Can growth in captivity alter the calcaneal microanatomy of a wild ungulate?

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Abstract

16   Reduced mobility associated with captivity induce changes in biomechanical stress on the

17   skeleton of domesticated animals. Due to ~~bone~~ plasticity, ~~the~~ morphology and ~~the~~ internal

18   structure ~~of the bones~~ can respond to these new biomechanical stresses over individuals'

19   lifetime. In a context where documenting early process of animal domestication is ~~a real~~

20   ~~zooarchaeological challenge,~~ this study will test the hypothesis that change in mobility patterns

21   during ~~the life of~~ a wild ungulate will alter internal structure of its limb bones and provide a

22   proof of concept for ~~its~~ application in Zooarchaeology. Using the calcaneus as phenotypic

23   marker through qualitative and quantitative 3D microanatomical analyses, we relied on a

24   comparative study across wild boars (*Sus scrofa*) populations from controlled experimental

25   conditions with different mobility patterns (natural habitat, large pen and stall) and

26   archaeological specimens collected from middle and late Mesolithic as surrogate for the norm

27   of reaction in European wild boar phenotype before the spread of agriculture and domestic pigs.

28   Results provide evidence for compressive and tensile forces as main elements affecting the

29   variation in the cortical thickness along the calcaneus. Furthermore, changes in the internal

30   structure of the calcaneus between mobility patterns are observed but their intensity is not

31   directly associated with the degree of mobility restriction and only weakly impacted by the size

32   or weight of the individuals. Despite having greater bone volume, the calcaneus of the

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33   Mesolithic wild boars displays a very similar microanatomy compared to the present-day
34   hunted or captive wild boars, these results suggest that calcaneus microanatomy is more
35   affected by population differences than by differences in locomotor contexts. For all these
36   reasons, this preliminary study doesn't support the use of microanatomy of the calcaneus as an
37   indicator of change in locomotor behaviour induced by captivity in the archaeological record.

38

39   Keyword : calcaneus; microanatomy; captivity; domestication; *Sus scrofa*; functional
40   morphology

41

Introduction

43

44   The bones that make up the vertebrate skeleton are plastic organs whose morphology, and to a
45   larger extent, internal structure, adapt in response to the stresses placed on individuals (Roux,
46   1881; Wolff, 1986; Hall, 1983, 2005; Ruff et al., 2006; Du et al., 2020), beyond the predominant
47   influence of heredity (Hall, 1989; Cubo et al., 2005). Muscle mobilization applies stresses that
48   influence bone growth, development and remodeling (Marcus, 2002). Numerous studies on
49   human skeletons have shown that intensive physical activity has characteristic consequences
50   on skeletal microanatomy that differ pending on the activity (Zanker & Swaine, 2000;
51   Modlesky et al., 2008; Maïmoun & Sultan 2011; Maïmoun et al., 2013). Conversely, the bone
52   resorption observed for astronauts (Lang et al., 2004) and bedridden individuals (Krølner &
53   Toft., 1983) illustrates bone accommodation to the absence of gravity (Carmeliet & Bouillon,
54   2001) and inactivity. Therefore, differences in mobility between individuals, that engenders
55   differences in biomechanical stresses, affect the organization of the skeleton.

56   Captive animals may grow in limited areas, which does not involve the same range of
57   movement and forces applied on their bones compared to their free-ranging relatives. Analysing
58   the biomechanical bone plasticity is a major asset for paleoanthropologists and
59   archaeozoologists trying to decipher individual-scale lifestyle changes from bones anatomy in
60   order to document change in activity patterns of past humans and other animals (Trinkaus et
61   al., 1994, Agarwal, 2016). In paleoanthropology, this biomechanical component has been used
62   to better understand the evolution towards bipedalism in hominids (Ruff, 2018) and to observe
63   the morphological consequences of the transition from a nomadic hunter-gatherer to a farmer-
64   herder lifestyle (Pinhasi & Stock, 2011). In archaeozoology, changes between a domestic and
65   a wild lifestyle related to locomotion and gait changes, have been estimated as a prevalent factor
66   over load-carrying on structural changes of domestic donkeys limb bones (Shackelford et al.,

67 2013). Despite this, archaeozoologists have always considered that the first observable

68 morphological changes occurred only late in the domestication process and could not perceive

69 its initial stages since they would not involve genetic isolation or selective reproduction (Vigne

70 et al., 2011; Colledge et al., 2016). To develop new phenotypic proxy of the first steps of animal

71 domestication, an experimental project (DOMEXP) has been performed to test whether growth

72 in captivity of a wild ungulate would leave a morpho-functional imprint distinguishable from

73 the its natural habitat norm of reaction. Hence, early process of cultural control (Hecker 1982)

74 could be traced from bones anatomy long before the so-called "domestication syndrome". To

75 test the hypothesis, the DOMEXP project used a genetically homogeneous wild boar (*Sus*

76 *scrofa*) population to control

77 the genetic and environmental

78 factors influencing skeletal

79 variation. From this

80 populations 6 months old

81 piglets were captured after

82 weaning to be reared until the

83 age of two years under two

84 mobility reduction regimes.

85 Geometric morphometrics'

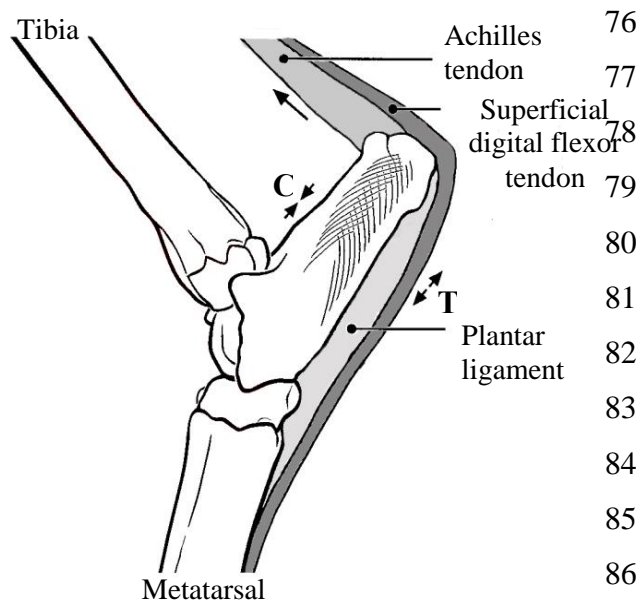86 analyses have provided proof

87 of concept that this mobility

Fig. 1. Lateral view of the tarsal region of a skeletally mature artiodactyl, showing the calcaneus with other associated bones, ligaments and tendons. The large arrow in the dorsal direction indicates the orientation of the force exerted by the Achilles tendon during paw extension, loading the dorsal cortex in compression ("C") and the plantar cortex in tension ("T"). Modified from Su et al. (1999).

reduction in a wild ungulate population could leave morphometric prints on skulls (Neaux et al., 2021) and calcaneus (Harbers et al.,

93 2020a). Microanatomical investigations have also revealed changes in the 3D topography of

94 the cortical thickness in the humeral shaft (Harbers et al., 2020b). However, the impact of

95 mobility reduction on the micraoantomical features of the skeleton remained to be explored.

