1   **TITLE**
2   Human femur morphology and histology variation with ancestry and behaviour in an ancient
3   sample from Vietnam.
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5   **RUNNING TITLE**
6   Human femur morphology and histology in ancient Vietnam.
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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 (Mán 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 Mán 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 Australo-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

91 this study, we overcome this limitation by turning to an anthropological collection of human

92 skeletal remains with known ancestry to test the extent to which it links with bone robusticity

93 and underlying remodelling. Following the 2013 recommendations of the Scientific Working

94 Group for Forensic Anthropology, we define 'ancestry' following Tallman et al. (2021: 74):

95 "biogeographically patterned, clinal, genetic variation that is often continentally derived and

96 defined".

1.1. Human ancestry and bone (micro)morphology

98   Evidence exists that there are inherited and inter-population differences in measures of bone

99   function and health (Jepsen et al. 2010; Kuipers et al., 2012; Pollitzer & Anderson, 1989;

100 Wallace et al., 2010, 2012; Zmuda et al., 2009). Jepsen et al. (2010) found that inheriting gracile

101 bones can be compensated for structurally on a microscale to provide additional bone strength.

102 Wallace et al. (2010; 2012) conducted genetic experiments with mice noting that ancestral bone

103 adaptation is retained in diaphyseal cross-sectional geometry, and that short-term rigorous

104 physical activity does not override genetic influences on bone structure. Bone mineral density

105 (BMD) varies across human populations today (Brennan-Olsen et al., 2017; 2019a, b), and so

106 does our cranial and post-cranial morphology and robusticity but over longer timescales

107 (Pearson, 2000). The latter is likely due to long term adaptations to environmental conditions

108 and lifestyle during global population diffusion (Martin et al., 2015; Matsumura et al., 2014;

109 Pearson, 2000; Robling et al., 2014; Shaw & Stock, 2013; Trinkaus & Ruff, 2012). The

110 fundamental ancestry-related variation in human skeletal morphology is that of stature, which

111 is a highly heritable trait (Feldesman & Fountain, 1996; Stulp & Barrett, 2016). Thus, bone

112 length (particularly the femur, which closely correlates with stature) variation plays a role in

113 the robusticity and mechanical adaptation of long bones (Eveleth & Tanner, 1990; Porter, 1999;

114 Stock & Shaw, 2007), and can be linked to climate, geographical variables, and lifestyle,

115 (Pearson, 2000), manifesting as between-population differences in cross-sectional geometry

116 indicating biomechanical properties vastly modelled earlier in ontogeny (e.g. Holt, 2003;

117 Huffer & Oxenham, 2015; Kubicka et al., 2018; 2022; Macintosh et al., 2014; Rainville, 2001;

118 Stock, 2006).

119 Less documented has been inter-population variation in microscopic markers of bone

120 remodelling in adult cortical bone though histological approaches. Cho et al. (2002) noted that

121 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

216 recognised that these features are highly variable within a population and a degree of overlap
217 of phenotypes between the ancestral groups is expected.

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

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

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

239 Standard histology preparation methods were followed (Bancroft & Gamble, 2008). Extracted
240 samples were embedded in an epoxy resin solution (4:1 resin and hardener). Once set, each
241 sample was laterally reduced using a Kemet MICRACUT 151 Precision Cutter using a
242 diamond cutting Disc 150mm. Residual samples were kept for repatriation or further analysis.
243 Once mounted onto microscope slides with epoxy resin adhesive, each sample was ground and
244 polished using a Buhler® histolic precision grinding fixture on a Buehler® EcoMet 300
245 Grinder-Polisher pad to achieve an even thickness between 100+/-60μm. Using a Buehler
246 MicroPolish II 0.3μm powder and Buehler polishing cloth scratches were removed. Slides were

247 cleaned in an ultrasonic tub, a series of ethanol baths, and dipped in xylene to remove micro-
248 debris and water prior to adding a glass cover slip.

