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# Methodological implications of intra- and inter-facet microwear texture variation for human childhood paleo-dietary reconstruction: Insights from the deciduous molars of extant and medieval children from France

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A B S T R A C T

The present study concerns occlusal dental microwear texture variation on the deciduous molars of children. A description and evaluation of microwear texture variation within facet 9 and a comparison of microwear textures between grinding facets 9 and 11 are presented. The relationship between wear facet surface area and intra-facet microwear texture variability is evaluated. The sample is composed of naturally-exfoliated, taphonomy-free deciduous second molars from twelve extant children and four archaeologically-derived medieval children (for a total of 51 surface measurements). Dental microwear texture analysis (DMTA) was performed using a confocal microscope and scale-sensitive fractal analysis (SSFA) at three standardized locations on facet 9, and one location on facet 11. Facet shape was visually assessed and scored using a headset magnifier (3×) and composite images (20× confocal microscopy). Individuals were assigned to two groups based on a qualitative assessment of facet surface area. Microwear texture variability within facet 9 was high relative to the variability of microwear textures between individuals. No significant inter-facet variation between facets 9 and 11 was detected. No clear differences in microwear and variabilities within facet 9 were found between individuals assigned to small and large facet groups. Our study shows the existence of important intra-facet microwear variation in a sample of children. Intra-facet microwear variation can affect the ability of DMTA to distinguish between diets in contexts with small sample sizes and subtle differences in diet – such as those characterizing dietary transitions in children. Results also suggest non-dietary factors may influence microwear formation during dental exfoliation. A better understanding of intra-facet microwear variation, and when and how to account for it, can improve the application of occlusal DMTA in similar contexts.

## 1. Introduction

During mastication, microscopic alteration of enamel surface texture occurs as hard or abrasive particles are pushed against the tooth surface and tooth-to-tooth contacts occur. Since the 1970s researchers have noticed that the microscopic surface of teeth differs between species with different diets and so a number of methods have been devised to describe and quantify these differences, most commonly using scanning electron microscopy (Gordon, 1988; Grine, 1984; Walker et al., 1978). Dental microwear texture analysis (DMTA), using a confocal microscope and scale-sensitive fractal analysis (SSFA), is a more recent method by which the microscopic surface of teeth can be quantitatively described using microwear texture variables (Scott et al., 2006, 2005; Ungar et al., 2003). Over the last two decades, microwear texture variables have proven useful as dietary proxies for exploring dietary variation between mammalian species and populations (Green and Croft, 2018; Ungar et al., 2007) and even within populations (Merceron et al., 2010; Percheret al., 2018). Numerous studies have

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investigated group-level differences in occlusal molar microwear textures using permanent human teeth (El-Zaatari, 2010; Schmidt et al., 2016; Schmidt et al., 2019; Ungar et al., 2019). These studies have identified some general tendencies in the mean and distribution of microwear texture variables between different subsistence groups and populations (Schmidt et al., 2019). Mahoney et al. (2016) suggest that DMTA applied to deciduous and permanent upper molars has the potential to detect differences in diet between children of different age groups and social backgrounds within a community as well as the age at which weaning is initiated (an idea further developed in Scott and Halcrow, 2017). However, DMTA has rarely been applied to the deciduous dentitions of juvenile individuals. Furthermore, there are differences between deciduous and permanent dentitions in enamel structure, composition, dental organization, and masticatory biomechanics that may influence microwear formation (Gentile et al., 2015; Kamegai et al., 2005; Low et al., 2008; De Menezes Oliveira et al., 2009; Ubelaker, 1987).

In humans, occlusal microwear is generally measured on phase II grinding facets 9 and 11 (Krueger et al., 2008; Maier and Schneck, 1981). In DMTA studies, measurements are usually carried out on the same tooth and wear facet to facilitate comparisons of microwear textures from different individuals (Green and Croft, 2018). However, measurements from different facets of the same phase (e.g., facets 9 and 11) or the use of multiple tooth types are sometimes employed to increase modest sample sizes (El-Zaatari, 2010; Mahoney et al., 2016; Schmidt et al., 2016). Since different facets and teeth are exposed to different biomechanical forces (Maier & Schneck, 1981), these strategies of increasing sample size may serve to decrease DMTA sensitivity at the individual scale (Merceron et al., 2017).

One study identified intra-facet dental microwear variation using a scanning electron microscope (SEM) in adult humans (Mahoney, 2006). Microwear was examined at the upper and lower portion of facet 9 on mandibular second molars of adult Natufian hunter-gatherers and early Neolithic individuals. Mahoney (2006) found greater pitting frequency in the lower part of facet 9 among Neolithic farmers, no intra-facet differences among the Natufians, and hypothesized an increase in hardness between the groups to explain the farmer intra-facet variation. Two conclusions from the above research must be emphasized: first, both the uniformity (Natufian) and significant variation (early Neolithic) in intra-facet dental microwear can be quantified and compared. Second, subtle intra-facet variation can improve our understanding of the biomechanical properties of masticated foodstuffs, thus contributing to paleodietary reconstruction.

Animal experiments using domestic sheep models have also been used to explore intra-facet dental microwear texture variation (Merceron et al., 2017). Merceron et al. (2017) show that combining multiple measurements across a facet is detrimental for distinguishing between dietary groups and using a single central measurement was preferable, but requires a sufficiently large surface. In herbivores, in addition to standardizing measurement locations, researchers advise using a sufficiently large sample size (ten minimum, twenty recommended) to estimate the microwear signature of a given group (Green and Croft, 2018; Merceron et al., 2017). However, bioarchaeological, paleontological, and paleoanthropological studies using DMTA are frequently hindered by small sample sizes (Merceron et al., 2017). Sample sizes are further reduced by taphonomic factors limiting the measurable surface areas on individual teeth, and by the grouping of individuals by age, sex, social status or other categorical variables.

Human DMTA studies are generally limited by the availability of specimens and sample conservation. For instance, El-Zaatari (2010) published group sizes that are generally greater than 20, but others were as low as 13 and 4 individuals. Schmidt et al. (2016) opted to group individuals by broad categories corresponding to subsistence strategies, which created groups in excess of 40 individuals. Nevertheless, recent studies indicate that DMTA can also be useful for investigating differences within populations and at the scale of individuals (Schmidt et al., 2019; Ungar et al., 2019). Likewise, DMTA studies of weaning and the changes in diet throughout childhood within a population are expected to employ smaller sample sizes than studies comparing species, populations, or other macrogroup categories, since individuals are typically further subdivided into biologically-relevant age groups. However, subdivisions by age are crucial to answer questions of subtle dietary changes throughout childhood, and because there are specific age-related ontogenetic changes in the composition of the dental row and masticatory biomechanics throughout childhood growth and development (Mahoney et al., 2016; Scott and Halcrow, 2017). For example, Mahoney et al. (2016) subdivided 40 individuals into four age groups of ~10 individuals each for a study of childhood DMTA.

