

The Patterning Cascade Model and Carabelli's trait expression in metameres of the mixed human dentition: exploring a morphogenetic model

Kathleen S. Paul¹ | Claudia M. Astorino^{2,3} | Shara E. Bailey^{3,4}

¹Center for Bioarchaeological Research, School of Human Evolution and Social Change, Arizona State University, Tempe, Arizona 85287

²The Graduate School and University Center, The City University of New York, New York, New York 10016

³New York Consortium in Evolutionary Primatology, New York, New York 10028

⁴Center for the Study of Human Origins, Department of Anthropology, New York University, New York, New York 10012

Correspondence

Kathleen Paul, School of Human Evolution and Social Change, 900 S. Cady Mall, Arizona State University, Tempe, AZ 85387.

Email: Kathleen.Paul@asu.edu

Abstract

Objectives: The Patterning Cascade Model (PCM) provides an evolutionary developmental framework for exploring diversity in tooth crown form. According to the model, proximity of secondary enamel knots and tooth germ size track underlying developmental processes that dictate ultimate crown morphology (i.e., cusp number, accessory cusp presence/size). Previous research has shown the model to successfully predict variation in Carabelli's trait expression between antimeric and metameric pairs of human permanent molars. In this study, we quantify Carabelli's trait expression for metameres of the mixed dentition (dm2 and M1) and assess the PCM's potential for explaining differences in expression between the two elements.

Materials and Methods: Crown dimensions, intercusp distances, and Carabelli's trait expression were collected from 49 subadults possessing observable dm2/M1 pairs. Wilcoxon signed-rank tests and paired *t*-tests were performed to assess whether metameres differ significantly in morphometric variables. We explored the relationships between relative intercusp distances (RICDs) and Carabelli's trait expression using proportional odds logistic regression.

Results: Intra-individual dm2/M1 pairs differed significantly in Carabelli's trait expression ($p = 0.01$), with dm2 exhibiting higher grades of expression more commonly despite its smaller crown size. Paired molars differed in only one statistically significant RICD: metacone-hypocone ($p < 0.01$). Most RICDs shared the predicted negative relationship with Carabelli's trait expression, but this relationship was only statistically significant for three RICDs in the dm2 (mean, protocone-paracone, metacone-hypocone).

Conclusions: We found mixed support for the PCM's ability to explain differences in Carabelli's trait expression between metameres of the mixed molar row. Results suggest that protocone-paracone enamel knot spacing has the greatest influence on Carabelli's trait expression. Lack of statistical significance for many of the relationships explored may reflect limitations related to sample composition and sample size.

KEYWORDS

ASUDAS, dental anthropology, dental development, dental morphology, primary teeth

1 | INTRODUCTION

Dental crown traits feature prominently in physical anthropology, playing an important role in both bioarchaeological and phylogenetic research. Traits commonly used to estimate biological or phylogenetic distance include crown dimensions, cusp number, and cusp size. Histor-

ically, such studies have rested on two fundamental assumptions. First, it has been accepted that dental trait variation tracks both micro- and macro-evolutionary events reliably. Second, these analyses often assume independence of crown characters. The latter assumption has been called into question by recent studies of dental development, which suggest that small changes to underlying developmental

programs yield predictable alterations in crown form (e.g., Bernal, Gonzalez, & Perez, 2013; Evans et al., 2016; Jernvall, 2000; Jernvall, Keränen, & Thesleff, 2000; Kangas, Evans, Thesleff, & Jernvall, 2004; Kavanagh, Evans, & Jernvall, 2007; Renovoisé et al., 2009; Salazar-Ciudad & Jernvall, 2002, 2010). As such, exploration of how developmental processes affect tooth crown morphology can provide essential insight into the foundations of dental variation and, ultimately, in determining which traits are informative for reconstruction of phylogenetic relationships and population histories.

In this study, we compare aspects of crown size and configuration between metameric elements of the mixed dentition: paired deciduous second and permanent first molars (henceforth, dm2 and M1).¹ We use these morphometric data to test predictions generated from a well-known developmental model—the Patterning Cascade Model (PCM)—and in so doing, explore the model's potential for explaining intra-individual variation in dental trait expression. Because our study is observational and not experimental, it relies on the use of proxies for various parameters of the model. For this reason, we begin with a brief review of current understandings of dental development, as well as the biological basis of the PCM and its previous application to dental anthropological research.

2 | MODELS OF DENTAL MORPHOGENESIS

Much of what we know about mammalian tooth morphogenesis comes from research in experimental genetics and embryology. The development of molar shape and morphology proceeds in a relatively predictable sequence that has been detailed in various reviews of tooth morphogenesis (e.g., Jernvall & Thesleff, 2000, 2012; Maas & Bei, 1997; Peters & Balling, 1999; Thesleff & Jernvall, 1997; Thesleff & Nieminen, 1996; Thesleff & Sharpe, 1997; Tucker & Sharpe, 2004; Weiss, Stock, & Zhao, 1998). Studies of mice and voles have provided a general pattern of morphogenesis that begins early in embryonic development when the epithelial tissue in the oral cavity begins to thicken (Hay, 1961; Lumsden, 1979; Ruch, 1987). Soon after, the epithelial tissue invaginates into the mesenchymal tissue, forming a tooth bud (Hay, 1961; Jernvall & Thesleff, 2012; Lumsden, 1979; Mina & Kollar, 1987; Ruch, 1987; Thesleff & Sharpe, 1997).

The stage that follows—the cap stage—is particularly important, because it is at this time that a primary enamel knot emerges as a cluster of epithelial cells within the inner enamel epithelium (Butler, 1956; Jernvall & Thesleff, 2012). This primary enamel knot acts as a signaling center for cell division, advancing tooth morphogenesis to cusp formation (Butler, 1956; Jernvall, Åberg, Kettunen, Keränen, & Thesleff, 1998; Jernvall, Kettunen, Karavanova, Martin, & Thesleff, 1994; Jernvall & Thesleff, 2012; Vahtokari, Åberg, Jernvall, Keränen, & Thesleff, 1996). Enamel knots are thought to be equivalent to other vertebrate signaling centers or “organizers” (Spemann & Mangold, 1924), such as those present in forming limb buds, hair follicles, and scales (Niswander & Martin, 1992; Thesleff & Nieminen, 1996; Thesleff, Vahtokari, & Partanen, 1995; Vahtokari et al., 1996). The enamel knots stimulate proliferation among nearby cells and express a variety of signaling pro-

teins, the literature on which is extensive (e.g., Åberg, Wozney, & Thesleff, 1997; Coburn & Sharpe, 2003; Jernvall et al., 1994, 1998; Kassai et al., 2005; Kettunen & Thesleff, 1998; Kettunen et al., 2000; Mitsiadis & Smith, 2006; Nadiri, Kuchler-Bopp, Haikel, & Lesot, 2004; Thesleff, 2003). Enamel knots are essential to successful tooth formation, as knockout studies of various genes have shown that tooth development arrests if there is a failure in primary enamel knot formation (Kratchowil, Dull, Fariñas, Galceran, & Grosschedl, 1996; Satokata & Maas, 1994; Thesleff & Jernvall, 1997; Tucker & Sharpe, 2004).

During the next phase—the bell stage—secondary enamel knots form at the site of future cusp tips and play a central role in directing morphogenesis in multicuspid teeth (Coin, Lesot, Vonesch, Haikel, & Ruch, 1999; Jernvall et al., 1994; Jernvall & Thesleff, 2000, 2012; Thesleff & Nieminen, 1996). Importantly, positioning of the secondary enamel knots initiates taxon-specific differences in crown morphology (Jernvall & Thesleff, 2012; Keränen, Åberg, Kettunen, Thesleff, & Jernvall, 1998). For example, studies of mice (*Mus musculus*) and voles (*Microtus rossiaemeridionalis*) show that differences in the location of secondary enamel knots correspond to differences in cusp positioning, which in turn yield the difference in final cusp number observed between these two taxa (Keränen et al., 1998). Within a tooth, cusps that form later in the course of development derive from later-forming secondary enamel knots, although the same signaling proteins appear to regulate the formation of all cusps (Jernvall & Thesleff, 2000; Keränen et al., 1998).

