Elliptical Fourier Analysis of Maxillary Molars in the Belgian Neolithic

Brandon Cory Bryan

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ABSTRACT

Dental morphology, and specifically molar shape, is a genetically stable marker of affinity and can be used to conduct studies of relatedness of past populations. Maxillary first molars from four cave sites of the Belgian Neolithic were compared using elliptical Fourier analysis (EFA) in order to understand the impact of both chronology and geographic distance on differences in molar crown shape within and between caves. Principal components analysis (PCA) revealed that individuals tended to cluster together based on cave burial as well as time period between sites, regardless of geographic distance. These findings contribute to the growing academic literature surrounding the use of dental morphology to understand human population dynamics of early farmers at the brink of the northern European Bronze Age.

INDEX WORDS: Elliptical Fourier analysis, Neolithic, Belgium, Molar morphology
ELLiptical fourier analysis of maxillary molars
in the belgian neolithic

by

brandon cory bryan

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ELLIPTICAL FOURIER ANALYSIS OF MAXILLARY MOLARS
IN THE BELGIAN NEOLITHIC

by

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May 2020
DEDICATION

I would like to dedicate this thesis to my mom, Denise, who has always provided a positive and nurturing light in my life, as well as my dad, Mark, who has encouraged my scientific curiosity throughout my life and supported all of my academic endeavors. Without the continuous assistance that you have provided me over the years, I would not be where I am today. You both have taught me so much about how to be a better person and a better scholar, and I have always followed your example and looked to you for guidance in all facets of life. I am forever grateful to have such incredibly supportive parents.

I would also like to dedicate this to my bunny, Albie. Although you cannot understand English as far as I can tell, you are my best friend and have always been my silent, fluffy companion, providing me with warmth and love in exchange for bites of banana and apple. You have been with me since I came to college and have never left my side through thick and thin, although that might be due to the fact that you don’t have thumbs and therefore can’t open the door to leave.
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1 INTRODUCTION

The Neolithic was a time of increased population density, greater variability in settlement patterns, and noteworthy anthropogenic alterations of the landscape (Woodbridge et al 2014). However, this revolutionary period of human history is largely centered around the creation and evolution of agricultural practices. The agricultural transition in the Neolithic transformed human activity and left profound impacts on flora and fauna in the physical environment. Plant and animal domestication, woodland clearing, slash-and-burn techniques, and other practices created an anthropogenic footprint that has had a cascading effect on the present day (Redman et al 2004).

The purpose of this study is to analyze two dimensions – space and time – that separate four Belgian Late Neolithic cave sites from one another. These four sites were occupied by groups living just on the cusp of the Bronze Age, and by understanding how kin, or related groups of individuals, were clustered along those two dimensions, valuable inferences can be made into the population structures of that time. The Neolithic-Bronze Age transition is a crucial transformative period in human development which has been noted to exhibit a shifting dynamic of burial styles as well as settlement types (McLaughlin et al 2016).

In order to understand relatedness to the extent it can be reconstructed, molar crown shape will be approximated in a manner that allows them to be compared using principal components analysis. Due to the heritable nature of dental remains, their use in biodistance analysis is important for understanding relatedness among ancient populations (Stojanowski et al 2018; Paul and Stojanowski 2017).

1.1 The Neolithic in Northern Europe
Generally speaking, the marked change in population dynamics during the Neolithic can be broken into two broad categories: internal and external. In this case, internal aspects consist of social and political dynamics while external aspects are related to climatic and environmental fluctuations. Scholars have long debated over the relative importance of one dynamic over the other, with some positing that Neolithic population expansion correlates strongly with a fluctuation of warmer and wetter winter climates that would have favored the subsistence practices of that time (Sánchez Goñi et al 2016). However, others have speculated that internal sociopolitical dynamics would have led to so-called “cultural cycles” throughout time which would have altered population structure. The author describes these cycles as “developments occurring in demography, economy, and social relations” (Zimmermann 2012).

Climatic events during the Neolithic transition, whether human-induced or naturally occurring, would have had serious implications for human settlements. These events would have created social and economic unrest, perhaps prompting food shortages or leading to abandonment of sites, and ultimately would have led to a restructuring of social patterns. Gronenborn (2009) presents a model of “climate-induced cultural change” that moves from equilibrium to fluctuations as a result of climatic changes, and then subsequently to social reorganization until a new equilibrium can be reached. This model sees climate crises as a catalyst for eventual increases in socio-political complexity, which may have included political cycling or migration events (Gronenborn 2009).

During the Neolithic transition, there is a noted correlation between higher population density and decline in woodland cover (along with an increase in semi-open pasture), indicating a link between agricultural practices and population structure (Woodbridge et al 2014). The Neolithic Demographic Transition (NDT) was a major long-term event in the process of human
history due to the large increase in demographic density, environmental impacts, and marked increases in fertility and mortality rates (Bocquet-Appel 2011). The shift from foraging and hunting-gathering to an agricultural system corresponded with what would eventually become sedentary village life. Nomadic lifeways made way for permanent and semi-permanent settlements containing designated farmlands, housing structures, walls (and other defensive measures), and domesticated animals. In some cases, this shift in sedentary lifestyle would have led to hundreds of years of continuous occupation, resulting in deep kinship ties and cultural traditions (Hofmann and Bickle 2009).

With that being said, it is important to understand the shortcomings of utilizing a cultural ecology theoretical model to analyze the nuances of human behavior. These models have been criticized by some scholars for minimizing the role of human agency in the production of culture, while instead relying too heavily on overarching environmental and ecological structures. While there is certainly a compelling correlation between human activity and the environment that they both live in and manipulate, it is important not to take a reductionist approach to the subject (Brumfiel 1992).