96 For this reason, this study investigates the microanatomical variation of the calcaneus from the

97 experimental specimens of the DOMEXP project. Indeed tarsal bones like the calcaneum could

98 be considered as more informative than others in investigating the mechanical forces that apply

99 to individuals during locomotion (Lovejoy et al., 1999; Carter & Beaupré, 2001; Pearson &

100 Lieberman, 2004). Proximally articulated to the fibula and distally to the cuboid bone, the

101   calcaneus is ~~particularly~~ stressed during locomotion in terrestrial tetrapods. It acts as a lever

102   arm for the ankle extensors, and is subject to high tensile, flexural, and compressive forces (Fig.

103   1; Hussain, 1975; Carrano, 1997; Bleefeld & Bock, 2002; Bassarova et al., 2009; Barone, 2017).

104   The calcaneus is therefore a particularly interesting object of study to investigate differences in

105   mobility between individuals (Harbers et al., 2020a). Moreover, the high compactness of the

106   calcaneus makes it resistant to taphonomic alterations, which ~~gives it a good general~~

107   ~~preservation~~ in archaeological contexts (Binford, 1978) ~~and highlights its archaeozoological~~

108   ~~interest.~~

109   This study compares the calcaneus microanatomy variation between adult captive-bred and

110   ~~hunted~~ wild boars to assess ~~how much~~ captivity can induce microanatomical variation in a wild

111   ungulate. We also include ~~8000 years old~~ wild boars from a Mesolithic context of hunter-

112   gatherers in northern France to observe how much the norm of reaction in wild boar

113   microanatomy has changed since the neolithisation of Europe and whether this proxy can be

114   used to infer change in locomotor behaviour from archaeological ~~bones~~.

115

116   **Material and method**

117

118   ## Experimental protocol

119   24 wild boars aged 6 months were sampled from a control population living in a fenced forest

120   from Northern France ~~of 100,000 m²~~. These specimens were then divided in two equal samples

121   ~~of~~ same sex ratio and reared until 24th in two different contexts of reduced mobility: (1) a stall

122   of 100 m², where males and females were separated (each group had 45 m²) and (2) a wooded

123   pen of 3000 m². These two contexts represent respectively 99.9% and 97% of range reduction

124   compared to the control population which prevent the average daily distances measured in free

125   boar populations (Palencia et al. 2019; Russo et al. 1997). The stall presented no opportunity

126   for foraging. In the wooded enclosure, this opportunity was limited due to space limitations.

127   Dry feed pellets suitable for feeding domestic pigs were provided to both groups.

128

129   ## Specimens studied

130   Our dataset includes calcaneus bones from 47 specimens: 26 from the DOMEXP experimental

131   farm - 11 stall specimens (stall), 10 pen specimens (pen), 5 from the control population (control)

132   - 15 hunted wild boars from two Northern France forests: 8 from the Chambord forest and 7

133   from the Compiègne forest (Natural habitat) and 6 archaeological specimens from the

134 Mesolithic contexts (Meso) of Noyen sur Seine (Mordant et al., 2013, Marinval-Vigne et al.,

135 1989) in Northern France (Table 1).The six archaeological specimens from Meolithic deposits

136 have been accumulated by hunter-gatherer living in Western Europe before the Neolithic

137 dispersal via the Mediterranean and Danubian pathways which have introduced domestic pigs

138 from the Near East (Larson 2006) and interbred with local populations of wild boars (Frantz et

139 al. 2020). Two specimens have been collected from the Middle Mesolithic radiocarbon dated

140 between -8000 and -7300 cal. BP and four specimens from the Final Mesolithic dated between

141 -7000 and -6200 cal. BP (Mordant et al., 2013). All the specimens bar the archaeological

142 specimens could have associated age and sex informations. 3 of the archaeological calcanei

143 belong to adults specimens since they have their proximal epiphysis known to fuse around 2

144 years in wild boars (Bridault et al., 2000).

Data acquisition

146 All the calcaneus of the dataset have been scanned by high-resolution microtomography

147 (EasyTom 40-150 scanner, RX Solutions) at the MRI platform, hosted at ISEM, University of

148 Montpellier (UMR 5554); reconstructions were then performed using X-Act (RX Solutions).

149

Virtual thin sections

151 For qualitative comparisons, three virtual sections (Fig. 2, Appendix 1,2 and 3) were made for

152 each calcaneus, following Barone (2017) for terminology and orientations. The bones were

153 oriented as follows: in dorsal view (Fig. 2a), bone's axis is vertical and the fibular trochlea is

154 oriented toward the observer, its dorsal part aligns with the contour of the bone's medial border;

155 in medial view (Fig. 2b) the *sustentaculum tali* is directed towards the observer, the observation

156 angle is fixed when the anterior edge of the fibular trochlea is no longer visible upon rotation

157 from the anterior view to the medial view. Sagittal sections (SS) run in dorsal view from the

158 distal end tip and the midpoint of the thickness at the proximal epiphysis base (Fig. 2). The

159 frontal sections (FS) extend from the distal end tip to the midpoint of the proximal epiphysis

160 base (see purple arrows on Fig. 2b). Cross-sections (TS) are taken perpendicular to the FS plane

161 at 1/3 of the total length of the bone (from the proximal epiphysis tip to the distal tip) (Fig. 2).

162 These sectional planes were chosen to depict large portions of the bone in order to analyze the

163 microanatomical structure (e.g., trabecular network, cortical thickness) while being easily

164 created with good reproducibility for all specimens. Virtual sections were created using

165 VGSTUDIO MAX, versions 2.2 (Volume Graphics Inc.).

166

Calcaneus 3D mapping

To observe and measure the thickness variations of the compact cortex along the calcaneus, bone tissue was segmented (excluding soft tissue and cavities) using image data reconstructed with Avizo 9.4 (VSG, Burlington, MA, USA). Then, we isolated the outer cortex from the trabecular bone, limited by the inner surface of the cortex for each bone. This segmentation step was done manually with a combination of Avizo's "remove islands" (to eliminate isolated volumes that are too small) and "smooth labels" (to connect the slices selection more realistically) functions to optimize the segmentation repeatability and consistency. Then, the distances between the inner and outer surfaces of the cortex were calculated in Avizo 9.4 using the "surface distance" function. Finally, a distance isosurface was obtained with a colour gradient that appears on the external surface of the bone (Fig. 4; Appendix 4). This colour gradient showing the relative variation of cortical thickness within each bone, is specific to each specimen since it varies between the minimum and maximum cortical thicknesses, warmer colours being used for higher thicknesses, and colder colours for lower thicknesses. Therefore, two specimens with similar colorimetry may have different absolute cortical thicknesses.