Cusps form through cell proliferation and the mechanical folding of successive cell layers (Jernvall & Thesleff, 2012; Thesleff & Jernvall, 1997; Thesleff & Sharpe, 1997; Weiss et al., 1998). Ameloblast and odontoblast cells secrete enamel and dentine, respectively, resulting in tooth crown mineralization (Butler, 1956; Caton & Tucker, 2009; Ruch, 1987; Thesleff & Hurmerinta, 1981). Gene expression directs each stage of odontogenesis via proteins that act as signaling molecules (Dassule, Lewis, Bei, Maas, & McMahon, 2000; Kollar & Baird, 1969, 1970; Kollar & Mina, 1991; Kratchowil et al., 1996; Lumsden, 1988; Mina & Kollar, 1987; Thesleff, 2003). Substantial deviations in gene expression or signaling protein activity may result in anomalous tooth morphology (e.g., Kangas et al., 2004; Miletich & Sharpe, 2003; Pispas et al., 1999; Satokata & Maas, 1994), but even slight changes affecting secondary enamel knot timing and positioning can yield observable and quantifiable variation in final crown morphology (Jernvall et al., 2000; Salazar-Ciudad & Jernvall, 2002, 2010).

2.1 | The Patterning Cascade Model

The PCM has emerged as a means of explaining and predicting variation in cusp number and cusp size in multicuspid teeth (Jernvall, 2000). The PCM predicts that teeth are more likely to develop a greater number of cusps and that later-forming (or accessory) cusps are more likely to be larger in size when: (a) earlier-forming cusps (and therefore, earlier-forming secondary enamel knots) are closely spaced within the developing tooth germ; and to some extent, (b) when the tooth germ is of relatively large size (Jernvall, 2000). Jernvall (1995) proposed that secondary enamel knot formation is dependent on the number of cells

created by cell proliferation that are available to support the formation of a new cusp. When a secondary enamel knot forms at the site of a new cusp tip, the number of available cells that can be allocated to additional, later-forming enamel knots decreases. Consequently, a “zone of inhibition” surrounds each secondary enamel knot. Any subsequent enamel knots must form outside these inhibition zones where cells are available that can be allocated to forming a new cusp (Jernvall, 1995; Weiss et al., 1998). When earlier-forming enamel knots are tightly spaced, their inhibition zones are consolidated in the tooth germ. As a result, a greater number of cells are available outside of these zones circumferentially to support later-forming enamel knots and, eventually, more and/or larger accessory cusps (Jernvall, 2000). This also means that a tooth germ that creates more cells via cell proliferation is expected to form more and/or larger accessory cusps.

The PCM has received substantial support. In the original study, Jernvall (2000) found that Lake Ladoga ringed seal (*Phoca hispida ladogensis*) postcanine teeth were more likely to develop more and larger accessory cusps when the three earliest-forming primary cusps were relatively similar in height and more closely spaced. Jernvall (2000) also found that absolute crown size played a limited role in accessory cusp expression. Salazar-Ciudad and Jernvall (2010) later produced simulated seal teeth whose resulting morphology conformed to PCM predictions using a computational model that encompassed nine genetic parameters and ten cellular developmental parameters. They found that few changes were required to alter resulting tooth morphology substantially and that these changes did not affect a single tooth but impacted the entire postcanine tooth row serially (Salazar-Ciudad & Jernvall, 2010). The PCM's predictions have also been supported by studies of rodent molar development (Jernvall, 2000; Jernvall et al., 1994; Keränen et al., 1998). Gene expression patterns for several proteins expressed by enamel knots of mice and voles (Jernvall, 2000) are concordant with computer models simulating mouse molar development (Salazar-Ciudad & Jernvall, 2002). The fact that the predictions of the PCM are met in these taxa is significant, because it suggests that the model is applicable to the development of multicusp teeth among mammals in general, regardless of specific cusp arrangement.

In sum, these findings suggest that minor changes in developmental trajectory can substantially alter tooth morphology—including both cusp number and size (Alberch, Gould, Oster, & Wake, 1979; Jernvall et al., 2000; Keränen et al., 1998; Salazar-Ciudad & Jernvall, 2002). Although separate dental elements/fields/structures may be subject to semi-independent sets of controls (see e.g., Hlusko, Do, & Mahaney, 2007; Hlusko, Maas, & Mahaney, 2004; Hlusko & Mahaney, 2009), these findings support the concept that the “dentition develops, varies, and evolves as a single functional complex” (Moormann, Guatelli-Steinberg, & Hunter, 2013, p. 400; see also Jernvall & Jung, 2000; Kangas et al., 2004; Salazar-Ciudad & Jernvall, 2002, 2010). These conclusions may have broad-ranging implications for anthropological research, particularly for phylogenetic and biological distance approaches that often treat dental characters as independent (Kangas et al., 2004).

It is not surprising then that the predictions of the PCM have also been applied to explorations of crown variation in some primate taxa,

including *Pan* (Skinner & Gunz, 2010). Researchers have also examined whether the predictions outlined by the PCM can be applied to variation in Carabelli's trait expression in recent modern humans (Durner, 2011; Guatelli-Steinberg et al., 2013; Hunter, Guatelli-Steinberg, Weston, Durner, & Betsinger, 2010; Jernvall & Jung, 2000; Moormann et al., 2013). Carabelli's trait is an accessory feature of the maxillary molars that forms on the mesiolingual aspect of the crown (protocone) (Harris, 1977; Korenhof, 1960; von Carabelli, 1842). Similar mesiolingual features also occur in Plio-Pleistocene fossil hominins (e.g., Irish & Guatelli-Steinberg, 2003; Korenhof, 1960; Robinson, 1956; Sperber, 1974; Van Reenen & Reid, 1995; Wood & Engleman, 1988) and may be homologous to traits observed in the lingual cingulum of extant and fossil apes (Korenhof, 1960; Ortiz, Skinner, Bailey, & Hublin, 2012). Carabelli's trait can be observed at both the external enamel surface and the internal enamel-dentine junction (EDJ) (Korenhof, 1960; Ortiz et al., 2012; Skinner et al., 2008) and has been used to test the PCM for several reasons. First, when present, Carabelli's trait varies in expression from a weak vertical groove to a fully formed cusp with a free apex (Dahlberg, 1956; Scott & Turner, 1997; Turner, Nichol, & Scott, 1991). In humans, Carabelli's trait expression varies along the molar row (Dahlberg, 1951; Turner et al., 1991; von Carabelli, 1842), as well as among populations (e.g., Alvesalo, Nuutila, & Portin, 1975; Dahlberg, 1951; Irish, 1997; Kraus, 1951; Pedersen, 1947; Scott, 1980; Townsend & Brown, 1981; Tsuji, 1958; Turner, 1967). Carabelli's trait forms relatively late in crown development, initiating after the molar's main cusps (i.e., protocone, paracone, metacone, and hypocone) (Jernvall & Jung, 2000; Kraus, 1963; Kraus & Jordan, 1965). If human postcanine tooth development conforms to the PCM, we would expect Carabelli's trait presence and degree of expression to be directly impacted by secondary enamel knot configuration (as approximated by the size and spacing of earlier forming cusps) (Jernvall, 2000; Jernvall & Jung, 2000) and to some extent by overall crown size (Jernvall, 2000).

Previous studies have provided support for the PCM in humans, finding positive associations between Carabelli's trait expression and crown length/width (Harris, 2007; Kondo & Townsend, 2006) and/or crown area (Kondo & Townsend, 2006; Reid, Van Reenen, & Groeneveld, 1991). Researchers have concluded that large crown size and pronounced Carabelli's trait expression are likely linked via: (a) long duration of tooth growth, and (b) reduced inhibition during enamel knot formation (Harris, 2007; Kondo & Townsend, 2006). In an Ohio orthodontic dental cast sample, Hunter et al. (2010) found the predicted statistically significant negative relationship between Carabelli's trait expression and mean relative intercusp distance (RICD), which reflects proximity of secondary enamel knots. First molars exhibiting cuspal forms (i.e., high expression grades) of Carabelli's trait possessed significantly smaller RICDs than M1s with low Carabelli's trait expression. Interestingly, no significant difference in crown size was found between M1s exhibiting high and low expressions of Carabelli's trait. This result suggests that intercusp spacing—as it reflects secondary enamel knot placement—has a greater influence on accessory cusp size and number than does tooth germ size (Jernvall, 2000). Importantly, Hunter et al. (2010) found that asymmetry in Carabelli's trait

expression between individually paired M1s (left and right antimeres) was associated with asymmetry in intercuspal spacing, indicating that variation in developmental parameters can yield differences in crown morphology even within an individual (and genotype).

