Biomechanical modelling of Human Femora: a comparison between Agriculturalists and Hunter-Gatherers using FEA, GMM and Beam Theory

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Abstract

Any behavioural reconstruction based on skeletal morphology relies on the principle that bone is functionally adapted to its mechanical environment during life. Traditional beam theory has commonly been applied in biomechanics to model the behaviour of past lifestyles of human populations. Despite its usefulness, the application of beam theory to long bones makes several unrealistic assumptions. The most noticeable one is the fact that long bones are modelled as simple beams, without taking into account their complex shapes. To this day few studies have assessed the amount of error inherent in simplification as compared with other techniques. One such alternative is the use of finite element analysis (FEA), a mechanical engineering technique that allows for more complex forms, but is much more complicated to perform. The aim of the present dissertation was to contrast the two techniques by applying both to a classical bioanthropological example: the comparison of skeletal performance between agriculturalists and hunter-gatherers. 20 male Native American left femora belonging to two distinct populations a) Norris Farm (n=10): agriculturalists and b) Black Earth (n=10): hunter-gatherers, were compared by applying both classical beam theory and FEA. The femora were scanned using a Medical CT-Scan and the data volumes were manually segmented. Cross-sectional properties were then estimated in the model and each dataset was converted into a finite element mesh to perform FEA. Statistical analyses were applied to the resultant datasets to test which technique was better differentiating between hunter-gatherers and agriculturalists. Geometric morphometrics (GMM) analyses were also carried out, in order to compare their competence when distinguishing between past lifestyles as compared with the other techniques applied. These results are expected to contribute in a better insight when assessing lifestyle from femoral shaft morphology.

Contents

Figures

Tables

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Author's declaration

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Do what you love. Know your own bone; gnaw at it, bury it, unearth it, and gnaw it still.

Henry David Thoreau. Letter to Harrison Blake, March 27, 1848.

Chapter 1: Introduction

1.1 First Section: Generalities

1.1.1 Introduction

Biomechanics is the discipline that applies mechanical principles to biological systems (Ethier and Simmons 2007). Its scope encompasses an enormous range of studies from the rotatory movement of bacterial's flagella to the design of tree trunks (Wainwright et al. 1982; Vogel 2003). The application of biomechanical principles to vertebrate skeletons has a long history that can be traced back to Galileo (1638) and Borelli (1680) (Fig. 1). However major development in biomechanics occurred in the 1800s and early 1900s with several investigations carried out by anatomists and orthopaedic surgeons (Evans 1953; Martin et al. 1998; Mow and Huiskes 2004; Bartel et al. 2006).

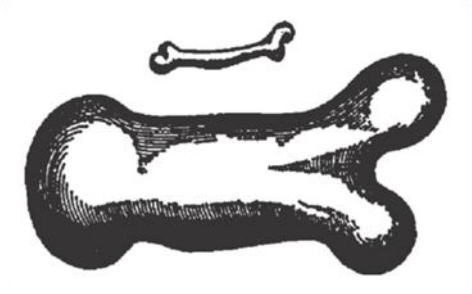

Figure. 1 Galileo was one the first scholars to concern about biological scaling in his "*Dialogues Concerning Two New Sciences*" (1638). His illustration of small and large animal bones is reproduced here.

Also interested in biomechanics but from different perspective, biological anthropologists have long been concerned with how mechanical principles can be applied to explain human and primate skeletal morphological variation among extant and extinct populations (Washburn 1951). Biomechanical theory is regularly used in physical anthropology to investigate diverse issues such as primate and human locomotion (Crompton et al. 2010; D'Août and Vereecke 2010; Sellers et al. 2010), masticatory function (O'Higgins et al. 2011; O'Higgins et al. 2012) and morphological variation due to bone functional adaptation (Ruff and Higgins 2013). One of the areas where traditional biomechanical methods have been widely applied is in bioarchaeology, where structural analysis of long bones has been used to infer past patterns of lifestyle and mobility (Ruff 2008).

1.1.2 Bone functional Adaptation

Any behavioural reconstruction based on skeletal morphology relies in the idea that bone is functionally adapted to its mechanical environment during life (Lieberman 1997). If bone does not morphologically respond to mechanical loadings, its morphology will not reflect the particular loadings that it was subjected to during life. This would prevent any attempt to infer the past behaviours that produce the loadings experienced by a bone.

The notion that bone is functionally adapted to the mechanical demands that are imposed during its ontogeny has been recognised for a long time. The general notion that mechanical loadings affect bone structure has been known for almost a century as "Wolff's Law", although many authors have pointed out that its original meaning was to certain extent different (Lieberman et al. 2004; Ruff et al. 2006; Ruff 2008). "Wolff's Law" referred initially to the proposal that trabecular bone tend to develop in orientations that parallel the principal mechanical stresses that acted on bone (Wolff 1892; Martin et al. 1998). On the other hand, nowadays is common to invoke this principle as a vague rule to denote bone functional adaptation to mechanical stimuli. Thus, the so-called "law" has evolved into a "black-box" explanation to relate skeletal form and function (Pearson and Lieberman 2004). In spite of these semantic issues, diverse evidence supports the idea that bone undergoes mechanical adaptation: a) studies with athletes that show an increased bone strength; b) experimental studies with animals that are submitted to vigorous exercise regimens or movement constraints; c) studies of limb disuse (e.g. pathology, zero gravity, immobility) (Trinkaus 1983; Haapasalo et al. 2000; Robling et al. 2000; Shaw and Stock 2009a; Shaw and Stock 2009b). Thereby an increased strain produced through for example, an increase in body size or muscle activity, will stimulate deposition of new bone tissue which strengthen the bone and consequently reduces strain. On the contrary a decreased strain (e.g. paralysis; inactivity) will lead to bone resorption, which weakens the bone. Additionally, experimental evidence has shown that the relationship between bone structure and mechanical loading is not as linear as initially thought, for example systemic factors such as disease, age, circulating hormones, genetic background and diet can modulate the bone response (Pearson and Lieberman 2004; Riggs et al. 2004). The general model has been supported by diverse experimental and observational studies, although some caution is required, therefore it has been proposed to replace the term "Wolff's Law" with the more exact "bone functional adaptation" (Ruff et al. 2006).

Bone is not an inanimate material but a dynamic tissue in which different cells respond to their microenvironment in order to ensure balanced growth, development, maintenance and repair of the tissue. The overall process is regulated by multiple factors, mechanical and molecular, that interrelate in a highly complex cascade of interactions. Despite the inherent complexity of this process, it is increasingly more known the detail of which hormones and biochemical signals participate actively in response to genetic and epigenetic influences (White and Folkens 2005; Carter and Beaupré 2007). The major factors that modulate bone modelling and re-modelling are mechanical stimuli (i.e. stretch; pressure; flow) (Ethier and Simmons 2005). This adaptive behaviour allows the bones to withstand repeated loadings without breaking or risking extensive damage. Nonetheless it has been evident for long time that bone responds adaptively to mechanical demands, the mechanisms by which bone senses and responds to these mechanical loads are still relatively unknown (Burr et al. 1985; Han et al 2004).

It seems that the loads applied to bones at the tissue level are experienced by the cells (osteocytes) that lie within the bone matrix (Han et al 2004). Mechanical loads deform the bone matrix, and that stimulates cell function. It has been shown that a subtle deformation of the bone matrix, triggers DNA synthesis, enzyme production, synthesis of collagen and non-collagenous proteins, and mineral production in the osteocytes (Kawata and Mikuni-Tagaki 1998; Mullender et al. 2004; Wang et al. 2007). This evidence demonstrates that bone cells are highly sensitive to even subtle mechanical stimuli, and they respond to local mechanical pressures in one or two ways: by generating new bone tissue or by reabsorbing old tissue (Ethier and Simmons 2005). This process is not random, but instead it is regulated in such manner that fulfils the local mechanical requirements of the organism's skeleton.

There are various well used illustrations about the mechanobiology and functional adaptation of bone, especially regarding adaptations to exercise, vigorous lifestyles, sexual dimorphism and so on. Using beam theory as proxy for the mechanical properties of the bone, it has been established that different loading regimens modify, not only the shape of long bones, but also the proportion between cortical and trabecular bone (Ruff 2008). In simple terms the idea is that long bone diaphyses behave much like engineering beams when they are mechanically loaded (Huiskes 1982). Comparisons of these data with those for earlier human ancestors and with nonhuman primates (Ruff 2003a, 2008), demonstrate a decline in relative bone strength among modern humans. Interestingly the relative cortical thickness of long bones has decreased during human evolution, probably because of the diminution of daily life loadings (Ruff 2000, Ruff 2008).

1.2 Second Section: Robusticity

1.2.1 What does robusticity mean?

Robusticity is a widely used term in biological anthropology; however it has multiple assessing ways, depending on the author and research area. Different kind of analyses examining long bone robusticity has been proposed to understand the relationship between skeletal morphology and behaviour (Hawkey and Merbs, 1995; Robling et al. 2000; Daly et al. 2004; Warden et al. 2005; Ruff et al. 2006; Weiss 2007). All of them are based on the principle of bone functional adaptation (usually referred as Wolff's Law; Wolff 1892), stating that bone form reflects the cumulative effects of *intra vitam* mechanical loadings (Ruff et al. 2006). Even though the effects of many other aspects, such as sex, body size, age at death and handedness are incompletely understood when related to robusticity, most of the authors agree on the fact that bone reacts to loading changing its form in order to consequently bear the strains exerted on it.

a) **Musculo-skeletal markers (MSM)**: During an individual's lifetime, intense, repetitive activity is known to produce responses in bones at the sites where ligaments, muscles and tendons attach. Activity-induced changes to ligament and muscle attachment sites on the skeleton, increased muscular activity augments strain on attachment sites, thus stimulating osteoblastic activity and as a result affecting bone morphology on the attachment location (i.e. increasing rugosity, furrowing, or ossification). Therefore, prominent MSM are typically the result of extensive ligament or muscle use, during an individual's lifetime (Weiss 2003, 2004, 2007).

These loadings stimulate bone growth, producing a hypertrophic bone response and distinct markings (Hawkey and Merbs 1995; Weiss 2007). Usually these features are analysed as discrete characters, (Fig 2.), even if there is a continuous gradient between the different states.

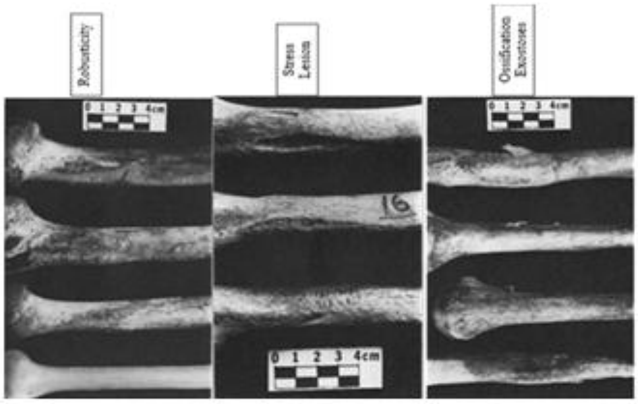

Figure 2. Example of a coding system for musculo-skeletal stress markers in long bones. Modified from Hawkey and Merbs 1995.

b) **Development of characteristics associated with sexual dimorphism**: Robusticity has also been used to assess sexual dimorphism both at intra and inter-specific levels and/or has been considered as a secondary sexual characteristic (Carlson et al. 2007).

c) **Morphometric definition**: The diameter of a long bone relative to its length, or in other words the thickness of a long bone shaft relative to the latter (Bräuer 1988, Martin and Saller 1957). The word has also been defined as the size of articular surfaces relative to length, torsional strength, and cortical area of shaft sections in relation to bone length (Endo and Kimura 1970; Lovejoy and Trinkaus 1980; Bridges 1989; Kimura and Takahashi 1992; Trinkaus et al. 1994).

d) **Beam theory definition**: When analysing long bones, usually changes to skeletal robusticity, have been defined as an increased thickness of the shaft relative to its length (Martin and Saller 1957) and more recently quantified by cross-sectional cortical geometry (Ruff 2000). Despite the fact that the process of bone functional adaptation is complicated and not completely understood (Lanyon 1992; Pearson and Lieberman, 2004), there is substantial evidence to establish a connection between mechanical loads and the geometric characteristics of long bone diaphyses (Robling, et al. 2000; Daly et al. 2004; Warden et al. 2005; Ruff et al. 2006). Different measurements for long bones skeletal robusticity have frequently been applied to interpret patterns of prehistoric behaviour such as the transition from hunting and gathering to agriculture (Stock and Pfeiffer 2001, 2004). More recent research has provided evidence linking long bone diaphyseal robusticity and shape with some general patterns of habitual locomotion amongst athletes (Shaw and Stock 2009a, 2009b). As an operational definition, robusticity has been described as the biomechanical the strength or rigidity of a structure relative to the mechanically relevant measure of body size (Ruff et al. 1993).

None of these definitions is free of limitations or problems, thus some authors have proposed combine some of these traits as interrelated indicators of “musculoskeletal massiveness” (Trinkaus et al. 1991).In this dissertation, robusticity will be defined in operational terms depending on the applied analytical technique:

Beam Theory: Cortical area (CA) and polar second moment of area (J) were used as proxies for compressive and bending strength, respectively. Augmented values in either of these two variables could be regarded as an increased robusticity.

FEA: Robusticity was considered as the resistance (degree of deformation; strains or global changes in form) to loading as assessed by Finite Element modelling. Therefore a lesser degree of deformation could be considered as a major robusticity.

1.2.2 Evolutionary trends in long bone robusticity

It is interesting to study long-term trends in long bone skeletal biomechanical properties, because these allow us to gain insights into past behavioural patterns and contributes to interpretation of modern skeletal variation. In spite of the well-known fact that modern humans tend to be more gracile in comparison to other hominids, there are few analyses of temporal trends in skeletal robusticity among humans (Ruff et al. 1993). Based on these it has been suggested that a 15 % decline occurs on average in relative bone strength from 2 million to 8000 years ago (the ability to resist breaking is referred to as strength). Indeed this decline has continued during the Holocene, by approximately another 15% on average. Consequently, a loss of around a third in bone strength has taken place within our own genus, with a half of that process happening over the past few thousand years. The most plausible explanation for this evident reduction is that the mechanical loadings exerted on the appendicular skeleton have also decreased during this period, due to the technological enhancements that have gradually protected the body from physical demands (Ruff et al. 1993).

When this data is compared with those for other hominoid primates and other hominids, an even greater post-cranial robusticty reduction is apparent over the course of hominin evolution (Ruff 2000, 2003a; Ruff et al. 1993; Trinkaus and Ruff 1999) (Fig. 3.). Modern humans correspond to the final stage of a long lineage of reductions in bone strength that can likely also parallel to a decline in muscle strength and physical activity.

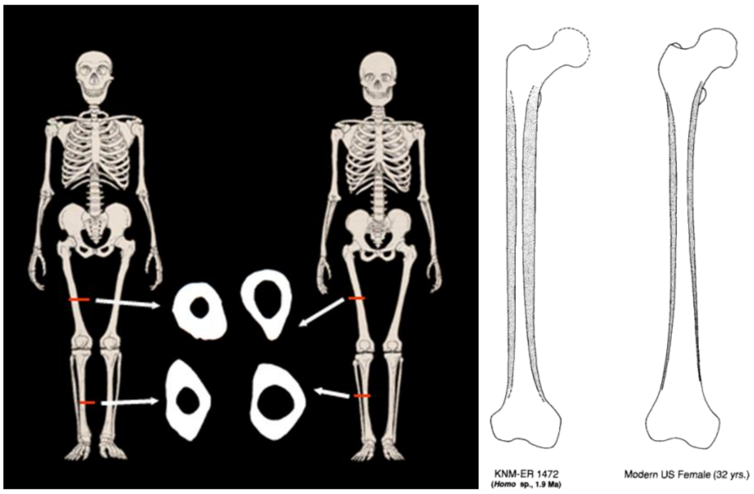

Figure 3. Left: differences in lower limb cross-sectional geometry and cortical thickness between Neanderthal (Amud 1) and *H. sapiens* (Skhül 5). Right: differences in cortical thickness between *H. rudolfensis* and *H. sapiens* (Ruff et al. 1993; Trinkaus and Ruff 1999).

1.2.3 The transition to agriculture

The relevance of the transition to agriculture was recognised by Gordon Childe who coined the term ‘Neolithic Revolution’. This concept refers to the change from hunting and gathering to agricultural during the Holocene. It has been regarded as possibly one of the most significant socio-cultural and eco-biological changes since the origin of our own species, initiating the human control over the reproduction and evolution of plants and animals (Childe 1936). A hunting and gathering subsistence strategy characterised much of our own genus evolutionary history of (Lee and De Vore 1968; Brain 1983), predominantly prior to the late Holocene when domestication of animal and plant resources along with critical technological innovations enabled the adoption of agricultural subsistence lifestyles.

Normally the transition to agriculture is regarded as a continues series of important transformations in human organisation, based on the increase of food production due to domestication and the storage of food surpluses (Pinhasi and Stock 2011). This rise on production and change in the mode of production has been interpreted as a required step towards social hierarchy, property ownership, task specialisation and technological evolution (Diamond 1997). From an ecological point of view, the transition to agriculture can be considered as a form of niche construction and inheritance, allowing human population to manipulate actively their environments and in the same fashion being subjected to the selected pressures derived from these new milieus (Odling-Smee et al. 2003; Lewontin and Levins 2007; Odling-Smee 2009). Despite the fact that several lines of evidence have shown that it seems that the transition to agriculture probably implied a downgrade in life quality and health (Armelagos et al. 1991), the majority of world's population relies on agriculture which could be considered as an indication of positive aspects of the agriculturalist lifestyle. It has been proposed that this transformation in the mode of production allowed an increased birth stacking associated with alloparenting, increased fertility and reduced interbirth intervals (Wells and Stock 2007).

It has long been speculated if the demographic shifts amongst hunter-gatherer societies motivated this cultural modification, since greater population numbers could not be maintained on the basis of hunter-gatherer subsistence (Cohen 1977). Irrespective of whether demography was the main causal influence in the agriculture development, it is nowadays relative clear that drastic demographic transformations were major consequences of the transition to agriculture (Bocquet-Appel and Bar-Yosef 2008). Regardless if population size was the main promoter for, or the key consequence of, the transition, there are almost no doubts that a positive feedback was establish between demography and cultural complexity, visible on how the agricultural shift went by hand with urbanisation and social hierarchisation. Agriculture is still today the principal means of production sustaining global population and economy. The importance of assessing robusticity in human long bones associated with the transition to agriculture is derived from the fact that human remains comprise a privileged glimpse for human biology in the course of this process.

1.2.4 Subsistence Strategy: Robusticity patterns in Hunter-Gatherers vs. Agriculturalists

Probably behavioural changes associated with changes in subsistence and technological advances influenced long-term changes in general robusticity patterns during human evolution. Actually several lines of bioarchaeological evidence have confirmed that both the physical environment (terrain) and subsistence strategy contributed to modifications in long bone diaphyseal structure (Stock and Pfeiffer 2004; Stock 2006; Shackelford 2007; Marchi et al. 2011; Pinhasi and Stock 2011). The shift from hunting and gathering to agriculture has traditionally been associated with a gracilisation in human form, especially in long bones (Ruff et al. 1984; Ruff 1987; Larsen 1999; Pearson 2000).

Nonetheless these factors are often complex and difficult to clarify. Various studies of Native American remains have shown that the relative bone strength of the femur and humerus tend to decrease from pre-agricultural to agricultural populations (Larsen 1999; Pinhasi and Stock 2011). This has been interpreted as result of an increasingly sedentary lifestyle and reduced workload in the agriculturalist populations (Ruff et al. 1984; Larsen 2001) (Fig. 4.).

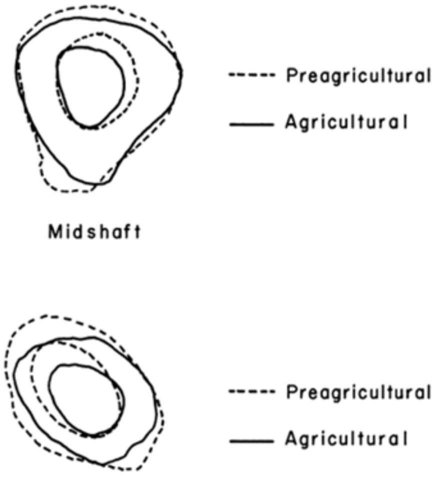

Figure 4. Changes in the cross-sectional shape of the femoral diaphysis between pre-agricultural and agricultural populations (Ruff et al. 1984).

Although, it is important to keep in mind that this relationship is far from being well-understood and almost certainly is dependent on the specific context of each human group. In fact several other studies have revealed that the relationship between the transition to agriculture and long bone robusticity is not always straightforward (Brock and Ruff 1988; Bridges 1991; Bridges et al. 2000). Interestingly meta-analyses based on studies of several different prehistoric North American samples have failed to show a consistent effect of subsistence strategy on femoral robusticity (Ruff 1999; Wescott 2006). However is possible to argue that those studies have suffered from the problem of encompassing very diverse groups under generic labels such as “Hunter-gatherer subsistence strategy”. Attempts to finely categorise activity have only been partially successful (Wescott, 2006). It is likely that depending on the particular culture as well as on the terrain changes in subsistence strategy will have varying effects on the activity of the skeleton.

1.2.5 Terrain

Interestingly, one of the same meta-analyses mentioned above have shown that there is a marked effect of the geographical terrain on femoral robusticity (Ruff 1999) (Fig. 5).

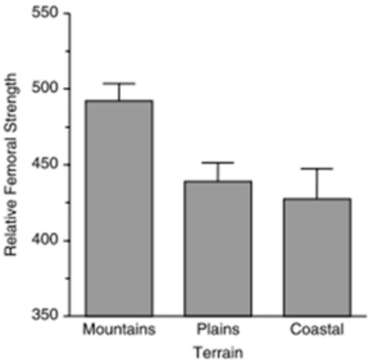

Figure 5. Effects of different terrains on femoral midshaft robusticty (polar second moment of area, standardized for bone length and controlled for subsistence strategy and sex).Mean + 1 SE. (Data from Ruff 1999).

The greater relative strength of the femur in samples from mountainous regions is consistent with the predicted mechanical cost of travelling through this more rough terrain. The upper limb does not reflect the same pattern, which is again consistent (since the humerus has hardly any locomotor function in humans during normal walking or running) (Ruff 1999). Another interesting study compared relative strengths of the upper and lower limb bones in populations who had primarily maritime versus terrestrial lifestyles (Stock and Pfeiffer 2001). Various studies have shown an increase in humeral robusticty in canoeist prehistoric populations (Oliver 1988; Ruff et al. 1993; Wanner et al. 2007).