Furthermore, deciduous teeth often exhibit small overall dimensions and facet areas, especially in younger children. Restricting studies to teeth in which multiple, and/or larger and more standardized measurements can be taken may increase the reliability of results, but will likely reduce sample size further. If significant differences between groups or individuals are only found at specific locations on a wear facet (e.g., Mahoney, 2006), using non-standardized measurement locations may misrepresent biologically meaningful variation in microwear (Merceron et al., 2017). Given the limitations inherent to DMTA sampling strategies in general, and sub-grouping by age for DMTA analyses of children specifically, it is of the utmost importance to understand whether intra-facet variation is present in deciduous molars and if a standardized protocol can account for this variation. Furthermore, the contrasts in the biomechanical properties of foods – namely, changes in hardness – that may be responsible for noted intra-facet microwear variation between adult hunter-gatherers and farmers (Mahoney, 2006) provide a compelling analogy for thinking about dietary changes between age-groups within populations that correspond to the introduction of hard and abrasive foods throughout dietary maturation (e.g., weaning and the transition to an adult diet).

### 1.1. Objectives

1) We describe for the first time, using DMTA, intra-facet microwear variation of facet 9 (the most commonly analyzed phase II grinding facet) of deciduous maxillary second molars in a sample of extant and Early-Medieval children. 2) We examine inter-facet variation between two commonly grouped phase II facets (9 and 11) on the same tooth. Thus, we will address whether the practice of pooling microwear from these facets to increase sample size (e.g., El-Zaatari, 2010; Mahoney et al., 2016) is a valid approach. 3) Microwear patterning may vary with age in part because facet shape and surface area change greatly as children get older. Therefore, we also compare microwear variation between smaller and larger wear facets.

## 2. Material and methods

### 2.1. Description of samples

A summary of the individuals studied can be found in Table 1.

The Tooth Fairy collection ("Petite souris" in French) encompasses 882 naturally exfoliated deciduous teeth from 89 extant individuals belonging to 36 families born in France during the last 70 years (Le Luyer and Bayle, 2016). For each individual in the collection, there is an associated data form with biological and life history information, from which the sex of the individual was considered relevant for consideration in this study. From the collection, we selected individuals that had an upper second deciduous tooth suitable for dental microwear texture analysis, free from cracks or tooth decay, with well-defined wear facets, and an unworn or slightly worn occlusal surface (scores 1–3: Molnar, 1971) and we only used one tooth per individual. For this study, 12 teeth from the Tooth Fairy collection complied with our selection criteria, belonging to 12 individuals from nine different families

**Table 1**
Description of individuals in the study.

| Individual | Age estimation (yrs) | Sex |
|---|---|---|
| Individuals from the Tooth Fairy collection | | |
| AF2 | 10 to 12 | Male |
| F1 | 10 to 12 | Female |
| H1 | 10 to 12 | Female |
| I1 | 10 to 12 | Female |
| I2 | 10 to 12 | Male |
| J2 | 10 to 12 | Female |
| J3 | 10 to 12 | Female |
| K2 | 10 to 12 | Female |
| N2 | 10 to 12 | Male |
| O1 | 10 to 12 | Female |
| O2 | 10 to 12 | Female |
| R1 | 10 to 12 | Female |
| Individuals from Jau-Dignac et Loirac | | |
| JAU210 | 1 to 4 | Undetermined |
| JAU217 | 5 to 9 | Undetermined |
| JAU410 | 1 to 4 | Undetermined |
| JAU458 | 5 to 9 | Undetermined |

(Table 1). Upper deciduous molars were selected because they have thicker enamel than their mandibular isomers and hence are less likely to have experienced too much gross wear to exclude them from DMTA studies (Mahoney et al., 2016). Second deciduous molars were used because they are larger in size than first deciduous molars, thus presenting better-defined wear facets. Differences in tooth wear between left and right teeth can occasionally occur due to para-masticatory activity, abnormal occlusion or various oral pathologies that can lead to lateralized differential tooth loss, however there is no a priori reason to expect systematic differences in microwear texture of dietary origin between left and right teeth in our sample, left and right teeth were therefore analyzed together. Deciduous molar grinding (Phase II) facets 9 and 11 (Maier and Schneck, 1981) were targeted because they are most likely to register meaningful variation in diet (Mahoney, 2006) and are the most commonly analyzed in DMTA studies of humans (Scott and Halcrow, 2017). These facets are located on the buccal slope of the protocone (mesiolingual cusp), facet 9 towards the center of the occlusal surface and facet 11 towards the mesial edge. On upper second deciduous molars facet 11 is much smaller than facet 9 and is not always present.

Although the sample is limited to 12 individuals, the sample size is sufficient for intra- and inter-facet analyses of each individual. Furthermore, the analyses provide a critique of largely untested DMTA sampling strategies and inform our understanding of microwear texture variation. Thus, dietary reconstruction is not the goal of this paper; instead, we provide relevant information that can be used to refine such research and interpretations. Deciduous maxillary second molars are naturally shed between 10 and 12 years of age. A further advantage of using this sample is that the teeth present no taphonomic alterations typical of archaeologically- or paleontologically-derived human teeth.

Four individuals from an archaeological context were also included. They originate from the Early-Medieval site of Jau-Dignac-et-Loirac, an isolated insular community living in a large wetland environment on the shores of the Garonne estuary in the Medoc, France (Cartron and Castex, 2010). These individuals provide an example of intra- and inter-facet microwear variation in children from a pre-industrial agrarian setting. The archaeological children are younger than their counterparts from the Tooth Fairy collection (Cartron and Castex, 2010), which may correspond to a weaker bite force (Kamegai et al., 2005), but macrowear (score 1–2: Molnar, 1971) is similar across the two samples. Although the samples are generally well preserved, it was occasionally necessary to avoid taphonomic surface alterations during measurement (El-Zaatari, 2010). Since taphonomic alterations reduce measurable surfaces, fewer than three measurements per facet were made in a few cases.

Dietary information around the time of tooth loss or death is scarce for members of both groups. Dietary variation in the Tooth Fairy collection is assumed to be very high as individuals are members of an industrialized and globalized western society, with wide latitude to cater to individual dietary preferences, however this diet contains relatively few abrasives. Microwear variation in the group is expected to reflect this high degree of dietary variation as well as individual dental attrition (D'Incau, 2004). We expect greater inter-individual variation within the Tooth Fairy group than that observed in a study of medieval children by Mahoney et al. (2016). The medieval diet is expected to be

