanced approach to human variation and resulting dental development.

2.4 – Community Service Learning Centers

Community Service Learning Centers are intended to serve those that live in rural communities that otherwise have limited access to dental care. The CSLC are designed to allow fourth-year dental students at East Carolina University School of Dental Medicine (SoDM) to complete clinical rotations and understand the needs of those that live in each region and provide high quality dental care to their patients (<https://dental.ecu.edu/fast-facts/>). Importantly, each CSLC provides unique access to developmental data throughout the state of North Carolina, allowing one to address developmental variation across the different communities associated with each CSLC. The centers below were chosen to capture as much demographic, geographic, and resulting ontogenetic variation across communities in North Carolina (Table 1).

2.4.1 – Ahoskie

Ahoskie is a small town located in northeastern North Carolina, encompassing 4.35 square miles with a population of approximately 4,891. The town features a core of light to medium commercial development along main street, expanding out into residential zones and industrial sites, focusing on the railroad and central areas. It has an extraterritorial jurisdiction that extends one mile beyond the official boundaries of the city, with zoning ordinance guiding

land use. Approximately 60% of the land in Ahoskie falls between three historic districts that reflect the heritage of the town. Despite its small size, it serves as an important economic center, drawing consumers from up to 40 miles away (Bracy et al., 2022).

The economy of Ahoskie is supported by industrial and agricultural distribution centers, including Nucor Steel, Perdue Farms, the Golden Peanut Company, and Georgia Pacific. However, despite its large economic hub, it has several challenges that impact the development of the community. There are limited job prospects and a lack of affordable housing. Due to this, the town has experienced a marginal population decline since 2010, a trend that seems overall consistent with many rural communities across North Carolina, despite the population of the state increasing by approximately 5%. The town's employment rate stands at 48.4%, though 24.4% of residents live below the poverty line. Of that 24.4%, 25.3% of those in poverty are under the age of 18. Additionally, 9.1% of the population lacks health insurance coverage (Bracy et al., 2022; U.S. Census Bureau, n.d.).

2.4.2 – Greenville

Greenville spans approximately 39.09 miles and serves as the cultural, educational, and economic hub of eastern North Carolina. With a population standing at approximately 87,521, it is the largest municipality in the region. It has a large academic presence due to the presence of East Carolina University's spread throughout the city. This includes larger facilities such as Brody School of Medicine and the ECU Medical Center, a major healthcare facility that has a total of 974 licensed beds (*About Greenville | Greenville, NC*, n.d.).

The leading industries in Greenville focus on its medical and educational institutions, drawing students and professionals from across the state and patients from the nearby counties.

Despite its strengths in education and industry, 24.8% of the population lives below the poverty line, with 21.0% being under the age of 18. Additionally, 7.1% of residents are left without healthcare (U.S. Census Bureau, n.d.).

2.4.3 – Lumberton

Lumberton is the largest municipality in Robeson County and serves as its county seat since 1787. It has been crucial in the cultural and economic development of North Carolina's southern coastal plains, as it sits right along I-95, and is intersected by several key highways for travel (US 301, NC 211, 41, 72, and 711). Historically, the city has benefitted from its accessibility and transportation networks and is a prominent regional hub that is strengthened by its access to the railroad, proximity to major airports, and diverse industries (Davis, n.d.).

The city spans approximately 15.7 square miles with a population of 19,025. Like many rural communities in North Carolina, Lumberton experiences high rates of poverty, as 28.9% of its population lives below the poverty line, with a significant portion of those living in poverty being below the age of 18 at 37.0%. Despite economic challenges, it is experiencing ongoing revitalization efforts, with a balanced approach between development and the increase of quality of living for its residents (Davis, n.d.; U.S. Census Bureau, n.d.).

2.4.4 – Spruce Pine

Spruce Pine is a small town in the Blue Ridge Mountains of western North Carolina, covering approximately 4.7 square miles, the town has a population of 2,194. Though small, the town is known for its rich mineral deposits and its recent increase in momentum as transportation infrastructure improves (*About Our Town*, 2025). The widening of US 19E has provided four-lane access throughout the mountains, connecting Spruce Pine to Interstate 26 and its

surrounding counties (NCDOT, 2020). These developments have allowed Spruce Pine residents easier access to work, schooling, and recreation. Additionally, the Avery County Airport is located just four miles northeast of Spruce Pine, allowing for easy travel (Avery County Administration, n.d.).

Despite the small, rural population, and an average income of \$47,583, 18.2% of residents live below the poverty line, with 28.3% of those below the age of 18. 16.6% of individuals within the town also do not have health insurance (U.S. Census Bureau, n.d.). However, Spruce Pine continues to grow as the recent infrastructure change has created more access and appeal to visitors, increasing revenue from tourism that is put toward developing the town.

2.4.5 – Sylva

Sylva is a small town, covering approximately 3.2 square miles with a total population of 2,578 residents. It serves as the county seat of Jackson County and is a hub for tourism and festivals (Town of Sylva, 2025). Despite the high number of residents holding a bachelor's degree or higher (58.9%), 26.6% live below the poverty line with 28% of those individuals being below the age of 18. Additionally, 25.5% of residents have no health insurance (U.S. Census Bureau, n.d.). Sylva and all of Jackson County are served by Harris Regional Hospital, a small hospital licensed for a total of 86 beds. A smaller hospital, Swain Community Hospital, also takes in Sylva residents with a total of 48 beds (Harris Regional Hospital, n.d.).

Compared to the other communities, Sylva strongly relies on its role as a tourism center, as it is surrounded by the Great Smoky Mountains National Park, the Blue Ridge Parkway, and the Nantahala National Forest. It boasts outdoor activities such as hiking, fishing, and camping.

Throughout the year, Sylva also has many festivals including “Greening Up the Mountains Festival”, and “WNC Pottery Festival”. Additional activities for visitors include exploring the Jackson County Historical Society Museum and the Appalachian Cultural Museum (*Sylva - Discover Jackson NC*, 2025).

Chapter 3: Materials and Methods

3.1 – Materials

Dental development was scored on a contemporary sample of subadult individuals (6 to 16 years of age) across the state of North Carolina. Five Community Service Learning Centers (CSLCs) were chosen out of the nine total that span the state to capture as much demographic, geographic, and resulting ontogenetic variation across the diverse communities of North Carolina (Table 1). These CSLC are as follows: Ahoskie, Ross Hall (East Carolina University School of Dental Medicine), Robeson (Lumberton), Spruce Pine, and Sylva. Each CSLC is highlighted in Figure 1 below.

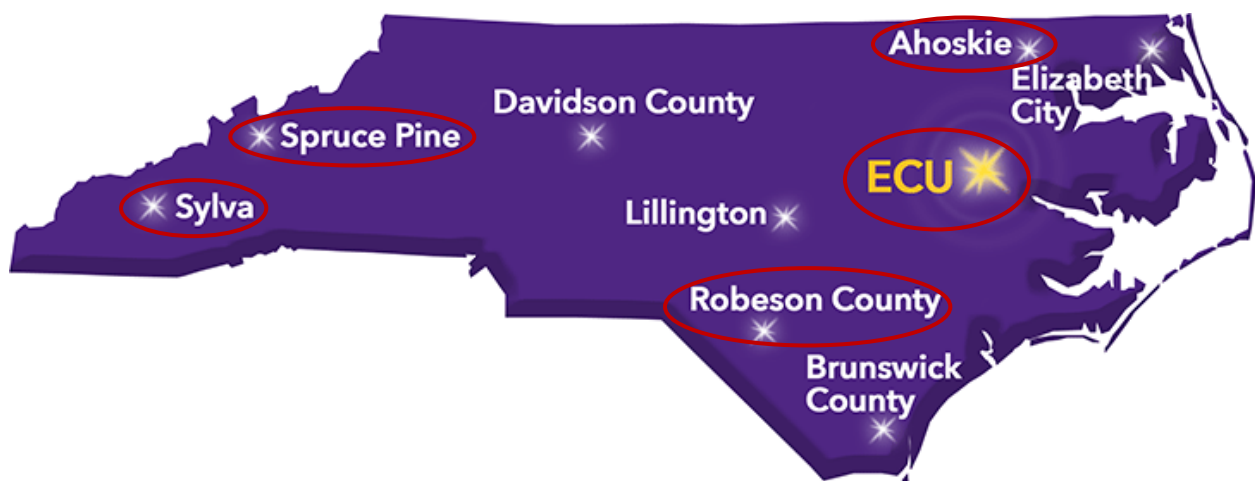


Figure 1. Image of all 9 CSLC. Those circled in red are the ones selected for this study. (East Carolina University School of Dental Medicine, 2025).

Table 1. Demographic information from each location. (U.S. Census Bureau, n.d.)

Location	Population	Median Income	% in Poverty	Race and Ethnicity (%)	% without Health Coverage
Ahoskie	4,891	\$40,762	24.4	White: 24% Hispanic or Latino: 2.2% Black or African American: 68% American Indian and Alaskan Native: 1% Asian: 1% Native Hawaiian and Other Pacific Islander: 0.2% Some Other Race: 1%	9.1
Lumberton	19,025	\$45,078	31.0	White: 35.5% Hispanic or Latino: 10% Black or African American: 35.7% American Indian and Alaskan Native: 15.2% Asian: 2% Native Hawaiian and Other Pacific Islander: 0.1% Some Other Race: 6.3%	13.6
Spruce Pine	2,194	\$47,583	18.2	White: 84.5% Hispanic or Latino: 11% Black or African American: 0.7% American Indian and Alaskan Native: 0.4% Asian: 0.5% Native Hawaiian and Other Pacific Islander: 0% Some Other Race: 6.7%	16.6
Sylva	2,578	\$41,926	26.6	White: 75% Hispanic or Latino: 13% Black or African American: 3% American Indian and Alaskan Native: 3.7% Asian: 3% Native Hawaiian and Other Pacific Islander: 0% Some Other Race: 8.2%	25.5
Greenville	87,521	\$51,628	24.8	White: 46.8% Hispanic or Latino: 6% Black or African American: 41% American Indian and Alaskan Native: 0.4% Asian: 3% Native Hawaiian and Other Pacific Islander: 0.06% Some Other Race: 3.1%	7.1

All data was collected from panoramic radiographs retrieved and accessed through axiUm and Aycan Medical System's OsiriX Pro workstation software provided by East Carolina University School of Dental Medicine (SoDM). AxiUm is an Electronic Health Record (EHR) program that provides easy access to patient information (<https://henryscheinone.ca/products/axium>), while OsiriX Pro is a software designed to analyze radiographic images (<https://www.aycan.com/product/aycan-workstation/>). Data collected resulted in a total sample size of n = 976 individuals, with a breakdown of the full sample size presented in Table 2. Collection of developmental data from SoDM underwent review and approval as an expedited study from the Institutional Review Board (IRB) prior to data collection (UMCIRB 24-000748).

Table 2. Number of individuals in the sample, separated by location, age, and biological sex.

	CSLC Ahoskie		CSLC Robeson		CSLC Spruce Pine		CSLC Sylva		Ross Hall	
Total #	220		167		220		149		220	
Age	M	F	M	F	M	F	M	F	M	F
6	10	10	4	3	10	10	2	4	10	10
7	10	10	5	6	10	10	7	6	10	10
8	10	10	5	4	10	10	8	6	10	10
9	10	10	8	3	10	10	7	9	10	10
10	10	10	6	7	10	10	7	2	10	10
11	10	10	11	9	10	10	11	6	10	10
12	10	10	9	8	10	10	12	3	10	10
13	10	10	10	9	10	10	12	3	10	10
14	10	10	10	10	10	10	4	2	10	10
15	10	10	10	10	10	10	8	10	10	10
16	10	10	10	10	10	10	10	10	10	10
Total	110	110	88	79	110	110	88	61	110	110

The age range of the sample was from 6 to 16 years of age, with equal distributions of biological males and females where possible. On average, dental panoramic radiographs are not routinely taken in younger children, with exceptions permitting earlier imaging due to deviations in dental development or dental health concerns (Thevissen et al., 2024). Due to this, the most accessible youngest age was 6 years, with the maximum age being 16, as most teeth have completed development at this stage (excluding the third molar). Twenty individuals were selected from each age group, with an equal number of each biological sex (10 males, 10 females). Cases in which sample size was less than twenty per age group, all individuals were included despite biological sex. SoDM provided demographic information for each individual including location, age, biological sex, race, and ethnicity. Age was calculated mathematically using the birth date of each individual and the date in which the radiograph was taken. Prior to data collection, each radiograph underwent visual examination to confirm image quality and the presence of most teeth. Data that included inconsistencies, such as variations in birth date, were removed to maintain uniformity in the sample. Information gathered in this study was subject to comparison with the Subadult Virtual Anthropology Database (SVAD) for the purpose of assessing rates of dental development. SVAD is a cross-sectional repository of subadult growth and development indicators (including dental development) from global samples (Stull & Corron, 2021). The total number of individuals in SVAD is represented in Table 3.

Table 3. Number of individuals in the SVAD sample separated by age and biological sex.

	SVAD	
Total #	1315	
Age	M	F
0	139	123
1	64	37
2	40	24
3	23	18
4	20	20
5	13	19
6	9	6
7	10	11
8	9	6
9	17	8
10	6	4
11	10	14
12	19	9
13	17	13
14	21	19
15	45	18
16	66	25
17	53	28
18	70	39
19	66	47
20	65	45
Total	782	533

3.2 – Methods for Data Collection

Mineralization of the permanent dentition was scored by stages of development, with every individual tooth scored on an ordinal scale of 1–13. All scoring was done by the author with an intra-observer linear weighted kappa = 0.97 and a range of 0.95–1.00 indicating appropriate levels of intra-observer error. The 13-stage scoring system is a combination of work developed by Stull & Corron (2021), with the original descriptions provided by AlQahtani et al. (2010), and Moorrees et al. (1963). (Figures 1 & 2). Previously published data from SVAD (Stull

& Corron, 2022), using the same standardized methods, were used as a comparison for dental development outside of North Carolina. This study uses the United States sample (New Mexico) from SVAD. Collected data was deidentified prior to use, using a set of two letters and a number to represent geographic location and the order in which it was deidentified (i.e., SY001).

Stage	Monoradicular teeth	Pluriradicular teeth	Description
1			Initial cusp formation
2			Coalescence of cusps
3			Cusp outline complete
4			Crown half completed with dentine formation
5			Crown three quarters completed
6			Crown completed with defined pulp roof
7			Initial root formation with diverge edges
8			Root length less than crown length
9			Root length equals crown length
10			Three quarters of root length developed with diverge ends
11			Root length completed with parallel ends
12			Apex closed (root ends converge) with wide periodontal ligament
13			Apex closed with normal periodontal ligament width

Figure 2. Image that shows the ordinal scoring method from AlQahtani et al. (2010), shown through radiographic images from Stull & Corron (2021).

	1 ci: initial cusp formation		7 Ri: initial root formation with diverge edges		Ci: initial cusp formation		
	2 Cco: Coalescence of cusps		8 R 1/4: root length less than crown length		Cco: Coalescence of cusps		8 R 1/4: root length less than crown length with visible bifurcation area
	3 Coc: Cusp outline complete		9 R 1/2: root length equals crown length		Coc: Cusp outline complete		9 R 1/2: root length equals crown length
	4 Cr 1/2: crown half completed with dentine formation		10 R 3/4: three quarters of root length developed with diverge ends		Cr 1/2: 4 crown half completed with dentine formation		10 R 3/4: three quarters of root length developed with diverge ends
	5 Cr 3/4: crown three quarters completed		11 Rc: root length completed with parallel ends		Cr 3/4: 5 crown three quarters completed		11 Rc: root length completed with parallel ends
	6 Crc: crown completed with defined pulp roof		12 A 1/2: apex closed (root ends converge) with wide PDL		Crc: crown completed with defined pulp roof		12 A 1/2: apex closed (root ends converge) with wide PDL
			13 Ac: apex closed with normal PDL width		Ri: initial root formation with diverge edges		13 Ac: apex closed with normal PDL width

Figure 3. Image of the modified stages for identifying tooth development present in AlQahtani et al. (2010). Red numbers added to image so that ordinal stages from Stull & Corron (2021) can be followed.