249 Thin sections were imaged using an Olympus BX53 high powered microscope with an inbuilt
250 DP74 camera using transmitted and polarised light. The Olympus cellSens 2018 imaging
251 software was used to scan each thin section completely at 4x magnification (Figure 3). Six
252 regions of interest (ROI) from the subperiosteal region of bone samples were determined from
253 the *linea aspera* outwards medially and laterally (Figure 4). Each ROI measured approximately
254 1.8 mm^2 and was scanned at 10x and 20x magnification. Only individuals that presented a
255 minimum of 25–50 intact osteons were required across each section to be viable for
256 histomorphometric analysis (Stout & Crowder, 2011). Many ROIs presented poor preservation
257 of histological features due to taphonomic alterations. This was assessed using the standard
258 Oxford Histological Index (OHI, see further below) (Hedges et al., 1995; Millard, 2001).

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

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

279　number of osteons counted using the 'multi-point' tool. Any histology features cut off at the

280　border of the ROI were not recorded.

281　**2.2. Data analyses**

282　Standard descriptive statistics were documented for each variable per individual. Distributions

283　for each variable across the sample population were established and analysed. Intra-observer

284　error was minimised by re-assessing histological features across a randomly selected sub-

285　sample that formed 10% of the entire sample. We elected to not run inferential statistical tests

286　because of the uneven and small sample size in some of the ancestry sub-groupings exacerbated

287　once age-at-death and sex are taken into account. Additionally, OPD was not tested against

288　N.On or N.On.Fg as the latter are components of the derived OPD variable. Data were analysed

289　descriptively using IMB SPSS 28 software by comparing all bone data between ancestry

290　groups. *Ex ante* evaluation of bone data variation with age-at-death and sex is included as

291　supplementary material.

292　**3. RESULTS**

293　Ancestry was defined here in three categories: Migrant (n = 8), Admixed (n = 5), and Local (n

294　= 4). An intra-observer error test yielded no major differences when comparing repeated

295　measures of data (Supplementary Table 2). The preservation of bone histology was within the

296　50% OHI mark, with some thin sections showing better preservation (up to 70–80%, but

297　not >85%), and others slightly lower than 50% but never less than ~45%. Bioerosion was

298　concentrated on the periosteal and endosteal borders of bone as is common in archaeology

299　(Hackett, 1981), but large areas of well-preserved cortical bone histology could be examined

300　for morphometric characteristics. Femoral robusticity and histology measurements sub-divided

301　by ancestry groupings are reported in Table 3. Robusticity and histology data across ancestry

302　groups are illustrated in Figure 6. For between group comparisons, we pooled age-at-death and

303　sexes mainly due to the small sample size (we will account for this when interpreting data), but

304　also because we assume ancestral history would override sexual dimorphism expression across

305　the entire sample (but not within each ancestry category, based on population-specific variation

306　in sexual dimorphism, see Ubelaker & DeGaglia, 2017; and also see Supplementary Tables 3–

307　5 for all data for 18 individuals sub-divided by age-at-death and sex; raw data can be accessed

308　via our figshare dataset at Walker et al., 2022b).

309   There was a clear variation in all the variables with our three ancestry categories. The longest

310   femora (mean 427.20 mm) along with the greatest stature estimates (mean 162.30 cm) were for

311   the Local individuals, whereas the Admixed individuals had the lowest values (mean femur

312   length = 402 mm, mean stature = 155.03 cm). The data for cortical width (mean 8.90 mm) and

313   shaft circumference (mean 86.60 mm) were also the greatest in the Local group, with the

314   Admixed (mean cortical width = 7.77 mm, mean shaft circumference = 80.67 mm) and Migrant

315   (mean cortical width = 7.44 mm, mean shaft circumference = 81.25 mm) categories showing

316   very similar values. This translated to the robusticity indices wherein the Local group still

317   showed the largest values (mean Ct.Wi.RI = 2.08, mean Shaft.Circ.RI = 20.32) compared to

318   the Migrant (mean Ct.Wi.RI = 1.71, mean Shaft.Circ.RI = 19.55) and Admixed (mean Ct.Wi.RI

319   = 1.83, mean Shaft.Circ.RI = 19.57) groupings. However, the robusticity index based on

320   cortical width values showed the largest range in the Admixed group indicating a relatively

321   wide degree of data variation (see **Figure 6**).

322   In terms of cortical bone remodelling indicators, it was the Admixed group that showed the

323   highest values of OPD (mean 23.68/mm^2), though it was followed closely by the Local group

324   (mean OPD = 22.27/mm^2) (**Table 3**). The Local individuals showed the largest range in OPD

325   values (see **Figure 6**), but it was somewhat alike in both the Migrant and the Admixed groups.

