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The mandibular molar pit-tubercle (MMPT) dental nonmetric trait: comprehensive analysis of a large sample

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Abstract

Dental nonmetric traits are quasicontinous variables, mostly of genetic origin. Thus, sets of such traits allow biological distance estimation between samples. Mandibular molar pit-tubercle (MMPT) is a buccal trait defined by Weets (2009) in Irish samples. This study aims to analyze a) trait frequencies, b) grade definitions, c) intraobserver error, d) sexual dimorphism, e) asymmetry, and f) trait associations for MMPT in a Portuguese sample.

The first (LM1), second (LM2) and third (LM3) lower molars of 600 identified individuals from the Coimbra collections were scored for MMPT in three scoring sessions. Intraobserver error, bilateral asymmetry and trait correlations were tested using Kendall's τ-b, while sexual dimorphism was verified using Pearson's χ^2.

Frequencies (LM1: 2.1%; LM2: 3.5%; LM3: 30.3%) were similar to previous reports. However, a new free apex cusp form (grade 3+) was detected. Considering three scoring sessions, intraobserver precision was above 85%, and correlation coefficients between observations were positive and moderate to very strong ($0.291 < \tau$-b < 0.835). Intrasample variation was low, since only LM3 showed sexual dimorphism (Female: 30.2%; males: 16.2%; $\chi^2 = 15.512$; $p < 0.001$; df = 1; n = 556) and large asymmetry (27.1%). There were no strong (τ-b > 0.3) intertrait associations involving MMPT.

MMPT shows low trait presence, sexual dimorphism and asymmetry in stable teeth (LM1 and LM2). Intraobserver precision is high, so scoring should be reliable, although a plaque and better threshold grade definition is needed. After further research on non-European samples, MMPT can be useful in biodistance research.

Introduction

The mandibular molar pit-tubercle, or MMPT, is a dental nonmetric trait described by Weets (2004, 2009). Although the MMPT was originally considered as a weak expression of the protostylid which could not be incorporated in the Arizona State University Dental Anthropology System (ASUDAS: Turner et al., 1991), it was soon evident the trait was expressed in a different location and could coexist with the protostylid (Weets, 2009). So Weets initially identified a much more mesial and

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occlusal pit or tubercle, as the Irish Mandibular Molar Pit, IMMP, and later renamed it MMPT (Weets, 2004, 2009).

In late 2009, during the scoring of dental morphology variables for a Master's thesis (Marado, 2010), there was difficulty scoring the protostylid using ASUDAS (Turner et al., 1991). The protostylid is described in ASUDAS as "A paramolar cusp found on the buccal surface of cusp 1. It is normally associated with the buccal groove separating cusps 1 and 3." (Turner et al., 1991:23) and almost all described grades include direct or indirect reference to the buccal groove (Turner et al., 1991). The presence of protostylid-like pits, grooves and tubercles in the mesiobuccal corner of lower molars (several millimeters apart from the buccal groove) confounded the first author. So, Weets's 2009 work was read and MMPT was added to the second observation of the sample (frequencies reported in Marado, 2010, 2012), as suggested by the second author. This motivated the authors to include the trait in further work and investigate its relevant properties and frequencies.

Scott (2008), who contributed to establish some of the ASUDAS dental plaques, proposes a set of principles to follow when defining a trait classification. One of them is defining trait presence across populations. Great care in defining equidistant grades is also pointed out as important by Scott (2008). This should help diminish observer error. Precision in reproducing results obtained by scientific methods is a concern of all researchers (Harris and Smith, 2009; Nichol and Turner, 1986; Scott, 1977; Scott and Turner, 1997), yet a degree of inconsistency is unavoidable (Harris and Smith, 2009); in dental morphology it is caused by the use of a graded scale to measure quasicontinuous variables (Turner et al., 1991), providing an inherent subjectivity (Hillson, 2005). Therefore, the need to calculate scoring errors for each research involving dental nonmetric traits is essential to give them validity (Hillson, 1996). New traits should not be an exception, as also exemplified by Scott (1977).

