

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

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Abstract

Reduced mobility associated with captivity induces changes in biomechanical stress on the skeleton of domesticated animals. Due to bone plasticity, ~~the bone's~~ morphology and ~~the~~ internal structure ~~of the bones~~ can respond to these new biomechanical stresses over individuals' lifetime. In a context where documenting early process of animal domestication is a real zooarchaeological challenge, ~~†~~ This study will test the hypothesis that change in mobility patterns during ~~the life of~~ a wild ungulate's life will alter ~~the~~ internal structure of its limb bones and provide a proof of concept for ~~its~~ ~~the~~ application ~~of this knowledge~~ in Zooarchaeology. Using the calcaneus as ~~a~~ phenotypic marker through qualitative and quantitative 3D microanatomical analyses, we relied on a comparative study across wild boars (*Sus scrofa*) populations from controlled experimental conditions with different mobility patterns (natural habitat, large pen, and stall) and archaeological specimens collected from middle and late Mesolithic as surrogate for the norm of reaction in European wild boar phenotype before the spread of agriculture and domestic pigs. Results provide evidence for compressive and tensile forces as ~~the~~ main elements affecting the variation in the cortical thickness along the calcaneus. Furthermore, changes in the internal structure of the calcaneus between mobility patterns are observed but their intensity is not directly associated with the degree of mobility restriction and only weakly impacted by the size or weight of the individuals. Despite having greater bone

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

39
40 Keywords: calcaneus; Bone structure; captivity; domestication; *Sus scrofa*;
41 functional morphology
42

43 **Introduction**

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

58 Captive animals may grow in limited areas, which does not involve the same range of
59 movement and forces applied to their bones compared to free-ranging
60 individuals. Analysing the biomechanical bone plasticity is a major asset for
61 paleoanthropologists and archaeozoologists trying to decipher individual-scale lifestyle
62 changes from bones anatomy in order to document changes in activity patterns of past humans
63 and other animals (Trinkaus et al., 1994, Agarwal, 2016). In paleoanthropology, this
64 biomechanical component has been used to better understand the evolution of
65 bipedalism in hominids (Ruff, 2018) and to observe the morphological consequences of the
66 transition from a nomadic hunter-gatherer to a farmer-herder lifestyle (Pinhasi & Stock, 2011).

67 In archaeozoology, changes between a domestic and a wild lifestyle related to locomotion and
 68 gait changes, have been estimated as a prevalent factor over load-carrying on structural changes
 69 of domestic donkeys limb bones (Shackelford et al., 2013). Despite this, archaeozoologists have
 70 always considered that the first observable morphological changes occurred only late in the
 71 domestication process and could not perceive its initial stages since they would not involve
 72 genetic isolation or selective reproduction (Vigne et al., 2011; Colledge et al., 2016). To
 73 develop new phenotypic proxy of the first steps of animal domestication, an experimental
 74 project (DOMEXP) has been performed to test whether growth in captivity of a wild ungulate
 75 would leave a morpho-functional imprint distinguishable from its natural habitat norm of

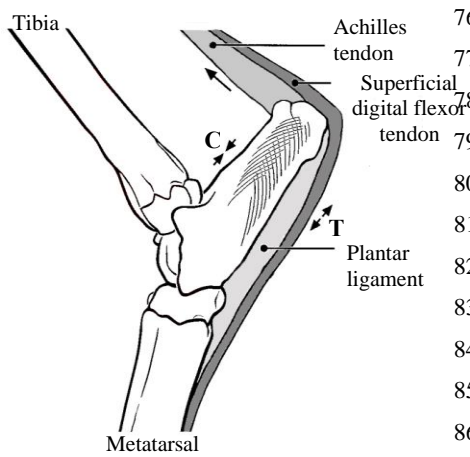


Fig. 1. Lateral view of the tarsal region of a skeletally mature artiodactyl, showing the calcaneus with other associated bones, ligaments and tendons. The large arrow in the dorsal direction indicates the orientation of the force exerted by the Achilles tendon during paw extension, loading the dorsal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

76 reaction. Hence, early process
 77 of cultural control (Hecker
 78 1982) could be traced from
 79 bones anatomy long before the
 80 so-called "domestication
 81 syndrome". To test this
 82 hypothesis, the DOMEXP
 83 project used a genetically
 84 homogeneous wild boar (*Sus*
 85 *scrofa*) population to control
 86 the genetic and environmental
 87 factors influencing skeletal
 variation. From this
 populations, 6 months old
 piglets were captured after
 weaning to be reared until the
 age of two years under two

93 mobility reduction regimes. Geometric morphometrics' analyses have provided proof of
 94 concept that this mobility reduction in a wild ungulate population could leave morphometric
 95 prints on skulls (Neaux et al., 2021) and calcaneus (Harbers et al., 2020a). Microanatomical
 96 investigations have also revealed changes in the 3D topography of the cortical thickness in the
 97 humeral shaft (Harbers et al., 2020b). However, the impact of mobility reduction on the
 98 microanatomical features of the skeleton remained to be explored.

99 For this reason, this study investigates the microanatomical variation of the calcaneus from the
 100 experimental specimens of the DOMEXP project. Tarsal bones like the calcaneum

101 are particularly considered as more informative than others in investigating the
102 mechanical forces that apply to individuals during locomotion (Lovejoy et al., 1999; Carter &
103 Beaupré, 2001; Pearson & Lieberman, 2004). Proximally articulated to the fibula and distally
104 to the cuboid bone, the calcaneus is intensely stressed during locomotion in
105 terrestrial tetrapods. It acts as a lever arm for the ankle extensor muscles, and is subject to high
106 tensile, flexural, and compressive forces (Fig. 1; Hussain, 1975; Carrano, 1997; Bleefeld &
107 Bock, 2002; Bassarova et al., 2009; Barone, 2017). The calcaneus is therefore a particularly
108 interesting object of study to investigate differences in mobility between individuals (Harbers
109 et al., 2020a). Moreover, the high compactness of the calcaneus makes it resistant to taphonomic
110 alterations, which gives it a good general preservation in archaeological contexts (Binford,
111 1978).