A more recent study by Moormann et al. (2013) explored the relationship between Carabelli's trait expression and RICD in metamerer of the permanent molar row (M1 and M2) (Moormann et al., 2013). Using the same Ohio dental cast sample, this study found that unlike M1, M2 did not follow the predicted relationship between Carabelli's trait expression and RICD. Moormann and colleagues noted that fewer M2s than M1s exhibited cuspal forms of Carabelli's trait (Guatelli-Steinberg et al., 2013; Moormann et al., 2013). Weaker Carabelli's trait expression in M2 may be explained by differences in RICD between M1 and M2. For its size, M1 exhibited smaller intercuspal distances than M2 (Guatelli-Steinberg et al., 2013; Moormann et al., 2013); this result is consistent with the PCM, where M1's smaller RICDs should allow for more frequent and pronounced expression of Carabelli's trait compared to M2. Nevertheless, these metameric patterns fail to explain why variation in Carabelli's trait expression among M2s is inconsistent with PCM predictions. Moormann and colleagues did not include the M2/M3 metameric pair in their study due to insufficient M3 data. That said, modern dental cast samples often lack observable M3s, and M3s rarely exhibit Carabelli's trait (Bailey, unpublished data). Thus, dm2/M1 represents another metameric pair that we feel is particularly worthy of study (see Objectives Section).

3 | OBJECTIVES

This study builds on previous research by testing the ability of the PCM to predict observed variation in Carabelli's trait expression between individually paired maxillary deciduous second (dm2) and permanent first (M1) molars.² There are a number of reasons why dm2 and M1 pairs may provide a more appropriate test of the PCM than permanent metamerer. The dm2 has been identified as the most stable tooth of the deciduous postcanine dentition, while the M1 has been identified as the most stable tooth of the permanent molar field (Butler, 1939; Dahlberg, 1945; Liversidge & Molleson, 1999; Margetts & Brown, 1978; Townsend, Harris, Lesot, Clauss, & Brook, 2009). The dm1, while also a primary postcanine element, exhibits an irregular and often premolariform crown shape; previous studies have shown dm1 to be more variable in size and morphology than dm2 (Edgar & Lease, 2007; Farmer & Townsend, 1993; Liversidge & Molleson, 1999). For this reason, the deciduous second molar is considered the anteriormost member of a molar district comprised of four meristic elements derived from the primary dental lamina: dm2, M1, M2, and M3 (Edmund, 1962; Kraus & Jordan, 1965; Saunders & Mayhall, 1982). Although dm2 and M1 differ in size, they are strikingly similar in crown morphology. The M2, on the other hand, is often reduced in size and cusp number, especially in recent humans (Butler, 1939; Dahlberg, 1945; Harris & Dinh, 2006; see also Moormann et al., 2013).

The dm2 and M1 are also more similar in their developmental timing and growth duration than are M1 and M2. Both dm2 and M1 begin

crown formation *in utero* and approach crown-completion within the first few years of life (Butler, 1967; Kraus & Jordan, 1965; Liversidge & Molleson, 1999; Ubelaker, 1978). Earlier formation times may buffer the dm2 and M1 from environmental influences to a greater extent than the later-forming members of the molar district (M2 and M3). If so, crown size and shape for dm2 and M1 may reflect an individual's underlying genotype more directly (Saunders & Mayhall, 1982; Smith, Gomorri, Spitz, & Becker, 1997; Smith & Tillier, 1989; c.f., Guatelli-Steinberg, Scullini, & Edgar, 2006). Indeed, it has been argued that the dm2 is less susceptible to environmental influence than are the permanent molars, because it completes calcification earlier during the perinatal period (Butler, 1967; Kraus, 1959; Kraus & Jordan, 1965; Turner, 1963). In a similar vein, others have noted that the dm2 exhibits the most evolutionarily "conservative" morphology of all molar row elements (Alberch, 1980; Alberch et al., 1979; Bailey, Benazzi, & Hublin, 2014; Bailey, Benazzi, Paul, Astorino, & Hublin 2016b; Smith et al., 1997; Smith, Koyoumdjisky-Kaye, Kalderon, & Stern, 1987; Sofaer, 1973; Townsend & Brown, 1981).

Carabelli's trait is more often expressed as a cuspal form on both dm2 and M1 than on M2 (Kieser, 1984; Saunders & Mayhall, 1982; Thomas, Kotze, & Nash, 1986). Consequently, dm2 and M1 are considered focal teeth for Carabelli's trait observation (Dahlberg, 1956, 1963; Hanihara, 1961, 1963; Lease, 2003; Scullini, 1998; Turner et al., 1991). Previous studies have found that, within individuals, when present, Carabelli's trait expression is more pronounced on dm2 than on M1 (Bermúdez de Castro, 1989; Dahlberg, 1963; Edgar & Lease, 2007; Saunders & Mayhall, 1982; Smith et al., 1987; Townsend & Brown, 1981). This may be unexpected given the deciduous molar's absolutely smaller crown size and the positive relationship between crown size and trait expression predicted by the PCM. On the other hand, if cusp tips are positioned relatively closer in dm2 than they are in M1, this finding would confirm that intercuspal spacing has a greater influence on accessory cusp expression than absolute crown size (see Hunter et al., 2010; Jernvall, 2000).

3.1 | Hypotheses

This study tests three hypotheses. The first two concern variation between metamerer within individuals. Within individuals, M1 is always larger in size than dm2 (Astorino, Paul, & Bailey, 2015; Bailey, Benazzi, Buti, & Hublin, 2016a; Bailey et al., 2014; Butler, 1967). Because M1 possesses a larger tooth germ than dm2 during development, it possesses absolutely more epithelial cells from which additional enamel knots may be created. Therefore, we expect that M1 will form a greater number of accessory cusps than dm2 (Jernvall, 2000).

If the difference in Carabelli's trait expression within individuals is driven primarily by tooth germ size, we predict that in dm2/M1 pairs M1 will exhibit higher grades of Carabelli's trait expression. It is worth noting that this hypothesis—based on tooth germ size alone—runs counter to observations in the literature, which suggest that dm2 often expresses Carabelli's trait more frequently and in higher grades of expression than M1 (Bermúdez de Castro, 1989; Dahlberg, 1963; Edgar & Lease, 2007; Saunders & Mayhall, 1982; Smith et al., 1987;

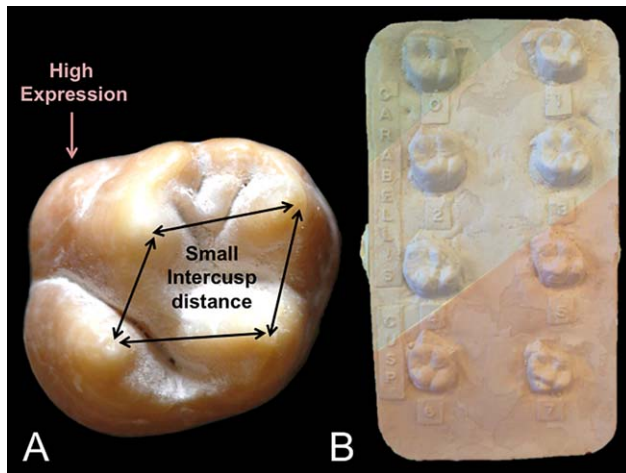


FIGURE 1 Occlusal surface image of a maxillary molar exhibiting a “high expression” of Carabelli’s trait. (A) Following predictions generated from the Patterning Cascade Model, smaller intercusp distances should be associated with higher expressions of Carabelli’s trait. (B) Carabelli’s trait expression is often quantified using Arizona State University Dental Anthropology System standards, which include eight ordinal grades (0–7). For the purposes of the present study, these grades have been collapsed into a three character state system that includes “absent” (grade 0) (blue), “low expression” (grades 1–4) (white), and “high expression” (grades 5–7) (pink). (Maxillary permanent first molar pictured)

Townsend & Brown, 1981). Therefore, we predict that if variation in expression is driven primarily by secondary enamel knot configuration (as approximated by intercusp spacing) and not by absolute size, the metamere with smaller RICDs, regardless of absolute crown size, will exhibit higher grades of Carabelli’s trait expression (Figure 1).

