TITLE
Human femur morphology and histology variation with ancestry and behaviour in an ancient sample from Vietnam.

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Human femur morphology and histology in ancient Vietnam.

AUTHORS
Meg M. Walker¹*, Marc F. Oxenham¹,², Melandri Vlok³, Hirofumi Matsumura⁴, Nguyen Thi Mai Huong⁵, Hoang Hiep Trinh⁶, Tran T Minh⁵, Justyna J. Miszkiewicz¹,⁷*

AFFILIATIONS
¹School of Archaeology and Anthropology, Australian National University, 0200 Canberra, ACT, Australia
²Department of Archaeology, University of Aberdeen, AB24 3FX Aberdeen, UK
³Sydney Southeast Asia Centre, The University of Sydney, Camperdown, 2050, NSW, Australia
⁴School of Health Sciences, Sapporo Medical University, Sapporo, Japan
⁵Anthropological and Palaeoenvironmental Department, The Institute of Archaeology of Vietnam, Hanoi, Vietnam
⁶Institute of Archaeology, Vietnam Academy of Social Science, Hanoi, 61 Phan Chu Trinh, Hanoi, Vietnam
⁷School of Social Science, University of Queensland, 4072 St Lucia, QLD, Australia

*corresponding authors: meg.walker@anu.edu.au & j.miszkiewicz@uq.edu.au
ABSTRACT

Background

There is a genetic component to the minimum effective strain (MES)—a threshold which determines when bone will adapt to function—which suggests ancestry should play a role in bone (re)modelling. Further elucidating this is difficult in living human populations because of the high global genetic admixture. We examined femora from an anthropological skeletal assemblage (Mấn Bạc, Vietnam) representing distinct ancestral groups. We tested whether femur morphological and histological markers of modelling and remodelling differed between ancestries despite their similar lifestyles.

Methods

Static histomorphometry data collected from subperiosteal cortical bone of the femoral midshaft, and gross morphometric measures of femur robusticity, were studied in 17 individuals from the Mấn Bạc collection dated to 1906 to 1523 cal. BC. This assemblage represents agricultural migrants with affinity to East Asian groups, who integrated with the local hunter-gatherers with affinity to Austra–Papuan groups during the mid-Holocene. Femur robusticity and histology data were compared between groups of ‘Migrant’ (n = 8), ‘Admixed’ (n = 4), and ‘Local’ (n = 5).

Results

Local individuals had more robust femoral diaphyses with greater secondary osteon densities, and relatively large secondary osteon and Haversian canal parameters than the migrants. The Migrant group showed gracile femoral shafts with the least dense bone made up of small secondary osteons and Haversian canals. The Admixed individuals fell between the Migrant and Local categories in terms of their femoral data. However, we also found that measures of how densely bone is remodelled per unit area were in a tight range across all three ancestries.

Conclusions

Bone modelling and remodelling markers varied with ancestral histories in our sample. This suggests that there is an ancestry related predisposition to bone optimising its metabolic expenditure likely in relation to the MES. Our results stress the need to incorporate population genetic history into hierarchical bone analyses. Understanding ancestry effects on bone morphology has implications for interpreting biomechanical loading history in past and modern human populations.

Keywords: bone histomorphometry; minimum effective strain; anatomical variation; Haversian systems; bone functional adaptation; bioarchaeology
1. INTRODUCTION

Bone modelling and remodelling are processes actioned by bone-depositing osteoblasts, bone-resorbing osteoclasts, and the receptive osteocyte network that adapt bone to function (Beck, 2022; Pivonka et al., 2018; Ruff et al., 2006). These cells form and rework (modelling) bone morphology during early ontogeny and under periods of extreme loading (Cambra-Moo et al., 2012; Maggiano, 2012; Pearson & Lieberman, 2004; Walker et al., 2022a; see Supplementary Note 1), and maintain skeletal physiology by remodelling bone throughout the lifespan in response to loading changes; localised damage; and systemic physiological signals (Burr, 2002; Drapeau & Streeter, 2006; Parfitt, 2002; Robling et al., 2006). In cortical bone, osteoclasts and osteoblasts are linked temporally and spatially in the Basic Multicellular Unit (BMU) which excavates a tunnel and refills it centripetally with lamellae around a blood vessel known as a Haversian canal (Allen & Burr, 2014; Boyce & Xing, 2008). In cross section, the product of the BMU is a secondary osteon (hereafter ‘osteon’) (Figure 1). Anatomists and biological anthropologists study long bone modelling and remodelling markers through robusticity measures (shaft diameter, thickness, cross-sectional geometry) (e.g. Shaw & Stock, 2013; Skedros, 2012; Trinkaus & Ruff, 2012), and histomorphometric variables (e.g. densities and geometric parameters of osteons, e.g. Britz et al., 2009; Burr et al., 1990; Mulhern, 2000; Stout & Lueck, 1995; Miszkiewicz & Mahoney, 2016), respectively, and combined (e.g. Miszkiewicz, 2016; Miszkiewicz et al., 2022; see Supplementary Note 2).

