Discriminating metapodials in female cattle using 3D geometric morphometrics: a preliminary study

Nőstény szarvasmarhák láközépcsontjainak vizsgálata 3D-s morfometrikus eljárással: előzetes tanulmány

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Kulcsszavak: szarvasmarha, fajta, nem, kor, mértani morfometrika, állatrégészet, lábközépcsont

Abstract

The identification of cattle breeds without a modern reference collection is a challenging task when conducting zooarchaeological studies. This preliminary study was carried out on modern Hungarian Grey and Charolais cattle to establish a methodology for a larger study and to investigate shape and size differences of metacarpal and metatarsal bones between breeds. Female specimens of varied ages were examined to investigate the effects of allometric and age variations and of breed using metapodial size and shape. The results show that morphological differences between the studied cattle breeds were not a result of allometric and age variations, but instead are likely to be breed-related. This study provides the basis for applying geometric morphometrics to zooarchaeological specimens.

Összefoglaló

Állatrégészeti kutatások során modern referenciagyűjtemény hiányában a szarvasmarhafajták azonosítása nehéz feladat. Ezt az előzetes kutatást mai szürke magyar és charolais marhákon végeztük el a célból, hogy megvizsgáljuk a fajták közötti méret- és alakkülönbségeket a mellső és hátsó lábközépcsontokat illetően. Különböző korú nőstény egyedek vizsgálata történt meg az allometrikus és kor szerinti változásokat fajtánként kutatva, a lábközépcsontok alakja és mérete alapján. Az eredmények azt mutatják, hogy a vizsgált fajták közötti morfológiai különbségeket nem allometrikus vagy kor szerinti variációk okozzák, hanem a tenyésztési különbségek. A tanulmány kiindulási alapot jelent az állatrégészeti egyedek mértani morfometrikus elemzéséhez.

Introduction

This paper presents the preliminary results of an ongoing study carried out on modern cattle reference collections using geometric morphometrics. The purpose of this study was firstly, to develop the methodology for the larger study, which will incorporate a greater sample size. Secondly, to investigate size and shape variations of cattle metacarpal and metatarsal bones between the Hungarian Grey and Charolais breeds. Thirdly, to examine the effects of allometry and age on metapodial size and shape variations within and between each breed group. Allometry is a biological factor that occurs as proportional changes in shape that are directly associated with changes in size as a response to growth, developmental or physical activities⁴. This size-shape association can be strong or weak, with the former having the greatest effect on the overall shape. There are two agerelated patterns that must be considered. First, young animals may produce asymmetrical patterns in bone shape during their ontogenetic growth. Second, as mature animals grow older the live weight may become heavier, affecting the bones, which in turn may undergo some degree of structural shape change. As a result, metapodials may demonstrate broadening or asymmetry, or both⁵. In both cases this allometric component is closely associated with age.

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⁴Morey 1994, 347, Levine 1982, 242, Rohlf – Slice 1990

The importance of this study using modern reference collections lies in the establishment of a baseline against which archaeological specimens can be compared, as part of an ongoing wider study examining changes in cattle husbandry over time.

Several methods can be used to determine cattle type from skeletal remains. The estimated withers height, or stature of livestock, can be established by measuring the long bones using the method developed by Matolcsi⁶. Pronounced differences in heights can then indicate different types of cattle. Distinctions can also be made by measuring the size and shape of horn cores, with large differences indicating different types of cattle⁷. Despite the widespread use of these methods there are some limitations. Differences in size and shape can be due to such factors as sex, age, castration, breed, nutrition, functional morphology, asymmetry as well as individual variation. The effects of these factors on cattle teeth, metapodials and horn cores are widely discussed in published zooarchaeological literature. For example, when determining the age of cattle using teeth, Andrews⁸ highlighted that first incisors not only erupt earlier in males than in females but also earlier in some breeds than in others. The author suggested that investigation of sex, age and breed variations between modern cattle should be carried out prior to the use of age determination techniques on archaeological specimens. Albarella9 raised the importance of using modern cattle of known age, sex and breed in order to understand the effects of these variables on the shape of metapodials. He observed that differences between the breeds were greater than differences between the sexes. Also, metatarsals were more useful as indicators of breed, while metacarpals were more useful as indicators of sex. Molecular analysis has also been employed for the identification of modern and ancient cattle breeds and this has greatly improved the overall understanding of cattle genotypes¹⁰. Unfortunately, this analysis remains prohibitively expensive for routine zooarchaeological research. Bartosiewicz¹¹ found that asymmetry or broadening, or both, in trochleas of the distal epiphyses of metapodials increases with the size and age of cattle.