The third hypothesis examines variation within specific elements across individuals. We predict a negative relationship between RICD and Carabelli’s trait expression for both dm2 and M1 separately. That is, Carabelli’s trait expression will be greater when RICDs between the four primary cusps are smaller (Figure 1A). This hypothesis follows Jernvall (2000) and is consistent with previous results showing a significant relationship between mean RICD and Carabelli’s trait expression for M1 (although not for M2) (Guatelli-Steinberg et al., 2013; Hunter et al., 2010; Moormann et al., 2013). An important distinction between this study and previous studies is that we include a geographically diverse sample. This is important because Carabelli’s trait varies, both in overall frequency and in expression by grade, across different world populations (Scott & Turner, 1997). Yet if the biological phenomenon outlined by the PCM alone accounts for variation in Carabelli’s trait expression, we would expect the predictions of the model to be upheld across populations.

4 | MATERIALS AND METHODS

Morphological and metric data were collected from maxillary dm2 and M1 pairs in subadults curated in archaeological collections (Table 1). The dm2 and M1 are concurrent in the tooth row for a period of up to seven years (Anderson, Thompson, & Popovich, 1976; Moorrees,

Fanning, & Hunt, 1963a, 1963b; Schour & Massler, 1944; Smith, 1991; Ubelaker, 1978, 1987). This limits relevant samples to those individuals aged between approximately 5–12 years (Anderson et al., 1976; Schour & Massler, 1944; Smith, 1991; Ubelaker, 1978, 1987). In addition, because of the differences in eruption timing of dm2 and M1 (approximately four years), a critical period exists during which dm2 is present but not so worn as to preclude morphometric observation (Liversidge & Molleson, 1999; Schour & Massler, 1944; Ubelaker, 1978, 1987). We collected data from the side possessing the least worn dm2 and M1 in each individual. Ultimately, 49 individuals representing eight major geographic regions were included in our analysis (Table 1).

4.1 | Data collection methods

Intercusp distances (ICDs) and crown base areas were collected from calibrated images of occlusal crown surfaces (procedures adapted from Bailey, 2004; Morris, 1986; Wood & Abbott, 1983; Wood & Engleman, 1988) by KSP. Following Bailey (2004), we photographed dm2 and M1 occlusal surfaces using a leveled Nikon-D80 digital camera with a macro setting (KSP) or a Canon Rebel XT fitted with a macro lens (SEB). A previous study showed that, using similar methods, independent photography by different researchers resulted in a level of inter-observer error that was no greater than the level of intra-observer error (Bailey, Pilbrow, & Wood, 2004). Therefore, images collected by KSP and SEB were pooled into a single sample. Each image included a

TABLE 1 Sample composition for PCM study

Region of Origin	Sample Size ^a	Housing Location ^b
East Asia		
Thailand	1	AMNH
Mediterranean		
Greece	1	AMNH
Turkey	2	AMNH
North America		
Arizona	3	AMNH
British Columbia	5	AMNH
Mexico	1	AMNH
Pacific Islands		
Marquesas Islands	3	AMNH
South Africa	5	UW
South America		
Peru	5	AMNH
West Africa		
Ghana	1	AMNH
Western Europe		
Austria/Germany	6	AMNH
England	16	NHML

^aSample size refers to the skeletal individual count and not to tooth count. Each individual is represented by both dm2 and M1.

^bAMNH = American Museum of Natural History, Biological Anthropology Collections in New York, U.S.A. NHML = Natural History Museum in London, U.K. UW = University of Witwatersrand in Johannesburg, S.A.

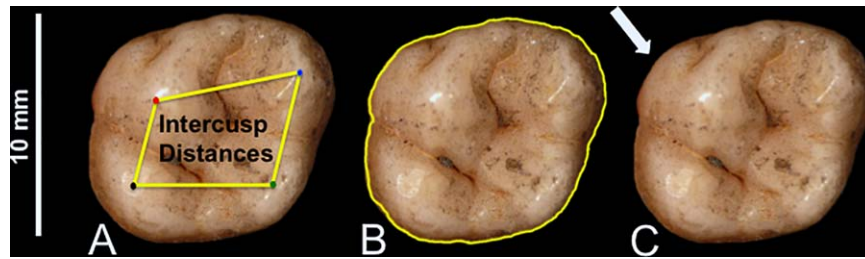


FIGURE 2 Dental data collected from scaled 2D images and primary observation included: (A) distances between cusp tips of the protocone (red dot), paracone (blue dot), metacone (green dot), and hypocone (black dot); (B) crown base area; and (C) Carabelli's trait expression. (Maxillary deciduous second molar pictured.)

millimeter scale mounted at a plane leveled between the cusp tips and the occlusal basin (Bailey, 2004; Gómez-Robles et al., 2007; Suwa, Wood, & White, 1994; Wood & Abbott, 1983; Wood & Engleman, 1988). Cusp apices were marked directly on digital images in PhotoShop 6.0 and iPhoto editor while referencing the original tooth morphology for accurate placement (Figure 2).

Crown base areas and ICDs were measured by KSP using the digital data capture program ImageJ (Rasband, 1997–2015; Schneider, Rasband, & Eliceiri, 2012). For each photograph, a measurement (in pixels) was taken of the scale three times in ImageJ. The average of these three measurements was used to calibrate the photos prior to data collection. Following previous work, ICDs were measured between pairs of cusp tips on the crown surface using ImageJ's line tool (Figure 2A). ImageJ's trace function was used to measure crown area. Crown base area was calculated by tracing the crown outline at cross-sectional maximum (Figure 2B). When encountering interproximal wear, the original outlines were estimated with reference to overall crown form (see Bailey, 2004; Gómez-Robles et al., 2007; Suwa et al., 1994; Wood & Engleman, 1988).

We (SEB and KSP) scored Carabelli's trait expression using the Arizona State University Dental Anthropology System (ASUDAS) (Turner et al., 1991; see Figures 1B and 2C). In this system, Carabelli's trait expression is scored according to eight grades of expression: from grade 0 (mesiolingual aspect of protocone is smooth) to grade 7 (large cusp with free apex present). Grades 1 to 4 represent non-cuspal forms manifested as grooves, divots, and V-shaped impressions, while grades 5 to 7 represent cuspal forms (Turner et al., 1991). We also converted the ordinal ASUDAS data to three character states representing absence (ASUDAS grade 0); low expression (ASUDAS grades 1–4); and high expression (ASUDAS grades 5–7) (Figures 1B and 3). These protocols were chosen to remain consistent with previous studies (Hunter et al., 2010; Moormann et al., 2013).

4.2 | Analytical methods

A subset of the original sample (approximately 68.0%) was re-measured and re-scored by KSP to quantify intra-observer error. To assess inter-observer error, Carabelli's trait expression was scored by KSP and SEB on 21 individuals. Intra- and inter-observer error estimates were calculated following Kieser (1990, p. 14) for all metric data. Observer error for Carabelli's trait scores was examined by assessing

pairwise difference between subsequent scoring sessions (intra-) and between observers (inter-), respectively (Hillson, 1996, p. 99).