Analyzing the evolution of anthropogenic change across the Neolithic landscape has proved to be a powerful marker for cultural variation and settlement use patterns. Scholars have noted that changes (or lack thereof) of wood usage, wood selection, and wood working technologies across temporal and geographic areas indicate cultural change or continuity of settlement occupation. This shows the powerful long-term impact of human activity on the natural landscape, especially as it relates to wood exploitation and agricultural ramifications (Out 2017).
Archaeological analysis of the Neolithic period in Northern Europe specifically has resulted in data in line with what would be expected, including the development of animal husbandry, cereal cultivation (and other agricultural practices), permanent housing structures, and long-term occupation. A temporal perspective on these practices aligns with expectations as well, with an increasing intensification of these marked subsistence strategies during the Neolithic (Kooijmans 2007).

The Late Neolithic to Early Bronze Age period is a critical transition that deserves focus due to the large-scale changes that occurred in terms of settlement patterns, burial practices, and population dynamics. Archaeological analysis of the Irish Neolithic-Bronze Age transition has provided meaningful answers in this regard. Late Neolithic burial styles were varied, and included cremations, single burials, group burials, and re-use of previously dominant megalithic burials. The same dynamic applies to the settlement types, with a variety of timber-based structures and pit complexes. The authors argue that it is possible that mass migration into parts of Ireland during the Bronze Age transition could have been a factor in the relatively rapid change in material culture patterns, though other evidence suggests that perhaps the population dynamics remained relatively stable over this time period. One interesting hypothesis, which would explain both the population stability and the material culture shift, is the idea that this area experienced “conquest” rather than migration. Therefore, the influx of new groups would match the decrease in occupied groups, which would then result in a marked cultural shift but not a noteworthy population density change (McLaughlin et al 2016).

1.2 Mortuary Patterns in the Neolithic

Analysis of burial patterns is key to understanding population dynamics and spatial relationships in Neolithic village life. Archaeological studies of Neolithic burial patterns have
resulted in a common theme of group burials, although regional and temporal variation clearly exists as well as deviations from the norm. Extensive research has been conducted on the spatial significance of various types of communal burials across the Neolithic period with results indicating a range of possibilities. Across the European Neolithic, there is evidence of deliberate placement of burials, presence of valuable grave goods, and a variety of body positions (Shroeder et al 2019; Sparacello et al 2019). Burial trends appear to vary temporally, with evidence of differences across the Early, Middle, and Late Neolithic (Murphy and Le Roy 2017).

A case study of mortuary patterns in the Early Neolithic in Italy indicate a combination of single and double burials containing a mix of features and presence/absence of materials. Body positioning was inconsistent with these materials, with some laying supine and others deposited onto their left side in a crouching position, but the majority were aligned spatially and were buried in contraposition. One burial chamber contained two individuals – one adult and one subadult – with radiocarbon dates suggesting that they were not placed at the same time. Some individuals were buried with their head propped on top of large stones, although the intentionality of this observation is not confirmed. The same could be said of the various grave goods that were found in some but not all burials, including potsherds and flint flakes, which may have been accidental debitage deposition. Despite the variety, general consistency in body and grave orientation combined with a range of radiocarbon dates imply a continuity of funerary tradition within a culture (Sparacello et al 2019). Interestingly, the relative simplicity of these single and double burials is a feature that has also been noted in analyses of Early Neolithic burial patterns in France.

Moving on to the French Neolithic, there appear to be large scale patterns that define the Early, Middle, and Late periods in terms of funerary behavior and practices. Generally speaking,
this begins with the aforementioned simple single and small group burials, followed by an uptick in larger burials with the presence of some megaliths and a variety of body positions, and then finally an exclusive focus on collective burials in only one position (supine) in the Late Neolithic.

A spatial analysis of burial trends across the period indicate a potential shift in perspective regarding the relationship between the living and the dead. The early period was marked by an association of burial contexts with habitation contexts, which opens up the potential for philosophical and psychological interpretation of how closely these groups thought of life and death. However, as the Neolithic progressed, this trend decreased, with an increase in intentional, isolated burial areas. Perhaps this was preempted by a cultural shift towards a distinct dichotomy between the living and the dead (Murphy and Le Roy 2017).

Along with spatial analysis of individuals buried in mass graves, there is also variation of materials and style of the graves themselves. The Late Neolithic in Scandinavia exhibits a variety of stone cist burials of varying sizes, shapes, chambers, and unique features such as portholes, as well as wood coffin burials (Fraser et al 2018). Studies of Neolithic graves of the Low Countries have noted the potential for symbolic value of materials associated with the individuals within. One study notes the use of non-local wood for a particular burial chamber, perhaps indicating some ritual or symbolic significance (Out 2017). A main advantage to research involving Late Neolithic collective burials is that many of the largest and best known are associated with radiocarbon dates, allowing for the formulation of hypotheses concerning whether chronology or geography can account for the variation observed within and among caves.

1.3 The Belgian Early Neolithic
The Early Belgian Neolithic is noted for generally corresponding to the *Linearbandkeramik* (LBK) culture that pervaded much of Central and Northern Europe. Broadly speaking, LBK settlements are clustered into groups of 30-40, known as *siedlungskammern*, or “settlement cells” which are separated from one another by 2-10 kilometers, although regional variability is certainly present. Sites have been known to be located near river bends or streams in linear arrangements with farmsteads and village longhouses (Golitko 2010).

Genetic and material analysis of early LBK culture unfortunately provides conflicting answers. Regarding Central Europe, some scholars have argued that the continuity between late Mesolithic and early LBK cultures indicate some level of slow, longer term diffusion rather than the rapid changes that might arise during major migration events (Golitko 2010). However, the spread of LBK culture throughout the Neolithic into Northwest Europe deviates from the gradual diffusion model, and instead appears to be more in line with the migration theory. The spread of LBK culture in this context is much more rapid and distinct, with marked differences between the Mesolithic culture groups in Belgium compared to the LBK. It is theorized that these groups moved into Northwest Europe rapidly and settled into areas of agricultural importance (Golitko 2010).