Materials and methods

Bones of female Hungarian Grey and Charolais cattle were used in this study. The Hungarian Grey cattle came from the reference collection of the Hungarian Museum of Agriculture in Budapest. This collection was developed from just over 70 individuals, mostly slaughtered in 1963¹². The bones of the Charolais breed came from the Bólacht Gaelach reference collection in Ireland, which has been developed by the first author of this paper from approximately 25 individuals, mostly slaughtered in 2012. The Hungarian Grey cattle were reared for both draught and beef, whereas the Charolais specimens were reared primarily for beef production.

Some limitations were found within each reference collection. The age of the Hungarian Greys ranged between 48 and 144 months while specimens of the Charolais breed were represented by much younger animals aged between 18 and 39 months. This is due to modern animal welfare and food safety regulations, which mean that beef cattle should be slaughtered by c. 3 years old. Within the Charolais group there was an age gap between 29 and 39 months meaning that no specimens between these dates were available for the analysis.

Table 1. Sample details of Charolais and Hungarian Grey metacarpal bones

	Age	Females	
	Range	Average	
Hungarian Grey	48-144 months	87 months	16
Charolais	18-39 months	26 months	15
Total			31

Table 2. Sample details of Charolais and Hungarian Grey metatarsal bones

	Age	Females	
	Range	Average	
Hungarian Grey	48-144 months	89 months	15
Charolais	18-39 months	26 months	15
Total			30

This pilot study included a total of 31 metacarpals and 30 metatarsals that came from the same individuals (tables 1–2). All Charolais metacarpals were fused whereas metatarsals were represented by fused and just-fused bones, indicating that the fusion of the latter element occurs slightly later than those of the metacarpals. The average age of the Charolais cattle was 2 years and 2 months for both metacarpal and metatarsal bones. Specimens of Hungarian Grey were represented by a wider age range, with the average age for metacarpals being 7 years 2 months and for metatarsals being 7 years 4 months. These factors make the examination of the effect of age on size and shape of the metapodials somewhat problematic, however these will be further investigated in the completed study, where a more satisfactory age structure will be available.

All bones were scanned using the 3D HD Next Engine surface laser scanner. Once scanned, a threedimensional model of each bone was created on com-

⁵ Bartosiewicz et al. 1997a, 62

⁶ Matolcsi 1970

⁷ Armitage 1989, Luff 1994, Sykes – Simmons 2007

⁸ Andrews 1982

⁹ Albarella 1997

¹⁰ Edwards et al. 2003

¹¹ Bartosiewicz et al. 1993, 1997a, 43

puter. The shape of the distal ends of the metacarpal and metatarsal bones was described by 15 landmarks, or anatomically-defined points (fig. 1; table 3). The landmarks were selected on the principle that they all can be found in anatomically-defined locations in both modern and zooarchaeological specimens. The landmarks used in this study were based on those established by Bignon et al.¹³ in their study of horse metapodials.

Each landmark has Cartesian x, y and z coordinates. The geometrical size and shape of the distal epiphyses were extracted from these coordinates



Figure 1. Landmarks used in this study based on right metatarsal bone

Table 3. Landmark definition based on anatomical orientation of metacarpals

Landmark	Definition
0	Axial end of the medial condyle
1	Trochlea ossis metacarpalis iii
2	Medial condylar ridge
3	Trochlea ossis metacarpalis iii
4	Abaxial end of the medial condyle
5	Abaxial end of the lateral condyle
6	Trochlea ossis metacarpalis iv
7	Lateral condylar ridge
8	Trochlea ossis metacarpalis iv
9	Axial end of the lateral condyle
10	Axial aspect of the physis of the lateral condyle
11	Proximal aspect of the lateral condyle
12	Incisura intertrochlearis middle point
13	Proximal aspect of the medial condyle
14	Axial aspect of the physis of the medial condyle

using Landmark software¹⁴. The shape of the metapodials was described using bones from the right side, with all left-sided bones being mirrored using Generalised Procrustes Analysis.