3.3 – Methods for Statistical Analysis

Sixteen teeth were selected for analysis (8 upper teeth and 8 lower teeth) and were determined by which tooth (right or left) had the most recorded values. For example, the lower left first incisor had 1264 teeth total, and the lower right first incisor had 1265 teeth total, thus, the lower right first incisor was chosen. Of these sixteen teeth analyzed, four teeth were selected for in depth results below. These teeth are the lower right first incisor, the lower right canine, the

lower left first premolar, and the lower right second molar. This is to incorporate differences that may exist per tooth type, and to differentiate between teeth that begin and end development at different times throughout an individual's life. All results are available at:

<https://doi.org/10.5281/zenodo.15073983>

3.3.1 – Cumulative Probit Model

To properly assess variation in dental development, cumulative probit models have been commonly utilized to model ordinal categorical data, notably as a method for age estimation techniques (Konigsberg, 2015). The cumulative probit model asks the question: How is the ordinal tooth score (Y) influenced by location, age, biological sex, race, and ethnicity (X) through the coefficient(s) (β)? When lower and upper 95 percent confidence interval values do not cross zero (i.e., 0.3 lower CI – 0.9 upper CI), it suggests that X directly influences the outcome of Y (Hemming & Taljaard, 2021). The cumulative probit model links the ordinal response variable, Y , to an unobserved and continuous latent variable, Z , through established threshold parameters that correspond to the age of transition between developmental stages. This transformation allows the estimation of the probability that Z falls within an age range based on the variable, X . The following equations outline this process:

$$Y = \phi(\beta_{Location}X_{Location} + \beta_{Age}X_{Age} + \beta_{Sex}X_{Sex} + \beta_{Race}X_{Race} + \beta_{Ethnicity}X_{Ethnicity})$$

$$\Rightarrow Z = (\beta_{Location}X_{Location} + \beta_{Age}X_{Age} + \beta_{Sex}X_{Sex} + \beta_{Race}X_{Race} + \beta_{Ethnicity}X_{Ethnicity})$$

Above, ϕ represents the translation to the standard normal scale and each β is a parameter associated with the association (or lack thereof) of a given predictor with the latent continuous outcome Z . Given that this study addresses the variation in dental developmental timing between

individuals at each CSLC, the relationship between tooth score and each covariate (including age) can be written probabilistically:

$$P(\text{Tooth Score} | \text{Age} + X)$$

Where X is any covariate of interest (i.e., CSLC Location). This conditional probability is the driving factor behind the results highlighted below.

The cumulative probit model was fit using the brms package (Bürkner, 2017), a front-end model development platform that utilizes the Stan probabilistic programming language (Stan Development Team, 2024). Stan is a software designed for complex Bayesian data analysis (<https://mc-stan.org/>). Brms allows a user to benefit from the computational efforts of Stan, while remaining easily interpretable to audiences familiar with regression modeling practices. The Bayesian approach in this work allows for a robust accounting of uncertainty and a straightforward approach to missing data, as is common in biological and ontogenetic research (Konigsberg, 2015; Konigsberg & Frankenberg, 2013; Stull et al., 2023). Parameter estimation results from the implementation of Stan's Hamiltonian Monte Carlo (HMC) approach with its extension, the NO-U-Turn Sample (NUTS). Previous research in human growth and development suggests that growth variables are often heteroskedastic – model error increases as a function of age. To test this assumption on human dental development, a homoskedastic and heteroskedastic model were fit to the data (Stull et al., 2023). Model selection was performed using leave-one-out cross validation (loo) (Vehtari et al., 2024), and all priors remained default according to the brms package. The default priors are relatively non-informative ($\beta \sim \text{"Student } T(3, 0, 1)"$) and are meant to regularize the parameter space without biasing the inference downstream. Model selection indicated that both models were nearly identical, and thus the simpler model (homoscedastic) was used for analysis.

All models were run with 4 chains, 1000 warmup iterations, and 1000 sampling iterations, resulting in 4000 posterior samples per parameter. The tuning parameter, `max_treedepth`, was set to a range of 12–13, and the acceptance probability, `adapt_delta`, at a range of 0.95–0.99 to account for complexity in the data. Contemporary Bayesian model diagnostics assess the robustness and precision of each sample through effective sample size (ESS) and evaluate the mixing of independent chains using R-hat. All ESS scores were above 400, and R-hat scores were all below 1.01, indicating that the model is statistically valid and reliable, and that all chains converged into a stable model. Additional data wrangling and cleaning was done through `tidyverse` (Wickham et al., 2019) and `tidybayes` (Kay, 2024). All plots were completed through `ggplot2` (Wickham, 2016), and analyses were completed in the R programming environment (R Core Team, 2024). Values present in the cumulative probit model used reference categories based on alphabetical order.

3.3.2 – Probability Graphs

Probability graphs serve as a means to visualize and interpret probabilistic results such as models fit through `brms`. These graphs provide a physical representation of the predictions and parameters from Bayesian models and allow for a clearer understanding of how each individual location may play a role in influencing the rate of dental development. Variation present in the data can be highlighted through this probability graph, and visualizing the data in this way is an effective means of communicating and understanding the results provided through the cumulative probit model.

3.3.3 – Scatter Plots

Scatter plots were also used as a method of visualizing the data. Rather than using the probability, it transforms the thresholds in the cumulative probit model into an estimated age at transition from one stage to another. The thresholds represent the “baseline” age without influence by other variables. Age and location influence these thresholds to create the mean “true age” at transition from one ordinal category to the next. This provides a visualization to show how age is related to the development of the teeth. The x-axis represents the posterior age, with a line that shows the mean age at transition for each location. From the cumulative probit model, the formula outlined below represents how the model was used to create these plots:

$$\text{Age at Transition} = \text{Threshold Value} + \text{Age} - \text{Location}$$

Chapter 4: Results

Results suggest that there is variation in dental development across North Carolina with each specific tooth type displaying differential patterns of development within each CSLC. That said, regardless of tooth type, the general pattern in all analyses is that CSLC Sylva experiences developmental delay when compared to all other locations in North Carolina. While Ahoskie does at times experience a degree of delay, Sylva's delay suggests that individuals that visit the dental clinic in Sylva and live in that geographic location have their dental development impacted negatively. In other words, an individual's dentition takes longer to *fully develop* in Sylva as compared to other locations. However, broadly speaking, dental development across North Carolina (all CSLCs) is similar to that found in the comparative New Mexico sample associated with SVAD, suggesting a degree of stability between dental developmental processes regardless of broad location.

4.1 – North Carolina

Dental development in North Carolina was compared with multiple covariates to determine possible relationships that may impact an individual's development. The covariates are as follows: biological sex, race, ethnicity, and location. Age was used to compare each of these covariates, as age has a direct influence on the rate of dental development (i.e., growth and development are a function of age). Sex, race, and ethnicity had very little influence on the rate of dental development (Table 4) and will not be shown on further tables, while geographic differences identified by CSLC location demonstrates a robust association with tooth development. Results below show an in-depth analysis of four of the sixteen total teeth examined: the lower right first incisor, the lower right canine, the lower left first premolar, and the lower right second molar. Additional results and analysis for the twelve remaining teeth can

be found here: <https://doi.org/10.5281/zenodo.15073983>

Table 4. Cumulative probit model results for the lower canine. Results show little influence for biological sex, race, and ethnicity.

Parameter	Estimate	l-95% CI	u-95% CI
Threshold 4 - 5	3.19	1.95	4.26
Threshold 5 - 6	3.47	2.36	4.49
Threshold 6 - 7	3.72	2.72	4.66
Threshold 7 - 8	5.67	4.96	6.39
Threshold 8 - 9	7.26	6.51	8.03
Threshold 9 - 10	8.62	7.84	9.41
Threshold 10 - 11	10.51	9.64	11.41
Threshold 11 - 12	11.81	10.87	12.75
Threshold 12 - 13	14.32	13.25	15.4
Age	0.93	0.87	1.00
Sex – Male	-0.52	-0.67	-0.37
Asian	-0.62	-1.61	0.37
Black or African American	0.35	-0.05	0.78
Mixed	-0.13	-0.94	0.73
Native Hawaiian/Pacific Islander	-0.51	-1.82	0.75
Other	-0.23	-0.74	0.29
White	0.10	-0.30	0.51
Not Hispanic or Latino	-0.19	-0.53	0.16

4.1.1 – Lower First Incisor

Initial data exploration in the form of box plots suggests that Sylva develops at a slower rate, with an average delay of approximately one developmental tooth score. Though there is a degree of variation present for the first incisor per location, it is overall consistent with few outliers. By age 11, all locations complete development with a developmental score of 13.

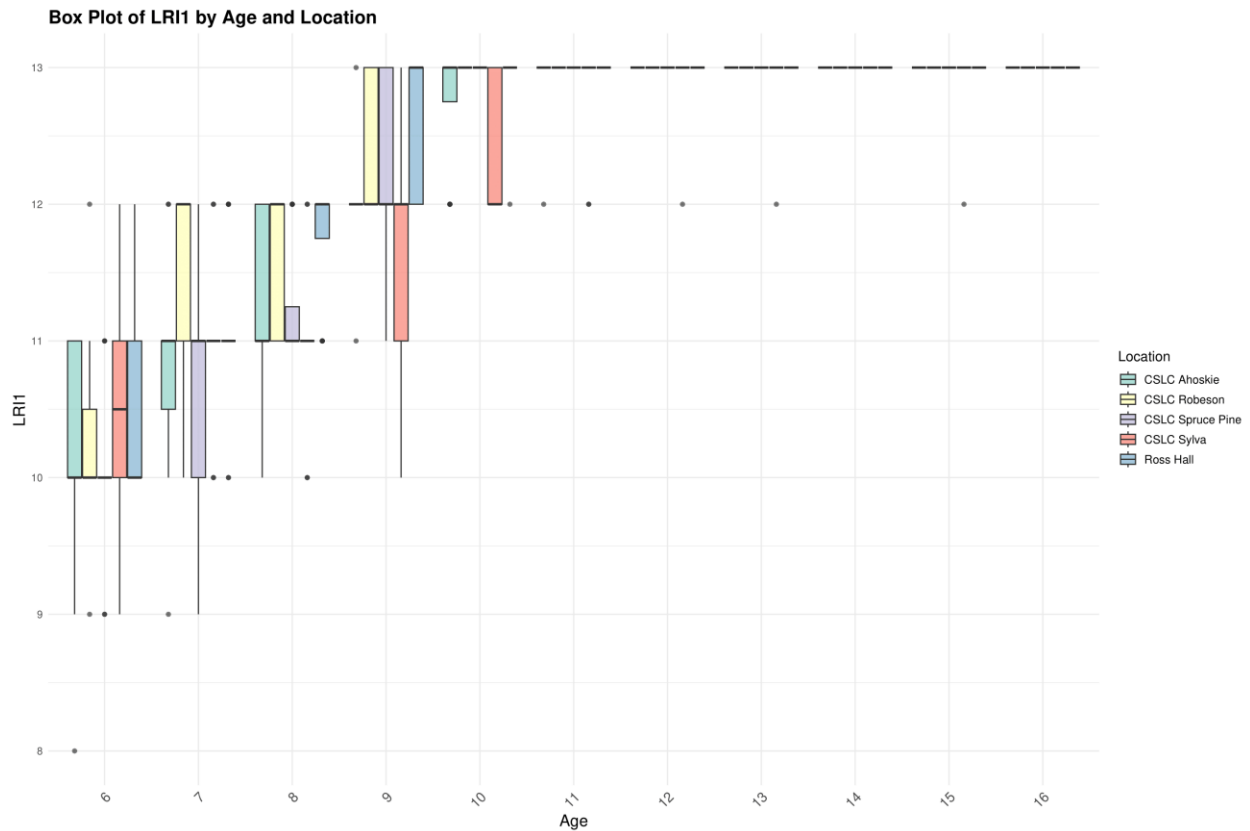


Figure 4. Box plot for ordinal tooth development scores for the lower right first incisor. Grouped by location and sorted by age.

Table 5 shows regression coefficients of the lower first incisor model. Individuals that visit the Robeson and Ross Hall CSLCs demonstrate a positive association with dental development, indicating a more advanced state for age as compared to the baseline Ahoskie sample. On the other hand, the wide confidence interval associated with the Spruce Pine coefficient suggests that Spruce Pine is relatively similar in developmental trajectory as compared to Ahoskie. Sylva, by comparison, has a considerable amount of delay with a negative coefficient value. This suggests that individuals that visit the Sylva CSLC are more delayed in dental development compared to the other locations.

Table 5. Cumulative probit model results for the lower right first incisor separated by location. Ahoskie is the reference group.

Parameter	Estimate	l-95% CI	u-95% CI
Threshold 8 - 9	5.65	4.16	7.05
Threshold 9 - 10	6.84	5.60	8.12
Threshold 10 - 11	8.57	7.29	9.87
Threshold 11 - 12	10.44	9.06	11.87
Threshold 12 - 13	12.29	10.78	13.84
Robeson	1.03	0.52	1.54
Spruce Pine	0.11	-0.28	0.48
Sylva	-0.60	-1.01	-0.17
Ross Hall	0.76	0.43	1.10
Age	1.19	1.08	1.31

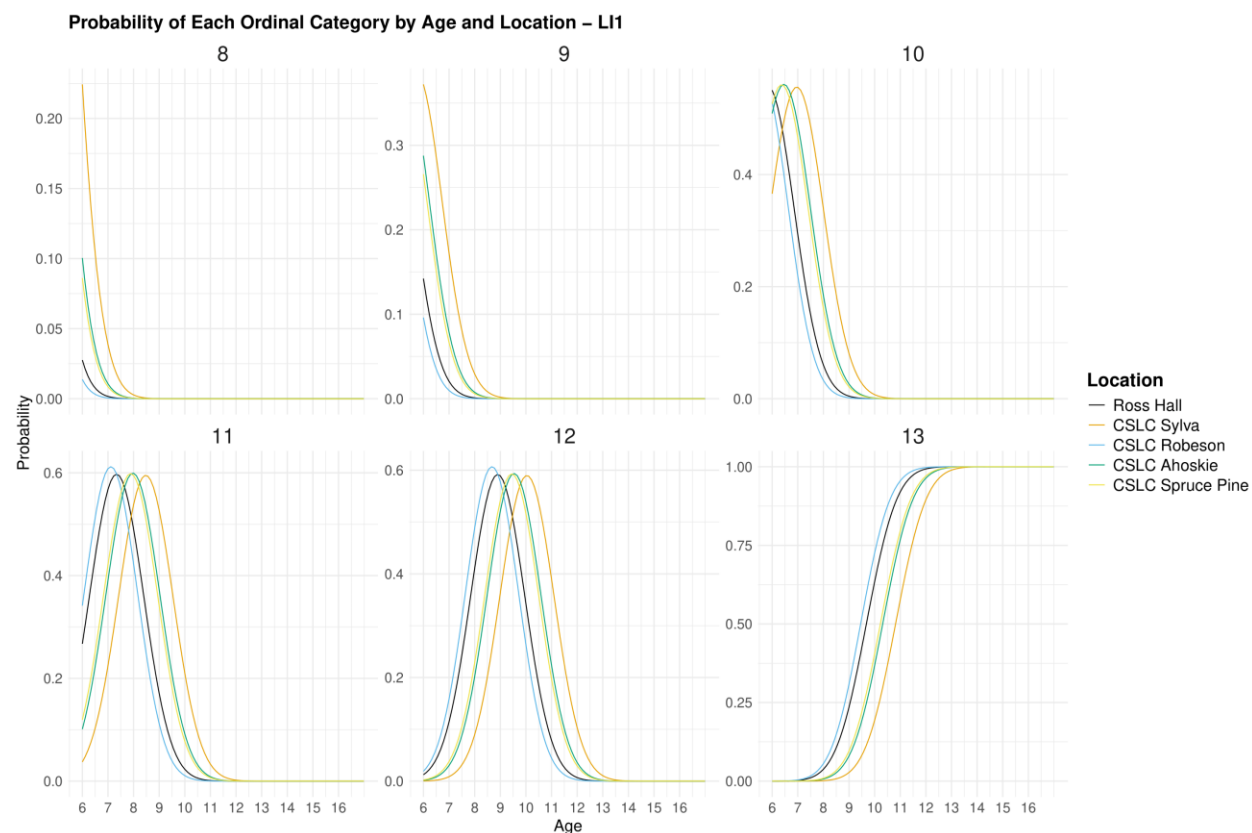


Figure 5. Probability graph for ordinal tooth development scores for the lower right first incisor, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 8–13 from ages 6 to 16.

Figure 5 visualizes the above results, showing how location affects the likelihood of achieving a certain tooth stage by a given age. The probability for each ordinal tooth category varies by location, with strong overlap between Robeson and Ross Hall, and additional overlap between Ahoskie and Spruce Pine. Robeson and Ross Hall are advanced when compared to Ahoskie and Spruce Pine by approximately 6 months. Sylva is delayed by nearly an entire year when compared to Robeson and Ross Hall, and nearly 6 months when compared to Ahoskie and Spruce Pine. Overall, Figure 5 suggests that when compared to the remainder of the locations, Sylva experiences considerable developmental delays.