1.3 Third Section: Beam Theory and Finite Element Analysis

1.3.1 Traditional Beam Theory

Long bone diaphyses have been conventionally modelled as beams because of their relative shape similarity and because the techniques involved are simple and straightforward (Huiskes 1982). For a beam it is possible to use its cross-sectional geometric properties to calculate stresses (pressures inside of a material) resulting from externally applied loadings. When stresses reach a critical point the structure fails; the resistance of a structure to deformation, prior to failure, is referred to as rigidity while the ability to resist breaking is referred to as strength. For a bone, both characteristics are highly important, because it has to be rigid enough to support the body and not break under load. Furthermore bones are subjected to different kinds of loads (Fig.6.) (e.g. Gere and Timoshenko1990; Larsen 1999). Table I. Also lists the cross-sectional geometric properties that have usually been applied to evaluate the rigidity and strength of beam or long bones (Fig. 7).

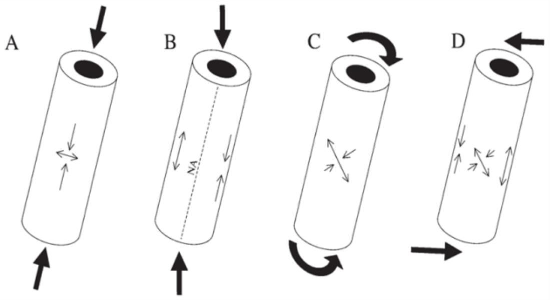

Figure 6. Cross-section and beam views of applied forces and strain distributions in: a) compression; b) bending; c) torsion; d) shear. Modified from Pearson and Lieberman 2004.

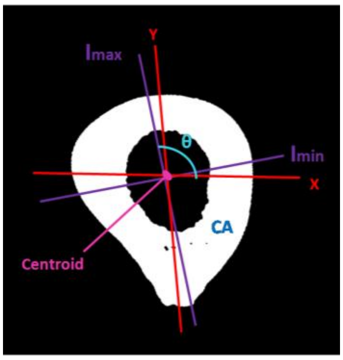

Figure 7. Some of the cross-sectional properties listed in Table properties *in situ*: Y and X are the anatomical axes, **θ** is the orientation of the maximum bending rigidity, Imax and Imin are maximum and minimum bending rigidity axes respectively from which J is calculated and CA is the cortical area.

Both strength and rigidity are proportional to material cross-sectional areas in a beam when it experiences pure axial compression; this is equivalent to the cortical area (CA) in long bone diaphyses. Although for diverse reasons, bones under physiological loading are hardly ever subjected to pure compression or tension. Thus more realistic models also evaluate bone rigidity and strength under bending and torsion. Bone behaviour under these two loading scenarios are proportional to second moments of area (SMAs), which are calculated about, either an axis through the section (bending) or about the section centroid (torsion). SMAs are defined as the product of small units of area of material multiplied by the squared distances of these areas to this axis or point; therefore their units are length to the fourth power:

$$I = \sum_{i}^{n} x_i^2 \cdot y_i^2$$

In bone biomechanics SMAs are normally estimated with respect to bone's anatomical axes (A-P: antero-posterior; M-L: medio-lateral) or as the maximum and minimum values over all orientations. Bending rigidities are proportional to SMAs in the A-P and M-P axes, and usually the angle of orientation of maximum bending rigidity (**θ)** is also calculated. To estimate torsional rigidity and (twice) the average bending rigidity, the polar second moment of area (J) is calculated as the SMA with respect the section centroid. Several biomechanical studies have used J as an indicator of the overall bone rigidity, however experimental evidence have shown that under physiological loading conditions loading axis rarely passes through the section centroid (Lieberman et al. 2004), therefore caution is needed when interpreting beam theory results.

Strength is estimated using section modulus rather than second moment of area. Since strength relates to breaking, which in turn relates to stress which, under bending or torsion, is maximal at the surface, all the strength related properties must be estimated by dividing SMAs by the distance from the appropriate axis (e.g. centroid axis) to the surface. These estimates are known as section moduli (Z) given in linear units to the third power. Normally section moduli are estimated by dividing SMAs by half the appropriate diameter of the section (external breadth).

Table I. Cross-sectional geometric properties traditionally used when modelling long bones as beams (Extracted from Ruff 2008).

Property	Abbreviations	Units	Definition
Cortical Area	CA	mm^2	compressive/tensile strength
Total Subperiosteal Area	TA	mm^2	area within outer surface
Medullary Area	MA	mm^2	area within medullary cavity
Percent Cortical Area	%CA	%	(CA/TA) x 100
Second Moment of Area about M-L (x) Axis	Ix	mm^4	A-P bending rigidity
Second Moment of Area about A-P (y) Axis	Iy	mm^4	M-L bending rigidity
Maximum Second Moment of Area	Imax	mm^4	maximum bending rigidity
Minimum Second Moment of Area	Imin	mm^4	minimum bending rigidity
Polar Second Moment of Area	J	mm^4	torsional and (twice) average bending rigidity
Theta	θ	degrees	orientation of maximum bending rigidity
Section Modulus about M-L (x) axis	Zx	mm^3	A-P bending strength
Section Modulus about A-P (y) axis	Zy	mm^3	M-L bending strength
Maximum Section Modulus	Zmax	mm^3	maximum bending strength
Minimu Section Modulus	Zmin	mm^3	minimum bending strength
Polar Section Modulus	Zp	mm^3	torsional and (twice) average bending strength

Other cross-sectional properties that are widely considered but from a morphological perspective rather than from a biomechanical point of view are the medullary area (MA) which is the area of the medullary cavity and the total sub-periosteal area (TA), which refers to the area included by the outermost perimeter of the section. Many authors tend to estimate an index based on these two morphological measurements in order to evaluate the relative cortical thickness with respect to the medullary canal. This measurement is known as per cent cortical area (%CA) and is normally calculated as (CA/TA)*100, though this index has no mechanical significance on its own.

In plain terms and based on the above description of SMAs and section moduli, it is possible to show that the distribution of bone, in addition to the amount of bone in a cross-section, are important in biomechanical analyses. In this regard it is important to notice that this change in geometry can have unexpected results for bone. A relatively thin-walled long bone may even be stronger than a relatively hick-walled bone, if the bone tissue is placed far enough from the bending axis or centroid (thus greatly increasing Second Moment of Area) (Ruff 2005; Turner 2006) (Figure 8.). However there are limits to far cortical bone cane be placed away from the neutral axis, therefore bones achieve loading resistance by a balanced combination of increasing relative cortical thickness and "moving away" their walls from the neutral axis (Turner 2006; Ruff 2008).

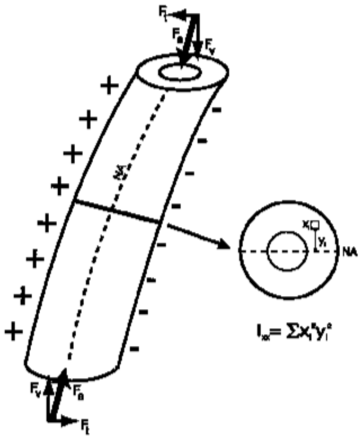

Figure 8. Cylindrical beam subject to loading at its proximal and distal ends. Axial forces (Fa), which generate compression, can be resolved into transverse (Ft) and vertical (Fv) components. NA is the neutral axis around which compressive and tensile strains occur. Bending moment at the midshaft cross-section is counteracted by Second Moment of Area, I. Extracted from Lieberman et al. 2004.

It also should be recognised that besides geometry, bone material properties such as bone density affect rigidity and strength. Although these properties are almost impossible to estimate from archaeological or paleontological remains, due the effect of diverse and complex taphonomic and diagenetic processes that act on bones after death. Fortunately, it has been shown that most of bone functional adaptation both in ontogenetic and evolutionary terms seems to occur mostly as geometrical changes than in material properties (Woo et al. 1981; Erickson et al. 2002; Robling et al. 2002). In fact diverse evidence suggests that most of bone material properties are highly conserved among vertebrates (Currey 2006). Based on these arguments some authors assumed that stress and strain are therefore proportional in bones, as such can be considered interchangeable. This is a useful assumption in, relating beam theoretical properties (defined on the basis of stress) to strain which is the proximate stimulus for bone functional adaptation (Ruff 2008).

Despite the massive amount of research that has been carried out using beam theory as a proxy for the biomechanical performance of bone, it is now clear that true mechanical loadings during an organism's life are more complex than those simplistically modelled using classical beam theory (Lieberman et al. 2004; Ruff et al. 2006). In spite of these limitations, this approach continues being used especially in bioarcheology, probably due its straightforwardness and simple applicability. Various authors have argued that cross-sectional properties estimated from beam theory are still the best available method for evaluating mechanical performance, despite the fact that cumulative evidence has shown that these properties are not linearly related to relative rigidity and strength *in vivo* (Lieberman et al. 2004; Ruff et al. 2006).

1.3.2 Finite Element Analysis

Traditionally, morphologists have applied the classical toolkit from comparative-anatomy and armchair biomechanics to study the functional significance of anatomical variation (Kupczik 2008). Normally researchers apply a combination of *in vivo* or *in vitro* experimentation in addition to morphological description. Although the incredible development and increased availability of high performance computers have propelled the birth of new morphological sub-disciplines: virtual anatomies and virtual biomechanics (Richmond et al. 2005; Weber and Bookstein 2011). Both start with 3D data collected by different means (e.g. CT-Scan; IMR; Laser scanners; etc.), but differ later on their goals and analytical approaches. One of the most popular techniques that have been applied to study the biomechanical importance of different morphologies is Finite Element Analysis (FEA) (Rayfield 2005; Panagiotopoulou 2009). This technique is a numerical analysis that acts by dividing a system into a finite number of discrete elements with well-known properties (e.g. triangles, tetrahedrons or cubes) (Ross et al. 2005) (Fig 9.).

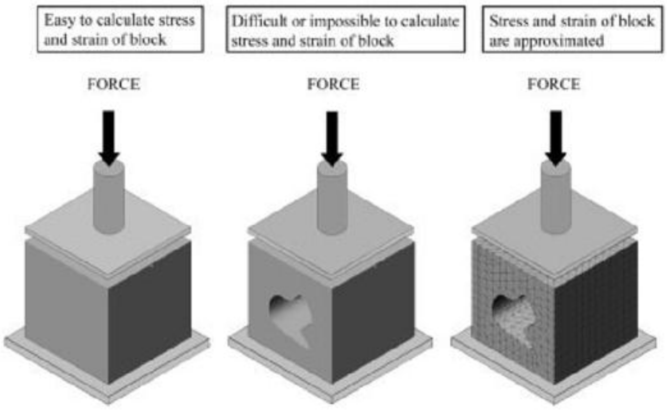

Figure 9. Problems with simple geometry (left) can be solved analytically. More complex shapes (centre) are impractical or impossible to solve in a similar fashion. The problem can be approximated (right) by subdividing the complex geometry into small elements of simple geometrical forms with well-known behaviour. Extracted from Richmond et al. 2005.

Strain and stress can be solved by finding analytical solutions if the geometry of the object is simple enough. However, more complex forms may be difficult or even impossible to solve using analytical means, especially if the loading regimens and/or material properties are complex (Beaupré and Carter 1992). This situation is the most common when dealing with realistic representations of biological structures. FEA offers an alternative approach, approximating the solution by subdividing complex geometries into multiple finite elements of simple geometry (usually millions). These elements are interconnected by nodes forming a mesh. In a structural analysis, typical mechanical parameters of interest are strain which is the deformation within a structure (change in length/original length; unitless) and stress, the applied force per unit area (Nm-2), which are obtainable as result of FEA (Kupczik 2008). Figure 10. and the following paragraphs briefly summarise some of the most relevant aspects of finite element modelling.

Pre-processing: This step involves the generation of a virtual model prior to its conversion into a FE mesh. CAD software allow the generation of 3D or 2D representations to easily manipulate and modify the virtual model. As outlined before, the procedure of extracting the anatomical locations of interest from digitised structure and assigning them to discrete label-fields is known as segmentation (Spoor et al. 1993; Spoor et al. 2000; Bruner and Manzi 2005; Zollikofer and Ponce de León 2005). There are various and diverse segmentation techniques available, ranging from manual to automatic algorithms that are easily accessible in numerous software. It is always important to bear in mind that the utilisation of each particular segmentation algorithm will vary depending on the specificities of the research problem. For example, different anatomical locations have distinct densities and material properties, therefore depending on the objectives diverse segmentation procedures may be followed.

Once the image segmentation is finished, it is necessary to convert the 3D model into a FE mesh, consisting of millions of polygons. It is also possible to generate a FE mesh transforming each one of the voxel from a 3D volume stack into cubic finite elements. The FEA software used in this dissertation Vox-FE (Fagan et al. 2007), is a non-commercial voxel-based programme, although the majority of the commercial obtainable software do not allow the direct transformation from voxels into elements.

Material Properties and boundary conditions: After the construction of the finite element mesh, it is necessary to specify the mechanical properties of the elements composing the specimens. The most common material properties used in biomechanics are the Young's modulus of elasticity, Poisson's ratio and shear modulus. The magnitude and orientation of these values in each one of the elements, and their spatial orientation within model, influence substantially the FEA results, therefore especial caution is needed when defining this properties in order to generate accurate simulations (Strait et al. 2005; Kupczik et al. 2007). Several studies provide values for the material properties of different biological tissues and from different anatomical locations, although more specific research question could require further inquiries.

Following this step, is required to set up the boundary conditions, such as displacement constraints and applied loadings (e.g. muscle forces, joint reaction forces, etc.). The FEA mathematical foundations establish that all the reaction forces within the model must achieve equilibrium. Nonetheless, in many situations the model could be displaced, therefore it is necessary to define constraints as anchorage for the model in the space. In similar fashion as for the material properties, force estimations for different muscles and organisms are available in the literature (e.g. Ross et al. 2005; Strait et al. 2005, 2007)

Solution and post-processing: After defining the material properties and establishing the boundary conditions, the model is prepared to its submission into an FE solver. Normally this implies a high-performance computer that calculates the displacements of the nodes and resulting strains and stresses. These results can be visualised as colour-maps representing the desired values (e.g. strain or stress), or even as animated deformations.

Validation and sensitivity: Sometimes FEA results are compared against experimental data, in order to validate them and thus test their reliability (Marinescu et al. 2005; Richmond et al. 2005; Ross et al. 2005; Strait et al. 2005, 2007; Kupczik et al. 2007). Basically the underlying idea of validation is to replicate the simulation conditions defined in an FEA but applying an experimental design and so doing, test for consistency between the results of both analyses. A complementary approach is to carry out a sensitivity analysis, which refers to replicate the FEA analysis, although changing some of the original settings (e.g. material properties, loading magnitudes or directions, shape and/or size of the model, etc.) until the obtained results converge towards consistency (Ross et al. 2005; Strait et al. 2005; Kupczik et al. 2007; Curtis et al. 2008).

Recently FEA has gained prominence in the biomechanical study of specimens that for different reasons are not suitable for more physical experimentation (Richmond et al 2005). FEA has the potential to uniquely advance our understanding of mechanical behaviour of living and extinct taxa, hence several research groups have now devoted their efforts to the application of FEA to vertebrate palaeontology, palaeoanthropology, comparative zoology and clinical studies (Papini et al. 2007; Strait et al. 2009; Dumont et al. 2011; O'Higgins et al. 2011; Brassey et al. 2013). Despite its potential in many questions in vertebrate biomechanics and evolution remained largely unexplored (Richmond 2005). Probably this is due to the many assumptions and uncertainties that surrounded this method when applied in population based studies and/or to extinct remains. Actually some authors have argued that one the greatest concerns with a broader application of FEA is that there are simply too many problems and assumptions associated with modelling biological materials (especially paleontological data) effectively and reproducibly with FEA (Rayfield 2005). In summary, is possible to say that virtual biomechanics and in particular FEA provide the opportunity to investigate functional morphology in a non-invasive way and where traditional empirical methods cannot be applied. It is still vital to validate FEA results against experimental data whenever is possible.

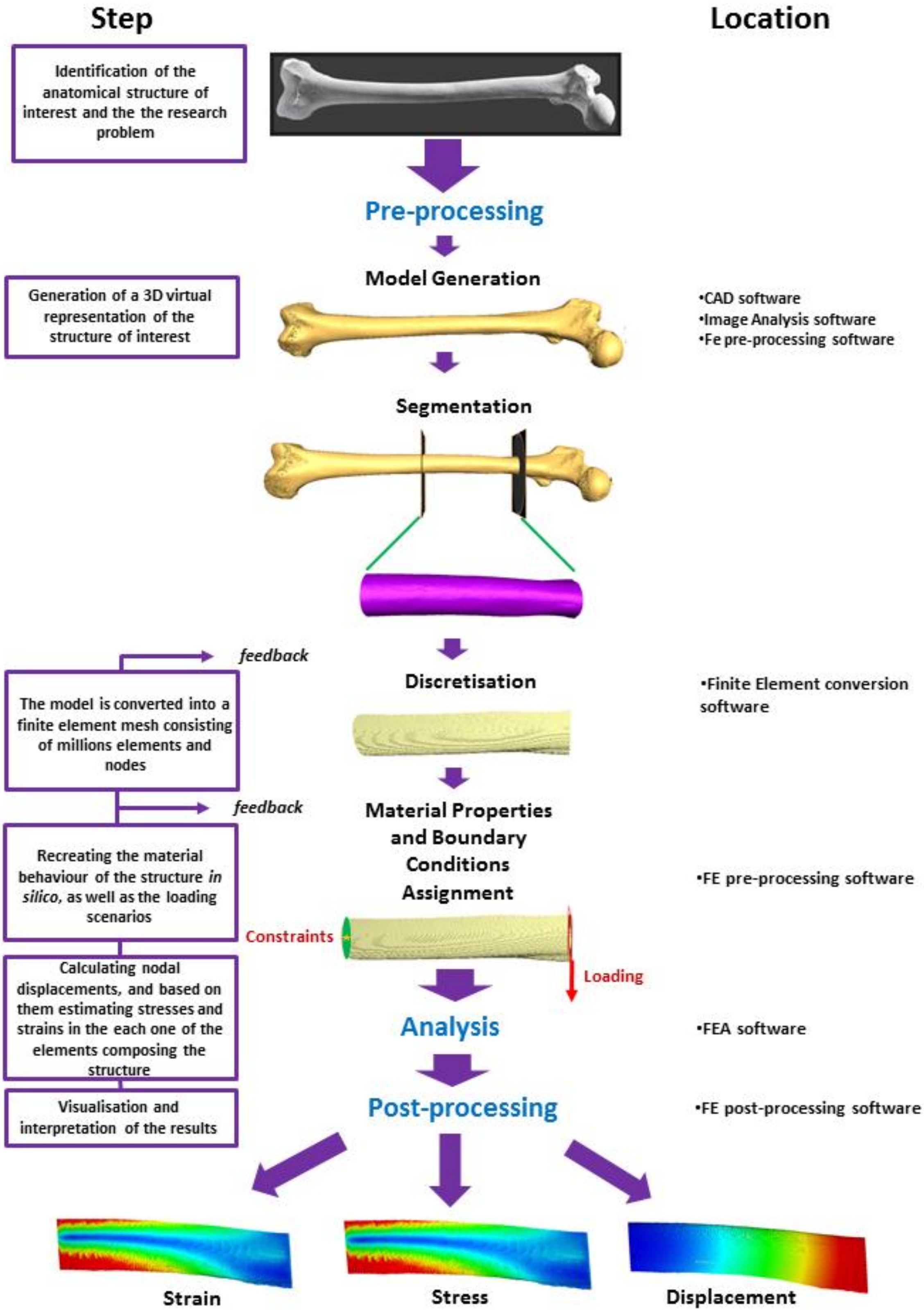

Figure 10. Workflow chart of the different steps involved in FEA. Adapted from Rayfield 2005.

Chapter 2: Problem Statement and Objectives

2.1 Problem Statement

Based on the previous antecedents reviewed in this dissertation, it is logical to wonder how different techniques perform in a classical bioanthropological problematic: distinguishing between agriculturalists and hunter-gatherers from femoral shaft morphology. As discussed earlier, any behavioural reconstruction based on skeletal morphology relies on the principle that bone is functionally adapted to its mechanical environment *intra vitam*. Therefore it is expected that both the femoral morphology, as well as its mechanical performance would express differences due to past lifestyles. Different methods measure different shaft characteristics related to ways of life, although it is still not clear which one is better when discriminating agriculturalists and hunter-gatherers. While Beam Theory measures static cross-sectional properties derived from shaft geometry and therefore bending and compression rigidity, FEA estimates simulated mechanical performance as stress and strain and hence deformation resistance. In other hand, GMM uses transformed Cartesian coordinates (via GPA), to describe general morphology.

The present dissertation applied these three different techniques: Beam Theory, GMM and FEA to use their outputs to perform multiple Discriminant Analyses in order to test the discriminatory ability of each one these methods. These results are expected to contribute in a better insight when assessing lifestyle from femoral shaft morphology.

2.2 Objectives

2.2.1 General Objective:

The aim of the present dissertation was to contrast beam theory and FEA by applying both to a classical bioanthropological example: the comparison of skeletal performance between agriculturalists and hunter-gatherers. GMM analyses were also carried out, in order to compare their competence when distinguishing between past lifestyles as compared with the other applied techniques. These results are expected to contribute in a better insight when assessing lifestyle from femoral shaft morphology.

2.2.2 Specific Objectives:

- Estimate stature and body mass from osteometric measurements.
- Calculate the cross-sectional properties of the femora based on beam theory.
- Perform the virtual segmentation of the femora.
- Collect 3D coordinates to represent the form of the femora sections and to extract FEA strain values.
- Convert the models into a finite element mesh and simulate three different loading scenarios: a) compression; b) medial bending; c) posterior bending.
- Carry out GMM analyses.
- Correlate the results from the beam theory to those obtained from FEA.
- Distinguish between agriculturalists vs. hunter-gatherers by statistical testing for difference using the results from beam theory, GMM and FEA.
- Apply GMM to the new coordinates obtained from the FEA outputs.
- Test the concordance between the results obtained from GMM applied to FEA and beam theory cross-sectional properties.
- Contribute in a better understanding when elucidating past lifestyles from femoral shaft morphology.

2.3 Hypothesis

h0: There are no significant differences in the discriminatory capability between beam theory, FEA and GMM when distinguishing between agriculturalists and hunter-gatherers.