Quantitative parameters

Quantitative parameters used to characterize the internal structure of the bones are: (1) cortex/medullary area volumes, (2) overall bone and trabecular tissue compactness, and average
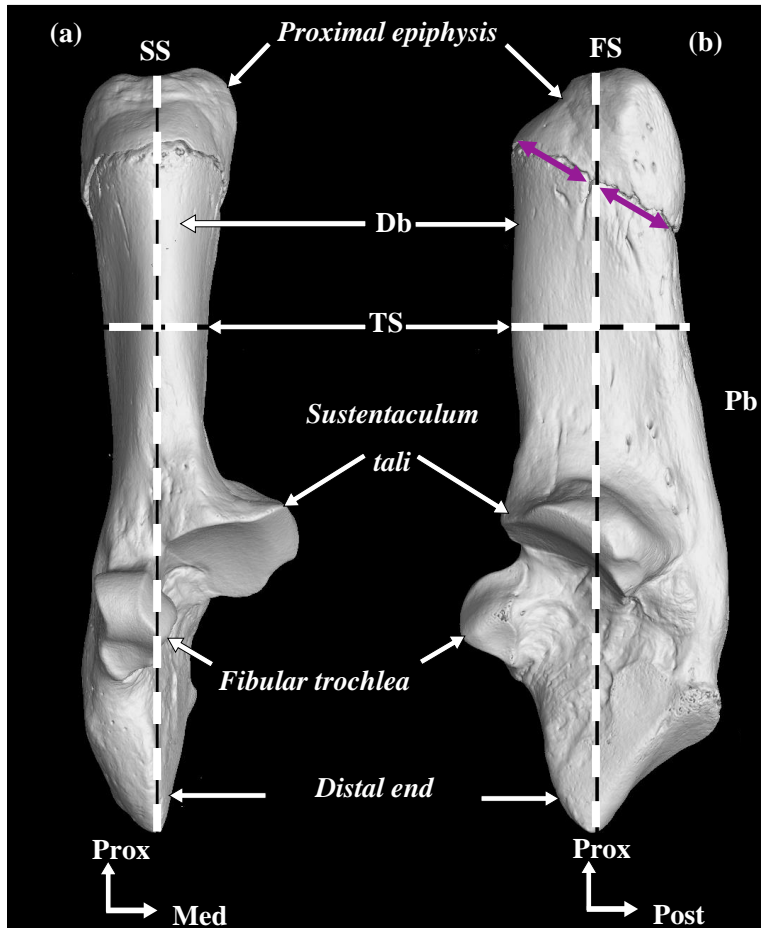
Fig. 2 Calcaneum of *Sus scrofa,* specimen 2017-? illustrating the planes of the virtual sections: SS, Sagital section; FS, Frontal section; TS, Transverse section. Db, Dorsal border; Pb, Plantar border. Purple arrows indicate the midpoint of the proximal epiphysis base.

and (3) maximum cortical thicknesses following Houssaye et al. (2021). Most are ratios produced using the volume values obtained from the "material statistics" function in Avizo after the segmentation and cortex isolation steps. The parameters used in the statistical analyses in this study are:

1) whole bone volume in mm^3 (WBV), as an indicator of size; 2) bone compactness (C= bone tissue volume*100/WBV); 3) relative trabecular bone tissue fraction (%Trab = trabecular bone tissue volume*100/bone tissue volume); 4) trabecular compactness (Tc= trabecular bone tissue*100/trabecular volume). From the mean (MeanT) and maximum (MaxT) cortical thicknesses that were obtained directly in Avizo 9.4 using the 'distance' function were calculated 5) RMaxT and 6) RMeanT, relative maximum and mean thicknesses, by dividing MaxT and MeanT by a mean radius r, obtained from the whole bone volume and considering that the calcanea are cylinders (as v=πr² h, r= √(v /hπ)).

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Table. 1 List of material and different parameters used in this study where each row corresponds to a specimen. Ctxt, Context; Prov, Provenance; WBV, total bone volume mm3; C, compactness ratio; %Trab, percentage of trabecular bone volume to cortical bone volume; TC, trabecular compactness; RMeanT, mean relative cortex thickness; RMaxT, maximum relative cortex thickness. Nh, Natural habitat; St, Experimental stall; Co, Experimental control; M, Mesolithic; P, Experimental pen; Comp, Compiègne; Cham, Chambord; Noy2/3, Noyen-sur-seine 2/3; Urc, Urcier.

Ctxt	Prov	Sex	Age (month)	Weight (kg)	WBV (mm3)	C	%Trab	TC	RMeanT	RMaxT
Nh	Comp	F	10	77	15252	81	28	57	0,08	0,34
Nh	Comp	M	13	64	18441	73	43	56	0,05	0,19
Nh	Comp	M	18	113	20011	83	27	61	0,10	0,44
Nh	Comp	F	12	61	16112	85	31	67	0,09	0,39
Nh	Comp	M	17	86	20892	82	35	65	0,08	0,32
Nh	Cham	F	36	52	11552	81	40	64	0,07	0,19
Nh	Cham	M	72	123	19932	87	30	68	0,10	0,31
Nh	Comp	F	18	61	14069	86	35	69	0,08	0,34
Nh	Comp	.	2	.	866	82	56	72	0,04	0,13
Nh	Cham	F	17	.	8541	65	51	51	0,07	0,21
Nh	Cham	F	18	.	13125	70	35	49	0,07	0,28
Nh	Cham	F	20	.	11478	78	36	59	0,07	0,22
Nh	Cham	F	18	.	13698	71	41	53	0,05	0,15
Nh	Cham	F	19	.	12480	78	26	50	0,09	0,17
Nh	Cham	F	19	NA	10870	77	31	55	0,08	0,29
St	LHT	F	25	91	13301	73	59	78	0,06	0,19
St	LHT	F	25	62	9179	75	42	60	0,06	0,20
St	LHT	F	25	67	13416	78	42	62	0,06	0,18
St	LHT	F	25	89	15853	73	39	53	0,05	0,19
St	LHT	F	25	5	15059	73	39	54	0,07	0,17
St	LHT	F	25	91	12971	79	34	59	0,08	0,30
P	LHT	M	25	84	14344	75	34	53	0,07	0,22
P	LHT	F	25	92	15051	84	36	68	0,09	0,26
P	LHT	F	25	54	16201	85	33	69	0,09	0,36
P	LHT	M	25	67	14028	76	34	54	0,08	0,22
St	LHT	M	25	84	20258	79	49	66	0,06	0,18
St	LHT	M	25	89	22432	73	37	57	0,07	0,22
St	LHT	M	25	78	20155	81	44	70	0,07	0,21
St	LHT	M	25	96	22497	74	41	58	0,07	0,21
St	LHT	M	25	73	19239	72	42	55	0,06	0,18
P	LHT	M	25	91	19452	82	37	66	0,07	0,24
P	LHT	F	25	67	15750	77	33	54	0,07	0,22
P	LHT	M	25	84	21659	74	37	55	0,08	0,27
P	LHT	M	25	90	18859	71	31	47	0,07	0,25
P	LHT	M	25	87	19942	75	37	54	0,07	0,22
P	LHT	M	25	92	20783	77	33	56	0,08	0,26
Co	Urc	M	20	53	13733	76	46	61	0,05	0,19
Co	Urc	M	8	35	13711	71	55	60	0,04	0,17
Co	Urc	M	20	52	14446	77	50	64	0,05	0,22
Co	Urc	F	84	110	24561	65	64	56	0,04	0,14
Co	Urc	F	96	60	16299	79	49	66	0,06	0,19
M	Noy3	.	.	.	15154	73	43	55	0,04	0,27
M	Noy3	.	.	.	16109	67	50	54	0,05	0,23
M	Noy3	.	.	.	16144	76	54	79	0,07	0,26
M	Noy3	.	.	.	34372	75	36	55	0,06	0,26
M	Noy2	.	.	.	23577	88	20	64	0,13	0,48
M	Noy2	.	.	.	25740	76	30	51	0,08	0,26