Biomechanical loading, amongst other factors (e.g., dietary, hormonal, disease) determines bone (micro)morphology (Heaney, 1995; Heaney et al., 2000; Robling et al., 2006). The Mechanostat model (Frost, 1987) builds on stress and strain theory to explain that a minimum effective strain (MES) determines when bone will adapt to function (Currey, 2012; Martin et al., 2015; Sugiyama et al., 2012). Remodelling is stimulated in both underloaded and overloaded bone, but resorption or formation dominate over one another in the respective mechanical states (Robling et al., 2006; Sugiyama et al., 2012). Ongoing efforts have identified genetic components to bone functional adaptation (Lu et al., 2022; Jepsen et al., 2010; Sawakami et al., 2006; Saxon et al., 2011; Suva et al., 2005; Wallace et al., 2017), and it has been suggested that the MES is genetically determined, essentially serving as a setpoint for bone response to load (Lanyon, 1987; Sugiyama et al., 2012; Rubin & Lanyon, 1984). Little is still known about ancestry effects on mechanically induced bone remodelling in humans. Elucidating this in modern living populations is difficult due to our high genetic admixture. In
this study, we overcome this limitation by turning to an anthropological collection of human skeletal remains with known ancestry to test the extent to which it links with bone robusticity and underlying remodelling. Following the 2013 recommendations of the Scientific Working Group for Forensic Anthropology, we define ‘ancestry’ following Tallman et al. (2021: 74): “biogeographically patterned, clinal, genetic variation that is often continentally derived and defined”.

1.1. Human ancestry and bone (micro)morphology

Evidence exists that there are inherited and inter-population differences in measures of bone function and health (Jepsen et al. 2010; Kuipers et al., 2012; Pollitzer & Anderson, 1989; Wallace et al., 2010, 2012; Zmuda et al., 2009). Jepsen et al. (2010) found that inheriting gracile bones can be compensated for structurally on a microscale to provide additional bone strength. Wallace et al. (2010; 2012) conducted genetic experiments with mice noting that ancestral bone adaptation is retained in diaphyseal cross-sectional geometry, and that short-term rigorous physical activity does not override genetic influences on bone structure. Bone mineral density (BMD) varies across human populations today (Brennan-Olsen et al., 2017; 2019a, b), and so does our cranial and post-cranial morphology and robusticity but over longer timescales (Pearson, 2000). The latter is likely due to long term adaptations to environmental conditions and lifestyle during global population diffusion (Martin et al., 2015; Matsumura et al., 2014; Pearson, 2000; Robling et al., 2014; Shaw & Stock, 2013; Trinkaus & Ruff, 2012). The fundamental ancestry-related variation in human skeletal morphology is that of stature, which is a highly heritable trait (Feldesman & Fountain, 1996; Stulp & Barrett, 2016). Thus, bone length (particularly the femur, which closely correlates with stature) variation plays a role in the robusticity and mechanical adaptation of long bones (Eveleth & Tanner, 1990; Porter, 1999; Stock & Shaw, 2007), and can be linked to climate, geographical variables, and lifestyle, (Pearson, 2000), manifesting as between-population differences in cross-sectional geometry indicating biomechanical properties vastly modelled earlier in ontogeny (e.g. Holt, 2003; Huffer & Oxenham, 2015; Kubicka et al., 2018; 2022; Macintosh et al., 2014; Rainville, 2001; Stock, 2006).

Less documented has been inter-population variation in microscopic markers of bone remodelling in adult cortical bone though histological approaches. Cho et al. (2002) noted that rib osteon population density (OPD) and osteon area used in age-at-death estimation should be
population-specific because they differ between individuals of European-American and African-American ancestry. Increasingly, publications reporting population-specific histomorphometry data for age-at-death estimation purposes are accumulating for populations including Albania (Kranioti et al., 2020), Switzerland and the US (Stout et al., 1996), Korea (Lee et al., 2014), and Poland (Sobol et al., 2015) based on clavicle histology; Malaysia (Nor et al., 2014) based on humerus, ulna, radius, femur, fibula, and tibia histology; Australia (non-Indigenous) (Maggio & Franklin, 2019), the Netherlands (Maat et al., 2006), South Africa (Botha et al., 2019), Japan (Yoshino et al., 2014) based on femur histology; and South Africa (Pratte & Pfeiffer, 1999) and the US (Stout & Paine, 1992) based on rib histology. A handful of direct comparisons of bone remodelling based on cortical bone histology parameters in temporally and spatially diverse samples has reported inconsistent results (summarised in Table 1), likely confounded by the multifactorial nature of bone remodelling. In our study, we consider the Mán Bạc population of ancient Vietnam which is represented by individuals of at least two distinct ancestries but who occupied the same region and likely engaged in similar cultural behaviours (Oxenham et al., 2021).