112 This study compares the calcaneus microanatomy variation between adult captive-bred and
113 free-ranging wild boars to assess if captivity can induce microanatomical
114 variation in a wild ungulate. We also include wild boar calcanei from a
115 Mesolithic (ca. 8000 years old) context of hunter-gatherers in northern France to observe how
116 much the variation in wild boar microanatomy has changed since the neolithisation of Europe
117 and whether this proxy can be used to infer change in locomotor behaviour from archaeological
118 calcanei.

120 **Material and method**

122 **Experimental protocol**

123 24 wild boars aged six months were sampled from a control population living in a fenced
124 forest of about 100,000 m² in northern France. These specimens were
125 then divided into two equal samples with the same sex ratio and reared until 24 month in
126 two different contexts of reduced mobility: (1) a stall of 100 m², where males and females were
127 separated (each group had 45 m²) and (2) a wooded pen of 3000 m². These two contexts
128 represent respectively 99.9% and 97% of range reduction compared to the control population,
129 which prevents the average daily distances measured in free boar populations (Palencia et al.
130 2019; Russo et al. 1997). The stall presented no opportunity for foraging. In the wooded
131 enclosure, this opportunity was limited due to space limitations. Dry feed pellets suitable for
132 feeding domestic pigs were provided to both groups.

134 Specimens studied

135 Our dataset includes calcaneus bones from 47 specimens: 26 from the DOMEXP experimental
136 farm - 11 stall specimens (stall), 10 pen specimens (pen), five from the control population
137 (control) - 15 hunted wild boars from two Northern France forests: eight from the Chambord
138 forest and seven from the Compiègne forest (Natural habitat) and six archaeological
139 specimens from the Mesolithic contexts (Meso) of Noyen sur Seine (Mordant et al., 2013,
140 Marinval-Vigne et al., 1989) in Northern France (Table 1). The six archaeological specimens
141 from Mesolithic deposits have been accumulated by hunter-gatherer living in Western Europe
142 before the Neolithic dispersal via the Mediterranean and Danubian pathways, which
143 introduced domestic pigs from the Near East (Larson 2006) that interbred with local
144 populations of wild boars (Frantz et al. 2020). Two specimens are
145 radiocarbon dated from the Middle Mesolithic (between -8000 and -7300
146 cal. BP) and four specimens to the Final Mesolithic dated between -7000 and -6200 cal.
147 BP (Mordant et al., 2013). All the specimens bar the archaeological samples
148 have associated age and sex informations. Three of the archaeological calcanei belong to
149 adult individuals since their proximal epiphysis is fused
150 which is known to happen around 2 years in wild boars (Bridault et al., 2000).

151

152 Data acquisition

153 All the calcanei have been scanned with high-resolution microtomography
154 (EasyTom 40-150 scanner, RX Solutions) at the MRI platform, hosted at ISEM, University of
155 Montpellier (UMR 5554); reconstructions were then performed using X-Act (RX Solutions).

156

157 Virtual thin sections

158 For qualitative comparisons, three virtual sections (Fig. 2, Appendix 1,2 and 3) were made for
159 each calcaneus, following Barone (2017) for terminology and orientations. The bones were
160 oriented as follows: in dorsal view (Fig. 2a), bone's axis is vertical and the fibular trochlea is
161 oriented toward the observer, its dorsal part aligns with the contour of the bone's medial border;
162 in medial view (Fig. 2b) the *sustentaculum tali* is directed towards the observer, the observation
163 angle is fixed when the anterior edge of the fibular trochlea is no longer visible upon rotation
164 from the anterior view to the medial view. Sagittal sections (SS) run in dorsal view from the
165 distal end tip and the midpoint of the thickness at the proximal epiphysis base (Fig. 2). The
166 frontal sections (FS) extend from the distal end tip to the midpoint of the proximal epiphysis

167 base (see purple arrows on Fig. 2b). Cross-sections (TS) are taken perpendicular to the FS plane
168 at 1/3 of the total length of the bone (from the proximal epiphysis tip to the distal tip) (Fig. 2).
169 These sectional planes were chosen to depict large portions of the bone in order to analyze the
170 microanatomical structure (e.g., trabecular network, cortical thickness) while being easily
171 created with good reproducibility for all specimens. Virtual sections were created using
172 VGSTUDIO MAX, versions 2.2 (Volume Graphics Inc.).

173

174 **Calcaneus 3D mapping**

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

190

191 **Quantitative parameters**

192 Quantitative parameters used to characterize the internal structure of the bones are: (1)
193 cortex/medullary area volumes, (2) overall bone and trabecular tissue compactness, and average

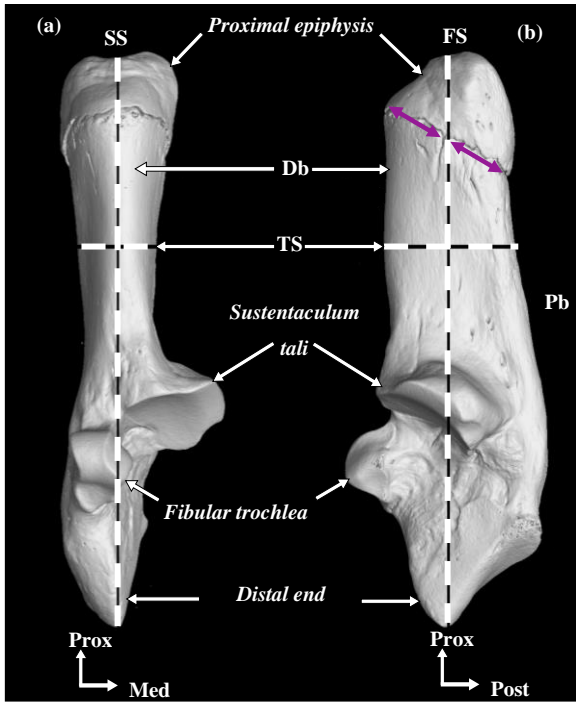


Fig. 2 Calcaneus of *Sus scrofa*, specimen 2017-218 illustrating the planes of the virtual sections: SS, Sagittal section; FS, Frontal section; TS, Transverse section. Db, Dorsal border; Pb, Plantar border. Purple arrows indicate midpoint of the proximal epiphysis base.