Chapter 3: Materials and Methods

3.1 Sample:

The sample comprised 20 male Native American left femora belonging to two distinct populations a) Norris Farm (n=10): agriculturalists and b) Black Earth (n=10): hunter-gatherers.These two populations of Native North Americans included in this study hail from the same geographic region, yet are temporally and behaviorally distinct. The Norris Farms site is a late Prehistoric cemetery site from the central Illinois River Valley dating to approximately AD 1300 with graves containing individuals associated with the Oneota cultural tradition of village agriculturalists (Santure et al. 1990). By contrast, Black Earth is a multi-component site in the Carrier Mills Archaeological District in southern Illinois that dates to the Middle Archaic. The Black Earth modern human hunter-gatherers relied upon white tailed deer and to a lesser extent a wide variety of aquatic avifauna, while also gathering an array of nuts and seeds (Jefferies et al. 1982).These skeletal materials are curated at the Pennsylvania State University and their scans were provided by Dr. Colin Shaw (University of Cambridge).

Sex was estimated using standard osteological techniques (Buikstra and Ubelaker 1994).Stature and body mass were estimated using the regression equations for males proposed by Sciulli and Giesen (1993) and Ruff et al. (1991) respectively:

$$Stature = 2.433\ \times Femoral\ maximum\ length + 42.805$$

$$Body\ mass = (2.741\ \times Femoral\ head\ diameter - 54.9) \times 0.9$$

Bone length, the estimated stature and body mass were compared between hunter-gatherers and agriculturalists by using both a two-sample test statistic and a 10000 rounds permutation test. This permutation test uses the absolute difference in means as statistical probe and has few assumptions as compared with its parametric counterpart (Hammer and Harper 2006).

3.2 Method:

3.2.1 Segmentation: Some individuals had small missing segments of the bone diaphysis due old sampling procedures. Hence a virtual reconstruction was necessary in some of them to continue with the analysis. The specimens were CT-scanned using a High-resolution Medical CT-Scan at Penn State University. The femora were scanned using a Medical CT-Scan and the data volumes were imported as DICOM stacks (1024 16-bit TIFF images) with an isotropic voxel size of 0.113 mm. Manual segmentation of the femora was carried out using AVIZO 7.0.1 (VS.G, USA), selecting only a small portion of the bone (between 50% and 80% of the biomechanical length *sensu* Ruff 2002) to resemble the properties of a beam and to minimise excessive assumptions. In brief reconstruction methods involved three main steps: volume creation by means of the imaging method; classification of the tissues represented in each voxel; and finally the projection of the classified volume data (Fishman et al 1991). Allocation of material geometry by image segmentation, separating the image into non-overlapping regions that are homogeneous based on for instance grey scale (Pal and Pal 1993; Pham et al. 2000). In this study bone was be separated from non-biological materials and surrounding air by defining a density threshold. The objective was to maximise the amount of bony material represented without losing to many details of more dense materials. The segmentation process began using thresholds and the subsequent results were refined by applying a manual segmentation (Clarke et al. 1995).

3.2.2 Estimation of Cross-Sectional Properties

There exist a variety of approaches to collecting and analysing long bone cross-sectional images. Images should be first oriented with respect to standard anatomical reference planes or axes (Ruff 2003b). Normally sections are obtained at standardised anatomical locations defined at predetermined percentages of bone length. Bone lengths are defined according to the anthropometric literature (Bräuer 1988; Ruff 2003b). Images can be obtained in a direct manner from broken bones and cut sections, by digitising them using digital photography or simple optical scanners. Some authors have just used external moulds of long bone diaphysis; however this method does not allow a full representation of the medullary cavity. Therefore cross-sectional properties will be estimated from non-invasive medical imaging techniques, in this case CT-scans (Ruff and Leo 1986) (O'Neill and Ruff 2004). Several software tools are available to estimate cross-sectional geometric properties from digitised images. All are based on the same basic underlying engineering principles and in the present study they will be estimated using either the JohnHopkins Macro for ImageJ, or the BoneJ package (Ruff 2008; Doube et al. 2012).

In the present study, images were first oriented with respect to standard anatomical reference axes and then the BoneJ package was applied (Doube et al. 2012) (Fig 11).

Cortical area (CA) and polar second moment of area (J) were used as proxies for compressive and bending strength, respectively. These variables were standardised against body weight (CA) and body weight x bone length^2 (J). These variables were used in a discriminant analysis comparing hunter-gatherers vs. agriculturalists. The significance level for the differences between means was estimated using both a parametric Hotelling's T^2 and a two-group permutation test. The equality of means of the two groups was tested using permutation with 2000 rounds.

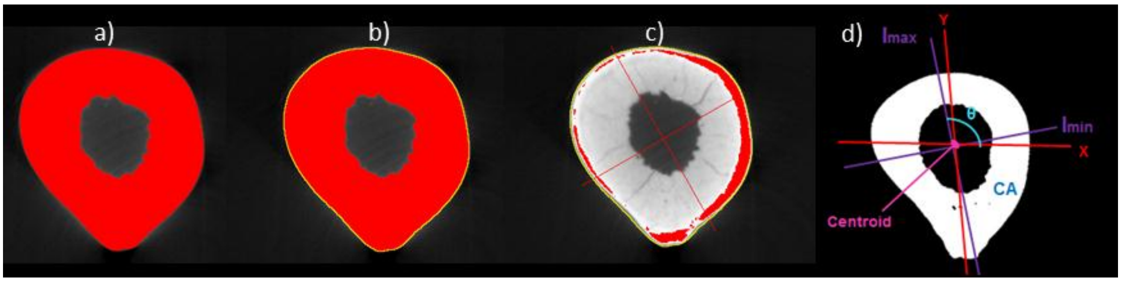

Figure 11. Cross-sectional properties estimation procedure applying beam theory: a) the cortical bone of the selected slice is selected; b) the contour of the slice is defined; c) cross-sectional properties are estimated using the appropriate software and Imax and Imin are drawn; d) some of the calculated cross-sectional properties are presented in situ: Y and X are the anatomical axes, **θ** is the orientation of the maximum bending rigidity, Imax and Imin are maximum and minimum bending rigidity axes respectively from which J is calculated and CA is the cortical area.

3.2.3 FEA:

Finite element modeling: The 3D volume data (n= 20 femora) were down-sampled to an isotropic voxel size of 0.339 mm in order to simplify the models. They were exported as a stack of bitmap images and each dataset was converted into a finite element mesh consisting of millions of eight-node cubic elements using custom software.

Material properties: The FEA was performed using the non-commercial FEA software *VOX-FE* (Fagan et al. 2007). Bone material properties are directly associated to the composition of its extracellular matrix, water and mineral content (Reilly and Burstein 1975; Currey 2006; Carter and Beaupré 2007; Panagiotopoulou 2009). Only cortical bone was modelled, because of resolution and negligible trabecular volume in the shaft. Isotropic material properties were assigned to the models, applying conventional cortical bone material properties (Young’s modulus: 17 Gpa; Poisson’s ratio: 0.3).

Boundary conditions: Conventional FEA studies tend to simulate muscular constraints measured directly from experimental data (e.g. Electromyography [EMG]) or estimated from muscle physiological cross-sectional areas (PCSA) (Duda et al. 1996; Viceconti et al. 2003; Panagiotopoulou and Cobb 2011). However due to objectives and restrictions of the present study, a non-physiological loading regimen was preferred. The sample is composed of archaeological specimens, and the objective is to compare general loading resistance, rather than simulating complex locomotion scenarios. This experiment allowed assessing different ways to compare long bones in terms of function, without having a full knowledge of real-life loadings (i.e. unknown EMG, maximal force, muscle cross-sectional areas, etc.) and so test whether it is possible to generate realistic and more simple models under standardised tests.

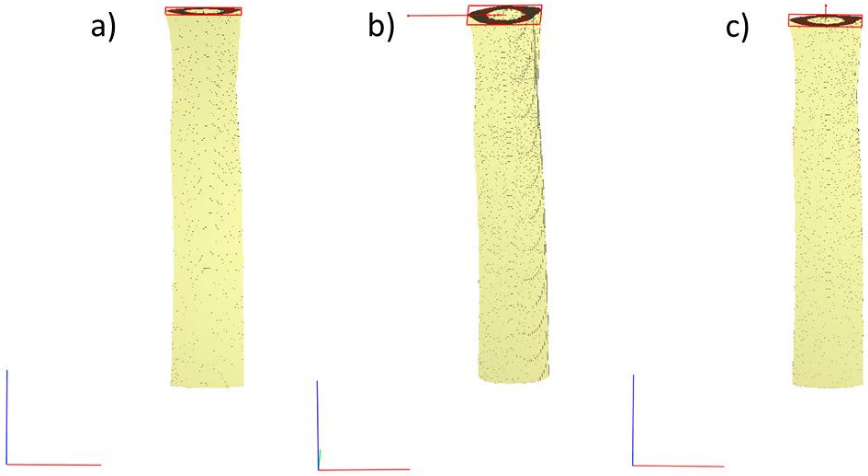

Figure 12. Different loading scenarios applied on the femoral sections: a) Compression; b) Medial bending; c) Posterior bending.

Each model was constrained at its distal surface (i.e. the midshaft cross-section was constrained in the three axes) and loaded by applying 10 N at its proximal portion under three non-physiological scenarios: a) compression; b) medial bending; c) posterior bending (Fig. 12).

Compression was simulated by applying the force vector on the upper cross-sectional portion of the femur and by directing it through the center of the constrained shaft section. Posterior bending was simulated by applying the force vector from the centre of the proximal section, orthogonal to the z axis and towards the posterior aspect. Finally, medial bending was computed directing the force vector from the centre of the proximal section, orthogonal to the z axis and towards the medial portion of the shaft.

Then all the models (n=60; 20 individuals and three loading scenarios per each one) were submitted to the FE solver restricting the maximum number of iteration to 60000. None of the models reached this boundary, but the majority solved relatively quickly instead. Principal and von Mises strains were measured at 112 equidistant landmarks over the cortical surface and the medullary cavities of the models in the same slices where the cross sectional properties were measured (Fig. 13).

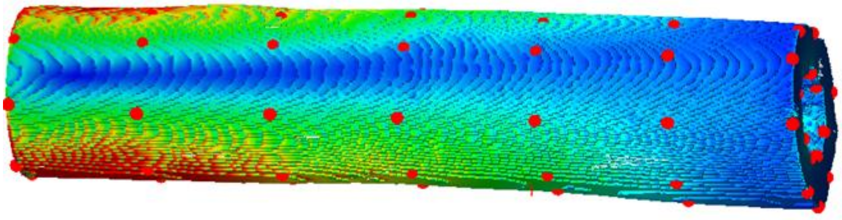

Figure 13. Strains were measured at 112 equidistant landmarks over the cortical surface and medullary cavity in the same slices where cross-sectional properties were estimated.

These values were scaled to bone length, in order to standardise them by one size measurement. Posteriorly, they were subjected to a Principal Component Analysis (PCA) (each loading regimen separately), and then the components that accounted for ca. the 90% of the variance in the sample were used in a Discriminant analysis. The significance level for difference between means was estimated using both a parametric Hotelling's T^2 and a two-group permutation test. The equality of means of the two groups was tested using permutation with 2000 replicates.

3.2.4 Concordance between FEA and beam Theory: A Pearson's correlation coefficient was calculated between the average result of both analyses: beam theory and FEA (CA vs. compression and J vs. medial bending/posterior bending) in the same slices where the 112 landmarks where collected.

3.2.5 GMM Analysis:

Standard GMM analyses were conducted in order to test for differences in shape between the two groups. This branch of shape analysis has been usually understood as the quantitative study of shape and its covariates (O'Higgins 2000). This method consists mainly in three steps: a) collecting primary data through the acquisition of Cartesian coordinates, b) obtaining shape variables by generalised Procrustes analysis and c) the multivariate statistical analysis of the shape variables. Geometric morphometrics methods were preferred in this research because as compared with traditional morphometrics, because they allow an easier visualisation of the results, and since they are grounded in a coherent and well developed statistical theory of shape (Zelditch et al. 2004; Slice 2007).

Landmark acquisition was carried out in Landmark Editor software v. 3.0.0.6 (IDAV) (Wiley et al. 2005), collecting 112 equidistant 3D type III landmarks by defining seven curves of eight landmarks on each one along the cortex and medullary cavity of the femoral shaft (Bookstein 1991) (Fig 14.) Geometric morphometrics and statistical analysis were carried out in MorphoJ v.1.0.5d, PAST v. 2.17c, Morphologika v.2.5. and EVAN toolbox v.1.62 (O'Higgins and Jones 1998; Hammer et al. 2001; Klingenberg 2011).

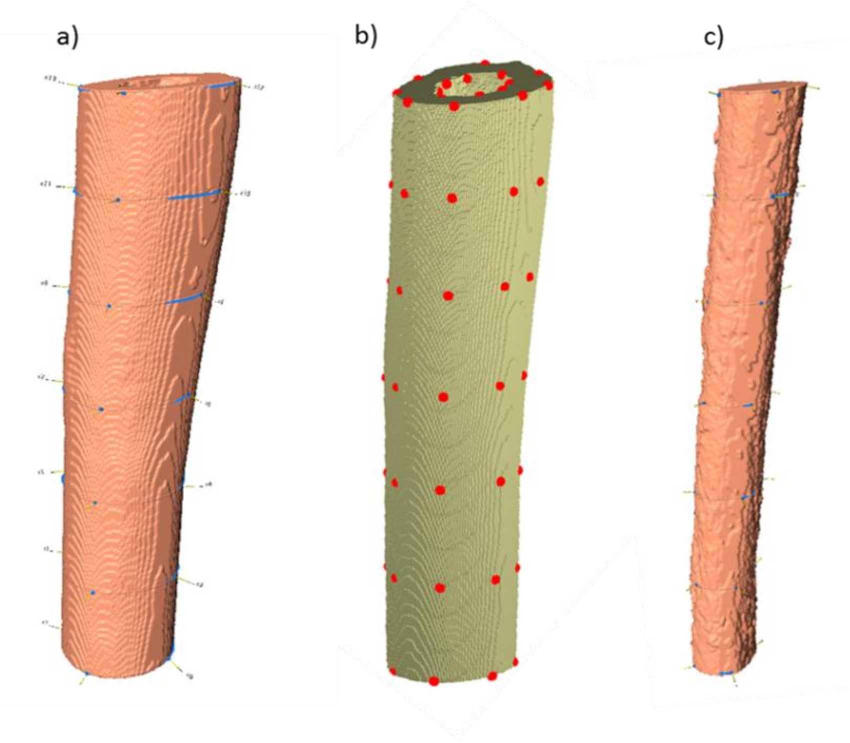

Figure 14. 112 type III landmarks collected both on a) the femoral cortex and c) the medullary cavity as seven curves of eight landmarks on each one. These landmarks were collected on the same slices where the cross-sectional properties were estimated. These landmarks were placed onto the b) FEA model to extract strain values and carry out GMM analyses.

A generalised Procrustes analysis (GPA) was performed in MorphoJ, removing the differences due rotation, scale and translation and thus obtaining shape variables (remaining geometric properties) (Bookstein 1991). These shape variables were used in the subsequent analysis. Because shape variation refers to the relative displacement of landmarks in many directions, it was strictly necessary to use multivariate methods

(Klingenberg and Monteiro 2005). Centroid size was used as geometric size estimator. It is defined as the square root of the sum of squared distances of a set of landmarks from their centroid (Bookstein 1991). This size estimator was compared between the two groups by means of a t test and the significance level was calculated by performing a 10000 replicates permutation.

Several PCA's were carried out to elucidate morphometric associations. A cross-validated discriminant analysis (using permutation to test for significance) was applied to test whether it was possible to distinguish between hunter-gatherers and agriculturalists. These procedures were sequentially repeated using different landmarks subsets: a) femoral cortex landmarks; b) medullary cavity landmarks; c) joined subsets.

Additionally, in order to study the degree of covariation between the medullary cavity and the femoral cortex a Partial Least-Squares (PLS) was applied. The PLS method basically tries to find the principal components of the covariation between the sets of variables by means of decomposing the covariance matrix of two blocks, into two sets of eigenvectors (one for each set of variables) and eigenvalues, using singular value decomposition (SVD) in order to generate a matrix of singular values (the square-roots of the eigenvalues), a matrix of eigenvectors for the first set of variables and the transpose of the matrix of eigenvectors for the second set of variables (Rohlf and Corti, 2000; Mitteroecker and Bookstein 2007; Goswami and Polly 2010). The PLS was carried out within one configuration, which means that the analysis considered all the existent covariation between blocks, including the part related to relative positions, orientations and sizes of the blocks (Klingenberg 2008). This approach is best suited for morphological integration studies that considered the covariation between parts in the context of an entire structure, where the relative arrangement of the components could produce a significant contribution to the covariation patterns (Klingenberg 2008). As overall strength of association between blocks of variables the RV-coefficient was applied (a multivariate analogue of a correlation) (Escoufier 1973).

Finally, in order to understand the relationship between shape and the cross-sectional properties of the femora, a multivariate regression was carried out. Shape variables (dependent variables) were regressed onto the averages of standardised CA and J separately (independent variables). A regression score was calculated for each independent variable (CA and J) for all the observations in the sample, mainly as a visualisation tool to simplify the relationship interpretation (Drake and Klingenberg 2008).

Additionally, a Discriminant Analysis was performed using the residuals from these two regressions of shape on the cross-sectional properties (CA and J). The essential aim was to separate the part of variation in the dependent variables (shape coordinates) that was predicted by the independent variable (CA or J in each regression), from the residual part of variation. These new variables (the residuals) were uncorrelated with the independent variables, and therefore suitable to perform a Discriminant Analysis focused on the discriminatory capability of the shape variation that does not depend on the cross-sectional properties.

3.2.6 GMM analysis applied to FEA results:

GMM can be applied to virtual biomechanical analysis especially to compare biomechanical performance form a population perspective. GMM can potentially be used in diverse but useful way, for example as O´Higgins et al. (2012) propose: a) to create and alter functional models, b) to measure the results of remodelling studies by comparing the outcomes with the real morphological changes during an individual's ontogeny, and c) to compare the results of FEA within a multivariate framework. This latter use of GMM applied to FEA was applied in the present dissertation.

The 112 landmarks registered on the models were used to perform the GMM analysis of the FEA outputs. These type III landmarks have no biological homology criterion (Bookstein1991) but biomechanical homologies instead, therefore are suited to this kind of analysis (Oxnard and O'Higgins 2009; O'Higgins et al. 2011).These3D coordinates from the unloaded model and each load-cases were submitted to GMM analyses. Micro-deformation of the shaft between the unloaded average shape and the three loading scenarios was quantitatively compared using a PCA of the Procrustes coordinates in the size-and-shape space. This analysis based on landmarks displacements do not consider forces, energies or material properties but rather lead to a visual comparison of the deformation depending on the loading regimen (Fitton et al. 2012; Milne and O'Higgins 2012).

As mentioned earlier the analysis was carried out in the size-and-shape space that means that the scaling step of a standard GPA was not performed (Dryden and Mardia 1998). The resulting variables combined both size and shape information. It is important to bear in mind that the omission of the scaling step to centroid size, leads to a space different from Kendall's shape space. Nonetheless, the fact that FEA deformations are really small alleviates the possible distortions (O'Higgins and Milne 2013).

Basically the procedure was as follows. Starting from the original geometric configurations of both undeformed and deformed, a Procrustes superimposition was performed (Generalised Procrustes Analysis or GPA) in the size-and-shape space (Bookstein 1991; Dryden and Mardia 1998; O'Higgins 2000; Slice 2005). Procrustes residuals were then extracted from the unloaded cases with respect to the three different loading scenarios. These residuals were added to the mean undeformed shape in order to facilitate visualization of the different deformation due to the loading regimens. Scale was re-introduced by multiplying these latter results by a standardised centroid size for each one the three different loading scenarios (mean undeformed centroid size x [loaded centroid size/unloaded centroid size]) (O'Higgins et al. 2011; Fitton et al. 2012; Milne and O'Higgins 2012). These novel landmarks coordinates from the unloaded mean and each individual loading scenario were transformed via a non-scaled GPA. The resultant Procrustes coordinates were later subjected to a series PCA's, including all loading regimens altogether and separately. Results were presented as PCA plots and deformations were visualised by using a warped femoral shaft 3D surface obtained from the first specimen. Morphologika^2 v.2.5. (O'Higgins and Jones 1998) and EVAN toolbox v.1.62 were used to carry out the GMM analysis.

3.2.7 Concordance between GMM analysis applied to FEA and Beam Theory: A Mantel test was calculated between the first two PC's of the size-and-shape PCA of the three loading scenarios and the Beam Theory cross-sectional properties (CA and J independently). The statistical significance was assessed by using the Mantel test statistic (observed Z values compared to their permutation distribution after 5000 replicates).

Chapter 4: Results

4.1 Preliminary Comparisons

Femoral length and the estimations of stature and body mass were compared between agriculturalists and hunter-gatherers (Fig 15.), using both parametric and non-parametric tests.

4.1.1 Bone length: There were no significant differences between the two groups in femoral maximum length (t: -0.55406; p-value parametric: 0.58636; p-value permutation test 10000 replicates: 0.5894).

4.1.2 Stature: There were no significant differences between Agriculturalists and Hunter-Gatherers (t: -0.55767; p-value parametric: 0.58394; p-value permutation test 10000 replicates: 0.58754).

4.1.3 Body mass: There were no significant differences between the two groups in body mass estimation (t: 0.52019; p-value parametric: 0.60927; p-value permutation test 10000 replicates: 0.6108).

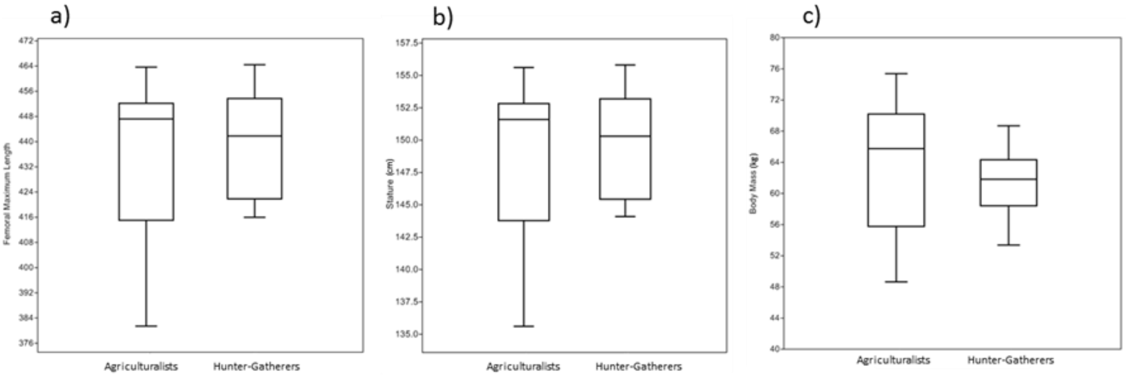

Figure 15. Box-plots of the preliminary comparisons between Agriculturalists and Hunter-Gatherers: a) Femoral maximum length (mm); b) Stature (cm); c) Body mass (kg).

