Int. J. Morphol.,
32(2):627-633, 2014.

Femoral Functional Adaptation: A Comparison Between Hunter Gatherers and Farmers Using Geometric Morphometrics

Adaptación Funcional del Fémur: Una Comparación entre Cazadores-Recolectores y Agricultores usando Morfometría Geométrica

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PÜSCHEL, T. A. & BENÍTEZ, H. A. Femoral functional adaptation: a comparison between hunter-gatherers and farmers using geometric morphometrics. ***Int. J. Morphol., 32(2)***:627-633, 2014.

SUMMARY: The transition from hunting and gathering to agriculture has been associated with a gracilization in human form, especially in long bones. This has been interpreted as result of an increasingly sedentary lifestyle and reduced workload in the farming populations. However the majority of these evidences have been based on the application of different biomechanical techniques or the qualitative assessment of distinct morphological traits on attachment sites. Hence, this study tried to address whether is possible to distinguish between two different populations (hunter-gatherers and farmers) by quantifying their femoral morphology applying geometric morphometrics. We compared 16 male left femora belonging to two populations of Native Americans from the same geographic region, yet temporally and behaviorally distinct, in order to test if geometric morphometrics were able to differentiate these based solely on the quantitative analysis of shape. The femora were scanned and then small portions of them were segmented in order to generate comparable 3D models. Hundred twelve equidistant landmarks were collected over the whole femoral sections and a Procrustes analysis was performed in order to obtain shape variables. Several PCAs were carried out to elucidate morphometric associations and cross-validated DAs were applied to distinguish between hunter-gatherers and farmers. These procedures were sequentially repeated using different landmarks subsets in order to try to establish the anatomical locations where bone functional adaptation mostly occurs: a) femoral cortex; b) medullary cavity; c) complete femoral section. The results here presented, showed that geometric morphometrics were able to distinguish between the two distinct lifestyles. Moreover, the contrasting results obtained from the analysis of the femoral cortex and the medullary cavity, suggest that long bone remodelling caused by lifestyle differences, mostly occurs on the outer surface of the femoral shaft. This study also showed a relative gracilization of the farmer as compared to the hunter-gatherers.

KEY WORDS: Bone Functional Adaptation; Geometric Morphometrics; Hunter-Gatherers; Farmers; Principal Component Analysis; Discriminant Analysis.

INTRODUCTION

Bone is not an inanimate material but a dynamic tissue that responds to its environment in order to ensure balanced growth, development, maintenance and repair of the tissue. The idea that bone is functionally adapted to the mechanical demands that are imposed during its ontogeny has been documented for a long time. The general notion that mechanical loadings influence 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, 2008). Several evidences support 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) (Haapasalo *et al*., 2000; Robling *et al*., 2000; Shaw & Stock, 2009a, 2009b). Thereby an increased strain will stimulate deposition of new bone tissue which strengthen the bone and consequently reduces strain, while decreased strain (e.g. paralysis; inactivity) will lead to bone resorption. Additionally, experimental evidence has shown that the relationship between mechanical loading and bone structure is not as simple as initially conceived, for instance systemic factors such as age, disease, circulating hormones, diet and genetic background can modulate bone response (Pearson & Lieberman, 2004; Riggs *et al*., 2004).

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As outlined above, there are diverse well-known examples relating mechanobiology and bone functional adaptation. It has been established applying beam theory as proxy for bone mechanical properties, that different loading regimens modify, not only the shape of long bones, but also the proportion between cortical and trabecular bone (Ruff, 2008). Comparisons of these data with those for earlier human ancestors and with nonhuman primates (Ruff, 2008), have demonstrated a constant 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, 2008).

It is well established that behavioral shifts associated with changes in subsistence and technological advances influenced long-term changes in general robusticity patterns during human evolution (Ruff, 2008; Pinhasi & Stock, 2011). Several lines of bioarchaeological research have confirmed that both physical environment (i.e. terrain) and subsistence strategy contributed to modifications in long bone diaphyseal structure (Stock & Pfeiffer, 2004; Stock, 2006; Marchi *et al*., 2011; Pinhasi & Stock). The transition from hunting and gathering to farming has been associated with a gracilization in human form, especially in long bones (Ruff *et al*., 1984; Ruff, 1987; Larsen, 1999; Pearson, 2000). 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 & Stock). This has been interpreted as result of an increasingly sedentary lifestyle and reduced workload in the agricultural populations (Ruff *et al*.; Larsen, 2001).