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 ($C = \text{bone tissue volume} * 100 / \text{WBV}$);
- 3) relative trabecular bone tissue fraction ($\% \text{Trab} = \text{trabecular bone tissue volume} * 100 / \text{bone tissue volume}$);
- 4) trabecular compactness ($T_c = \text{trabecular bone tissue} * 100 / \text{trabecular volume}$). 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 $v = \pi r^2 h$, $r = \sqrt{(v / h\pi)}$).

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 (mm ³)	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

220 **Statistical analysis**
221 Statistical tests and graphical representations were performed in R (R Core Team, 2017) using
222 the Rstudio software (see appendix 5 and 6 for the script and the data Table). A linear regression
223 model ("lm" function of the "stats" package) as well as a regression coefficient ("cor" function
224 of the "stats" package) were used to assess the linear relationships between the variables (1)
225 whole bone volume (WBV; considered as an estimate of bone size), (2) weight, and (3) age of
226 each individual with all the other quantitative parameters used (Table 2). To explore the
227 distribution of specimens based on their quantitative microanatomical parameters and the
228 variation patterns, we performed a standardized Principal Component Analyses (PCA;
229 "dudi.pca" function of the ade4 package; David and Jacobs 2014).

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

245
246
247

248 **Results**

249 **Qualitative descriptions**

250 *General microanatomical pattern*

251 The general microanatomical pattern of the wild boar calcaneus is close to the structure of long
252 bones of terrestrial mammals, with the cortex forming a tubular diaphysis but with a very short
253 diaphyseal part (sections Fig. 3). The proximal epiphysis is not fused to the rest of the bone for

254 the most part of the specimens, except for the oldest. The cortex surrounds cancellous bone
255 (trabecular bone and intertrabecular spaces), which is essentially quite dense, and a small open
256 medullary cavity, about 1cm (Pradat187; Fig. 3c,h) to 2 cm long (2013-1286; Fig. 3d,i) and 1
257 cm wide, at the level of the *Sustentaculum tali* (FigXa-j). The thickness of the trabeculae is
258 relatively homogeneous along the calcaneus, except around the medullary cavity, where they
259 are generally thicker (Fig. 3a-j). Also, the trabecular density is heterogeneous, with some
260 specimens having many trabeculae (2017-570; Fig. 3b,g,l) while others have twice as less but
261 thicker trabeculae (2013-1286; Fig. 3 d,I,n). Finally, the bone density in the distal part strongly
262 varies between individuals from compact (2017-570; Fig. 3b,g) to spongy (Calc2139; Fig.
263 3e,j).

264 *Sagittal sections*

265 In all specimens, the dorsal and plantar margins (Db and Pb) show a relatively high cortical
266 thickness, especially at mid-diaphysis. Spongy bone shows anisotropic
267 trabeculae (i.e. with a preferential direction) above the medullary cavity in the bone center (Fig.
268 3a-e). Anisotropic trabeculae follow the two main directions that are represented with
269 green/outline arrows in the figure 3a and schematized with intersecting lines in figure 1. The
270 cortical thicknesses of the plantar and dorsal margins vary from thick (1 cm in specimen 2017-
271 570; Fig. 3b) to proportionally twice as thin (0.5 cm in Pradat187; Fig. 3c). A variation in the
272 cortical thickness of the plantar border of the proximal epiphysis is also observed, it is very thin
273 (1mm) in some specimens like Pradat 187 (Fig. 3c) while others, such as 2017-555 (Fig. 3a),
274 show a clear thickening of the cortex (2mm) in this area. Similarly, bone is highly
275 compact next to the fibular trochlea for numerous specimens (Calc2139; Fig. 3e)
276 or rather spongy for some individuals (2013-1286; Fig. 3d).

277

278 *Frontal sections*

279 All individuals have a cortex that varies relatively little in thickness (about 2-3mm) on the
280 medial and lateral sides (Fig. 3f-e). In contrast, the cortical thickness in the proximal epiphysis
281 varies from thin (0.5 cm in specimen 2013-1286; Fig. 3i) to twice thicker (0.3 cm in specimen
282 2017-555; Fig. 3f). Similarly, the compactness and the cortical thickness of the *sustentaculum*
283 *tali* varies between individuals, some of which show compact bone and thick cortex (Pradat187;
284 Fig. 3h) while others show spongy bone and thin cortex (2013-1286; Fig. 3i).

285

286 *Transverse sections*

287 The transverse sections' shapes are generally oval and elongate (2017-555; Fig. 3k) but
288 several specimens present a rounder section (2017-570; Fig. 3l). The cortical thickness is fairly
289 constant across the sections, but some specimens s show a cortical thickening at the plantar
290 border (2013-1286; Fig. 3n).

291
292 None of the variation observed between specimens is clearly qualitatively associated to any
293 main parameter of the study, namely the context, provenance, sex, size or weight.