4.2 Beam Theory

As proxy for compressive resistance, cortical area (CA) was used, while for bending rigidity the Polar Second Moment of Area (J) was selected. The femoral section was compared by selecting seven slices from the bottom to top of the bone. The comparison between agriculturalists and hunter-gatherers was not significant for CA using a parametric test (Hotelling's T2: 16.562; F: 1.5774; p-value: 0.2327), nor by performing a two-group permutation test (2000 rounds; Euclidean distance: 176.24; p-value: 0.093) (Fig. 16a). Similarly, when comparing the two groups for J there were not significant differences neither using a parametric test (Hotelling's T2: 23.11; F: 2.2009; p-value: 0.1099), nor for the permutation test (2000 rounds; Euclidean distance: 4.1152E05; p-value: 0.699) (Fig. 16b).

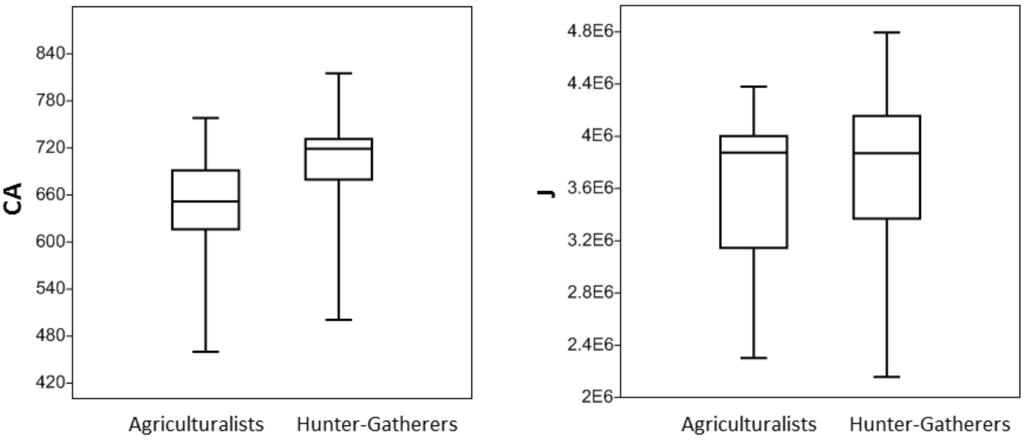

Figure 16. Box-plots of the cross-sectional comparisons between Agriculturalists and Hunter-Gatherers: a) Average Standardised Cortical Area (CA); b) Average Standardised Polar Second Moment of Area (J).

One hunter-gatherer individual was an extreme outlier, presenting an enlarged medullary cavity and a thinner layer of cortical bone. Hence, it was decided to remove this particular individual and repeat the analyses described above. The results for CA were now significant for both the parametric test (Hotelling's T2: 33.933; F: 3.1367; p-value: **0.0443**), as well as for the permutation test (2000 rounds; Euclidean distance: 235.36; p-value: **0.01**). However, the results of the comparison for J remained non-significant neither using the parametric test (Hotelling's T2: 19.941; F: 1.8433; p-value: 0.1751), nor for the permutation test (2000 rounds; Euclidean distance: 8.3416E05; p-value: 0.287).

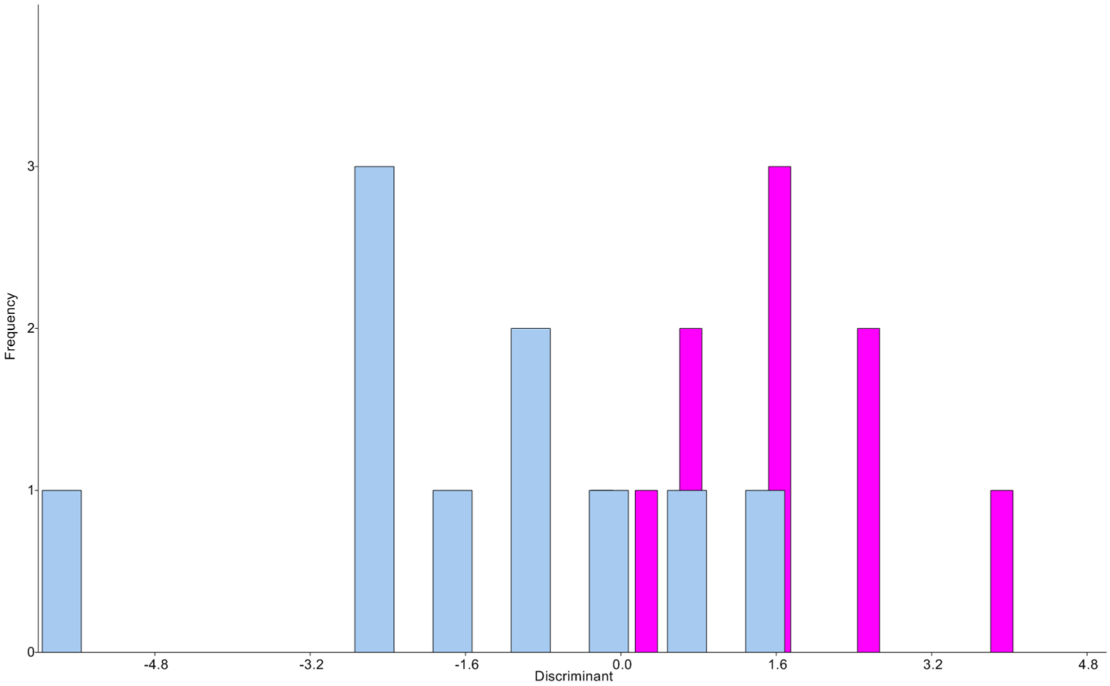

Figure 17. Discriminant analysis of CA. Fuchsia: Agriculturalists; Light blue: Hunter-Gatherers.

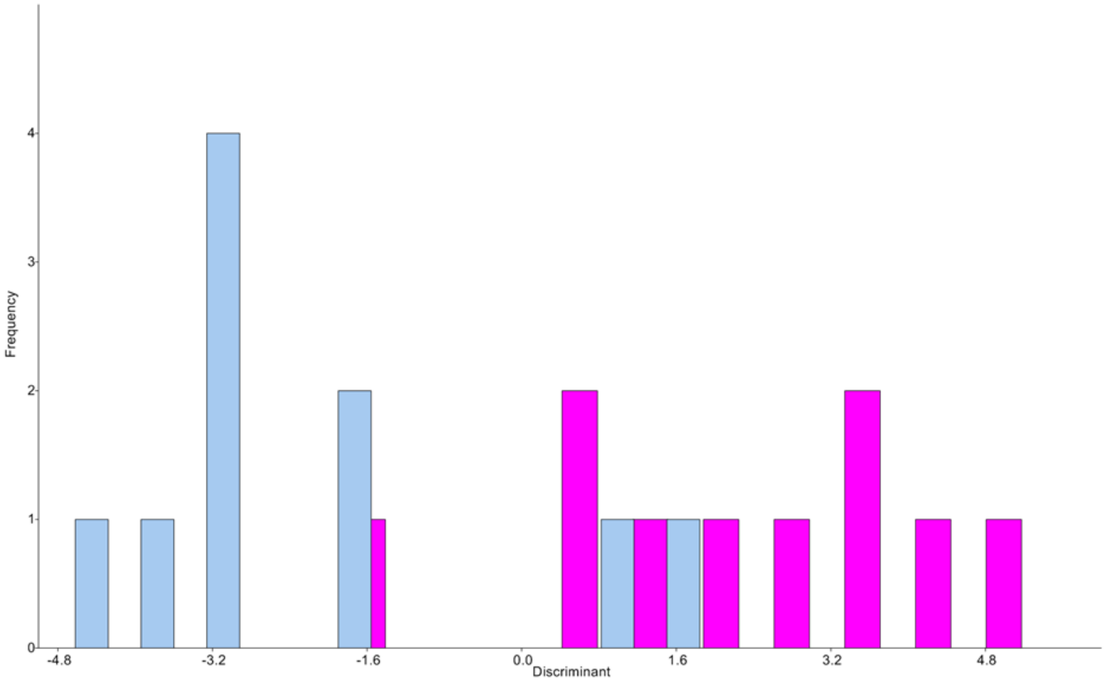

Figure 18. Discriminant analysis of J. Fuchsia: Agriculturalists; Light blue: Hunter-Gatherers.

4.3 GMM

4.3.1 Centroid Size:

The comparison between agriculturalist and hunter-gatherers using centroid size (CS) was not significant (t: -0.65254; p-value (10000 perm. rounds): 0.5363).

The following GMM analyses were divided into three steps using different landmarks subsets: a) femoral cortex landmarks; b) medullary cavity landmarks; c) complete subsets.

4.3.2 Femoral Cortex: 56 landmarks on the surface of the femoral section were used to perform a PCA (Fig. 19). There was a relatively good distinction between the two groups explained by PC2 with the hunter-gatherers on top. Although, there was some overlap between the confidence intervals of the two groups.

The cross-validated discriminant analysis for the subset of landmarks showed a clear distinction between the two lifestyles, with a level of correct classification was of 90% for agriculturalists and 80% for hunter-gatherers (Procrustes distance: 0.02493151; p-value (500 perm. rounds): **0.0440**). (Fig. 20).

4.3.3 Medullary cavity: 56 landmarks on the central cavity of the femoral shaft were used to perform a PCA (Fig. 21). There was a high overlap between the confidence intervals of the two groups due to a higher variability in the hunter-gatherer sample.

The cross-validated discriminant analysis reflects this low separation for this subset of landmarks, with a level of correct classification of only 70% for agriculturalists and 50% for hunter-gatherers (Fig. 22.), although there were significant differences between their multivariate means (Procrustes distance: 0.02362927; p-value (500 perm. rounds): **0.0320**).

4.3.4. Complete dataset: 112 landmarks comprising both the femoral cortex and the marrow cavity coordinates were used to perform a PCA (Fig. 23). There was a high overlap between the confidence intervals of the two groups, although there was a clear distinction between the dots representing the two lifestyles. The cross-validated discriminant analysis presented a marked distinction between the two groups, correct classification was of 80% for agriculturalists and 90% for hunter-gatherers, with highly significant differences between their multivariate means (Procrustes distance: 0.03307833; p-value (500 perm. rounds): **0.0080**) (Fig.24).

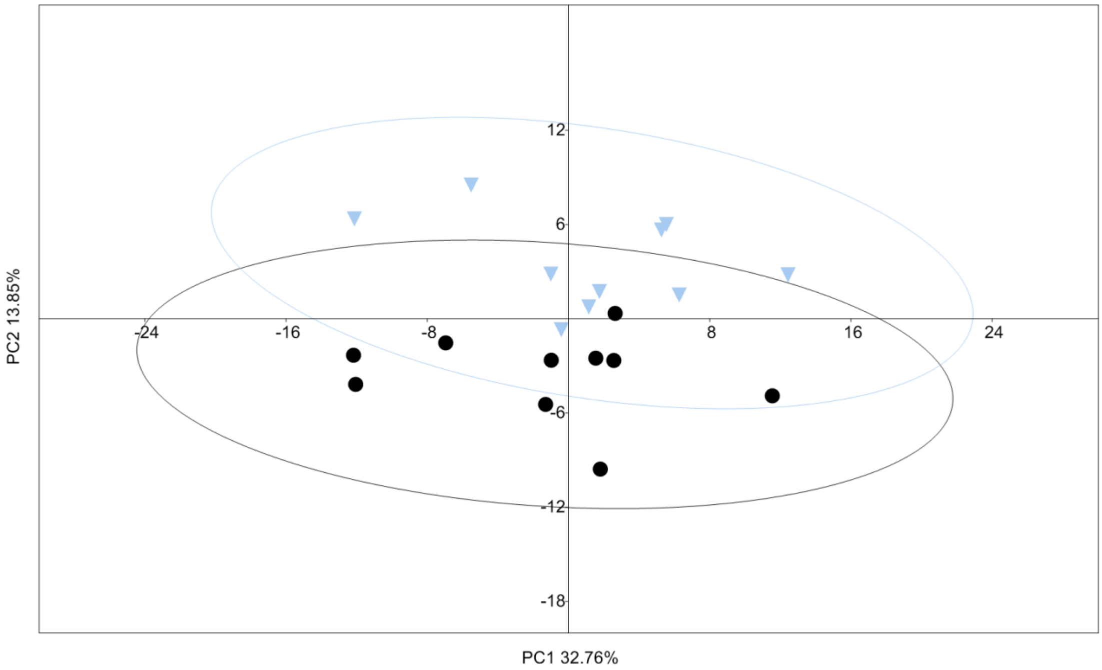

Figure 19. PCA plot of the first two principal components of the cortex landmarks. ▼ = Hunter-Gatherers; ● = Agriculturalists. The ellipses represent the 95% confidence intervals.

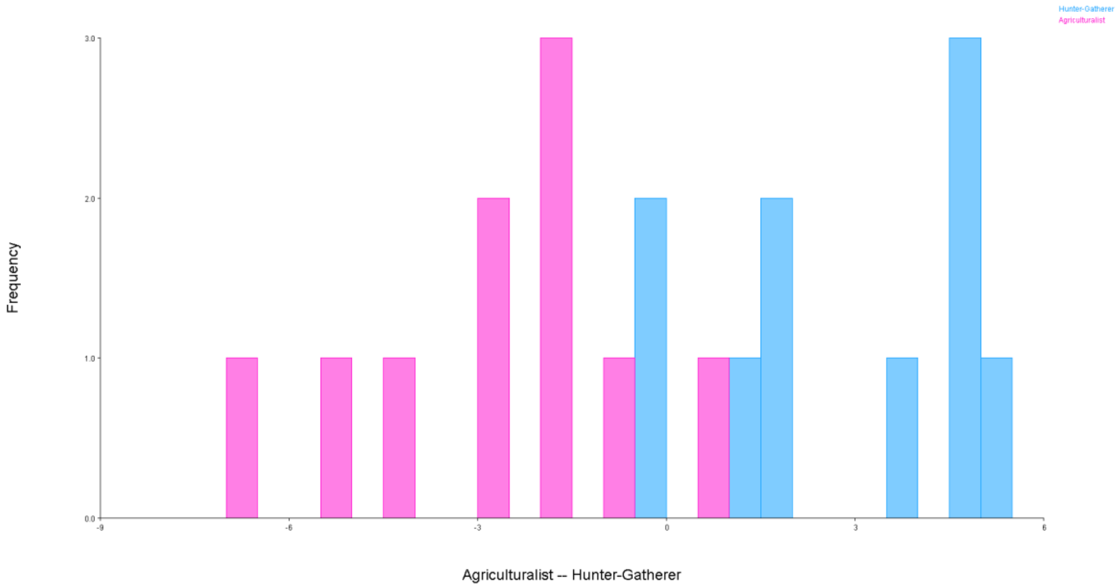

Figure 20. Cross-validated Discriminant Analysis of the cortex landmarks. The level of correct classification was of 90% for agriculturalists and 80% for hunter-gatherers.

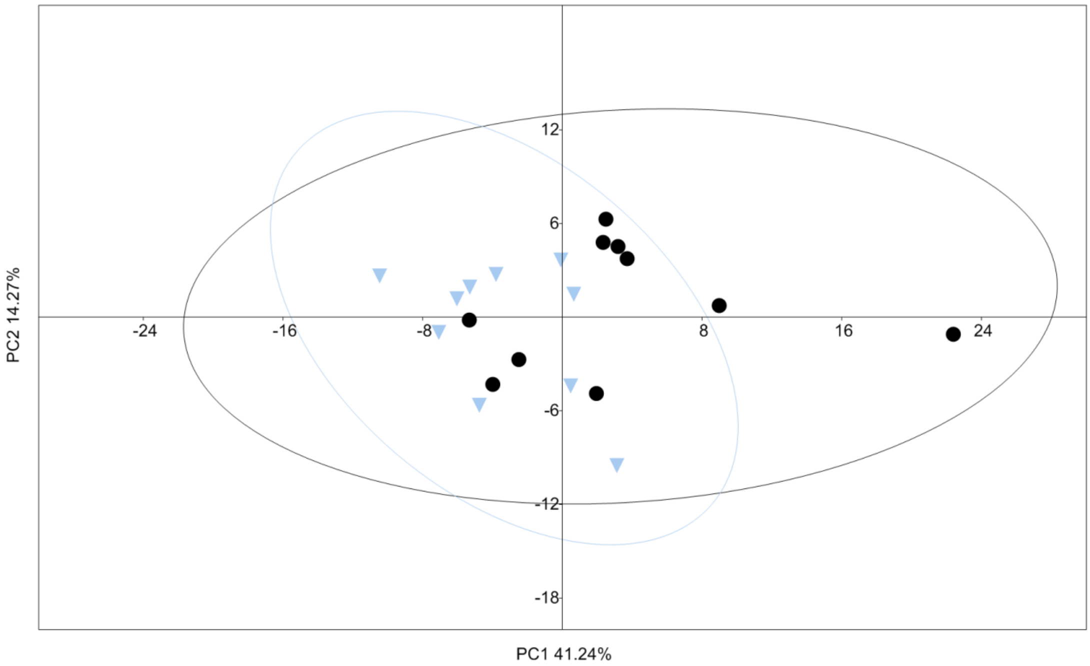

Figure 21. PCA plot of the first two principal components of the medullary cavity landmarks. ▼ = Hunter-Gatherers; ● = Agriculturalists. The ellipses represent the 95% confidence intervals.

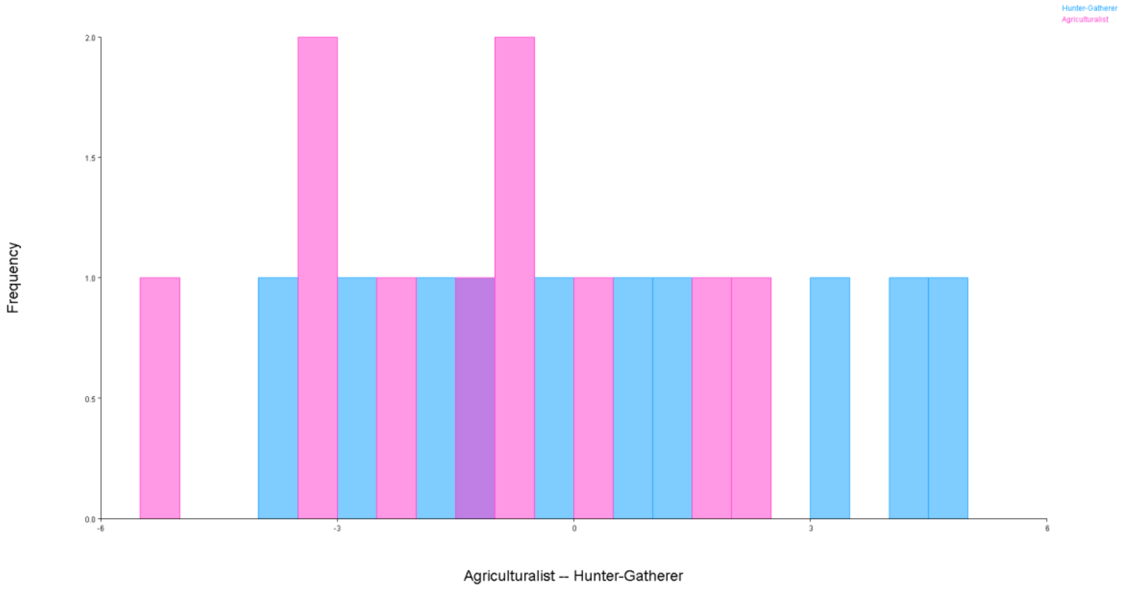

Figure 22. Cross-validated Discriminant Analysis of the medullary cavity landmarks. The level of correct classification was of only 70% for agriculturalists and 50% for hunter-gatherers.

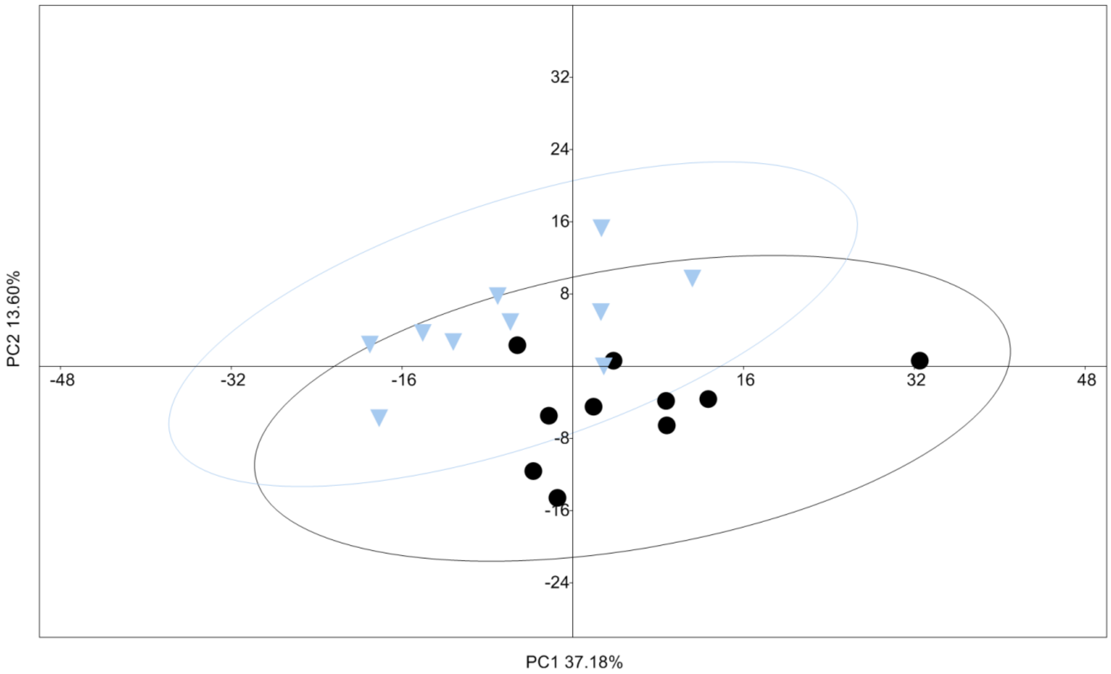

Figure 23. PCA plot of the first two principal components of the complete dataset. ▼ = Hunter-Gatherers; ● = Agriculturalists. The ellipses represent the 95% confidence intervals.