211

8

Statistical analysis

A linear regression model ("lm" function of the "stats" package) as well as a regression coefficient ("cor" function of the "stats" package) were used to assess the linear relationships between the variables (1) whole bone volume (WBV; considered as an estimate of bone size), (2) weight, and (3) age of each individual with all the other quantitative parameters used (Table 2). To explore the distribution of specimens based on their quantitative microanatomical parameters and the variation patterns, we performed a standardized Principal Component Analyses (PCA; "dudi.pca" function of the ade4 package; David and Jacobs 2014).

In order to estimate the role of factors such as Sex (male/female/indeterminate), origin (Compiègne/ Chambord/ Urcier/ La Haute Touche/ Noyen sur Seine) and mobility status (Natural habitat /Experimental control/ Experimental pen/ Experimental stall/Mesolithic wild boars) in the variation of the quantitative microanatomical parameters, we used analyses of variance (ANOVA; function "anova_test" of the "rstatix" package) after checking the conditions of normality (function "shapiro_test" of the "rstatix" package) and homogeneity of variances (function "levene_test" of the "rstatix" package). When overall difference is significant, we computed pairwise comparison tests using Tukey hsd tests ("tukey_hsd" function of the "rstatix" package). When the variables did not meet the conditions of homogeneity of variances and/or normality, we used the kruskal-Wallis test (function "Kruskal_test" of the "rstatix" package), a non-parametric alternative to ANOVA. When this test is significant, a Dunn's test ("dunn_test (p.adjust.method="bonferroni")" function of the "rstatix" package) is used to compare pairwise differences between the groups concerned.

MANOVA ("res.man" function) was also used to test the overall difference in microanatomical variables between mobility contexts. Statistical tests and graphical representations were performed in R (R Core Team. 2017) using the Rstudio software (see appendix 5 and 6 for the script and the data table).

Results

Qualitative descriptions
General microanatomical pattern

The general microanatomical pattern of the wild boar calcaneus is close to the structure of long bones of terrestrial mammals, with the cortex forming a tubular diaphysis but with a very short diaphyseal part (sections Fig. 3). The proximal epiphysis is not fused to the rest of the bone for the most part of the specimens, except for the oldest. The cortex surrounds cancellous bone (trabecular bone and intertrabecular spaces), which is essentially quite dense, and a small open

246 medullary cavity, about 1cm (Pradat187; Fig. 3c,h) to 2 cm long (2013-1286; Fig. 3d,i) and 1

247 cm wide, at the level of the *Sustentaculum tali* (FigXa-j). The thickness of the trabeculae is

248 relatively homogeneous along the calcaneus, except around the medullary cavity, where they

249 are generally thicker (Fig. 3a-j). Also, the trabecular density is heterogeneous, with some

250 specimens having many trabeculae (2017-570; Fig. 3b,g,l) while others have twice as less but

251 thicker trabeculae (2013-1286; Fig. 3 d,I,n). Finally, the bone density in the distal part strongly

252 varies between individuals from compact (2017-570; Fig. 3b,g) to spongious (Calc2139; Fig.

253 3e,j).

Sagittal sections

255 In all specimens, the dorsal and plantar margins (Db and Pb) show a relatively high cortical

256 thickness, especially at mid-diaphysis. Spongiosa show anisotropic trabeculae (i.e. with a

257 preferential direction) above the medullary cavity in the bone center (Fig. 3a-e). Anisotropic

258 trabeculae follow the two main directions that are represented with green/outline arrows in the

259 figure 3a and schematized with intersecting lines on figure 1. The cortical thicknesses of the

260 plantar and dorsal margins vary from thick (2017-570; Fig. 3b) to proportionally twice as thin

261 (Pradat187; Fig. 3c). A variation in the cortical thickness of the plantar border of the proximal

262 epiphysis is also observed, it is very thin (1mm) in some specimens like Pradat 187 (Fig. 3c)

263 while others, such as 2017-555 (Fig. 3a), show a clear thickening of the cortex (2mm) in this

264 area. Similarly, bone density is compact like as cortex next to the fibular trochlea for numerous

265 specimens (Calc2139; Fig. 3e) or rather spongious for some individuals (2013-1286; Fig. 3d).

266

Frontal sections

268 All individuals have a cortex that varies relatively little in thickness (about 2-3mm) on the

269 medial and lateral sides (Fig. 3f-e). In contrast, the cortical thickness in the proximal epiphysis

270 varies from thin (2013-1286; Fig. 3i) to twice thicker (2017-555; Fig. 3a). Similarly, the

271 compactness and the cortical thickness of the *sustentaculum tali* varies between individuals,

272 some of which show compact bone and thick cortex (Padat187; Fig. 3h) while others show

273 spongious bone and thin cortex (2013-1286; Fig. 3i).

274

Transverse sections

276 The sections shape is generally oval and elongate (2017-555; Fig. 3k) but several specimens

277 present a rounder section (2017-570; Fig. 3l). The cortical thickness is fairly constant across

278   the sections, but some specimen show a cortical thickening at the plantar border (2013-1286;

279   Fig. 3n).

280

281   None of the variation observed between specimens is clearly associated to any main parameter

282   of the study, namely the context, provenance, sex, size or weight.

283

Fig. 3 Virtual thin sections of the calcaneus of boars, a,f,k, 2017-555 (Experimental stall); b,g,l 2017-570 (Experimental pen); c,h,m Pradat187 (Experimental control); d,i,n, 2013-1286 (Natural habitat); e,j,o, Calc2139 (Mesolithic). Db, Dorsal border; De, Distal end; Ft, Fibular trochlea; Pb, Plantar border; Pe, Proximal epiphysis; St, *Sustentaculu tali*. Scale bars of sagittal and frontal sections equal 2 cm; Scale bars of transverse sections equal 1 cm.