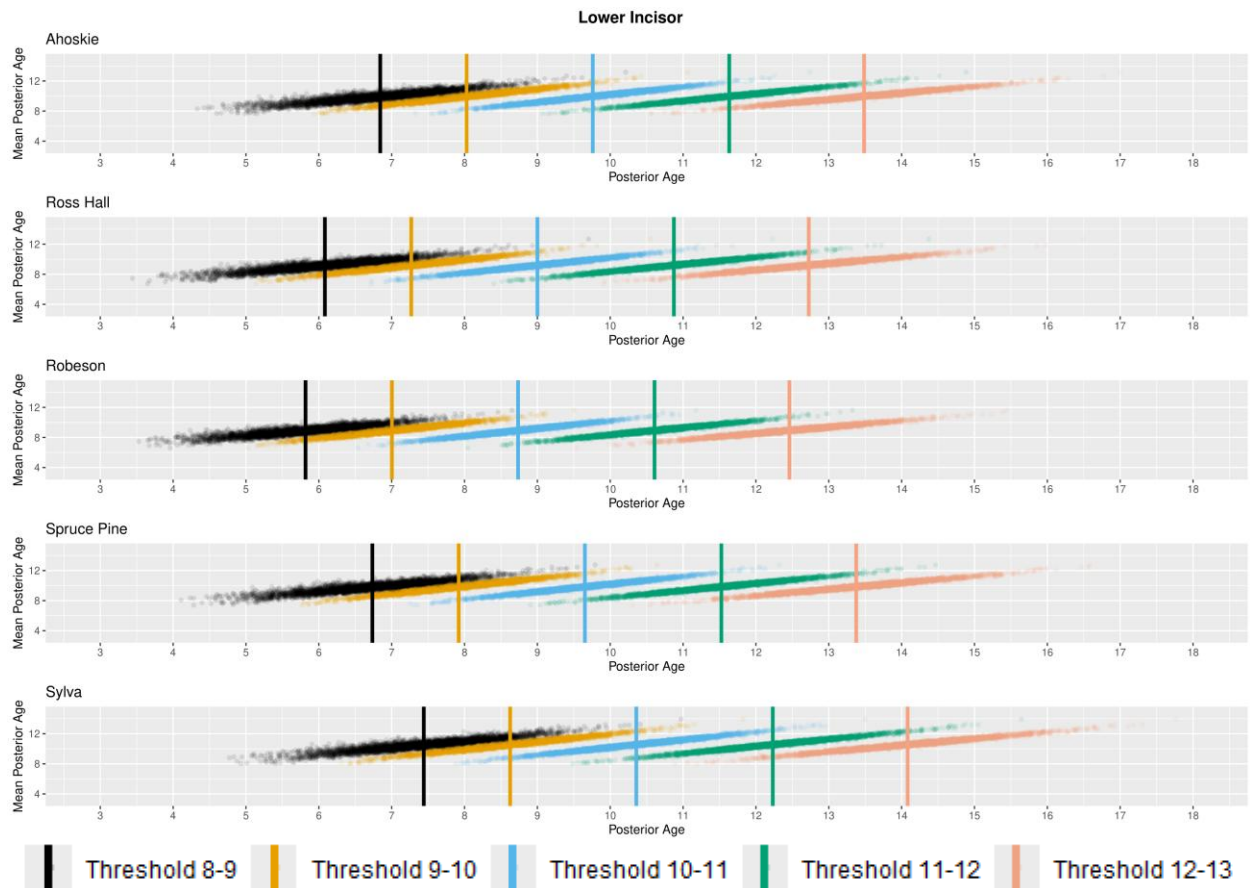


Figure 6. Posterior age from each location for the lower right first incisor, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition.

As shown in Figure 6, the mean age at transition per group indicates the time at which each location transitions from one score to the next. Every location, despite having some degree of overlapping due to model uncertainty, has a mean age that suggests a large developmental range is present. Overall, development in Robeson and Ross Hall begins earlier, with Spruce Pine following close behind. Ahoskie and Sylva seem to experience the most delay in dental development, with Sylva's delay comparatively larger than the remaining locations. This suggests, on average, an individual takes longer to go from one developmental stage to the next in Sylva as compared to other locations.

4.1.2 – Lower Canine

Compared to the first incisor, Figure 7 shows that the canine is more variable overall (i.e., a wider range of probable tooth scores). That said, there is still evidence of developmental delay in Ahoskie and Sylva. Comparatively, Robeson, Ross Hall, and Spruce Pine all display similar levels of developmental variation.

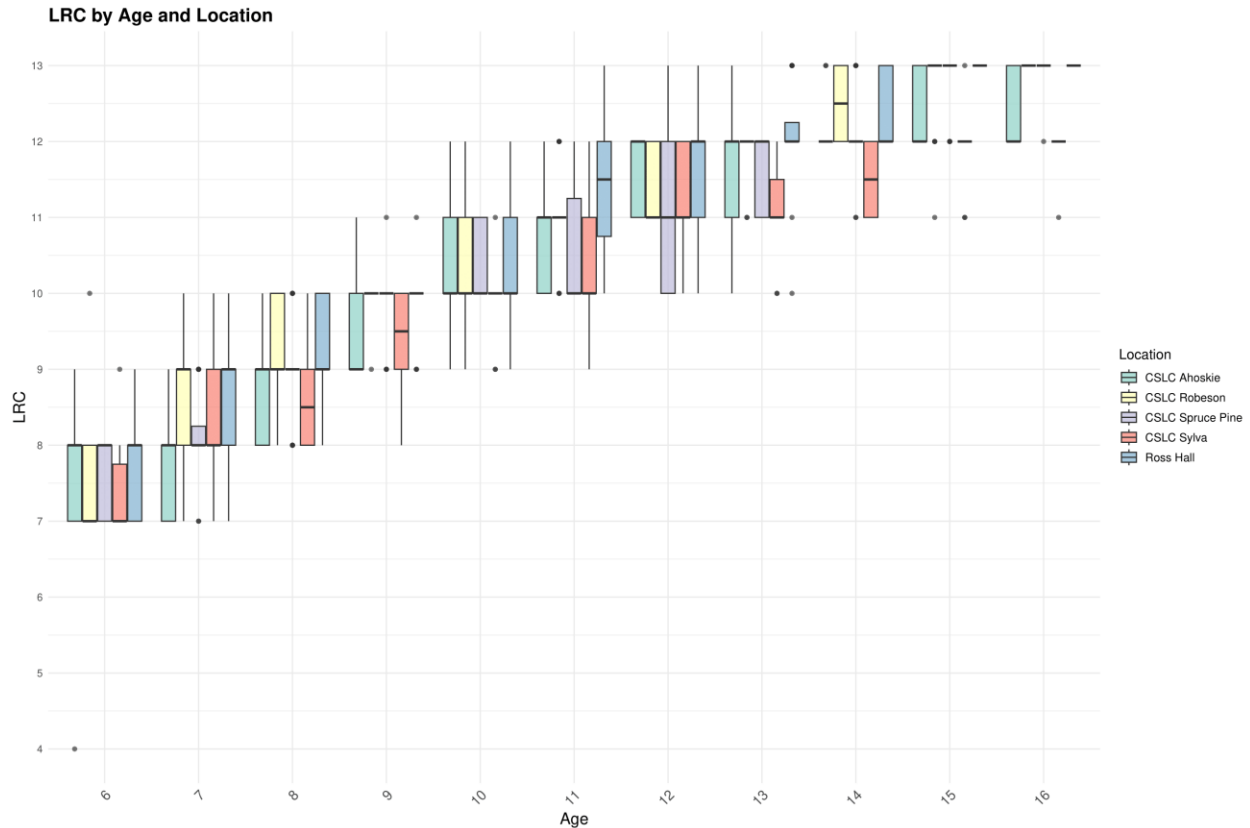


Figure 7. Box plot for ordinal tooth development scores for the lower right canine. Grouped by location and sorted by age.

Table 6 shows the regression results for the lower canine model. Robeson and Ross Hall share nearly identical rates of development, as both locations have a value of 0.84. This suggests that both locations have nearly identical rates of development when compared to the reference sample Ahoskie. Robeson, Ross Hall, and Spruce Pine also have lower and upper confidence intervals that do not cross zero, suggesting that individuals that visit these three CSLC have an overall advanced rate of development, though Spruce Pine's influence is not as strong. In contrast, Sylva demonstrates a negative coefficient, indicating a delay in development.

Table 6. Cumulative probit model results for the lower right canine separated by location. Ahoskie is the reference group.

Parameter	Estimate	l-95% CI	u-95% CI
Threshold 4 - 5	3.19	1.95	4.26
Threshold 5 - 6	3.47	2.36	4.49
Threshold 6 - 7	3.72	2.72	4.66
Threshold 7 - 8	5.67	4.96	6.39
Threshold 8 - 9	7.26	6.51	8.03
Threshold 9 - 10	8.62	7.84	9.41
Threshold 10 - 11	10.51	9.64	11.41
Threshold 11 - 12	11.81	10.87	12.75
Threshold 12 - 13	14.32	13.25	15.4
Robeson	0.84	0.53	1.14
Spruce Pine	0.43	0.18	0.67
Sylva	-0.47	-0.74	-0.19
Ross Hall	0.84	0.61	1.07
Age	0.93	0.87	1.00

The canine expresses a wider range of variation between locations as shown by Figure 8. While Robeson and Ross Hall still experience heavy overlaps, Spruce Pine, Ahoskie, and Sylva are nearly equidistant from one another by around 5 months. When compared to Ahoskie's 10 month delay, Sylva has a nearly 1.3 year difference with Robeson and Ross Hall.

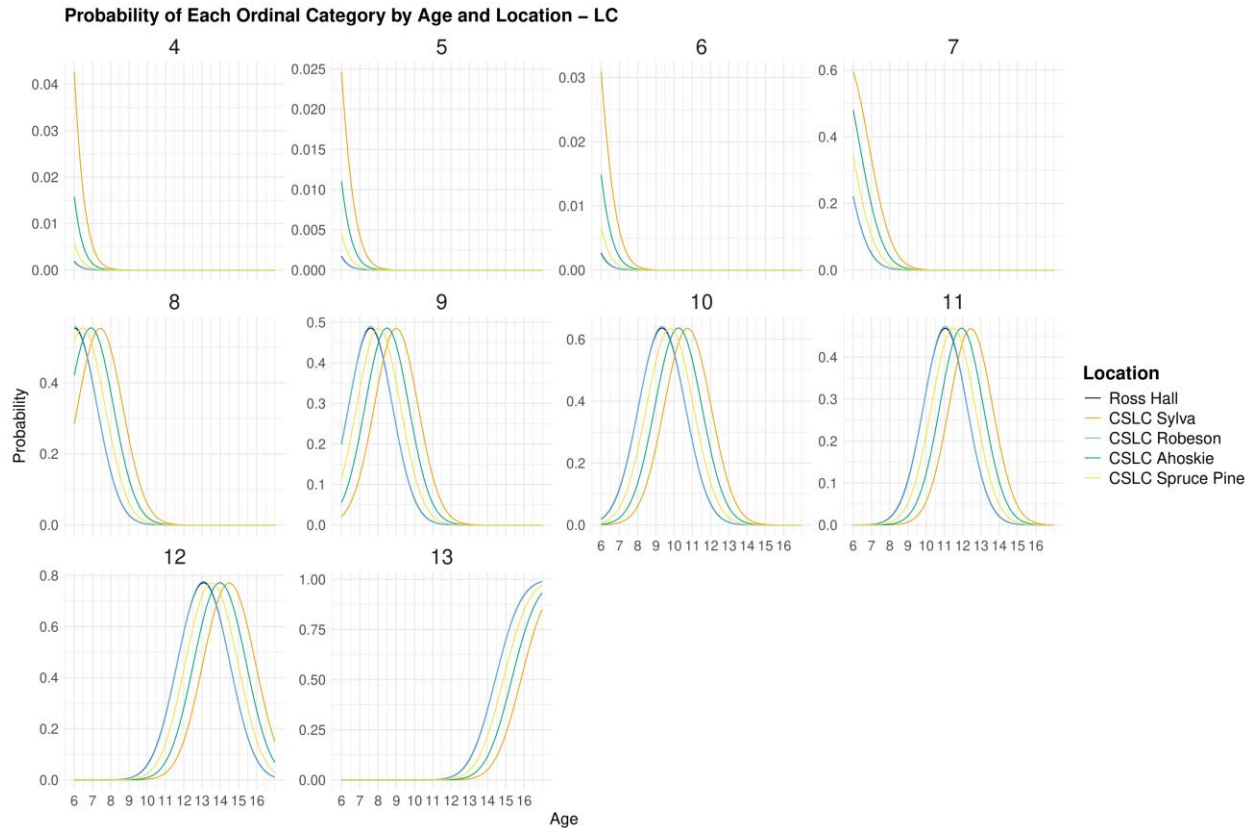


Figure 8. Probability graph for ordinal tooth development scores for the lower right canine, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 4–13 from ages 6 to 16.

Figure 9 shows the posterior age at transition for the canine, ranging from stages 4–13. Ross Hall and Robeson enter developmental states 4–7 prior to the age of 3, while Spruce Pine follows approximately 5 months later and through the age of 4. Ahoskie does not enter stage 4 until the later months of 4 years old, but prior to the age of 5. Sylva, however, enters stage 4 while Ahoskie is transitioning into stage 7. When examining the earlier stages of development (between ages 6–11), Sylva is consistently delayed compared to the remainder of the locations, as the other four have a developmental range of approximately 6 months for each transition.

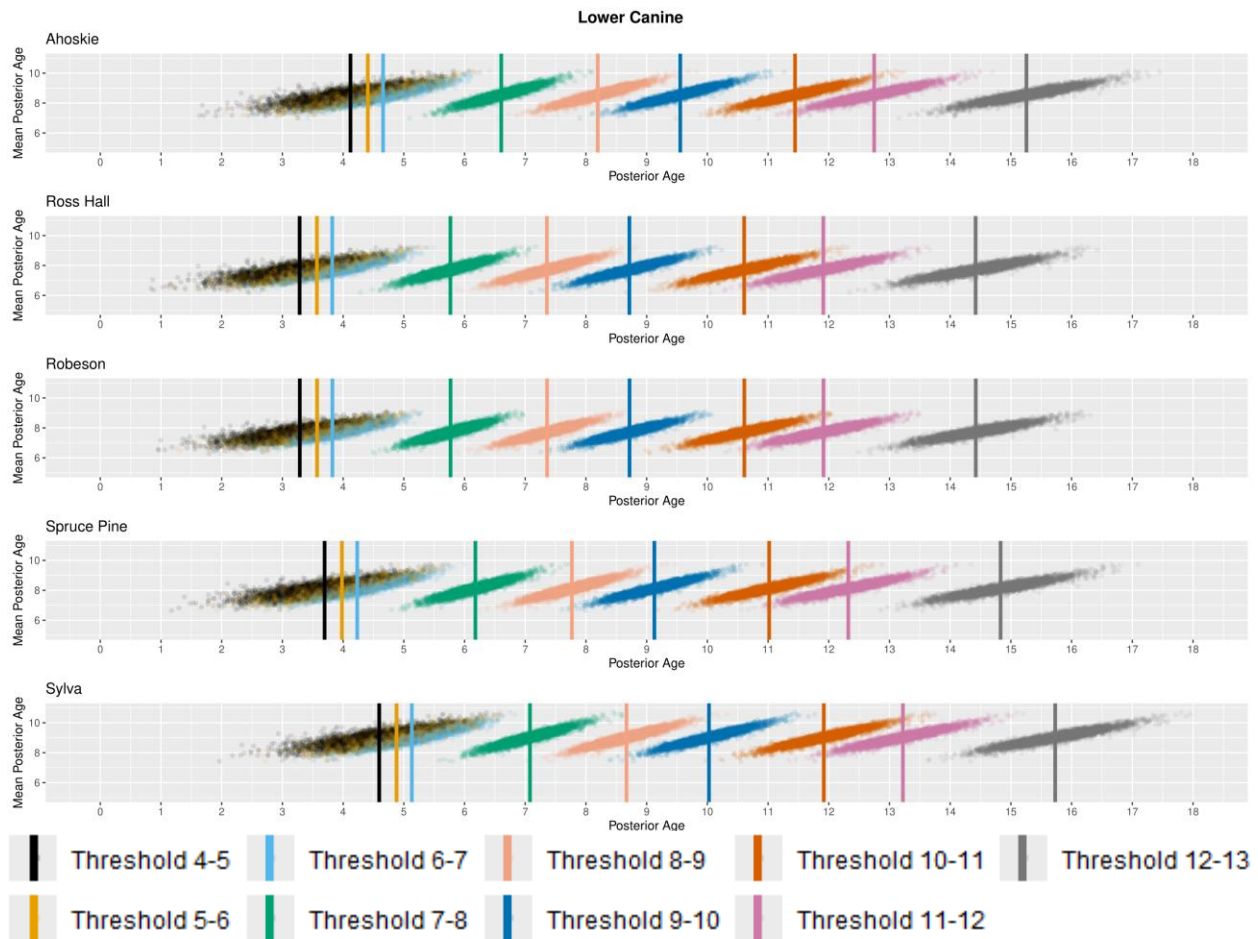


Figure 9. Posterior age from each location for the lower right canine, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition.