Early Neolithic burial styles in Belgium are generally sorted into two types: semi-flexed and cremated (though this represents less than 10% of the known samples). Analysis of grave artifacts indicate an association between material goods and age, as well as a dichotomy between male and female grave goods. The association of age and material goods is indicative of a status-based cultural system in some way (Golitko 2010).

1.4 The Belgian Late Neolithic
The Late Neolithic of Belgium is the terminus of a long process of humans adapting to agricultural subsistence and away from foraging. Four different sites from the Belgian Neolithic, encompassing several hundred years, have been identified for analysis (Figure 1). They are located within several of the karst caves found along the Meuse River, a major North European feature that extends into France and the Netherlands. These cave sites represent mortuary locations for early Belgian farming communities during the Late Neolithic period. More specifically, Sclaigneaux and Bois Madame have been dated to the final/late Neolithic \([4,110 \text{ years before present (BP)}\) for the former, and \(4,075 \pm 38 \text{ years BP and } 3,910 \pm 40 \text{ years BP}\) for the latter] while Hastière Caverne M and Trou Garçon C can be considered early/late Neolithic \((4,345 \pm 60 \text{ years BP and } 4220 \pm 45 \text{ years BP})\). The radiocarbon date from Sclaigneaux was rendered using conventional methods at the University of Gent (UG), while the latter three were radiocarbon dates acquired through accelerator mass spectrometry (AMS) at the University of Oxford (Table 1) (Bronk-Ramsey et al. 2002; de Paep 2007; Toussaint 2007).

Bois Madame and Hastière rockshelter are located the closest to one another (~15 kilometers), while Sclaigneaux is about 35 km away from Hastière (Williams et al 2018; Table 1). These three sites are only a few of several hundred karst caves preserving human burials.

Broadly speaking, these cave sites exhibit a variety of mortuary features in terms of number of individuals buried, orientation, method of burial, and other noteworthy postmortem features. As is standard for the Neolithic period, there is a mix of single, double, and mass burials, with the majority consisting of communal graves. More specifically, evidence of maceration, cremation, commingling, and deliberate manipulation of remains has been noted. Graves at Bois Madame feature pottery fragments, animal fragments, and stone tools (Dumbruch 2003; Williams and Polet 2017). Unfortunately, the majority of the sites were excavated in the
19th century, providing incomplete field notes and excavation data from which to extract material culture information (Polet 2011; Williams et al 2018).

Figure 1 Map of Belgian cave sites. This map shows the general distance between the Hastière rockshelter (containing both Trou Garçon C and Caverne M) and the other two sites, Bois Madame (Burnot) and Sclaigneaux, as well as the distance from the capital city, Brussels. The inclusion of the four cave sites provides the opportunity to analyze relatedness at a variety of geographic scales. Image adapted from Williams et al (2018).
### Table 1 Cave site chronology and distance

This table is an overview of the chronology and distance of the four cave sites used in the sample, as well as the sample individual used for dating. While each site is separated by ~100 years, there is some overlap, especially between Hastière Trou Garçon C and Sclaigneaux. Meanwhile, Bois Madame is by far the most recent site, occupying a period of time that encroaches upon the start of the Bronze Age.

<table>
<thead>
<tr>
<th>Cave site</th>
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<th>Date range</th>
<th>Distance from Hastière (km)</th>
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<tr>
<td>Hastière Caverne M</td>
<td>AMS OxA-6558</td>
<td>4345 ± 60</td>
<td>0</td>
</tr>
<tr>
<td>Hastière Trou Garçon C</td>
<td>AMS OxA-6853</td>
<td>4220 ± 45</td>
<td>0</td>
</tr>
<tr>
<td>Sclaigneaux</td>
<td>UG (de Paepe 2007)</td>
<td>4110</td>
<td>35</td>
</tr>
<tr>
<td>Bois Madame</td>
<td>AMS OxA 10831</td>
<td>4,075 ± 38</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>AMS OxA 10830</td>
<td>3910 ± 40</td>
<td></td>
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1.5 Brief History of Discerning Affinity using Dentition

The relationship between genotype and phenotype in human populations has a rich history in the academic literature, including dental morphology, with studies as early as the 1920s and 1930s regarding the dentition of identical twins (Bachrach and Young 1927; Ashley-Montagu 1933). The concept of “biodistance,” coined in bioarchaeology, further emphasized the importance of understanding phenotypic expression as a proxy for genetic data (Hafner et al 2016). Dentition is a highly valuable tool in biodistance analysis due to its relative prevalence at bioarchaeological sites as well as its lack of environmental plasticity and secondary growth as compared to skeletal anatomy (Stojanowski et al 2018; Paul and Stojanowski 2017). These features, combined with the early development of much of the dental arcade, make it an important candidate for understanding the role genes play in phenotypic expression. This is not
to say that the environment is immaterial with respect to dentition, as its impact can be seen in the form of linear enamel hypoplasias, dental caries, and occlusal attrition among others, let alone the fundamental relationship between diet and dental morphology in evolutionary biology. However, its genetic stability in modern human groups make it a valuable indicator of relatedness.

A variety of dental traits and characteristics have been identified by scholars as markers of heredity which can be compared across bioarchaeological populations to understand relatedness and variability. Before the 1990s, many of these morphological variations did not have systematic methods to categorize them based on their size or significance. However, once Turner et al (1991) quantified and systematized these traits using a number scale in the Arizona State University Dental Anthropology System (ASUDAS), the analysis of dental morphology became standardized in a way that could allow for statistical comparison and interobserver repeatability. Some of the most highly used traits include: Carabelli’s cusp, parastyle, shovel shaped incisors, incisal winging, and extranumerary cusps and roots (Turner et al 1991).