It is standard practice to pool the sexes in population studies using dental morphology (Irish and Guatelli-Steinberg, 2003; Scott and Turner, 1997; Turner et al., 1991), since most traits are not consistently dimorphic -- except the canine distal accessory ridge (see Scott, 1977) -- and small samples, which would be even smaller samples if considered separately, benefit from the combination of the two sexes (Scott and Turner, 1997). Besides, in some cases, loose teeth from archaeological or paleontological contexts cannot be sexed. However, if the results are significantly and

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systematically different between sexes, it may be unwise to pool sexes in samples with uneven sex ratios (Turner et al., 1991).

Symmetry in trait expression is expected, since theoretically dental morphology is mirrored in antimeric teeth (Bollini et al., 2009; Groeneveld and Kieser, 1991). However, teeth on opposite sides of the jaw can exhibit asymmetry in size, morphology and hypodontia (Scott, 2008; Bollini et al., 2009; Scott and Turner, 1997), which can occur in three types, as fluctuating asymmetry or more systematic forms, directional asymmetry and antisymmetry (Bollini et al., 2009; Gawlikowska et al., 2007; Graham et al., 1993; Klingenberg and Nijhout, 1999; Van Dongen et al., 1999). While fluctuating asymmetry is a constant phenomenon in dental morphology throughout populations (Scott and Turner, 1997), it is influenced by environmental conditions which, if extreme, can also cause more systematic forms of asymmetry (Graham et al., 1993). Those forms, directional asymmetry and antisymmetry, should be detected, because they affect the choice of scoring method (Scott and Turner, 1997). For example, the tooth count method considers all the scored data, and can be useful to characterize small samples (Scott, 1980) -- although the expression count method is helpful in such situations (Turner, 1985) -- yet cannot be used when there is systematic asymmetry, because it misrepresents the underlying genetic information (Scott and Turner, 1997). Besides this, the investigation of a new trait's susceptibility to environmental stressors can be helpful to determine its usefulness in biodistance analyses.

Trait correlation between all teeth of the same morphogenetic field where a dental nonmetric trait was scored is expected (Scott and Turner, 1997) and has been found in several instances (e.g. Scott, 1977; Tocheri, 2002). These trait associations were also found for different traits and morphogenetic fields on occasion (e.g. Moormann et al., 2013; Scott, 1979). The existence of correlations among traits hinders the use of some statistical tests which assume trait independence, particularly the common Mean Measure of Divergence (MMD) (Edgar, 2004; Irish, 2010). Dental nonmetric traits are useful as phenetic expressions of an underlying genomic variation; if a new trait is systematically correlated to other trait(s), its usefulness is questionable. Correlation between MMPT and protostylid should be tested, since Skinner and colleagues (2008, 2009) suggest that all cingular expressions in the buccal surfaces of lower molars are developmentally related and often are found simultaneously in enamel-dentine junctions of hominids. These suggestions also point to the possibility that protostylid and MMPT are different expressions of the same trait, in which case an update to the ASUDAS

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definition of the protostylid would be necessary. Testing the correlation between these two lower molar buccal features will also help understand if they are independently expressed traits.

The aims are to a) report trait frequencies, b) discuss grade definitions, c) provide intraobserver precision, d) measure sexual dimorphism, e) analyze trait symmetry, and f) investigate trait correlations for MMPT (and therefore clarify its relationship with protostylid) on a 19th/20th centuries' identified skeletal sample curated at the Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Portugal.

Materials

The Department of Life Sciences (Faculty of Sciences and Technologies, University of Coimbra) has three large identified osteological collections. Two of these collections, "Trocas Internacionais" (International Exchange Collection) and "Escolas Médicas" (Medical Schools Collections), exclusively comprised of crania and mandibles, were the source of the present sample. That sample includes 600 individuals, 300 males and 300 females (with 13892 teeth, 2373 of which were lower molars). "Escolas Médicas" was gathered by Bernardino Machado (from 1896 to 1903) from Medical Schools in Lisbon, Oporto and Coimbra (Cunha and Wasterlain, 2007; Fernandes, 1985; Rocha, 1995), and is represented by 31 individuals in the present sample. "Trocas Internacionais" was collected by Eusébio Tamagnini (between 1932 and 1942) from the Conchada cemetery in Coimbra (Cunha and Wasterlain, 2007; Fernandes, 1985; Rocha, 1995), and contributed 569 individuals to the sample. Machado and Tamagnini were two of the most prominent early physical anthropologists in Portugal (Santos, 2012).

