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Protostylid variation in *Australopithecus*

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Abstract

Recent advances in computed tomography (CT) and genetics provide new insights into the morphology and biology of anatomical traits, particularly in the dentition. As we move towards a fuller understanding of the genetic and developmental bases for dental traits, we need to reassess the taxonomic and evolutionary variation of established characters. Quantitative genetic analyses indicate that the degree of expression of upper and lower primate cingular remnants are genetically interdependent. This has serious evolutionary implications that need to be explored for fossil hominids. Studies of Carabelli's cusp, a cingular remnant on hominid upper molars, have been advanced through both genetic and CT analyses setting the stage for such an investigation. But its mandibular morphological homologue, the protostylid has not been similarly studied. This paper represents the first step towards a quantitative understanding of the variation and evolution of this trait in early hominids.

Since the first discoveries of *Australopithecus* specimens in South Africa more than sixty years ago, cingular features on lower molars have played a significant role in the description and comparison of hominid taxa. This largely qualitative history is reviewed. Because the modern human classification system for protostylid variation does not adequately describe the variation seen in *Australopithecus* samples, a quantification scheme with six expression states is established.

Using this new protocol, protostylid variation in six species of *Australopithecus* is assessed. Results from these analyses show that the distribution of the degree of protostylid expression in these species is highly varied. When first, second, and third molar samples are considered separately, the distribution of expression states is found to differ considerably within the same species. These results provide a foundation for further genetic and developmental research on the evolutionary history of the hominid dentition.

Keywords: *Australopithecus anamensis*; *Australopithecus afarensis*; *Australopithecus africanus*; *Australopithecus robustus*; *Australopithecus boisei*; *Australopithecus aethiopicus*; Cingulum; Dental variation; Dental evolution; Paleontology

Introduction

Cingular remnants have long been studied by primate paleontologists interested in phylogenetic and functional aspects of the evolving dentition

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(see historical review below). These features, including Carabelli's cusp and the protostylid are regularly employed in the study of early hominids and modern humans to assess relationships. Reid and Van Reenen (1995) and Van Reenen and Reid (1995) formally evaluated Carabelli's cusp variation in South African hominids and found that the human classification system does not adequately describe *Australopithecus* variation. They developed a new classification system based on the South African morphologies (Van Reenen and Reid, 1995). Using their new classification system, they found no correlation between Carabelli's cusp and tooth size, and that the overall trend in the South African hominid group appears to be towards a reduction of the Carabelli feature (Reid and Van Reenen, 1995).

Building on this research, Schwartz et al. (1998) explored the internal morphology of early South African hominid teeth using high-resolution computed tomography (CT), assessing the relationship between enamel thickness and Carabelli's cusp. They found that Carabelli features affect linear measurement of enamel thickness, confounding the assessment of enamel thickness variation in hominids with prevalent Carabelli features. This study of Carabelli's cusp variation using CT scanning has enhanced our understanding of both the trait in question and its relationship to other traits, such as enamel thickness and molar crown size.

In this paper, I report the results of a detailed study of another cingular remnant, the protostylid, in order to lay the foundation for a more comprehensive understanding of its evolutionary history.

Historical background

Early in the history of fossil hominid studies, Broom (1937: 681) noted that in *Australopithecus*: "there are clear indications of a rudimentary external cingulum such as we find in *Dryopithecus*.… Indications of the cingulum are usually seen in the molars of the gorilla, but they are usually lost or only represented by pits in the chimpanzee and man." Dart (1948a: 74) described "… a definite cingular furrow and cuspular enamel ridge on each antero-lateral molar cusp" of the lower molars of MLD 2 from Makapansgat, South Africa. This feature clearly distinguished MLD 2 from the Taung specimen, as well as from all other known *Australopithecus* species (Dart, 1948a,b).

The early recognition of cingular effects on the buccal surfaces of lower molars and considerations of their phylogenetic importance extended beyond South Africa. Weidenreich (1937: 86) interpreted the presence of a cingulum "or its differentiations" on the buccal side of *Sinanthropus* lower molars from China as indicating its more apelike affinities compared to recent human molars that lack these cingular effects.

Prior to Dahlberg's (1950, 1956) formalization of the protostylid, features like these buccal cingula or cuspular enamel ridges on lower molars were thought to be quite rare in modern humans. The protostylid is defined as "an elevation or ridge of enamel on the anterior part of the buccal surface of the lower molars, which ascends from the gingival end of the buccal groove and extends mesio-occlusally" (Dahlberg, 1950). Analysis of the Pima population from Arizona demonstrated the first evidence that the protostylid is fairly common in some human populations and its frequency varies at the population level (Dahlberg, 1950). Continuing research confirmed this, and provided a more complete understanding of its variation across modern human populations (Scott and Turner, 1997).

Dahlberg (1956), Hanihara (1961), and Turner et al. (1991) formalized expression types for the protostylid to provide consistency in studies of modern humans. The protostylid was divided into eight categories, ranging from a smooth buccal surface to a cusp with a free apex (Turner et al., 1991). This formalization of expression types for numerous morphological features of the human dentition led to considerable improvements and advances in modern human odontology (Scott and Turner, 1997).

Samples of fossil hominids continued to grow and by 1956 Robinson had further demonstrated the phylogenetic importance of this feature in early hominids. A highly developed protoconidal cingulum, as he called it, differentiated *Australopithecus*

from *Paranthropus* (=*A. robustus*). Robinson (1956: 120) believed that the presence of the morphologically similar protostylid in modern humans probably reflected a "fading remnant of man's australopithecine heritage". Robinson (1956) was careful to note that the morphology of the early hominid protoconidal cingulum was somewhat different from that of the modern human protostylid. Sperber (1974) categorized the expression of this feature as slight, moderate, or prominent. His analyses agreed with Robinson's (1956) conclusions that the frequency and degree of expression in the protoconidal cingulum differed between the Swartkrans/Kromdraai specimens and those from Sterkfontein/Makapansgat.

Subsequent to these analyses, the focus of early hominid paleontology shifted to East Africa and the protostylid/protoconidal cingulum began to play a lesser role in alpha taxonomy and phylogenetic assessment. For example, it was not included as a character in Strait et al.'s (1997) comprehensive study of early hominids, nor was it mentioned in the initial description of *Australopithecus anamensis* (Leakey et al., 1995). However, it is a feature described in the fuller treatment of this species (Ward et al., 2001) and in other early hominid studies (Wood and Abbott, 1983; Wood, 1991). These three latter descriptions use Dahlberg's terminology, calling this feature a "protostylid". As the trait's expression in eastern African and South African specimens is clearly homologous, it is evident that Robinson's "protoconidal cingulum" and Dahlberg's "protostylid" have been used interchangeably in early hominid studies. These three studies also indicate that the frequency and degree of expression of this feature are potentially taxonomically and phylogenetically informative for eastern African hominids.