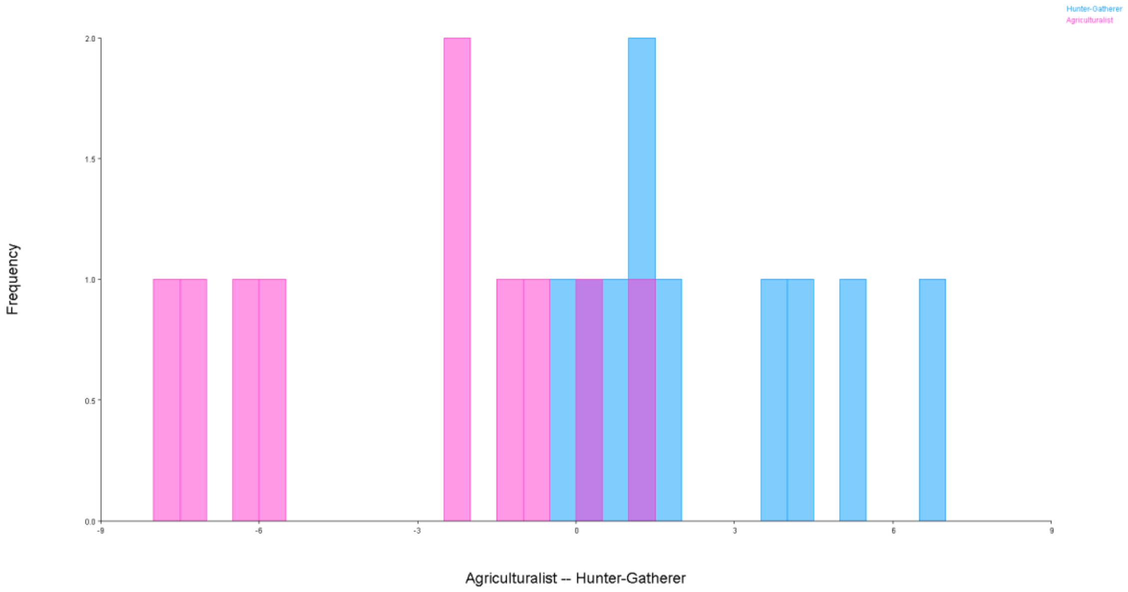

Figure 24. Cross-validated Discriminant Analysis of the complete dataset. The level of correct classification was of 80% for agriculturalists and 90% for hunter-gatherers.

4.3.5 Morphological Integration:

The null hypothesis of complete independence between the two blocks of variables (femoral cortex and medullary cavity) was rejected (RV-coefficient: 0.608548; p-value (500 perm.): **<0.001**) (Table II). The pairs of PLS axes that accounted for the majority of the total covariance of the sample were significantly correlated. The analysis identified the characteristics of shape variation that most covary between the two blocks and highlighted their relative contribution to the total amount of covariation between blocks. The correlation of the PLS1 for the two black was particularly elevated and highly significant (*r* : 0.90202; p-value: **<0.001**) (Fig. 25.).

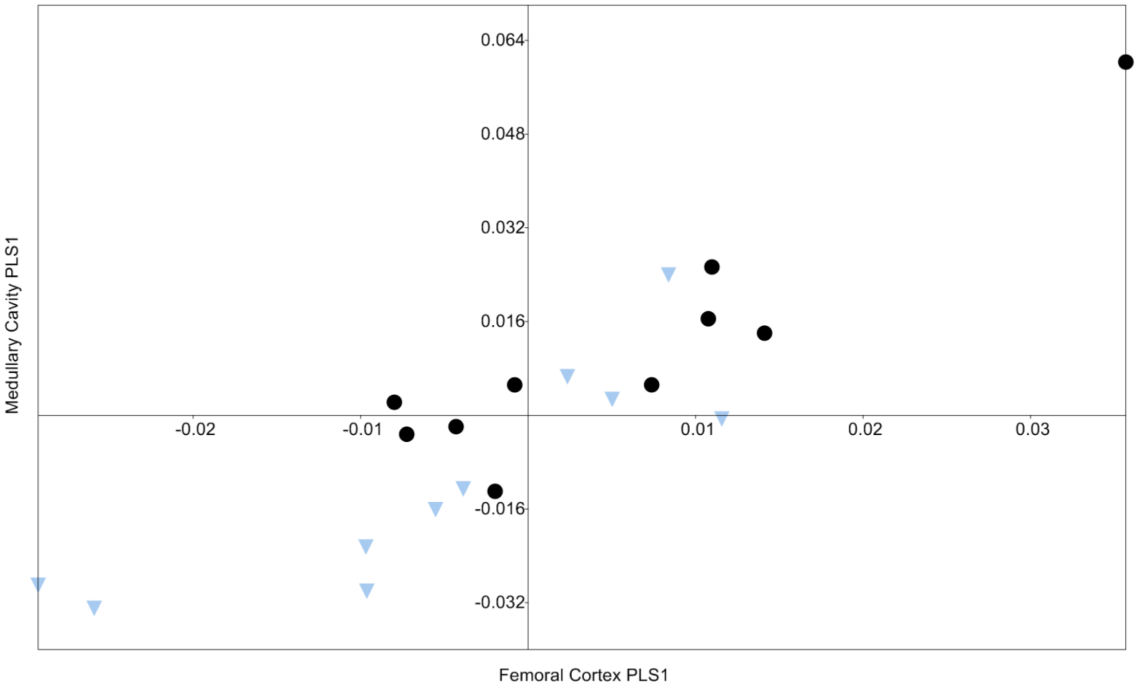

Figure 25. Plot of the first two PLS axes of the two blocks: femoral cortex and medullary cavity.

Table II. Singular values and pairwise correlations of PLS scores between blocks:

	Singular value	P-value (perm.)	% total covar.	Correlation	P-value (perm.)
PLS1	0.00028685	**<0.001**	77.847	0.90202	**<0.001**
PLS2	0.00009429	0.084	8.41	0.87096	**0.008**
PLS3	0.00007908	**<0.001**	5.916	0.87107	**0.024**
PLS4	0.00004824	0.36	2.202	0.8678	**0.04**
PLS5	0.00004326	0.088	1.77	0.85462	**0.048**
PLS6	0.00003673	0.064	1.276	0.84964	0.096
PLS7	0.00002681	0.452	0.68	0.88529	0.016
PLS8	0.0000234	0.3	0.518	0.86325	0.1
PLS9	0.00001971	0.38	0.367	0.91598	**0.004**
PLS10	0.00001642	0.616	0.255	0.88978	0.064
PLS11	0.00001472	0.552	0.205	0.82388	0.544
PLS12	0.00001248	0.704	0.147	0.88127	0.128
PLS13	0.00001234	0.096	0.144	0.80462	0.808
PLS14	0.00000839	0.996	0.067	0.94728	**0.004**
PLS15	0.00000789	0.992	0.059	0.89228	0.304
PLS16	0.00000702	0.984	0.047	0.93951	**0.02**
PLS17	0.00000648	0.928	0.04	0.94954	**0.02**
PLS18	0.00000567	0.912	0.03	0.93615	0.112
PLS19	0.00000457	1	0.02	0.95023	**0.04**

Significant P-values in bold; α: 0.05; 500 permutation rounds.

4.3.6 Multivariate Regression of shape (complete dataset) on cross-sectional properties:

The multivariate regression of shape on CA was significant (10000 perm. rounds; p-value: **0.0447**) (Fig. 26). The relative amount of variation for which the regression accounted was calculated as sum of squares (SS) (Total SS: 0.03446566; Predicted SS: 0.00362305; Residual SS: 0.03084261). Therefore the proportion of variation for which the regression accounted as a percentage of the total variation was of only 10.51%. It was possible to interpret from the figure, that those individuals with enlarged medullary cavities and more gracile morphologies have less cross-sectional cortical area. Interestingly, there was a visually evident distinction between hunter-gatherers and agriculturalists, excepting some individuals already identified as outliers by the preliminary analyses.

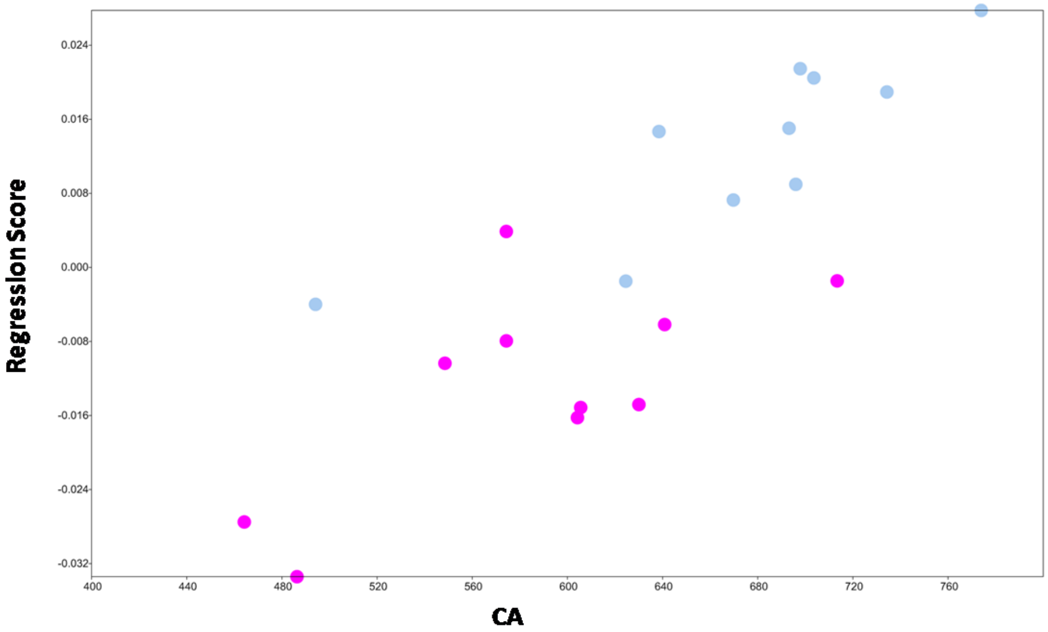

Figure 26. Regression plot of shape variables on standardised cross-sectional cortical area (CA). Fuchsia: Agriculturalists; Grey: Hunter-Gatherers.

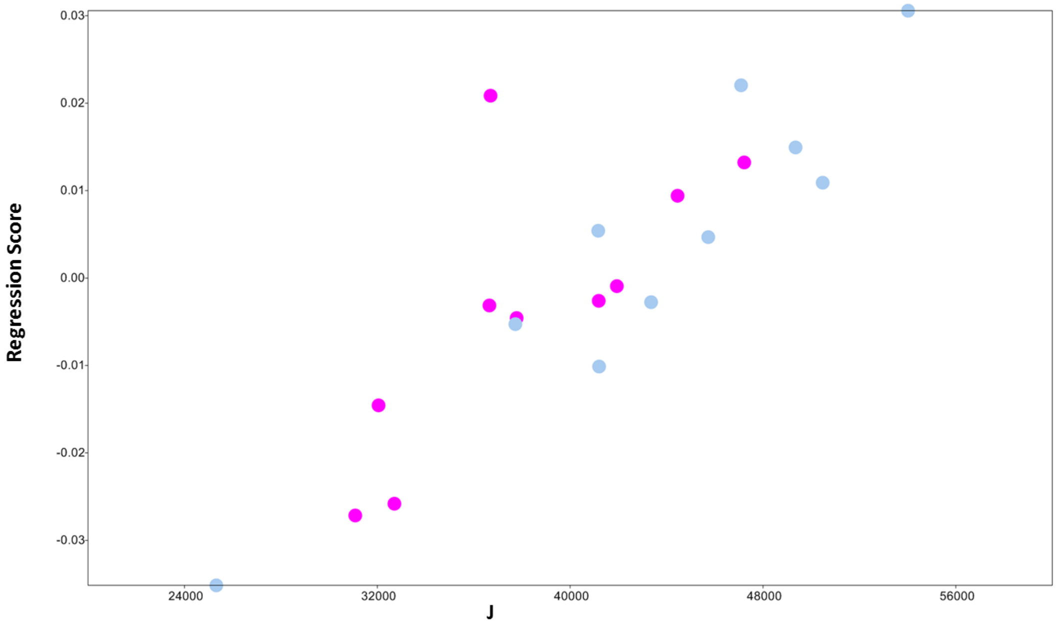

Figure 27. Regression plot of shape variables on standardised Polar Second Moment of Area (J). Fuchsia: Agriculturalists; Grey: Hunter-Gatherers.

The same procedure already described was followed in the multivariate regression of shape variables on Polar Second Moment of Area (J). This multivariate regression was also significant (10000 perm. rounds; p-value: **0.028**) (Fig. 27). The relative amount of variation for which the regression accounted was calculated as sum of squares (SS) (Total SS: 0.03446566; Predicted SS: 0.00404822; Residual SS: 0.03041744). Therefore the proportion of variation for which the regression accounted as a percentage of the total variation was of only 11.75%. From a morphological perspective the individuals at the bottom of the regression trend are more gracile and have enlarged medullary cavities, while those at the upper portion of the graph presenting the opposite characteristics. One individual was highlighted as an outlier, because although having a relatively robust femoral cortex, it had an extremely enlarged medullary cavity as well.

It was decided to perform a Cross-validated Discriminant Analysis on the residuals of these two regressions. There was not a significant difference between groups for the Shape on CA regression residuals (Procrustes distance: 0.02368811; p-value 500 perm. rounds: 0.0760), although the correct classification was almost perfect, being 90% for agriculturalists and 100% for hunter-gatherers (Fig.28). In the case of the Shape on J regression residuals the difference was highly significant (Procrustes distance: 0.03253624; p-value 500 perm. rounds: <**0.0001**). The correct classification was of 90% both for agriculturalists and hunter-gatherers (Fig. 29).

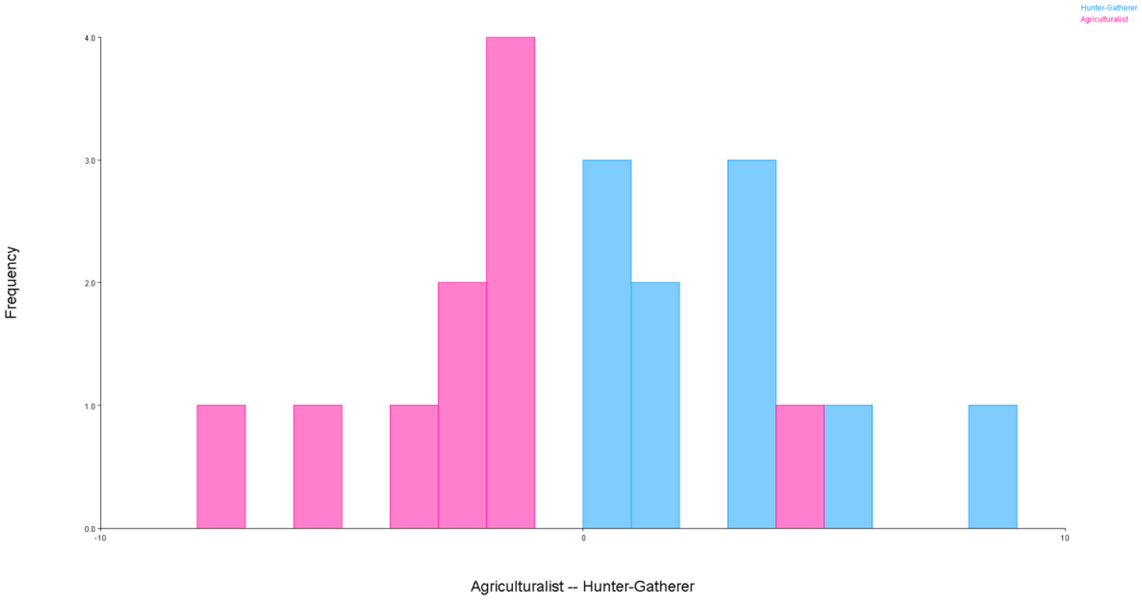

Figure 28. Cross-validated Discriminant Analysis of the Shape on CA regression residuals. The level of correct classification was of 90% for agriculturalists and 100% for hunter-gatherers.

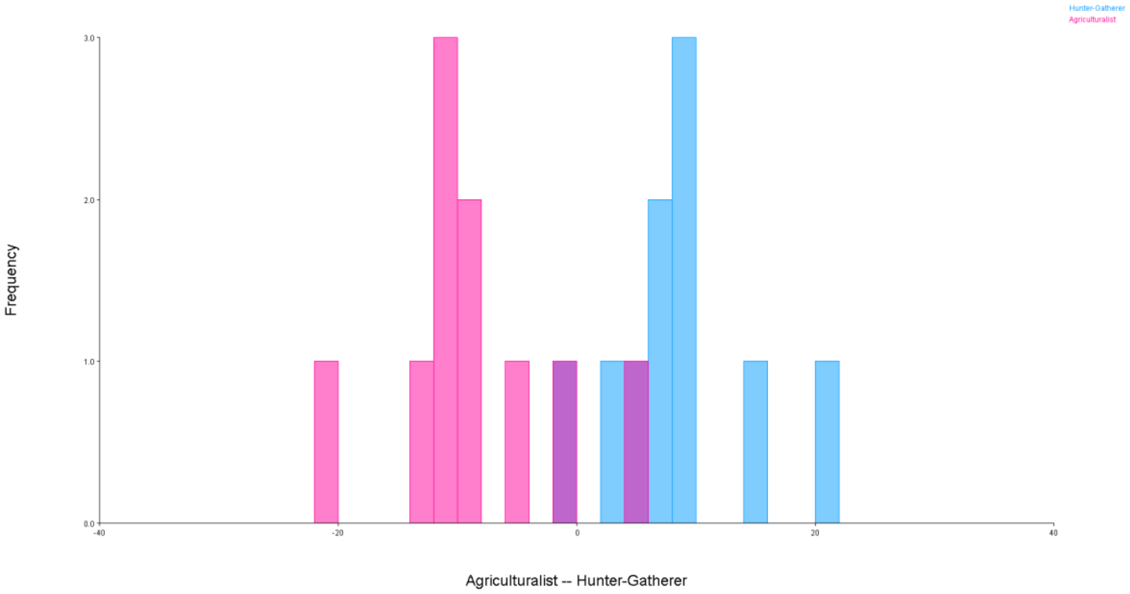

Figure 29. Cross-validated Discriminant Analysis of the Shape on J regression residuals. The level of correct classification was of 90% for agriculturalists and 90% for hunter-gatherers..

4.4 FEA:

The strain distribution over the bones was relatively the same for all the femora, with more noticeable differences among the more gracile femora with enlarged medullary cavities. The strain values for compression were much lower than those obtained under the bending scenarios. Three different values were extracted from each one of the 112 landmarks: a) von Mises strains, b) first principal strains (PS1) and c) second principal strains 2 (PS2). This procedure was repeated for each one of the three different loading scenarios: a) compression, b) medial bending and c) posterior bending (Fig 30).

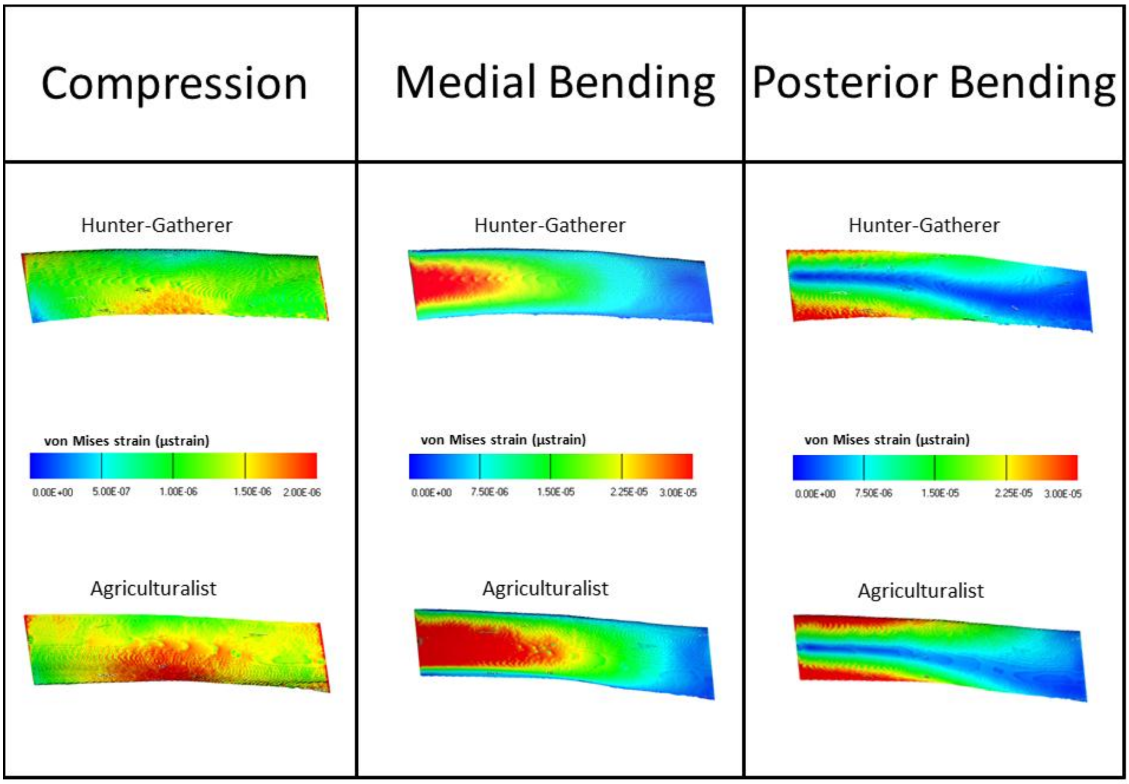

Figure 30. FEA colour-map of the three loading scenarios.

These extracted values were scaled by bone maximum length and then used to perform a PCA. The principal components that accounted for ca. 90% of the variance of the sample were extracted and utilised to compare by means of a Discriminant Analysis. Statistical significance was assessed by using a parametric Hotelling's T^2 and a two-group permutation test (2000 rounds) of the Euclidean distance between groups. Table III summarises the results and Figures 31, 32 and 33 show the Discriminant Analysis visual classification graphs.

The majority of the results did not show significant differences, despite the fact that some of them almost achieved the required α level. The only comparison that was significantly different for both the parametric and the permutation p-values was the PS1 of the medial bending scenario. Interestingly, PS1 was also significant for compression and almost significant for the posterior bending scenario.