3D mapping of the cortical thickness

284

285 The 3D mappings of the cortical thickness and its variation across each bone, are quite similar

286 between specimens (Appendix 1). In agreement with the observation of the virtual sections,

287 there is a fairly extensive area with greater cortical thickness at the plantar border of the

288 calcaneus (Pb; Fig. 4A). Although generally less extensive, thickenings of the cortex can also

289 be noted on the dorsal margin (Db) and on the dorsal base of the *sustentaculum tali* (Sb; Fig.

290 4B). There is little thickening of the cortex on the proximal end (Pe). Finally, there is no lateral

291 or medial thickening noted.

292 25 of the 47 specimens show strong cortical thickening at the plantar margin (Pb; Appendix 1),

293 like in 2017-568 (Fig. 4A), this is less visible in the 22 others, like Pradat 185 and 2013-1264

294 (Fig. 4D; Fig. 4G). 23 specimens have thick cortical bone at the dorsal margin (Db; Table 1;

295 Appendix 1), like in Pradat 185 and 2017-568 boars (Fig. 4B and Fig. 4E), whereas the others

296 do not show such thickening like the calcaneus of boar 2013-1264 (Fig. 4H). 28 specimens

297 show a relative thickening at the base of the sustentaculum (Sb; Table 1), this widening is

298 particularly noticeable in boar 2013-1264 (Fig. 4H) and absent in others like Pradat 185 (Fig.

299 4E). A slight recurrent cortical thickening on the distal end is observed for a part of the

300 specimens like 2017-568 (Fig. 4a). 27 specimens have a slightly thicker cortex on the proximal

301 end (Pe) such as 2017-568 (Fig. 4C), 15 show no or very little thickening, such as Pradat185

302 and 2013-1264, respectively (Fig. 4F and Fig. 4I). 4 of the proximal ends of the Mesolithic

303 specimens were not found because they were not fused to the rest of the bone.
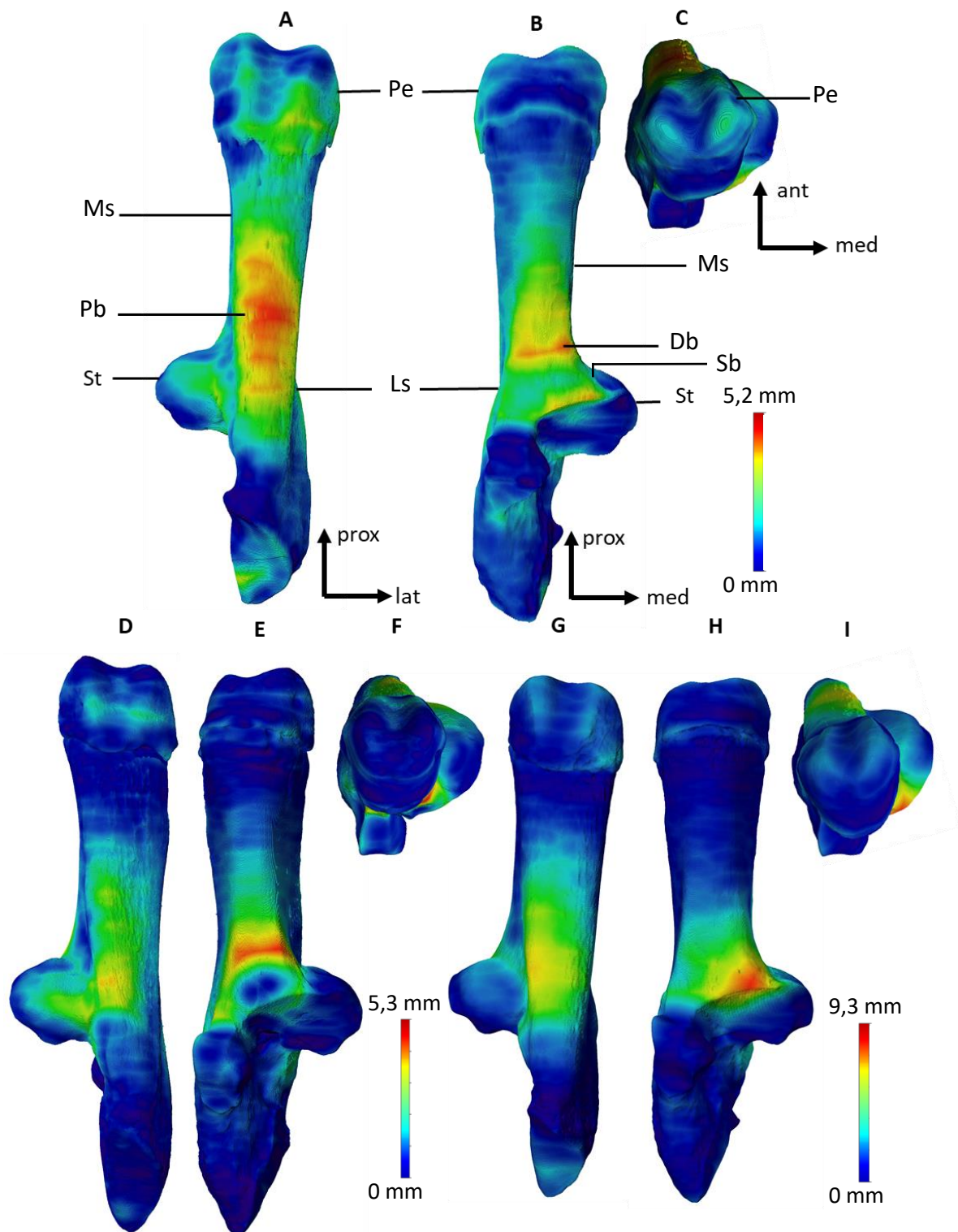
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Fig. 4 3D mappings of the *Sus scrofa* calcaneus relative cortical thickness. A-C specimen 2017-568; D-F Pradat 185; G-I 2013-1264. Anatomical abbreviations: Db, dorsal border; Ls, lateral side; Ms, medial side; Pb, plantar border; Pe, proximal epiphysis; Sb, Sustentaculum base; St, *Sustentaculum tali*. D, A and G are in plantar view; E, B and H are in dorsal view; F and I and C are in posterior view. Abbreviations for orientations, prox, proximal; lat, lateral; med, medial; post, posterior; ant, anterior.

307

Quantitative analyses

Microanatomical covariation with Weight, age size and sex.

The Whole bone volume (WBV) is expectedly only correlated to the body weight and the PCA axes (Table. 2). Only %Trab is significantly correlated with specimen age with a slight increase in the proportion of trabecular bone over cortical bone as boars get older (r=0.32).

Variables C, %Trab, RMeanT, RMaxT, weight, and TC did not differ between males and females. Only whole calcaneus volume (WBV) variation differs with sex (Kruskal Wallis: p<0.01) with males larger than females (Fig. 6). Mesolithic specimens (sex unknown) have larger calcanea than present-day males (Dunn's test: p<0.01).