326   Measures of osteon area differed substantially between all the groups, with the Local

327   individuals having the largest osteons (mean On.Ar = 40,961.28 μm^2). The Admixed (mean

328   On.Ar = 34,805.65 μm^2) and Migrant individuals showed smaller osteons compared to the

329   Locals, with the smallest osteons found in the Migrants (mean On.Ar = 31,597.28 μm^2). The

330   Haversian canal data constitute the smallest number of canals represented out of all the

331   histology variables we considered, so we make a cautious observation that the Locals had the

332   largest canals (mean H.Ar = 1,022.03 μm^2) when compared to the Admixed (mean H.Ar =

333   849.92 μm^2) and Migrants (mean H.Ar = 593.17 μm^2).

334   In summary, the Local individuals exhibited the most robust diaphyseal circumference and

335   width along with the densest osteon densities accompanied by relatively large osteon and

336   Haversian canal parameters. The Migrants had the most gracile femoral shaft circumference

337   and width packed with least dense bone of small osteons and Haversian canals. The Admixed

338   individuals consistently fell between the Migrant and Local categories in terms of their femoral

339   robusticity and histology data. However, we highlight that the OPD data, which measure how

340   densely bone is remodelled per unit area, were in a tight range (mean OPD range = 20.88/mm^2
341   – 23.68/mm^2) across all three groups (**Table 3**).

342   **4. DISCUSSION**

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

347   **4.1. Mechanical and bone anatomical constraint interpretations**

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

361   Robust femora with evidence for slower remodelling in adulthood has been observed in other
362   anthropological and clinical studies (e.g. Miszkiewicz et al., 2022; Zebaze et al., 2010) because
363   bone will modify its physiological response depending on function (and other factors)
364   throughout the entire human lifespan. So, it is not surprising that bone robusticity markers
365   forming at childhood/adolescence may differ from remodelling markers operating in later
366   decades. For example, geometrically well-developed femoral cross-sections showing
367   substantial porosity and trabecularisation of cortex are commonly reported in modern patients
368   (e.g. Zebaze et al., 2010). This is also mirrored in the archaeological record, such as in a recent
369   study of behaviour in Bronze Age Iran where nomadic individuals showed robust femoral
370   cross-sections experiencing slower remodelling in older individuals (Miszkiewicz et al., 2022).

371 Thus, the separation between femoral robusticity characteristics, and the geometric histological
372 parameters, in our study, can relate to the effect of ancestry because all our three groupings
373 engaged in similar behaviours. Yet, the Local individuals clearly show different values from
374 the Admixed and Migrant categories of ancestry.

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

390 However, ancestry-based subsistence roles, where Locals experienced fewer or less strained
391 mechanical loads, would also produce larger strain related osteons. Similar amounts of osteon
392 densities were previously found in genetically distinct samples, such as in archaeological
393 Native American and modern European-Americans (though in the anterior femur cortex; Burr
394 et al., 1990); and in femoral and tibial cross-sections in a Native Americans, Late Archaic, and
395 Early Modern humans (Streeter et al., 2010); which matches our findings. An alternative
396 interpretation of the OPD data at Mán Bạc is that they simply reflect an average of tissue age
397 accumulated per ancestry. Our sample does contain a range of younger and older individuals,
398 so age-related progression in osteon formation can be confounding our results. Therefore, our
399 remarks here regarding OPD should be treated cautiously. It is possible that locals were
400 engaged in different behaviours—in other words, rather than a gendered division of labour
401 there was and ancestrally mediated division of labour. Alternatively, as we do not have

402 generational level resolution in dating, some local individuals may have been born and grew to
403 adulthood prior to integration with the migrants.