The majority of the individuals included in the sample were Portuguese citizens born between 1820 and 1924; although nine were born abroad and five had no exact birth records (they were "expostos", a Portuguese term referring to children who were abandoned by their parents -- direct English translation: "exposed"). All of the individuals analyzed died between 1896 and 1938 in Portugal, between the ages of 7 and 97 years, with a mean age of 35.93 (sd=16.79) years.

Methods

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The provisional trait expression score sequence suggested by Weets (2009) was adapted, since a further grade (3+; see Figure 1, displaying grades 1 to 3+) was added, to account for cusps with free apices, detected by the first author during early observations (Marado, 2010) and found again in the present sample. Weets (2009) indicated there was no cuspal form of MMPT in the Irish diachronic samples he studied.

Calculation of trait frequencies used the individual count method, and the most expressive side was used when there was asymmetry; side differences and intraobserver error were reported using the tooth count method (Scott, 1980; Scott and Turner, 1997; Turner, 1985). Associations between MMPT and other traits were calculated using Kendall's τ-b rank correlation coefficient, to infer if one trait presence or absence influences other trait's expression. Those dental and oral nonmetric variables were scored using ASUDAS and other methods or descriptions (Hauser and De Stefano, 1989; Irish, 1998; Scott and Turner, 1997).

There was a preliminary observation of 100 individuals (50 of each sex, chosen from the final 600 individuals) and two full observations (each scoring session occurred one month apart from the next). Intraobserver precision was calculated for each lower molar as percentages of correct correspondence between paired observations (Pre-1st and Pre-2nd n = 100 individuals; 1st-2nd n = 600 individuals). Kendall's τ-b was also used to calculate the degree of correlation between paired observations, since correlation coefficients provide information on the trend of paired observations, even when scores are not exactly the same between them. The fields analyzed were overall precision, overall correlation (considering the full trait expression score sequence), precision within one grade ("±1"), dichotomized precision, dichotomized correlation (considering correspondence in trait absence or presence), and observable precision. "Overall" refers to data by grades, "dichotomized" concerns data divided by trait absence and presence, and "observable" refers to the reliability of the decision to score MMPT or not (because of tooth wear or destruction of surfaces) on paired observations.

Sexual dimorphism was investigated by comparing the MMPT frequencies of both sexes with a Pearson's χ^2 test of independence. MMPT grade frequencies were reported for each sex independently for comparative purposes.

The impact of asymmetry was inferred by the calculation of the rank correlation coefficient Kendall's τ-b, since fluctuating asymmetry is expected to show positive correlation between sides, while antisymmetry would show null coefficients and directional asymmetry would show negative results. The frequency (as percentages) of

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bilateral asymmetry was also calculated for each lower molar, as overall asymmetry (considering every scored surface) and comparable asymmetry (excluding bilateral trait absence).

Results and Discussion

Trait frequencies

When the MMPT was described (Weets, 2004, 2009), very low frequencies were found on LM1 on the diachronic (Neolithic to Early Christian) Irish sample where the trait was identified. On the Museum of Natural History, University of Porto sample (Marado, 2010, 2012) the trait was absent on LM1; however, the presence breakpoint excludes grade 0-1, which is present in the sample (LMM personal observation). LM2 shows a slightly higher frequency, and LM3 shows frequencies around 30% on the Irish and Portuguese Museum samples (Marado, 2012; Weets, 2009).

The MMPT presence frequencies for the present sample correspond to this pattern (see Table 1): a) low frequency on LM1 (2.1%), b) low frequency on LM2 (3.5%), and c) relatively high frequency on LM3 (30.3%). These Coimbra frequencies are intermediate, between Irish and Portuguese Museum results, for all three lower molars.