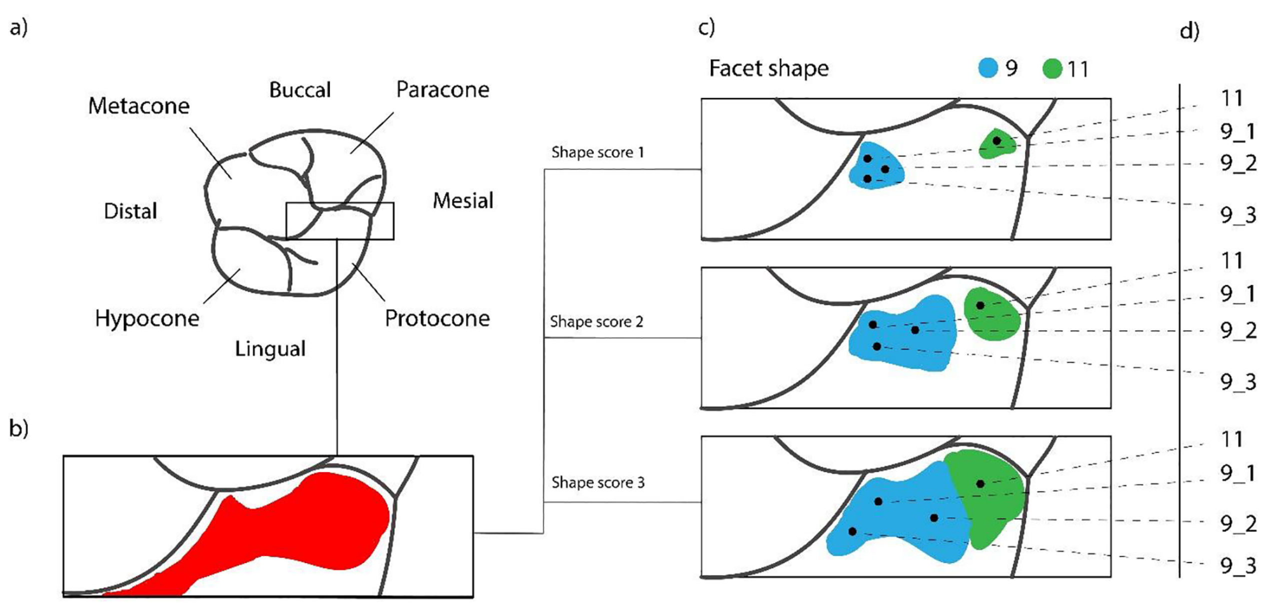


**Fig. 1.** Overview of measurement locations: a) area studied on the occlusal surface of the right second upper deciduous molar (occlusal view), b) area of development of the grinding wear facets on the protocone, c) facet shape scores of facets 9 and 11, note that the total surface area increases with the score, facets with a shape score of 2 and 3 having a much larger surface area than those with a facet shape score of 1, d) standardized measurement positions of microwear textures on the facets. Note that not all individuals presented a facet 11 in which case the facet 9 usually formed more mesially, facet location and the relative size of facets 9 and 11 are subject to individual variation in occlusion.

much more restricted and abrasive (Mahoney et al., 2016) than the modern child diet. As stated above, further dietary interpretations are unnecessary as they go beyond the methodological focus of this research.

### 2.2. Measurement procedure

All measurement positions on the wear facet are illustrated in Fig. 1. In practice, the exact position of the measurements varied slightly with the facet shape from individual to individual as they were located visually under the microscope (5x objective). It cannot be demonstrated that measurement positions correspond to homologous facet areas from one individual to the next; therefore, no analysis was made grouping measurements by position. However, defining these positions contributes to making measurements of intra-facet microwear variation more comparable between individuals. Measurements were not made close to the edge of the wear facet as surface texture can be different around the facet periphery and it is sometimes hard to determine visually the edge of the facet. In smaller facets, measurement areas were close, sometimes overlapping. Surface area and preservation were sufficient to measure microwear of facet 11 for eight out of the sixteen individuals (see Table 2).

Occlusal surface impressions were taken using President MicroSystemTM (Coltene) regular body polyvinylsiloxane material after cleaning the occlusal surface of the teeth using cotton swabs soaked with water or acetone when needed. The first and second impressions were used to adhere to and remove any particles remaining on the surface, thus only third impressions were used for analysis.

Microwear surface measurements were obtained using a standard DMTA methodology (see Ungar et al., 2003; Scott et al., 2005, 2006; Mahoney et al., 2016). Acquisitions were made on impressions of facet 9 or 11, using a Sensofar S Neox microscope and the acquisition software SensoSCAN 6.2. Facets were placed perfectly flat under the microscope and their orientation was standardized so that the distal side of the facet was closest to the observer and the mesial side furthest away. An initial surface of 333.21 × 250.78 µm was recorded for every measurement at the locations described above, corresponding to four fields of view with the 100x objective. Digital Surf surface imaging and metrology software (Mountains Map 7.2) was used to mirror the surface along the z axis, as the measurements were made on a negative, and to correct or remove any features identified as artifacts from measurement or molding (i.e., morphological filter and outlier removal). Surfaces with too many defects from the molding process or measurement errors were excluded. Post-treatment extraction of a 240 × 180 µm surface was made from the total surface of 333.21 × 250.78 µm, so as to include as few surface corrections as possible. Only this 240 × 180 µm surface was used for scale-sensitive fractal analysis. Toothfrax and Sfrax software (Ungar et al., 2003) were used to generate four surface texture variables that quantitatively describe the microscopic surface texture (see Scott et al., 2006): Complexity (Asfc) is a measurement of surface roughness at multiple scales and is generally greater when consuming hard foodstuffs as the increased bite force during mastication pushes particles deeper into the enamel surface. Anisotropy (epLsar) relates to the alignment of features across the surface and is generally greater with mastication of tough foodstuffs and repetitive jaw movements as particles are dragged across the surface along the same trajectory. Textural fill volume (Tfv) is an estimation of the volume of matter removed from the surface based on the highest point correcting for surface curvature. Heterogeneity of complexity (HAsfc) is a measurement of the heterogeneity of Asfc calculated independently, here over nine subsections of the measurement surface (3 × 3). HAsfc was calculated over nine subsections because HAsfc correlates with Asfc, and it was found that using nine subsections correlates least with complexity and was therefore considered less redundant with Asfc and hence offers the potential to provide more information about the surface texture. There are many other variables that can be used to describe microscopic texture such as scale of maximum complexity (Smc) and the ISO 25,178 parameters, however these variables are currently very difficult to interpret in terms of dietary habits and the physical properties of foods consumed. Some recent anthropological studies use only complexity (Asfc) and anisotropy (epLsar) as these two variables are the best understood. Given our sample we preferred not to multiply un-necessarily the number of parameters tested and opted instead to focus only on the most commonly employed and best-described variables.

The facet shape is defined in this study on the basis of a qualitative description in which facets with a higher score have a greater surface area. This score was determined on the dental silicone mold of the occlusal surface using a headset magnifier (3x) with frontal LED light, and using composite images created with a 20x objective and the confocal microscope. A three-stage scoring system was designed for this