Table III. FEA Discriminant Analysis results

Loading Scenario	**Strain values**	**Hotelling's T^2**	**P-value**	**Euclidean Distance**	**P-value ***	**Figure**
Compression						
	von Mises	13.156	0.068	9.46E-09	0.067	31a
	PS1	15.574	0.088	4.49E-09	**0.036**	31b
	PS2	21.058	0.281	9.36E-09	0.079	31c
Medial Bending						
	von Mises	8.483	0.095	1.28E-07	0.098	32a
	PS1	11.88	**0.039**	1.17E-07	**0.039**	32b
	PS2	8.085	0.206	9.61E-07	0.085	32c
Posterior Bending						
	von Mises	22.71	**0.003**	1.01E-07	0.119	33a
	PS1	17.53	0.064	8.27E-08	0.077	33b
	PS2	22.12	**0.013**	7.10E-08	0.175	33c

Significant P-values in bold; α: 0.05; PS1: First Principal Strain; PS2: Second Principal Strain; *:2,000 perm. rounds

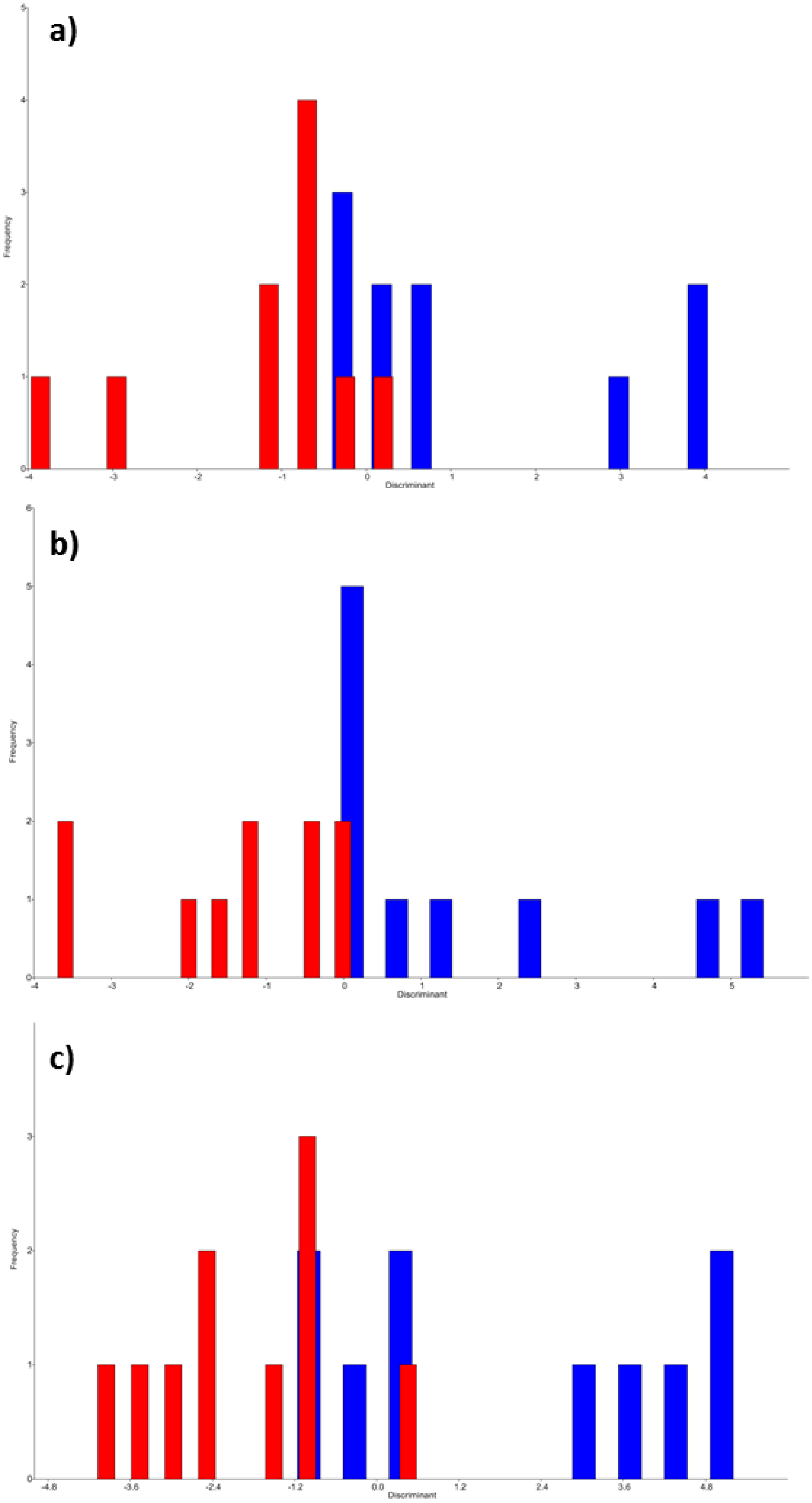

Figure 31. Discriminant Analyses of the Principal components (90% of the variance) of the scaled strain values extracted from the Compression scenario: a) von Mises, b) PS1 and c) PS2. Red: Hunter-gatherers; Blue: Agriculturalists

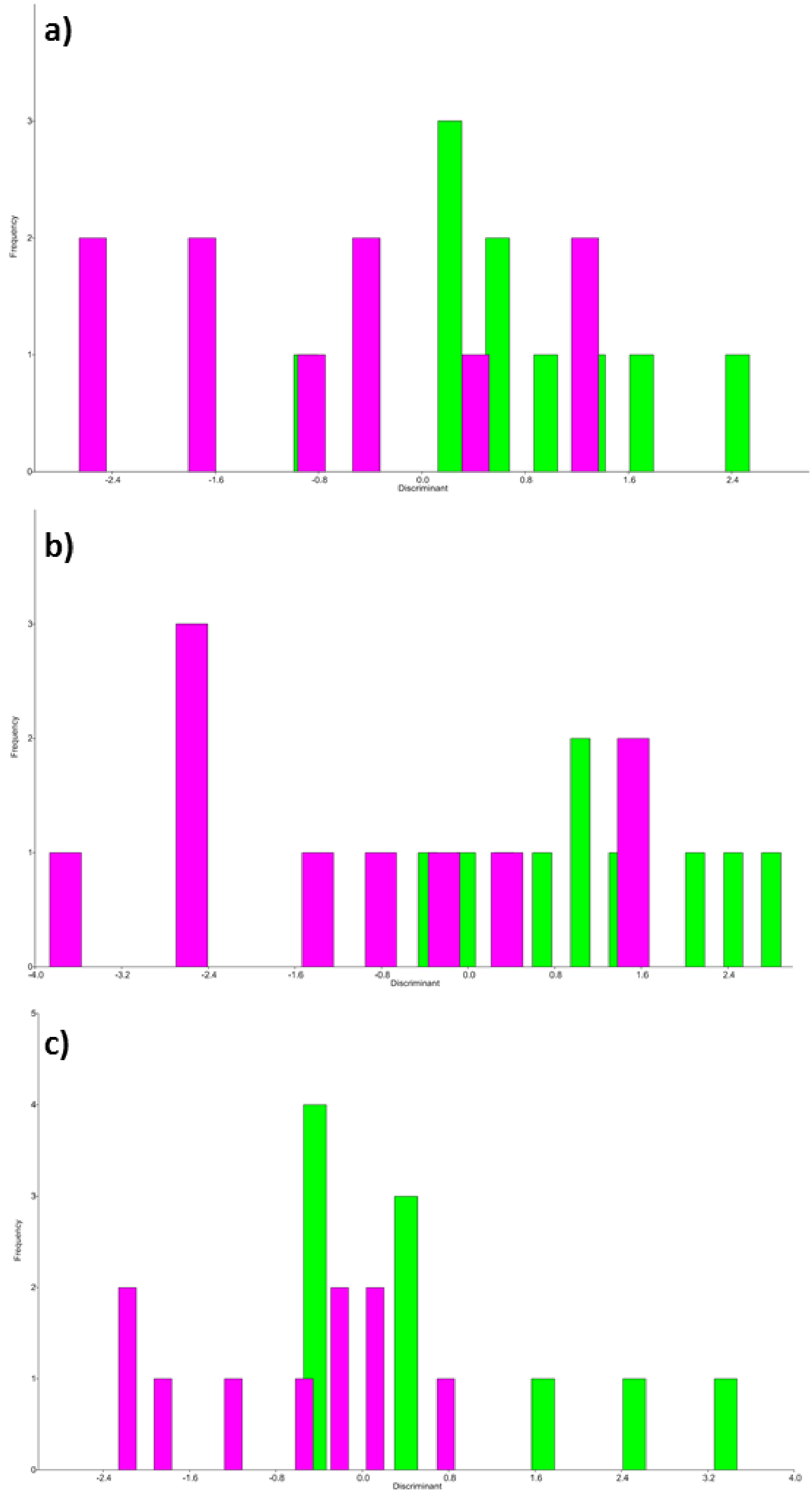

Figure 32. Discriminant Analyses of the Principal components (90% of the variance) of the scaled strain values extracted from the Medial Bending scenario: a) von Mises, b) PS1 and c) PS2. Fuchsia: Hunter-gatherers; Green: Agriculturalists.

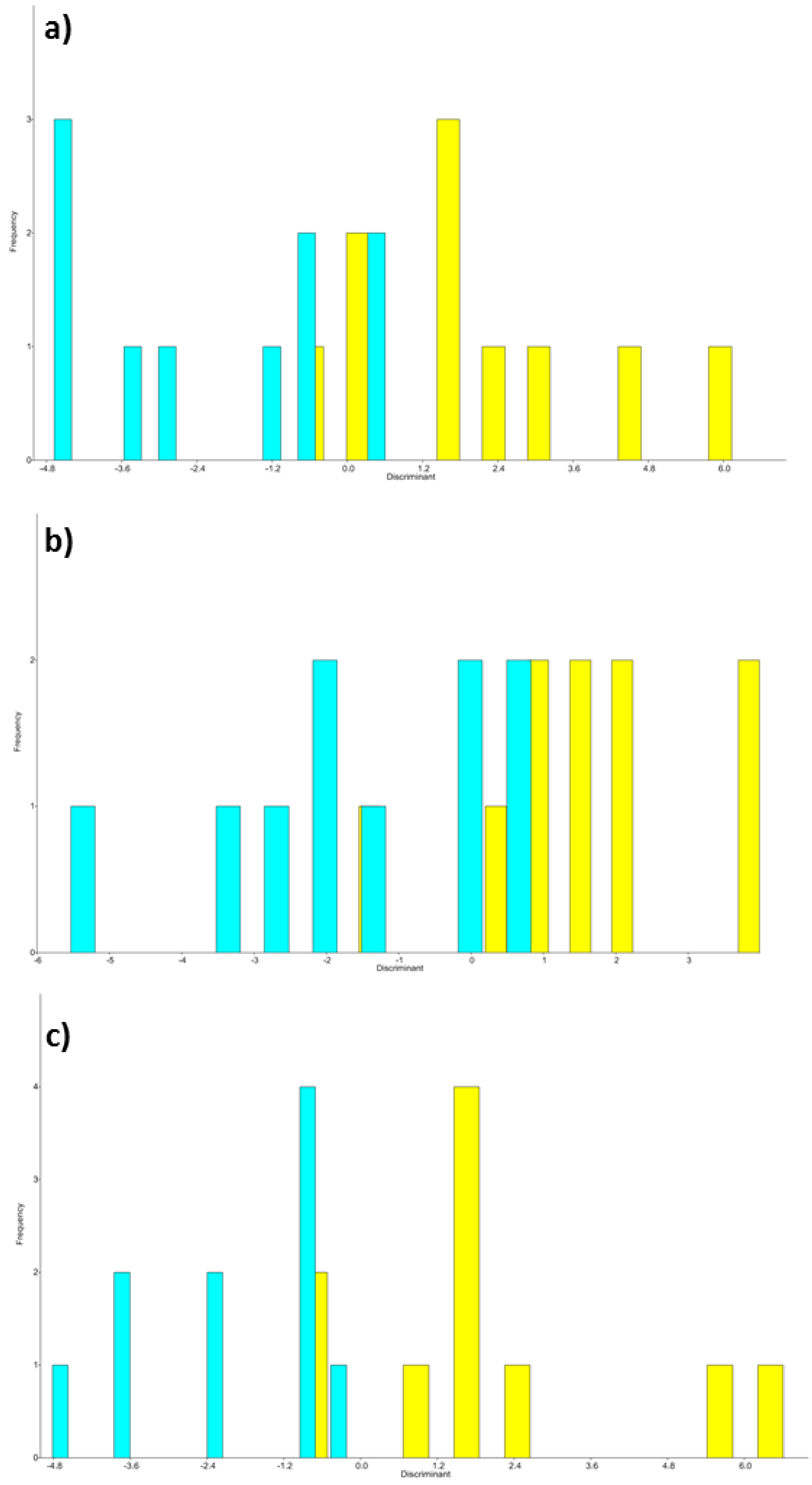

Figure 33. Discriminant Analyses of the Principal components (90% of the variance) of the scaled strain values extracted from the Posterior Bending scenario: a) von Mises, b) PS1 and c) PS2. Cyan: Hunter-gatherers; Yellow: Agriculturalists.

4.5 Concordance between FEA and Beam Theory

Interestingly, when the average strain distribution of the models was correlated with their average cross-sectional properties, there were highly significant and relatively strong negative correlations both for CA and compression strains and J and posterior/medial bending strains when selecting von Mises and PS1 strains (Table IV.). This means that when increasing CA and J, compression and medial/bending strains diminish accordingly (von Mises and PS1). On the other hand, when the same procedure was repeated but using PS2 strains, the correlation was positive, which is logical because of the negative sign of these strains.

Table IV. Person's correlation between the average values of the results obtained from Beam Theory and FEA.

Cross-sectional Property	Loading Scenario	Strain Value	Pearson's *r*	p-value
CA	Compression	von Mises	-0.72186	**0.00033**
CA	Compression	PS1	-0.75185	**0.00013**
CA	Compression	PS2	0.71768	**0.00037**
J	Medial Bending	von Mises	-0.63343	**0.00271**
J	Medial Bending	PS1	-0.62324	**0.00333**
J	Medial Bending	PS2	0.59698	**0.00545**
J	Posterior Bending	von Mises	-0.70164	**0.00057**
J	Posterior Bending	PS1	-0.64786	**0.00201**
J	Posterior Bending	PS2	0.71598	**0.00038**

Significant P-values in bold; α: 0.05; CA: Cortical Area; J: Polar Second Moment of Area: PS1: First Principal Strain; PS2: Second Principal Strain.

4.6 GMM applied to FEA results:

GMM analyses were applied to compare the results of FEA within a multivariate framework. Initially the average of each one of the loading regimens was calculated, as well as the undeformed multivariate mean shape. These averaged values were submitted to a size-and-shape PCA and the first two PC's were plotted (Fig. 34). The chart shows that the deformation due to the bending regimens is much greater (some orders of magnitude higher) than the one produced by the compression regimen. Actually this latter, is almost in the same position as the undeformed average. Due to the fact that 10N were applied in the three loading scenarios, it is possible to state that bone is more resistant to compression than to bending. As expected the two bending regimen points plot on opposite sides, which is logical due to the fact that both were bended in orthogonal ways. The small discrepancies in their relative locations are explained by the morphological differences in both axes of the femoral shaft (if the femoral sections were perfect tubes, the points would be located in exact opposite positions).

Later, the different loading scenarios were plotted separately along with the undeformed average (Fig. 35, 36 and 37). The three separate plots showed some overlap between the two groups. In all the cases, some individuals stood out as being more dissimilar to their own groups. These specimens were always the same ones in the three loading scenarios. They can be characterised from a morphological point of view, as having a more gracile femoral cortex, enlarged medullary cavities and a thinner layer of cortical bone. Later on, the three loading scenarios were combined and plotted on graph along with the undeformed average (Fig.38). There was a considerable overlap between agriculturalists and hunter-gatherers, although the formers showed a greater variability. One hunter-gatherer individual was particularly different, biomechanically behaving like some of the more distinct agriculturalists.

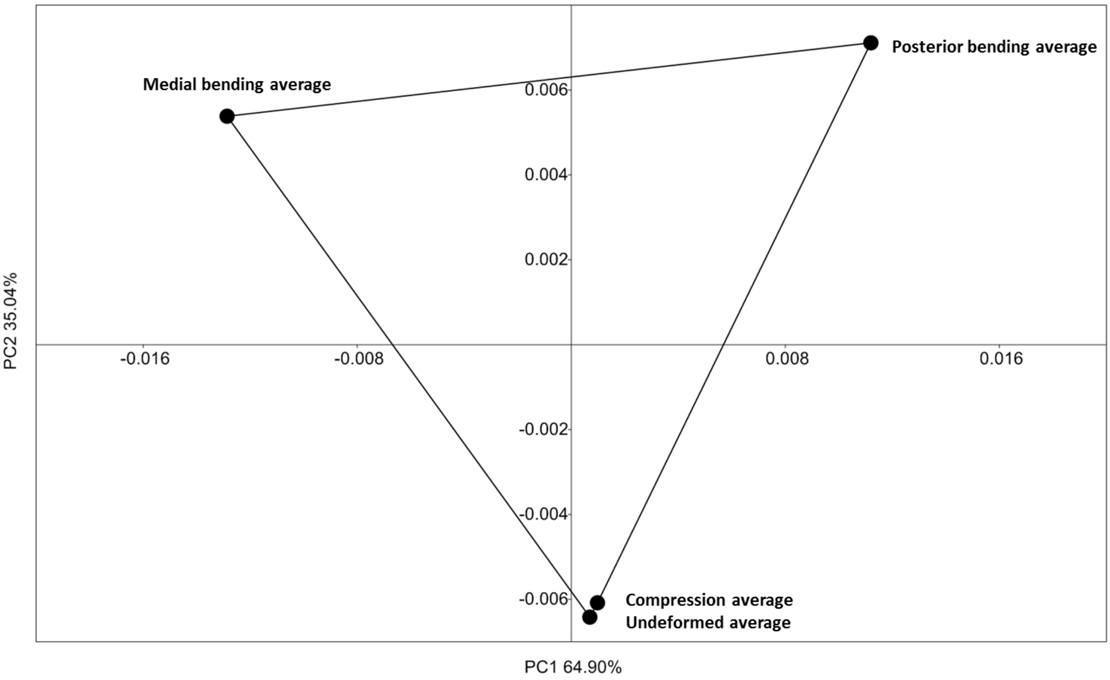

Figure 34. Size-and-Shape PCA of the average 112 3D coordinates of the three loading scenarios and the undeformed average.

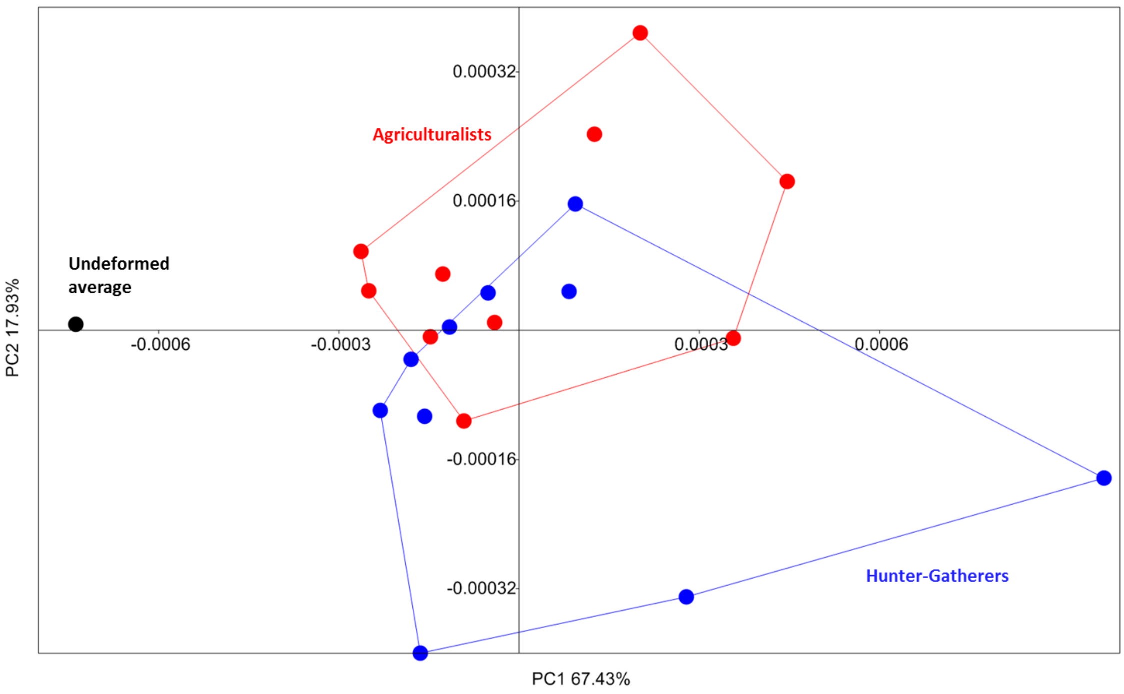

Figure 35. Size-and-Shape PCA of the 112 3D coordinates of the compression scenario and the undeformed average. Red: Agriculturalists; Blue: Hunter-Gatherers.

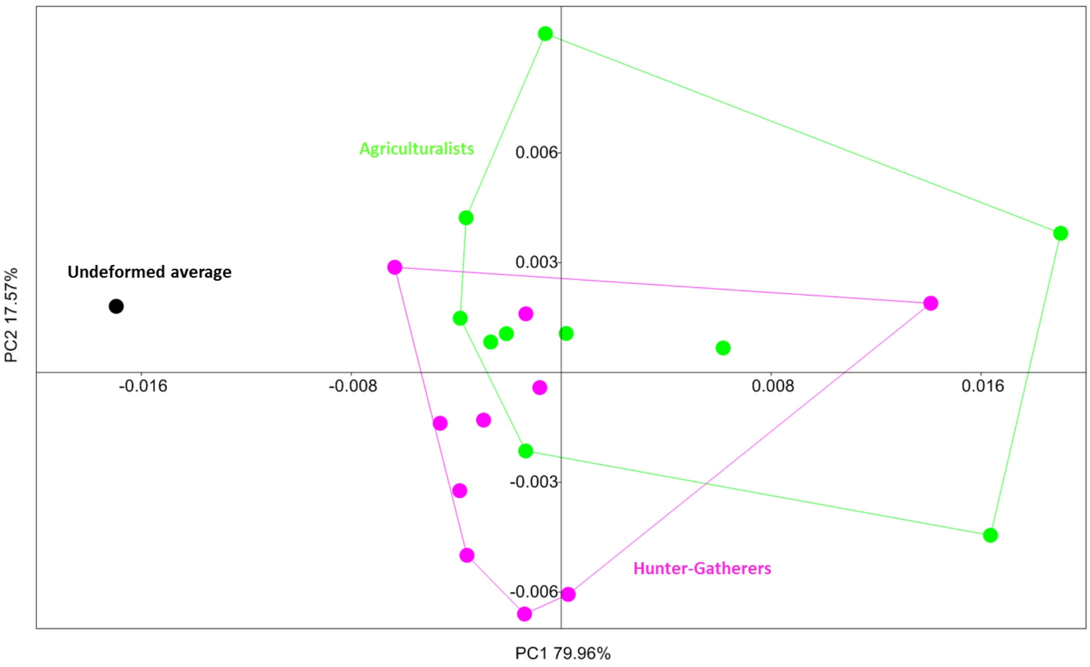

Figure 36. Size-and-Shape PCA of the 112 3D coordinates of the medial bending scenario and the undeformed average. Green: Agriculturalists; Fuchsia: Hunter-Gatherers.