404 Collectively, our results do point in the direction of some behavioural influences on bone
405 morphology and microstructure, but for those to be possibly underlain by long term inherited
406 predisposition to certain morphology, because the local Mán Bạc populations stem from
407 distinct hunter-gatherers, whereas the migrant populations were predominantly farmers
408 (Matsumura, 2011b; Oxenham et al., 2018). We do know that hunter-gatherer and farmer long
409 bone robusticity, both in the lower and upper limb, has been subject to a marked decline,
410 exacerbated by sexual dimorphism (Ruff, 2018). It is likely that evolutionary mechanisms
411 (such as natural selection) selecting for optimal bone remodelling occurred in response to
412 divergent subsistence and behavioural strategies at Mán Bạc, ultimately favouring survival
413 over a long period of time. Disparate experiences amongst the farmers and hunter-gatherers of
414 Mán Bạc include adaptation to two different climates (warm vs. cold) over the span of tens of
415 thousands of years (Oxenham & Buckley, 2015). More recently, adaptive changes may have
416 occurred with the adoption of agriculture by the ancestors of the migrant population who had
417 begun domesticating animals as early as 9,000 years ago in modern day China (Bellwood,
418 2005). Human self-domestication, with the adoption of agricultural subsistence driving
419 increased gracility of the skeleton (Leach, 2003), may have further contributed to
420 microscopically observable bone changes, as seen in the Mán Bạc Migrant data.

421 The human self-domestication hypothesis proposes that, as was the case for domestication of
422 animals, selection for lower aggression in agricultural communities requiring co-operation for
423 success led to biological phenotypical change in humans as a secondary consequence
424 (Sánchez- Villagra & Van Schaik, 2019). This would explain the increased gracility of the
425 human skeleton, including the skull, alongside agricultural transitions as reflected in the
426 Migrant sub-sample in our study. Huffer and Oxenham (2015) who investigated long bone
427 cross-sectional geometry and entheseal morphology in the Mán Bạc individuals found data
428 trends changes towards sedentary activities along with increasing adoption of agriculture and
429 a decline in mobility over time. Their findings are somewhat mirrored in our data for robusticity
430 indices based upon cortical width data, where the Local and Migrant individuals showed
431 smaller ranges of variability compared to the Mixed individuals (**Figure 6**). The femora of
432 Mixed individuals had both very gracile and very robust shafts, which still likely reflect
433 possible ancestry-specific underpinning to femoral morphology. The Migrant individual

434 gracile skeletal profiles added to the agricultural behavioural changes. Having said this, Huffer
435 and Oxenham (2015) also found distinct sex-specific differences in long bone activity markers,
436 which might also play a role in the combination of our histology and femoral robusticity
437 measures per ancestry.

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

446 **4.2. Limitations and suggestions for future research**

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

463 **5. CONCLUSIONS**

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

492 **CONFLICT OF INTEREST STATEMENT**

493 Authors declare no competing interests.

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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943 **FIGURE CAPTIONS**

944 **FIGURE 1**. Right human femur (posterior view, left side of the image) with marked midshaft
945 cross-section (black rectangle) illustrated at the histological level (right side of the image).
946 Secondary osteons are the circular structures tightly packed in the adult cortical wall of the
947 long bone. A posterior 'quadrant', separated from the remainder of the cross-section, is the
948 subject of the present study.
949

950 **FIGURE 2**: Differences in phenotypic cranial features distinguishing local from migrant
951 groups at Mán Bạc: (A) round versus square orbits; (B) moderate versus prominent glabella;
952 (C) broad and rounded versus tear drop nasal aperture; (D) high versus low and wide face; (E)
953 mesocephalic versus dolichocephalic cranium; (F) flat versus prognathic maxilla; (G) thin and
954 elongated versus short and broad mandible.
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956 **FIGURE 3.** Examples of posterior femur thin sections removed from each Mán Bạc individual,
957 showing a range of sizes and preservation. All images are shown with the posterior *linea aspera*
958 aspect oriented towards the top of the image. Individual accession numbers are shown in the
959 'empty' medullary space.
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961 **FIGURE 4.** Imaging method for selecting six regions of interest across each thin section at
962 10x magnification. Adapted from Miszkiewicz (2016: 180).
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964 **FIGURE 5.** Cortical bone histology images (A: transmitted light, B: linearly polarised light)
965 from femoral samples in individual MN0741M5, illustrating counts of secondary osteons (A);
966 and Haversian canals (HC) and cement lines (indicated with black arrows) traced for the
967 collection of area measurements.
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969 **FIGURE 6.** Femur morphology and histology across ancestry groups. Graphs A and B show
970 femur robusticity measure distribution with Ancestry, whereas graphs C and D focus on
971 Haversian bone histomorphometric variables.
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979 **TABLES**

980 Table 1. Examples of studies where comparisons of bone remodelling based on cortical bone
981 histology parameters were made between spatially and temporally diverse populations.