**Table 2**
Facet shape score and all microwear textures measured (Asfc, epLsar, Tfv and HAsfc) for each individual.

| | | Microwear variables | | | | | |
|---|---|---|---|---|---|---|---|
| Individual | Facet shape score | Facet | Measurement | Asfc | epLsar | Tfv | HAsfc |
| AF2 | 2 | 9 | 9_1 | 5.0 | 0.0016 | 54,250 | 0.35 |
| | | 9 | 9_2 | 4.6 | 0.0021 | 37,084 | 0.78 |
| | | 9 | 9_3 | 2.5 | 0.0007 | 51,183 | 0.65 |
| | | 11 | 11 | 4.7 | 0.0015 | 41,408 | 0.45 |
| F1 | 2 | 9 | 9_1 | 1.3 | 0.0025 | 19,042 | 0.46 |
| | | 9 | 9_2 | 2.0 | 0.0016 | 44,614 | 0.42 |
| | | 9 | 9_3 | 4.8 | 0.0029 | 58,436 | 0.64 |
| H1 | 1 | 9 | 9_1 | 8.3 | 0.0013 | 46,068 | 1.18 |
| | | 9 | 9_2 | 5.0 | 0.0028 | 50,020 | 0.80 |
| | | 9 | 9_3 | 10.3 | 0.0030 | 52,876 | 0.89 |
| | | 11 | 11 | 3.1 | 0.0043 | 55,665 | 1.48 |
| I1 | 2 | 9 | 9_1 | 1.2 | 0.0027 | 45,153 | 0.50 |
| | | 9 | 9_2 | 0.6 | 0.0024 | 50,292 | 0.42 |
| | | 9 | 9_3 | 0.7 | 0.0024 | 42,577 | 0.94 |
| | | 11 | 11 | 0.4 | 0.0053 | 47,989 | 0.50 |
| I2 | 3 | 9 | 9_1 | 0.3 | 0.0037 | 31,869 | 0.43 |
| | | 9 | 9_2 | 0.6 | 0.0026 | 41,743 | 0.30 |
| | | 9 | 9_3 | 0.4 | 0.0014 | 3194 | 0.48 |
| | | 11 | 11 | 0.4 | 0.0005 | 37,164 | 0.25 |
| J2 | 2 | 9 | 9_1 | 1.3 | 0.0036 | 47,002 | 0.98 |
| | | 9 | 9_2 | 0.6 | 0.0003 | 38,607 | 0.38 |
| | | 11 | 11 | 0.6 | 0.0045 | 33,053 | 0.58 |
| J3 | 1 | 9 | 9_1 | 0.4 | 0.0073 | 38,723 | 0.36 |
| | | 9 | 9_2 | 6.9 | 0.0017 | 43,949 | 1.74 |
| | | 9 | 9_3 | 4.3 | 0.0021 | 54,176 | 0.58 |
| | | 11 | 11 | 4.6 | 0.0063 | 48,668 | 0.55 |
| K2 | 2 | 9 | 9_1 | 7.5 | 0.0016 | 55,225 | 1.30 |
| | | 9 | 9_2 | 14.8 | 0.0010 | 59,882 | 0.65 |
| | | 11 | 11 | 5.8 | 0.0023 | 36,747 | 1.44 |
| N2 | 2 | 9 | 9_1 | 1.1 | 0.0046 | 43,783 | 0.79 |
| | | 9 | 9_2 | 0.6 | 0.0047 | 39,904 | 0.43 |
| | | 9 | 9_3 | 0.8 | 0.0024 | 47,640 | 0.29 |
| O1 | 1 | 9 | 9_1 | 1.8 | 0.0027 | 37,078 | 1.45 |
| | | 9 | 9_2 | 1.1 | 0.0006 | 49,099 | 0.71 |
| | | 9 | 9_3 | 0.6 | 0.0016 | 36,565 | 0.42 |
| O2 | 1 | 9 | 9_1 | 1.6 | 0.0017 | 33,375 | 0.24 |
| | | 9 | 9_2 | 1.4 | 0.0022 | 41,921 | 0.57 |
| R1 | 3 | 9 | 9_1 | 6.3 | 0.0004 | 46,021 | 0.36 |
| | | 9 | 9_2 | 1.2 | 0.0029 | 39,759 | 0.58 |
| | | 9 | 9_3 | 2.9 | 0.0024 | 28,647 | 0.32 |
| | | 11 | 11 | 1.9 | 0.0014 | 36,257 | 0.33 |
| JAU210 | 2 | 9 | 9_1 | 1.2 | 0.0035 | 27,584 | 0.18 |
| | | 9 | 9_2 | 1.7 | 0.0037 | 49,720 | 0.12 |
| | | 9 | 9_3 | 1.9 | 0.0010 | 55,306 | 0.19 |
| JAU217 | 3 | 9 | 9_1 | 3.6 | 0.0006 | 45,446 | 0.33 |
| | | 9 | 9_2 | 2.9 | 0.0015 | 47,198 | 0.29 |
| | | 9 | 9_3 | 2.9 | 0.0007 | 33,626 | 0.19 |
| JAU410 | 1 | 9 | 9_1 | 2.5 | 0.0003 | 34,024 | 0.59 |
| | | 9 | 9_2 | 2.5 | 0.0033 | 38,979 | 0.35 |
| JAU458 | 1 | 9 | 9_1 | 2.0 | 0.0033 | 35,760 | 0.31 |
| | | 9 | 9_2 | 2.6 | 0.0008 | 28,425 | 0.30 |
| | | 9 | 9_3 | 2.0 | 0.0033 | 47,741 | 0.40 |

study and is illustrated in Fig. 1. The stages are visually distinctive from one another and were scored by two authors (MB and PB) and no inter-observer errors were found. If facets 9 and 11 are small and rounded the score is one, if the facets are larger and elongated but do not touch each other the score is two, and if the facets are large enough to touch each other the score is three.

### 2.3. Statistical analysis

The statistical analysis was carried out using IBM SPSS 25. Descriptive statistics were employed to describe the intra-facet microwear variability. We note that the coefficient of variation (CV) is not an ideal statistic of intra-facet variability because it is invariant to the number of replicate measurements for a single individual for facet 9 (N = 3 or 2). For a better reflection of measurement variation within facet nine, we decided to use the standard error (SE) in percent of the mean (standard error/mean*100; SE%) (Eisenberg et al., 2015). The SE (SD/√N), unlike the CV, decreases with increasing numbers of replicates. Regarding comparability for the calculation for the SE of all measurements, the average number of measurements for the single individual was used (N = 2.8). Due to group sizes, non-parametric statistics were employed.

Differences between facets 9 and 11 were tested for each variable, for the eight individuals for whom this was possible, using a non-parametric Wilcoxon signed ranks test (paired difference test). Individuals were separated into two groups to increase group sizes using facet shape score for analysis of the relationship between facet shape and microwear in the sample. The "small facet" group consisted of all individuals with a score of 1, and the "large facet" group consisted of all individuals with a facet shape score of 2 or 3. Groups were compared by rank-sum using a non-parametric Wilcoxon–Mann–Whitney *U* test.

## 3. Results

All results are summarized in Table 2. Before analysis, we tested whether extant and medieval individuals can be pooled for the statistical investigation of variability within facet 9 using a Wilcoxon–Mann–Whitney *U* test for all SE% variables. The test revealed significant differences in the variation for the Asfc_SE% ($p = 0.008$) of facet 9 for the extant versus the medieval group. The three other variables did not show significant differences at the $\alpha = 0.05$ level (epLsar_SE%, $p = 0.170$; Tfv_SE%, $p = 0.446$ and Hasfc_SE%, $p = 0.133$). Nevertheless, we decided to pool the data of the extant and the medieval individuals for further statistical analysis as the Asfc SE% calculated for the medieval individuals are equivalent to some individuals in the Tooth Fairy collection. The difference in Asfc SE% can be explained by a few individuals from the Tooth Fairy collection displaying very high Asfc variation across facet 9.

