SEPARATING CAPRINE (*CAPRA/OVIS*) DISTAL TIBIAE: A CASE STUDY FROM THE POLISH NEOLITHIC

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“The tibia is the most difficult of all the limb bones to distinguish” (Boessneck et al., 1964, 99, translated)

Abstract

Distinguishing the skeletal remains of sheep (*Ovis aries*) from goats (*Capra hircus*) is a fundamental and habitual problem in zooarchaeology. Such a distinction is desirable because the taxa can be raised to serve variable purposes, are behaviourally different, and are biologically dissimilar; all factors influencing their role in past human economies. Whilst considerable effort has been spent exploring various methods for their distinction in the Near East, the Mediterranean world, and in modern populations, the rarity of Neolithic caprines north of the Alps has meant distinction has not been attempted on any systematic basis. In this study, we present the first attempt to do so, using one of the more problematic elements, the distal tibia, to investigate the caprine remains from Racot 18, Poland, a settlement of the Neolithic Late Lengyel culture. The assemblage is characterised by an unusual proportion of caprines, and ZooMS results indicate relative parity in numbers of sheep and goats. Standard and new biometrics and morphological identifications were also applied. Morphological identifications did not reliably distinguish the taxa. Nonetheless, both standard and new biometrics demonstrate significant size differences between the taxa in this assemblage. This study represents the first step required of establishing effective criteria for differentiation of caprines in Neolithic Poland, and by extension other Neolithic contexts north of the Alps.

Introduction

As Neolithic agriculture spread from its centre of independent origins in the Near East, its pathway bifurcated in the eastern Mediterranean, with one expansion north through Eastern Europe, and another westward along the European shores of the Mediterranean (McClure, 2015; Rowley-Conwy, 2011). The westward expansion maintained the pattern of predominantly caprine husbandry seen in the eastern Mediterranean, whilst the northward expansion gave rise to an increasing frequency of cattle (Manning et al. 2013), probably as an adaptation to continental life north of the Alps (McClure, 2015; Ethier et al., 2017). Despite this adaptive change to temperate ecosystems (Ivanova et al. 2018) in favor of cattle (Manning et al., 2013), caprines (*Ovis* sp./*Capra* sp.) served some purpose in Neolithic economies north of the Alps, attested to by their near ubiquitous, albeit much smaller proportional representation in faunal assemblages. What were the caprines for? What purpose did they serve? Why were they maintained despite a clear preference for domestic cattle husbandry?

Answering these fundamental questions necessitates reliable differentiation of sheep (*Ovis aries*) and goats (*Capra hircus*), but also large enough numbers of samples which provide more than just anecdotal evidence. The fundamental problem with understanding northern European caprine husbandry is that only small numbers of the taxa are present at most sites. It is therefore imperative that targeted analyses of the exceptions to this pattern be undertaken to provide a frame of reference, and methodological basis for understanding broad-scale patterns of use.

In this paper, we present biometric, morphology-based and zooarchaeology by mass spectroscopy (ZooMS) analyses of caprine tibiae from the Neolithic of the Polish lowlands, attributable to the Late Lengyel Culture of the latter half of the fifth millennium BC. The faunal assemblage recovered at the site is sizeable, and contains a large number of caprine remains. In order to test the applicability of metric and morphometric means of caprine differentiation established for other populations, to further explore the role of caprines at the site and by extension in Neolithic economies north of the Alps, and given the utility of such a combined approach elsewhere (Pilaar Birch et al. 2018; Prendergast et al., 2019), multiple methods for the differentiation of sheep and goats were employed. In so doing, we demonstrate that conventional methods for morphological differentiation of tibiae established for research elsewhere are unreliable in this setting. Nonetheless, new and established biometric methods show promise, and this contribution represents a first attempt at exploring the potential for caprine-based zooarchaeological research in temperate Neolithic Europe.

Differentiating Caprines on the Basis of Tibiae

Attempts to distinguish sheep from goats have a long history in zooarchaeology. Both morphological and metrical methods have been put forward for a variety of elements. The problem was first addressed systematically by Boessneck at al. (1964), who considered almost all skeletal elements, almost entirely from a morphological perspective (also Boessneck, 1969). Metrical methods were subsequently put forward for the metapodials (Payne, 1969, Rowley-Conwy, 1998; see also Boessneck et al., 1964: 115-116; Boessneck, 1969: 354-355), and more recently for the astragalus (Davis, 2017). A major recent survey has examined most skeletal elements using discriminant analysis, and demonstrated various degrees of success (Salvagno and Albarella, 2017). Morphological methods have been applied to the dentition, both deciduous (Payne, 1985) and permanent (Halstead et al., 2002); these have been examined by Zeder and Pilaar (2010). For postcranial elements, various morphological methods have been discussed (reviewed by Zeder and Lapham, 2010).