We tested the assumption of normality for all metric data by referencing normal probability plots and, more formally, the results of Shapiro–Wilk tests. As the assumption of normality was not violated for the final dataset, parametric statistical tests were applied to all metric data. Relative inter-cusp dimensions were included in the analysis to control for size differences between the dm2 and M1. Relative inter-cusp distance (RICD) was quantified as absolute ICD divided by the square root of the crown area (SQRTA) (i.e., $RICD = ICD/SQRTA$). These relative dimensions serve as a proxy measurement of the proximity of secondary enamel knots and final positioning of cusp tips in relation to the crown perimeter (Bailey, 2004; Wood & Engleman, 1988; and following Hunter et al., 2010; Moormann et al., 2013). We first examined the relationship between the size-standardized crown

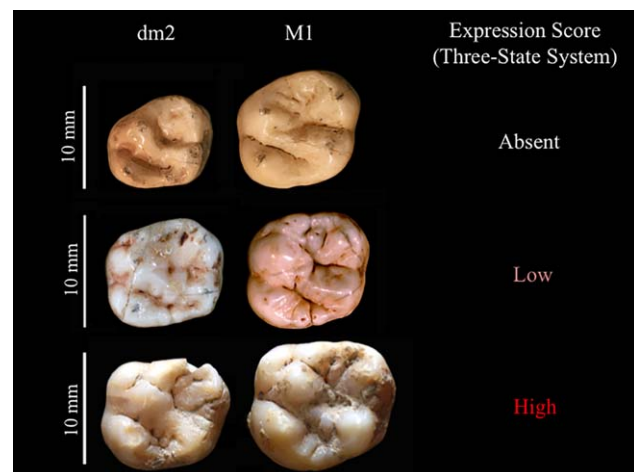


FIGURE 3 Paired metameres exhibiting a range of Carabelli's trait expression, as quantified under the three-state system: absent (Individual BC-99/1622); low expression that includes grooves, divots, and V-shaped impressions (Individual SA-1320); and high expression that includes attached cusplules and cusplules with free apices (Individual PB-601). We note that Individual BC-99/1622 was scored as absent for Carabelli's trait expression on the permanent molar, while the deciduous molar exhibited low expression. Courtesy of the Division of Anthropology, American Museum of Natural History, the University of Witwatersrand, and the Natural History Museum, London. Individual BC-99/1622 corresponds to AMNH Anthropology catalog number 99/1622.

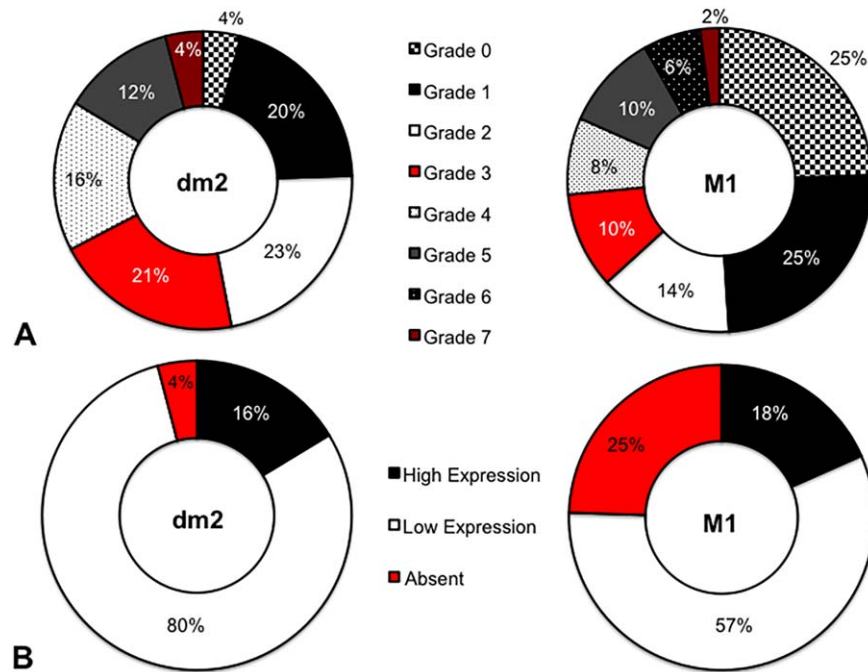


FIGURE 4 Distribution of Carabelli's trait expression for the dm2 and M1 samples, as quantified under (A) the Arizona State University Dental Anthropology System, and (B) the three-state system.

area and Carabelli's trait expression to ensure that the RICD metric reflects solely intercuspal spacing and adequately controls for crown size. To do this, we reduced both our dm2 and M1 sample to only those teeth (19.4% total sample) possessing Carabelli's trait at either extreme of the ASDUAS expression range (ASUDAS grade 0 versus ASUDAS grades 6–7). Grades 6 and 7 were pooled because few teeth (5.1% total sample) exhibited cuspal form Carabelli's trait. We then used *t*-tests to compare mean square root crown area between teeth at separate ends of the ASDUAS expression range. To ensure that these results were robust, we repeated these analyses with a larger sample (31.6% total sample), where the upper extreme of the ASDUAS range included grades 5 through 7.

We conducted paired *t*-tests by metric variable to test for significant differences in RICDs between dm2 and M1, and we used Wilcoxon signed-rank tests to test for significant differences in Carabelli's trait expression between dm2 and M1. Following Hunter et al. (2010) and Moormann et al. (2013), we also used proportional odds logistic regression to explore the relationship between RICD (independent variable) and Carabelli's trait expression (dependent variable). These analyses were run separately by tooth type (dm2 versus M1) and by scoring system (Carabelli's trait expression as quantified using the eight-grade system versus the three-grade system). To avoid multicollinearity, multiple RICDs were never included as co-predictors within the same model. Likelihood ratio chi-square was used to test the significance of model fit improvement over a null model. For each model, we tested the assumption of proportionality using a parallel lines test where the null hypothesis states that slope coefficients are equal across all Carabelli's trait expression grades, and significance was set at $p < 0.10$. We used more relaxed standards to test this assumption due to our fairly small sample size, as

well as the composition of our dataset, which included few individuals exhibiting high cuspal-form expressions of Carabelli's trait. All analyses were conducted in SPSS v. 22 (IBM Corporation, 2013).

5 | RESULTS

Intra-observer error was calculated using metric data (i.e., crown area, ICD) collected by the first author (KSP). Intra-observer error results fell within an acceptable range for all metric traits (mean absolute difference between observations < 0.20 mm) (Kieser, 1990; Kieser & Groeneveld, 1991). Mean intra-observer error was 0.6% (range: 0.0–4.7%) for the metric dataset. It is difficult to accurately score Carabelli's trait expression from two-dimensional images, because low expression grades are not clearly observable in occlusal view. Therefore, the morphological dataset included ASDUAS scores recorded by either KSP or SEB during primary observation. Average intra- and inter-observer error for Carabelli's trait expression fell within 0–1 grade. Conflicting error altered presence/absence dichotomization at a rate of 2.9% when a breakpoint was set at ASDUAS grade 0 (grade 0 versus grades 1–7), and at a rate of 3.9% when this breakpoint was adjusted to ASDUAS grade 5 (grades 0–4 versus grades 5–7).

Results of *t*-tests revealed no significant difference in mean square root crown area for teeth exhibiting the lowest and highest degrees of Carabelli's trait expression for both the dm2 ($p = 0.15$) and M1 ($p = 0.33$). When we repeated these analyses with a larger sample (where the upper extreme of the ASDUAS range included grades 5–7), the *t*-test results again revealed no significant difference in square root crown area between teeth on which Carabelli's trait was minimally and maximally expressed (dm2: $p = 0.93$; M1: $p = 0.78$). Therefore, we concluded that

TABLE 2 Results of the intra-individual metameric comparative analyses

Crown Trait	dm2 Mean (SD) ^a	M1 Mean (SD) ^a	Paired Test Statistic ^b	<i>p</i> -value ($\alpha = 0.05$) ^c
Area				
Base Crown Area ^d	73.19 (7.88)	96.77 (10.08)	-24.28	<0.01
Square Root Crown Area	8.55 (0.45)	9.83 (0.51)	-26.08	<0.01
Carabelli's Trait ^e				
ASUDAS	2.82 (1.65)	2.18 (2.03)	214.00	0.01
Three-State	1.13 (0.42)	0.94 (0.66)	4.00	0.03
Absolute Intercusp Distance ^d				
Protocone-Paracone	5.57 (0.56)	6.55 (0.61)	-12.04	<0.01
Paracone-Metacone	4.46 (0.48)	6.41 (0.48)	-7.10	<0.01
Metacone-Hypocone	5.58 (0.60)	6.35 (0.05)	-6.27	<0.01
Hypocone-Protocone	4.12 (0.42)	4.85 (0.60)	-7.96	<0.01
Mean	4.98 (0.38)	5.69 (0.45)	-11.97	<0.01
Relative Intercusp Distance				
Protocone-Paracone	0.65 (0.05)	0.67 (0.05)	-1.91	0.06
Paracone-Metacone	0.52 (0.05)	0.51 (0.04)	1.65	0.11
Metacone-Hypocone	0.68 (0.06)	0.65 (0.05)	3.54	<0.01
Hypocone-Protocone	0.48 (0.04)	0.49 (0.05)	-1.16	0.25
Mean	0.58 (0.03)	0.58 (0.03)	0.83	0.41

^aSD = standard deviation; metric variables in mm or mm².