In particular, Carabelli’s cusp has an especially significant abundance of literature surrounding it due to its high frequency, perceived association with particular populations, and its highly variable expression, ranging from a small bump to a full cusp-esque structure (Stojanowski et al 2018; Scott and Irish 2013). Variation in dental morphology has been analyzed at a number of spatial scales, which shows its flexibility as a tool for understanding relatedness at the local, regional, or even global level (Hanihara and Ishida 2005; Hájek et al 2008).

A case study by Cucina et al (2017) highlights some of the valuable conclusions that can be drawn using the ASUDAS system in order to trace migration and bioaffinity in particular
populations. In their study, three sample groups, one from the Classic period and two from the Postclassic period of Mexico, were compared to one another and to several modern populations in the region. Several of the previously mentioned dental non-metric traits formed the basis of the analysis, including shoveling, Carabelli’s trait, and extranumerary cusps. While the authors expected differences based around the geographic isolation of some of the groups, the results indicated that this was not strictly true, indicating a greater level of gene flow than previously thought (Cucina et al 2017).

Along with these traits, dental size and shape is also a valuable indicator of relatedness in bioarchaeological contexts. Studies with humans and non-human primates have shown that molar crown shape, cusp size, and occlusal outline are closely associated with heritability (Hlusko et al 2006). Dental measurements such as these, along with non-metric traits mentioned previously, are generally considered to be genetically neutral in terms of selection pressures and therefore should be stable (Turner et al 1991). Typically, this type of analysis is conducted with the first molar, as it is shown to be more heritable than the second or third molar (Hlusko et al 2006). With crown outlines, the method employed here, photographs of the teeth are taken and converted into binarized black and white images that make shape comparable across teeth using elliptical Fourier analysis. This method will be assessed further in the latter portion of this thesis.

Several case studies will be highlighted to illustrate the degree to which dental characteristics can be analyzed in order to understand affinity and population dynamics. In a 2018 study by Khudaverdyan, a variety of groups ranging from the early Bronze Age to the modern day within the Armenian Highlands were analyzed according to sixteen different non-metric traits. Using this sample, the author intended to understand the relative prevalence of these genetically based traits in a temporal manner, while keeping the spatial element constant.
She found that there was a general continuity of traits among each sample, indicating at least some permanent occupation by descendants of the groups that have lived in the area over the last several thousand years (Khudaverdyan 2018).

Hanihara (2008) provides a meaningful comparative case study to the Khudaverdyan (2018) article in terms of variables analyzed. In Hanihara’s paper, the temporal element is constant (as the sample groups are all modern humans), but the spatial element ranges according to geographic area. The author was interested in understanding large scale variation among populations and achieved this by comparing non-metric dental traits for twelve different geographic population samples. Due to the scale of the materials, the results that Hanihara landed on were wide-ranging. He found that some geographic groups displayed a high degree of intra-group variation while others fell on the opposite end of the spectrum. In particular, nonmetric traits, such as the ones used to compare geographic groups, are relatively stable regarding selection pressures in recent history and display regional variation in a consistent manner. This finding further cements the techniques utilized in the article as theoretically sound for assessing group membership using the dentition (Hanihara 2008).

Williams et al (2017) utilizes occlusal outline analysis in their comparison of Neanderthal and Neolithic human groups in Belgium. Using principal components analysis, a statistical technique that will be explored later, the authors assessed occlusal shape variability between the two distinct groups. Several interesting conclusions were gleaned from the principal component (PC) scores. First, the Neanderthal group exhibited higher intra-group variation than did the Neolithic cave groups in terms of crown and occlusal area. Two of the Neanderthal groups displayed larger crown size than the other two, but the PC scores indicated that differences in shape are more important than differences in size when comparing them to the Neolithic sample.
Overall, the results indicated that the two groups were substantially different in dental morphology (Williams et al 2017).

Another technique that can be used to approximate affinity based on morphological traits is geometric morphometrics. This type of analysis utilizes 3D scanning technologies to take digital models of skeletal features and compares virtual “landmarks” in order to understand shape and size differences. A 2018 study by Yong et al. uses geometric morphometrics to assess premolar occlusal size and shape of European- and Aboriginal-Australian individuals. The results indicated a significant difference between the two groups, with the authors suggesting that upper premolars may be a more reliable genetic proxy than lowers based on their evolutionary history (Yong et al 2018).

It is important to understand the degree to which human dentition varies according to location, position, and age, even within one individual (Edgar and Lease 2007; Paul et al 2017). Deciduous and permanent teeth are fundamentally different in terms of their morphology and size. The relative accuracy of their heritability is debated, but some research has postulated that, while permanent teeth are adequate at assessing affinity, the deciduous dentition may be more reliable due to its lack of environmental influence on the expression of traits (Paul and Stojanowski 2017). Williams et al (2018) echoes similar sentiments. They argue that due to the shorter crown formation period and lesser degree of agenesis, they may better a representation of affinity than the permanent dentition. It is possible that the deciduous dentition consists of fundamentally distinct processes of development regarding non-metric traits such that particular features that appeared on the primary teeth are not expressed on the permanent counterparts (Williams et al 2018). This raises important questions about the developmental processes of dental elements and how they may be affected by both genetics and the environment.
Maxillary and mandibular dentition also differ in their morphology and expression of traits. Molars of the opposing jaws in particular differ fundamentally in root number, cusp number, and occlusal shape (Folkens and White 2005). Evidence for greater genetic stability of maxillary or mandibular dentition is lacking but morphological differences between the two may be an indication. Evidence regarding third molar eruption suggests that mandibular third molars finish root formation earlier than their maxillary counterparts (Trakiniere et al 2019); the same is often true of the other molars. Studies of the emergence of deciduous incisors have indicated that they are strongly influenced by hereditary factors in a general sense, but emergence times between maxillary and mandibular, as well as central and lateral incisors are asymmetrical (Hughes et al 2007). This study provides evidence for the genetic component of dental development as well as the seemingly differential influence on each tooth in the arcade.