Grade frequencies demonstrate that not only is MMPT rarer on LM1 and LM2, it is also less expressive (i.e. the higher trait grades are only observed on LM3). This pattern is also found on the Irish sample, with the exception of the right LM2, which displays lesser frequencies on grades of intermediate expression (Weets, 2009). Diminished trait expression on LM1 and LM2 is also found on the Portuguese Museum sample, when compared to LM3 (LMM personal observation). The proposed grade 3+ was only found on LM3 in the Coimbra sample (Table 1), yet it was first identified on a right LM2, in the Portuguese Museum sample (LMM personal observation).

It should be noted that Weets (2009) reported individual count results on both sides of the mandible, while Marado (2010, 2012) used the tooth count method. Since the present results are reported as individual count method considering each individual's greatest trait expression, the results of these three samples may not be directly comparable. However, a) the pattern of frequencies and of grade distribution is very similar between these samples nonetheless, and b) Table 4 displays tooth count method frequencies for each side.

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Intraobserver reliability

Precisions and correlations for the comparisons between the preliminary observation ("Pre") and the full observations ("1st" and "2nd") are presented on Table 2.

LM1 MMPT observations were very precise in all calculated fields for all sets of observations. The LM1 "1st-2nd" set was the most reliable, with the highest results for overall and dichotomized precision, as well as precision within one grade. "1st-2nd" was not the most precise observable trait comparison by 0.2%, yet was still very reliable. This set of compared observations ("1st-2nd") is of particular relevance, since it pertains to the full sample (600 individuals) and the second observation was used to calculate trait frequencies (Tables 1, 3 and 4).

The only results under 90% are found for LM3 MMPT overall precisions on all sets of observations (e.g. "1st-2nd": 89.2%). This may be related to the greater variability found in MMPT grade distribution, when LM3 is compared to the other scored molars. The greater number of trait presences increases the likelihood of error in scoring the same grade of presence repeatedly. The lowest dichotomized precision found in this sample was also on LM3 ("1st-2nd": 92%). The lowest present expression (grade 1) was found on 21.2% of individuals, with the other present grades distributed along only 9.1% of the sample. This corroborates the interpretation suggested by Nichol and Turner (1986): the least expressive grades are difficult to score correctly, since they represent threshold expressions that can be deemed absent. The inexistence of grade 0-1 in the present sample (Table 1) suggests grade 1 can also be considered a threshold expression of MMPT.

The correlations found are generally positive and moderate to strong, which suggests the scoring guideline adapted from Weets (2009) can be reproduced without systematic error. This is underlined by the relatively high to very high precisions obtained for all fields, which suggest the guideline is clear and covers the extent of variation in MMPT expression. The use of this guideline, preferably after the creation of a plaster cast plaque to exemplify each grade tridimensionally (as is typical for ASUDAS), will likely insure high precision even from relatively inexperienced scorers, after some training.

Precision calculations used the tooth count method. If the individual count method was used, precisions are likely to have been higher, since only the most expressive grade is considered (in individuals with scorable molars of both sides), and less likely to be unreliably scored.

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Sexual dimorphism

While sexual dimorphism is very noticeable in simians (Wood et al., 1991) and in the human evolutionary line, it is slight in *H. sapiens* (Rodríguez, 2003). Hormonal (Saunders et al., 2007) and chromosomal (Alvesalo, 2013) influences cause sexual dimorphism in enamel and dentine formation, yet dental nonmetric traits are usually not dimorphic and, when they are, the sexual dimorphism is usually inconsistent from sample to sample (Scott and Turner, 1997). Canine distal accessory ridges are the exception (Scott, 1977; Turner et al., 1991), yet the authors of the ASUDAS guidelines only mention the possibility of considering males and females separately in the presence of different sex ratios. Pooling the sexes is a standard practice in dental morphology population studies, because of the usually low sexual dimorphism (Irish and Guatelli-Steinberg, 2003; Scott and Turner, 1997; Turner et al., 1991).

Marado (2010) did not find significant sexual dimorphism for MMPT in the Portuguese Museum sample; however, the sample was sexed using the mandibles (the only available material), and the female sample was very small (23 individuals). Weets (2004, 2009) did not report any sexual dimorphism for the Irish sample.