However, the majority of these evidences have been based on the application of different biomechanical techniques (e.g. beam theory), the qualitative assessment of distinct morphological traits on attachment sites (e.g. musculoskeletal stress markers) and linear morphometric measurements. Therefore, the present study tried to address whether is possible to distinguish between two different populations (hunter-gatherers and farmers) by quantifying their femoral morphology applying Geometric Morphometrics (henceforth GMM). The femurs of these populations probably experienced different biomechanical loads due to the dissimilar lifestyles that each one of these groups had. GMM is able to quantify shape variation and how it co-varies with other factors such as biomechanics (Benítez & Püschel, 2014). Even though GMM probably describes broader aspects of variation as compared to analyses more focused on bone functional adaptation (e.g. beam theory), it is logical to wonder if these methods are able to differentiate between these two behaviorally distinct groups based solely in the quantitative analysis of shape.

MATERIAL AND METHOD

Sample. The sample comprised 16 Native American male left femora belonging to two distinctive populations a) Norris Farm (n=8): farmers and b) Black Earth (n=8): hunter-gatherers.These two populations of Native Americans included in this study are from the same geographic region, although they are temporally and behaviorally distinct. The Norris Farms site is a late Prehistoric cemetery site from the central Illinois River Valley, comprising individuals associated with a cultural tradition of village farmers (Santure *et al*., 1990). By contrast, Black Earth is site in the Carrier Mills Archaeological District in southern Illinois that dates to the Middle Archaic. They were hunter-gatherers that relied upon white tailed deer and wide variety of aquatic avifauna, while also gathering an array of seeds and nuts (Jefferies *et al*., 1982).

Method. The different methodological stages applied in this study are schematically described in Figure 1. Data acquisition, segmentation and analytical procedures are straightforwardly described in successive steps.

Segmentation: 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) in order to simplify the subsequent analyses and to test GMM methods under a complex scenario. 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 too many details of more dense materials. The segmentation process began using thresholds and the subsequent results were refined by applying a manual segmentation.

GMM Analysis: 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). Geometric morphometrics and statistical analysis were carried out in PAST v. 2.17c and MorphoJ v.1.0.5d (Hammer *et al*., 2001; Klingenberg, 2011).

A generalised Procrustes analysis was performed, removing the differences due rotation, scale and translation and thus obtaining shape variables (remaining geometric properties) (Bookstein). These shape variables were used in the subsequent analysis.

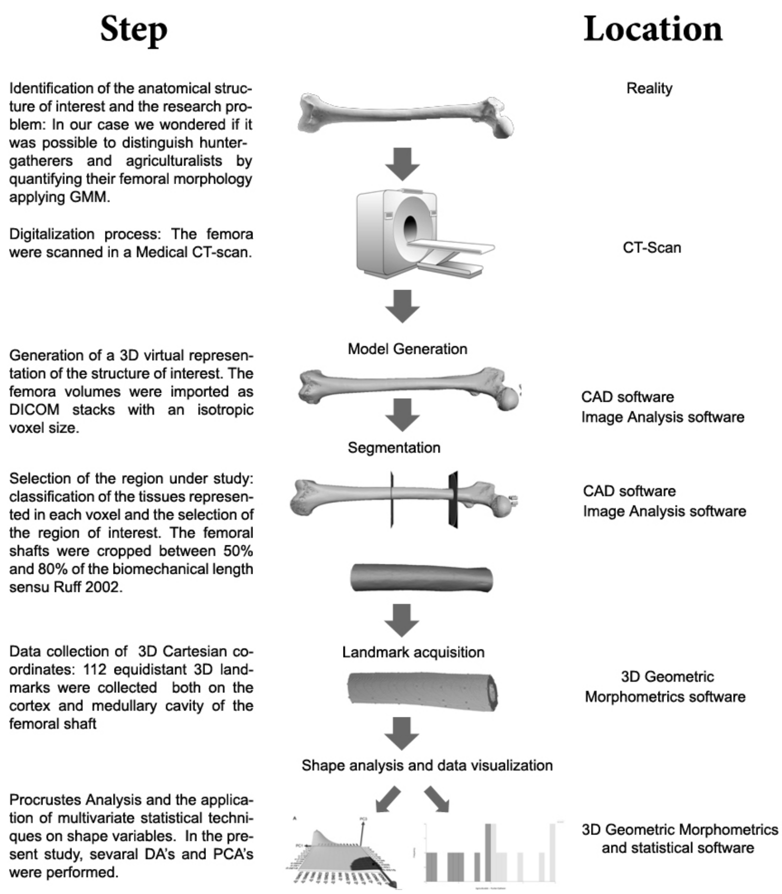

Fig. 1. Workflow applied in this study.