Other genetic quirks persist in dental morphology. Studies have noted that cusps forming later during development tend to be more variable in size, with the paracone being the least variable cusp in the maxilla (Kondo and Townsend 2005). Interestingly, a study of baboons found buccal cusps to be more closely linked in pedigreed lineages than the lingual cusps (Hlusko et al 2007). The authors indicated that the fashion in which the individual cusps occlude with their counterpart may play a role in how they develop. They write that the protocone and protoconid are the cusps that the others “pattern” around, so it may follow that the cusps that occlude with these are more genetically conservative than the others. Though the latter results apply to a non-human primate group, the authors argue that the “genetic architecture” is potentially a significant proxy for human (and other primate) dentition (Hlusko et al 2007). This also raises interesting questions regarding the factors that impact various features of the dentition.
Analysis of dental morphology is also a highly valuable noninvasive genetic proxy that is often preferred over the process of extracting DNA biomarkers (which results in destruction of the tooth). Information regarding the migration patterns of past populations, kinship ties, and other crucial research questions within bioarchaeology can be gathered from these noninvasive techniques.

1.6 Previous uses of Elliptical Fourier Analysis

There are several examples of the application of elliptical Fourier analysis in the field of bioarchaeology that show its importance in understanding shape variation in population groups. Letham et al (2017) present two straightforward case studies to showcase its effectiveness. In one example, the shape of the obturator foramen of the pelvis was assessed in a sample of ~200 modern humans in order to understand sources of variation. Principal components analysis revealed that PC1 (based on height:width ratio and medial border angle) is most reflected by sex and continent of origin. Similar results can be seen for PC2 (longitudinal axis angle) which is highly correlated with continent of origin.

In another example, the shape of the nasal aperture was analyzed based on a sample of ~850 skulls. The authors noted that population was strongly correlated with shape. They concluded that EFA and PCA used in combination with one another have led to meaningful results in regard to complex anatomical traits, and can provide some general answers regarding the source(s) of variation in shape between groups (Latham et al 2017).

1.7 Expected Results

Due to the strength of the background research regarding the use of molars in assessing affinity, it is expected that some convincing clusters of individuals based around the cave sites will be evident. The combination of time period of occupation and geographic distance between
sites should allow for a variety of compelling results to arise from the data. The oldest cave site, Hastière Caverne M, as well as the most recent, Bois Madame of the Burnot valley, are expected to show the most variable results with respect to the rest of the sample. As the furthest cave site geographically speaking, Sclaigneaux should stand out to a degree, but its chronological closeness to multiple sites may temper these expectations.

Previous research conducted in the Belgian caves has indicated that time may be the most important factor. Williams et al (2018) noted that Hastière Caverne M was unique in its strong expression of Carabelli’s trait, as well as being the only site with a protostylid present on the deciduous molars. Therefore, I would postulate that crown outline analysis will present similar results such that a difference is likely to exist between Hastière Caverne M and the Final/Late. However, it is important to note that Williams et al (2018) used deciduous dentition in their analysis.

Based on the sample sizes, it is entirely possible that variation within one cave will equal or even exceed the variation seen across the caves as groups. For methodological consistency, only maxillary first molars are utilized for crown outline analysis, and only well-preserved molars with minimal attrition were included in order to glean a reliable occlusal surface. I expect considerable noise in the data due to the small sample size. If the sample is small enough, the shape representations of the standard deviations during the elliptical Fourier analysis will appear quite extreme. In my particular example, the graphical visualization of the standard deviations appears normal (Figure 5), though with a total sample of 27 individuals, there will still be an expectation of variation.

Genetic expression is a highly complex manifestation and trying to understand affinity strictly using phenotypic representation leaves the possibility of perplexing outliers. Some
molars are simply oddly shaped, and individual variability in the skeletal record is a common occurrence. I expect to see a few individuals in the sample which exhibit unusual characteristics in their dental morphology. This could be due to the aforementioned catchall of human variation, or it could be indicative of something more intriguing, such as an immigrant to the population, an outside marriage, or an individual who assimilated into the group for any number of reasons. While these possibilities lie well outside of the scope of the project, the chances for this sort of outcome create interesting future research directions.
2 MATERIALS

A total of 27 individuals from four Late Neolithic cave burials were examined (Table 2). More individuals were available for Bois Madame and Sclaigneaux compared to the two cave burials from the Hastière rockshelter. Hastière Trou Garçon C is represented by the smallest number of individuals.

<table>
<thead>
<tr>
<th>Cave site</th>
<th>Number of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hastière Caverne M</td>
<td>6</td>
</tr>
<tr>
<td>Hastière Trou Garçon C</td>
<td>4</td>
</tr>
<tr>
<td>Sclaigneaux</td>
<td>8</td>
</tr>
<tr>
<td>Bois Madame</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>27</strong></td>
</tr>
</tbody>
</table>

*Table 2 Total sample sizes. This table shows the number of individuals sampled from each individual cave site, as well as the overall sample size.*

3 METHODS

The steps of data collection broadly include the photography and binarization process, and the statistical analysis. Two different methods can be used to capture images of the teeth. In one method, the dentition is casted in a plastic mold in order to create an accurate impression of the crown surface of the teeth without damaging or degrading them in any way. First, a layer of polyvinylsiloxane is applied to the surface of the tooth and allowed to dry. This step creates an impression of the crown surface which can then be utilized any number of times to create high-fidelity replicas of the teeth. Once the impression has been hardened, it is then filled with a
mixture of epoxy resin and hardener and left for at least 24 hours to set. Then, the dental cast can be removed and will be ready for microscopic analysis (Williams et al 2017). The other method (which was used in this thesis) involves using actual photographs of the crown surfaces of the teeth to conduct the outline tracing (Figure 2).