The Coimbra sample presents significantly different LM3 MMPT frequencies between the sexes (Table 3). On these 200 female individuals and 224 males, 39.2% of females and only 22.0% of males presented MMPT. The two higher grades (3 and 3+) were only present in the female sample. Both samples display greater frequencies of grade 1; yet, while the males' grade distribution shows a steady drop from grade to grade, females' distribution is more irregular, with slight increases from grades 1-2 to 2, and 3 to 3+.

LM1 and LM2 have differences in MMPT presence between female and male individuals, with greater female frequency, but with no statistical significance. There are also sex differences in MMPT grade distribution on LM1 and LM2, mainly because on these molars males show absence of grade 2 and above.

The (significant and not significant) sexual dimorphism found in the present sample and its direction (greater female MMPT frequencies) may be sample-specific. There is no statistically significant sexual dimorphism in the Portuguese Museum sample, as noted above, and on that sample's LM2 there is only MMPT presence in male individuals (6.1%) (Marado, 2010). However, this greater male frequency may be due to the fact that only 14 female LM2's were scorable for the museum sample (compared to 66 male LM2's) (Marado, 2010).

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The existence of sexual dimorphism on LM3 MMPT could be related to the known morphological variability that characterizes said molar (Scott and Turner, 1997), or to the fact LM3 is the most affected by fluctuating asymmetry (Table 4), and may therefore be subjected to the effects of high developmental instability (Bollini et al., 2009; DeLeon, 2007; Klingenberg and Nijhout, 1999). Further research on MMPT sexual dimorphism in different samples will clarify if this trait is consistently dimorphic on LM3 or any of the lower molars.

Asymmetry

Dental morphology is theoretically equal on either side of the dental arches, since the genetic information that guides odontogenesis is the same for both antimeres (Bollini et al., 2009; Groeneveld and Kieser, 1991; Scott, 1977). However, bilateral asymmetry is commonly found in dental nonmetric traits (Scott and Turner, 1997). The definition of the type of asymmetry is of relevance, since it can clarify if the bilateral asymmetry present is fluctuating or systematic (Van Dongen et al., 1999). The more systematic forms of asymmetry (affecting most individuals in a population) are directional asymmetry (a specific side is different in shape or size from the other on most individuals) or antisymmetry (a random side presents a different shape or size from the other in the majority of a population) (Bollini et al., 2009; Klingenberg and Nijhout, 1999; Van Dongen et al., 1999). Fluctuating asymmetry is likely related to environmental factors (lack of nutrients, high viral loads, and other internal or external disturbances) impacting development, or developmental instability (Bollini et al., 2009; DeLeon, 2007; Klingenberg and Nijhout, 1999; Van Dongen et al., 1999). The differentiation between types of asymmetry is important for dental nonmetric traits because of count method selection: if a tooth count method is used, large asymmetry will downplay trait frequencies; if an individual count method is used and a side is preferred, directional asymmetry can greatly impact sample frequencies. Finally, large asymmetry can indicate a trait is very susceptible to developmental instability, and therefore its phenetic expression may not strongly correlate to the underlying genetic information.

Marado (2010) reported no significant bilateral asymmetry for the Portuguese Museum sample on any lower molar for MMPT. No statistical tests were performed by Weets (2009), who reported MMPT frequencies for each side of all lower molars. LM1 and LM2 have similar trait frequencies for both sides, while LM3 has greater MMPT

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presence on the right LM3, and greater frequency of high grades on the left LM3 (Weets, 2009).

Table 4 displays the results for the asymmetry calculations. Overall asymmetry is low on LM1 and LM2, and higher on LM3 (27.1%). Comparable asymmetry (teeth with bilateral MMPT absence are ignored) is high on all lower molars. Correlation coefficients are null (very weak negative) and not significant for LM1, which indicates MMPT is antisymmetric for this sample. This coefficient is probably due to the small number of (all asymmetric) individuals displaying LM1 MMPT. The correlation of both sides is positive, strong and significant for LM2. As for LM3, the weak to moderate correlation is related to both high overall and comparable asymmetry frequencies.