^bPaired *t*-test results for all metric variables and non-parametric Wilcoxon signed-rank test results for Carabelli's trait (d.f. = 48).

^cStatistically significant results ($p < 0.05$) bolded.

^dArea unit of measurement = mm²; Absolute intercuspal distance unit of measurement = mm.

^eASUDAS = expression scored according to Arizona State University Dental Anthropology System standards. Three-State = expression scored according to the three character state schema (i.e., absent, low expression, high expression). Continuity corrections were applied to account for the structure of the ordinal datasets. Sample size adjustments were made to account for pairs with equal expression scores; *p*-values were approximated based on two-tailed critical values for these adjusted sample sizes at $\alpha = 0.05$.

by using SQRTA as the standardization factor to calculate RICD, analyses exploring the relationship between RICD and Carabelli's trait expression address primarily the influence of intercuspal spacing.

5.1 | Morphological results

We first compared Carabelli's trait expression for the dm2 and M1 samples without regard to metameric pairing (Figure 4). Across our entire dataset, dm2s exhibited Carabelli's trait with higher average ASUDAS grades of expression (2.82) than M1 (2.18) (Table 2). When scored via the three-state system (absent = ASUDAS grade 0; low expression = ASUDAS grades 1–4; high expression = ASUDAS grades 5–7), high expression was observed in 16.3% of dm2s and 18.4% of M1s (Figure 4B). However, under the same system, Carabelli's trait was more often scored as absent for M1s—24.5% versus 4.1% in the dm2 sample (Figure 4B). In other words, while the dm2 and M1 samples were comparable in their incidence of high expressions (i.e., cuspal forms of Carabelli's trait), low expressions (i.e., grooves, divots, and V-shaped impressions) were less frequent in M1.

We now turn to the results of the paired analyses. Twenty percent of individuals (10) in the sample possessed a dm2 and M1 that exhibited identical ASUDAS scores for Carabelli's trait expression (Figure 5). When the ASUDAS was collapsed to the three-state sys-

tem, concordance between metameres increased to 65.3% (Figure 5; for examples see Figure 3). Figure 5 presents intra-individual metameric variation in Carabelli's trait expression across the entire sample. However, if we considered only the paired molars that differed in their ASUDAS scores, the higher score was recorded for the deciduous molar in 69.2% of cases (76.5% when scored under the three-state system). Results of Wilcoxon signed-rank tests indicate that paired dm2s and M1s differ significantly in Carabelli's trait expression when quantified using ASUDAS standards ($T = 214.00$,

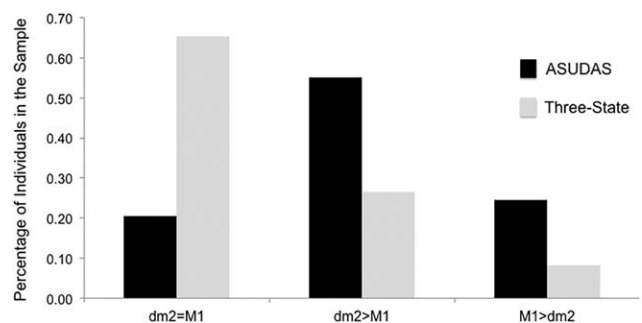


FIGURE 5 Intra-individual metameric comparison of Carabelli's trait expression under the Arizona State University Dental Anthropology System (ASUDAS, eight ordinal grades) and the three-state system across the sample.

TABLE 3 Proportional odds logistic regression results: Carabelli's trait (ASUDAS) and relative interscup distances

	Coefficient ^b	Threshold Value ^a							LR χ^2 ^c	p-value ^c	Odds ^d
		1	2	3	4	5	6	7			
dm2											
Proto-Para ^e	-13.31	-12.07	-9.95	-8.79	-7.82	-6.86	-	-5.32	7.01	0.01	0.26
Para-Meta ^{e*}	-1.57	-3.98	-1.94	-0.93	-0.83	0.83	-	2.34	0.09	0.76	-
Meta-Hypo ^e	-11.16	-10.90	-8.76	-7.63	-6.71	-5.80	-	-4.25	5.88	0.02	0.33
Hypo-Proto ^e	-1.01	-3.65	-1.61	-0.61	-0.24	1.15	-	2.67	0.03	0.86	-
Mean	-21.17	-15.69	-13.55	-12.39	-11.45	-10.51	-	-8.99	6.04	0.01	0.12
M1											
Proto-Para ^e	-10.17	-8.02	-6.88	-6.27	-5.77	-5.28	-4.31	-2.82	3.70	0.06	0.36
Para-Meta ^e	-1.51	-1.90	-0.81	-0.23	0.25	0.73	1.66	3.11	0.62	0.80	-
Meta-Hypo ^e	-2.18	-2.54	-1.45	-0.87	-0.40	0.08	1.01	2.49	0.24	0.63	-
Hypo-Proto ^e	0.06	-1.09	-0.01	0.58	1.05	1.52	2.45	3.90	0.00	0.99	-
Mean	-6.49	-4.91	-3.82	-3.22	-2.74	-2.26	-1.32	0.14	0.83	0.36	-

^aProjected cutoff value of underlying latent trait expression used to differentiate between grades of observed Carabelli's trait expression (i.e., response variable in our model where ASUDAS grades range from 0 to 7) when predictor values equal zero (i.e., relative interscup distance = 0). For example, it is estimated that M1s characterized by latent trait expression greater than 3.90 would be classified as grade 7 when their relative hypocone-protocone distance equals zero.

^bEstimates of ordered log-odds regression coefficients.

^cLikelihood ratio (LR) chi-square test used to assess model fit. Model fit only considered in comparison to a null model with no predictors ($\alpha = 0.05$, d.f. = 1). Significant model results bolded.

^dFor interpretability, odds ratios were scaled to 0.10, which is equivalent to approximately one-fourth of the range of all observed relative interscup distances (0.37–0.80) in the sample or equivalent to one-half of the range of all relative interscup distance averages (0.48–0.68; see reported means in Table 2). Odds are also presented for the M1 protocone-paracone relative interscup distance predictor model, because this model approached significance ($p = 0.06$).

^eProto = protocone; para = paracone; meta = metacone; hypo = hypocone. Models violating the assumption of proportionality (parallel lines test $p < 0.10$) are marked with asterisks. These results should be interpreted with caution.

$p = 0.01$) as well as when quantified using a three character state system (absent, low expression, high expression) ($T = 4.00$, $p = 0.03$) (Table 2).

5.2 | Dimensional results

As expected, paired t -test results confirmed that dm2s and M1s differed significantly in absolute crown area ($t = -24.28$, $p < 0.01$; see Table 2). Paired dm2s and M1s also differed in mean absolute ICD ($t = -11.97$, $p < 0.01$) and all individual absolute interscup (e.g., protocone-paracone) distances (t -range = -12.04 to -6.27 , $p < 0.01$; see Table 2). As expected, based on its smaller size, dm2 consistently possessed smaller absolute interscup dimensions than did M1. On average, the M1 was 15.0% larger in square root crown area than dm2, and 14.2% larger in absolute ICD than dm2. Interestingly, for individual absolute ICDs involving the protocone—the primary cusp from which Carabelli's trait originates—we observed a different pattern. In this case, the M1 showed a disproportionately larger increase ($\sim 18.0\%$) in ICD between both protocone-paracone and hypocone-protocone. These distances are greater than would be expected if dm2/M1 crown scaling were completely isometric. This leads to the dm2 possessing relatively smaller protocone-paracone and hypocone-protocone distances, which falls in line with the predictions of the PCM.