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

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

ANCESTRY	AGE AND SEX		N
Migrant	Young adult	Female	1
		Male	1
	Middle-aged adult	Male	1
	Mature adult	Female	2
		Male	2
	Adult	Indeterminate	1
		Total	**8**
Admixed	Mature adult	Female	1
		Male	2
	Adult	Female	1
		Total	**4**
Local	Young adult	Male	2
	Middle-aged adult	Male	2
	Mature adult	Male	1
		Total	**5**

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990 **Table 3.** Cortical bone histology variables examined in the present study.

VARIABLE	DEFINITION
Cortical area (Ct.Ar) in mm^2	Total area of bone excluding trabeculae struts (Dominguez & Agnew, 2019). Transmitted light, magnification 4x.
Cortical Width (Ct.Wi) in mm	Maximum distance between the periosteal and endosteal envelope at the linea aspera. Measured between two points parallel to one another (Miszkiewicz & Mahoney, 2019). Transmitted light, magnification 4x.
Oxford Histological Index (OHI)	Percentage of bone area impacted by biodegradation as per Hedges (2002) and Millard (2001). Transmitted light, magnification 4x.
Total sampled bone area (B.Ar) in mm^2	Sum of cortical bone area measured across ROIs (Stout & Crowder, 2012). Transmitted light, magnification 20x.
Oxford Histological Index (OHI)	Bone areas impacted by bioerosion scored on a 0-5 scale based on percentage (Hedges et al., 1995; Millard, 2001). Transmitted light, magnification 20x.
Osteon population density (OPD)/mm^2	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 & Crowder, 2012). OPD is (N.On + N.On.Fg)/ /B.Ar (Drapeau & Streeter, 2006). Transmitted light, magnification 20x.
Osteon area (On.Ar) in μm^2	Mean area of intact secondary osteons with a complete cement line (Stout & Crowder, 2012). Transmitted light, magnification 20x.
Haversian canal area (H.Ar) in μm^2/canals	Mean area of complete canals with no evidence of resorption. Transmitted light, magnification 20x.

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1001 **Table 4.** Descriptive statistics outlining the parameters of robusticity indices, and histological
1002 features of ancestry groups.

VARIABLE	N	MIN.	MAX.	MEAN	SD
Migrant					
Stature	6	152.20	169.40	160.07	6.15
Femur length (mm)	6	397.00	467.00	420.83	28.55
Ct.Wi (mm)	7	6.51	8.89	7.44	0.84
Shaft Circ. (mm)	8	76.00	91.00	81.25	4.62
Ct.Wi.RI (*unitless)	5	1.39	1.92	1.71	0.22
Shaft.Circ.RI*	6	18.74	20.91	19.55	0.84
OPD/mm^2	8	11.30	28.45	20.88	4.92
On.Ar (μm^2)	7	21,792.46	40,049.52	31,597.28	7,205.71
H.Ar (μm^2)	2	591.47	594.87	593.17	2.40
Admixed					
Stature	4	152.50	156.90	155.03	2.10
Femur length (mm)	3	396.00	412.00	402.00	8.72
Ct.Wi (mm)	4	4.59	9.88	7.77	2.35
Shaft Circ. (mm)	3	77.00	84.00	80.67	3.51
Ct.Wi.RI (*unitless)	3	1.11	2.49	1.83	0.69
Shaft.Circ.RI*	2	18.69	20.45	19.57	1.23
OPD/mm^2	4	21.13	26.64	23.68	2.67
On.Ar (μm^2)	3	34,562.39	35,201.09	34,805.65	345.48
H.Ar (μm^2)	2	846.35	853.50	849.92	5.06
Local					
Stature	5	155.50	171.50	162.30	6.59
Femur length (mm)	5	388.00	481.00	427.20	38.31
Ct.Wi (mm)	5	7.85	10.55	8.90	1.16
Shaft Circ. (mm)	5	82.00	96.00	86.60	5.59
Ct.Wi.RI (*unitless)	5	1.99	2.19	2.08	0.08
Shaft.Circ.RI*	5	19.33	21.39	20.32	0.99
OPD/mm^2	5	14.32	27.17	22.27	5.76
On.Ar (μm^2)	5	31,190.46	48,733.71	40,961.28	8,290.73
H.Ar (μm^2)	3	760.30	1,368.52	1,022.03	312.84

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