The importance of protostylid/protoconidal and other cingular remnants in early hominid taxonomy continues to be prevalent in descriptive accounts of new and previously known hominids. Tobias' (1991) monographic study of *Homo habilis* includes the cingulum on the buccal face of the protoconid of lower molars. The newly discovered and named *Sahelanthropus tchadensis* is diagnosed, in part, by the presence of cingular remnants on the upper molars (Brunet et al., 2002).

Evolutionary biology of the protostylid

Primate molars are derived from the tribosphenic, primitive mammalian molar. One of the characteristics of these primitive molars is a shelf of enamel that encircles the entire base of the crown, known as the cingulum. In general, the fossil evidence suggests that the cingulum in primates reduced over time, resulting in differentially expressed remnants on the buccal side of lower molars and the lingual side of upper molars (James, 1960; Hartwig, 2002). Compared to strepsirhines, the cingulum is all but lost in many extant anthropoids. However, the ancillary features that sometimes flank the sides of anthropoid molar crowns are considered to be remnants of the primitive cingulum (Swindler, 1976; Hillson, 1986; Scott and Turner, 1997). These remnant features include the parastyle, protostyle, Carabelli's cusp, mesostyle, and interconulus of upper molars, and the protostylid and interconulid of the lower molars (Dahlberg, 1950; Swindler, 1976; Aiello and Dean, 1990; Hillson, 1996; Scott and Turner, 1997; Butler, 2000).

The developmental mechanisms of early tooth organogenesis are now understood to some degree due to gene expression and knock-out studies performed over the last decade on mouse models (Zhao et al., 2000; Peters and Balling, 1999; Weiss et al., 1998). These studies are important in providing a general understanding of how a tooth is formed, and in identifying genes that may underlie normal variation in crown morphology. Despite the rapid advances in the field of developmental dental genetics, virtually nothing is known about the specific mechanisms that result in the minor phenotypic dental variation seen between closely related species or populations. As such, little is currently known about the genetic mechanisms underlying variation in the expression of cingular remnant features.

Some advances are being made on this front through quantitative genetic analyses of modern humans (Lasker, 1950; Potter et al., 1981; Nichols, 1989; Corruccini et al., 1990) and baboons (Hlusko, 2000; Hlusko et al., 2002; Hlusko and Mahaney, 2003). Variation in these traits is heritable, meaning that a significant proportion of

the variance results from the additive effects of genes and therefore is responsive to selection. Baboon upper and lower molar expression of morphologically similar traits, the interconulus and interconulid, appear to result from overlapping but not identical sets of genes (Hlusko and Mahaney, 2003). Therefore, upper and lower molar cingular remnants are neither completely dependent nor independent, and consequently, may respond differently to selective pressures. Given that genetic mechanisms are conserved between distantly related taxa such as mice and humans, it is reasonable to assume that the genetic mechanisms underlying baboon dental variation are relevant to human dental variation, and their common ancestors.

As noted by Robinson (1956), the morphology of the protostylid in early hominids differs from that seen in modern humans. The range in degree of expression in early hominids extends beyond that seen in modern humans, and therefore exceeds the stages established for humans (Turner et al., 1991). Though the written descriptions of human protostylid stages (Dahlberg, 1950; Turner et al., 1991) accord with the lower degrees of *Australopithecus* protostylid expression, the author's experience with the human protostylid indicates that it is usually located on the buccal-most surface of the protoconid, whereas the *Australopithecus* protostylid is more centrally located on the buccal side of the crown with a stronger relationship to the buccal groove. In humans, the protostylid is a groove on the protoconid that extends mesioapically from the buccal groove along the cusp's distobuccal surface. The human protostylid does not extend onto the mesial portion of the protoconid. This differs from the early hominid condition, where the protostylid extends around the entire buccal face of the protoconid and parallels the occlusal rim rather than slanting mesioapically.

A second mandibular cingular remnant also found on early hominid molars is a ridge of enamel that runs almost vertically on the mesiobuccal corner of the protoconid (see molars 1 and 2 in Fig. 1). Similarly, the South African hominids have two components to the upper molar lingual cingular remnants (Van Reenen and Reid, 1995). Though no data were presented to demonstrate their covariance, Van Reenen and Reid (1995) included both in their Carabelli's cusp classification system. For the mandibular molars, this shelf does not covary with the protostylid in the larger sample studied here. Fig. 1 demonstrates this, as the strongest protoconid ridge is seen on Type 2 (Stw 133) and the highest level of protostylid expression, Type 6 (MLD 2) has no evidence of a protoconid ridge. In addition to the lack of covariance within these *Australopithecus* samples, a survey of 30 modern human mandibular dentitions from the Native Californian Early Horizon skeletal collection housed at University of California at Berkeley did not reveal any evidence of this protoconid shelf (although it is fairly common in baboons). Preliminary quantitative genetic analyses of morphological homologues in baboon mandibular molars provide inconclusive evidence for genetic covariance between these two traits (Hlusko, 2000). Therefore, the conservative approach is to use a restrictive definition of the protostylid until further evidence is found for genetic and developmental interrelatedness between these two features. The mesial protoconid ridge is not considered part of the protostylid complex in this paper.

The buccal cingular remnant in baboon lower molars is not predominantly associated with the protoconid or hypoconid, even in its higher expression states, unlike in early hominids or modern humans. Following terminology from elsewhere (Swindler, 1976; Hlusko, 2002), the "*interconulid*" is the most appropriate term for the baboon, so as to not suggest a link with the protoconid. Although the *Australopithecus* protostylid is less associated with the protoconid and has a higher degree of expression than modern humans, maintaining the term "*protostylid*" is the simplest approach, although the morphological differences between the modern human and *Australopithecus* forms do need to be taken into account.