Several PCAs were carried out to elucidate morphometric associations. A cross-validated discriminant analysis (DA) was applied to test whether it was possible to distinguish between hunter-gatherers and farmers. In order to avoid problems due to the small sample size, permutation tests were used to assess significance. All these procedures were sequentially repeated using different landmarks subsets: a) femoral cortex landmarks; b) medullary cavity landmarks; c) complete dataset.

RESULTS

Femoral Cortex: 56 landmarks on the surface of the femoral section were used to perform a PCA (Fig. 2). There was a clear distinction between the two groups explained mainly by PC2. The cross-validated DA for the subset of landmarks showed a clear distinction between the two lifestyles, with a level of correct classification of 87.5% for farmers and 75% for hunter-gatherers (Procrustes distance: 0.03015881; p-value (1000 perm. rounds): 0.011).

Medullary cavity: 56 landmarks on the central cavity of the femoral shaft were used to perform a PCA (Fig. 3). There was a high overlap between the two groups due to the presence of farming individuals that clustered more closely with the hunter-gatherer sample. The cross-validated DA reflects this subtle separation, with a level of correct classification of only 62.5% for farmers and 50% for hunter-gatherers and without significant differences between their multivariate means (Procrustes distance: 0.01942070; p-value (1000 perm. rounds): 0.09).

Complete dataset: 112 landmarks comprising both the femoral cortex and the marrow cavity coordinates were used to perform a PCA (Fig. 4). There was a clear distinction between the

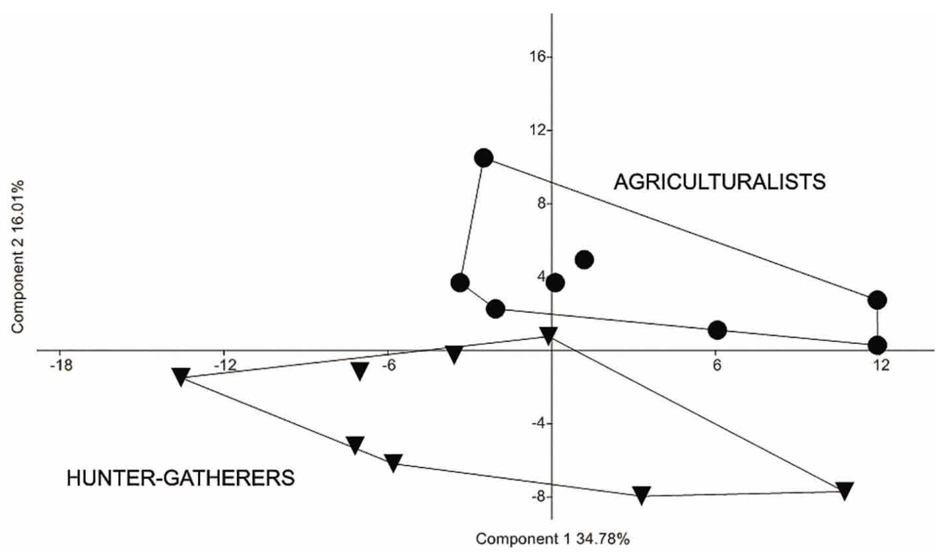

Fig. 2. PCA plot of the first two principal components of the femoral cortex landmarks. Dots represent farming individuals, while the inverted triangles are hunter-gatherers.

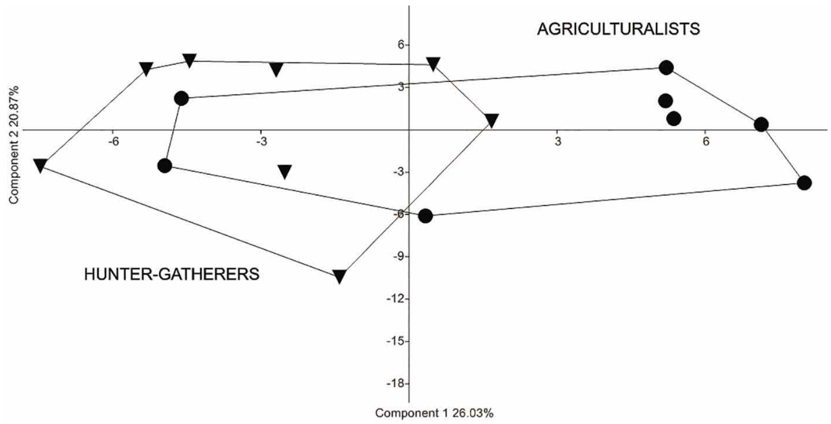

Fig. 3. PCA plot of the first two principal components of the medullary cavity landmarks. Dots represent farming individuals, while the inverted triangles are hunter-gatherers.