Patterns of calcanei microanatomical variations and mobility contexts

Specimen 2013-1287, corresponding to the youngest individual (2 months), was removed before performing PCA (n=46; Fig. 5) and other quantitative analyses. Axes 1 and 2 of the PCA explain 75.3% of the total variance. Furthermore, contribution of the variables to the axes (Fig.
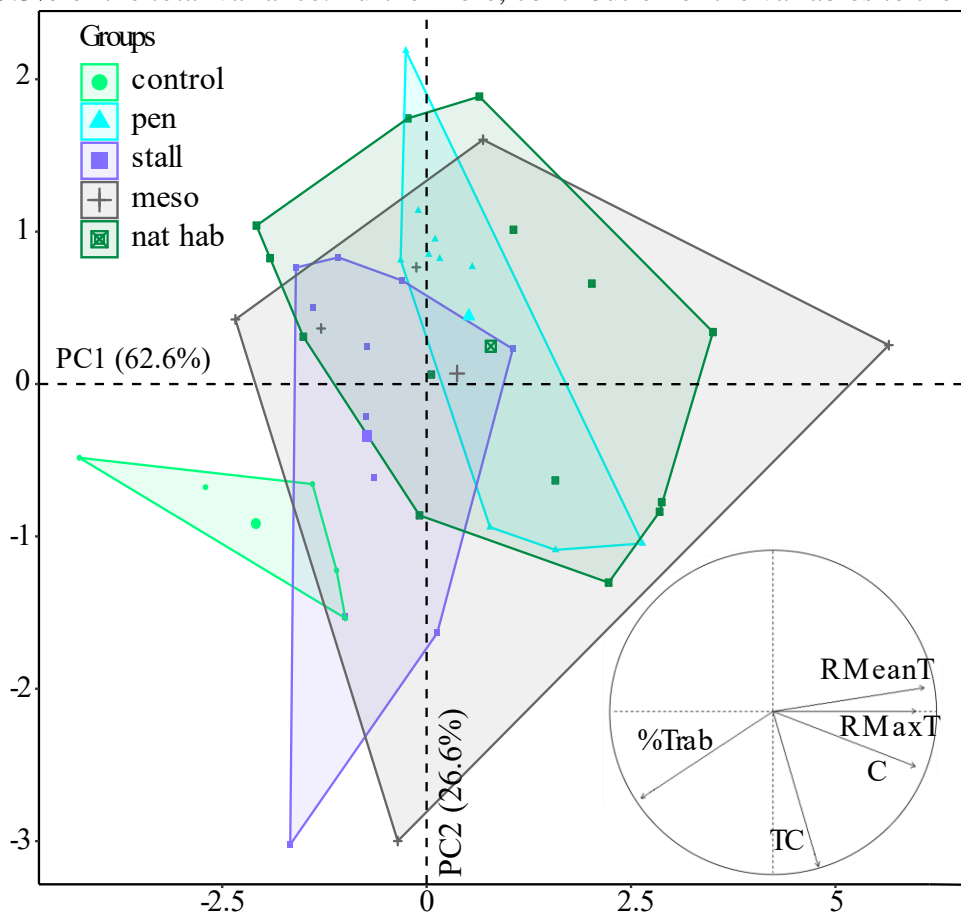

Fig. 5 Distribution of the 46 specimens and their associated context groups on the first two axes of the PCA computed on: WBV, total bone volume; RMeanT, relative mean bone thickness; RMaxT, relative maximum bone thickness; C, compactness ratio; TC, trabecular compactness ratio; %Trab, percentage of trabecular bone volume to cortical bone volume. meso, Mesolithic; nat hab, Natural habitat.

322 5), show that RMeanT and RMaxT covary while WBV and %Trab vary in opposite ways. The

323 first axis is influenced by the cortical thickness (TC), %Trab and C, while only TC greatly

324 structures the second axis. WBV is correlated with the PCA axes whereas age, weight and the

325 microanatomical parameters are not (table 2). The different mobility contexts induce significant

326 microanatomical differences (MANOVA: p-value<0.01). On the PCA (figure 5), experimental

327 penned, stalled and control wild boars are quite distinct along PC1, whereas the Mesolithic

328 specimens of Noyen-sur-Seine covers almost all variation along PC1. Differences across the

329 locomotor contexts are observed for RMeanT (Kruskal Wallis: p<0.01), RMaxT (Kruskal

330 Wallis: p<0.01) and %Trab (Kruskal Wallis: p<0.01). RMeanT differences are significant

331 between wild boars from the Natural habitat and the Experimental control groups (Dunn test:

332 p<0.01) and between Experimental control and Experimental pen groups (Dunn's test: p<0.01).

333 RMaxT is significantly different between Experimental control and Mesolithic wild boars

334 (Dunn's test: p<0.01). %Trab is significant different between Experimental control and natural

335 habitat (Dunn's test: p<0.01) and between Experimental pen and control (Dunn's test: p<0.01).

336 Thus, the pen-reared boars have a higher average cortex thickness (RMeanT) than the control

337 group, for which, conversely, it is the percentage of trabecular bone (%Trab) that is higher (Fig.
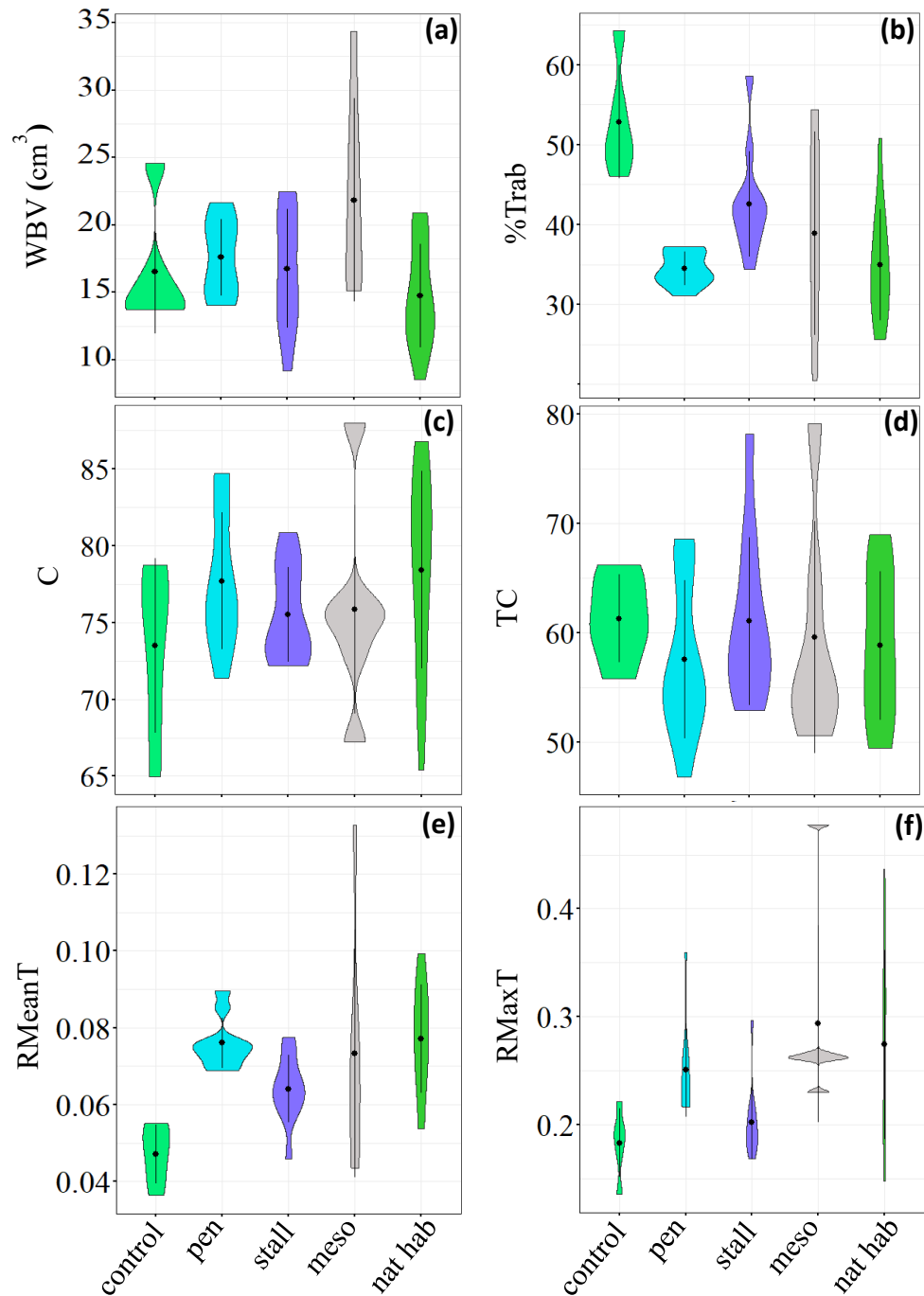
Fig. 6 Calcaneus microanatomical variation in *Sus scrofa* from experimental populations and Mesolithic contexts. WBV, whole bone volume; RMeanT, relative mean cortical thickness; RMaxT, relative maximum cortical thickness; C, compactness ratio; TC, trabecular compactness ratio; %Trab, percentage of trabecular bone volume to cortical bone volume. meso, Mesolithic; nat hab, Natural habitat.