Materials

Some hominid paleontologists have previously developed categories for expression of the protostylid rather than relying on purely descriptive

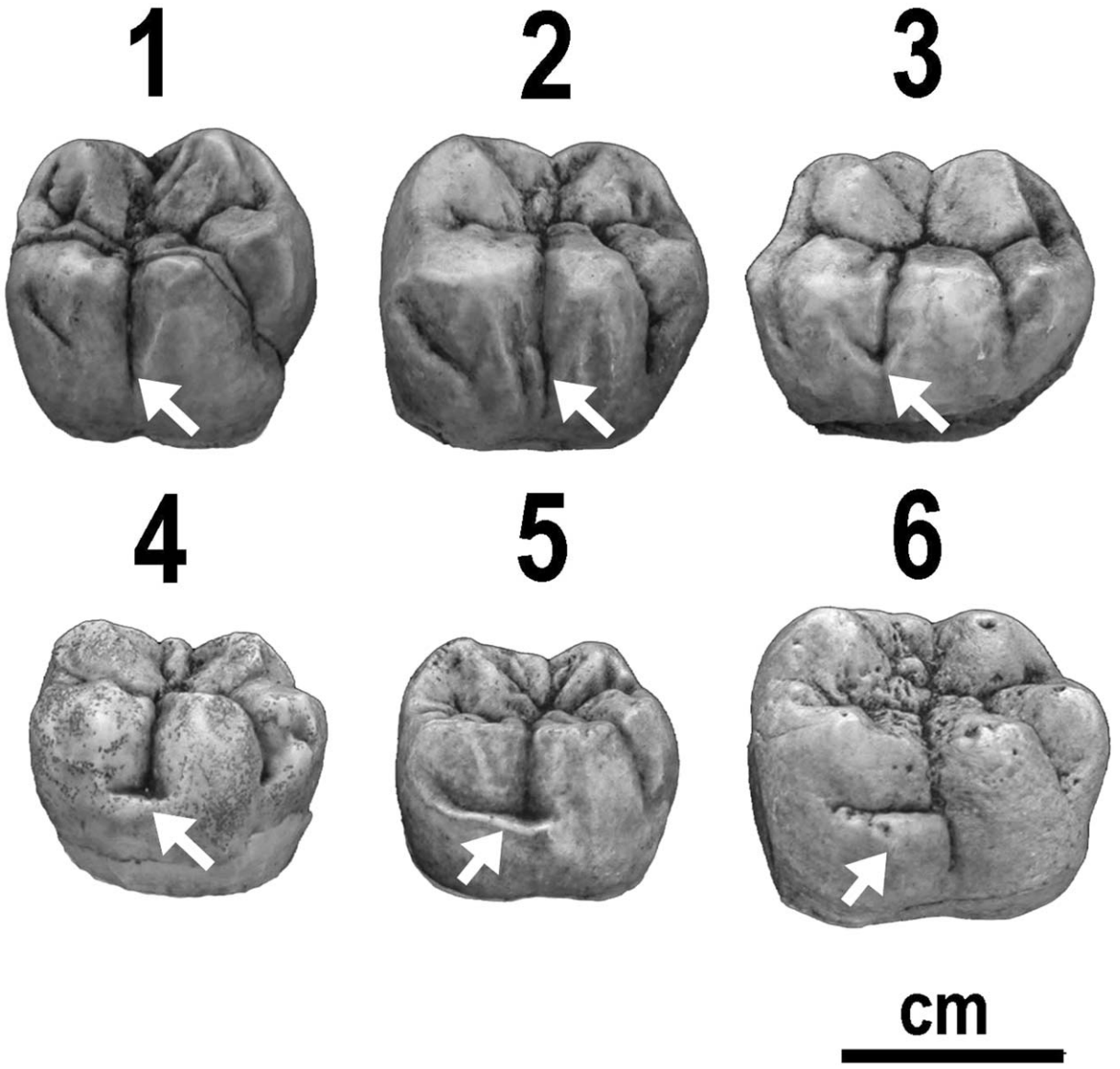

Fig. 1. Early hominid protostylid expression types. Buccally oriented occlusal views of the six expression types for the protostylid in early hominids are shown with the protostylid highlighted with a white arrow. Mesial is to the left for all crowns. TYPE 1 (Stw 309a RM1, shown in mirror image), no evidence of any protostylid deriving from the buccal groove. TYPE 2 (Stw 133 LM3), buccal groove ends in a small wrinkle of enamel not distinct from the tooth crown. TYPE 3 (Stw 246 LM1), buccal groove ends in a V-shaped cleft expanding onto both cusps. TYPE 4 (Stw 151 LM1), buccal groove ends in a linear cleft that expands onto both cusps and bulges slightly outwards. TYPE 5 (Stw 123b LM1), buccal groove ends in a deep linear cleft that primarily expands onto the protoconid almost to the mesiobuccal corner of this cusp. TYPE 6 (MLD 2 LM2), buccal groove ends in a deep cleft that expands onto the protoconid but differs from the previous type in that an ancillary cusplet is formed that protrudes buccally beyond the buccal-most aspect of either the protoconid or hypoconid. Note the variable groove, or protoconid shelf, on the mesial aspect of the protoconid. The protoconid shelf does not covary with the protostylid and therefore is not included in these expression states. Photographs are of high-resolution plaster dental casts.