Distal tibia has been considered by various authorities. Boessneck et al. (1964) concluded that separation was not possible (see the quote at the start of this paper). A major attempt came from Kratochvil (1969), who identified various points of distinction based on modern skeletons. Many of these are problematic, for example a small prolapse in the posterior edge of the articulation (Kratochvil criterion B2d): “this prolapse is variable enough and is not present in all the sheep…. In one case it was also observed in goat” (Kratochvil, 1969: 485). Other criteria are qualified with expressions such as “usually” or “very often,” or present “in the majority of cases” (op. cit., 485, 488). Only two of Kratochvil’s criteria have been much used: (a) the sulcus malleolaris; and (b) the articulation on the distal projection of the medial malleolus (see Figure 1).

Figure 1: Key morphological features of the distal tibia.

The *sulcus malleolaris* is a groove on the distal shaft. Kratochvil (1969, criteria Ba, Da) states that the groove is well-developed in goat, fainter in sheep. In a subsequent review based on modern domestic animals, Prummel and Frisch (1986, their criterion 4) reiterate this.

However, we believe that the degree of development is at least partly dependent on the age and weight of the individual animal; and its archaeological utility is limited because our material is usually fragmentary, most of the shaft usually being missing. More recently, a major review by Zeder and Lapham (2010, fig. 4) based on numerous modern wild and domestic animals, does not mention this criterion.

The *articulation on the distal projection of the medial malleolus* is stated to be different in the anterior view (Kratochvil criterion Aa): in sheep only about half of it is visible (the shaded portion in Figure 1), while in goats the projection is twisted, so that more of it is visible (as far as the dotted line in Figure 1). Prummel and Frisch (1986), despite stating that Kratochvil's criteria are generally helpful (1986, 567), do not even consider this criterion. Zeder and Lapham (2010, criterion 1) do consider it, but conclude after extensive blind testing that it is among the least reliable anywhere in the skeleton: 23.8% of sheep were misidentified as goats, 16.9% of goats as sheep (op. cit., table 3). Our own experience bears this out. In the major Neolithic assemblage from Arene Candide (Italy) this was the criterion most commonly used, and the sheep:goat proportion based on distal tibia was the most divergent of any element from that established on reliable criteria (Rowley-Conwy, 1997, 164, tables 2.1 and 2.2).

Several other criteria are listed by Prummel and Frisch (1986) which are not identified by Kratochvil. They state that the sharp edge of the medial malleolus continues round to form the edge of the articulation in sheep, while in goat it diverges to join the edge of the *sulcus malleolaris* (op. cit., criterion 4). We have found this very difficult to apply in practice. Zeder and Lapham (2010, table 2) state that their criterion 3 corresponds to this, but their description and drawing (op. cit., fig. 4) actually refers to the degree of curvature of the articular edge, not to its junction with the *sulcus malleolaris*. With regard to Prummel and Frisch's criterion 4, Zeder and Lapham's drawing of sheep in fact resembles Prummel and Frisch's goat more closely than their goat does (cf. Zeder and Lapham, 2010, fig. 4, and Prummel and Frisch, 1986, fig. 9). Prummel and Frisch (op. cit., criterion 2) state that when viewed from the distal end the outline of the bone is nearly rectangular in goat, more trapezoidal in sheep. Neither Kratochvil (1969) nor Zeder and Lapham (2010) mention this. Salvagno and Albarella (2017, fig. 9) examined this metrically, but found that there was a major metrical overlap between the two species (op. cit., figs. 24, 44).

There has thus been neither much clarity nor much agreement as to which if any criteria are useful for distinguishing the species based upon distal tibia. Furthermore, all the studies cited above have been based on modern specimens. It is not clear how relevant these might be to prehistoric assemblages, nor how much regional and/or temporal variation there might have been in the past.

Materials and Methods

Racot 18 is a settlement of the Late Lengyel culture of the Polish Lowlands, located south of modern-day Poznań. The site consists of a series of trapezoidal longhouses on either side of what was a small stream, and is well-dated to the latter half of the fifth millennium BC

(Czerniak et al., 2016). The faunal assemblage totaled 5,229 bones, of which the majority were identifiable to species (87.9%) (Marciniak, 2014). Notably, the proportional representation of caprines (*Ovis* sp./*Capra* sp.) (31.1%) reaches near parity with cattle (33.0%), an atypical situation in a Neolithic context north of the Alps where assemblages are most often dominated by bovids (Manning et al., 2013).