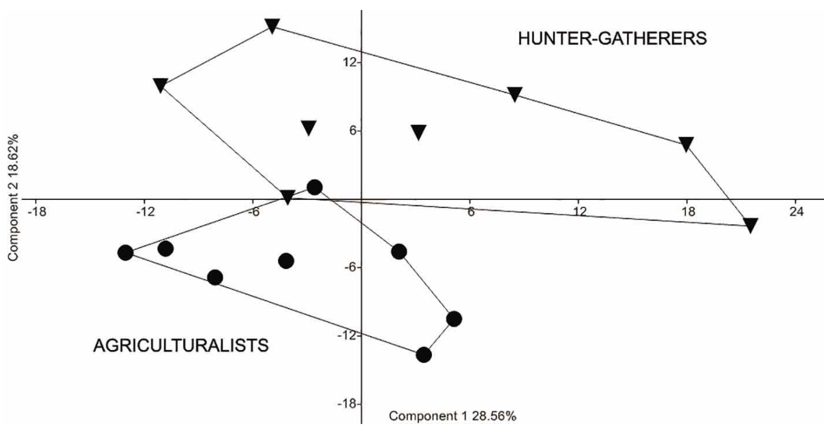

Fig. 4. PCA plot of the first two principal components of the complete dataset. Dots represent farming individuals, while the inverted triangles are hunter-gatherers.

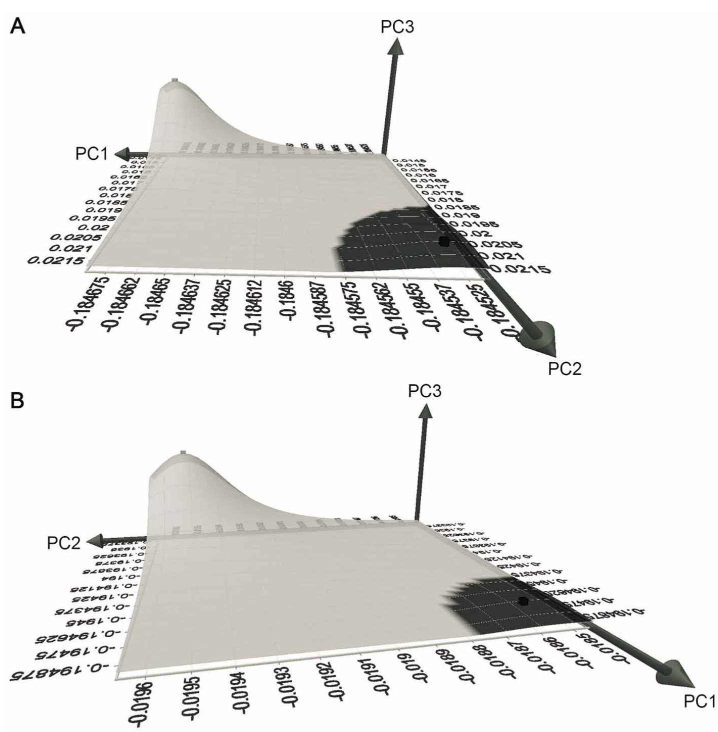

Fig. 5. PCA graph of the average shape of both a) the femoral cortex, and b) the medullary cavity. The three axes represent ca. 60.8% and 58.9% of the total shape variation, respectively. The point in the darker area represents the average shape of the farmers sample, while the other one on the clearer area represents the average shape of the hunter-gatherer sample. The whole volume simulates the morphospace of variation.

dots representing the two lifestyles. The cross-validated DA presented a noticeable distinction between the two groups, with a level of correct classification of 75% for farmers and 75% for hunter-gatherers, with highly significant differences between their multivariate means (Procrustes distance: 0.03215611; p-value (1000 perm. rounds): 0.003).

The PCA of femoral cortex and the medullary cavity shape variation showed that the first three PCs accounted for 60.8% (PC1 = 35.83%; PC2 = 14.766 %; PC3 = 9.482 %) (Fig. 5a) and 58.9% (PC1 = 27.051%; PC2 = 19.493%; PC3 = 12.360%) (Fig. 5b) of the total shape variation, therefore providing a reasonable approximation of the total femoral shape variation.

DISCUSSION

Any behavioural reconstruction based on skeletal morphology relies on the notion that bone is functionally adapted to its mechanical environment intra vitam (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 behaviors that produce the loadings experienced by a bone. Hence the present study contributes to elucidate if GMM methods are suitable to study bone functional adaption not from a biomechanical perspective, but by quantifying shape differences.