1.2. Mán Bạc ancestry background and research question

Mán Bạc is a Neolithic archaeological site from northern Vietnam, with the cemetery dated to 1906 to 1523 cal BC (Vlok et al., 2020), which is the early transition to agriculture in the region (Oxenham et al., 2011; Matsumura et al., 2011). Skeletal remains recovered at Mán Bạc testify to a period of cohabitation between local Indigenous groups and incoming migrant farmers (see Supplementary Note 3). There is marked ancestrally derived morphological variation (Figure 2) observed at Mán Bạc which appears to be related to the very recent event of both cohabitation and some degree of admixture (Lipson et al., 2018; Matsumura & Oxenham 2014). During the early Neolithic in Vietnam, farming populations from southern China migrated into the warmer and more humid regions of Vietnam and interacted with local hunter-gatherers (Oxenham & Buckley, 2015). This interaction resulted in both social and genetic changes to the populations of mainland Southeast Asia. The Mán Bạc community appears to include first generation immigrants living with an indigenous population. The indigenous people of Southeast Asia are archaeologically present in the Southeast Asia-Pacific region from approximately 60,000 years ago (Oxenham & Buckley, 2015). As such, the Mán Bạc indigenous group shares affinity to Aboriginal Australians, Papuan and Negrito communities (Matsumura, 2011b; Matsumura & Oxenham, 2014; Matsumura et al., 2011). The immigrant
population shares morphological affinity to individuals of Siberian and Northeast Asian
descent (Lipson et al., 2018). The morphological traits observed reflect physically cold adapted
people who dispersed from Africa to Eurasia approximately 40,000 years ago and eventually
extended as far as China and the Americas (Oxenham & Buckley, 2015). Modern Southeast
Asians share greater affinity to the immigrant group than the indigenous group due to major
demographic and genetic shifts that occurred following the agricultural transition in Southeast
Asia (Oxenham & Buckley, 2015). Additionally, there are individuals who share traits from
both the immigrant and indigenous population. These individuals may be of mixed immigrant
and local descent, or they may represent inter-group overlap in the morphological traits
assessed. Nevertheless, as the individuals represent two extremes of morphological traits, the
confidence of assigning an individual to that of the immigrant or the local population is high at
Mán Bạc.

This is a unique sample of human remains with distinct ancestry. We thus hypothesised that
femur bone remodelling and exterior robusticity measures should differ between these distinct
categories of ancestry at Mán Bạc.

2. MATERIALS AND METHODS

Mán Bạc is a coastal habitation and burial site located in Ninh Binh province, northern Vietnam
(Supplementary Figure 1). The inhabitants of Mán Bạc represented a sedentary year-round
cosmopolitan community with a mixed farming and foraging economy (Jones et al., 2019b;
Oxenham et al., 2011). The population was under some demographic stress with a high fertility
rate of 6.8 births per woman as well as an estimated elevated rate of natural population increase
of 4.3% per annum (McFadden & Oxenham 2018; McFadden et al., 2018). Furthermore,
extreme physiological stress and poor health were evidenced by treponemal disease, malaria,
thalassaemia, anaemia and scurvy identified in both adults and children (Adams et al., 2021;
McDonell & Oxenham, 2014; Vlok et al., 2020; 2021a; 2021b). Walker et al. (2022a) recently
examined humeral and femoral bone histology in an individual from Mán Bạc afflicted with
paraplegia (possibly quadriplegia), describing microscopic changes in skeletal tissue in
response to muscular dysfunction. Otherwise, no other bone histology data are currently
available for this site, and the broader region.

Excavations of the Mán Bạc burials were undertaken over three excavation seasons (1999,
2005 and 2007), resulting in the recovery of 101 individuals (Oxenham et al., 2011).
Radiocarbon dates of the burials and of charcoal within habitation layers indicate occupation between 4,000–3,500 years ago (Oxenham et al., 2011; Vlok et al., 2020). Today, the human remains are curated at the Vietnamese Institute of Archaeology (VIA) in Hanoi, Vietnam. Following extensive local consultation, the VIA granted permission and certification for sampling and export of bone examined in this study for a total of $n = 18$ Mán Bạc individuals. All analyses were conducted following ethics guidelines stipulated by the Australasian Society for Human Biology Code of Ethics.

### 2.1. Osteological analyses, femoral robusticity, and bone histology

Following standard methods (Brickley & McKinley, 2004; Buikstra & Ubelaker, 1994), biological profiles were reconstructed for each individual to estimate their biological sex, age-at-death, stature, and ancestral groups (Table 1). Sex was determined using morphological analysis of the skull and pelvis (Brickley & McKinley, 2004; Buikstra & Ubelaker, 1994). Stature estimates were based on physiological femoral length (Martin & Saller, 1957). Age-at-death estimates were based upon pubic symphyseal morphology, late fusion of epiphyses stages, and/or molar wear (Brickley & McKinley, 2004; Buikstra & Ubelaker, 1994; Scott, 1979).

Biological ancestry was previously determined by Oxenham et al. (2021) and those data are used in this study (see Supplementary Table 1). In summary, both qualitative and quantitative results of cranial and dental morphology (Matsumura 2011a; Dodo, 2011; Matsumura 2011b; Matsumura & Oxenham 2014) in combination with ancient DNA (aDNA) haplogroup data (Lipson et al., 2018) were used to assign geographic or biological ancestry. Further corroboration of ancestry determination includes the observation that this formed an important component of both biological kin groupings and also body modification (e.g., tooth ablation) practices at Man Bac (see discussion in Oxenham et al. 2021). Each individual was estimated to express one of three categories based upon morphological traits: ‘Migrant’, ‘Local’, or ‘Mixed’. Those who exhibited Siberian/Northeast Asian traits are defined as ‘Migrants’, those who affiliate with Australo-Papuans are here termed ‘Locals’, and those with mixed traits are termed ‘Admixed’ individuals. The Local populations displayed extremely robust cranial features, whereas the Migrant populations had gracile features. This allowed for an unusually high confidence in assigning ancestral affinity from phenotypic skull traits (Figure 2, Matsumura 2011a, b; 2014; Matsumura & Oxenham, 2013; 2014). Nevertheless, it is
recognised that these features are highly variable within a population and a degree of overlap of phenotypes between the ancestral groups is expected.

Although haplogroup data from aDNA were also available for a sample of these individuals (Lipson et al., 2018) it can only indicate biological maternal ancestry not associated with a particular region or ancestral group. For this reason, one of the individuals (MB05M09) is excluded from our ancestry analyses (their bone data are still reported in the Supplementary Material) lowering our main sample size to 17 individuals.