Frequencies for left and right antimeres on LM1 are relatively similar (left: 1.7%; right: 0.9%). The same is true for LM2 (left: 2.9%; right: 2.3%). LM3 has greater frequency differences between sides, and the right side (25%) has greater MMPT frequency than the left (21.1%), contrary to LM1 and LM2.

The bilateral correlations found on LM2 and LM3 rule out antisymmetry, since one side tends to correspond to the other in MMPT expression. Correlations and lack of large differences in frequency between the sides also put aside directional asymmetry. Therefore, LM1 results notwithstanding, the bilateral asymmetry in MMPT expression is characteristically fluctuating, as usual for dental nonmetric traits.

When compared to a large set of traits (results not shown), MMPT asymmetry is only particularly high on LM1 comparable asymmetry (100%). The remaining results correspond to the typical trait asymmetry of this sample, which seemed to be very affected by fluctuating asymmetry (personal observation), due to demographic (Veiga, 2004) and economic (Reis, 1987; Roque, 1982) crises affecting Portugal at the time. Scott's (1977) results regarding the canine distal accessory ridge are considered normal and surpass those found for MMPT, at least overall asymmetry on LM1 and LM2.

MMPT asymmetry should be further studied in different populations, to better understand the typical disruption of symmetry for this trait. The present results suggest care should be taken when comparing frequencies calculated with other than the individual count method, since they might not reflect the true phenetic variation of MMPT, due to large asymmetry. The asymmetry in this sample could however be heightened by poor environmental conditions in 19th and early to mid-20th century Portugal, as noted.

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Trait associations

Trait associations can limit the functions of some statistical tests and impair the analysis of phenetic variation between samples (Edgar, 2004; Harris, 2008; Irish, 2010). These associations have been described before (Moormann et al., 2013; Scott, 1979), and a single protein was found to affect dental nonmetric trait expression, so this relation between variables should be investigated for all samples (Kangas et al., 2004).

MMPT correlations present results below a positive Kendall's τ-b rank correlation coefficient of 0.3 when associations with 59 other dental and oral nonmetric traits (results not shown), including protostylid are considered. The strongest associations were positive, between UP4 odontome and LM2 MMPT ($\tau = 0.261$; $p = 0.000$; $n = 424$), and LP4 odontomes and LM2 MMPT ($\tau = 0.259$; $p = 0.000$; $n = 430$), but only moderate and likely related to the very rare occurrence of odontomes, which coincided with MMPT. The results of the correlations between and within MMPT and protostylid for all lower molars are shown in Table 5. All coefficients -- of MMPT in any of the lower molars, of MMPT with protostylid in any tooth, and of protostylid associations with manifestations of the same variable in other teeth -- are weak positive, and the majority are not significant. Such results in a large sample (the smallest number of individuals compared was 257 for associations involving MMPT, and 254 overall), indicate that MMPT does not covary with protostylid, and will not interfere with statistical analyses, at least in this sample.

While MMPT trait correlations have not been reported before, some works address the possibility of its association with protostylid and other buccal cingulum manifestations (Hlusko, 2004; Skinner et al., 2008; Skinner et al., 2009). The enamel-dentine junction images and the protostylid descriptions found in two articles (Skinner et al., 2008; Skinner et al., 2009) propose the trait later described as MMPT (Weets, 2009) as a part of the protostylid morphological continuum in several taxa. The authors consider these cingular crests as developmentally linked, and therefore non-independent. Hlusko (2004) had previously detected a cingular trait mesial to the protostylid, which she considered not to covary with protostylid, and so deemed it as an independent trait. Present results agree with Hlusko's (2004) perspective, since protostylid and MMPT can co-occur (which is evidenced by the positive coefficients) but are not correlated. Developmentally, cusps are formed during the bell stage, through secondary enamel knots that promote epithelial and mesenchymal proliferation, while assuring folding of the epithelium around the enamel knot and the dental mesenchyme,