4.1.3 – Lower First Premolar

The box plot for the premolar shows a generally wider range of variation as compared to the teeth above. Initial data exploration suggests that Sylva has some degree of delay, though not as extreme. Ahoskie shows a small amount of delay, but it is much less than the delay that Sylva shows.

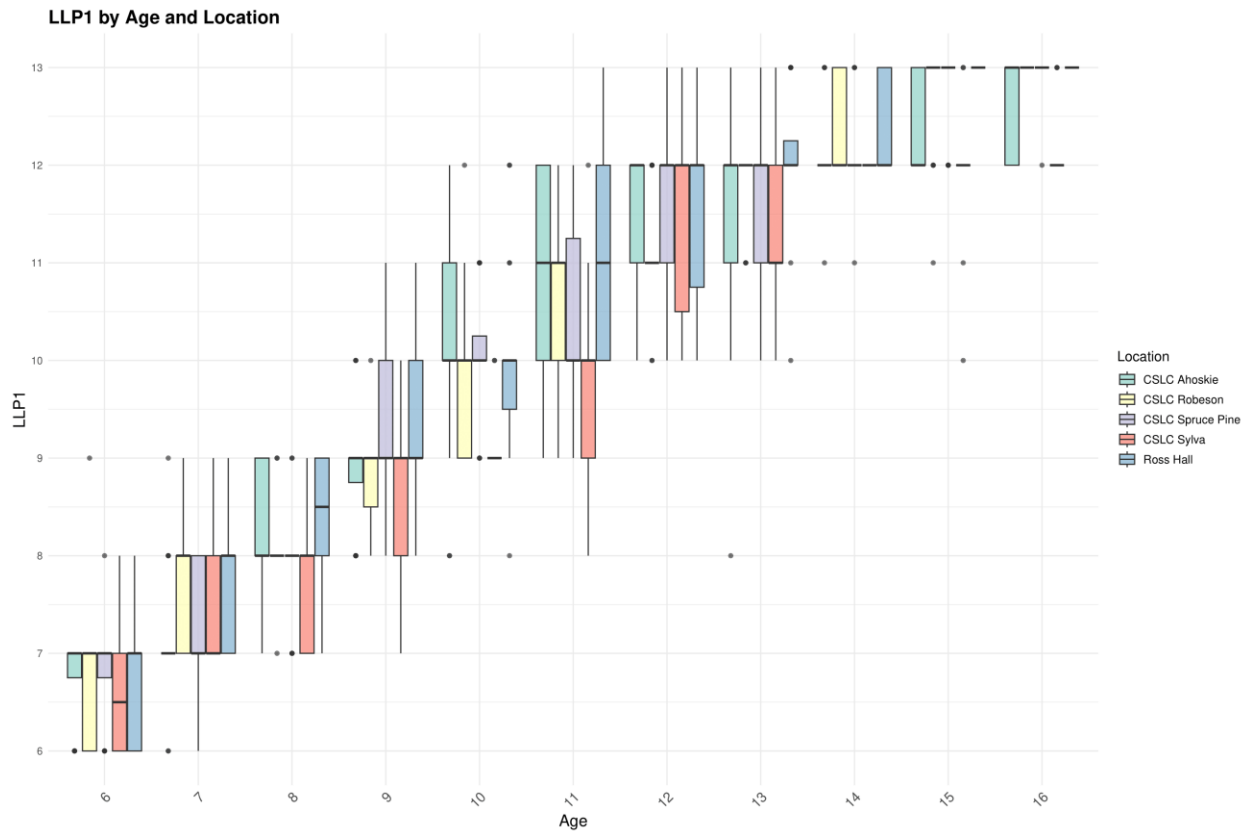


Figure 10. Box plot for ordinal tooth development scores for the lower left first premolar. Grouped by location and sorted by age.

Table 7 summarizes regression results for the premolar model. Robeson, Ross Hall, and Spruce Pine all have a significant impact on the development of the tooth itself, as those that live in the location have a positive influence. All locations, excluding Sylva, have a relatively close developmental window. Similar to the above, the negative coefficient for Sylva indicates a delayed degree of development as compared to the other locations.

Table 7. Cumulative probit model results for the lower left first premolar separated by location. Ahoskie is the reference group, indicated by the thresholds plus the age category.

Parameter	Estimate	l-95% CI	u-95% CI
Threshold 4 - 5	3.19	1.95	4.26
Threshold 5 - 6	3.47	2.36	4.49
Threshold 6 - 7	3.72	2.72	4.66
Threshold 7 - 8	5.67	4.96	6.39
Threshold 8 - 9	7.26	6.51	8.03
Threshold 9 - 10	8.62	7.84	9.41
Threshold 10 - 11	10.51	9.64	11.41
Threshold 11 - 12	11.81	10.87	12.75
Threshold 12 - 13	14.32	13.25	15.4
Robeson	0.84	0.53	1.14
Spruce Pine	0.43	0.18	0.67
Sylva	-0.47	-0.74	-0.19
Ross Hall	0.84	0.61	1.07
Age	0.93	0.87	1.00

The probability graph for the lower first premolar indicates that Robeson, Ross Hall, and Spruce Pine are developmentally close, while Ahoskie and Sylva are delayed by 6 months and nearly one year respectively.

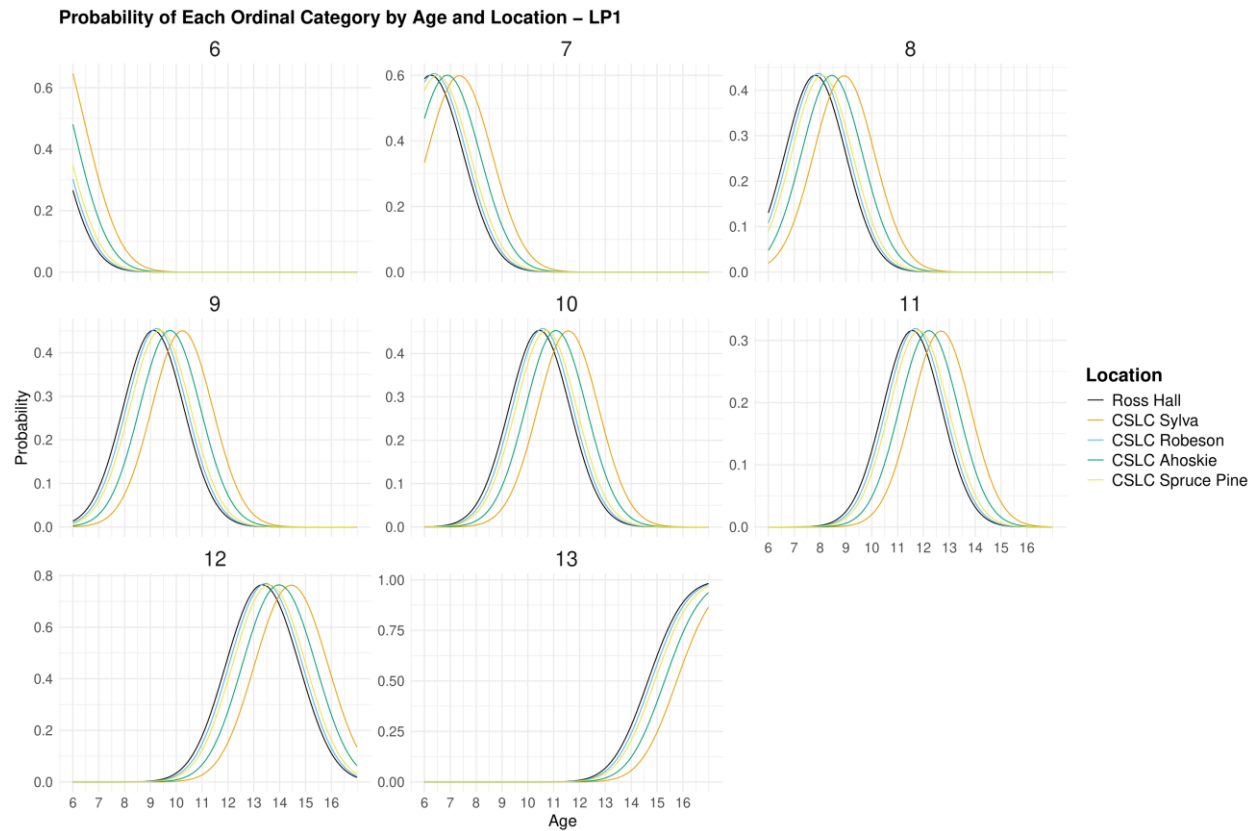


Figure 11. Probability graph for ordinal tooth development scores for the lower left first premolar, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 6–13 from ages 6 to 16.

When examining Figure 12, Sylva is developmentally delayed by approximately one year, while the remainder of the four locations are all within an approximate 6-month time frame. Robeson, Ross Hall, and Spruce Pine are relatively equal in their developmental times, while Ahoskie is slightly delayed, though smaller than Sylva's nearly one year delay.

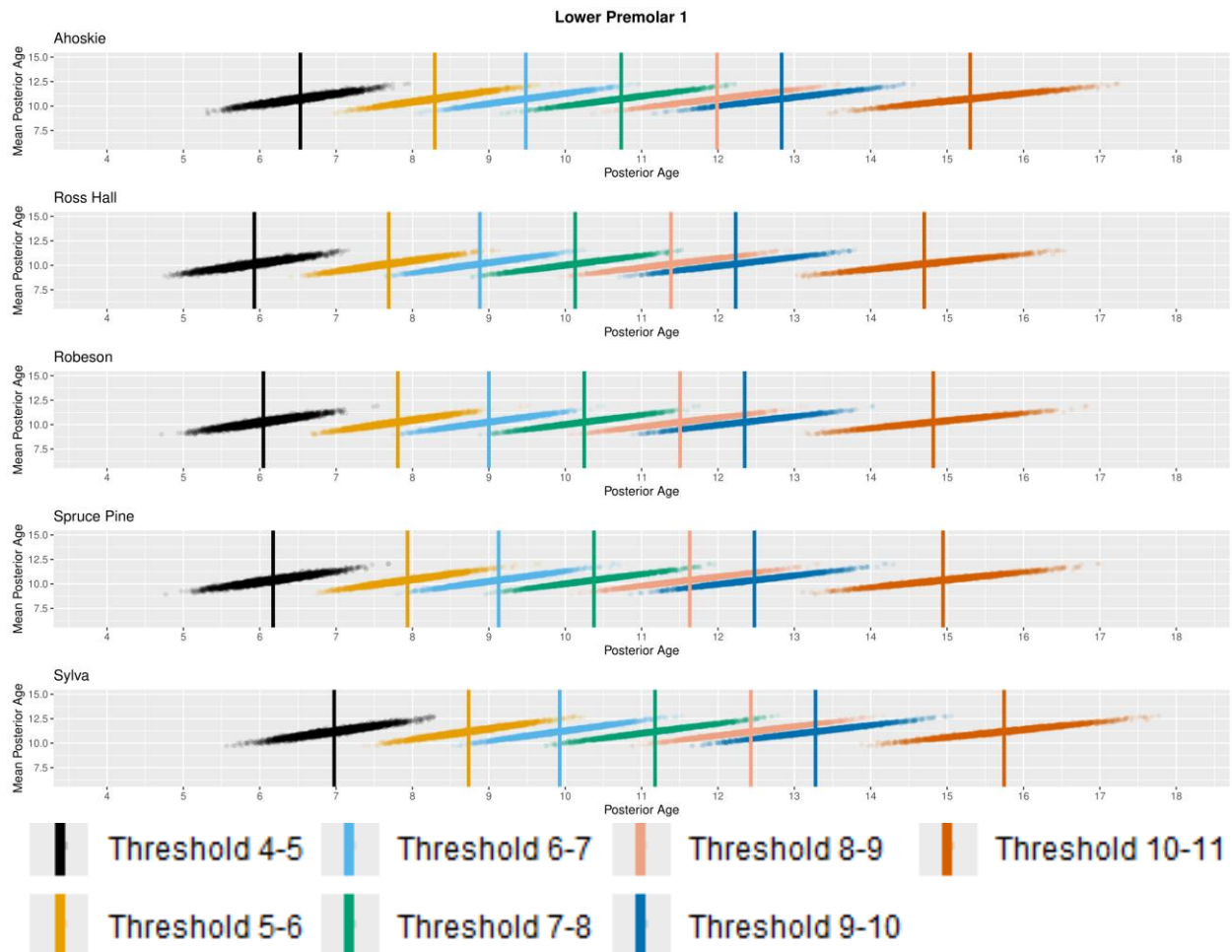


Figure 12. Posterior age from each location for the lower left first premolar, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition.

4.1.4 – Lower Second Molar

Visually, the lower second molar has a higher rate of variability when compared to the other teeth as shown by the box plot. The boxes are longer and many have whiskers that extend outward in either one direction or both, with more outliers than seen in the box plots of other teeth. Overall, Sylva at times does seem to have a slight amount of delay, particularly between the ages of 12 and 13, but all groups show similar growth trajectories.

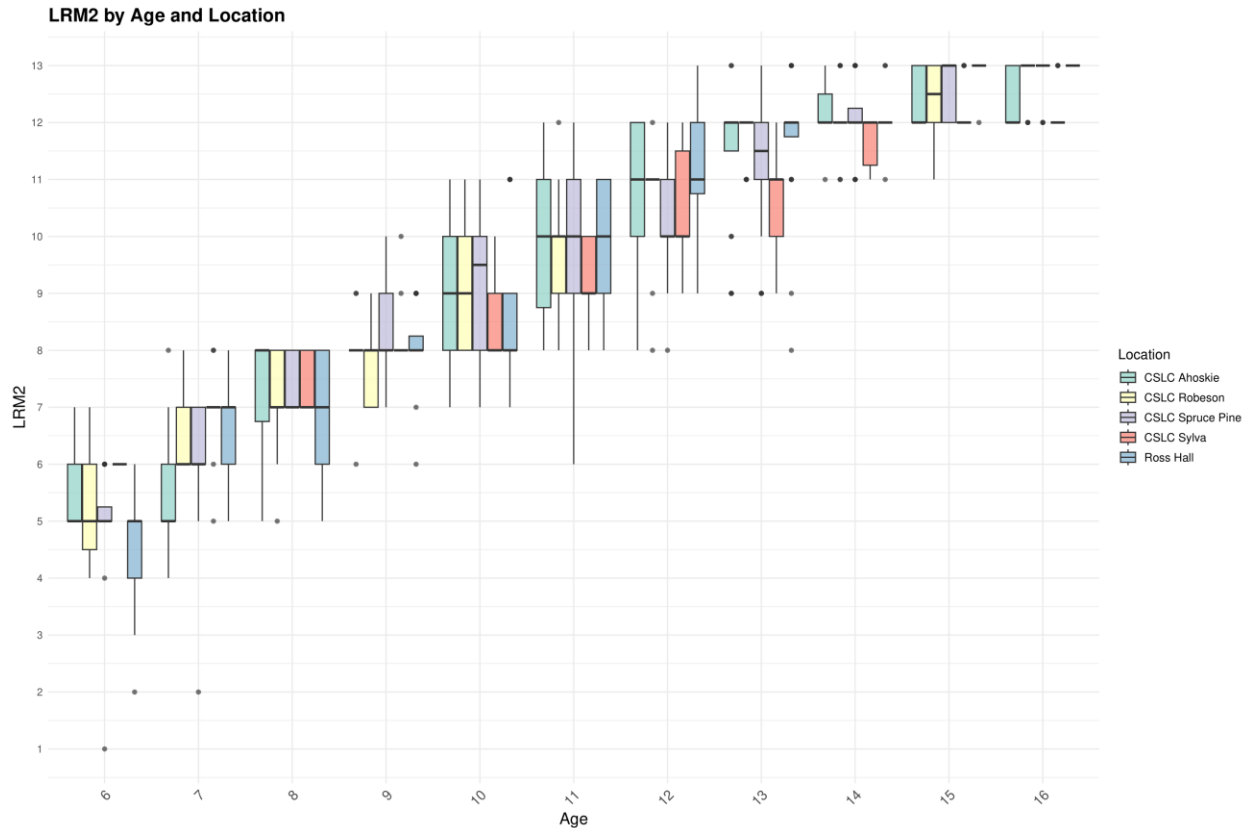


Figure 13. Box plot for ordinal tooth development scores for the lower right second molar. Grouped by location and sorted by age.