Table 1
Specimen list and data

Specimen number	Tooth	Expression state
A. aethiopicus (*n*=7)		
L28-31	RM2	1
L62-17	RM2	3
L157-35	LM2	1
Omo F22-1b	RM3	1
Omo 18-1968-34	LM1	4
Omo 33-1969-9	RM3	4
Omo 33-1974-6172	RM3	1
A. afarensis (*n*=37)		
AL 128-23	RM1	2
AL 128-23	RM2	3
AL 145-35	LM1	1
AL 145-35	LM2	4
AL 188-1	RM2	2
AL 188-1	RM3	2
AL 200-1b	RM1	1
AL 241-14	LM?	2
AL 266-1	LM1?	1
AL 266-1	RM3	2
AL 266-1	RM2	3
AL 288-1	RM1	1
AL 288-1	RM2	2
AL 288-1	R/LM3	2
AL 333w-1	R/LM1	2
AL 333w-1	R/LM2	3
AL 333w-32	RM3	1
AL 333w-48	RM2	3
AL 333w-57	LM3	1
AL 333w-59	LM3	2
AL 333w-60	LM1	1
AL 333w-60	LM2	1
AL 333w-60	LM3	1
AL 400-1	R/LM2	2
AL 400-1a	L/RM1	2
AL 400-1a	RM3	4
LH 15	LM3	1
LH 2	LM1	3
LH 3t	LM1	3
LH 4	LM2	1
LH 4	RM3	1
MAK VP 1/2	RM3	1
MAK VP 1/4	RM2	4
MAK VP 1/12	R/LM1	1
MAK VP 1/12	RM3	1
W7-508	RM1	5
W8-752	RM1	1
A. africanus (*n*=63)		
MLD 2	R/LM1	6
MLD 2	R/LM2	6
Sts 9	RM2	1
Sts 18	R/LM1	1
Sts 18	RM2	1
Sts 24	RM1	2
Sts 41	LM3	4
Sts 52	R/LM2	5
Sts 52	R/LM3	2
Sts 556	LM3	2
Stw 3	LM3	2
Stw 14	RM2	2
Stw 14	RM3	1
Stw 54	LM2	6
Stw 61	RM2	3
Stw 96	LM3	2
Stw 106	RM1	1
Stw 109	RM2	1
Stw 109	RM3	1
Stw 120	LM2	2
Stw 123	R/LM1	5
Stw 131	RM1	4
Stw 133	LM3	2
Stw 135	LM2	5
Stw 145	RM1	2
Stw 151	R/LM1	4
Stw 196	LM3	5
Stw 213	R/LM2	2
Stw 234	RM2	2
Stw 237	LM3	1
Stw 246	LM1	3
Stw 276	LM1	6
Stw 278	RM3	2
Stw 285b (286)	LM2	6
Stw 308	RM2	1
Stw 309a,b (409)	R/LM1	1
Stw 327	LM1	3
Stw 327	LM2	2
Stw 364	RM1	4
Stw 384	RM2	6
Stw 384	RM3	6
Stw 404	RM2	6
Stw 404	RM3	6
Stw 412a,b (419)	R/LM2	1
Stw 421a,b (429)	R/LM1	6
Stw 424	LM2	6
Stw 487	RM3	3
Stw 491 (518)	LM3	1
Stw 491 (519)	LM2	1
Stw 498	LM2	1
Stw 498	LM3	1
Stw 520	RM3	5
Stw 529 (531 and 532)	R/LM3	1
Stw 529 (534)	LM2	1
Stw 537 (269 and 540)	R/LM2	5
Stw 537 (541)	LM1	5
Stw 551	R/LM3	6
Stw 555	LM2	4
Stw 560a,b	R/LM3	5

Table 1 (continued)

Specimen number	Tooth	Expression state
Stw 560d,e	R/LM2	6
Taung	R/LM1	3
TM 1518	RM3	3
TM 1520	LM3	1
A. anamensis (*n*=20)		
KNM ER 20422	LM1	3
KNM ER 20423	LM2	6
KNM ER 30201	LM1	1
KNM ER 35232	LM1	2
KNM ER 35233	LM2	2
KNM KP 29281	LM1	1
KNM KP 29281	RM3	1
KNM KP 29287	LM1	3
KNM KP 29287	R/LM2	2
KNM KP 30500	LM1	6
KNM KP 30500	R/LM2	5
KNM KP 30500	LM3	6
KNM KP 30502	LM3	6
KNM KP 31712	RM1	1
KNM KP 31728	LM1	3
KNM KP 34725	RM1	4
KNM KP 34725	LM2	5
KNM KP 35838	LM3	1
KNM KP 35847	LM2	6
KNM KP 37522	LM1	2
A. boisei (*n*=14)		
KNM ER 729	RM3	2
KNM ER 802	RM3	1
KNM ER 810	LM3	1
KNM ER 1171	R/LM2	4
KNM ER 1816	LM2	4
KNM ER 1820	LM1	1
KNM ER 3230	LM1	1
KNM ER 3230	R/LM2	1
KNM ER 3230	R/LM3	3
KNM ER 6080	RM2	3
KNM ER 8020	RM2	4
OH 3d	LM1	1
OH 26	RM2 or 3	1
Peninj (WN 64)	RM3	1
A. aff. *boisei* (*n*=7)		
L427-7	RM2	5
L628-3	LM3	1
L628-9	LM1	4
Omo 47-1973-1500	RM2	1
Omo 76-1972-37	LM3	6
Omo 136-1972-1	LM3	6
Omo 136-1972-2/3	R/LM2	6
A. robustus (*n*=40)		
KB 5223	R/LM1	4
SK 1	LM2	4
SK 6	R/LM2	4

Table 1 (continued)

Specimen number	Tooth	Expression state
SK 6	R/LM3	2
SK 22	RM3	4
SK 23	R/LM3	4
SK 25	R/LM1	4
SK 25	RM2	4
SK 34	R/LM3	2
SK 37	LM2	5
SK 55	RM2	3
SK 61	RM1	4
SK 63	R/LM1	4
SK 75	RM3	4
SK 81	LM3	3
SK 104	RM1	5
SK 841b	LM3	3
SK 843	LM2	3
SK 843	LM3	3
SK 844	LM3	3
SK 846a	RM1	2
SK 851	RM3	4
SK 858	RM3	2
SK 862	RM3	3
SK 871	LM2	4
SK 876	RM3	1
SK 880	LM3	4
SK 1587	R/LM2	6
SK 3974	RM1	4
SK 3976	LM2	6
SKW 5	LM3	4
SKX 4446	RM1	2
SKX 4446	RM2	4
SKW (4)767	RM1	2
SKX 5002	LM3	4
SKX 5014	RM3	4
TM 1517	RM2	3
TM 1517	RM3	3
TM 1536	RM1	3
TM 1600	LM3	2
Omo non-robust (*n*=6)		
L26-1g	RM1	1
L28-30	RM3	1
L45-2	RM1	4
L51-1	LM1	3
Omo 75-1969-14	R/LM2	6
Omo 75-1969-14	LM3	5

terms. These range from three (Sperber, 1974) to five stages of expression (Wood and Abbott, 1983; Wood, 1991). However, these schemes have not received wide recognition and were not depicted graphically, making them difficult to replicate.

Using the large lower molar sample of *Australopithecus africanus*, I developed a six-stage expression series for the protostylid in early hominids (Fig. 1) following the standard classification procedures established in dental anthropology (Turner et al., 1991; Scott and Turner, 1997).