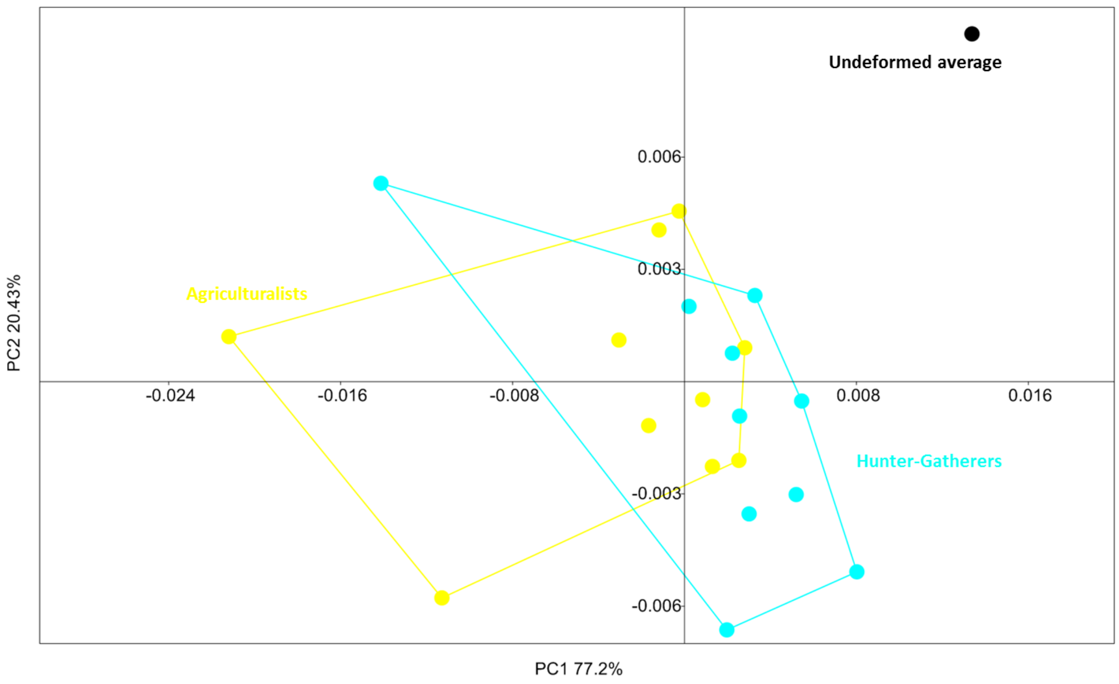

Figure 37. Size-and-Shape PCA of the 112 3D coordinates of the posterior bending scenario and the undeformed average. Yellow: Agriculturalists; Cyan: Hunter-Gatherers.

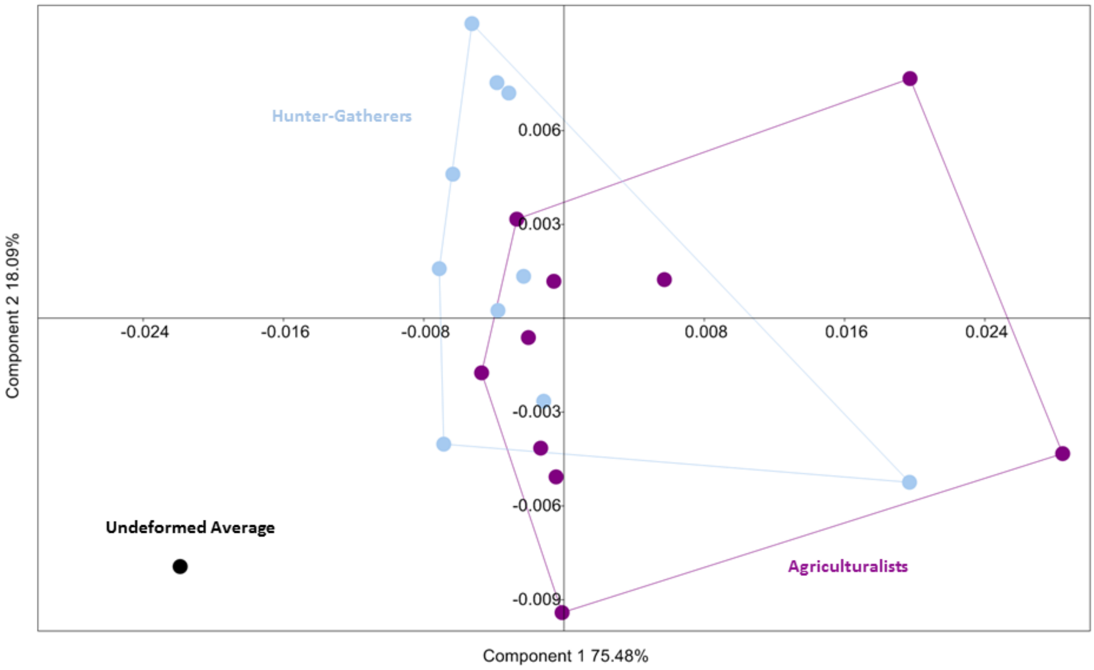

Figure 38. Size-and-Shape PCA of the 112 3D coordinates of the combined three loading scenarios and the undeformed average. Purple: Agriculturalists; Light-Blue: Hunter-Gatherers.

Later, the undeformed average was removed and another Size-and-Shape PCA was performed with the combined three loading scenarios (results not shown). The first two PC's (93.2% of the variance) were then used in a Discriminant Analysis using lifestyle as classifier. The results were not significant and showed a noticeable level of overlap (Hotelling's T^2: 3.9699; p-value: 0.1838; Mahalanobis distance: 0.0070; p-value (2000 perm. rounds): 0.177) (Fig.39).

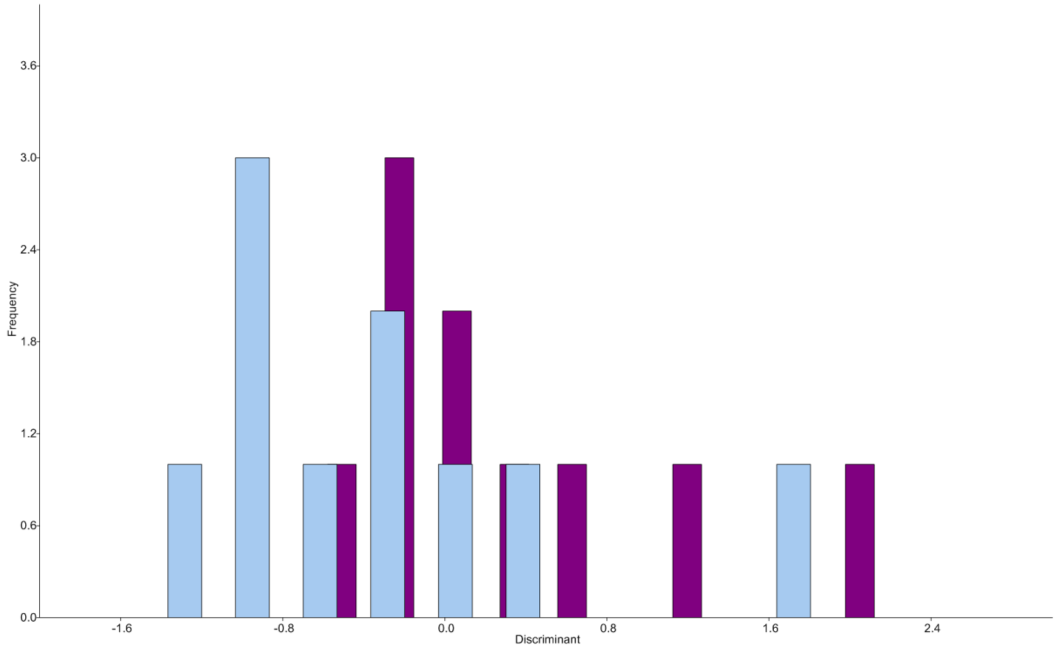

Figure 39. Discriminant Analysis of the first two PC's of the Size-and-Shape PCA of the combined three loading scenarios. Purple: Agriculturalists; Light-Blue: Hunter-Gatherers.

However one hunter-gatherer individual was clearly an extreme outlier, hence it was decided to re-run the same analysis removing this particular individual. After that removal, there was a significant difference between the two groups (Hotelling's T^2: 10.35; p-value: **0.022**; Mahalanobis distance: 0.0096; p-value (2000 perm. rounds): **0.015**). (Fig. 40.).

Afterwards the same procedure outlined above was repeated on each one of the loading scenarios separately (although without removing any outlier). Figures 41, 42 and 43 show the Discriminant analysis classification results for the different loading cases. Only the compression scenario achieved significant results, with a slight degree of overlap (Hotelling's T^2: 13.738; p-value: **0.025**; Mahalanobis distance: 2.7476, p-value (2000 perm. rounds): **0.017**). The Medial Bending Scenario almost reached significance, but showed a greater level of misclassification (Hotelling's T^2: 6.2502, p-value: 0.079; Mahalanobis distance: 1.25; p-value (2000 perm. rounds): 0.074).

Finally, the Posterior Bending Scenario also did not exhibit significant differences between the two groups (Hotelling's T^2: 2.6288; p-value: 0.314; Mahalanobis distance: 0.52575; p-value (2000 perm. rounds): 0.339).

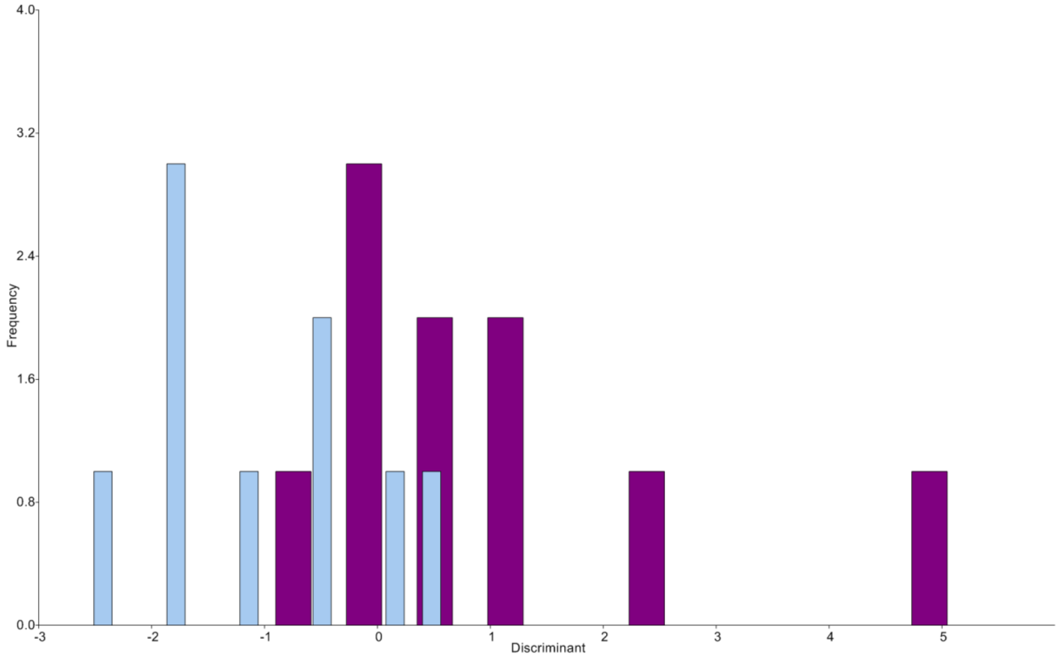

Figure 40. Discriminant Analysis of the first two PC's of the Size-and-Shape PCA of the combined three loading scenarios with the outlier removed. Purple: Agriculturalists; Light-Blue: Hunter-Gatherers.

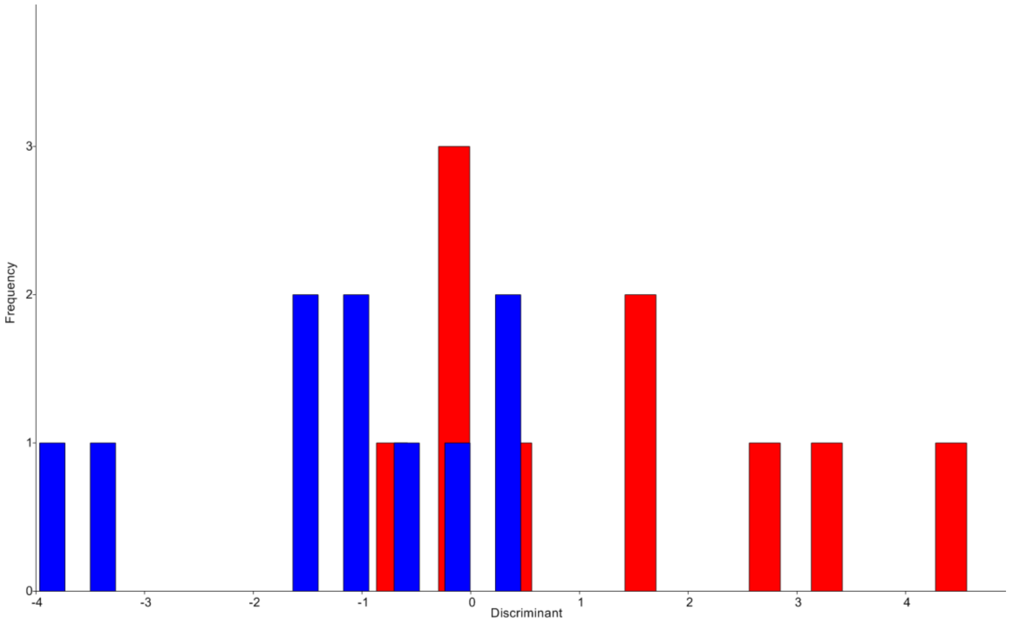

Figure 41. Discriminant Analysis of the first PC's of the Size-and-Shape PCA of the Compression Scenario. Red: Agriculturalists; Blue: Hunter-Gatherers.

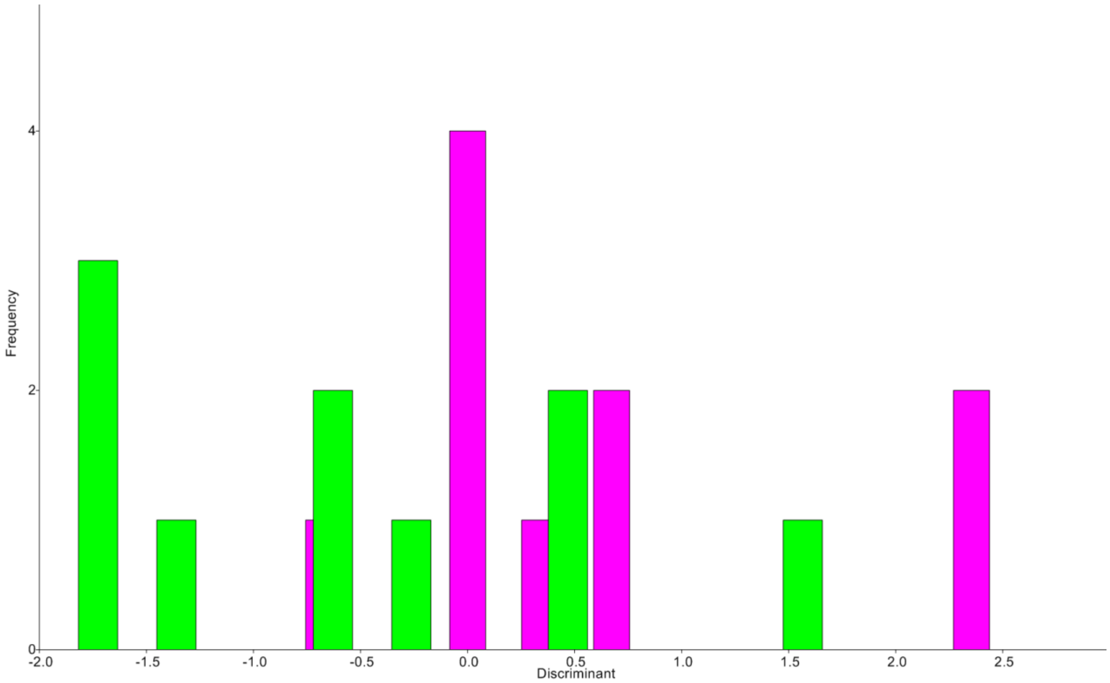

Figure 42. Discriminant Analysis of the first PC's of the Size-and-Shape PCA of the Medial Bending Scenario. Purple: Agriculturalists; Green: Hunter-Gatherers.

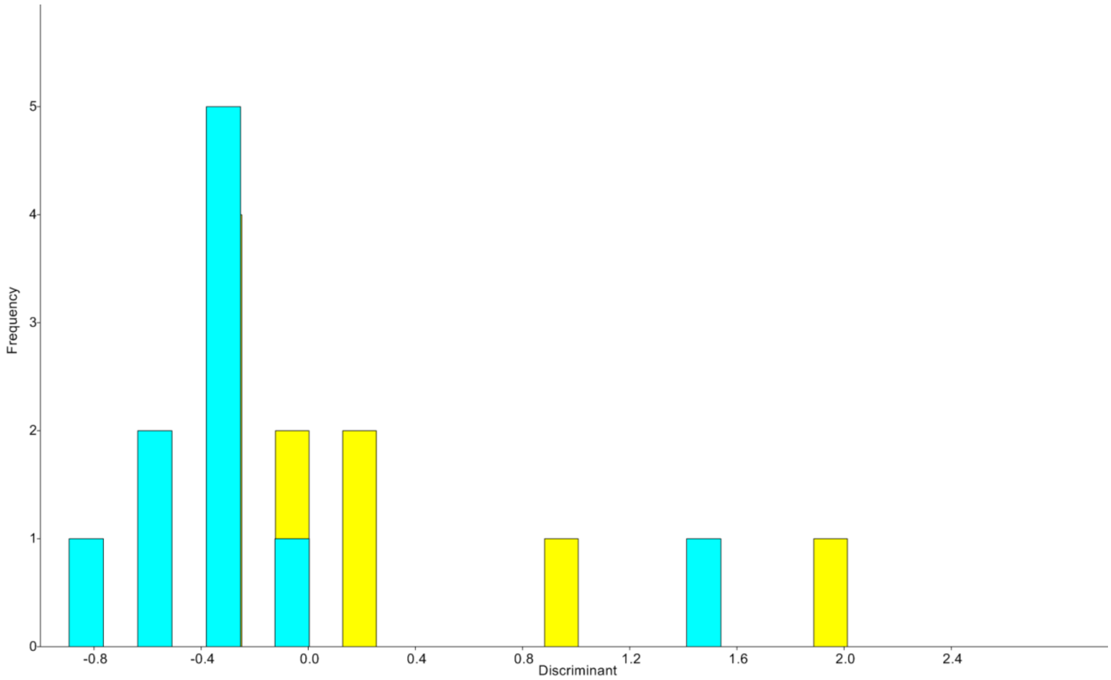

Figure 43. Discriminant Analysis of the first PC's of the Size-and-Shape PCA of the Posterior Bending Scenario. Yellow: Agriculturalists; Cyan: Hunter-Gatherers.

4.7 Concordance between of GMM applied to FEA and Beam Theory:

The simulated responses to loadings of the finite element models of the femoral shaft denote functional changes probably related to differences in *intra vitam* locomotion. Therefore, two specimens nearby in the morphospace (represented by a shape-and-size PCA) are expected to have similar biomechanical responses to loading. Due to the fact that scale and material properties were almost invariant, it was possible to consider that changes in gross morphology and parameters such as CA or J could explain the variation in the morphospace. A Mantel test was carried out between the PC's of the size-and-shape PCA of the three loading scenarios that accounted for ca. 90% of the sample variance and the cross-sectional properties (CA and J separately) (Table V.). Statistical significance was computed by using the Mantel test statistic (observed Z values compared to their permutation distribution after 5000 replicates). There were highly significant medium to high positive correlations between the two matrices. This means that the femoral form under loading is relatively related to the cross-sectional properties of the bones.

Table V. Mantel test of the first PC's of the size-and-shape PCA of the three loading scenarios on CA and J separately.

Size-and-Shape first PC's	Cross-sectional Property	R	P-value *
Compression	CA	0.5716	**0.0018**
Medial Bending	J	0.6355	**0.0002**
Posterior Bending	J	0.6026	**0.0009**

Significant P-values in bold; α: 0.05; CA: Cortical Area; J: Polar Second Moment of Area; *:5000 perm. rounds.

Chapter 5: Discussion and Conclusion

5.1 Discussion

Based on the majority of the results presented, it is possible to reject the initial null hypothesis. There were significant differences between the discriminatory capabilities of the different methods here applied. The findings suggest that when discriminating between past lifestyles based on femoral shaft morphology and biomechanical response, different techniques achieve distinct discrimination results. This was particularly patent in the results obtained after performing several Discriminant Analyses (Table VI.). When comparing the three techniques it was surprising that one of the best discriminatory capability was achieved applying only a traditional GMM analysis.

This result is intriguing, because it would mean that when discriminating by function/robusticity/ecology/lifestyle (i.e. basically those traits directly related to the way of life of the individuals), shape seems to be a better descriptor and hence have an increased discriminatory capability. Because GMM analyses are suited describing morphological variation, they are probably taking into account many different factors that distinguish between groups, including those related to function. On the other hand, functional analyses such as FEA, only consider some specific and delimited aspects of functional variation. Thus, it is possible that GMM differentiates in an enhanced way as compared with methods that use measures of load resistance or performance under loads, because by describing morphological variation, different traits and aspects of form that better distinguish between different lifestyles contribute.

Table VI. Cross-Validated Classification/Misclassification Table of the Discriminant Functions resulting from the different techniques applied.