Two types of bone morphology data were collected: femoral robusticity and static cortical bone histomorphometry (Miszkiewicz & Mahoney, 2017; 2019; Stock & Shaw, 2007). Femoral physiological length (cm), shaft circumference (mm), medio-lateral and postero-anterior diameter (mm) were measured following established guidelines for bone morphometry (Buikstra & Ubelaker, 1994). Derived robusticity was then calculated following Stock and Shaw’s (2007) recommendations. Femoral shaft circumference robusticity index (Circ.RI) was calculated by dividing circumference (mm) by femoral physiological length, multiplied by 100. Cortical width RI (Ct.Wi.RI) was calculated by dividing the cortical width (mm) by femoral physiological length, multiplied by 100.

Only individuals who did not display lesions indicative of systemic and/or localised disease were selected for histology. Extraction of bone samples for histology followed standard methods (Miszkiewicz & Mahoney, 2016; 2017). A 1x1 cm cortical bone samples were removed from the posterior mid-third femoral diaphysis spanning the linea aspera (Miszkiewicz & Mahoney, 2016) using a Dremel® Variable-Speed Rotary Tool 3000 equipped with a Dremel® blade. We targeted the posterior femur in line with prior studies assessing biomechanical influences in this anatomical landmark (Miszkiewicz & Mahoney, 2016).

Standard histology preparation methods were followed (Bancroft & Gamble, 2008). Extracted samples were embedded in an epoxy resin solution (4:1 resin and hardener). Once set, each sample was laterally reduced using a Kemet MICRACUT 151 Precision Cutter using a diamond cutting Disc 150mm. Residual samples were kept for repatriation or further analysis. Once mounted onto microscope slides with epoxy resin adhesive, each sample was ground and polished using a Buhler® histologic precision grinding fixture on a Buehler® EcoMet 300 Grinder-Polisher pad to achieve an even thickness between 100+/-60µm. Using a Buehler MicroPolish II 0.3µm powder and Buehler polishing cloth scratches were removed. Slides were
cleaned in an ultrasonic tub, a series of ethanol baths, and dipped in xylene to remove micro-
debris and water prior to adding a glass cover slip.

Thin sections were imaged using an Olympus BX53 high powered microscope with an inbuilt
DP74 camera using transmitted and polarised light. The Olympus cellSens 2018 imaging
software was used to scan each thin section completely at 4x magnification (Figure 3). Six
regions of interest (ROI) from the subperiosteal region of bone samples were determined from
the linea aspera outwards medially and laterally (Figure 4). Each ROI measured approximately
1.8 mm² and was scanned at 10x and 20x magnification. Only individuals that presented a
minimum of 25–50 intact osteons were required across each section to be viable for
histomorphometric analysis (Stout & Crowder, 2011). Many ROIs presented poor preservation
of histological features due to taphonomic alterations. This was assessed using the standard
Oxford Histological Index (OHI, see further below) (Hedges et al., 1995; Millard, 2001).

Components of cortical bone histology (osteons and Haversian canals) were examined across
each ROI using the ‘line’, ‘free hand’, and ‘multi-point’ tools in FIJI/ImageJ vol. 1.52a
(Schindelin et al., 2012). The ‘ROI Manager’ tool was used to save the measurements as
discrete layers for replicability purposes. Table 2 presents a full list of all histological variables
measured and their definitions following nomenclature standards for two-dimensional bone
histology (Dempster et al., 2012; unless otherwise stated). Figure 5 illustrates some examples
of these variables. The open-source macro for FIJI/ImageJ developed by Dominguez and
Agnew (2019) was adapted to standardise the calculation of Cortical Area (Ct.Ar). Scans of
complete sections were processed by removing image background in Adobe Photoshop 2018
using the ‘Magic Wand’ tool. The image was then uploaded to FIJI/ImageJ vol. 1.52a,
converted to 8-bit greyscale, and analysed using the ‘Threshold’ function. The total bone area
could then be measured accurately with the ‘area’ function.

Demarcation of the transitional zone, between the cortical bone and trabeculae struts, was
defined for each image prior to processing Ct.Ar through FIJI/ImageJ vol. 1.52a (Dempster et
al., 2013; Dominguez & Agnew, 2019). Cortical width was measured using the ‘straight line’
tool. Total area impacted by bioerosion was measured using the ‘free hand’ tool and included
items such as Wedl tunnelling, dissolution, and other areas of bone that simply did not present
as well-preserved histology (Hedges, 2002). Geometric variables were measured by tracing
around the cement line of intact osteons or Haversian canals with the ‘free hand’ tool. Density
variables, such as intact and fragmentary osteon population density, were recorded from the
number of osteons counted using the ‘multi-point’ tool. Any histology features cut off at the border of the ROI were not recorded.

### 2.2. Data analyses

Standard descriptive statistics were documented for each variable per individual. Distributions for each variable across the sample population were established and analysed. Intra-observer error was minimised by re-assessing histological features across a randomly selected sub-sample that formed 10% of the entire sample. We elected to not run inferential statistical tests because of the uneven and small sample size in some of the ancestry sub-groupings exacerbated once age-at-death and sex are taken into account. Additionally, OPD was not tested against N.On or N.On.Fg as the latter are components of the derived OPD variable. Data were analysed descriptively using IBM SPSS 28 software by comparing all bone data between ancestry groups. Ex ante evaluation of bone data variation with age-at-death and sex is included as supplementary material.