338   6). The stall group had intermediate RMeanT, RMaxT and %Trab values compared to the pen

339   and control groups. In addition, there is a generally greater standard deviation in the Natural

340   habitat and Mesolithic groups for all parameters, except TC, for which individuals in the stall

341   group are slightly more dispersed than those in the Natural habitat group. On the other hand, no

342   group is distinguished along PC2, the variables C and TC did not vary according to context

17

343   (ANOVA; p-value C =0.39; p-value TC =0.82; Fig. 6); Finally, the whole volume (WBV) of

344   the calcaneus differed between groups (ANOVA: p=0.04) but only the difference between

345   hunted Mesolithic and modern wild boars in their natural habitat was significant (Tukey hsd

346   test: p=0.02; Fig. 6), with Mesolithic individuals being larger.

347

Discussion
348

349

1.Overall calcaneal microanatomy in wild and captive wild boars
350

351   The qualitative description of the 3D maps and virtual thin sections identified a strong cortical

352   thickening on the plantar and dorsal borders (Pb and Db; Fig. 4). These observations are

353   consistent with the main constraints identified in the calcaneus of artiodactyls with significant

354   compression, bending, and tension forces on the plantar and dorsal borders Lanyon (1973; Fig.

355   1). Thickenings of the plantar and dorsal margins have also been identified for wild deers

356   (Skedros et al., 2001) and pasture-raised domestic sheeps (Skedros et al., 2007). Moreover, the

357   anisotropric properties of the boar specimens trabeculae to follow an antero-posterior

358   orientations (outline arrows Fig. 3a) are congruent with the distribution of constraints

359   mentioned above, as internal bone structure organized itself to better respond to stress (Wolff,

360   1986; Ruff et al., 2006; Van der Meulen et al., 2006).

361   Although the entheses (i.e., areas of ligament and tendon insertion (Djukic et al., 2015)) are

362   regions of stress concentration, these areas show little or no effect on the microanatomy of boar

363   calcaneus. The loads applied to the calcaneus of artiodactyls are primarily shared between the

364   long plantar ligament and the Achilles tendon, which connects the calcaneus to the

365   gastrocnemius and soleus muscles, the latters forming the sural triceps (Lanyon,1973; Woo et

366   al., 1981; Skedros et al., 2001; Skedros et al., 2007; Barone, 2020). However, while the long

367   plantar ligament attaches all along the plantar border, only a part of this edge is thickened in

368   the boar calcaneus. Furthermore, an important thickening of similar proportion is also observed

369   on the dorsal edge, whereas this bone side shows no enthesis. The Achilles tendon insertion at

370   the proximal end of the calcaneus is itself covered by a tendinous structure, the calcaneus cap

371   of the superficial flexor digitorum muscle (*m. flexor superficialis*), taking an attachment point

372   from the tip of the proximal end of the calcaneus to phalanges (Su et al., 1999; Barone, 2017;

373   Fig. 1). A slight cortical thickening on the proximal epiphysis of our specimens coincides with

374   the insertion of the tendons of the *m. flexor superficialis* muscles of the toes and of the *m.*

375   *gastrocnemius* muscles (Bénévent & Bressot, 1968; Barone 2017).

376 Regarding the articular surfaces, the *sustentaculum tali* is a strong protuberance on the medial

377 side forming an articular surface with the talus, the fibular trochlea is an articular surface for

378 the malleolar bone and the end of the distal part articulate with the cuboid bone (Barone, 2017),

379 recurrent slight cortical thickening ~~are~~ also observed ~~on~~ those regions.

380 Overall, the microanatomy seems to reflect the tension and compression forces with a strong

381 cortical thickening on Pb and Db as well as the anisotropy of the trabeculae. The areas of contact

382 with other bones are also represented with important bone density on the fibular trochlea, the

383 *sustentaculum tali* and the end of the distal part.

384

2.Intra individual variation in calcaneal Microanatomy

386 Beyond the general pattern, no clear relationship is observed between the variability of the

387 microanatomical parameters and the factors explored in this study (context, sex, weight, size).

388 However, we found important inter-individual differences, notably on the extent and depth of

389 the cortical thickness of the plantar and dorsal edges, and on the proximal epiphysis; the length

390 of the medullary cavity; the number and thickness of the trabeculae; the bone tissue density of

391 the *sustentaculum tali*, at the distal part of the bone and next to the fibular trochlea; and the

392 transverse sectional shape. This suggest that other factors influencing ~~the~~ bone development

393 during ~~the~~ growth must be explored to further understand ~~this~~ inter-individual disparity.