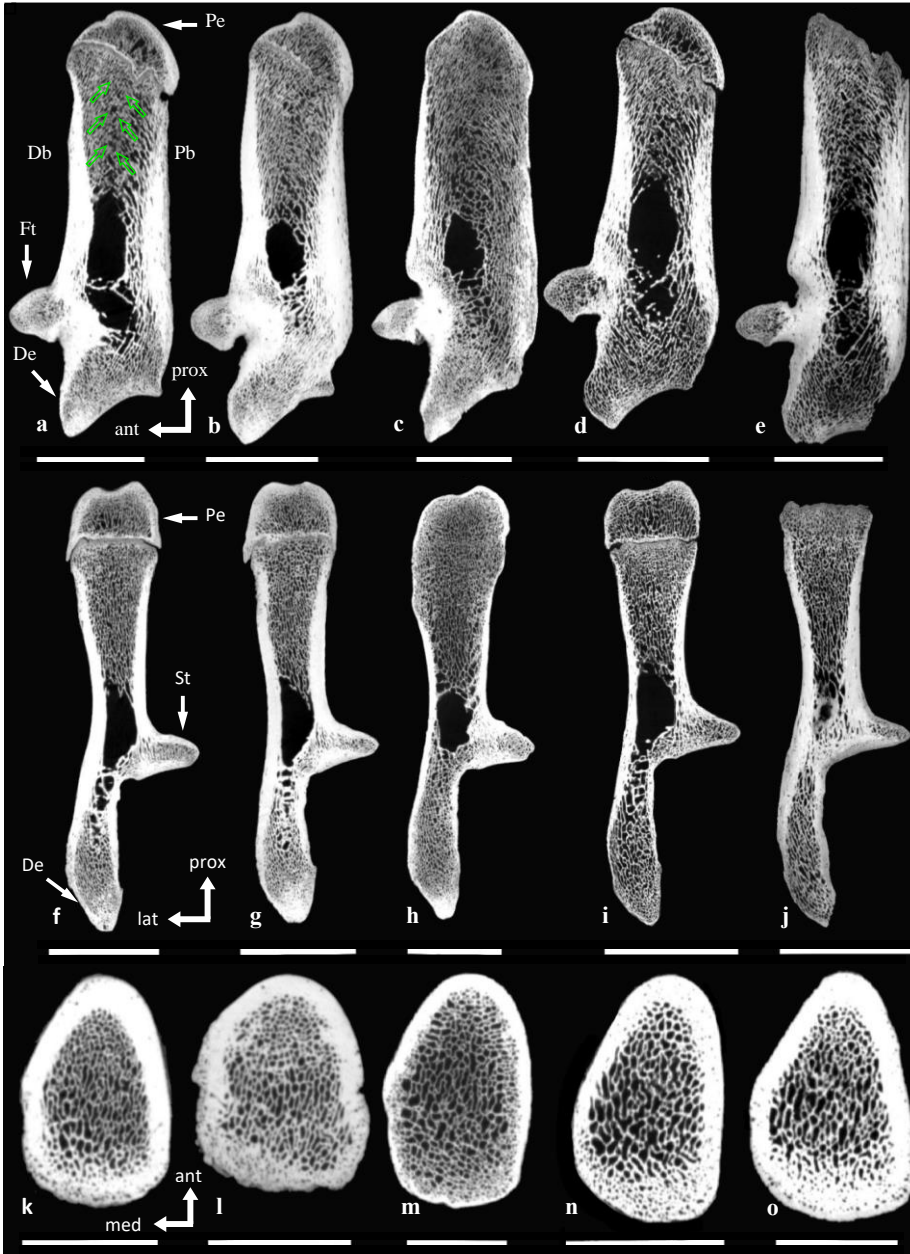


Fig. 3 Virtual thin sections of the calcaneus of boars, a,f,k, 2017-555 (Experimental stall); b,g,l 2017-570 (Experimental pen); c,h,m Pradat187 (Experimental control); d,i,n, 2013-1286 (Natural habitat); e,j,o, Calc2139 (Mesolithic). Db, Dorsal border; De, Distal end; Ft, Fibular trochlea; Pb, Plantar 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.

295 **3D mapping of the cortical thickness**

296 The 3D mappings of the cortical thickness and its variation across each bone, are quite similar
297 between specimens (Appendix 1). In agreement with the observation of the virtual sections,
298 there is a fairly extensive area with greater cortical thickness at the plantar border of the
299 calcaneus (Pb; Fig. 4A). Although generally less extensive, thickenings of the cortex can also
300 be noted on the dorsal margin (Db) and on the dorsal base of the *sustentaculum tali* (Sb; Fig.
301 4B). There is little thickening of the cortex on the proximal end (Pe). Finally, there is no lateral
302 or medial thickening noted.

β03 Twenty-five of the 47 specimens show strong cortical thickening at the plantar margin (Pb;
β04 Appendix 1), like in 2017-568 (Fig. 4A); this is less visible in the 22 others, like Pradat 185
β05 and 2013-1264 (Fig. 4D; Fig. 4G). Twenty-three specimens have thick cortical bone at the
306 dorsal margin (Db; Table 1; Appendix 1), like in Pradat 185 and 2017-568 boars (Fig. 4B and
307 Fig. 4E), whereas the others do not show such thickening like the calcaneus of boar 2013-1264
β08 (Fig. 4H). Twenty-eight specimens show a relative thickening at the base of the
309 sustentaculum (Sb; Table 1), this widening is particularly noticeable in boar 2013-1264 (Fig.
310 4H) and absent in others like Pradat 185 (Fig. 4E). A slight recurrent cortical thickening on the
β11 distal end is observed in part of the specimens like 2017-568 (Fig. 4a). Twenty-seven
312 specimens have a slightly thicker cortex on the proximal end (Pe) such as 2017-568 (Fig. 4C),
313 15 show no or very little thickening, such as Pradat185 and 2013-1264, respectively (Fig. 4F
β14 and Fig. 4I). Four of the proximal ends of the Mesolithic specimens were not found because
315 they were not fused to the rest of the bone.