A further Wilcoxon–Mann–Whitney U for means of facet 9 between extant and medieval individuals found a significant difference for heterogeneity of complexity (HAsfc) ($\alpha = 0.05$ level, HAsfc: $p = 0.008$). A non-parametric Wilcoxon–Mann–Whitney U was also carried out between male and female individuals (from the Tooth Fairy collection) for means and SE% of facet 9 that revealed no significant differences between the two sexes. Additionally, microwear textures and variabilities from the two individuals with a Molnar (1971) score of 3 (AF2 and K2) were not found to differ from the rest of the group.

### 3.1. Microwear variability within facet 9

In Table 3, results of the statistical description of variabilities within facet 9 are summarized.

The mean variabilities within facet 9 for anisotropy (epLsar) and textural fill volume (Tfv) are higher than the mean inter-individual variability. For anisotropy (epLsar) the intra-facet variability is very high. For heterogeneity of complexity (HAsfc) the mean intra-facet variability is almost the same as the mean inter-individual variability, whereas for complexity (Asfc) the mean variability within facet 9 is only half that of the inter-individual variability. For all four variables within this sample, the SE% within facet 9 can be considered high relative to the inter-individual SE%. Some individuals present a generally low variation within facet 9 and some individuals, such as J3, display consistently high intra-facet variation equivalent to the entire group (Tables 1 & 2). Examples of surface variation within facet 9 and between individuals can be visualized in Fig. 2 and the values measured for all individuals and variables in Fig. 3.

**Table 3**
Summary of intra-facet nine and inter-individual microwear variation (SE%) by variable. SE% refers to the standard error in percent of the mean used to quantify variation.

| Variable | Intra-facet nine variation = Mean of individual SE% (N = 16) [%] | | | Inter-individual variation = SE% of individual Mean (N = 16) [%] |
|---|---|---|---|---|
| | Mean | Min | Max | Mean |
| Asfc(9) | 22.1 | 0.2 | 48.5 | 56.8 |
| epLsar(9) | 33.7 | 4.4 | 84.9 | 21.3 |
| Tfv(9) | 13.0 | 4.0 | 45.2 | 9.9 |
| Hasfc(9) | 25.1 | 9.8 | 48.0 | 26.4 |

### 3.2. Microwear variability between facets 9 and 11

Differences between measurements from facets 9 and 11 are described using the boxplots in Fig. 4. In the boxplots (Fig. 4) we can note that the dispersion of epLsar values appears greater for facet 11 than for facet 9, but no significant differences in rank-sum between facets 9 and 11 could be detected by the Wilcoxon signed ranks test ($\alpha = 0.05$; Asfc: $p = 0.138$; epLsar: $p = 0.132$; Tfv: $p = 0.596$ and HAsfc: $p = 0.983$).

### 3.3. Differences in microwear between small and large facets

The non-parametric Wilcoxon–Mann–Whitney *U* test detected no significant differences for all variables tested (facet 9 means and SE%) ($\alpha = 0.05$; Asfc: $p = 0.635$; epLsar: $p = 0.875$; Tfv: $p = 0.428$, HAsfc: $p = 0.562$, Asfc_SE%: $p = 0.492$, epLsar_SE%: $p = 0.313$; Tfv_SE%: $p = 0.562$, HAsfc_SE%: $p = 0.713$). However some small differences between both groups can be observed (Fig. 5.). Results suggest that the measured values and intra-facet variability in heterogeneity of complexity are higher in small facets relative to larger facets.

## 4. Discussion

Occlusal DMTA has demonstrated promise as a method for reconstructing diet in the past. However, further evaluation, standardization, and improvement of the methodology is needed (Green and Croft, 2018), especially in contexts where sample sizes are small (e.g., many paleontological or archaeological studies, and those requiring extensive subgrouping by site, sex, age, etc.) and/or where differences in diet are subtle. Intra-facet microwear variation has already been observed in sheep (Merceron et al., 2017) and humans (Mahoney, 2006), with implications for dietary interpretation and the sensitivity of dental microwear analysis methodology (Mahoney, 2006). The present study is the first to explore intra- and inter-facet microwear variation in children using DMTA, and to search for any effects of facet shape on the formation and variability of microwear on the facet surface.

In our sample, variability within facet 9 is generally large relative to the variation in microwear between individuals, especially for the anisotropy. Anisotropy is one of the most commonly used variables in DMTA for dietary reconstruction and has been related to the toughness

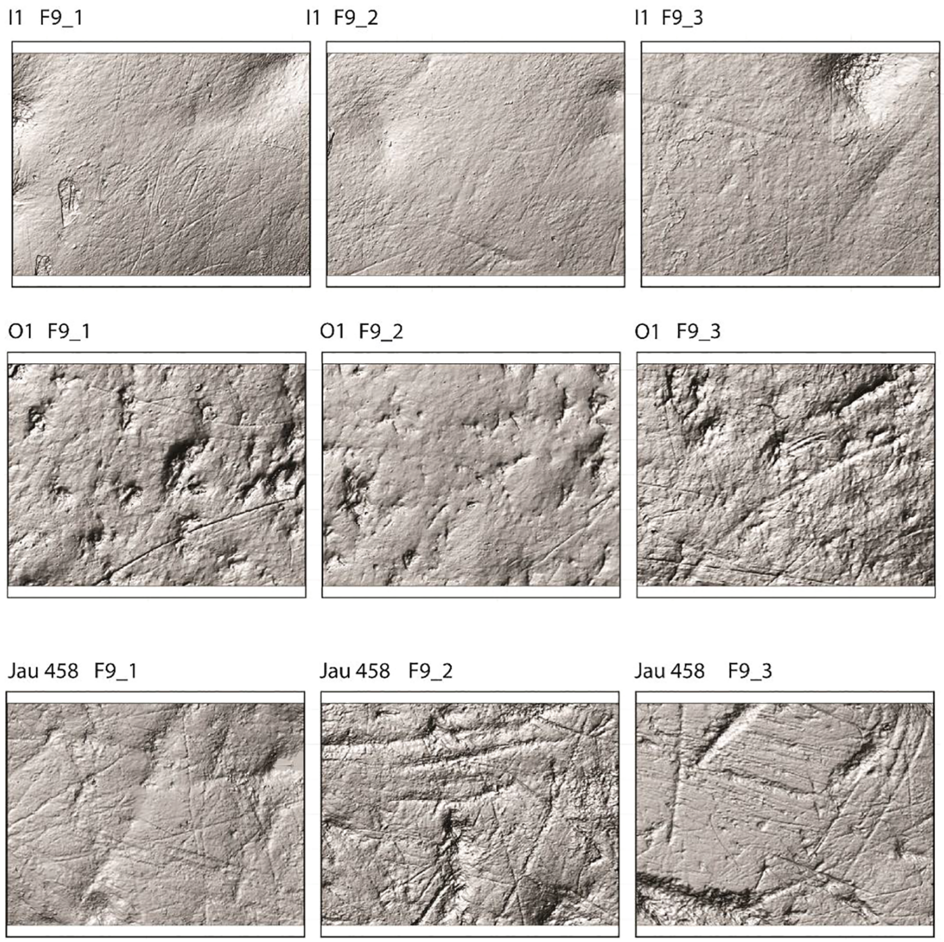


**Fig. 2.** Photo-simulations of the enamel surface (240 × 180 µm area) of individuals I1, O1 and Jau 458 illustrating the variation between measurements on facet 9 for a given individual and between individuals.