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by not proliferating themselves (Jernvall et al., 1994). The patterning cascade model of cusp development proposes the presence of secondary enamel knots inhibit other secondary enamel knots from forming immediately around them, creating a morphodynamic model which determines tooth size and the presence of enamel knots limit the possibility for the formation of extra cusps and tubercles (Jervall et al., 2000; Salazar-Ciudad and Jernvall, 2002, 2010). This process indirectly affects the formation of cusp patterns and crests. The MMPT and the protostylid apparently are cingular derivatives of cusp formation, crests forming indirectly by epithelial folding resulting from the interaction of secondary enamel knots. Protostylid and MMPT cusp forms could be exacerbations of such cingular remnants. Alternatively, these traits could correspond to separate enamel knots, forming outside inhibitory signaling, because of a (currently unexplained) lack of such inhibition, or even from differently timed enamel knot apoptosis. Regardless of these merely theoretical hypotheses, present results suggest protostylid and MMPT are independently formed, which could be explained by variables such as tooth size, the relationship between cusps (e.g., Moormann et al., 2013) or different interactions between the up to three secondary enamel knots (corresponding to protoconid, hypoconid and hypoconulid). Future research into the patterning cascade model of cusp development and its role in tooth crown morphology is necessary to test the proposed hypotheses.

The mandibular molar pit-tubercle is still an obscure dental nonmetric trait. Since early reports which mainly focused on characterizing it and conveying trait frequencies, it had been virtually absent from research in dental morphology.

The exclusion of grade 0-1, which expression can be included in grade 1, and the inclusion of at least one grade that corresponds to the described cusp form, should aid in further research of this trait. The production of a plaster cast representing each grade would also be a great contribution for future studies.

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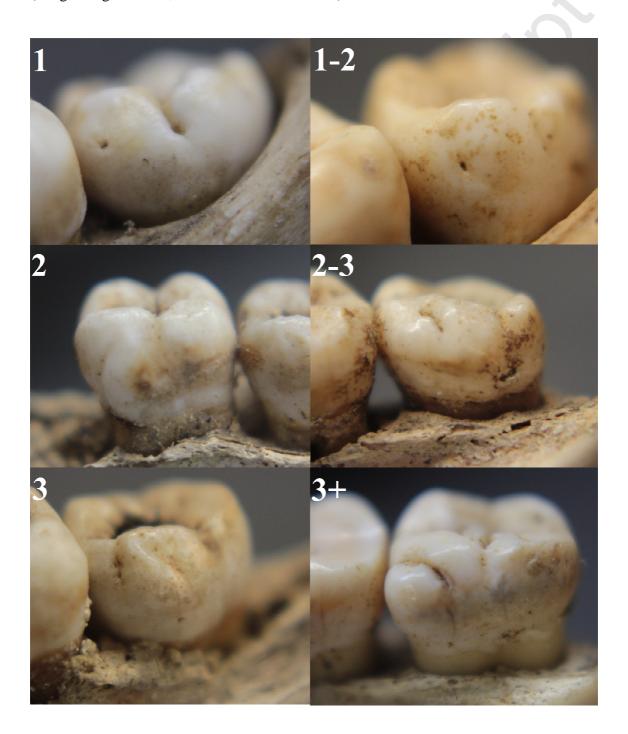
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Fig. 1. Buccal/mesiobuccal view of lower molars representing mandibular molar pit-tubercle (MMPT) grades 1, 1-2, 2, 2-3, 3 and 3+. Mesial is to the left of the image (images of grades 1-2, 2-3 and 3+ were inverted).

Table 1. Mandibular molar pit-tubercle grade and presence frequencies of all lower molars.

Molar	0-1	1	1-2	2	2-3	3	3+	Presence + = 0-1 - 3+
LM1 %	0.0	1.9	0.0	0.0	0.0	0.2	0.0	2.1
n	0	8	0	0	0	1	0	424
LM2 %	0.0	2.4	0.2	0.4	0.0	0.4	0.0	3.5
n	0	12	1	2	0	2	0	491
LM3 %	0.0	21.2	2.8	2.8	1.7	0.6	1.1	30.3
n	0	75	10	10	6	2	4	353

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Table 2. Mandibular molar pit-tubercle intraobserver precisions and correlations of all lower molars.