### 3. RESULTS

Ancestry was defined here in three categories: Migrant (n = 8), Admixed (n = 5), and Local (n = 4). An intra-observer error test yielded no major differences when comparing repeated measures of data (Supplementary Table 2). The preservation of bone histology was within the 50% OHI mark, with some thin sections showing better preservation (up to 70–80%, but not >85%), and others slightly lower than 50% but never less than ~45%. Bioerosion was concentrated on the periosteal and endosteal borders of bone as is common in archaeology (Hackett, 1981), but large areas of well-preserved cortical bone histology could be examined for morphometric characteristics. Femoral robusticity and histology measurements sub-divided by ancestry groupings are reported in Table 3. Robusticity and histology data across ancestry groups are illustrated in Figure 6. For between group comparisons, we pooled age-at-death and sexes mainly due to the small sample size (we will account for this when interpreting data), but also because we assume ancestral history would override sexual dimorphism expression across the entire sample (but not within each ancestry category, based on population-specific variation in sexual dimorphism, see Ubelaker & DeGaglia, 2017; and also see Supplementary Tables 3–5 for all data for 18 individuals sub-divided by age-at-death and sex; raw data can be accessed via our figshare dataset at Walker et al., 2022b).
There was a clear variation in all the variables with our three ancestry categories. The longest femora (mean 427.20 mm) along with the greatest stature estimates (mean 162.30 cm) were for the Local individuals, whereas the Admixed individuals had the lowest values (mean femur length = 402 mm, mean stature = 155.03 cm). The data for cortical width (mean 8.90 mm) and shaft circumference (mean 86.60 mm) were also the greatest in the Local group, with the Admixed (mean cortical width = 7.77 mm, mean shaft circumference = 80.67 mm) and Migrant (mean cortical width = 7.44 mm, mean shaft circumference = 81.25 mm) categories showing very similar values. This translated to the robusticity indices wherein the Local group still showed the largest values (mean Ct.Wi.RI = 2.08, mean Shaft.Circ.RI = 20.32) compared to the Migrant (mean Ct.Wi.RI = 1.71, mean Shaft.Circ.RI = 19.55) and Admixed (mean Ct.Wi.RI = 1.83, mean Shaft.Circ.RI = 19.57) groupings. However, the robusticity index based on cortical width values showed the largest range in the Admixed group indicating a relatively wide degree of data variation (see Figure 6).

In terms of cortical bone remodelling indicators, it was the Admixed group that showed the highest values of OPD (mean 23.68/mm²), though it was followed closely by the Local group (mean OPD = 22.27/mm²) (Table 3). The Local individuals showed the largest range in OPD values (see Figure 6), but it was somewhat alike in both the Migrant and the Admixed groups. Measures of osteon area differed substantially between all the groups, with the Local individuals having the largest osteons (mean On.Ar = 40,961.28 µm²). The Admixed (mean On.Ar = 34,805.65 µm²) and Migrant individuals showed smaller osteons compared to the Locals, with the smallest osteons found in the Migrants (mean On.Ar = 31,597.28 µm²). The Haversian canal data constitute the smallest number of canals represented out of all the histology variables we considered, so we make a cautious observation that the Locals had the largest canals (mean H.Ar = 1,022.03 µm²) when compared to the Admixed (mean H.Ar = 849.92 µm²) and Migrants (mean H.Ar = 593.17 µm²).

In summary, the Local individuals exhibited the most robust diaphyseal circumference and width along with the densest osteon densities accompanied by relatively large osteon and Haversian canal parameters. The Migrants had the most gracile femoral shaft circumference and width packed with least dense bone of small osteons and Haversian canals. The Admixed individuals consistently fell between the Migrant and Local categories in terms of their femoral robusticity and histology data. However, we highlight that the OPD data, which measure how
densely bone is remodelled per unit area, were in a tight range (mean OPD range = 20.88/mm² – 23.68/mm²) across all three groups (Table 3).

4. DISCUSSION

There was variation in bone histology and robusticity indices with designated ancestral groups in this Mán Bạc sample. Our key finding is that the Local individuals had the most robust limb bones with relatively large osteon morphology when compared with the groups assigned as Migrant and Admixed.

4.1. Mechanical and bone anatomical constraint interpretations

Firstly, it is possible that the femora of the Local individuals were built with substantial mechanical stimulus during the earlier phases of their ontogeny (hence their wider and thicker bone diaphyses; Ruff et al., 2006; Pearson & Lieberman, 2004; Carter et al., 1996), but experienced relatively slower remodelling events in later adulthood (as deduced from large osteon and canal areas; Seeman & Martin, 2019; van Oers et al., 2008). Alternatively, the relatively large size of the osteon and Haversian canals in relation to wider femoral diaphysis could be an indication of dimensional isometric or allometric relationships underlying bone growth in these individuals (Miszkiewicz & Mahoney, 2018; Felder et al., 2017). We cannot confirm either of these interpretations without a much larger sample size, and experimental data. However, we can propose that in both scenarios a genetically determined MES threshold may have been at play, such that it either predisposed the Local individuals to growing relatively larger femora, and/or set a lower threshold for response of bone to function early in ontogeny facilitating diaphyseal expansion with mechanical stimulation.