3.1 Outline Tracing and Binarization

Photographs of the crown outlines were first imported into GIMP, a free image editing software. From there, the crown outlines of the teeth were manually traced using the paths tool (Figure 2). Once the paths tool adequately captured the crown outline of the tooth, the shape was “binarized.” In the binarization process, GIMP essentially turns what was once a microscopic photograph of a tooth into a simple, highly contrasted black and white image. The polygon, which contains the outline of the crown surface, becomes entirely black, while the surrounding negative space becomes entirely white. This sharp contrast creates a controlled environment for shape analysis to be conducted accurately.

Figure 2 Outline tracing. This figure shows the results of the GIMP paths tool. The left image shows the individual anchor points that were manually placed, while the right image shows the final selection of the crown outline after the pen tool has successfully been used.
Figure 3 Binarization process. This progression of images illustrates the overall binarization process, beginning with a photograph of the original tooth (a), followed by a color-adjusted microscopic image of the dental cast with drawn crown outline (b), and the final black and white image which will then be converted into chain code (c). Image courtesy of Williams et al (2017).

3.2 SHAPE Software

After image binarization, a program suite called SHAPE was used to conduct the remainder of the analysis. SHAPE, a collection of free software, has a series of functions that ultimately analyzes shape using elliptical Fourier analysis. A program within SHAPE called ChainCoder assesses the closed contours of the image and stores this information as chain code, which is a lossless storage format for monochrome images (Iwata and Ukai 2002). Chain code visualizes an image as a series of vectors changing directions at fixed lengths to form the aforementioned contours. The changing directions are interpreted as “links” in the chain (Latham et al 2017).

The next step in the process uses elliptical Fourier analysis (EFA) to analyze shape. This process is a “curve fitting technology” which “allows a form’s outline to be approximated via the sum of multiple sine and cosine waves, permitting the profile perimeter of an object to be described in a dense (continuous) manner” (Caple et al 2017). In Iwata and Ukai’s (2002) explanation of EFA’s use within the SHAPE software, they write that it, “can delineate any type of shape with a closed two-dimensional contour.” A program within SHAPE called Chc2Nef
produces coefficients of the elliptical Fourier descriptors (EFDs) from the contours of the chain codes which can then be reduced to principal components scores from a principal components analysis (PCA). Elliptical Fourier analysis is central to the methodological process because it allows the shape to be assessed in a way in which PCA can effectively reduce the data to vectors that describe the variation among individuals.

Elliptical Fourier analysis gathers an accurate representation of the relevant shape and contours by utilizing harmonics. This process views the outline as a series of increasingly irregular “transitional shapes” which can progressively be mapped by ellipses. This series of harmonics depends on the overall irregularity of the shape being assessed, and each harmonic in the series analyzes a specific feature of the shape. For example, the first harmonic describes the shape’s size, the second harmonic describes its ellipticity, and the third describes its triangularity (Latham et al 2017).
Figure 4 Harmonic progressions. This progression of images illustrates a harmonic series surrounding a complex polygon shape. From left to right, the series shows 1-, 2-, 5-, and 7-harmonic progressions derived from the original chain code. Note the increasing complexity as the harmonics progress, beginning with a simple ellipsis and concluding with an outline much more representative of the original polygon. Image courtesy of Kuhl and Giardina (1981).
Figure 5 Elliptical Fourier analysis visualization. This illustration is a visualization of elliptical Fourier analysis taken from the SHAPE program suite. The left shows a fully binarized molar crown outline while the right shows an outline superimposed onto an ellipse. The differences between the ellipse and the tooth shape is what forms the basis of the analysis. This is quantified into numerical values used for the PCA.

### 3.3 Principal Components Analysis

Principal components analysis is the next step of the shape analysis process. After the harmonic coefficients are calculated using elliptical Fourier analysis, PCA variation among the different samples is broken down into principal component (PC) scores which are then assessed to understand the greatest degree of polarization of individuals along vectors. Each PC score is seen as one particular way in which the shapes differ, with the first being the most critical to the overall variation, and each subsequent score explaining less and less of the variation (Table 3).

Due to the highly complex nature of human skeletal anatomy, it is often difficult to gather data that accurately reflects morphology. While linear measurements and scoring standards (such as the Arizona State University Dental Anthropology System) are commonly used to understand size (and in some cases, shape), it is often unrealistic to rely on these types of measures to
understand the complex morphology of human anatomy. Meanwhile, the methodology laid out in this paper presents a mathematical framework for statistically analyzing complex shape in a fashion that bypasses several of the issues present in the other techniques (Latham et al 2017).

3.4 SPSS Statistics

The data output from the PCA was then analyzed in order to address whether spatial and temporal patterning exists between sample groups. SPSS Statistics by IBM was used to conduct the bulk of the statistical analysis as well as for the creation of the scatter plots. This assessment forms the core of the analysis as understanding the greatest degree of variation among samples is crucial in evaluating the expectations of the study.

In my particular sample group, the spatial variable is based on one cave (Sclaigneaux), located approximately 35 kilometers away from the three more closely positioned sites (Figure 1; Table 1). Meanwhile, the temporal variation hinges around the two Hastière sites, which are estimated to have been used several hundred years prior to the other two, and marks a difference between the Early/Late Neolithic and the Final/Late Neolithic period.