of food, and some studies rely only on data for anisotropy and complexity (Schmidt et al., 2019). Extensive variability within facet 9 is observed for both extant and medieval individuals. Intra-facet variability is different for each variable and varies between individuals. For some individuals intra-facet variation can be considered low but for some it is extremely high, equivalent to the variation within the entire group. Some individuals (AF2, F1, H1, J3, K2 and R1) display high complexity values, higher than those of the medieval children, and very high intra-facet variability of complexity. Other individuals (I1, I2, J2, N2, O1 and O2) display lower complexity values and intra-facet variability of complexity (see Fig. 3). It was observed that the surfaces of the individuals with high complexity and intra-facet variability present many large irregular pits spread unevenly across the surface. Usually, such features resulting in extremely high complexity values may be interpreted as taphonomic surface alterations, individuals K2 and H1 for instance display complexity values beyond the normal ranges for well-preserved surfaces, however due to the nature of the sample these surfaces are in fact most likely unchanged since exfoliation. Nonetheless, these pits may not be dietary in nature but rather more likely an artifact of dental exfoliation caused by changes to occlusion or biomechanics, or because the softer deciduous enamel is no longer suited for the bite force of children around the time the last deciduous teeth are shed. Although the origins of these complex uneven surfaces are unclear, these results would suggest that microwear texture analysis in children may reflect non-dietary ontogenetic factors, in this instance during the period of time around when teeth are shed. Intra-facet variability remains high even if we choose not to include these individuals with high variation of complexity under the assumption that their microwear textures are non-dietary in nature. If mean intra-facet variability is calculated for each variable without the individuals AF2, F1, H1, J3, K2 and R1 then SE% of complexity (Asfc) changes from 22% to 15.2%, for anisotropy (epLsar) from 33.7% to 36.2%, for textural fill volume (Tfv) from 13% to 13.8%, and for heterogeneity of complexity (HAsfc) from 25.1% to 25.4%. Naturally the mean intra-facet variability for complexity decreases but for the other variables it in fact increases subtly. These unusual surfaces are therefore not the main driver for the intra-facet variability described in this paper, although it must be noted they may increase the described variability of complexity somewhat.

In our whole sample, mean microwear variability within facet 9 is equivalent to the intra-facet variability measured in sheep (Merceron et al., 2017). Based on the raw data from Merceron et al. (2017), we compiled SE% for each variable: the mean SE% is 22.2% for complexity (Asfc) and 26.9% for anisotropy (epLsar), only 0.1% higher and 6.8% lower, respectively, than our data on juvenile humans. Furthermore, some mean SE% are even higher when compiled separately for certain dietary groups in their study.

No previous studies of microwear have been carried out in an extant sample of naturally shed teeth like the Tooth Fairy collection, so it is possible that the intra-facet variability observed here is somewhat specific to this sample. The absence of abrasive elements in the modern diet, or the prevalence of soft foods conducive to tooth-to-tooth contact that could lead to a dominance of attrition in the microwear signal. Behaviors such as tooth brushing with modern-day toothpaste may also explain the high intra-facet variability (D'Incau, 2004). Furthermore, as discussed above, the data suggest that non-dietary factors related to

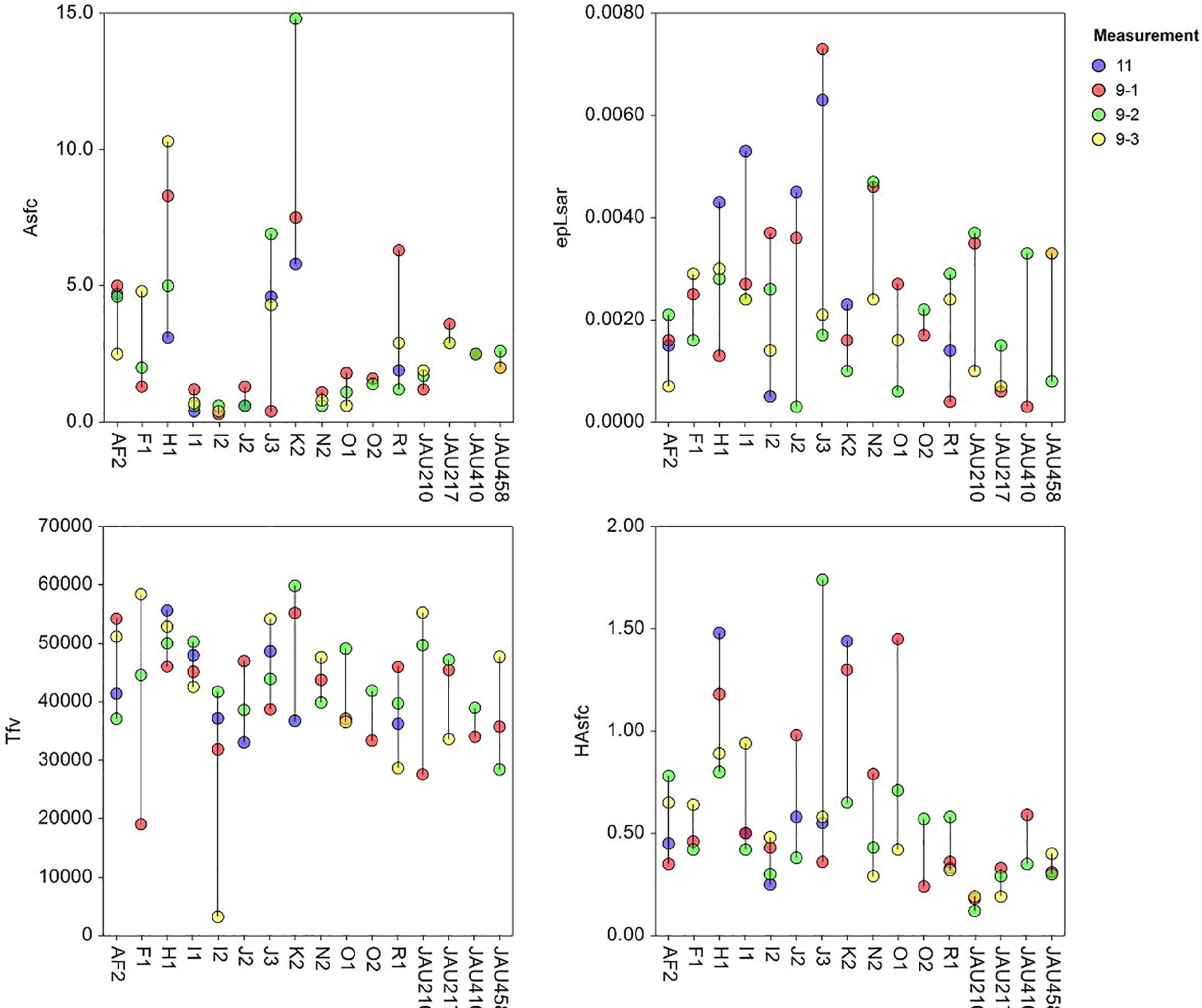


**Fig. 3.** Dropline graphics of measured values for each individual on facets 9 and 11, for each variable. This figure indicates relative measurement dispersion for a given individual and overlap of intra-individual variation between individuals.

dental exfoliation, and less common in archaeological samples, may have affected the surfaces of the extant individuals in this study. However, the similar microwear variation within facet 9 observed in our four medieval individuals when compared to some extant individuals suggest that intra-facet variation is not merely due to modern or ontogenetic factors (although these may increase observed intra-facet variations in complexity in this sample). Although microwear variation within facet 9 could be observed here in individuals from an industrialized and a medieval agrarian population, it remains to be explored in future studies if such variation can also be observed in individuals with more abrasive, tough and fibrous diets, and generally flatter macrowear angles, such as those from many foraging populations.