Our intra-individual comparisons between paired dm2s and M1s pairs provided somewhat mixed results. Contrary to expectations of the PCM, mean RICD did not differ significantly between metameres ($t = 0.83$, $p = 0.41$; see Table 2). For individual relative cusp distances, paired dm2s and M1s only differed significantly for the relative distance between the metacone and the hypocone (m-h) ($t = 3.54$, $p < 0.01$; dm2 mean relative m-h distance = 0.68, M1 mean relative m-h distance = 0.65), while the relative distance between the protocone and the paracone (p-p) approached statistical significance ($t = -1.91$, $p = 0.06$; dm2 mean relative p-p distance = 0.65, M1 mean relative p-p distance = 0.67; see Table 2).

5.3 | Regression results

Table 3 presents results of the proportional odds logistic regression analyses, which were performed separately for dm2 and M1. Results of parallel lines tests confirm that only one model (the dm2 paracone-metacone RICD predictor model) violated the assumption of proportionality. Results for this model, therefore, should be interpreted with caution. With the exception of hypocone-protocone RICD in M1, we found the predicted negative relationship between all RICDs and Carabelli's trait expression (Table 3). For dm2, this relationship was significant for mean RICD, protocone-paracone RICD, and metacone-hypocone RICD. For M1, no relationships between RICD and

TABLE 4 Proportional odds logistic regression results: Carabelli's trait (three-state system) and relative intercuspal distances

	Coefficient ^b	Threshold Value ^a		LR χ^2 ^c	p-value ^c
		Low	High		
dm2					
Proto-Para ^d	-6.79	-8.36	-2.75	0.82	0.37
Para-Meta ^d	8.76	0.61	6.28	1.58	0.21
Meta-Hypo ^d	-5.14	-7.39	-1.82	0.60	0.49
Hypo-Proto ^d	-1.74	-4.71	0.80	0.04	0.84
Mean	-3.18	-5.73	-0.22	0.07	0.80
M1					
Proto-Para ^d	-9.13	-7.30	-4.58	2.44	0.12
Para-Meta ^d	1.88	-0.17	2.46	0.08	0.78
Meta-Hypo ^d	-5.37	-4.62	-1.96	1.11	0.29
Hypo-Proto ^d	2.67	0.19	2.82	0.30	0.58
Mean	-5.17	-4.13	-1.50	0.41	0.52

^aProjected cutoff value of underlying latent trait expression used to differentiate between categories of observed Carabelli's trait expression (i.e., response variable in our model where absent = 0, low expression = 1, and high expression = 2) when predictor values equal zero (i.e., relative intercuspal distances = 0). For example, it is estimated that M1s characterized by latent trait expression less than 0.19 would be classified as "absent" when their relative hypocone-protocone distance equals zero.

^bEstimates of ordered log-odds regression coefficients.

^cLikelihood ratio (LR) chi-square test used to assess model fit. Model fit only considered in comparison to a null model with no predictors ($\alpha = 0.05$, d.f. = 1).

^dProto = protocone; para = paracone; meta = metacone; hypo = hypocone.

Carabelli's trait expression were found to be statistically significant, although the relationship involving protocone-paracone RICD approached significance ($p = 0.06$).

To improve the interpretability of the results obtained from proportional odds logistic regression, and following Hunter et al. (2010) and Moormann et al. (2013), we scaled our resulting odds ratios to 0.10. This value is equivalent to approximately one-fourth of the range of all observed RICDs in the sample or equivalent to one-half of the range of all RICD averages (see reported means in Table 2). We accomplished this by dividing the logit-scale coefficients by ten and exponentiating the quotients. Results indicate that if a dm2 (e.g., dm2-A) is characterized by a mean RICD value that is 0.10 unit greater than that of another dm2 (e.g., dm2-B), dm2-A is only 12.0% as likely to exhibit equal or greater Carabelli's trait expression than dm2-B (Table 3). Additionally, when dm2-A exhibits a 0.10 unit greater protocone-paracone RICD, it has approximately one-fourth (0.26) the odds of forming an equal-sized or larger Carabelli's trait than dm2-B. Where dm2-B exhibits a 0.10 unit smaller metacone-hypocone RICD, dm2-A has one-third (0.33) the odds of forming an equal-sized or larger Carabelli's trait (Table 3).

Proportional odds logistic regression analysis was performed again, using Carabelli's trait quantified under the three-state system (i.e., absent, low, or high). Again, the regression coefficients were negative for mean RICD and most individual RICDs (Table 4); the exceptions

were hypocone-protocone RICD in M1 and paracone-metacone RICD in both dm2 and M1 (Table 4). None of the resulting models or regression coefficients were statistically significant.

6 | DISCUSSION

The primary objectives of this study were: (a) to examine if and how dm2s and M1s differed in Carabelli's trait expression in a geographically diverse sample, and (b) to explore the PCM's potential for explaining any observed differences. Our results corroborate previous findings that paired dm2s and M1s differ significantly in Carabelli's trait expression, with dm2 tending to exhibit higher grades of expression (see Bermúdez de Castro, 1989; Dahlberg, 1963; Edgar & Lease, 2007; Saunders & Mayhall, 1982; Smith et al., 1987; Townsend & Brown, 1981). On the one hand, this result is unsurprising as Carabelli's trait diminishes in both frequency and grade of expression toward the distal end of the tooth row. On the other hand, given the smaller crown size of dm2, this finding runs counter to one of the predictions generated from the PCM: that the larger tooth germ (and hence, larger crowned tooth) should exhibit more and/or larger accessory cusps. In comparing M1 and M2, Moormann et al. (2013) stated that smaller crown size and more triangular crown shape (as well as greater relative intercuspal spacing) were among the likely explanations for diminished Carabelli's trait expression in M2 relative to M1. However, our comparison of dm2 and M1 suggests that crown size is not the primary driver of metacone disparities in Carabelli's trait expression.

The other prediction generated from the PCM is that the tooth with tighter cusp spacing (here, smaller RICDs) will exhibit higher grades of Carabelli's trait expression (see Hunter et al., 2010; Moormann et al., 2013). Our results provide mixed support for this hypothesis. On the one hand, and counter to expectations, we found no significant differences in *mean* RICD between dm2 and M1. However, significant differences were found between some of the *individual* intercuspal distances. We found that absolute ICDs involving the protocone (i.e., protocone-paracone distance and hypocone-protocone distance) were disproportionately (~4.0%) larger in the M1 than in the dm2 than would be expected based on crown size scaling between these teeth. That dimensions involving the protocone are generally involved in our significant findings—as well as those showing stronger relationships—is not surprising under the PCM given this cusp's proximity to Carabelli's trait. Based on the PCM, we would expect that the zone of inhibition surrounding the protocone's secondary enamel knot would have the greatest impact on potential Carabelli's trait expression. Small ICDs between the protocone and its neighboring cusps likely reflect a more restricted inhibitory zone, which would accommodate a larger circumferential area for an accessory cusp to form lingually. Previously, Jernvall and Jung (2000) and Guatelli-Steinberg et al. (2013) posited that Carabelli's trait expression might be particularly sensitive to protocone-paracone enamel knot spacing. Cusp initiation begins earliest in the paracone, followed directly by the protocone (Kraus and Jordan, 1965), so it is reasonable that—assuming enamel knot activation follows the same pattern—Carabelli's trait would be most directly affected by protocone-paracone

configuration (Guatelli-Steinberg et al., 2013; Jernvall & Jung, 2000). Our results provide some support for this hypothesis.