316

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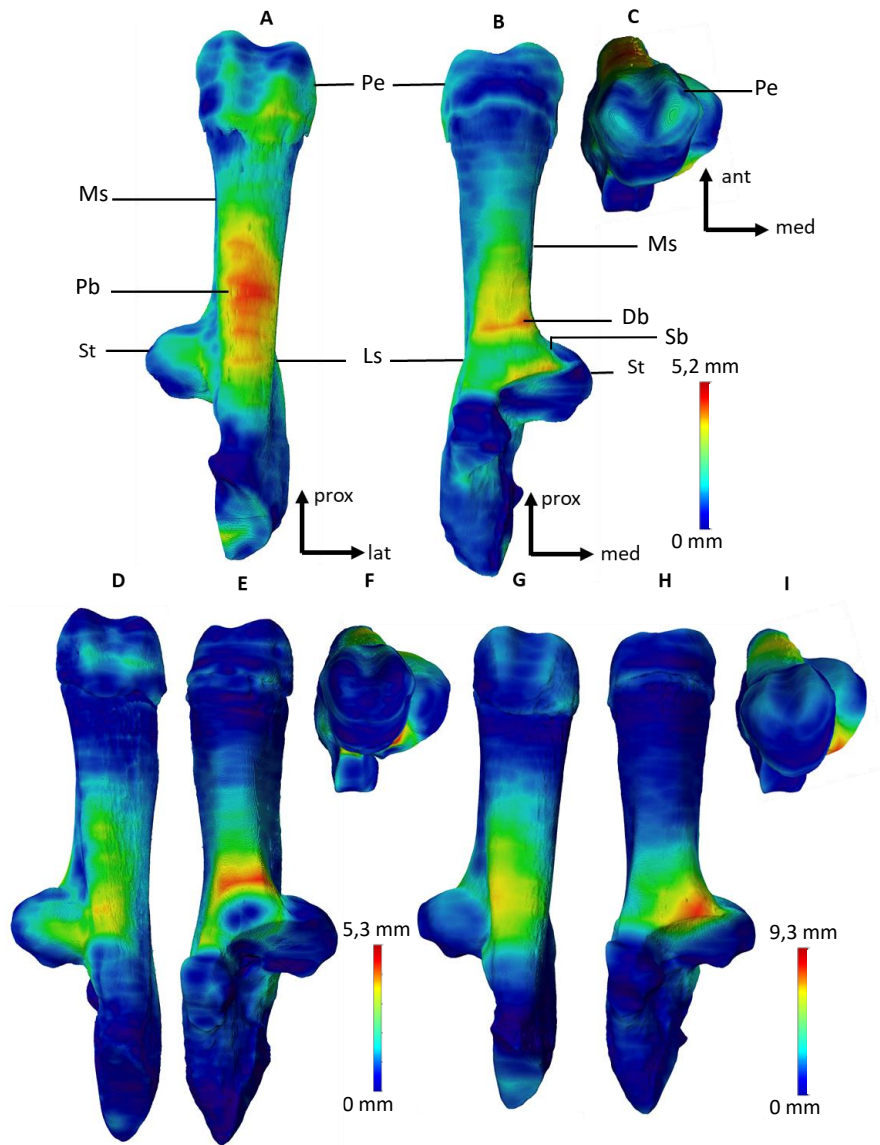


Fig. 4 3D mappings of the *Sus scrofa* calcaneus relative cortical thickness. A-C specimen 2017-568; D-F Pradat 185; G-I 2013-1264. Anatomical abbreviations: Db, dorsal border; Ls, lateral side; Ms, medial side; Pb, plantar border; Pe, proximal epiphysis; Sb, Sustentaculum base; St, *Sustentaculum tali*. 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.

320 **Quantitative analyses**

321 *Microanatomical covariation with Weight, age size and sex.*

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

325 Variables C, %Trab, RMeanT, RMaxT, weight, and TC did not differ between males and
 326 females. Only whole calcaneus volume (WBV) variation differs with sex (Kruskal Wallis:
 327 $p<0.01$) with males larger than females (Fig. 6). Mesolithic specimens (sex unknown) have
 328 larger calcanei than present-day males (Dunn's test: $p<0.01$).

Table. 2 Values obtained for tests of the effect of total volume, age, and weight on the different parameters and the PCA axes. r: correlation coefficient; p: p-value of the linear regression model; WBV: whole bone volume; RMeanT, mean relative cortical thickness; RMaxT, maximum relative cortical thickness; PC1, PC2: position of individuals on the first two axes of the PCA.

	WBV	Age	Weight	C	%Trab	TC	RMeanT	RMaxT	PC1	PC2	
WBV n=46	X	X	X		$r=0,07$ $p=0,63$	$r=-0,13$ $p=0,38$	$r=-0,05$ $p=0,75$	$r=0,13$ $p=0,37$	$r=0,20$ $p=0,18$	$r=-0,46$ $p<0,01$	$r=0,36$ $p=0,01$
Age n=40	$r=0,30$ $p=0,06$	X	X		$r=0,47$ $p=0,92$	$r=0,32$ $p=0,04$	$r=0,17$ $p=0,29$	$r=-0,19$ $p=0,23$	$r=-0,20$ $p=0,21$	$r=0,07$ $p=0,65$	$r=0,25$ $p=0,12$
Weight n=34	$r=0,56$ $p<0,01$	$r=0,27$ $p=0,12$	X		$r=0,08$ $p=0,65$	$r=-0,16$ $p=0,35$	$r=0,02$ $p=0,93$	$r=0,30$ $p=0,08$	$r=0,26$ $p=0,14$	$r=0,34$ $p=0,05$	$r=0,31$ $p=0,07$