The cumulative probit results shown below indicate that there are few differences between locations when examining the development of the lower second molar. There is a very slight positive association between patients at Ross Hall, but the results suggest that these associations are negligible. Overall, all locations show relatively consistent results with similar developmental patterns.

Table 8. Cumulative probit model results for the lower right second molar separated by location. Ahoskie is the reference group, indicated by the thresholds plus the age category.

Parameter	Estimate	l-95% CI	u-95% CI
Threshold 1 - 2	3.74	2.67	4.70
Threshold 2 - 3	4.32	3.48	5.10
Threshold 3 - 4	4.52	3.71	5.29
Threshold 4 - 5	5.13	4.41	5.82
Threshold 5 - 6	6.46	5.77	7.15
Threshold 6 - 7	7.28	6.55	7.99
Threshold 7 - 8	8.2	7.45	8.93
Threshold 8 - 9	10.0	9.17	10.83
Threshold 9 - 10	10.77	9.91	11.63
Threshold 10 - 11	11.5	10.62	12.39
Threshold 11 - 12	12.5	11.58	13.44
Threshold 12 - 13	14.79	13.77	15.86
Robeson	0.28	-0.01	0.56
Spruce Pine	0.24	0.00	0.47
Sylva	-0.14	-0.41	0.12
Ross Hall	0.29	0.08	0.51
Age	0.94	0.89	1.00

The probability corroborates the above results. Overall, the development of the lower second molar is similar across all locations with very little visual differences.

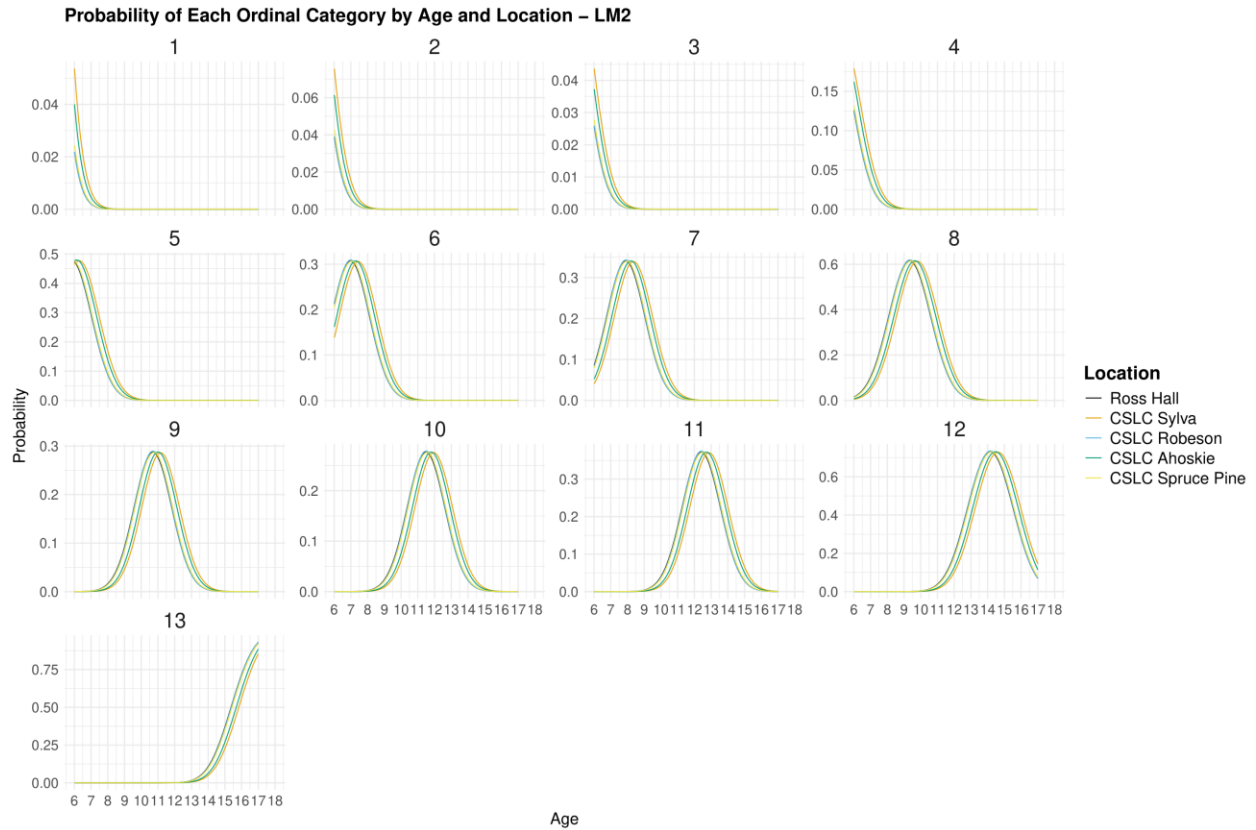


Figure 14. Probability graph for ordinal tooth development scores for the lower right second molar, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 1–13 from ages 6 to 16.

When examining the posterior age at transition, development of the lower second molar seems to express very little variation. While the general pattern remains with Robeson and Ross Hall being slightly accelerated compared to the remainder of the groups, and Sylva experiencing delay, the degree of delay is drastically smaller for this tooth when compared to the remainder of the teeth. Overall, development seems consistent between each geographic location with little notable difference.

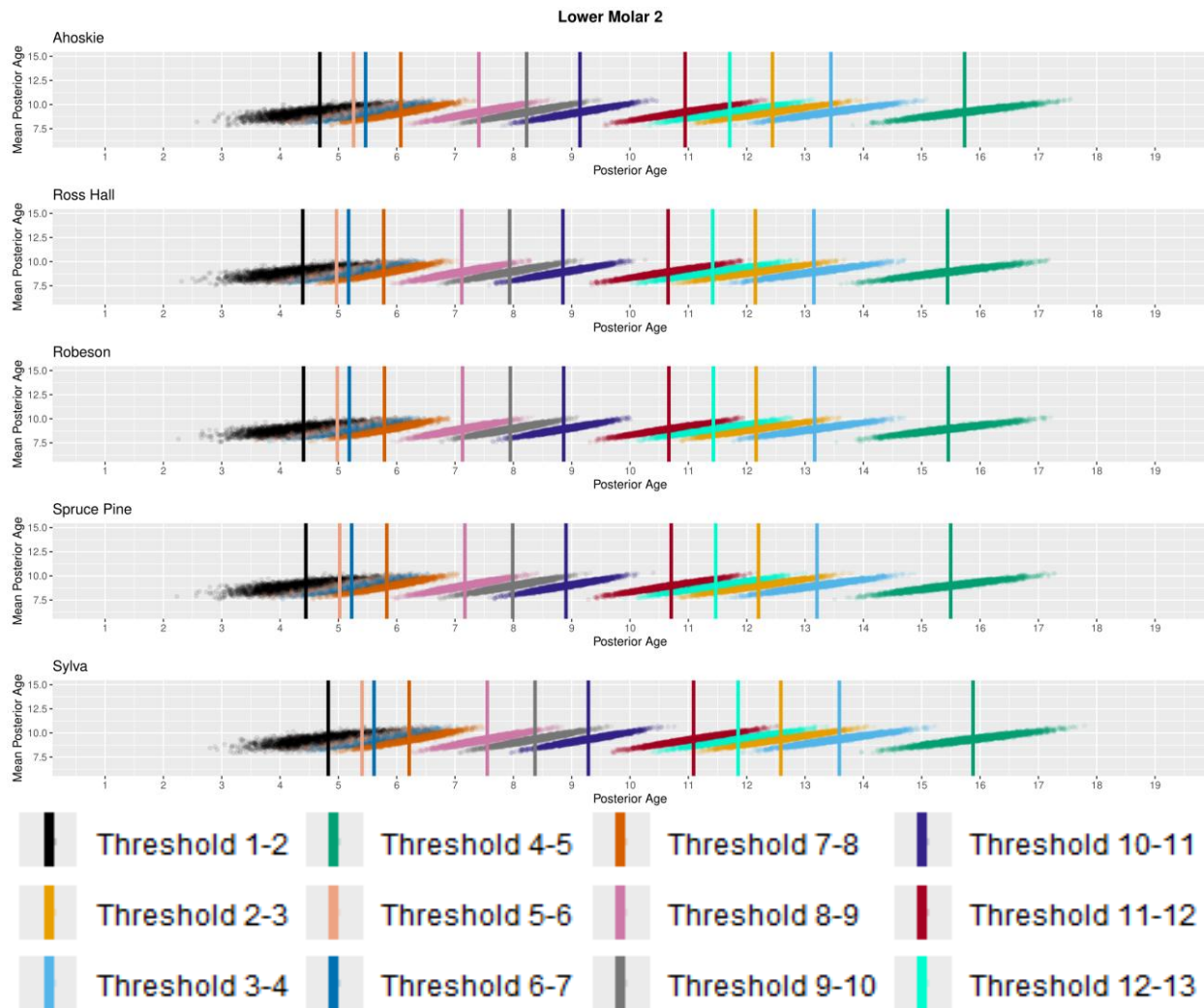


Figure 15. Posterior age from each location for the lower right second molar, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition.

4.2 – SVAD Comparison

All data from SVAD’s United States sample was used as a comparison to North Carolina. The age range of SVAD is between 0–20, incorporating a wide range of developmental stages. SVAD was compared to North Carolina’s five locations through probability plots and adding SVAD’s mean posterior age to each of the earlier scatter plots indicated by a dotted line. Overall, SVAD follows similar patterns to North Carolina, though SVAD seems to present tighter developmental windows between stages. The lower first incisor, canine, and premolar have

relatively consistent results, though the lower second molar has much higher rates of present variation.

4.2.1 – Lower First Incisor

When compared to the probability of North Carolina, SVAD has a degree of delay to nearly the entirety of the state. As shown in Figure 16, the rate of growth that SVAD experiences is similar to that of Sylva, with the remainder of the groups having an accelerated developmental time. For the earlier stages of the incisor, SVAD has more extreme developmental delays, but the gap closes as it reaches later stages and is well within the developmental time of North Carolina as it reaches the final stage of 13.

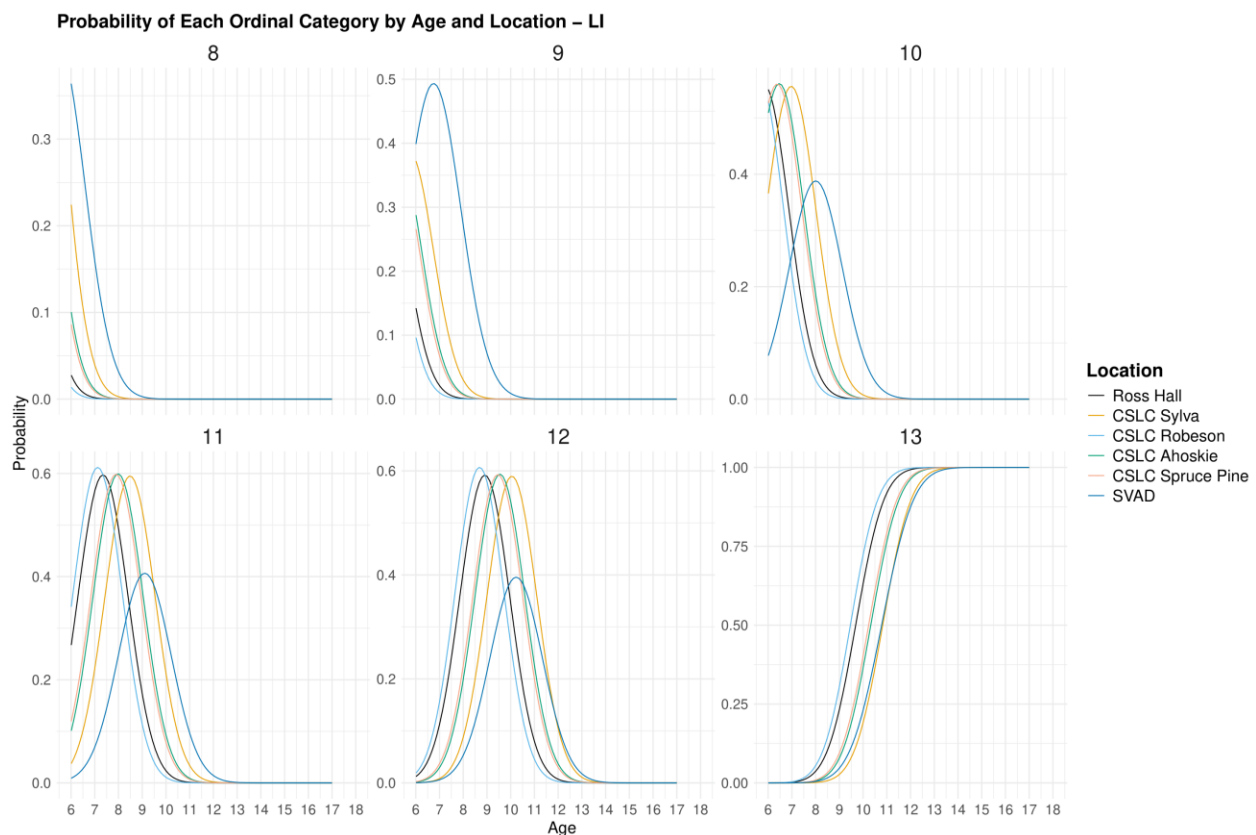


Figure 16. Probability graph for ordinal tooth development scores for the lower right first incisor, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 8–13 from ages 6 to 16. Data added from SVAD as a comparison.

The scatter plot, Figure 17, shows that SVAD has a developmental rate that is the closest to Ahoskie or Spruce Pine. Compared to all locations in North Carolina, SVAD has a faster developmental time in the later stages, and stages 12 and 13 are closer together when comparing each stage of development. Overall, early development of SVAD is closer to that of Ahoskie or Spruce Pine, and later stages are closer to that of Robeson and Ross Hall.

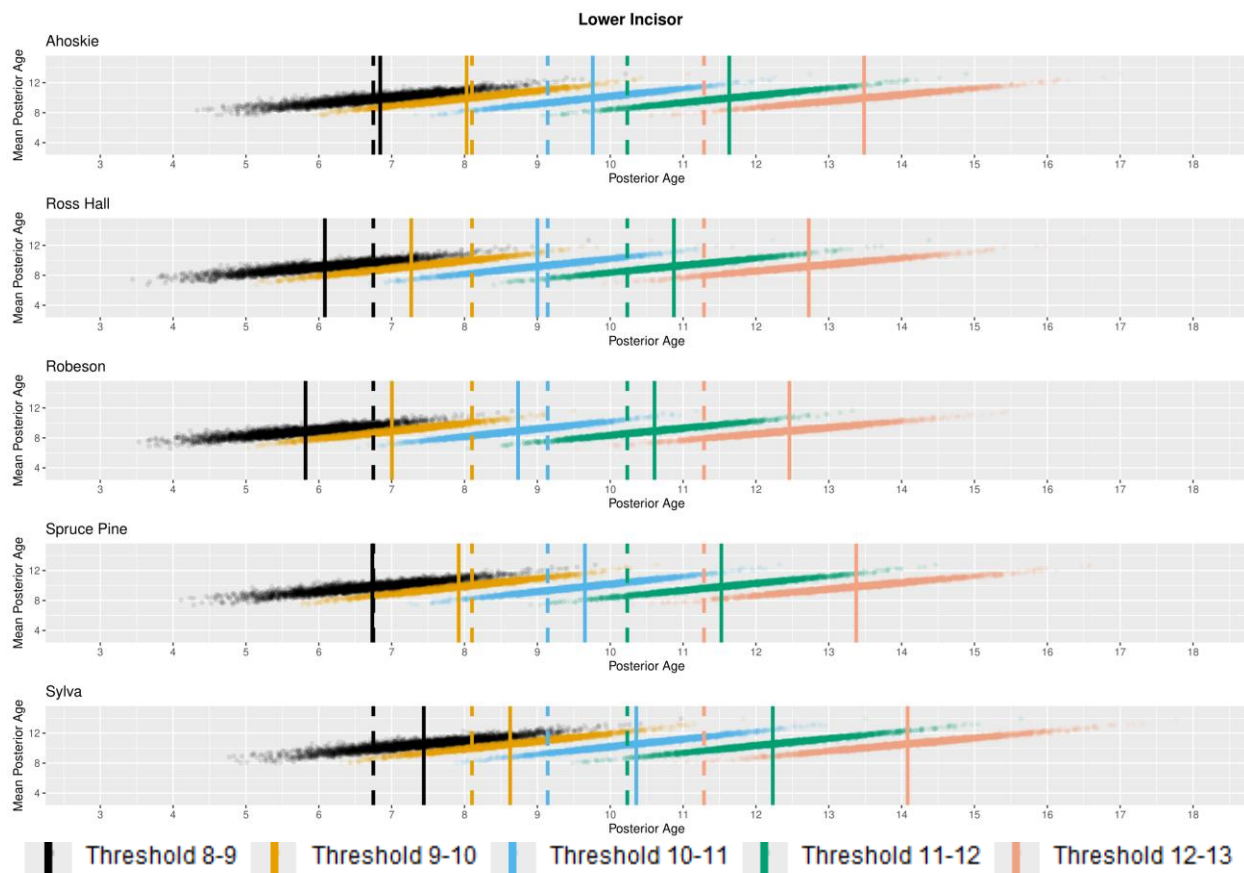


Figure 17. Posterior age from each location for the lower right first incisor, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition. Added dashed lines represent means from SVAD.