Yet, despite significant differences in absolute crown dimensions for dm2/M1 pairs, difference in *relative* protocone-paracone distance only approached significance at a *p*-value of 0.06. In fact, the only RICD to significantly differ between dm2 and M1 was metacone-hypocone RICD. Furthermore, nearly all RICDs shared the predicted negative relationship with Carabelli's trait expression, but this relationship was statistically significant for just three dimensions of the dm2, while none of the dimensions of the M1 were statistically significant (Table 3). It is important to note that these results only pertain to the regression models based on the ASUDAS data. All models based on the three-state system data yielded non-significant results. These findings conflict with those of previous studies, which showed M1 to strongly adhere to PCM predictions (e.g., Hunter et al., 2010; Moormann et al., 2013), yet they corroborate findings reported in a more recent study of Carabelli's trait expression at the EDJ (Ortiz, 2016). In a sample of 35 *Homo sapiens* permanent maxillary molars, Ortiz (2016) found a weak positive relationship between RICD and Carabelli's trait expression, counter to PCM predictions. When the sample was expanded to include multiple *Homo* species (*n* = 82 molars), the relationship was negative but failed to reach statistical significance (Ortiz, 2016). It is important to note that the small sample sizes in this and Ortiz's (2016) study may contribute to the lack of statistically significant relationships detected. Small samples are a frequent complication of studies that employ micro-computed tomography scanning like Ortiz (2016), as well as of those that focus on underrepresented demographic subsets in skeletal samples—in our case, subadult individuals possessing mixed and unworn dentitions.

It is unclear why the relationship between cusp spacing and Carabelli's trait expression is more consistent and stronger in dm2 than in M1 for our sample. It is possible that this is due to the more "conservative" nature of dm2, which develops more quickly and is thought to be subject to less environmental influence than M1. For example, M1's (relatively) extended period of crown development may provide greater opportunity for complex interactions between the inhibition factors directing enamel knot placement, external environmental factors, and final crown form. It is also possible that differences in enamel thickness are at least partially responsible. In our sample, Carabelli's trait was more often absent on M1 where dm2 was scored present for low expression (Figure 4). This could be due to thicker enamel in the M1 (Gantt, Harris, Rafter, & Rahn, 2001; Grine, 2002, 2005) that may mask expressions visible at the EDJ (see Bailey, Skinner, & Hublin, 2011; Morita, 2016; Ortiz et al., 2012; Skinner et al., 2008).

Another factor that may have influenced our results is the geographically diverse sample. Unlike populations of European ancestry, which show the highest frequency and expression of Carabelli's trait, our sample did not include a large number of individuals possessing cuspal forms of the trait (Figure 4). This aspect of sample composition might also explain why the relationship between Carabelli's trait expression and RICD was not significant when Carabelli's trait expression was quantified under the three-state system. Previous

work has indicated that there is some genetic component to Carabelli's trait variation (Alvesalo et al., 1975; Boraas, Messer, & Till, 1988; Goose & Lee, 1971; Kieser, 1984; Laatikainen & Ranta, 1996; Townsend & Martin, 1992) and that prevalence varies between populations (Dahlberg, 1951; Irish, 1997; Kraus, 1951; Meredith & Hixon, 1954; Scott, 1980; Townsend & Brown, 1981; Tsuji, 1958; Turner, 1967). That the relationships detected by this study were not as strong as those reported in previous work that focused on a single bioregional sample suggests that underlying gene expression—which may differ between populations—affects Carabelli's trait expression. Furthermore, it suggests that the PCM is likely not the sole predictor of cusp-related trait variation among populations. We are currently exploring whether or not differences in RICDs between populations predict their frequency and expression of Carabelli's trait (Astorino et al., in prep).

The ASUDAS is among the most commonly used scoring systems in dental anthropological research. Yet there has been some interest in how the system's standards reflect true variation in crown anatomy (e.g., Ortiz et al., 2012; Skinner et al., 2008). Previous investigations of Carabelli's trait at the EDJ of permanent molars suggest that low-grade expression does not always involve dentine horns—features that represent enamel knot positions during development (Ortiz, 2016; Ortiz, et al. 2012; Skinner et al., 2008). This raises two issues. First, because the PCM assumes that all accessory cusp expression follows from enamel knot activity, it is possible that only the cuspal forms of Carabelli's trait are appropriate to test the PCM. If true, this would account for the conflicting results of our study and of previous studies (e.g., Moormann et al., 2013; Ortiz, 2016), which included absence or low-grade expression as character states that might be predicted by the PCM. We would argue that more research involving a broader suite of accessory crown features is required to provide an adequate test of the PCM in humans (see Ortiz, 2016). Second, we might question whether the low expression grades (grades 1–4) included in the ASUDAS actually reflect variation along a continuous spectrum of Carabelli's trait expression or, instead, reflect variation in some negative feature (i.e., concavity) on the crown surface that is entirely separate from the Carabelli's trait complex. What our results show—at least in the dm2—is that ASUDAS scoring does track some aspect of crown variation that corresponds to intercuspal spacing in a manner predicted by the PCM. Therefore, one could argue that this scoring system does reflect the full range of expression of this single accessory cusp feature (although this is not fully supported by M1 results or previous studies of EDJ morphology). Yet this raises another issue and one that has important implications for phylogenetic and biodistance research. Because dm2 morphology was predicted by relative intercuspal spacing with some success, our results provide support for suggestions by previous researchers that we cannot always assume the independence of crown characters (Harris, 2007; Hunter et al., 2010; Kangas et al., 2004; Kondo & Townsend, 2006; Moormann et al., 2013; Salazar-Ciudad & Jernvall, 2002). In this way, more nuanced understandings of the bases of dental variation have the practical impact of informing dental anthropological "best practices," especially in the areas of data collection, data analysis, and interpretation of results.

Finally, previous studies have found low heritability estimates for human intercuspal dimensions, suggesting that a high degree of epigenetic and environmental influence contributes to their relatively high phenotypic variability among dental metric traits (Sharma, Corruccini, & Henderson, 1985; Townsend, 1985; Townsend & Alvesalo, 1995; Townsend & Brown, 1978; Townsend, Hughes, & Richards, 2006; Townsend, Richards, & Hughes, 2003). The dm2 and M1 are the two earliest-forming molariform teeth of an individual's tooth row and the two that are typically most similar in crown form. Therefore, as compared to paired M1s and M2s, we suggest that any observed differences in intercuspal spacing between the metameres of the mixed molar row are reflective of epigenetic/developmental-based variation rather than environmental "noise." We might test this hypothesis further by quantifying levels of fluctuating asymmetry versus directional asymmetry in a broader sample. Fluctuating asymmetry data are commonly interpreted as indirect reflections of environmental perturbation (e.g., Bailit, Workman, Niswander, & MacLean, 1970; Guatelli-Steinberg et al., 2006; Siegel & Doyle, 1975; Townsend & Brown, 1981), while directional correspondence in asymmetry between intercuspal spacing and accessory cusp expression is interpreted as supporting the notion that the PCM operates at an epigenetic level (e.g., Hunter et al., 2010). Unfortunately, the nature of our skeletal sample limited access to bilateral dm2/M1 pairs, as well as to a sample size large enough for an adequate assessment of asymmetry (Garn, Cole, & Smith, 1979; Smith, Garn & Cole, 1982). We highlight within-individual, antimeric comparisons of the mixed molar row as a fruitful direction for future research only in the appropriate sample.

7 | CONCLUSION

In this article, we reviewed current understandings of dental morphogenesis and tested the predictive power of the PCM for explaining intra-individual variation in Carabelli's trait expression in dm2/M1 pairs. This PCM study is the first to incorporate deciduous teeth, which is significant because deciduous teeth form *in utero* and may provide greater environmental control than later-forming elements of the molar row. We found that paired dm2s and M1s differed significantly in Carabelli's trait expression, and that dm2 exhibited greater degrees of expression compared to M1. However, contrary to predictions of the PCM we found that neither absolute crown size nor mean relative intercuspal spacing appear to be driving factors behind metameric differences in Carabelli's trait expression. It is worth noting that when examining specific ICDs, we found disproportionate reduction in absolute distances involving the protocone (protocone-paracone distance and hypocone-protocone distance) in the dm2, which provides some support for previous investigations of the model. Our study also supports previous work that identified negative relationships between Carabelli's trait expression (quantified using ASUDAS) and RICD as predicted by the PCM, although these relationships were not always statistically significant for our sample (and never significant for the M1). Sample composition and sample size may explain our mixed results; we suggest that future

studies include larger multiregional samples and a broader suite of accessory crown features, and/or explore PCM predictions at the population level focusing on groups with varying prevalence of Carabelli's trait, especially in the higher grade, cuspal form expressions.

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ENDNOTES

¹ We use "dm2" instead of "dp4" in this study to remain consistent with the dental anthropological literature relating to recent humans (see also Bailey et al., 2016b).

² See footnote¹.

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