329 *Patterns of calcanei microanatomical variations and mobility contexts*

330 *Patterns of calcanei microanatomical variations and mobility contexts*

331 *Patterns of calcanei microanatomical variations and mobility contexts*

332 Specimen 2013-1287, corresponding to the youngest individual (2 months), was removed
 333 before performing PCA (n=46; Fig. 5) and other quantitative analyses. Axes 1 and 2 of the PCA
 334 explain 75.3% of the total variance. Furthermore, contribution of the variables to the axes (Fig.
 335 5), show that RMeanT and RMaxT covary while WBV and %Trab vary in opposite ways. The
 336 first axis is influenced by the cortical thickness (TC), %Trab and C, while only TC greatly
 337 structures the second axis. WBV is correlated with the PCA axes whereas age, weight and the
 338 microanatomical parameters are not (table 2). The different mobility contexts induce significant
 339 microanatomical differences (MANOVA: $p\text{-value}<0.01$). On the PCA (figure 5), experimental
 340 panned, stalled, and control wild boars are quite distinct along PC1, whereas the Mesolithic
 341 specimens of Noyen-sur-Seine covers almost all variation along PC1. Differences across the
 342 locomotor contexts are observed for RMeanT (Kruskal Wallis: $p<0.01$), RMaxT (Kruskal
 343 Wallis: $p<0.01$) and %Trab (Kruskal Wallis: $p<0.01$). RMeanT differences are significant

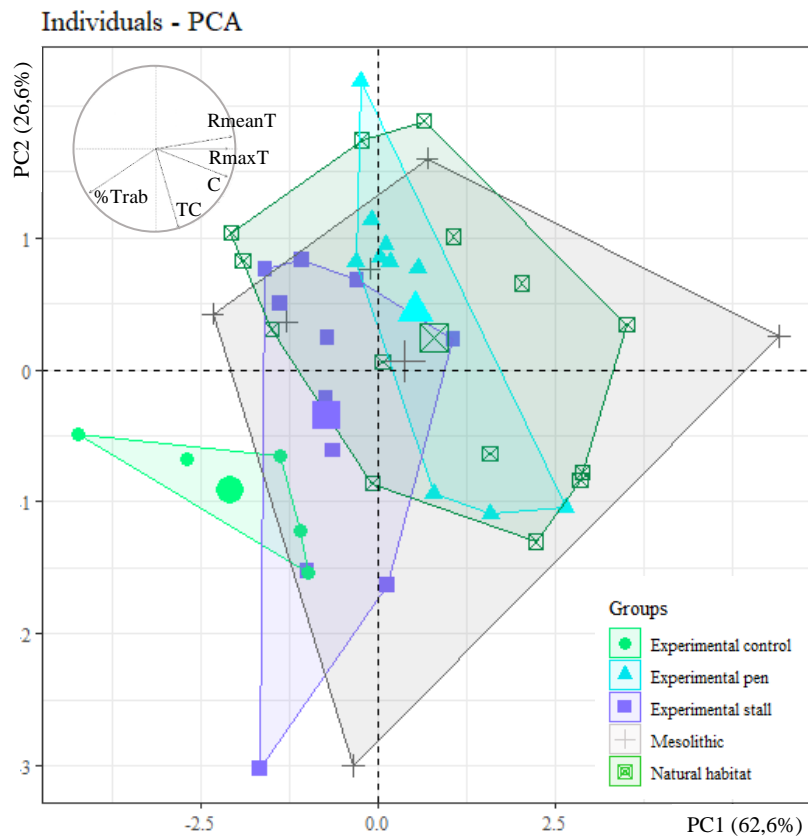


Fig. 5 Distribution of the 46 specimens and their associated context groups on the first two axes of the PCA computed on: WBV, total bone volume; RMeanT, relative mean bone thickness; RMaxT, relative maximum bone thickness; C, compactness ratio; TC, trabecular compactness ratio; % Trab, percentage of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

344 between wild boars from the Natural habitat and the Experimental control groups (Dunn test:
 345 $p < 0.01$) and between Experimental control and Experimental pen groups (Dunn's test: $p < 0.01$).
 346 RMaxT is significantly different between Experimental control and Mesolithic wild boars
 347 (Dunn's test: $p < 0.01$). % Trab is significant different between Experimental control and natural
 348 habitat (Dunn's test: $p < 0.01$) and between Experimental pen and control (Dunn's test: $p < 0.01$).
 349 Thus, the pen-reared boars have a higher average cortex thickness (RMeanT) than the control
 350 group, for which, conversely, it is the percentage of trabecular bone (% Trab) that is higher (Fig.