394 Only a few correlations (positive or negative) of the microanatomical variables with ~~the~~ bone

395 size, weight and age of the individuals (Table 2), are significant. These three parameters

396 therefore have a limited impact on the microanatomical organisation. ~~The~~ body weight is only

397 correlated with ~~the~~ bone size (WBV). WBV influences the PCA axes (Table 2) while,

398 paradoxically, bone size is not directly correlated with any of the microanatomic variables in

399 isolation, which is congruent with the observations of the sections and 3D maps that have not

400 found any link between specimens' size and their microanatomy. Thus, it is the covariation

401 between the variables that are themselves weakly correlated with whole volume that makes the

402 relationship between whole volume and all variables significant. Nevertheless, although age is

403 not one of the parameters on which this study focuses, a weak correlation was found between

404 age and the trabecular percentage (%Trab; r=0.32) but trabecular percentage is not significantly

405 correlated with bone size since age and bone size are neither significantly correlated. The

406 increase in the proportion of trabecular bone tissue with age is not related to an increase in

407 trabecular compactness (TC) because this parameter is not significantly correlated with age

408 (p=0.29); the volume of the medullary zone tends to increase since the cortex becomes

409 proportionally thinner. This result is surprising because the opposite phenomenon occurs in the

19

410   calcaneus of deer (Skedros et al., 2001) and sheep (Skedros et al., 2007), where a thickening of

411   the cortex is observed with size in relation to the medullary zone. Conversely, while it is

412   surprising that age does not correlate with whole bone volume (WBV; Table2), this shows that

413   age and volume do not follow a linear relationship or that intraspecific variability in calcaneus

414   size between specimens exceeds the effect of growth. However, our sample does not adequately

415   test the relationship between the variables and ontogeny because of the large proportion of

416   individuals of the same age (25 months).

417

418   3.Change in mobility regime and calcaneal Microanatomy

419   Despite the lack of directly observable influence of the mobility context over the calcaneal

420   microanatomy, we found quantitative effect of mobility differences in the cortical thickness

421   (RmeanT and RmaxT), trabecular percentage (%Trab) and in the overall variation patterns

422   (PCA), indicating that difference in mobility context influences the microanatomical

423   characteristics of the calcaneus, although not in a strongly discriminatory pattern. However, we

424   didn't find the expected microanatomical proximity of wild boars living in their natural habitat

425   and their dissimilarity from wild boars kept in captivity, as seen in previous studies on the

426   calcaneus 3D shape and form (Harbers et al.2020). The variations in relative mean cortical

427   thickness (RMeanT) illustrate the general trend of variations related to the mobility regime (Fig.

428   6). While we expected to observe similar average thicknesses between animals that had similar

429   mobility conditions (e.g. Natural habitat and Experimental control), we found that control

430   individuals have a much lower cortical thickness than wild individuals from natural habitat.

431   Thus, modern and Mesolithic wild boars hunted in their natural habitat display similar

432   microanatomy with the wild boars which grew in a very small living space (enclosure of 4m2

433   in a hangar of 100m2.). The most divergent microanatomy from the wild boar norm of reaction

434   have been observed in the control populations from the wild boar farm of Urcier, which have a

435   much lower cortical thickness than wild boars in their natural habitat. All these results suggest

436   that the microanatomy of the *Sus scrofa* calcaneus does not strongly reflect the mobility regime,

437   contradicting the strong microanatomical signal associated with locomotor restriction

438   evidenced in battery chickens, including osteoporosis related to inactivity (Rath et al., 2000).

439   However, the locomotor restrictions of these reared animals are generally greater than those

440   imposed on the animals of this study.

441

20

4.Greater microanatomical variability in modern and ancient wild boars hunted in their natural habitat

The six Mesolithic calcaneus from the archaeological site of Noyen-sur-Seine show similarity for all parameters and variables with the other groups (Fig. 6). Thus, the Mesolithic calcaneus shares the same micronatomy with their current relative. We also found greater microanatomical variability in hunted wild boars populations both modern and Mesolithic, external factors were much less controlled than for the DOMEXP groups, thus resulting in more elements to affect bone plasticity. In addition, the animals from Compiègne and Chambord (Natural habitat) and NoyenEns2 and NoyenEns3 (archaeology) are different populations for which genetic variability is more important, with a time lag of several centuries in the Mesolithic specimens. This genetic variability is much probably a substantial factor which have a greater impact on the observed phenotype than the intrapopulation variability related to motricity in this study. Consequently, when several populations are included in the same group (Natural habitat and Mesolithic), their variability exceeds that observed between the same population placed in different locomotor contexts (stall, pen, control). Thus, the microanatomy of the calcaneus appears to be more affected by population differences than by the locomotor context in which the animals grew.

In addition to this explanation, the wider locomotor regime of wild boars in their natural habitat would foster greater ecophenotypic variation compared to captive specimens with reduced mobility and more stereotypical locomotor behaviour. One the one hand, wild boars in nature have a locomotor repertoire that must respond to several problems that are not encountered in captivity, such as foraging or escape (Spitz & Janeau, 1990, 1995). Their daily travel is generally less than 10km but it could be up to 80 km in one night (Keuling et al., 2009), peak speeds of 40 km/h and high jumps up to 1.5 m have also been observed (Baskin & Danell, 2003). One the other hand, there is no study describing the skeletal repercussion of stereotypic behaviours. However, because these types of behaviours performed to compensate for lack of activity are induced during significant psychological depressions in individuals (Rushen, 1993; Andre, 2007), other experimental approaches involving living specimens are not desirable.

quantifying

Also, Diet plays an important role in the development of the skeleton (Randoin & Causeret, 1945). Experimental specimens were fed nutritionally balanced pellets (15% protein) intended for pig breeding to ensure consistent growth and bone formation. In their natural habitat, food availability, seasonal and geographical variations are major factors influencing food selection

475 by wild boar (Ballari, & Barrios-García, 2014), Thus, it is probable that the diet factor also

476 contributes to the high variability observed in hunted individuals. Furthemore, it is likely that

477 there are differences in substrates between the forests and areas in which these boars lived; this

478 may have important implications for the autopod (Kappelman, 1988). Indeed, the substrate

479 factor seems potentially important for the stall-reared group where the flat ground, was covered

480 with moss and straw mats, whereas the more irregular natural terrain of wild boars (like control

481 group) implies variable and multidirectional soil reaction forces (Hanot et al., 2017). The group

482 of pens was raised on a flat terrain covered with grass and a few trees.

483 The overview of the different studies related to the DOMEXP project provides arguments to

484 consider that the bone plasticity associated with domestication is expressed to a greater or lesser

485 extent depending on the bones studied, and the most surprising result is that it is more expressed

486 in the morphology of the calcaneus than in its microanatomy, whereas the latter is considered

487 to be more plastic than the morphology. Since the results of the study show that boars living in

488 completely different contexts can have the same microanatomical pattern/values, calcaneal

489 microanatomy cannot be used to infer a captive lifestyle. However, a strong diversity is noted

490 in the microanatomy between wild and Mesolithic specimens. A better understanding of the

491 factors that regulate this diversity would possibly allow inferences related to the habitat (type

492 of soil, open or closed environment) or what the animals are/were fed on.

493

Aknowledgements

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