Our results suggest that an individual microwear signature assigned on the basis of a single measurement made at any location on the wear facet is liable to not accurately represent microwear variation within facet 9 regardless of whether facet surface area is small or large. Furthermore, specific differences in microwear signatures may exist based on where it is analyzed on a given facet (Mahoney, 2006). Therefore, especially when working with small samples or trying to detect subtle dietary differences measures should be taken to capture, or negate the effects of, intra-facet variability. Some researchers argue that large enough samples will provide an accurate measure of group and macrogroup (e.g., subsistence practice) diet. However, when looking at intra-population variation (e.g., by age, sex, status, etc.), the variation on a single facet could place an individual into more than one subclassification. Paleoanthropological research concerning rare fossils with small subgroupings (by ecogeography, chronology, etc.) is a perfect example of how within facet variability could imply a drastically different conclusion regarding paleodietary and behavioral reconstructions. How consistent patterns of intra-facet microwear variation are in samples of different subsistence practices, ecogeographic origins, age, and degree of macrowear has yet to be explored. And yet, this intra-facet variation could have important implications in contexts such as the study of childhood diet.

Both facets 9 and 11 are phase II grinding facets; however, they are formed during different movements of the jaw and through contact with different opposing cusps (Kullmer et al., 2009). This difference in the biomechanical origin of the surface was not clearly reflected by the scores obtained for these four microwear variables. This result supports the common practice of pooling data from facets 9 and 11. However, it must be noted that facet 11 presents a greater distribution of anisotropy values in our sample. Once again, in future studies similar comparisons between facets 9 and 11 should be carried out with individuals from different subsistence practices (e.g., earlier agriculturists and foraging populations) as well, to further test the utility of pooling data from different facets of phase II.

When individuals are placed into two groups based on the visually

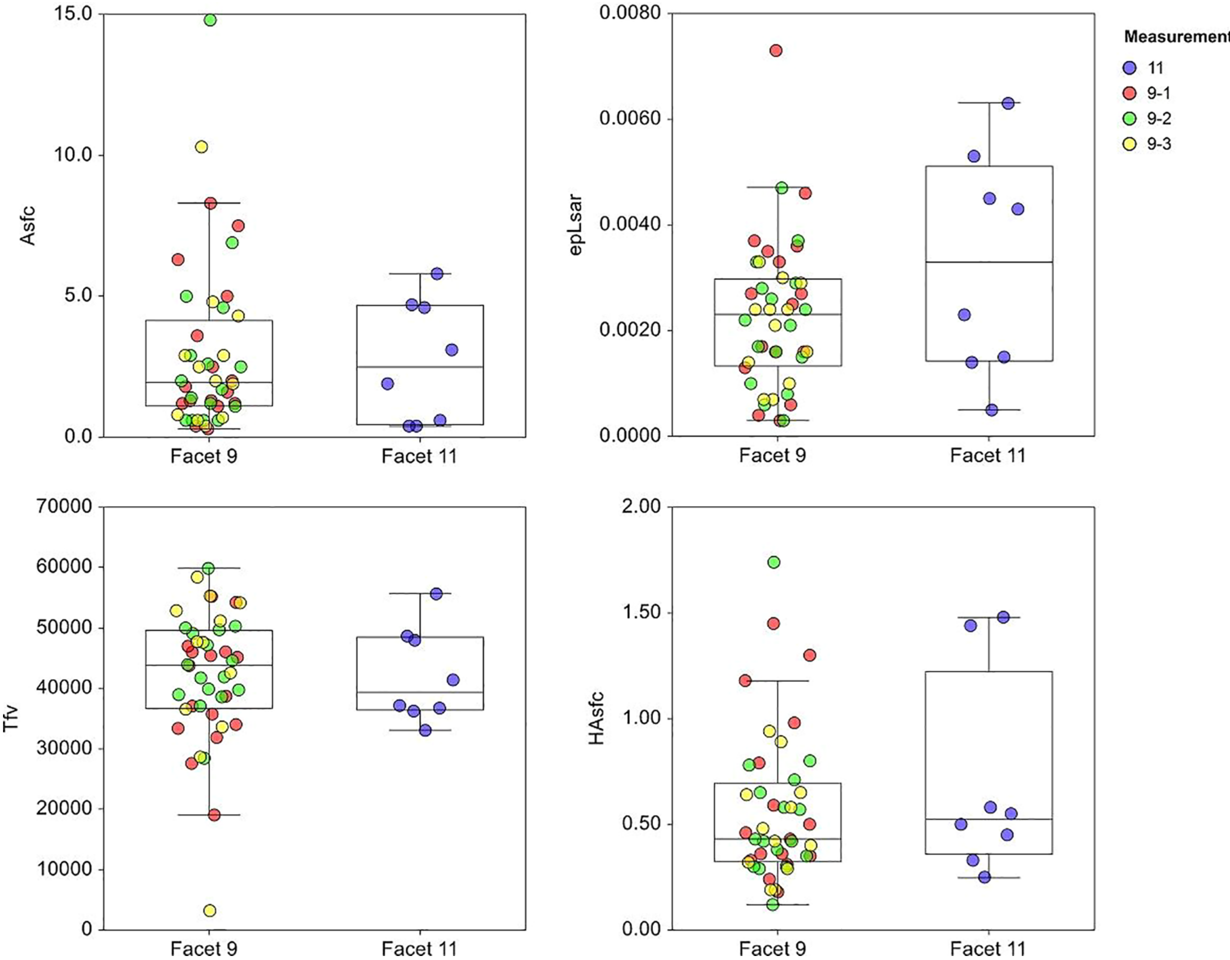


**Fig. 4.** Boxplot dot-plot combinations comparing facet 9 and facet 11 measurements for each variable.

assessed shape of the facet, no significant relationship was found between the shape of the facet and the microwear and its variability within facet nine. However, the use of a different sample and more precise quantitative descriptions of wear facets in future studies will help confirm or challenge the absence of a relationship between the wear facet shape and surface area, and the microwear texture recorded and measured on it.