The results presented support the fact that GMM methods are suitable to distinguish different lifestyles by quantifying morphology, in a similar fashion as other traditionally applied techniques (e.g. beam theory, musculoskeletal stress markers). Although, further inquiries are required in order to compare the accuracy of these different methods when comparing distinct lifestyles based on skeletal morphology. The fact that GMM is able to distinguish between lifestyles is interesting, because it implies that a shape analysis can differentiate functional differences, thus allowing the study of the relationship between form and function applying GMM techniques. Probably GMM captures broader aspects of variation, nevertheless 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 seems to encompass different aspects of morphological variation, including many that are related to function, while other specific functional analyses only compare more restricted aspects. However, it is evident that GMM clearly separates between groups, so it could be a useful tool when assessing lifestyle from morphology.

Bioarchaeological research has shown that there is an association between major transitions in subsistence and long-term changes in general robusticity patterns during human evolution (Stock & Pfeiffer; Stock; Marchi *et al*.; Pinhasi & Stock). Nowadays, it is relatively clear 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 gracilization of human long bones (Ruff *et al*.; Ruff, 1987; Larsen, 1999; Pearson). The results presented in this study follow grosso modo the same trend, showing a relative gracilization of the farmers sample with respect to the hunter-gatherers. GMM analyses were able to reflect this tendency, with the hunter-gatherers showing more robust femoral morphologies. On the other hand, farmers have thinner cortical layers, expanded medullary cavities and external cortices with less marked signs of muscle attachments (e.g. a smoother linea aspera).

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 on the outer surface of the shaft. The results from the PCAs and DAs 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 farmers when using the medullary cavity, while the femoral cortex landmarks allowed an almost complete separation between the groups. This result probably reflects the underlying process of bone remodelling induced by mechanical strains. If only the femoral cortex allows a clear distinction between the two populations, this possibly means that the remodelling process due to mechanical forces occurs mostly on the femoral surface. On the other hand, the fact that the medullary cavity does not separate the two groups, could reflect that the medullar surface morphology is more influenced by factors other than mechanical pressures. Further research is required to elucidate this interesting finding.

ACKNOWLEDGMENTS

I give great gratitude to Prof. Paul O'Higgins, who provided me useful comments and encouragement words. I am also grateful with Dr. Colin Shaw, who generously provided the sample used here. This work has been partially funded by Becas Chile, Conicyt-PCHA/2012/73130010.

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RESUMEN: La transición desde la caza y la recolección a la agricultura ha sido asociada con una gracilización en la forma humana, especialmente en los huesos largos. Esto ha sido interpretado como resultado de un estilo de vida más sedentario y con menor carga de trabajo en las poblaciones agricultoras. Sin embargo, la mayoría de estas evidencias se han basado en técnicas biomecánicas o en la evaluación cualitativa de rasgos morfológicos en los sitios de inserción muscular. Este estudio intentó distinguir entre dos poblaciones diferentes (cazadores-recolectores y agricultores) mediante la cuantificación de su morfología femoral aplicando morfometría geométrica. Dieciséis fémures masculinos pertenecientes a dos poblaciones nativo americanas de la misma región geográfica, aunque temporal y conductualmente diferentes, fueron comparadas con la finalidad de probar si la morfometría geométrica era capaz de distinguirlas. Los fémures fueron escaneados y pequeñas porciones fueron segmentadas para generar modelos 3D. Se colectaron 112 hitos equidistantes sobre toda la superficie femoral y luego se realizó un análisis de Procusto para obtener variables de la forma. Diversos análisis de componentes principales se llevaron a cabo para establecer asociaciones morfométricas, así como también análisis discriminantes con validación cruzada para distinguir entre cazadores-recolectores y agricultores. Estos procedimientos fueron repetidos utilizando diferentes subconjuntos de hitos para establecer donde ocurre mayoritariamente la adaptación funcional del hueso: a) corteza femoral; b) cavidad medular; c) sección femoral completa. Los resultados aquí presentados mostraron que la morfometría geométrica permite distinguir entre ambos modos de vida. Además, los contrastantes resultados obtenidos del análisis de la corteza femoral y cavidad medular, sugieren que el remodelamiento óseo debido a diferentes estilos de vida ocurren mayoritariamente en la superficie de la diáfisis del fémur. Este estudio también mostró una relativa gracilización de la población agricultora al ser comparada con los cazadores-recolectores.

PALABRAS CLAVE: Adaptación hcional ósea; Morfometría geométrica; Cazadores-Recolectores; Agricultores; Análisis de componentes principales; Análisis discriminante.

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Received: 05-02-2014
Accepted: 31-03-2014