Robust femora with evidence for slower remodelling in adulthood has been observed in other anthropological and clinical studies (e.g. Miszkiewicz et al., 2022; Zebaze et al., 2010) because bone will modify its physiological response depending on function (and other factors) throughout the entire human lifespan. So, it is not surprising that bone robusticity markers forming at childhood/adolescence may differ from remodelling markers operating in later decades. For example, geometrically well-developed femoral cross-sections showing substantial porosity and trabecularisation of cortex are commonly reported in modern patients (e.g. Zebaze et al., 2010). This is also mirrored in the archaeological record, such as in a recent study of behaviour in Bronze Age Iran where nomadic individuals showed robust femoral cross-sections experiencing slower remodelling in older individuals (Miszkiewicz et al., 2022).
Thus, the separation between femoral robusticity characteristics, and the geometric histological parameters, in our study, can relate to the effect of ancestry because all our three groupings engaged in similar behaviours. Yet, the Local individuals clearly show different values from the Admixed and Migrant categories of ancestry.

The above interpretation can be further corroborated by our OPD results, which did not vary much with ancestry. The resulting OPD values fell into a tight range, showing that all these individuals were remodelling similar amounts of bone per mm$^2$, despite their different ancestries (and despite the differences in the geometry of both osteons and femoral shaft structure). These similarities could suggest that the number of remodelling events activated at any one time was the same across all the ancestries, possibly because of all three groups of individuals participating in the same community behaviours stimulating the remodelling responses. However, the speed at which individual BMUs completed remodelling events, and the bone space across which they would have been operating, varied, as inferred above from the histomorphometry and femur robusticity data. As secondary osteons are mechanical in nature, it would suggest that densely distributed secondary osteons provide better fatigue resistant properties (Martin, 2002). As the experienced strain magnitudes vary based on robusticity (van der Meulen et al., 2001), the observed size differences may be a biomechanical function of perceived cellular strains (Jepsen et al., 2010; Jepsen 2009). Thus, larger strain magnitudes in Migrants would reduce osteon size to provide better fatigue resistant properties.

However, ancestry-based subsistence roles, where Locals experienced fewer or less strained mechanical loads, would also produce larger strain related osteons. Similar amounts of osteon densities were previously found in genetically distinct samples, such as in archaeological Native American and modern European-Americans (though in the anterior femur cortex; Burr et al., 1990); and in femoral and tibial cross-sections in a Native Americans, Late Archaic, and Early Modern humans (Streeter et al., 2010); which matches our findings. An alternative interpretation of the OPD data at Mán Bạc is that they simply reflect an average of tissue age accumulated per ancestry. Our sample does contain a range of younger and older individuals, so age-related progression in osteon formation can be confounding our results. Therefore, our remarks here regarding OPD should be treated cautiously. It is possible that locals were engaged in different behaviours—in other words, rather than a gendered division of labour there was and ancestrally mediated division of labour. Alternatively, as we do not have
generational level resolution in dating, some local individuals may have been born and grew to adulthood prior to integration with the migrants. Collectively, our results do point in the direction of some behavioural influences on bone morphology and microstructure, but for those to be possibly underlain by long term inherited predisposition to certain morphology, because the local Mán Bạc populations stem from distinct hunter-gatherers, whereas the migrant populations were predominantly farmers (Matsumura, 2011b; Oxenham et al., 2018). We do know that hunter-gatherer and farmer long bone robusticity, both in the lower and upper limb, has been subject to a marked decline, exacerbated by sexual dimorphism (Ruff, 2018). It is likely that evolutionary mechanisms (such as natural selection) selecting for optimal bone remodelling occurred in response to divergent subsistence and behavioural strategies at Mán Bạc, ultimately favouring survival over a long period of time. Disparate experiences amongst the farmers and hunter-gatherers of Mán Bạc include adaptation to two different climates (warm vs. cold) over the span of tens of thousands of years (Oxenham & Buckley, 2015). More recently, adaptive changes may have occurred with the adoption of agriculture by the ancestors of the migrant population who had begun domesticating animals as early as 9,000 years ago in modern day China (Bellwood, 2005). Human self-domestication, with the adoption of agricultural subsistence driving increased gracility of the skeleton (Leach, 2003), may have further contributed to microscopically observable bone changes, as seen in the Mán Bạc Migrant data.

The human self-domestication hypothesis proposes that, as was the case for domestication of animals, selection for lower aggression in agricultural communities requiring co-operation for success led to biological phenotypical change in humans as a secondary consequence (Sánchez- Villagra & Van Schaik, 2019). This would explain the increased gracility of the human skeleton, including the skull, alongside agricultural transitions as reflected in the Migrant sub-sample in our study. Huffer and Oxenham (2015) who investigated long bone cross-sectional geometry and enthesal morphology in the Mán Bạc individuals found data trends changes towards sedentary activities along with increasing adoption of agriculture and a decline in mobility over time. Their findings are somewhat mirrored in our data for robusticity indices based upon cortical width data, where the Local and Migrant individuals showed smaller ranges of variability compared to the Mixed individuals (Figure 6). The femora of Mixed individuals had both very gracile and very robust shafts, which still likely reflect possible ancestry-specific underpinning to femoral morphology. The Migrant individual
gracile skeletal profiles added to the agricultural behavioural changes. Having said this, Huffer and Oxenham (2015) also found distinct sex-specific differences in long bone activity markers, which might also play a role in the combination of our histology and femoral robusticity measures per ancestry.