4.2.2 – Lower Canine

The probability graph of the lower canine from SVAD is well within the same range as North Carolina. SVAD's development of the canine is consistent with each probability stage of North Carolina's sample, with little inconsistency. Developmental times on the earlier stages of

the canine are consistent with those of North Carolina, though the later stage, 13, has a slightly earlier developmental time. Overall, the canine from the SVAD sample follows the same pattern as North Carolina.

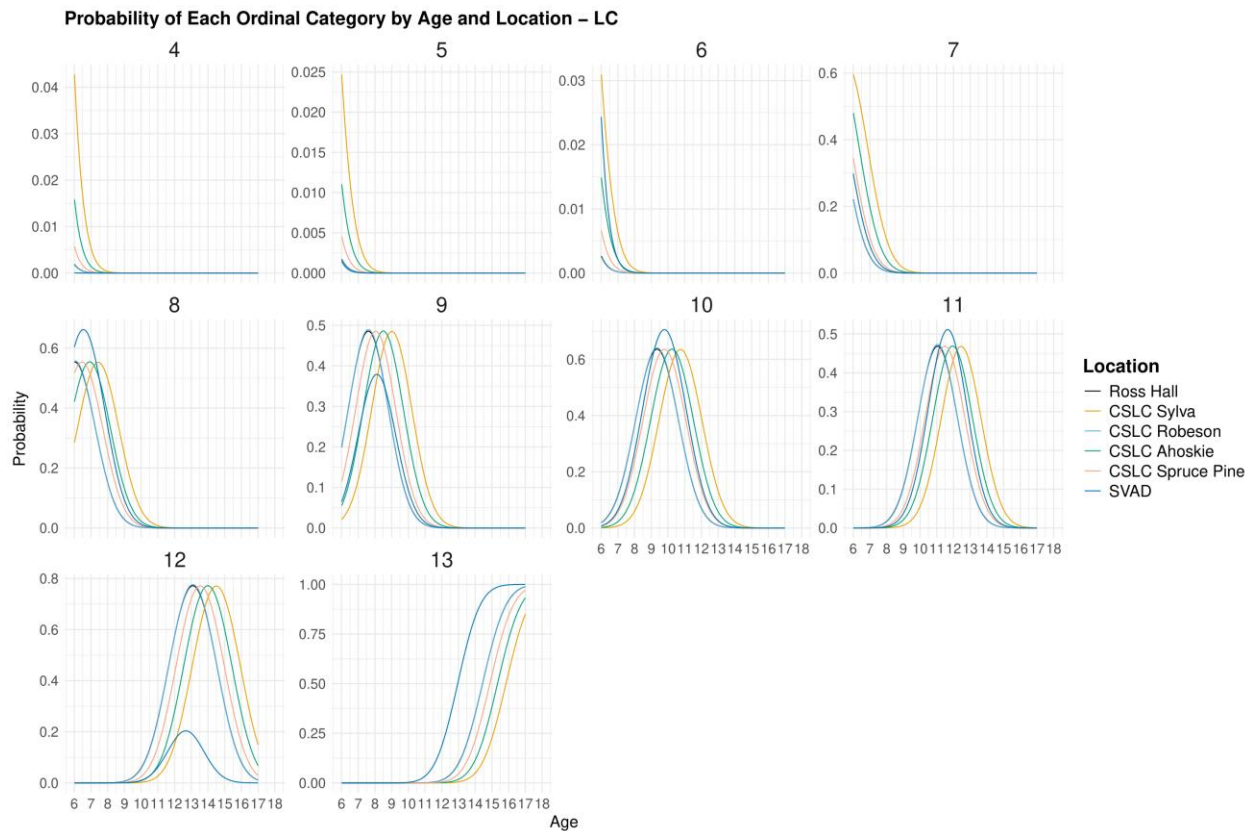


Figure 18. Probability graph for ordinal tooth development scores for the lower right canine, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 4–13 from ages 6 to 16. Data added from SVAD as a comparison.

The scatter plot shares a similar pattern to that of the probability graph. Figure 19 shows that the developmental age of SVAD follows that of North Carolina, with each threshold closely aligned with one of the five CSLC. In the earlier stages of development, the canine follows the rate of Robeson and Ross Hall. Comparatively, in later stages, the development of the canine is closer to that of Ahoskie or Spruce Pine. Sylva, on the other hand, seems consistently delayed to the SVAD sample, though not to the same extent as some of the other locations in North Carolina (Robeson and Ross Hall).

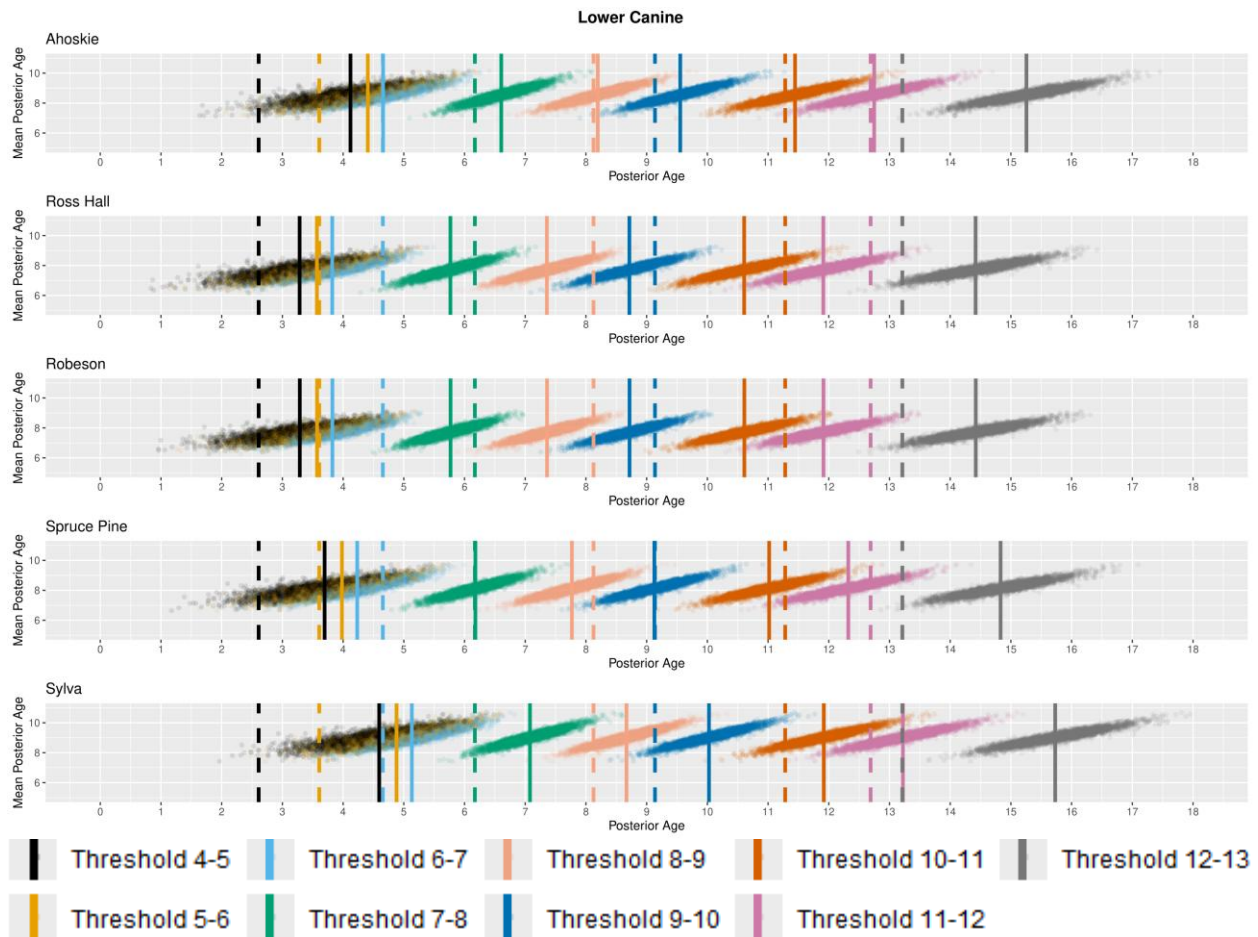


Figure 19. Posterior age from each location for the lower right canine, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition. Added dashed lines represent means from SVAD.

4.2.3 – Lower First Premolar

On average, the lower first premolar from SVAD develops earlier than the CSLC in North Carolina, however, the development slows at the later stages of 11–13. In earlier stages, the development is earlier than Robeson and Ross Hall by nearly 1 year, while later stages align closer to the results from North Carolina.

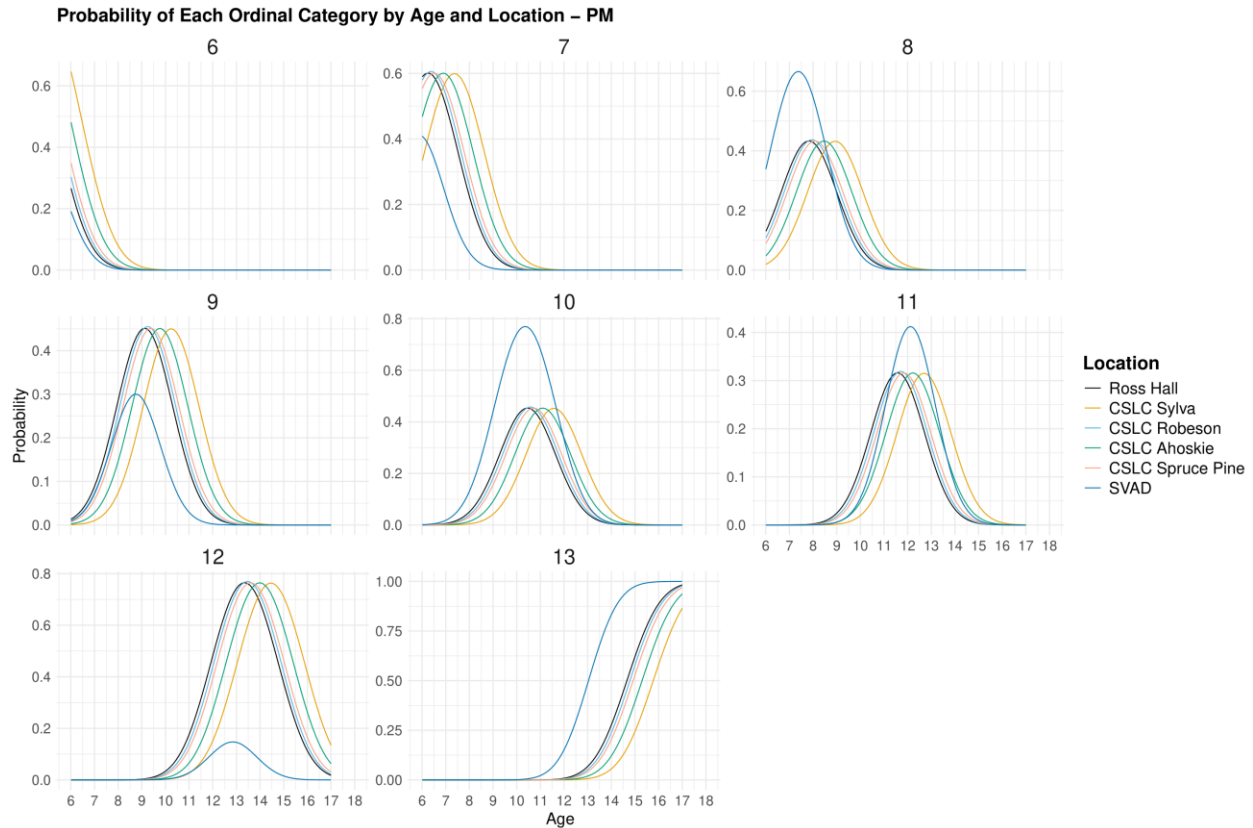


Figure 20. Probability graph for ordinal tooth development scores for the lower left first premolar, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 6–13 from ages 6 to 16. Data added from SVAD as comparison.

In the earlier stages of the first premolar, SVAD was well within the same range as North Carolina, especially Robeson and Ross Hall. As the first premolar enters later stages, the development of SVAD slows, with North Carolina having a faster developmental time than SVAD as it enters stage 11, though SVAD catches up as it enters stage 13 prior to that of North Carolina.

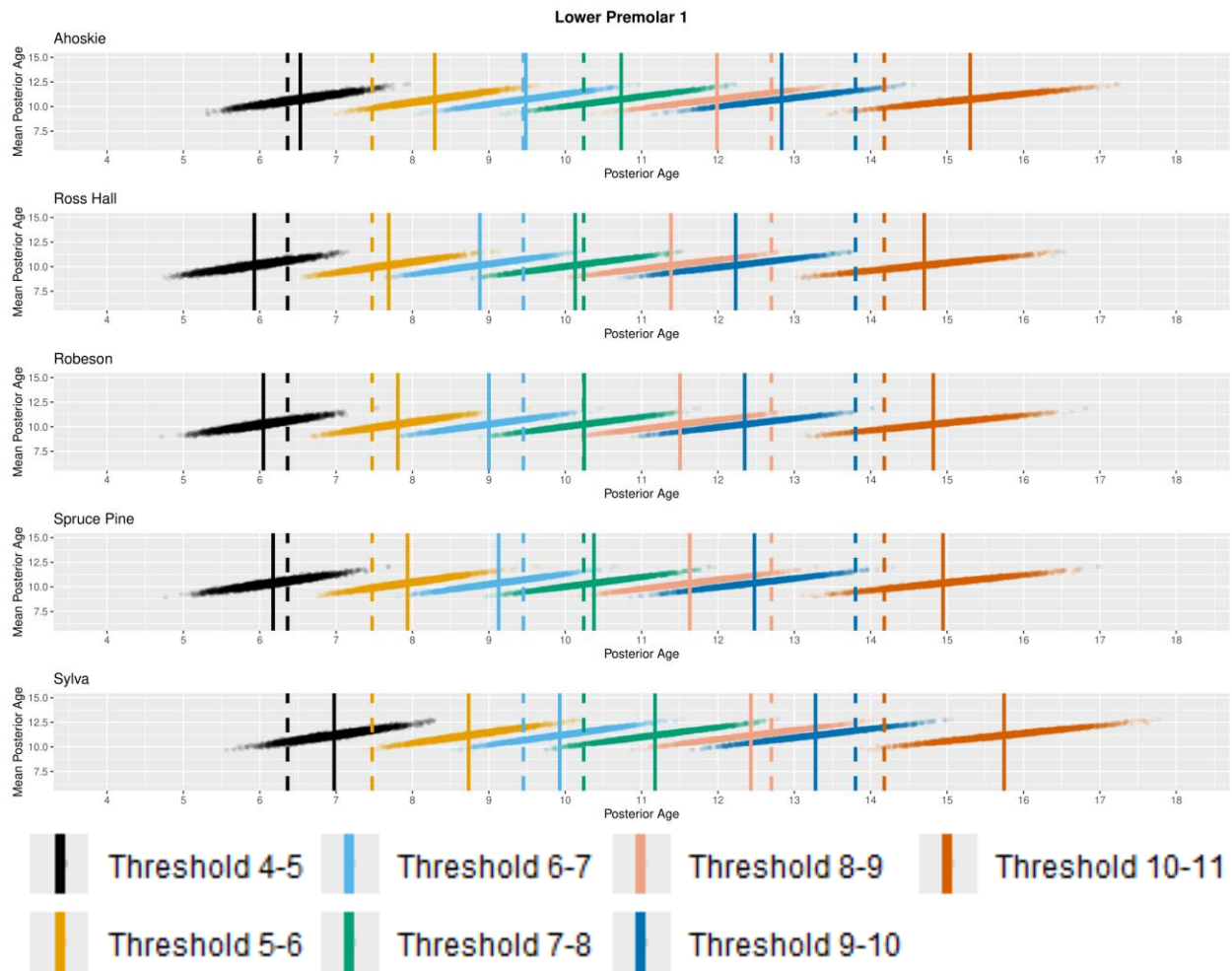


Figure 21. Posterior age from each location for the lower left first premolar, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition. Added dashed lines represent means from SVAD.