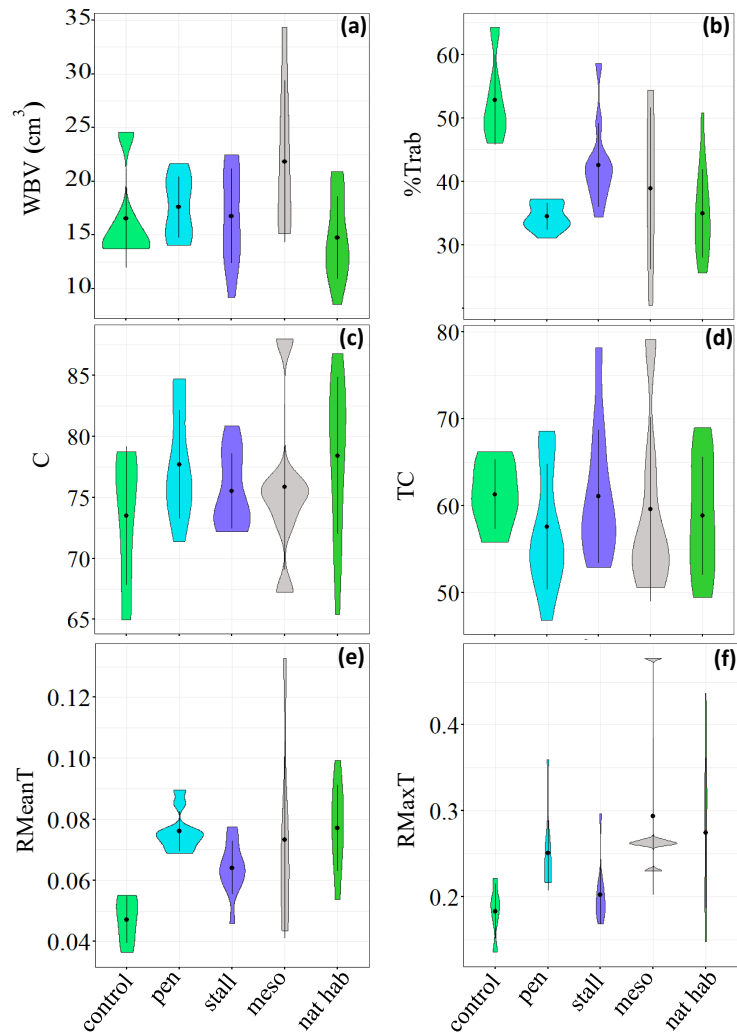


Fig. 6 Calcaneus microanatomical variation in *Sus scrofa* from experimental populations and Mesolithic contexts. WBV, whole bone volume; RMeanT, relative mean cortical thickness; RMaxT, relative maximum cortical thickness; C, compactness ratio; TC, trabecular compactness ratio; %Trab, percentage of trabecular bone volume to cortical bone volume. meso, Mesolithic; nat hab, Natural habitat.

351 6). The stall group had intermediate RMeanT, RMaxT and %Trab values compared to the pen
 352 and control groups. In addition, there is a generally greater standard deviation in the Natural
 353 habitat and Mesolithic groups for all parameters, except TC, for which individuals in the stall
 354 group are slightly more dispersed than those in the Natural habitat group. On the other hand, no
 355 group is distinguished along PC2, the variables C and TC did not vary according to context

356 (ANOVA; p-value C =0.39; p-value TC =0.82; Fig. 6); Finally, the whole volume (WBV) of
357 the calcaneus differed slightly between groups (ANOVA: p=0.04) but only the
358 difference between hunted Mesolithic and modern wild boars in their natural habitat was
359 significant (Tukey hsd test: p=0.02; Fig. 6), with Mesolithic individuals being larger.

360

361 **Discussion**

362

363 **1. Overall calcaneal microanatomy in wild and captive wild boars**

364 The qualitative description of the 3D maps and virtual thin sections identified a strong cortical
365 thickening on the plantar and dorsal borders (Pb and Db; Fig. 4). These observations are
366 consistent with the main constraints identified in the calcaneus of artiodactyls with significant
367 compression, bending, and tension forces on the plantar and dorsal borders (Lanyon 1973; Fig.
368 1). Thickenings of the plantar and dorsal margins have also been identified for wild deers
369 (Skedros et al., 2001) and pasture-raised domestic sheeps (Skedros et al., 2007). Moreover, the
370 anisotropic properties of the boar specimens trabeculae to follow an antero-posterior
371 orientations (outline arrows Fig. 3a) are congruent with the distribution of constraints
372 mentioned above, as internal bone structure organized itself to better respond to stress (Wolff,
373 1986; Ruff et al., 2006; Van der Meulen et al., 2006).

374 Although the entheses (i.e., areas of ligament and tendon insertion (Djukic et al., 2015)) are
375 regions of stress concentration, these areas show little or no effect on the microanatomy of boar
376 calcaneus. The loads applied to the calcaneus of artiodactyls are primarily shared between the
377 long plantar ligament and the Achilles tendon, which connects the calcaneus to the
378 gastrocnemius and soleus muscles, the latter forming the sural triceps (Lanyon, 1973; Woo et
379 al., 1981; Skedros et al., 2001; Skedros et al., 2007; Barone, 2020). However, while the long
380 plantar ligament attaches all along the plantar border, only a part of this edge is thickened in
381 the boar calcaneus. Furthermore, an important thickening of similar proportion is also observed
382 on the dorsal edge, whereas this bone side shows no enthesis. The Achilles tendon insertion at
383 the proximal end of the calcaneus is itself covered by a tendinous structure, the calcaneus cap
384 of the superficial flexor digitorum muscle (*m. flexor superficialis*), taking an attachment point
385 from the tip of the proximal end of the calcaneus to phalanges (Su et al., 1999; Barone, 2017;
386 Fig. 1). A slight cortical thickening on the proximal epiphysis of our specimens coincides with
387 the insertion of the tendons of the *m. flexor superficialis* muscles of the toes and of the *m.*
388 *gastrocnemius* muscles (Bénévent & Bressot, 1968; Barone 2017).

389 Regarding the articular surfaces, the *sustentaculum tali* is a strong protuberance on the medial
390 side forming an articular surface with the talus, the fibular trochlea is an articular surface for
391 the malleolar bone and the end of the distal part articulate with the cuboid bone (Barone, 2017),
392 recurrent slight cortical thickening is also observed in those regions.
393 Overall, the microanatomy seems to reflect the tension and compression forces with a strong
394 cortical thickening on Pb and Db as well as the anisotropy of the trabeculae. The areas of contact
395 with other bones are also represented with important bone density on the fibular trochlea, the
396 *sustentaculum tali* and the end of the distal part.

397

398 2. Intra individual variation in calcaneal Microanatomy

399 Beyond the general pattern, no clear relationship is observed between the variability of the
400 microanatomical parameters and the factors explored in this study (context, sex, weight, size).
401 However, we found important inter-individual differences, notably in the extent and depth of
402 the cortical thickness of the plantar and dorsal edges, and on the proximal epiphysis; the length
403 of the medullary cavity; the number and thickness of the trabeculae; the bone tissue density of
404 the *sustentaculum tali*, at the distal part of the bone and next to the fibular trochlea; and the
405 transverse sectional shape. This suggests s that other factors influencing bone development
406 during growth must be explored to further understand inter-individual disparity.