High-resolution plaster casts of *Australopithecus* lower molar specimens housed at the Laboratory for Human Evolutionary Studies, University of California, Berkeley, were studied. Six widely-recognized taxa were included in the analysis (*A. aethiopicus*, *A. afarensis*, *A. africanus*, *A. anamensis*, *A. boisei*, and *A. robustus*), as well as six molars from the Omo Shungura Formation that demonstrate affinities with *A. boisei* (Suwa et al., 1996). M_1, M_2, and M_3s were scored. Samples are as evenly comprised of M_1, M_2, and M_3s as was possible. When antimeric pairs were available, only the right side was scored. All observations were made under incandescent light. Each tooth was scored twice, separated by three days. When characters were deemed intermediate between stages, the lower state was chosen. In all, 190 molars were assessed twice, and 63 (33%) were scored a third time since the first and second scores did not match. This accords with the scorability of the protostylid and Carabelli's cusp in humans (Nichols and Turner, 1986) and the interconulus and interconulid in baboons (Hlusko, 2002). Errors were evenly distributed among the hominid taxa, so the types are not particularly more or less problematic for any of the species studied here.

All specimens included in this study and their taxonomic identifications are listed in Table 1. Taxonomy follows that of White et al. (1981), Suwa (1996), Suwa et al. (1996), Asfaw et al. (1999), Ward et al. (2001), and White (2002). Note that the Stw specimen numbers listed here agree with the recent analysis by Moggi-Cecchi and Tobias (in prep) and Moggi-Cecchi et al. (in prep), and that former Stw specimen numbers are noted in parentheses to the right of the updated catalogue number. For isolated molars, I relied on the identifications made by the above cited researchers. When I disagreed with the identification, the molar in question was used in the pooled sample but not the specific M_1, M_2, or M_3 comparisons and is not given a position designation in Table 1, explaining the discrepancy between the sub-samples and the pooled sample totals in Table 2. Although there is potential error introduced into the study by possibly mis-identified molar positions, I was conservative in molar attribution and therefore mis-identifications are minimal and not biased towards or against any one tooth position.

Methods

Species data were analyzed several ways. Homoscedasticity was assessed between paired samples of teeth along the tooth row and appropriate *t*-tests conducted to compare means applying the sequential Bonferonni's adjustment. The nonparametric Mann–Whitney test was also employed. Frequency distributions were compared using *k*-sample Kruskal–Wallis (k=6) and two-sample Kolmogorov–Smirnov tests, both nonparametric analyses, since the assumption of normality is typically violated by these samples even when the data are transformed.

Results

Histograms of all species samples are shown in Fig. 2. Frequencies are reported in Table 2. Student's *t*-test comparison of means revealed that three of the 15 pairs are significantly different at $p<0.05$ (Bonferroni's correction, $p<0.003$ applied for sequential comparisons). These are *A. afarensis* vs. *A. robustus*, *A. afarensis* vs. *A. africanus*, and *A. boisei* vs. *A. robustus*.

The Kruskal–Wallis test with k=6 samples estimated a χ^2=21.592 (df=5), significance=0.001, showing that one particular distribution (such as a normal distribution) cannot account for the variation in frequency distributions seen here. Two-sample Kolmogorov–Smirnov tests between the 15 species pairs show that the following frequency distributions are different at $p<0.05$: *A. afarensis* vs. *A. robustus* ($p<0.001$), *A. boisei* vs. *A. robustus* (p=0.007), *A. afarensis* vs. *A. africanus* (p=0.025), and *A. robustus* vs. *A. africanus* (p=0.031). Only the first two are significant when Bonferonni's correction ($p<0.003$) for sequential comparisons is applied.

Table 2
Frequencies of protostylid expression states for six *Australopithecus* species[a]

Expression state	*A. afarensis*	*A. africanus*	*A. anamensis*	*A. robustus*	*A. boisei*+	*A. aethiopicus*
Pooled sample[b]						
1	16	18	5	1	10	4
2	11	13	4	7	1	0
3	6	6	3	10	2	1
4	3	5	1	18	4	2
5	1	8	2	2	1	0
6	0	13	5	2	3	0
N=	37	63	20	40	21	7
M_1						
1	6	3	3	0	3	0
2	3	2	2	3	0	0
3	2	3	3	1	0	0
4	0	3	1	5	1	1
5	1	2	0	1	0	0
6	0	3	1	0	0	0
N=	12	16	10	10	4	1
M_2						
1	2	8	0	0	2	2
2	3	5	2	0	0	0
3	4	1	0	3	1	1
4	2	1	0	5	3	0
5	0	3	2	1	1	0
6	0	7	2	2	1	0
N=	11	25	6	11	8	3
M_3						
1	7	7	2	1	4	2
2	4	6	0	4	1	0
3	0	2	0	6	1	0
4	1	1	0	8	0	1
5	0	3	0	0	0	0
6	0	3	2	0	2	0
N=	12	22	4	19	8	3

[a]*A. boisei*+ includes specimens from Omo, Ethiopia designated *A.* aff. *boisei*.
[b]M_1, M_2, and M_3 samples do not include isolated molars with questionable serial position identifications. Therefore, pooled samples are larger than the total of the subsamples for *A. afarensis* and *A. boisei*+.

Separate M_1, M_2, and M_3 frequencies for all six taxa are reported in Table 2, and histograms for four species presented in Fig. 3. Data for *A. aethiopicus* and *A. boisei* are not presented in Fig. 3 because of the particularly small sample sizes for each molar category. For the largest samples (*A. afarensis*, *A. africanus*, and *A. robustus*) M_1, M_2, and M_3 protostylid expression averages were tested via *t*-tests for equality of means serially along the tooth row. No significant metameric variation was found for *A. africanus*. Only the M_2 and M_3 were found to be significantly different for both *A. afarensis* (p=0.027) and *A. robustus* (p=0.006), with the second molar consistently having the highest degree of protostylid expression. Nonparametric Mann–Whitney tests confirmed these results with only *A. afarensis* M_2 vs. M_3 (p=0.024) and *A. robustus* M_2 vs. M_3 (p=0.015) significantly differing from equality.