No differences in microwear were found between male and female individuals, this was expected as differences in bite force between boys and girls remain small before puberty, although it can differ significantly already in eleven and twelve-year-olds (Kamegai et al., 2005). Furthermore, diet is not expected to differ significantly between children of the two sexes in a modern western context. A small but significant difference for one variable (HAsfc) was observed between the extant and medieval individuals. If the individuals in Tooth Fairy collection with the large pits and high complexity, that may not be dietary in origin are removed from the comparison, the remaining Tooth Fairy individuals present lower overall complexity values then the medieval individuals. Measurements of anisotropy and complexity for the medieval individuals are similar to those of the medieval children presented by Mahoney and colleagues (2016). There is an overlap between the values for the extant and the medieval individuals. We conclude that these four microwear variables discriminate poorly between the diets of individual extant and medieval children in our sample using the mean of facet 9 measurements, due in part to the large intra-facet microwear variation relative to inter-individual differences, a conclusion that is also supported by experiments on mammalian herbivores (Merceron et al., 2017).

## 5. Conclusions

Microwear analyses have demonstrated their capacity to detect broad dietary variation at species and population levels, and prior studies suggest they are sensitive to dietary variation at the intra-population and individual level. There is great potential that microwear analysis may be employed to describe changes in diet throughout childhood, such as weaning and the introduction of harder and tougher foodstuffs, as well as the differential treatment of children within a community, yet very few DMTA studies have focused on the deciduous molars of juveniles. The present study found important microwear variation within facet 9 in a group of extant and medieval children. The intra-facet variation found here must be accounted for when discussing differences at the scale of individuals. Furthermore, our results suggest that dental microwear may be subject to non-dietary factors around the time the tooth is shed. Both the differences and similarities in results between extant and medieval individuals are in accord with results from prior studies in which the distribution of possible individual microwear signatures within groups with vastly different diets overlap. This suggests that, when comparing individuals or small groups, it is preferable to tailor the sample and the paleodietary hypotheses to the specific context. Our results also underline the fact that dental microwear only captures a limited aspect of diet linked to the physical properties of food and does not always reflect the real degree of differences between diets due to the equifinality of dental wear (e.g., two different diets can produce identical microwear signatures), whilst simultaneously being sensitive to non-dietary factors related to occlusion and ontogeny. Even when comparing groups of larger sizes (ten to twenty or more individuals), a high degree of intra-facet microwear

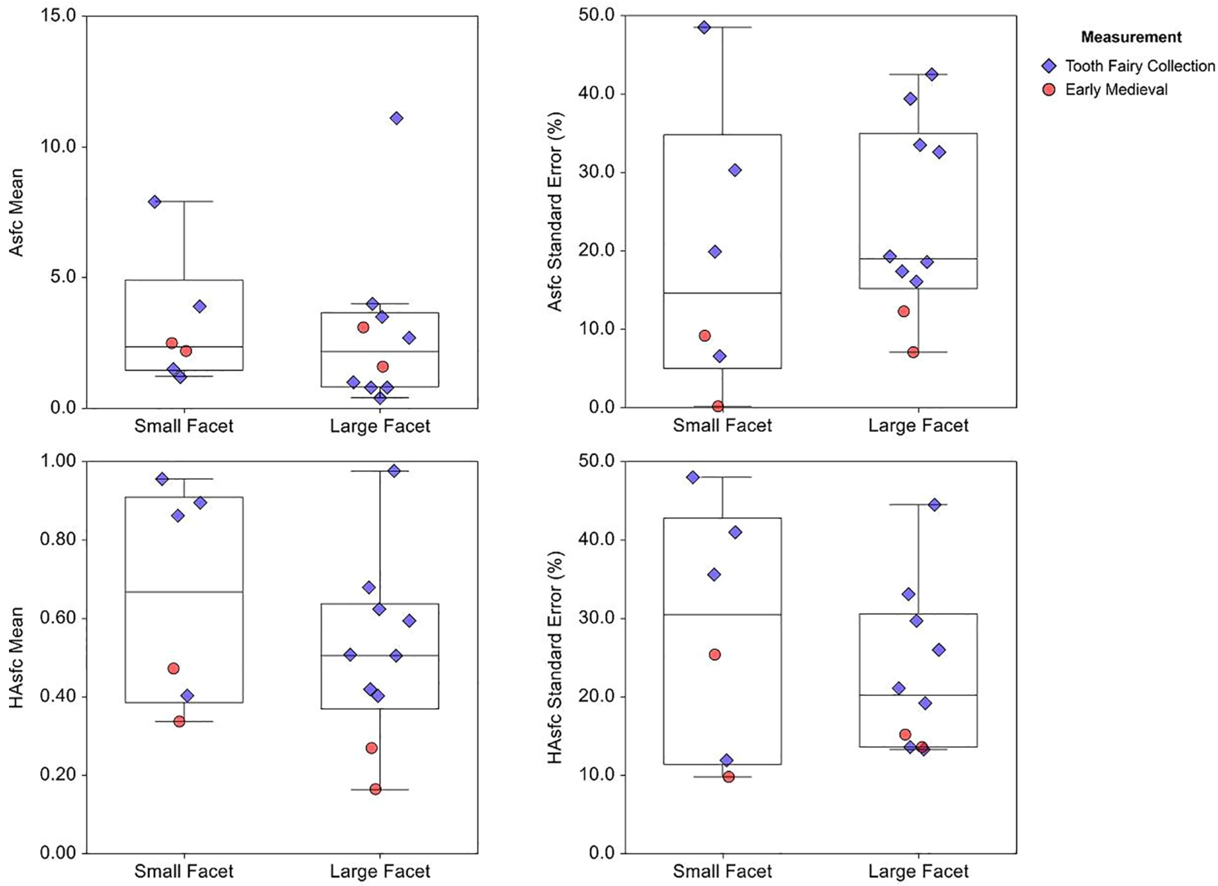


**Fig. 5.** Boxplots dot-plot combinations comparing intra-facet 9 mean values and variation (SE%) for complexity (Asfc) and heterogeneity of complexity (HAsfc), between small and large facet surface area groups based on the facet shape score (surface area). SE% refers to the standard error in percent of the mean used to quantify the variation of measurements within facet 9.

variability may still reduce DMTA sensitivity and hide subtle differences in diet. We did not find any clear differences between facets 9 and 11 measurements thus supporting the practice of pooling phase II facets, albeit this conclusion is yet to be examined in individuals with other subsistence practices (e.g., earlier agriculturalists and foragers). We found no significant differences between small and large facet surface areas, however our method for describing wear facets was relatively limited. Future developments in the procedures of data acquisition, treatment, and selection to better deal with intra-facet microwear variability and other factors that may influence the microwear texture measured will undoubtedly define the method's ability to tackle questions of (potentially subtle) dietary changes during childhood and other dietary differences between children within a population.

## CRediT authorship contribution statement

**Marlon Bas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Mona Le Luyer:** Conceptualization, Methodology, Supervision, Writing - original draft. **Fabian Kanz:** Formal analysis, Supervision, Writing - review & editing. **Katharina Rebay-Salisbury:** Writing - review & editing, Funding acquisition. **Alain Queffelec:** Methodology, Resources, Writing - review & editing. **Antoine Souron:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **John Willman:** Conceptualization, Visualization, Writing - review & editing. **Priscilla Bayle:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing - original draft.

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