There has been widespread use of geometric morphometrics for studies investigating size and shape variations in biological forms¹⁵. In comparison to traditional morphometrics, geometric morphometrics captures the form of the studied bone using Cartesian x, y, z coordinates instead of linear measurements. This allows adequate investigation of size and shape variations to be carried out. The methodology of geometric morphometrics is widely described in specialised literature¹⁶. In brief, Generalised Procrustes Analysis (GPA) consists of standardising the configurations of landmarks by superimposing or aligning them. This is done by scaling all shapes to the same size, translating them into the same location and then rotating them around the origin until the sum of squared distances between the corresponding points is minimised¹⁷. The aim of GPA is to ensure that the differences in shape are minimised. This procedure allows separation of shape from the overall size, with shape being represented by the Procrustes coordinates and size as a centroid size or its logarithm. Subsequently, both size and shape can be analysed independently from each other. Although shape is size-free it is not allometry-free and this biological factor should be investigated along with other types of variation.

A series of complementary analyses routinely used in geometric morphometrics were performed using MorphoJ¹⁸, Excel and SPSS to investigate size and shape variations. Configurations with raw coordinates of all 15 landmarks were aligned using Generalised Procrustes Analysis (GPA). The Procrustes coordinates and log centroid size were then used in univariate and multivariate analyses. Principal Component Analysis (PCA) was conducted using the covariance matrix of the Procrustes aligned coordinates to investigate shape variation. Univariate Regression Analysis was used to test the association of allometric and age components with the first two principal components. Multivariate Regression Analysis with a permutation test of 10,000 runs was used to test the significance of allometric and age components over metapodial size and shape within and between each group. Allometry and age-corrections were performed using Multivariate Regression Analysis pooled within-breed groups. Differences between size means of metapodials of the different groups were examined using the ANOVA test. The MANCOVA test was used to examine shape differences between the groups while controlling for the effect of allometry.

¹² Bartosiewicz 1997b

¹³ Bignon et al. 2005

¹⁴ http://www.idav.ucdavis.edu/research/EvoMorph

¹⁵ e.g. Bignon et al. 2005, Cucchi et al. 2011, Evin et al. 2013, Owen et al. 2014, Seetah et al. 2014

¹⁶ Bookstein 1991, 1996, Rohlf and Marcus 1993, Dryden and Mardia 1998, O'Higgins and Jones 1998, Klingenberg 2002, 2011, Rolhf 2000, Monteiro et al. 2000, Adams et al. 2004, Zelditch et al. 2004

¹⁷ Bookstein 1991

¹⁸ Klingenberg 2011, http://www.flywings.org.uk/MorphoJ_page.htm

Table 4. Hungarian Grey (HG) and Charolais (CH)
metacarpals with the values of log centroid size, age and
scores of the first two principal components

Id	Log	Age		DC1	DCO
	Centroid Size	months	fusion	PCI	PC2
HG_F_1	4.4122708	60	fused	-0.009108	0.018025
HG_F_2	4.4477267	120	fused	-0.053031	0.004963
HG_F_3	4.4103762	84	fused	-0.008281	-0.025570
HG_F_4	4.4027495	120	fused	-0.040298	0.009389
HG_F_5	4.3894809	144	fused	-0.037539	-0.030522
HG_F_6	4.4545562	42	fused	-0.013521	0.003682
HG_F_7	4.3609789	108	fused	-0.036538	-0.004175
HG_F_8	4.4582192	60	fused	0.005156	0.020659
HG_F_9	4.3711491	72	fused	-0.004290	-0.008908
HG_F_10	4.3535208	60	fused	-0.047815	-0.001351
HG_F_11	4.3572245	120	fused	-0.037250	-0.000003
HG_F_12	4.4254757	108	fused	-0.024483	-0.010479
HG_F_13	4.3485435	60	fused	-0.012765	0.026749
HG_F_14	4.4610359	132	fused	0.005689	0.011530
HG_F_15	4.3994548	48	fused	-0.010023	-0.035532
HG_F_16	4.4427291	48	fused	-0.018376	0.018702
CH_F_1	4.4848250	23	fused	0.023498	-0.010166
CH_F_2	4.5331614	26	fused	0.027283	0.020688
CH_F_3	4.5921835	27	fused	0.020039	0.004341
CH_F_4	4.5616435	29	fused	0.033367	-0.026702
CH_F_5	4.4762472	20	fused	0.049829	-0.019453
CH_F_6	4.4442859	25	fused	0.017134	-0.014961
CH_F_7	4.5668983	39	fused	0.014315	0.015651
CH_F_8	4.4443707	24	fused	0.013609	-0.004831
CH_F_9	4.5372016	26	fused	0.030657	0.009763
CH_F_10	4.5883118	27	fused	0.007240	0.019315
CH_F_11	4.5724295	29	fused	0.025763	0.006716
CH_F_12	4.5623852	18	fused	0.042083	0.016028
CH_F_13	4.4545501	25	fused	0.026230	-0.010033
CH_F_14	4.4393946	24	fused	0.017826	-0.014751
CH_F_15	4.5713008	39	fused	-0.006400	0.011237