Taken together, the data differences identified at Mán Bạc may highlight a complex relationship between ancestry and skeletal morphology shaping in response to behaviour whereby the Mán Bạc ancestral agriculturalists have more slender bone than ancestral hunter-gatherers. It can be inferred that an increase in porosity through remodelling and larger canal area in larger bone of the Local individuals may be a multi-hierarchical metabolic (Schlecht & Jepsen, 2013), structural (Miszkiewicz & Mahoney, 2018), and genetic (Jepsen et al., 2010) trade-off, that acted according to perceived biomechanical loads. This stresses the biomechanical and metabolic relationship in bone plasticity across bone hierarchical levels.

4.2. Limitations and suggestions for future research

The key limitation in our study is the small sample size in each ancestry category, so we elected to not perform inferential statistical analyses on the data. This also meant we could not analyse data change with age-at-death and sex groups. We could not secure a larger sample size as the histology technique is destructive to this unique skeletal assemblage. However, we hope our descriptive analysis will pave the way to future replication of our study design on any other larger assemblages. There is also the issue of localised biodegradation in thin sections, which meant we could not collect osteon data from the entire cortical bone captured in each histology sample. Further, the lack of complete chronology at Mán Bạc limits our inferences on potential chronological change in mobility or cultural practices through time. Similarly, other cultural factors, such as social status or potential ancestral-based roles, are simply unknown so we can only comment on inferences made from the behavioural markers in bone. Future bioarchaeological or anatomical research where sub-groups of distinct ancestries are available for analysis in larger samples, could validate our observations statistically. Further research should also incorporate three dimensional methods of bone microscopic examination so that larger volumetric bone regions are accounted for (for example, the relationship between volumetric cortical bone porosity and shaft size and shape).

5. CONCLUSIONS
We showed that relationships between femur robusticity and histological markers of remodeling appear to be ancestrally determined at Mán Bạc. We inferred that these defined femur structure and fatigue resistance properties of the leg bones of Mán Bạc individuals. In the archaeological past, the Mán Bạc site was home to a new wave of societies in Northern Vietnam that combined hunting and agricultural lifeways. We found that these lifeways were possibly reflected in femoral robusticity and histology as a result of long-term evolutionary change where bone metabolic activity, if related to ancestry, could be biological in nature due to long term variation in the behaviour and exposure to different climates (and subsequent survival strategies) in distinct ancestral groups, sub-divided here into Local, Admixed, and Migrant. Our key finding was that the femoral robusticity and cortical bone histology markers indicated robust bones with active remodelling in the Australo-Papuan Locals individuals, but gracile bones with similarly active remodelling in the Migrant individuals. Because bone microstructure is mechanically driven and responds to perceived strain magnitudes to accommodate genetically determined bone MES thresholds and structure, we inferred that: ancestry was a factor determining bone morphology in these Mán Bạc individuals; their bone might have been maximising metabolic efficiency by providing greater fatigue resistance properties across ancestrally determined bone structures; genetic differences between ancestries complicated microstructural responses to strain suggesting that complex, cellular mechano-sensing and transducing pathways alter bone microstructure to achieve metabolic homeostasis.

ACKNOWLEDGMENTS

We are indebted to the Vietnamese Institute of Archaeology in Hanoi, Vietnam for permissions to conduct this study, collaboration, and facilitating data collection in Hanoi. We thank Hallie Buckley and Dave McGregor for research support, the School of Social Science at the University of Queensland for access to microscopy facilities, and feedback during peer review which has improved this article. Funding was received from the Australian Research Council (DE190100068 to JJM), and the College of Arts and Social Sciences at the Australian National University.

CONFLICT OF INTEREST STATEMENT

Authors declare no competing interests.

FUNDING STATEMENT

This study was part of a research fellowship funded by the Australian Research Council (DE190100068). The College of Arts and Social Sciences at the Australian National University
ANU) funded histology laboratory equipment used in the ANU Histology laboratory of the School of Archaeology and Anthropology.

ETHICAL APPROVAL DETAILS

Approval to conduct this research was issued by the Vietnamese Institute of Archaeology (VIA) in Hanoi, Vietnam. The VIA granted permission and certification for sampling, and established the parameters of this research. This research examines archaeological human remains dated to antiquity. All analyses were conducted following ethics guidelines stipulated by the Australasian Society for Human Biology Code of Ethics. While this research uses an invasive technique (histology), the skeletal remains were treated with respect in all stages of the analyses. Research standards of objectivity, integrity and the open sharing of knowledge were respected. All data are available open access from the Figshare platform (see reference list).

AUTHOR CONTRIBUTIONS

MMW: Conceptualisation; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Writing—original draft; Writing—review & editing; MFO: Data curation; Funding acquisition; Investigation; Project administration; Resources; Supervision; Writing—review & editing; MV: Investigation; Methodology; Roles/Writing—original draft; Writing—review & editing; HM, MH, HHT, TTM: Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Writing—review & editing; JJM: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Roles/Writing—original draft; Writing—review & editing.

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FIGURE CAPTIONS

FIGURE 1. Right human femur (posterior view, left side of the image) with marked midshaft cross-section (black rectangle) illustrated at the histological level (right side of the image). Secondary osteons are the circular structures tightly packed in the adult cortical wall of the long bone. A posterior ‘quadrant’, separated from the remainder of the cross-section, is the subject of the present study.

FIGURE 2: Differences in phenotypic cranial features distinguishing local from migrant groups at Mán Bạc: (A) round versus square orbits; (B) moderate versus prominent glabella; (C) broad and rounded versus tear drop nasal aperture; (D) high versus low and wide face; (E) mesocephalic versus dolichocephalic cranium; (F) flat versus prognathic maxilla; (G) thin and elongated versus short and broad mandible.