Samples were selected in adherence with a strict Minimum Number of Individuals-based (MNI-based) sampling strategy (Casteel and Grayson, 1977). In so doing, twenty-two probable caprine distal tibiae were selected for analysis, ultimately representing an MNI of 15 (Table 1; Table S1; see Appendix S1 for MNI justification). No comparative collection was available for side-by-side specific identification at the time of sampling, so the strategy employed was chosen to mitigate this limitation. Therefore, this selection was made on the basis of a) the ease by which distal tibiae are identified and assigned side, b) their prevalence within the assemblage, c) the condition of the assemblage precluding the selection of other, more diagnostic elements, and d) the fact that there are morphological criteria, established for populations elsewhere, which ostensibly permit differentiation of sheep from goats using this element.

ZooMS

Twenty-two boneswere selected for biomolecular species identification. Samples were taken using a dental rotary saw, and manually cleaned of surface contamination using a diamond-tipped burr. Collagen was extracted using standard protocols based on a modified Longin (1971) method (Ambrose and DeNiro, 1986; Brown et al., 1988; DeNiro, 1985). ZooMS was applied to the collagen in order to test the fidelity of morphological specific identifications, and to differentiate the caprines. The rationale for doing so is a) some of the selected specimens were very fragmentary, and therefore had heightened chances of having been misidentified, b) sheep and goats are often raised for very different purposes and may feed on different foodstuffs (Arbuckle et al., 2009), c) to determine the composition of the caprine component of the Racot 18 faunal assemblage, and d) evaluate the validity and utility of morphometric and metric methods of caprine differentiation.

Peptide extraction for ZooMS was carried out at the Center for Evolutionary Genomics, Natural History Museum, University of Copenhagen, Denmark and MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) analysis was subsequently performed at the Centre for Excellence in Proteomics at the University of York, United Kingdom.

An average of 1 mg collagen was transferred to a 1.5 mL Protein Lowbind Eppendorf tube. 100 µL of 50mM NH_4HCO_3 (Sigma Aldrich) was added, and the samples were centrifuged at 13,000 rpm for 1 min. The samples were subsequently incubated at 65°C for 60 minutes to gelatinise the collagen. 50 µL of supernatant was transferred into a new 1.5 mL eppendorf tube. After reaching ambient temperature, 1 µL of sequence grade Trypsin (0.4 µg/µL) (Promega) was added to each sample followed by incubation at 37^{o}C for 16 hours. Following trypsin digestion, the samples were centrifuged at 13,000 rpm for 1 min before acidification to

<pH 2 using 5% (vol/vol) Trifluoroacetic acid (TFA, Sigma Aldrich). Peptides were then desalted and enriched using C18 reverse phase resin tips (PierceTM), and subsequently eluted in 50 µL of 50% acetonitrile (ACN)/0.1% TFA (vol/vol). 1 µL of the eluted peptides were spotted in triplicate and co-crystallised with α-cyano-4-hydroxycinnamic acid (Sigma Aldrich) matrix solution (50% ACN /0.1% TFA (vol/vol) at a ratio of 1:1 (1 µL : 1 µL). Spots were left to dry for three hours. Mass spectrometry was performed using a Bruker Ultraflex III (Bruker Daltonics) MALDI-TOF-MS instrument in reflector mode with laser acquisition set to 1200.

The triplicate spectral output was converted to TXT, merged and peak picked with a signal to noise threshold of 4 using the open-source software mMass v.5.5.0 (Strohalm et al., 2010).

Morphological Identification

Five conventional morphological techniques for differentiation were chosen to distinguish the tibiae to genus. For each criterion, a score was assigned between 1 and 5, which represents a graded scale between sheep and goats, and increasing or decreasing confidence of identification (Table 2). A score of 1 means the specimen can be assigned as *Ovis* with confidence, a score of 2 means the specimen is probably *Ovis*, 3 means no determination is possible, 4 means the specimen is probably *Capra*, and 5 means the observer is confident the specimen is *Capra*. Methods 1, 2 and 3 (Table 2) correspond to Zeder and Lapham's (2010: 2891) Criteria 1, 2, and 3 respectively. Method 4 corresponds to Prummel and Frisch's (1986: 573) criteria for the medial aspect of the distal tibia, whilst Method 5 corresponds to those presented for the anterior aspect of the element. All scores were assigned independently by KJG and PR-C (Table 2).