Technique	True Groups	Allocated to		% Correctly Classified	Permutation Significance
		Agriculturalists	Hunter-Gatherers		
Beam Theory CA	Agriculturalists	7	3	70%	0.086
	Hunter-Gatherers	4	6	60%	
Beam Theory J	Agriculturalists	6	4	60%	0.688
	Hunter-Gatherers	4	6	60%	
FEA Compression PS1	Agriculturalists	5	5	50%	**0.032**
	Hunter-Gatherers	7	3	70%	
FEA Medial Bending PS1	Agriculturalists	9	1	90%	**0.042**
	Hunter-Gatherers	3	7	70%	
FEA Posterior Bending PS1	Agriculturalists	8	2	80%	0.083
	Hunter-Gatherers	5	5	50%	
GMM Femoral Cortex	Agriculturalists	9	1	90%	**0.044**
	Hunter-Gatherers	8	2	80%	
GMM Femoral Medullary Cavity	Agriculturalists	7	3	70%	**0.032**
	Hunter-Gatherers	5	5	50%	
GMM Femoral Complete Dataset	Agriculturalists	8	2	80%	**0.008**
	Hunter-Gatherers	1	9	90%	
GMM residuals Shape on CA	Agriculturalists	9	1	90%	0.086
	Hunter-Gatherers	0	10	100%	
GMM residuals Shape on J	Agriculturalists	9	1	90%	**0.002**
	Hunter-Gatherers	1	9	90%	
GMM of the FE Displacements of the three Loading Scenarios Combined	Agriculturalists	5	5	50%	0.152
	Hunter-Gatherers	3	7	70%	
GMM of the FE Displacements of the three Compression Scenario	Agriculturalists	6	4	60%	**0.015**
	Hunter-Gatherers	2	8	80%	
GMM of the FE Displacements of the Medial Bending Scenario	Agriculturalists	6	4	60%	0.082
	Hunter-Gatherers	7	3	70%	
GMM of the FE Displacements of the Posterior Bending Scenario	Agriculturalists	4	6	40%	0.339
	Hunter-Gatherers	2	8	80%	

Significant P-values in bold; α: 0.05; CA: Cortical Area; J: Polar Second Moment of Area; PS1: First Principal Strains *:500 perm. rounds.

Additionally, the results obtained from the Discriminant Analysis performed using the residuals from the regression of shape on cross-sectional properties (CA and J) were interesting. The underlying idea was to separate the part of variation in the dependent variables (shape coordinates) that was predicted by the independent variable (CA or J in each regression), from the residual part of variation, which was uncorrelated with the independent variables. By removing the predicted part from the analysis and hence focusing utterly on the residuals, these new variables were uncorrelated with the independent variables (CA or J). Thus, it was possible to focus only on the shape variation not dependent on the cross-sectional properties. The results from these Discriminant Analyses allow a clear distinction between the two lifestyles, despite the fact that one of them was not significant. This result is intriguing, because it means that even when removing the part of shape variation due to the factors that supposedly best discriminate between lifestyles, the remaining variation differentiates even better between the two groups.

This is consistent with the prior GMM Discriminant Analyses, that showed increased discrimination as compared with functional analysis (e.g. FEA) and static estimation of stress resistance from geometry (e.g. Beam Theory). The fact that the residuals from the regression of shape on cross-sectional properties positively discriminate between groups, could mean that there are actually other shape features not necessarily related to the compression/bending strength that are also able to distinguish those traits directly related to an individual’s lifestyle. One possible explanation could be that when measuring biomechanical performance with different methods (e.g. Beam Theory; FEA), they only quantify a small aspect of the variation due to complex functions (e.g. locomotion). Therefore, it seems that GMM captures more of the variation related to these complex biological functions, nonetheless it is not possible to establish *a priori* what part of this variation is strictly related to function or to other possible sources (e.g. development, phylogeny, sexual dimorphism, etc.). GMM gives the impression of encompassing different aspects of morphological variation, including many that are related to function, while other specific functional analyses only compare more restricted aspects of this variation.

The contrasting results obtained from the GMM analysis of the femoral cortex and the medullary cavity, suggest that long bone remodelling caused by locomotion differences, mostly occurs in the outer surface of the shaft. The results from the PCA's and discriminant analyses showed that most of the variation that separates the two lifestyle groups occurs on the femoral cortex rather than on the medullary cavity. In fact, the cross-validated results showed an almost total overlap between the hunter-gatherers and agriculturalist when using the medullary cavity, while the femoral cortex landmarks allowed an almost complete separation between the groups. Nevertheless, the results from the two-block PLS between the femoral cortex and the medullary intriguingly showed a high morphological integration. Further inquiries regarding this specific subject are required to elucidate this matter.

Traditional beam theory could not differentiate between agriculturalists and hunter-gatherers when comparing standardised CA and standardised J. Perhaps this discriminatory limitation was related to the segmentation of only a small portion of the femoral shaft, which maybe does not vary that much in cross-sectional properties. Although when the hunter-gatherer outlier was removed, CA was able to significantly discriminate between the two groups; hence it seems it could be a relevant factor in this type of comparisons. The present study only analysed a small section of the femoral shaft, consequently future inquiries should include the whole femur, because there is the possibility that the analysed bony portions do not vary that much in cross-sectional properties. Additionally, as described before when the shape coordinates where regressed on these cross-sectional properties, the resulting residuals were able to clearly distinguish between the two lifestyles. Hence, it seems that actually there are other shape features not related to cross-sectional properties that better separates between hunter-gatherers and agriculturalists.

Initially the FEA results showed discriminatory potential, many of them were significant at the moment of discriminating between agriculturalists and hunter-gatherers. When these series of analyses were performed without scaling by bone length (results not shown), most of the comparison reached statistical significance, indicating that size is an important factor to take into account when comparing FEA results (Dumont et al. 2009). Nonetheless, after standardising all the results dividing them by bone length, only few results remained significant. This means that the significant differences were at least partially due to bone length, which seemed to reduce the strains in the hunter-gatherers sample, because the femora were a little bit bigger (though not statistically significant). Therefore, when scaling for one possible size measurement (bone length), femoral shafts tend to behave in biomechanically similar ways. This is visible in the strain distribution over the bones, which are pretty much the same for all the femora, with more noticeable differences with respect to the more gracile femora with enlarged medullary cavities (Fig. 44).

This result was expected because bone is adapted to bear loadings in a similar fashion at an intraspecific level once body mass, sex and mineral content are controlled (Brianza et al. 2007). It has been suggested that bone allometric change is the tactic that this tissue adopts to adjust its structure and function in order to compensate for the physical consequences of size differences (Brown et al. 2000). This adjustment occurs by redistributing the mount of tissue proportional to body mass and so doing the energy per unit of mass required to fracture a bone remains mass invariant (Brianza et al. 2007). Consequently, when modelling long bones as simplified beam models, similar stresses and strains on the cross-sections facing increasing loads are expected. In other words, if bone responds adaptively to its *intra vitam* loadings, it is expected the stresses and strains remain relatively the same.

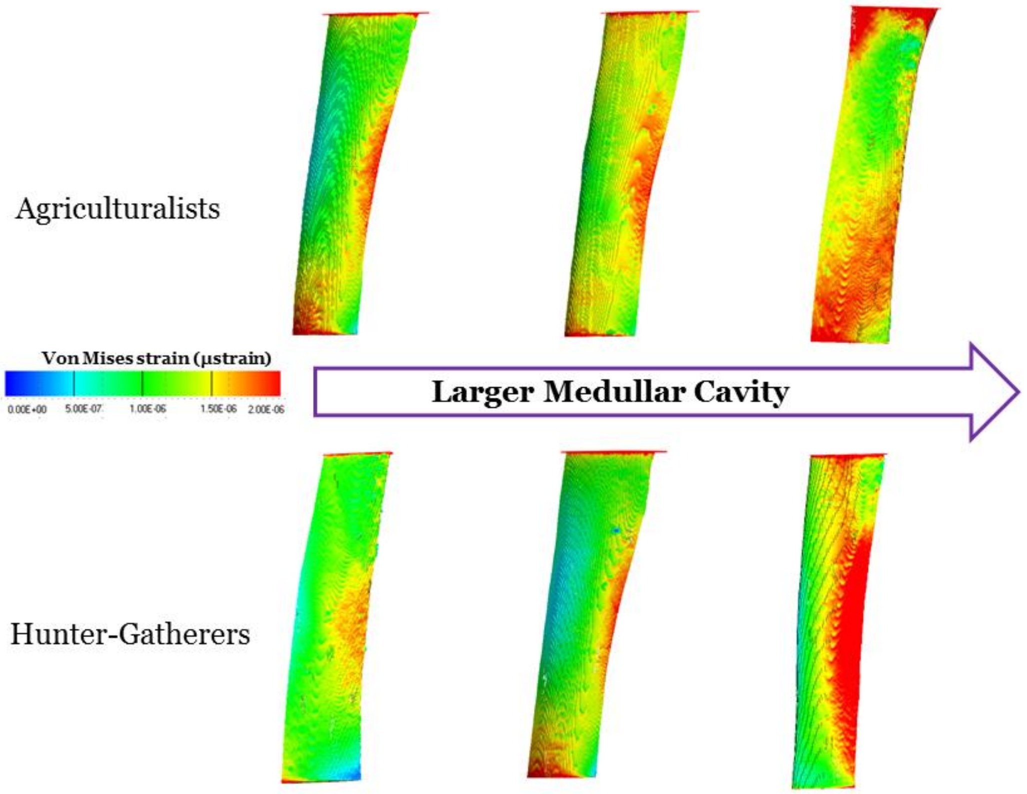

Figure 44. Example of the strain distribution under compression: Agriculturalists on top and Hunter-gatherers at the bottom. From left to right individuals present larger medullary cavities. Relatively irrespective of lifestyle, the strain distribution seems to depend more on the medullary cavity proportion.

As verified by many studies, it is evident that bones are adapted to the loads falling on them (Trinkaus 1983; Haapasalo et al. 2000; Robling et al. 2000; Shaw and Stock 2009a; Shaw and Stock 2009b). Bone is a dynamic tissue where different cells react to their environment (Kawata and Mikuni-Tagaki 1998; Mullender et al. 2004; Wang et al. 2007). Different lines of evidence showed that natural selection will tend to favour animals that can perform their locomotor functions with the greater efficiency (Carter and Beaupré 2007; Currey 2006). Hence, bone has to respond for two main contradictory selective pressures: it has to support body weight and the high forces resulting from locomotion, and simultaneously it must be light as possible, to allow organism motion and to diminish energy requirements (Ethier and Simmons 2007). If bones are made of material with the relatively invariant properties, the thicker they are, the stiffer and heavier they will be. Therefore, it might seem that there is a simple trade-off between stiffness and mass. Nevertheless, when the mass of a bone is reduced, that also weakens it and consequently its safety factors are reduced. For instance, the differential hollowness of long bones can be shown to depend on the specific mode of life of the animals (Currey and Alexander 1985). These reasons could explain why the strain distribution over the femoral sections was relatively the same, despite some morphological differences. This means that bones have to fulfil their function (i.e. mainly locomotion in femora) irrespective to these minor differences, keeping safety factors, bone weight and stiffness relatively similar. Thus, it was not unexpected that most of the comparison between strain values between hunter-gatherers and agriculturalists were not significant at all, being unable of differentiate between lifestyles. Only PS1's were able to discriminate between agriculturalists and hunter-gatherers, although the underlying reason is unclear.

Regarding the results obtained by the application of GMM on the coordinates obtained from FEA, the results were promising although further explorations are required. Because it is basically a form analysis of a functional/biomechanical test, it could be regarded as an appropriate way to deal with the issue that shape tends to better distinguish functional traits. However the obtained results when discriminating by lifestyle were limited, probably because the small sample size. Only the compression scenario allowed the separation between groups, perhaps related with the significant differences for CA. More research is needed to develop the application of this kind of analysis in bioarchaeological contexts.

The tests to establish the concordance between the different kinds of analyses showed that there is a highly significant and relatively strong negative correlation both for CA and compression strains and J and posterior/medial bending strains when selecting von Mises and PS1 strains. Hence, when increasing CA and J, compression and medial/bending strains diminish accordingly (von Mises and PS1). Although the two tests measure different properties, this relatively strong relationship is logical, because the biomechanical performance under compression is related to CA, and bending resistance depends on J. PS2's showed a relatively strong positive correlation with the cross-sectional properties, which is coherent due to the negative sign of this strain value.

The first PC's (that accounted for ca. 90% of the sample variance) of the size-and-shape PCA of the three loading scenarios were correlated with CA and J separately by means of a Mantel test. A positive medium significant correlation was found when this test was carried out in order to relate gross morphology under loading scenarios and cross-sectional properties. This suggests that the biomechanical performance assessed as shape deformation is related with the cross-sectional properties of the femoral sections. Probably the simulated responses to loadings of the FE models as well as their cross-sectional properties denote functional changes that are related to differences in *intra vitam* locomotion.

However, it is necessary to be aware that because robusticity has often been defined in different ways and thus measured differently, when comparing different methods it is likely that we are in fact contrasting dissimilar properties. Indeed the aim of the present work is not demonstrate that one technique is necessarily better than the other one, but insofar as they are all related to a common behaviour (locomotion), provide a broader perspective when analysing long bone form and biomechanics. If there is an incongruity between methodological approaches is probably based primarily on the different aspects that each technique is actually measuring. Beam Theory cross-sectional properties are measurements of compression/bending rigidity, while FEA outputs are proxies of biomechanical performance and deformation. GMM on the other hand, quantifies shape variation and how it covaries with other factors. The fact that actually biomechanical analyses poorly distinguish between the two lifestyles as compared with GMM, could mean that actually the former analyses only accounted a restricted aspect of variation. Moreover GMM probably describes broader aspects of variation that are able to differentiate between the two groups. Thus, studies integrating different approaches would be an enhanced way to analyse long bone robusticity trends, despite the fact that some of them could be better distinguishing between past lifestyles.

Several lines of bioarchaeological evidence have shown that there is an association between major transitions in subsistence and long-term changes in general robusticity patterns during human evolution (Stock and Pfeiffer 2004; Stock 2006; Shackelford 2007; Marchi et al. 2011; Pinhasi and Stock 2011). Nowadays is relatively clear that that both terrain and subsistence strategy contributed to modifications in long bone diaphyseal structure (Ruff 2008). The shift from hunting-gathering to agriculture has usually been connected to the gracilisation of human long bones (Ruff et al. 1984; Ruff 1987; Larsen 1999; Pearson 2000). The results presented in this dissertation follow *grosso modo* the same trend, showing a relative gracilisation of the agriculturalist sample with respect to the hunter-gatherers in some of the different analyses performed. The GMM analyses reflected this tendency, with the hunter-gatherers exhibiting more robust femoral morphologies. On the other hand, the agriculturalists have thinner cortical layers, expanded medullary cavities and external cortices with less marked signs of muscle attachments (e.g. a smoother *linea aspera*). Moreover, centroid size (CS) was used as a geometric size estimator and when it was compared between the two groups, did not separate them indicating that none of them have bigger femoral shafts. Similarly, when femoral maximum length, stature and body mass estimations were compared, there were no significant differences between the two lifestyles. Beam theory cross-sectional properties confirmed that hunter-gatherers have more cortical area (CA) than the agriculturalists but only when one hunter-gatherer outlier was removed. There were no significant differences when polar second moment of area (J) was compared. Despite the fact that many of the FEA comparisons were not significant, the few results that achieved significance showed that the hunter-gatherers presented a better biomechanical performance, with lower strain values under 10 N loadings. When the deformation caused by loadings was assessed by applying GMM to the new Cartesian coordinates resulting from FEA, it was noticeable that agriculturalist behave more variably, with the more deformed individuals belonging to this group. Summarising, it is possible to establish that some of the results obtained are consistent with previous observations that indicate that agriculturalist populations have more gracile long bones as compared with hunter-gatherers. In spite of the several criticisms on the concept of robusticity due to its unspecific meaning, the diverse results of this dissertation support *sensu lato* the traditional link established between the transition from hunting-gathering to agriculture and femora gracilisation, although further studies are needed.

It is important to bear in mind the limitations of this dissertation, in order to overcome them in future researches. One of the problems was the small sample size available. This problem was partially overcome by applying permutation tests, in order to avoid inconveniences related to deviations from the multivariate normal distribution. Although it still unknown how representative are these sample regarding the past lifestyles under study. Actually there was clearly one hunter-gatherer individual that behaved in most of the analyses as an agriculturalist, because of its gracile external femoral morphology and its enlarged medullary cavity. Further investigations will require a larger sample size; despite the fact that normally FEA is really time-consuming as compared with the other techniques here applied and hence more difficult to perform when having more individuals under analysis.

Another limitation was the analyses of only a small portion of the femoral shaft. Even though it was a tougher test for the discriminatory capability of the different methods, since the femoral shafts do not differ that much in that area, by using only that small portion of femur, its morphological total variation was reduced. The decision of just analysing that small femoral section was justified to simplify the models, reducing the required time and computational power, as well as to approximate the femoral morphology to a beam, in order to facilitate the technical comparison. However, future analysis should include more complete morphologies, as well as more complex loading scenarios with the aim of comparing more realistic results.

Finally, the present results lead to several subjects that remain to be explored. Perhaps one of the most exciting topics is the classical relation between form and function. Based on the analyses here presented, it was surprising that the best discriminatory capabilities were achieved by applying GMM analyses. As discussed earlier, it seems that the analysis of shape better distinguish between groups defined by function, which is really intriguing. A plausible explanation is that functional analyses only quantify a restricted portion of the variation between different lifestyles, while GMM seems to grasp more of it. A complex phenomenon such as the differences in locomotion due to lifestyle that are reflected in long bone morphology probably includes many factors that are only partially accounted when biomechanical analyses are performed. This possibly happens because these kinds of analyses simply focus on more specific and constrained facets of variation, whereas GMM can incorporate more diverse sources, although with the disadvantage of not knowing what part of this variation is strictly related to function. In order to properly address this problem further investigations are necessary, ideally including a more diverse range of species and morpho-functional aspects (e.g. masticatory function and diet, locomotion and terrain, shape and biomechanical performance, etc.).

Regarding the precise issue addressed in this dissertation, which was testing the discrimination competence of different methods when comparing between agriculturalists and hunter-gatherers, is necessary to establish that further research is needed. Not only a larger sample is required, but also a more diverse one. More populations with known lifestyles should be included to test the discriminatory capability of the different methods in more complex situations. Furthermore, the analyses could be replicated on other long bones, because it has been suggested that the more distal limb elements better reflect activity or functional adaptations in primates (Stock 2006; Buck et al. 2010; Fleagle 2013). Additionally, a more varied array of methods could be included, to test whether GMM still discriminates in an enhanced way in comparison with other techniques. In this manner, it is expected to contribute in a better insight when assessing lifestyle from long bone morphology.

5.2 Conclusion

The aim of the present dissertation was to contrast beam theory, GMM and FEA by comparing their discriminatory capability when analysing the skeletal performance between agriculturalists and hunter-gatherers. Regarding this specific subject it was established that there were significant differences in the discrimination results obtained from the different analyses. It was unexpected that the best discrimination outcomes were accomplished applying GMM. Shape analyses better differentiate between groups defined by function, probably because functional analyses only quantify a limited aspect of the variation between different lifestyles. GMM seems to include more variation aspects that distinguish between groups that are not necessarily related to function or activity (e.g. phylogeny, development, genetics, etc.). This situation probably arises as result of the fact that biomechanical analyses merely concentrate on more restricted variation features; while GMM is able integrate more diverse causes, although with the limitation of ignoring their origin.

The relevance of the present dissertation is related to a better understanding when assessing lifestyle from femoral shaft morphology. The different analyses that were carried out here, allow a better perspective of how different analyses measure different properties related to a common phenomenon denominated as lifestyle. Furthermore, this dissertation opens further questions regarding the classical topic about the relationship between form and function. Although many questions with respect to this subject remain unsolved, the present dissertation contributes in the comprehension of the relationship between femoral functional morphology and lifestyle.

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Glossary

Beam Theory: Euler–Bernoulli beam theory or “classical beam theory” is a simplification of the linear theory of elasticity developed as way to calculate load-carrying and deflection characteristics of beams. It is suitable for small deflections of a beam. It is therefore a specific case of Timoshenko beam theory that accounts for shear deformation and is applicable for thicker beams.

CS: Centroid Size is the square root of the sum of squared distances of a set of landmarks from their centroid, or in other words, the square root of the sum of the variances of the landmarks about that centroid in x- and y-directions.

Coordinates: Set of parameters that localise a point in certain geometrical space. Cartesian coordinates, for example, locate a point on a plane or in physical space by projection onto perpendicular lines through the origin.

DA: Discriminant analysis is a vast class of methods concerned with the development of rules for assigning unclassified specimens to *a priori* defined groups. See discriminant function.

Discriminant function: A discriminant function is applied in order to assign a specimen to one of a set of groups. For instance, a linear discriminant function takes a vector of observations from a specimen and multiplies it by a vector of coefficients to produce a score which can be used to classify the specimen into *a priori* defined groups.

FEA: Finite element analysis is a computational system for continuum mechanics that estimates the deformation (completely detailed positional changes of all elements) that are expected to result from a specified pattern of loadings upon a mechanical system.

Form: Form variables are measurements of a geometric object that are unchanged by translations and rotations.

GMM: Geometric morphometrics are a collection of approaches for the multivariate statistical analysis of Cartesian coordinates.

Invariant: An invariant is a quantity that is unchanged when one changes some inessential aspect of a measurement.

Isotropic: Invariant with respect to direction.

Landmark: Specific point on a biological form (or image/virtual representation) of a form positioned according to a certain rule.

Least-squares: Parameter estimates that minimize the sum of squared differences between observed and predicted sample values.

Multivariate Regression: The prediction of two or more dependent variables using one independent variable.

PLS: Partial Least Squares is a multivariate statistical method for evaluating associations among usually two or more sets of variables measured on the same entities. PLS analyses the covariances between the sets of variables rather than optimizing linear combinations of variables in the various sets.

PCA: The eigenanalysis of the sample covariance/correlation matrix. It is possible to define principal components (PC's) as the set of vectors that are orthogonal both with respect to the identity matrix and the sample covariance matrix. Therefore, PC1 is the linear combination with the largest variance of all those with coefficients summing in square to 1; PC2 has the largest variance (when normalized that way) of all that are uncorrelated with PC1; and so on.

Size-and-Shape Space: Morphometric space produced as result of a Procustes fit involving only translation and rotation, but no size standardization. In this kind of analysis the Procustes coordinates are in a space known as the size-and-shape space, which should not be confused with the form space (space generated after carrying out a PCA of shape data plus a size variable vector, such as log centroid size).

Shape: The geometric properties of a configuration of coordinates, that are invariant to changes in translation, rotation, and scale.