	n	Precision (%)				Correlation (τ-b)	
		Overall	$\pm1^{a}$	Dichot.	Observ.	Overall	Dichot.
LM1							
Pre-1st	112	98.2	98.2	98.2	99.5	0.572**	0.572**
1st-2nd	708	98.6	98.7	98.6	99.3	0.291**	0.292**
Pre-2nd	111	96.4	96.4	96.4	97.5	0.315**	0.315**
LM2							
Pre-1st	133	91.0	92.5	92.5	97.5	0.426**	0.459**
1st-2nd	838	97.6	97.6	97.9	97.8	0.628**	0.634**
Pre-2nd	135	94.1	96.3	96.3	98.0	0.672**	0.699**
LM3							
Pre-1st	94	87.2	91.5	92.6	95.5	0.781**	0.805**
1st-2nd	537	89.2	91.6	92.0	96.9	0.764**	0.777**
Pre-2nd	96	85.4	91.7	93.8	95.0	0.829**	0.834**

[a]-Precision within one grade; [b]-Dichotomized (results divided intro trait absence and presence); [c]-Observable trait (accounts the frequency of surfaces deemed observable on paired observations), with n = 200 for "Pre-1st" and "Pre-2nd", and n = 1200 for "1st-2nd"; **- P-values < 0.01.

Table 3. Pearson's χ^2 results for sexual dimorphism, and grade frequencies by sex of all molars.

Molar	Test of independence				+ = 0-1 - 3+		
	n	χ^2	df[1]	p	Females(%)	Males(%)	
LM1	424	1.403	1	0.236	3.0	1.3	
LM2	491	3.136	1	0.077	4.9	2.0	
LM3	353	12.352	1	<0.001	39.2	22.0	
Grade frequencies (%)[2]							
	n	1	1-2	2	2-3	3	3+
LM1 Females	200	2.5	0.0	0.0	0.0	0.5	0.0
LM1 Males	224	1.3	0.0	0.0	0.0	0.0	0.0
LM2 Females	243	3.3	0.0	0.8	0.0	0.8	0.0
LM2 Males	248	1.6	0.4	0.0	0.0	0.0	0.0
LM3 Females	171	25.1	2.9	4.7	2.9	1.2	2.3
LM3 Males	182	17.6	2.7	1.1	0.5	0.0	0.0

[a]- Degrees of freedom; [b]- Grade 0-1 frequencies are always 0%.

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Table 4. Mandibular molar pit-tubercle bilateral asymmetry, bilateral correlations (Kendall's τ-b), and bilateral grade frequencies of all lower molars.

Molar		Overall asymmetry		Comparable asymmetry[a]	
	n	Frequency(%)	Correlation	n	Frequency(%)
LM1	289	2.8	-0.014	8	100.0
LM2	365	3.3	**0.456	16	75.0
LM3	203	27.1	**0.285	70	78.6
Grade frequencies (%)[b]							
	n	1	1-2	2	2-3	3	3+
Left LM1	365	1.4	0.0	0.0	0.0	0.3	0.0
Right LM1	348	0.9	0.0	0.0	0.0	0.0	0.0
Left LM2	425	1.9	0.0	0.5	0.0	0.5	0.0
Right LM2	431	2.1	0.2	0.0	0.0	0.0	0.0
Left LM3	275	14.9	2.9	2.5	0.4	0.0	0.4
Right LM3	281	18.5	1.1	1.8	1.8	0.7	1.1

[a]- Bilateral absences excluded; [b]- Grade 0-1 frequencies are always 0%; **- P-value < 0.01.

Table 5. Kendall's τ-b rank coefficient correlations between mandibular molar pit-tubercle and protostylid of all lower molars. Coefficients are above the diagonal, and the number of individuals compared is below.

LM1 MMPT	-	**0.157	*0.119	0.061	0.077	0.065
LM2 MMPT	384	-	0.107	0.021	*0.101	0.020
LM3 MMPT	257	306	-	0.063	0.046	0.020
LM1 Prot.	419	385	259	-	**0.199	0.043
LM2 Prot.	383	484	304	386	-	**0.192
LM3 Prot.	252	301	342	254	300	-

[a]-Protostylid; *- P-value < 0.01; **- P-value < 0.05.