4 RESULTS

A total of 76 principal component (PC) scores were extracted from 20 harmonic descriptors using EFA. However, only the first six PC scores will be used for analysis, as they contain nearly 90% of the total variation. A table of the total variance explained by each PC score details the contribution of each of the six PC axes identified by SHAPE as representing the variation in morphology (Table 3).

The first two scores (which together explain ~60% of the variance) show the largest difference between mean and standard deviations of the eight axes explaining the lion’s share of the variation among individuals (Figure 5). The first column in Figure 5, which is an overlay of
all of the pictures within a row, becomes progressively more uniform as less variation is contained within each subsequent PC score.

<table>
<thead>
<tr>
<th>PC Score</th>
<th>Eigenvalue</th>
<th>Proportion</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.22E-03</td>
<td>38.48</td>
<td>38.48</td>
</tr>
<tr>
<td>2</td>
<td>6.75E-04</td>
<td>21.33</td>
<td>59.81</td>
</tr>
<tr>
<td>3</td>
<td>3.40E-04</td>
<td>10.74</td>
<td>70.55</td>
</tr>
<tr>
<td>4</td>
<td>2.67E-04</td>
<td>8.43</td>
<td>78.97</td>
</tr>
<tr>
<td>5</td>
<td>1.74E-04</td>
<td>5.51</td>
<td>84.49</td>
</tr>
<tr>
<td>6</td>
<td>1.45E-04</td>
<td>4.57</td>
<td>89.06</td>
</tr>
<tr>
<td>7</td>
<td>7.29E-05</td>
<td>2.3</td>
<td>91.37</td>
</tr>
<tr>
<td>8</td>
<td>5.81E-05</td>
<td>1.84</td>
<td>93.2</td>
</tr>
</tbody>
</table>

Table 3 Total variance explained. This table is a representation of variance explained by each PC score, as well as the cumulative effect of each subsequent score. While a total of 76 scores were calculated, only the first six were chosen due to their relative importance compared to the rest of the data. Here, the first eight are shown with their eigenvalues.

Tight groupings can immediately be noted on the x-axis corresponding to the first PC score (Figure 6). Hastière Caverne M is clustered exclusively on the positive side, while Sclaigneaux and Hastière Trou Garçon C overwhelmingly group onto the negative side. Meanwhile, Bois Madame fits in somewhat intermediate between the other three. Next, on the y-axis, corresponding to the second PC score, similar trends are noted, though the groupings are not nearly as tight. Sclaigneaux and Bois Madame appear to be the most closely grouped clusters, while Hastière Trou Garçon C is less clustered but still generally grouped. Most of the
major outliers are from the Hastière Caverne M sample, with two in particular (32 and 35) distinct from all others. Interestingly, Hastière Caverne M contains both the highest and lowest scores along the y-axis. Furthermore, the other two minor outliers belong to Trou Garçon C, making the rockshelter of Hastière the most variable site when considering the overall geographic location.
Figure 6 Principal components analysis visualization. This graph shows a representation of the crown outline analysis and how each PC score can be visualized as a physical shape, as well as the mean and two standard deviations in either direction. This can also be understood as a visualization of the total percentage of variation explained by each PC score.
Figure 7 PC1 vs PC2. This graph is a simple scatter plot containing the first two principal component scores, encompassing about 59% of the total variation. Each site is labeled by the shape indicated in the legend. The major outlier along the positive y-axis is Hastière Caverne M 32, while the most positive on the x-axis is Hastière Caverne M 25.

A comparison of the third and fourth PC scores displays a much more closely grouped set of data points across the cave sites, although some trends can still be noted (Figure 7). Hastière Caverne M appears to exhibit the most extreme outliers along the x-axis and in general has a loose grouping at best along this axis. However, along the y-axis, the sample is much more tightly grouped. Bois Madame is closely grouped along the third PC score, though it exhibits a large amount of variation along the y-axis. In fact, Bois Madame is somewhat isolated from the rest of the cave sites on the PC3 axis. Hastière Trou Garçon C and Sclaigneaux are intermediate
and are clustered together along both axes. When comparing the two sites from Hastière rockshelter, Hastière Caverne M appears to skew towards the positive side along PC4, while Hastière Trou Garçon C is more negatively projected, creating a partial separation of the two.

Figure 8 PC3 vs PC4. This graph is a simple scatter plot containing the third and fourth principal component scores, encompassing about 19% of the total variation. Each site is labeled by the shape indicated in the legend. The most negative outlier along the x-axis is Hastière Caverne M 25, while the most positive on the same axis is Hastière Caverne M 24. Meanwhile, the most negative individual on the y-axis is Bois Madame BM Mx 13.
The sixth principal component score strongly shows a clear divide between Hastière Trou Garçon C and the rest of the cave sites (Figure 8). Where Hastière Trou Garçon C and Sclaigneaux were previously clustered, here they are completely separated, with the Sclaigneaux sample projecting entirely towards the positive end. Bois Madame is generally entirely positive as well and overlaps with Hastière Caverne M strongly. PC5 does not provide as much interesting information, as most cave sites are highly variable and spread. Hastière Trou Garçon C does cluster onto the positive end other than one outlier, but unlike PC6, overlaps with all of the other sites. Sclaigneaux is perhaps the most negatively projected site on this axis.