None of the same-species serial samples (M_1 vs. M_2, M_2 vs. M_3, M_1 vs. M_3) were found to have significantly different frequency distributions when

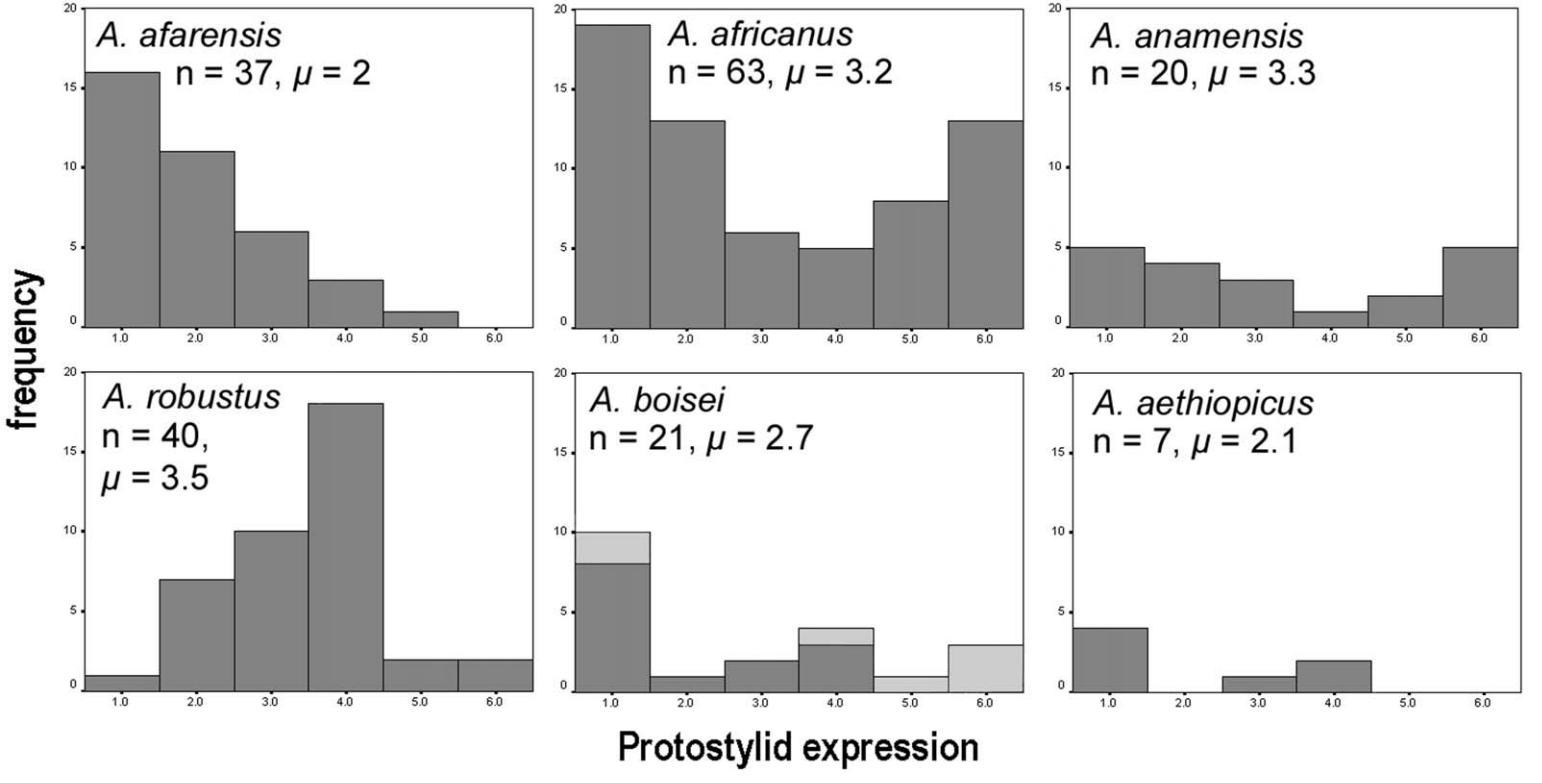

Fig. 2. Histograms of the protostylid scores for all taxa included in this analysis. The *A. boisei* group includes Omo specimens identified by Suwa et al. (1996) as having affinities with *A. boisei*. These less certain specimens are demarcated within the histogram by lighter gray blocks. See Table 1.