Results

The data for log centroid size, age, state of fusion and scores of the first two principal components per individual for each breed group can be seen in tables 4 and 5.

Size differences between the Charolais and Hungarian Grey sample groups were highly significant both in metacarpal (p < 0.0000036) and metatarsal (p < 0.0000012) bones, with the average size of Charolais metapodials found to be larger than those of the Hungarian Greys.

A total of 38 eigenvalues, or shape variables, were extracted representing 38 variances in the directions of the respective principal components. For metacarpal bones the first principal component accounted for 37% of the total variation, followed by the second principal component with 13%, the third prinTable 5. Hungarian Grey (HG) and Charolais (CH) metatarsals with the values of log centroid size, age and scores of the first two principal components

Id	Log	Age			
	Centroid Size	months	fusion	PC1	PC2
HG_F_1	4.348603	60	fused	-0.003232	0.014515
HG_F_2	4.394935	120	fused	-0.041137	-0.003049
HG_F_3	4.323118	84	fused	0.000575	0.002577
HG_F_4	4.380481	120	fused	-0.027449	0.038003
HG_F_5	4.335988	144	fused	-0.044174	-0.010728
HG_F_6	4.383463	42	fused	-0.013151	0.024656
HG_F_7	4.360949	108	fused	-0.011923	-0.000340
HG_F_8	4.412879	60	fused	-0.018893	-0.000766
HG_F_9	4.324733	72	fused	-0.019580	-0.012393
HG_F_10	4.271214	60	fused	-0.028758	0.001791
HG_F_11	4.389572	120	fused	-0.000036	-0.010875
HG_F_12	4.350473	108	fused	-0.001690	0.038035
HG_F_13	4.319767	60	fused	0.009349	-0.016214
HG_F_14	4.371400	132	fused	-0.058396	0.001516
HG_F_15	4.340790	48	fused	-0.024263	0.001953
CH_F_1	4.452755	23	fused	0.012532	0.000562
CH_F_2	4.389773	26	fused	0.039133	0.037693
CH_F_3	4.549767	27	fused	0.000303	-0.006384
CH_F_4	4.532734	29	fused	-0.001482	-0.018353
CH_F_5	4.471099	20	just- fused	0.025747	-0.048310
CH_F_6	4.414785	25	fused	0.012350	-0.021222
CH_F_7	4.547116	39	fused	0.024385	0.017561
CH_F_8	4.422579	24	fused	0.022888	-0.023107
CH_F_9	4.392825	26	fused	0.040318	0.030093
CH_F_10	4.534649	27	fused	0.007203	-0.007181
CH_F_11	4.525410	29	fused	0.001856	-0.012400
CH_F_12	4.497706	18	just- fused	0.043389	0.003412
CH_F_13	4.410198	25	fused	0.013968	-0.023910
CH_F_14	4.423518	24	fused	0.017851	-0.008468
CH_F_15	4.540994	39	fused	0.022317	0.011334

cipal component with 10% and decreasing thereafter. For metatarsal bones the first principal component accounted for almost 33% of the total variation, the second principal component for 20%, the third principal component for 8% and decreasing thereafter. From a total of 38 principal components, the first 13 accounted for 95% of the total variance in both metapodials, which is a very good approximation of the total shape variation of the Hungarian Grey and Charolais sample groups.

The shape variation of metapodials between the two groups can be observed by examining the first two principal components, PC1 and PC2 (figs 2–3). For both metacarpals and metatarsals, the majority of the Hungarian Greys clustered along the negative axis of PC1, whereas most of the Charolais specimens clustered along its positive axis.