Standard and New Measurements

Both standard and new biometric measurements were taken. KJG (Table S2) and PR-C (Table S3) took measurements independently, and the results were averaged (Table S4) and are the values considered further. Bd (Von den Driesch, 1976) corresponds to the greatest breadth of the distal end (Figure 2). Dd (Von den Driesch, 1976) corresponds to the depth of the distal end (Figure 2). Ma/Lp represents the greatest distal distance at the distal end of the element between the medial anterior part and the lateral posterior part of the element (Figure 2). La/Mp represents the greatest distance between the lateral anterior part and the mesial posterior part of the element (Figure 2). Cd is the greatest distance between the center of the element's anterior-posterior axis at the narrowest point of the distal end of the element between the anterior and posterior medial and lateral malleolae (Figure 2). This measurement was taken perpendicular to the element, that is, with the external jaws of the caliper pointing in a proximal direction up the skeletal element from its distal end.

Figure 2: Standard and new measurements taken. Bd and Dd follow Von den Driesch, 1976). The arrows provide clarity the measurement point for Cd.

ResultsZooMS

We performed ZooMS peptide mass fingerprinting on 22 samples preliminarily identified on a morphological basis as either sheep or goat. Spectral outputs showed series of high intensity tryptic peptide products, some of which are unique to species (i.e. the peptides with a m/z of 3077.3 and 3093.3 for goats or 3017.3 and 3033.3 for sheep; see Columns G1 and G2 in Table 1) (Buckley et al., 2010; Kirby et al., 2013). The assemblage showed a mixed proportion of sheep (n = 11) and goat (n = 8), and three were misidentified by osteological observations as they contained peptides that in combination are unique to roe deer (*Capreolus capreolus*) (Table 1).

Table 1: Peptides observed for species identification by MALDI-TOF-MS.

Morphological Identification

To assess the utility of established morphological criteria for the differentiation of distal tibiae, While there are numerous sources of inter-observer error, analyst experience in particular has been shown to affect higher-level species identification (Lyman 2019), and KJG and PR-C, while both experienced zooarchaeologists, have a difference in experience on the order of decades. Therefore, PR-C and KJG independently assessed the five criteria without knowledge of the ZooMS results (Table 2). Two of the three roe deer identified through ZooMS were highly fragmentary so no measurements could be taken and those taken on the third specimen were discarded. The deer will not be discussed further.

In the assemblage of distal caprine tibiae, Methods 1, 2, and 3 were most often correct when performed by PR-C, followed by Method 5 and lastly Method 4. Method 1 was also most often correct when performed by KJG, followed by Methods 4, 5, 3, and 2 respectively. Both zooarchaeologists were in agreement most often when using Method 1. Method 1 performed by KJG was correct eleven times out of fourteen and was the best performing method in this study. With this one exception, it is notable that on the basis of Methods 1, 2, and 3, all assessments by both analysts were less accurate than those previously performed (Zeder and Lapham, 2010), and often by a large margin. The data suggests that the applied methods are at best as unreliable and often substantially more unreliable on northern European Neolithic caprine specimens, as they have previously proven to be for modern specimens (Zeder and Lapham, 2010).

Table 2: Morphological determinations, inter-observer error, and percent correct. 1= Ovis, 2= cf. Ovis, 3= indeterminate, 4= cf. Capra, 5= Capra. Indeterminate in conjunction with any other value and measurement taken versus no measurement taken are counted as disagreement. Determinations and probable determinations of the same genus are considered agreement. Underlined values are incorrect. Blank measurements were not taken.

Standard and New Measurements

Two standard (Von Den Dreisch, 1976) and three new measurements were taken of the distal tibiae (Table S2: Figure 3). Measurements Bd (K-W Test (adjusted for ties), H=4.88, $p<0.05$), La/Mp (H=4.59, $p<0.05$), Ma/Lp (H=5.72, $p<0.05$), Dd (H=5.62, $p<0.05$) and Cd (H=11.89, $p<0.01$) (Figure 2; Von Den Driesch, 1976) all show a significant difference between the species. The Racot 18 sheep are therefore smaller than the goats. There is, however, overlap in all cases between the taxa (Figure 3).