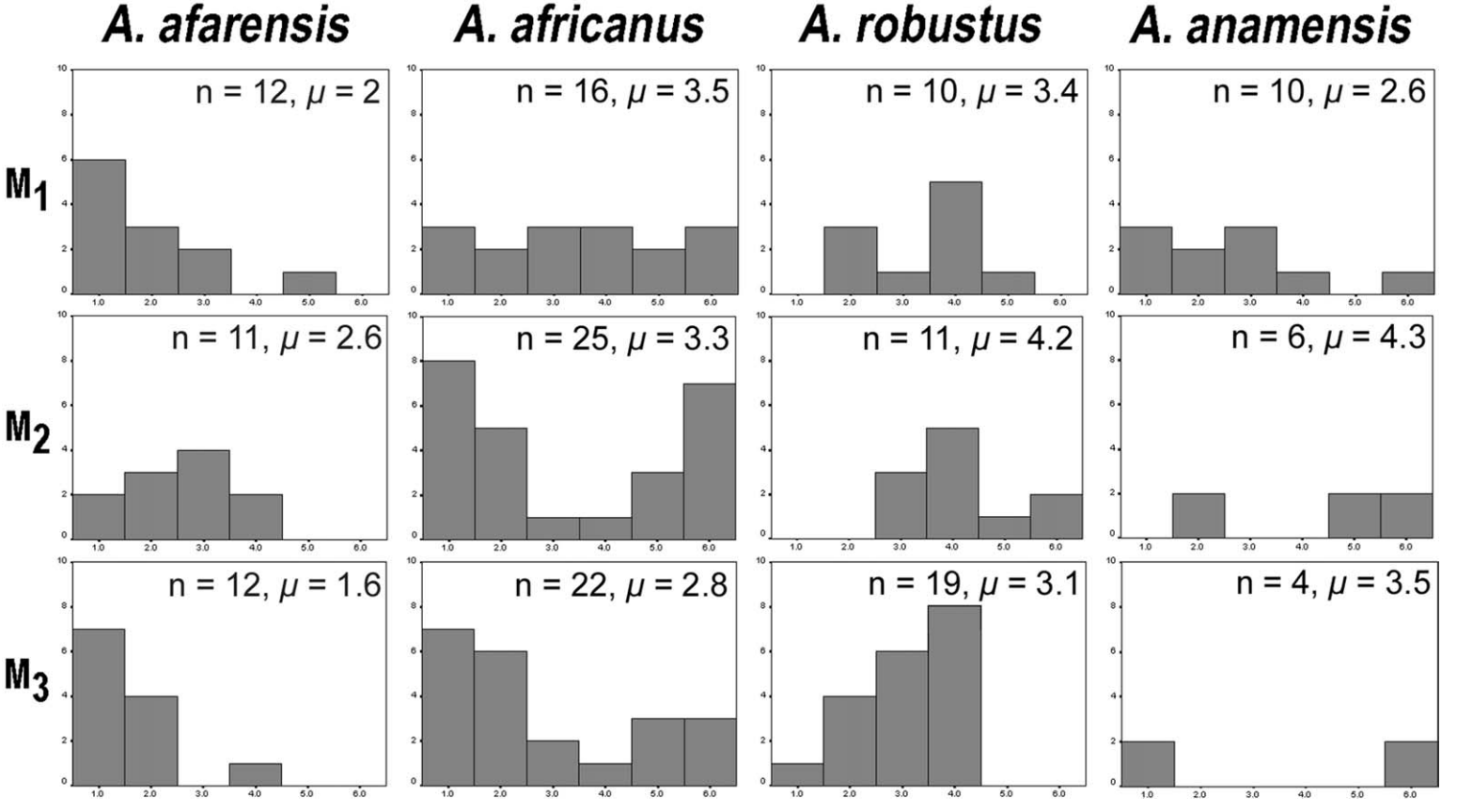

Fig. 3. Histograms of M_1, M_2, and M_3 samples for the four largest samples. Axes are the same as in Fig. 2.

two-sample Kolmogorov–Smirnov tests were employed. Kruskal–Wallis k=3 sample tests show that the assumption of equal distributions is not supported only by the *A. robustus* population (p=0.05).

Visual inspection of the frequency distributions shown in Fig. 3 suggests interesting differences, though these differences are not statistically significant. However, tentative comparisons can be made. For *A. africanus*, M_1 protostylid expression is evenly distributed among the six states. M_2 and M_3 expression is generally at either end of the extreme. The distribution differences between M_1 and the more distal molars stand in contrast with the lack of metameric variation indicated by the comparison of means. Based on a very small sample, *A. anamensis* M_1, M_2, and M_3 protostylid frequency appears to be similar to that seen in *A. africanus*. *A. robustus* M_1 expression states are centered around 3, whereas M_2s have higher and M_3s lower degrees of expression. *A. afarensis* M_2s have higher degrees of expression than M_1 or M_2. These latter two are in accord with the results from the metameric comparison of means reported above.

Discussion

Early in hominid evolutionary studies, Robinson (1956) determined that the frequency and degree of expression of the protostylid (protoconidal cingulum, in his terms) differentiated *A. robustus* from *A. africanus*. Using their quantification method of this trait, Wood and Abbott (1983) found no significant difference between samples of these South African taxa. However, they note "[o]ur observations on the protostylid suggest that though it is more common in the 'robust' australopithecines than the 'graciles', when it does occur it is more strongly expressed in the 'gracile' group" (Wood and Abbott, 1983: 217). Similarly, the present study finds that the mean score for each taxon is not necessarily as informative as is the pattern of distribution within each sample.

The Kruskal–Wallace (k=6) test shows that one distribution of protostylid expression does not characterize all six early hominid species. The histograms show that the *A. africanus* sample has a bimodal distribution, with most of the specimens showing either no expression or a high degree of expression, and few individuals falling in the middle-range. The *A. robustus* distribution is the exact opposite, approximating a normal distribution with most specimens in the center of the range and few at either end. *Australopithecus anamensis* has flat or platykurtic distribution, whereas *A. afarensis* is highly skewed to the left, where most specimens have little to no expression and few have high degrees of expression. *Australopithecus boisei* is similar to *A. afarensis* in this regard, and *A. aethiopicus* possibly shares this distribution as well, although sample size for the latter taxon is small.

Given these findings, t-test comparisons of protostylid means for populations of early hominids may be uninformative. A simple presence–absence scoring would likewise be inappropriate. This trait is best considered in terms of its population frequency distributions. For example, comparing *A. africanus* and *A. robustus* protostylid means results in support for an assumed equality of means. However, statistical tests of the frequency distributions show that these samples are significantly different (though conservative Bonferroni's correction suggests that this interpretation be tentative until further analyses confirm or refute these results).

Frequency distributions of protostylid expression may ultimately prove important to taxonomic and phylogenetic debates. For example, protostylid expression distributions provide information about the potential presence of *A. africanus* in eastern Africa. The largest sample of hominid specimens securely dated between 2 and 3 Ma is from Omo, Ethiopia, a time period critical to the origins of the genus *Homo*. Though numerically large, interpretation of the Omo sample is problematic because many of these specimens are fragmentary and isolated teeth. Initial descriptions stated that the non-robust dental specimens "cannot be separated on the basis of size, proportions, and crown morphology from *A. africanus* samples from Sterkfontein and Makapansgat Limeworks" (Howell and Coppens, 1976: 524).

Subsequent analysis of fossils from Hadar and Laetoli, and the description of *A. afarensis*

(Johanson et al., 1978), brought new information to the taxonomic interpretation of the Omo gracile specimens. Hunt and Vitzthum's (1986) analysis of dental metrics found that the Omo gracile sample is nearly identical in tooth size to the South African gracile sample, demonstrating the presence of a hominid with *A. africanus*-sized teeth in eastern Africa, making "it more likely than heretofore that *A. africanus* is an ancestor of later *Homo* species" (Hunt and Vitzthum, 1986: 153). Although it is recognized that metrics on small populations do not discriminate between *A. africanus* and *A. afarensis* (White et al., 1981), morphological features may shed light on the debate. Suwa et al.'s (1996) analysis of the Omo sample caution that "[c]haracterization of the East African nonrobust lineage is more difficult because of the polymorphic nature of detailed dental morphology, there being substantial overlap in ranges of variation among *A. afarensis*, *A. africanus*, and early *Homo*" (Suwa et al., 1996: 275). The most likely of the conclusions they propose is that the East African nonrobust lineage between 2.5 to 2.9 Ma represents a transition from *A. afarensis* to early *Homo* (Suwa et al., 1996). Frequency of protostylid expression in the Omo nonrobust sample provides additional evidence in support of this interpretation.

Protostylid expression on six of the non-robust Omo specimens from Hunt and Vitzthum's (1986) study was assessed (Table 1). The frequency distribution is shown in Fig. 4. Although this sample is small, it is interesting that none of these specimens has a high protostylid expression state. This suggests that protostylid frequency does not accord with the results from the above-mentioned metric study and that the most informative studies will consist of combined morphological and metric analyses.