Figure 2. Scatter plot of the scores for the first two principal components of metacarpal bones for Hungarian Grey (in circles) and Charolais (in squares) groups



Figure 3. Scatter plot of the scores for the first two principal components of metatarsal bones for Hungarian Grey (in circles) and Charolais (in squares) groups



Figure 4. Shape changes associated with the first two principal components of metacarpal bones for Hungarian Grey and Charolais groups (the overall mean shape with open circles and the shape change associated with the respective principal component with filled circles)



Figure 5. Shape changes associated with the first two principal components of metatarsal bones for Hungarian Grey and Charolais groups (the overall mean shape with open circles and the shape change associated with the respective principal component with filled circles)

In both types of metapodials the average shape of the distal epiphysis is shorter and broader in the Hungarian Grey, having a wider breadth at the articular crests, broader trochleas and a shallower incisura intertrochlearis than in the Charolais group (figs 4–5). Slight asymmetry was detected within the Charolais group. This may be due to the ontogenetic development of bone in animals of young age so that the Charolais are more affected whereas no effect is seen in the older Hungarian Grey group.

Regression Analysis shows that allometric adjustments within the Charolais group were significant in metacarpals (p < 0.050) explaining 12% of the shape variation (fig. 6) and insignificant in metatarsals (p = 0.223) accounting for 9%.

In metacarpals this allometric vector was highly correlated with the second principal component ($R^2 = 0.393$, p < 0.012) (fig. 7). No correlation was found between PC1 and log centroid size in metacarpals ($R^2 = 0.017$, p = 0.640) or in metatarsals ($R^2 = 0.042$, p = 0.462). Similarly no correlation was detected between PC2 and log centroid size in metatarsals ($R^2 = 0.007$, p = 0.765) for the Charolais group.



Figure 6. Metacarpal shape against log centroid size for the Charolais group



Figure 7. PC2 against log centroid size for the Charolais group metacarpals

Within the Hungarian Grey group Regression Analysis showed that allometric adjustments in shape variation were insignificant in both metacarpals (p =0.100) and metatarsals (p = 0.626) explaining 10% and 5% of the total shape variation respectively. There was no correlation between either of the first two principal components and log centroid size for metacarpals or for metatarsals. The same pattern of allometric trajectory was found for both Charolais and Hungarian Grey groups after analysis of covariance for metacarpals (p = 0.760) and for metatarsals (p =0.156). This indicated that shape differences found in metapodials between the two groups were not the result of allometry but instead, may be due to such factors as breed, age or geography.

Firstly examining size, the effect of age on metapodial size within the Charolais group was insignificant in both metacarpals (p = 0.897) accounting for 21% of the total variation and in metatarsals (p = 0.055) accounting for 25%. Within the Hungarian Grey group, age also did not influence the size of either metacarpals (p = 0.886) or metatarsals (p = 0.325), explaining only 0.155% and 7% of the total variation respectively. This means that age has no statistically significant effect on metapodial size in the studied groups.

Moving on to shape variation, the effect of age on metapodial shape within the Charolais group was sig-

nificant in metacarpals (p < 0.015) accounting for 14% of the total shape variation (fig. 8) and insignificant in metatarsals (p = 0.317) accounting for only 7%.



Figure 8. Metacarpal shape against age for the Charolais group. Graphic visualisation of the metacarpal shape changes with age in Charolais over 18 months (the overall mean shape with open circles and the shape change associated with the respective principal component with filled circles)

PC1 was highly correlated with the age of Charolais cattle for metacarpals (R^2 = 0.462; p < 0.005) (fig. 9) but not for metatarsals (R^2 = 0.042, p = 0.462). Graphic representation of metacarpal shape changes with age between 18 and 39 months showed that most changes were associated with vertical stretching of the dorsomedial part and lowering of the ventrolateral aspect of the epiphysis, narrowing of the articular crest of the medial trochlea and with the incisura intertrochlearis becoming shallower.



Figure 9. PC1 against age for the Charolais metacarpals

Within the Hungarian Grey group, age did not significantly influence the shape of either metacarpals (p = 0.328) or metatarsals (p = 0.381) explaining only 7% of the total variation in each. For the Hungarian Grey group no correlation was found between either of the first two principal components and age for metacarpals or for metatarsals. This means that age has no significant influence over metapodial shape in the Hungarian Grey sample group.