4.2.4 – Lower Second Molar

The lower second molar shown in Figure 22 has a larger developmental gap when compared to North Carolina. Variation is highly present throughout the sample, with North Carolina showing data that is consistent and comparable overall, the development of the second molar in SVAD shares little to no consistencies with the North Carolina sample. In some stages, SVAD begins development of each stage earlier, while in other stages, the development is later. Representative sample size and variation of the composition of the tooth scores causes changes in the overall kurtosis of the graph for SVAD, though data overall is still consistent.

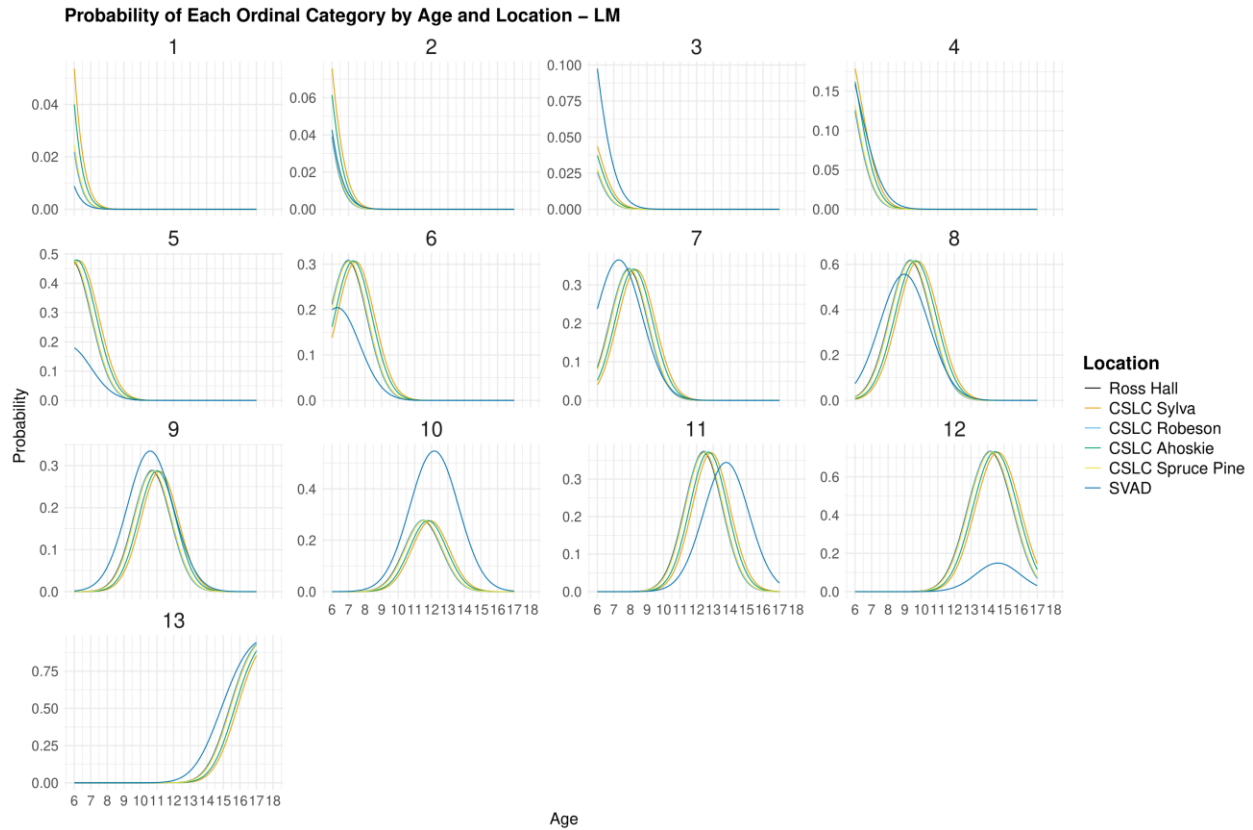


Figure 22. Probability graph for ordinal tooth development scores for the lower right second molar, shows the probability of each tooth score given age. Grouped by location and sorted by age. Shows stages 1–13 from ages 6 to 16. Data added from SVAD as comparison.

According to the scatter plot, the development of the lower second molar begins much earlier than that of North Carolina. As shown in Figure 22, the development of the second molar for SVAD ends near the age of 12, while development continues well into the age of 15 for North Carolina.

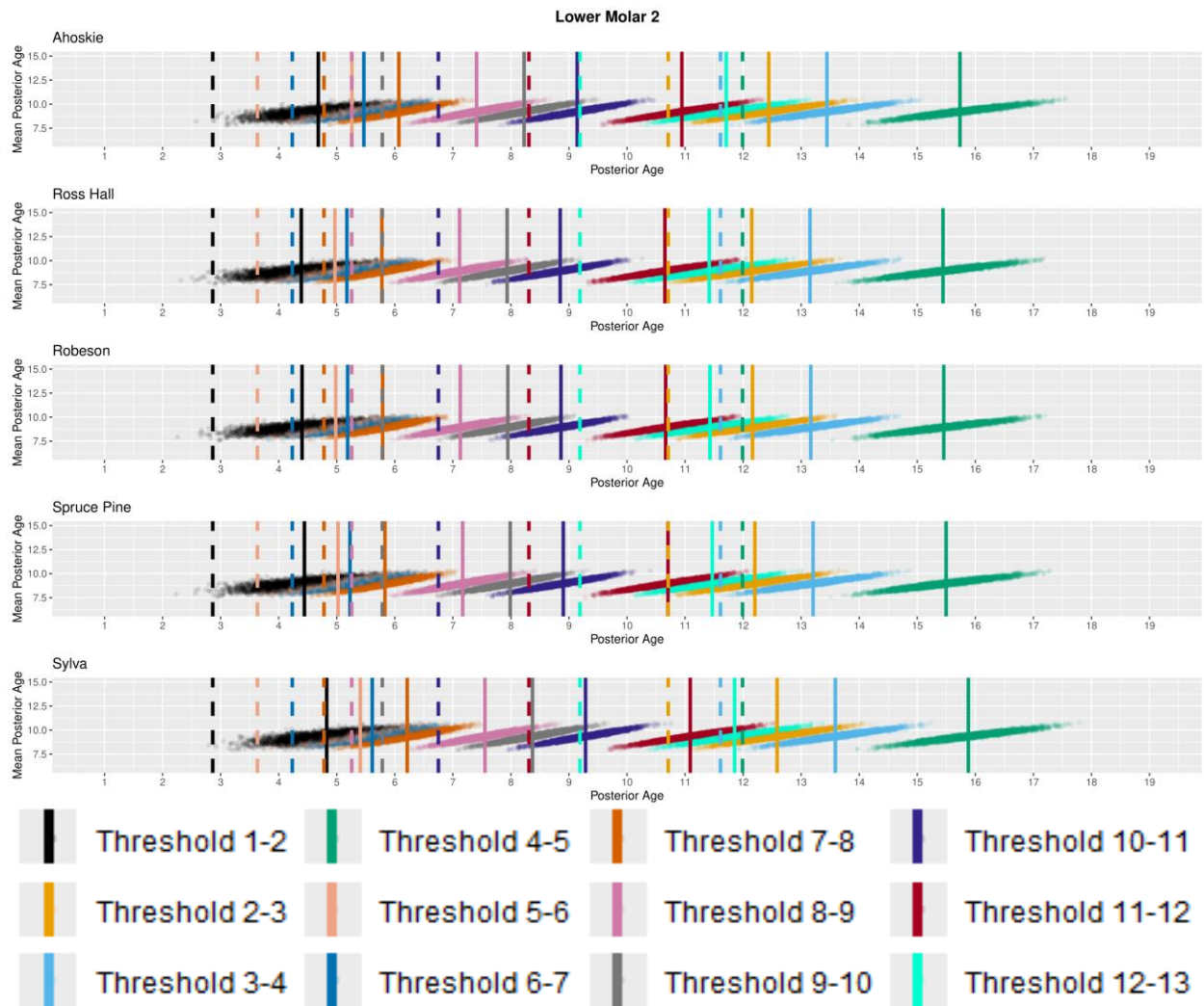


Figure 23. Posterior age from each location for the lower right second molar, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition. Added dashed lines represent means from SVAD.

Chapter 5: Discussion

5.1 – Summary

The results show that sex, race, and ethnicity have very little influence on dental development, while geographic location has a much stronger impact. Biological sex consistently showed that females were slightly advanced in developmental stages, with the exception being the third molars. This is expected, as most literature suggests that females on average develop at a slightly advanced pace compared to males (Scheuer & Black, 2004). Race and ethnicity had little influence on the presence of variation throughout the state, though groups with small sample sizes skewed the results and conclusions must be made with caution when examining the results of the cumulative probit models. The two groups “Native Hawaiian/Pacific Islander” (n = 3) and “Asian” (n = 6) cannot be reliably compared to the remainder of the samples due to their small size. These results are corroborated by Thevissen et al. (2024), as ethnicity had little influence on tooth developmental timing.

Comparably, the locations of all CSLC showed a wide range of variation in the developmental stages of the dentition. On average, between the four teeth discussed, the presence of variation in tooth development stages spans approximately 1.2 years when examining the mean transition age from one stage to the next. Crown formation and root formation display differing developmental trajectories with the crown developing at a faster rate on average when compared to the root (Scheuer & Black, 2004). The lower first incisor and lower canine seem to exhibit more variation between each developmental stage, while the lower first premolar and lower second molar express less variation between each CSLC. Given that the earlier forming teeth focus on root development as opposed to the crown and root development, this could also be indicative of higher rates of root variation when compared to the crown.

5.2 – Interpretations

The results highlight notable differences present in dental development across CSLC locations in North Carolina, suggesting that environmental and socioeconomic factors may play a role in the variation in tooth mineralization. Rural communities, such as Sylva, experience delays in dental development, with a potential link to limited healthcare access and resources. Comparably, Spruce Pine, though similar in size and location, has better access to external resources that may explain accelerated developmental timing (Caldas & Cardoso, 2021; Cardoso, 2007; Conceição & Cardoso, 2011; Nelson et al., 2021). Tooth plasticity also plays a role in the developmental variation present in the sample. Early forming teeth such as incisors and canines demonstrate a level of genetic stability, while later forming teeth such as premolars and molars are more susceptible to environmental influences. The ‘key tooth’ theory (suggests that the most mesial tooth has greater stability for each tooth type) and the ‘clone theory’ (each tooth is formed from cloned cells, which become less efficient with each tooth of the same type) offer possible explanations for why more distal teeth exhibit greater developmental variation (Morita et al., 2016). Results of this study align with previous research on race and ethnicity, emphasizing environmental conditions as key factors that contribute to variation seen in dental development (Thevissen et al. 2024). Additionally, this study supports the idea that patterns of dental development are more strongly influenced by the environment than previously thought. Further research is recommended to explore these observations and identify the exact causes of the variation present throughout North Carolina.

5.2.1 – Differences in Dental Development

Results show that different CSLC exhibit varied rates of dental development and average ages at transition. All the communities in this study that exhibit delayed dental development

come from rural locations (Ahoskie, Spruce Pine, and Sylva). In contrast, the communities that demonstrate considerably accelerated development have patients that live in a more urbanized environment (Ross Hall and Robeson). This pattern may reflect disparities in access to healthcare and other essential resources, as rural communities are often more isolated and underserved (Caldas & Cardoso, 2021; Cardoso, 2007; Conceição & Cardoso, 2011; Krause et al., 2012; Dharmo et al., 2018; Nelson et al., 2021; Skillman et al., 2010). It is worth noting that Spruce Pine and Sylva share similar ancestral backgrounds and geographic locationality, as both are rural mountain communities comprised of nearly identical ancestral makeups. Despite this commonality, notable differences in dental development are still present, suggesting that environmental and/or socioeconomic factors play a more prominent role than genetic history. Rural communities as a whole are known to have higher rates of dental carious lesions, and in turn these lesions may cause a delay in dental development (Dharmo et al., 2018; Skillman et al., 2010). Additionally, rural communities typically have fewer dentists and must travel greater distances to access proper dental care (Skillman et al., 2010). Compounding the above issues is an overall trend towards lack of access to health, social resources, and an overall lack of affluence that may impact immediate biological function (e.g., carious lesions) or lead to longer term stunting in dental development.

In addition to these factors, community water fluoridation, or the lack thereof, could influence developmental differences. Water fluoridation was introduced as a passive way to prevent dental caries and is considered one of the most effective public health strategies to prevent tooth decay (Krause et al., 2012). The CDC currently recommends fluoride levels of 0.7 ppm to mitigate the development of dental caries, yet many rural communities in North Carolina fall below this threshold. As shown in Table 9, Ahoskie, Spruce Pine, and Sylva do not meet that

threshold, with Ahoskie at 0.4 ppm, and both Spruce Pine and Sylva having non-detectable (ND) amounts of fluoride in their water supply (Environmental Protection Agency, 2021; Lassiter, 2022; Tuckasegee Water & Sewer Authority, 2022). According to Dhamo et al. (2018), untreated dental caries in deciduous and adult dentition can delay tooth development, suggesting that inadequate fluoride levels in combination with other factors may contribute to the observed delays in tooth mineralization.

Table 9. Detected fluoride in water by parts per million. Report years range from 2021 – 2024. (Environmental Protection Agency, 2021; Greenville Utilities, 2023; Harper, 2024; Lassiter, 2022; Tuckasegee Water & Sewer Authority, 2022).

Location	Fluoride (ppm)	Year Reported
Ahoskie	0.4	2022
Greenville	0.84 – 0.63	2023
Lumberton	0.68	2024
Spruce Pine	ND	2021
Sylva	ND	2022

Greenville and Lumberton have been large economic hubs throughout the history of North Carolina, serving as economic anchors within eastern North Carolina and the southern coastal plains respectively. Strong transportation infrastructure, in the form of highways, railroads, and air travel, provides residents with reliable and faster access to medical and dental care. Combined with proper levels of fluoride in the water, this accessibility may explain the comparatively accelerated rates of dental development observed in these two communities (*About Greenville | Greenville, NC*, n.d.; Davis, n.d.).

In contrast, Spruce Pine and Ahoskie share similar structural patterns and function as regional economic centers in their respective areas. Ahoskie has a strong agricultural and industrial base, and Spruce Pine’s mining facilities support local exports. Both rural communities are easily accessible and serve the surrounding populations, with Ahoskie spreading its reach up

to 40 miles away and Spruce Pine benefitting from improved highways and airport access. However, compared to Greenville and Lumberton, both rural communities have lower fluoride levels in their water and limited access to healthcare services, factors that may contribute to the slight delay present in their tooth development (*About Our Town*, 2025; Avery County Administration, n.d.; Bracy et al., 2022).

Sylva exhibits the most pronounced developmental delay among the communities studied, with an average delay in nearly one calendar year when compared to Greenville and Lumberton. Its small population size, elevated rates of poverty, and high percentage of residents without health insurance present unique challenges faced by this small mountain community. While other locations may have similar or higher poverty rates, Sylva stands out due to its limited health coverage and underdeveloped infrastructure. With nearly 30 percent of Sylva's population in poverty, estimating as approximately 774 people, it is likely that a significant portion of patients at the clinic fall within these underserved groups. Its non-detectable levels of fluoride in the water may increase susceptibility to dental caries, causing further delays in dental development. Unlike Ahoskie and Spruce Pine, which benefit from export-based economies and better infrastructure for travel, Sylva's economy relies heavily on tourism and lacks the same infrastructure for economic and healthcare access. This difference may further exacerbate the disparities shown in its rate of dental development (*Sylva - Discover Jackson NC*, 2025; (Town of Sylva, 2025; U.S. Census Bureau, n.d.).