A complex serial or metameric pattern is found when M_1, M_2, and M_3s are compared within each species. None of the molar sets alone appears to drive the species level differences. Therefore, cross-species comparison of M_1s only, for example, would not characterize the pooled-sample species distribution. Species samples that comprise equal numbers of M_1, M_2, and M_3s will be the most informative.

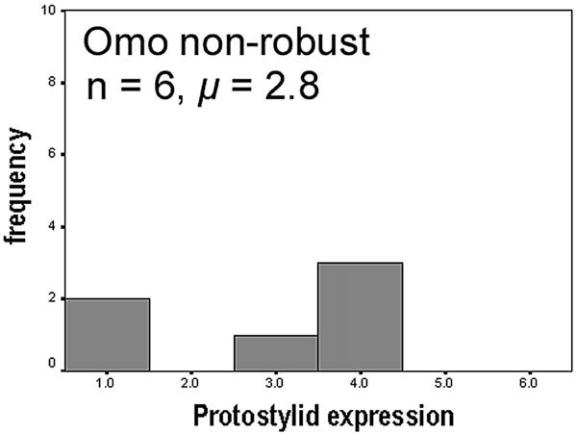

Fig. 4. Histogram of pooled M_1, M_2, and M_3 data for six non-robust lower molars from the 2–3 Ma deposits at Omo, Ethiopia.

Within species variation provides information about the developmental evolutionary history of the dentition. The identified serial differences between these frequency distributions provide insight into the evolution of the dental patterning mechanism of early hominids. Current knowledge of developmental genetics demonstrates that metameric variation, morphological differences between repeated segments of anatomy, often results from slight, genetically determined physiological differences, such as changes in regulatory genes (Weiss, 1990). Identification of the order and timing of the evolution of such variation elucidates the evolution of the underlying developmental mechanisms. A previous study has shown that metameric variation in the dentition of hominoids can shed light on the interrelatedness of various dental characters and on the sequence and timing of the evolution of these traits (Hlusko, 2002).

Protostylid expression similarly reveals metameric patterns. *Australopithecus afarensis* and *A. robustus* are both found to have a significantly higher degree of protostylid expression on M_2 compared to M_3. This contrasts with the serial relationships seen in modern humans. Humans typically have a higher degree and frequency of protostylid expression on M_1 and M_3 compared to M_2 (Hillson, 1996; Turner et al., 1991). Baboons also demonstrate a higher degree and frequency of

interconulid expression on M_1 compared to M_2 and M_3 (Hlusko, 2002), similar to the human relationship. Protostylid expression needs to be explored in the great apes, as well as earlier hominoids, in order to better understand the evolution of this pattern. However, it appears as though a shift occurred in the relationship between M_1, M_2 and M_3 relative protostylid expression during hominid evolution. *Australopithecus afarensis* is considered to be a likely ancestor to the genus *Homo* (White et al., 1981), suggesting that this metameric shift from M_2 protostylid dominance to M_1/M_3 dominance occurred sometime after the last appearance date for *A. afarensis* (2.95 ± 0.02 Ma, see Lockwood et al., 2000).

Because taxa as phylogenetically distant and morphologically dissimilar as mice and humans appear to have conserved dental developmental mechanisms (Davideau et al., 1999), it is reasonable to assume that the genetic mechanisms underlying normal variation will be similar between more closely related taxa such as baboons, humans and their ancestors. Salazar-Cuidad and Jernvall (2002) demonstrated through modeling experiments that minor modification of relatively well-known developmental pathways can produce known morphological variation between taxa as diverse as mice and voles. These results demonstrate that any advance in understanding the development of one organism greatly enhances our understanding of others, especially for taxa in the same order. Quantitative genetic analyses of the interconulus and interconulid in baboons suggest that degree of cingular expression in the upper and lower molars is determined by overlapping but non-identical sets of genes, i.e., incomplete pleiotropy (Hlusko and Mahaney, 2003). I suggest that incomplete pleiotropy is also likely to determine the incidence and expression of the protostylid in hominids.

Carabelli's cusp expression and frequency is greatest in modern human M_1s (Hillson, 1996). In contrast, the analysis of Carabelli's cusp in South African species of *Australopithecus* found that its expression and frequency increases from mesial to distal, with M_3 having the highest degree of expression (Reid and Van Reenen, 1995). Therefore, shifts in the relative expression of both upper and lower cingular remnants occurred during the course of human evolution.

Patterns of metameric variation suggest that a shift in dental patterning occurred within the last 3 Ma of hominid evolution. The polarity of expression is reversed for both the upper and lower cingular remnants between *Australopithecus* and modern humans. The *Australopithecus* upper molar pattern is $M_1<M_2<M_3$ and the lower molar pattern $M_1<M_2>M_3$. The human upper molar pattern is $M_1>M_2>M_3$ and the lower molar pattern is $M_1>M_2<M_3$ (Hillson, 1996). Although correlations between the protostylid and Carabelli's cusp in populations of Native Americans suggests interdependence (Scott, 1978), further investigation is needed to determine if this reversal occurred concomitantly as a result of the shared additive genetic effects or independently as a result of the unshared effects between these two traits. If the upper and lower molar expression patterns changed simultaneously during the course of evolution, then it is most likely the result of shared genetic effects.

Summary

The protostylid has played a significant role in early hominid studies, although it has traditionally been described qualitatively. Advances in biotechnology and genetics place renewed interest in dental traits such as the protostylid. To provide a strong quantitative foundation for further study of the evolutionary biology of the protostylid, six stages of expression are formalized. These stages are used for a taxonomic comparison of this trait in six species of *Australopithecus*.

These six species of *Australopithecus* demonstrate considerable intraspecific variation in protostylid expression. Though statistical comparisons of species means reveal differences between several of these taxa, the most informative aspect of this trait is in its distribution of expression states. Each species has a characteristic frequency distribution. Samples of M_1, M_2, and M_3 for each species show that no one tooth category fully describes the species distribution, and pooled samples need to contain equal numbers of M_1, M_2, and M_3.

As our understanding of the genetic and developmental bases for morphological traits, such as the protostylid, increases, the evolutionary and adaptive information they provide will grow. The first step towards this goal is a more complete understanding of the inter- and intraspecific variation of these features in the fossil record. This paper represents the first step in this direction by documenting the protostylid in early hominids.

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