Allometry and age-corrected data for the metacarpals and allometry-corrected data for the meta-

tarsals have improved breed separation. Using Principal Component Analysis conducted on the residuals obtained from the Regression Analysis for both metacarpals and metatarsals, all the Hungarian Grey specimens clustered along the negative axis of PC1 and all the Charolais specimens clustered along the positive axis (figs 10-11). For metacarpals, the first principal component accounted for 34% of the total variation, followed by the second principal component with almost 13%, the third principal component with 11% and decreasing thereafter. For metatarsals the first principal component accounted for 42% of the total variation, the second principal component for almost 18% and the third principal component for 7%. This demonstrates that both allometric and age variations were preventing accurate discrimination between the studied groups.



Figure 10. Allometry and age-free scores for the first two principal components of metacarpal bones for Hungarian Grey (in circles) and Charolais (in squares) groups



Figure 11. Allometry-free scores for the first two principal components of metatarsal bones for Hungarian Grey (in circles) and Charolais (in squares) groups

Discussion

The results show that for female cattle the Hungarian Grey and Charolais sample groups can be separated on the basis of shape using Principal Component Analysis, and that this separation occurs in the first principal component. When visualised, these differences in shape are clearly seen, with the distal end of the Hungarian Grey metapodials being shorter and broader than those of the Charolais. The size of the distal epiphyses of cattle metapodials was also found to be significantly different between the Hungarian Grey and Charolais samples, with latter being larger, despite the Charolais breed being represented by younger specimens than those of the Hungarian Grey.

Allometry and age-related shape changes were found to be associated with the Charolais group only, which was not surprising since this group was represented by younger specimens, thus ontogenetic development of the bones was recognised. These allometric and age components were preventing accurate differentiation between the two groups. The sample size for both breed groups was somewhat problematic but the outcome that age had very little influence on the overall shape is evident. This is in agreement with Bartosiewicz¹⁹ study where no significant asymmetry was found in mature Hungarian Grey female cattle. Overall, shape variation caused by allometric and age effects did not show breed-specific patterns. In fact, both components, except for creating a little overlap between the groups, contributed very little to the overall morphological variability.

Both cattle breeds are reared for beef production, however, traditionally Hungarian Grey were also bred for traction purposes, gaining an excellent reputation as draught cattle²⁰. The size and shape dissimilarities can be due to differences between the two phenotypes, geographic differences or a combination of these two factors. The phenotypic differences reflect selective pressures imposed on breeds by humans over many generations. This selective breeding has resulted in animals with certain traits and therefore the shape of the bones reflects these economic and social choices. Charolais are characterised as heavy, early-maturing and fast-growing cattle, valued for good muscling, wide back and loin. Their legs are strong in order to withstand heavy body weight from the early stages of their lives. They are reared predominantly for beef production, optimised to yield as much carcass meat as possible. This breed is valued for its great economic importance²¹. By contrast, Hungarian Greys are also described as heavy, but are late-maturing cattle, having long legs, a narrow back and loin, and poor muscling²². Differences may also

¹⁹ Bartosiewicz et al. 1993, 71

²⁰ Bodó et al. 2004

²¹ Mandell et al. 1997, Zahrádková et al. 2010

²² Bodó et al. 2004

be due to variation in terrain and environmental conditions that play a role in the development of the bone morphology of each breed group. Finally, it may be a combination of the two, so that phenotypic differences may have occurred because of the differences in both animal management and the geographical regions in which cattle have been herded. Ireland is known for its extensive grazing lands and it is common to keep herds outdoors all year round including during the mild, but wet winter months. High rainfall leads to the grazing grounds being wet throughout the year, making the terrain damp and even waterlogged in places. In Hungary, cattle are kept indoors most of the time but when kept outdoors the grazing grounds are drier because rain is less frequent than in Ireland²³. As a result, different geographical regions that have different climatic conditions determine the cattle management strategies employed so creating a variety of cattle phenotypes.

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Conclusion

Overall, this study has demonstrated the feasibility of the current methodology and its ability to discriminate cattle breeds using metapodials. Size and shape variations can be used to discriminate between the Hungarian Grey and Charolais breed groups for both metacarpal and metatarsal bones, although the effects of allometry and age on the bone shape did prevent complete differentiation between the studied breeds. While preliminary, the results obtained from the modern reference collections provide a positive background for zooarchaeological studies investigating cattle populations. However, a further study needs to be carried out incorporating more than two breeds in order to better understand the patterns of size and shape variation between breeds. Sexual dimorphism must also be fully examined in the completed study, which should incorporate a larger sample size with varied age ranges and balanced sex ratios. This will hopefully determine the effects of sexual dimorphism, age-related factors and allometric scaling on accurate breed discrimination.

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