Figure 9 PC5 vs PC6. This graph is a simple scatter plot containing the fifth and sixth principal component scores, encompassing about 10% of the total variation. Each site is labeled by the shape indicated in the legend.
5 DISCUSSIONS AND CONCLUSIONS

The major expectations of this study were that the individuals of each cave burial would cluster as a unit and that these units would be patterned with respect to temporality and geographic distance. Ultimately, these factors inform the degree to which Neolithic populations were closely related across the region at one time, and the degree to which kinship communities utilized a burial site exclusively across several hundred years. Certainly though, phenotypic analysis is highly complex and human variation is considerable, meaning a mix of factors are surely to be involved (Table 3). Along the first two PC scores, a clear clustering of Trou Garçon C and Sclaigneaux can be plainly observed. This can be seen less so in the third and fourth PC scores, where there is overlap but some separation, particularly on the y-axis. While PC2 and PC4 are not as informative as their counterparts, they have potential for interesting analysis. As the two sites with the closest overlap in occupation period, it is noteworthy Trou Garçon C and Sclaigneaux exhibit a relative lack of differentiation, perhaps informing the understanding of the population dynamics during this time. However, this connection is thrown to the wayside by the results of PC5 and PC6, which separates Trou Garçon C entirely from the rest of the sample, and particularly Sclaigneaux. However, as these represent a fractionally small portion of the variance in the overall data, their relevance has to be understood in its proper context. These two sites (along with Hastière Caverne M) represent the longest distance among all groups. It appears that, despite their distance of about 22 miles, the two sites present some similarities in crown outline shape.

The two most disparate sites in terms of chronology are Hastière Caverne M and Bois Madame, with possibly up to 600 years of difference in occupation period. The data present a mixture of conflicting results regarding their affinity. Bois Madame is very tightly clustered
along the first and second PC axes, while Hastière Caverne M is highly variable along PC2 but containing the majority of the positive PC 1 scores, giving itself a cluster along the x-axis. This presents two distinct regions on the graph, indicating a difference in molar crown outline morphology. This would make sense, given the substantial chronological distinction. However, PC scores 3-6 show less compelling results, leaving room for other interpretations. Hastière Caverne M in general varied across the board, and contained most of the major outliers in the data. When isolating Bois Madame, it tended to cluster by itself in some regards, such as on PC3 and to a lesser extent PC6.

Meanwhile, the strong clustering of Bois Madame and Sclaigneaux are significant indicators of a group of related populations existing at potentially a very similar period of time. One potential time range reported by Williams et al (2018) could place Bois Madame at a slightly earlier time period, as far back as 4,075 ± 38 years BP at its most extreme end. This lines up closely with Sclaigneaux’s proposed date of 4,110 years BP, indicating the potential for two closely related groups living at the same time. While the earliest Bois Madame date is not the likeliest scenario, it opens the door for a clear temporal link between these two sites.

Hastière Caverne M, as the oldest site in the sample, appears to be the most highly variable across all PC scores. It contains an outlier in every graph and has the least tightly clustered points, especially when looking at PC5 and PC6. Its distinct patterning (or lack thereof) is an interesting signal for its unique position as the earliest analyzed site. Due to the effects of gene flow, with the onset of the Bronze Age and beyond, there should theoretically be more genetic homogenization. Hastière Caverne M’s separation from some of the later sites as well as its outliers in the data indicate a distinctive population, removed from the others by its chronological position.
The two Hastière rockshelter sites provide an opportunity to assess the effects of chronology on two populations that occupied the same geographic area. Interestingly, the two sites are virtually separated in all aspects. In fact, along PC6, Trou Garçon C is by far the largest outlier in the data. In most cases, Hastière Trou Garçon C and Hastière Caverne M cluster separately and distinctly. With about 100 years of difference between the two, it raises questions over the genetic similarity between these two populations, despite their habitation in the same area.

The major outliers raise intriguing questions over their connection to the overall population with which they are grouped. Multiple interpretations are possible, and certainly the complexity of human variation is rife with situations such as these. As previously stated, Hastière Caverne M appears to have the most extreme outliers. Once the individuals are singled out, there does appear to be a few who are consistently on the periphery of the sample, although there is some variety in this, indicating that perhaps Hastière Caverne M is unique overall. Hastière Caverne M 25 appears as the most positive outlier along the PC1 axis, while also being the most negative along the PC3 axis. Meanwhile, Hastière Caverne M 24 lies along the most positive axis on both PC3 and PC5. On the other end of the chronological spectrum, Bois Madame also appears to contain some outliers, creating doubt over the theory that the earliest sites should display the largest outliers. This is especially true when isolating PC4, where Bois Madame BM Mx 13 is the most highly negative sample, while BM Mx 17 is the second most positive. However, as previously stated, their overall tight cluster along PC3 tempers some of this variation.

Overall, the analysis has indicated that chronology is perhaps the more important differentiating factor compared to geographic distance. The grouping of Sclaigneaux and
Hastière Trou Garçon C, despite their distance of roughly 35 kilometers, as well as the separation of the two Hastière sites, are compelling results for understanding population dynamics during the late Belgian Neolithic. The highly variable nature of Hastière Caverene M, with its extreme outliers, is another interesting finding that is logically sound in the context of population genetics during this time.
6 REFERENCES


6.1 APPENDICES

6.2 Appendix A: Complete list of individuals (n=27)

Appendix A.1 Hastière Caverne M (n=6)

24, 25, 32, 34, 35, 36

Appendix A.2 Hastière Trou Garçon C (n=4)

1, 4, 6, 9

Appendix A.3 Sclaigneaux (n=8)

92, 93, 97, 98, 99, 100, 103, 122

Appendix A.4 Bois Madame (n=9)

27, Mx 1, Mx 5, Mx 6, Mx 9, Mx 11, Mx 12, Mx 13, Mx 17
6.3 Appendix B: Complete list of binarized mages

Appendix B.1 Hastière Caverne M (n=6)
Appendix B.2 Hastière Trou Garçon C (n=4)
Appendix B.3 Sclaigneaux (n=8)
Appendix B.4 Bois Madame (n=9)