On a purely metric basis, if individuals falling into this zone of overlap are considered ambiguous, Bd measurements greater than 26.0mm can be considered goats, and measurements less than 24.2mm can be considered sheep. Similarly, Dd measurements greater than 20.2mm are goats, and those less than 20.0mm are sheep. Ma/Lp measurements greater than 27.1mm are goats, and those less than 25.1 are sheep. La/Mp values greater than 25.3mm are goats, and those less than 23.5 are sheep. Lastly, a Cd measurement of 16.0mm can be taken as the cut-off between the caprines. Cd values above the cut-off are goats, and those below it are sheep.

Figure 3: Standard and new biometric data. The quartile calculation was performed in MS Excel using an inclusive median implemented by the QUARTILE.INC function. The outer 'whiskers' are the range of the samples. The box depicts the interquartile range and the line is the median.

Discussion, Conclusions and Future Directions

In conjunction with the ZooMS results, it is possible to assess the correctness of the morphological identifications and standard and new measurements for differentiation of caprines. As discussed above, distal tibiae, despite being easily recognisable to skeletal element, are among the least reliable elements for caprine taxonomic identification (Wolfhagen and Price, 2017). Furthermore, whilst methods are designed to be as quantitative as possible, there is almost always a certain degree of inherent inter-observer error (Lyman and VanPool, 2009), including in observations where actual measurements are taken (Zeder and Lapham, 2010), but also in cases where the morphological methods rely on qualitative assessments of shape, prominence, and degree (Lyman, 2019).

One solution is to consider morphological identifications of caprines to fall upon a spectrum, and to consider identification as probabilistic (Wolfhagen and Price, 2017), but such approaches dilute the ultimate meaning behind relative proportions of sheep and goats in caprine assemblages. Another approach is to combine biomolecular and fundamental zooarchaeological methods, an approach that has proven useful even when inaccuracy of identification and inter-analyst variation is not substantial (Prendergast et al., 2019). In caprine populations, only through quantitative biomolecular means can accuracy of identification, the utility of different skeletal elements for this purpose, and inter-observer error be understood.

This study confirms that conventional morphological criteria for differentiation of sheep and goat distal tibiae are unreliable in this population and yield conflicting results due to inter-observer error. The applied morphological criteria do not separate the taxa reliably (Prummel and Frisch, 1986; Zeder and Lapham, 2010). Standard and new measurements discriminate sheep and goats, albeit with some overlap. As such, simple biometrics are better suited for caprine discrimination in this setting. Salvagno and Albarella (2017) concluded that a combination of the ratio depth of the medial (Dda) and lateral (Ddb) sides with the breadth of the distal end provided the best discrimination between modern sheep and goats, at best successful 89% of the time. Within this assemblage, a single measurement of Cd, taken between the condyles at the element's narrowest point discriminates sheep from goats with a demarcation at 16.0mm.

Such a large caprine assemblage is uncommon for Neolithic northern Europe, and we cannot therefore assume that these biometrics will discriminate as effectively in other populations. Furthermore, it is unclear what proportions of male and female are represented. Regardless, our results underscore the need to broaden this dataset to include other assemblages, and in particular to modern and reference material, to determine if biometric discrimination reflects morphologically consistent difference between the taxa, or simply size of breeds.

Little research regarding Neolithic caprine husbandry has been undertaken in cattle-focused agricultural temperate regions north of the Alps, and the basic methodological underpinnings required to do so are currently lacking. If we are to understand the purpose(s) for which caprines were raised, we first must understand whether sheep or goats were present, or both. In this assemblage, relative parity in numbers between sheep and goats is demonstrated, indicating that both taxa were raised as part of an integrated regime of agrarian practice, along with cattle, and presumably cereal agriculture. We hope that we have taken a step away from the archaeological chimera that is the sheep/goat in the Neolithic of northern Europe, and have underscored that even with minimal resources, a pair of calipers, productive results may be forthcoming.

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Figures

Figure 1: Key morphological features of the distal tibia.

Figure 2: Standard and new measurements taken. Bd and Dd follow Von den Driesch (1976). The arrows provide clarity the measurement point for Cd.

Figure 3: Standard and new measurements. The quartile calculation was performed in MS Excel using an inclusive median implemented by the QUARTILE.INC function. The outer 'whiskers' are the range of the samples. The box depicts the interquartile range and the line is the median.

Tables

Table 1: Peptides observed for species identification by MALDI-TOF-MS.

Table 2: Morphological determinations, inter-observer error, and percent correct. 1= Ovis, 2= cf. Ovis, 3= indeterminate, 4= cf. Capra, 5= Capra. Indeterminate in conjunction with any other value and measurement taken versus no measurement taken are counted as disagreement. Determinations and probable determinations of the same genus are considered agreement. Underlined values are incorrect. Blank measurements were not taken.