5.2.2 – Developmental Plasticity and the Dentition

The development of the dentition is a complicated process that involves many different growth factors that allow for the development of individual tooth germs (collection of tooth cells) and the entire dentition. Due to this, the characteristics of a given tooth are determined by a

cascade of events particular to each specific tooth type (Gulabivala & Ng, 2014). Each tooth has a different rate of stability with minimum genetic variation. These teeth are as follows: the canines, central incisors, and the first molars. Comparatively, the teeth that seem to have the largest degree of variation are the maxillary lateral incisors, the second premolars, and the second and third molars. As crowns reach the completion of formation, roots continue to develop well into dental eruption and may be influenced by outside factors (Gulabivala & Ng, 2014; Scheuer & Black, 2004).

It is well known that both bilaterally symmetrical teeth and their functional counterparts attain each stage of mineralization at nearly the same rate, indicating that these teeth are comparable with one another (Anderson et al., 1976). While the incisor and canine have a lower degree of intra-population variation and a higher degree of interpopulation differences, the premolar and second molar present a wide range of plasticity as shown by the range of ordinal scores for each age category. As such, early forming teeth such as the incisors, the canine, and the first molar show a degree of genetic stability, while later forming teeth are more plastic and are developmentally affected by the environment.

As shown in endodontic literature, the root and root canal of the premolar are strongly varied, with anatomical variations and developmental anomalies that require clinical examination prior to root canal procedures. In the maxillary premolar, root variation comes in many forms such as: single rooted, double rooted, and triple rooted, with root a variety of canals present in each root formation (Ahmad & Alenezi, 2016). Given this information, the variation that is observed in the scores and model results of the premolar shows a high rate of plasticity and variability, with scores having a much larger range than the incisor and canine. This suggests that the premolar is more susceptible to environmental factors. Because of this, the premolar presents

with a higher degree of variation when compared to earlier forming teeth, as the development of the premolar has a much larger developmental age range than the teeth that present stable and consistent variation by location. While the incisor and canine present high rates of variation between each CSLC, the premolar does not express these results to the same degree. Due to its broad range of expressions, location wide differences may be masked by the inherent consistency of the tooth.

While the first molar is considered more genetically stable and overall, less prone to high rates of variability during stages of formation, the second and third molar are prone to higher rates of variation. As the molars become more distal (first molar to third molar), the range of variability increases, as second and third molars are more plastic than the first molar. Two theories suggest the reasoning for this occurrence: the ‘key tooth theory,’ and the ‘clone theory.’ The key tooth theory assumes that the most mesial tooth in each tooth category has greater stability in both size and morphology. This implies that the first molar is the ‘key tooth,’ the second molar is the intermediate tooth, and the third molar is the most varied of all three of them and lacks the strong stability of the first molar, and the intermediate stability of the second molar (Morita et al., 2016). Comparatively, the second theory, the ‘clone theory,’ suggests that each tooth has a set of cloned cells that are preprogrammed in the same region that the key tooth exists, and as each additional tooth of that tooth type forms, they become less and less efficient at ‘cloning’ the tooth. This would continue to validate the point that the distal teeth experience more variation, and their developmental times are not as predetermined as the mesial teeth (Morita et al., 2016).

While these two theories focus on morphological variation such as cusp size, number of cusps, and overall size of the tooth, the results of the three molars (both maxillary and

mandibular) follow the same pattern as suggested by Morita et al. (2016). This suggests that a similar occurrence happens with the development of the roots of the molars, and as the teeth develop distally, they exhibit higher rates of variation in root formation time. Further research must be done to explore these findings; however, results of the cumulative probit model concur with the findings associated with variability in molar morphology.

5.2.3 – Current Literature

Literature that explores dental development is often done on homogenous samples that are easily available to the researcher (AlQahtani et al., 2010; Liversidge, 2003). Despite many studies that examine variation in dental development, the data often focuses on a single factor that may influence dental development, without exploring the possibility of it being a multitude of factors overall (AlQahtani et al., 2010; Alvarez et al., 1993; Caldas & Cardoso, 2021; H. Cardoso, 2007; H. F. V. Cardoso, 2007; H. F. V. Cardoso et al., 2010; Conceição & Cardoso, 2011; Demirjian et al., 1973; Dhamo et al., 2018; Elamin et al., 2017; Elamin & Liversidge, 2013; Fanning & Moorrees, 1969; H. Liversidge, 2003; H. M. Liversidge & Molleson, 2004; Nelson et al., 2021; Sgheiza & Liversidge, 2023, Thevissen et al., 2024). As such, most research focuses on a specific effect such as sex, socioeconomic status, race, or ethnic group, though each group lives within the same area and results often return as expected, with little to no notable differences. Comparatively, when exploring different groups based on location, differences are often noted but attributed to genetic variation rather than additional factors that could play a role in the differences that are seen (Liversidge, 2003).

A recent study by Thevissen et al. (2024) explores the possible variation in the timing of tooth mineralization between populations, focusing on a sample of Finnish and Somali individuals. The assumption, according to the study, is that differences in populations are not

only due to ethnic origin, but also nutritional and/or environmental conditions that may play a role in the development of the dentition. As stated above, many studies often focus on a single sample, and in this case, both ethnic groups were both born and lived in Finland. According to the study, when subject to similar environmental conditions, all ethnic groups develop at the same rate, suggesting that within a region, dental development will likely follow a similar pattern given most individuals in that area live similar lives. Overall, this study corroborates the results of Thevissen et al. (2024) where there are little to no developmental differences between self-described social race or ethnicity categories.

5.3 – Limitations

The limitations of this study are associated with overall population size leading to smaller sample sizes, the limitations in which dental panoramic radiographs are taken on living populations, and the use of ordinal data collection methods transforming a continuous process into discrete categories that limit precision in inference and prediction. Due to the limitations imposed radiographically, the earliest stage that was able to be reliably accessed is the age of 6, with very little data prior to that age. Dental panoramic radiographs pose risks associated with radiation and are not taken routinely in any particular age group. Rather, those that have dental panoramic radiographs taken at earlier ages are often associated with deviations in dental development, or concerns associated with dental health (Thevissen et al., 2024). This age limitation resulted in the inability to score some teeth in the crown stages, especially the early forming teeth such as the incisors and the first molar. Because of this, some teeth may have estimated ages that are earlier than expected, as the model fills in those gaps based on the known information from the collected data. A particular tooth that was affected by this limitation is the first molar, as the estimated age for each tooth score is much earlier than reported in other

literature (AlQahtani et al., 2010; Demirjian et al., 1973; Liversidge, 2003; Moorrees et al., 1963; Stull & Corron, 2022).

Population size played a role in limitations in the data as well. Originally, the intended sample size of each population was 220, with 20 individuals from each group within the age of 6–16 years. However, Sylva and Robeson did not have that many individuals with dental panoramic radiographs that fit into those age categories, and thus all data that could be collected from those CSLCs were collected. Though this limitation did reduce the sample size of Sylva and Robeson, results did not seem to be affected. Robeson, despite having a total sample size of $n = 167$, remained nearly identical in developmental stages when compared to Ross Hall. Due to this, despite the limited sample sizes present in Sylva and Robeson, the results of their statistical analyses are likely an accurate representation of the population.

The comparison of the North Carolina dataset with that of SVAD created some limitations as well. SVAD has limited demographic information. While North Carolina's dataset has age, biological sex, location, race, and ethnicity recorded, SVAD only has age and biological sex. Additionally, SVAD has limited sample sizes of similar aged groups, as SVAD contains data mostly from infants and older subadult individuals as indicated on Table 3. As such, the data from SVAD could be compared to some degree, though direct comparisons may not be as accurate as if the sample sizes were closer in size. The lack of demographic information from SVAD also limited the ability to examine if similar locational differences existed, as all SVAD's data has been merged into a single sample to represent New Mexico. Overall, despite the limitations presented in this study, results show that dental development in North Carolina is far more varied than originally expected within such a small region.

5.4 – Implications

The results of this study indicate that dental development does have a large degree of variation within the state of North Carolina, with patients having either accelerated or delayed dental development. As such, it is important to discern location wide differences in the collection and analysis of data. When data is collected within a wide range of locations (in this case, five different CSLC within the state of North Carolina), the development of a generalized “North Carolina” group results in an overall model that fits different developmental times of separate locations, while masking the full range of variation that exists throughout the state. As shown in Figure 24, the combination of all locations into a single sample result in a model that is not representative of the population, as each individual location is obscured. In the case of Sylva, for example, the data shifts to an accelerated rate of development, and all developmental data from Sylva is completely lost.

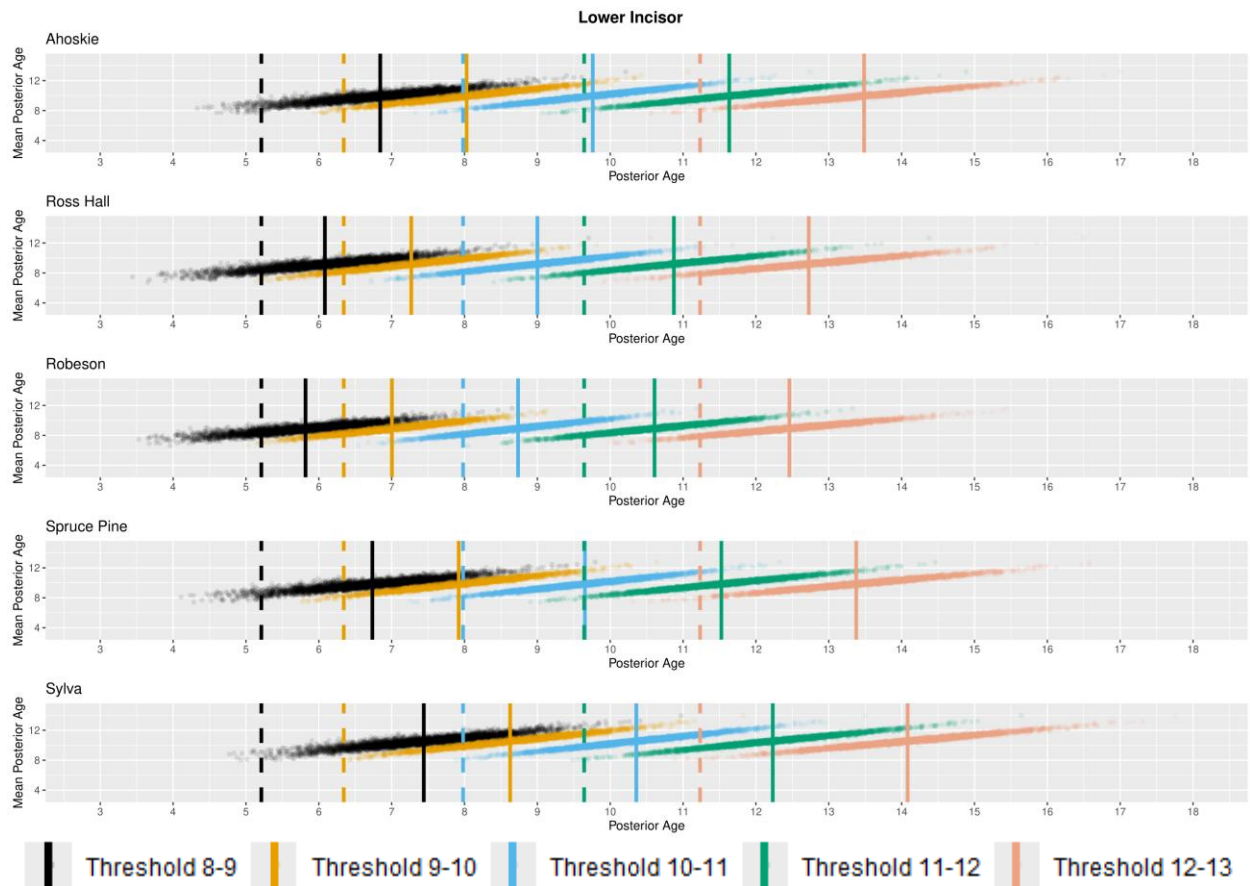


Figure 24. Posterior age from each location for the lower right first incisor, indicating mean transition age from one stage to the next. Vertical lines represent mean age at transition. Added dashed lines represent the North Carolina Sample.

The North Carolina sample predicts earlier ages at transition, and a majority of the variation present within the separate locations is lost. Robeson and Ross Hall are the closest when examining earlier developmental stages of the North Carolina sample, and Spruce Pine and Ahoskie are somewhat represented in the later stages. While creating models to fit every city and population is not possible, understanding what the data looks like when separated based on demographic information and geographic location may be important when developing models in the future, as the North Carolina sample pictured above does a poor job at fully encapsulating the data. Forensic and bioarchaeological methods rely on research done on homogenous populations that do not represent the true variability that is present in this study, and though all geographic locations in the sample do experience degrees of overlap, the means suggest that the generalization of data can create an inaccurate representation of the population in which the data is meant to represent.

5.5 – Future Research Directions

Future research may provide better evidence and information on how dental development may vary, especially when examining intrapopulation data such as the state of North Carolina. Though the lack of access to resources, healthcare, and high rates of poverty may cause delays as it does to the skeleton, further studies on how dental development may be affected in locations within close geographic distance may provide answers, and these answers may promote a better understanding of how models and future sampling methods can be adjusted to represent intended populations. Data exploration is an important tool in research, and identifying trends earlier in the steps of statistical analysis may point toward potential patterns in which variation can be further understood. Prior to developing methods using the dentition such as age estimation techniques, the data must first undergo examination to explore what variation may exist, and how

these different groups can be combined in a way that encapsulates the developmental trajectory of each unique sample and population, despite possible racial or ethnic differences.

Additional information must also be explored when examining the differences present between geographic locations, as human growth and development are strongly linked with the overall health of an individual and the population in which they live. If certain geographic regions experience developmental delay, exploring how lack of access to healthcare may affect these individuals may be a step forward in public health policy and strategies to reduce these factors. While no direct links have been made to health and developmental delays, exploring these avenues is important to limit the effects of health disparities in certain rural areas given this may be the cause for developmental delay shown in this study.

Chapter 6: Conclusion

This study explores the wide range of dental development across different geographic locations within the state of North Carolina, with notable differences observed between rural and urban areas. While other observed factors such as sex, race, and ethnicity expressed little to no influence on dental development, geographic location strongly influences the rate of dental development. Given that smaller, rural towns experience higher rates of developmental delay, this suggests that factors that are related to access to resources and healthcare may play a role in the rate of development in an individual.

The comparison of data between North Carolina and SVAD further explores the broader patterns of dental development across the United States, with SVAD expressing smaller developmental windows in dental development, while North Carolina shows a slower rate of development overall. Teeth such as the lower first incisor, the canine, and the first premolar exhibit relatively consistent developmental patterns with SVAD, while the second molar demonstrates a wide range of variability that may be due to the wide range of variation seen within the second molar morphologically. Findings suggest that the premolar and second molar are more plastic and susceptible to environmental influence, leading to increased developmental variation present in the samples that may mask the variation clearly indicated on early forming teeth.

This study also highlights the importance of further research into intrapopulation variation to deepen our understanding of how environmental, socioeconomic, and genetic factors may interact to directly influence the rate of dental development, and future studies should explore the relationship between these factors and the timing and progress of tooth mineralization.

Overall, this study shows the complex interplay between assumed genetic and environmental factors that shape how each tooth develops within each individual, and how patients at each CSLC experience a wide range of developmental patterns. Further research is needed to explore these findings and expand on the implications of this study, though results point to the importance of considering demographic factors when studying patterns of growth and development, as these patterns offer valuable insights into future healthcare accessibility and public health policies and strategies that may benefit those that experience delay. Additionally, understanding how geographic location may emerge as determinants in developmental patterns may allow us to explore the importance of considering both genetic and environmental factors in future dental research and healthcare.

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APPENDIX: IRB Approval Letter



EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
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600 Moye Boulevard · Greenville, NC 27834
Office 252-744-2914 · Fax 252-744-2284 · redc@ecu.edu/
umcibr/

Closure Notification

From: Biomedical IRB
To: [Liam Cline](#)
CC: [Christopher Wolfe](#)
Date: 4/16/2025
Re: [FR00002955](#)
2025 Final Report for UMCIRB 24-000748
An Examination of Variability in Dental Development Across North Carolina

I am pleased to inform you that your request to close this study has been approved on 4/15/2025.

It is your responsibility to ensure that you retain all research related documents, including the consent form(s), if applicable, for a period of no less than three years. If you have any questions or need for any reason to re-open this research study, please contact the UMCIRB office prior to implementing any research actions.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.