FIGURE 3. Examples of posterior femur thin sections removed from each Mán Bạc individual, showing a range of sizes and preservation. All images are shown with the posterior linea aspera aspect oriented towards the top of the image. Individual accession numbers are shown in the ‘empty’ medullary space.

FIGURE 4. Imaging method for selecting six regions of interest across each thin section at 10x magnification. Adapted from Miszkiewicz (2016: 180).

FIGURE 5. Cortical bone histology images (A: transmitted light, B: linearly polarised light) from femoral samples in individual MN0741M5, illustrating counts of secondary osteons (A); and Haversian canals (HC) and cement lines (indicated with black arrows) traced for the collection of area measurements.

FIGURE 6. Femur morphology and histology across ancestry groups. Graphs A and B show femur robusticity measure distribution with Ancestry, whereas graphs C and D focus on Haversian bone histomorphometric variables.
Table 1. Examples of studies where comparisons of bone remodelling based on cortical bone histology parameters were made between spatially and temporally diverse populations.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>FINDING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thompson &amp; Gunness-Hey, 1981</td>
<td>Archaeological native Yupik-Inupiaq individuals from North Alaska and Canada had higher OPD in their femora compared to modern European-American individuals.</td>
</tr>
<tr>
<td>Burr et al., 1990</td>
<td>Archaeological samples from Native American and modern European-American individuals had similar bone histology in the anterior femur cortex despite genetic and cultural differences.</td>
</tr>
<tr>
<td>Abbott et al., 1996; Streeter et al., 2010</td>
<td>Femoral and tibial osteon area in a Native American sample differed from Late Archaic and Early Modern human samples, but had similar OPD values.</td>
</tr>
<tr>
<td>Pfeiffer, 1998; Pfeiffer et al., 2006</td>
<td>Large variability in rib and femur osteon and Haversian canal area characterised archaeological samples from South Africa, and historical samples from England and Canada.</td>
</tr>
</tbody>
</table>

Table 2. Biological profile data for each Mán Bạc individual. Age-at-death (yrs; years): Young adult (17–25 yrs), Middle-aged adult (30–39 yrs), Mature adult (40–49, and 50+ yrs), Adult (unspecified, but >20 yrs).

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<th>ANCESTRY</th>
<th>AGE AND SEX</th>
<th>N</th>
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</tr>
<tr>
<td></td>
<td></td>
<td>Male 1</td>
</tr>
<tr>
<td></td>
<td>Middle-aged adult</td>
<td>Male 1</td>
</tr>
<tr>
<td></td>
<td>Mature adult</td>
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</tr>
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<td></td>
<td></td>
<td>Male 2</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>Indeterminate 1</td>
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<tr>
<td></td>
<td><strong>Total</strong></td>
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<tr>
<td>Admixed</td>
<td>Mature adult</td>
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<td>Adult</td>
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<td></td>
<td>Middle-aged adult</td>
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<tr>
<td></td>
<td>Mature adult</td>
<td>Male 1</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
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<td>DEFINITION</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------------</td>
<td></td>
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<tr>
<td>Cortical area (Ct.Ar) in mm$^2$</td>
<td>Total area of bone excluding trabeculae struts (Dominguez &amp; Agnew, 2019). Transmitted light, magnification 4x.</td>
<td></td>
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<tr>
<td>Cortical Width (Ct.Wi) in mm</td>
<td>Maximum distance between the periosteal and endosteal envelope at the linea aspera. Measured between two points parallel to one another (Miszkiewicz &amp; Mahoney, 2019). Transmitted light, magnification 4x.</td>
<td></td>
</tr>
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<td>Oxford Histological Index (OHI)</td>
<td>Percentage of bone area impacted by biodegradation as per Hedges (2002) and Millard (2001). Transmitted light, magnification 4x.</td>
<td></td>
</tr>
<tr>
<td>Total sampled bone area (B.Ar) in mm$^2$</td>
<td>Sum of cortical bone area measured across ROIs (Stout &amp; Crowder, 2012). Transmitted light, magnification 20x.</td>
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<tr>
<td>Oxford Histological Index (OHI)</td>
<td>Bone areas impacted by bioerosion scored on a 0-5 scale based on percentage (Hedges et al., 1995; Millard, 2001). Transmitted light, magnification 20x.</td>
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<tr>
<td>Osteon population density (OPD)/mm$^2$</td>
<td>Value derived from Intact osteon density (N.On) and Fragmentary osteon density (N.On.Fg). N.On is the total number of secondary osteons with at least 90% of cement line visible, divided by B.Ar. N.On.Fg is the total number of osteons with at least 10% of the osteon remodelled, divided by B.Ar (Stout &amp; Crowder, 2012). OPD is (N.On + N.On.Fg)/B.Ar (Drapeau &amp; Streeter, 2006). Transmitted light, magnification 20x.</td>
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<tr>
<td>Osteon area (On.Ar) in µm$^2$</td>
<td>Mean area of intact secondary osteons with a complete cement line (Stout &amp; Crowder, 2012). Transmitted light, magnification 20x.</td>
<td></td>
</tr>
<tr>
<td>Haversian canal area (H.Ar) in µm$^2$/canals</td>
<td>Mean area of complete canals with no evidence of resorption. Transmitted light, magnification 20x.</td>
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Table 4. Descriptive statistics outlining the parameters of robusticity indices, and histological features of ancestry groups.

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<td>169.40</td>
<td>160.07</td>
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<td>467.00</td>
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<tr>
<td>OPD/mm²</td>
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