407 Only a few correlations (positive or negative) of the microanatomical variables with bone
408 size, weight, and age of the individuals (Table 2), are significant. These three parameters,
409 therefore, have a limited impact on the microanatomical organisation. Body weight is
410 only correlated with bone size (WBV). WBV influences the PCA axes (Table 2) while,
411 paradoxically, bone size is not directly correlated with any of the microanatomic variables in
412 isolation, which is congruent with the observations of the sections and 3D maps that have not
413 found any link between specimens' size and their microanatomy. Thus, it is the covariation
414 between the variables that are themselves weakly correlated with whole volume that makes the
415 relationship between whole volume and all variables significant. Nevertheless, although age is
416 not one of the parameters on which this study focuses, a weak correlation was found between
417 age and the trabecular percentage (%Trab; $r=0.32$), but trabecular percentage is not significantly
418 correlated with bone size since age and bone size are neither significantly correlated. The
419 increase in the proportion of trabecular bone tissue with age is not related to an increase in
420 trabecular compactness (TC) because this parameter is not significantly correlated with age
421 ($p=0.29$); the volume of the medullary zone tends to increase since the cortex becomes
422 proportionally thinner. This result is surprising because the opposite phenomenon occurs in the

423 calcaneus of deer (Skedros et al., 2001) and sheep (Skedros et al., 2007), where a thickening of
424 the cortex is observed with size in relation to the medullary zone. Conversely, while it is
425 surprising that age does not correlate with whole bone volume (WBV; Table2), this shows that
426 age and volume do not follow a linear relationship or that intraspecific variability in calcaneus
427 size between specimens exceeds the effect of growth. However, our sample does not adequately
428 test the relationship between the variables and ontogeny because of the large proportion of
429 individuals of the same age (25 months).

430

431 3.Change in mobility regime and calcaneal Microanatomy

432 Despite the lack of directly observable influence of the mobility context over the calcaneal
433 microanatomy, we found quantitative effect of mobility differences in the cortical thickness
434 (RmeanT and RmaxT), trabecular percentage (%Trab) and in the overall variation patterns
435 (PCA), indicating that difference in mobility context influences the microanatomical
436 characteristics of the calcaneus, although not in a strongly discriminatory pattern. However, we
437 didn't find the expected microanatomical proximity of wild boars living in their natural habitat
438 and their dissimilarity from wild boars kept in captivity, as seen in previous studies on the
439 calcaneus_3D_external shape and form (Harbers et al.2020). The variations in relative
440 mean cortical thickness (RMeanT) illustrate the general trend of variations related to the
441 mobility regime (Fig. 6). While we expected to observe similar average thicknesses between
442 animals that had similar mobility conditions (e.g. Natural habitat and Experimental control), we
443 found that control individuals have a much lower cortical thickness than wild individuals from
444 natural habitat. Thus, modern and Mesolithic wild boars hunted in their natural habitat display
445 similar microanatomy with the wild boars which grew in a very small living space (enclosure
446 of 4m2 in a hangar of 100m2.). The most divergent microanatomy from the wild boar norm of
447 reaction have been observed in the control populations from the wild boar farm of Urcier, which
448 have a much lower cortical thickness than wild boars in their natural habitat. All these results
449 suggest that the microanatomy of the *Sus scrofa* calcaneus does not strongly reflect the mobility
450 regime, contradicting the strong microanatomical signal associated with locomotor restriction
451 evidenced in battery chickens, including osteoporosis related to inactivity (Rath et al., 2000).
452 However, the locomotor restrictions of these reared animals are generally greater than those
453 imposed on the animals of this study.

454

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

457 The six Mesolithic calcanei from the archaeological site of Noyen-sur-Seine show similarity
458 for all parameters and variables with the other groups (Fig. 6). Thus, the Mesolithic
459 individuals share the same micromorphology with their modern relative. We also found
460 greater microanatomical variability in wild boars populations both modern and
461 Mesolithic. External factors were much less controlled than for the DOMEXP groups, thus
462 resulting in more elements to affect bone plasticity. In addition, the animals from Compiègne
463 and Chambord (Natural habitat) and NoyenEns2 and NoyenEns3 (archaeology) are different
464 populations so they have greater genetic variability in comparison to the DOMEXP group.
465 Furthermore, the Mesolithic specimens belong to individuals from multiple generations
466 separated by several centuries.

467 This genetic variability is much a
468 substantial factor that has a greater impact on the observed phenotype than the
469 intrapopulation variability related to motricity in this study. Consequently, when several
470 populations are included in the same group (Natural habitat and Mesolithic), their variability
471 exceeds that observed between the same population placed in different locomotor contexts
472 (stall, pen, control). Thus, the microanatomy of the calcaneus appears to be more affected by
473 population differences than by the locomotor context in which the animals grew.

474 In addition to this explanation, the wider locomotor regime of wild boars in their natural habitat
475 would foster greater ecophenotypic variation compared to captive specimens with reduced
476 mobility and more stereotypical locomotor behaviour. On the one hand, wild boars in nature
477 have a locomotor repertoire that must respond to several problems that are not encountered in
478 captivity, such as foraging or escape (Spitz & Janeau, 1990, 1995). Their daily travel is
479 generally less than 10km but it could be up to 80 km in one night (Keuling et al., 2009), peak
480 speeds of 40 km/h and high jumps up to 1.5 m have also been observed (Baskin & Danell,
481 2003). On the other hand, there is no study describing the skeletal repercussion of stereotypic
482 behaviours. However, because these types of behaviours performed to compensate for lack of
483 activity are induced during significant psychological depressions in individuals (Rushen, 1993;
484 Andre, 2007), other experimental approaches involving living specimens are not desirable.
485 quantifying

486 Also, diet plays a role in the development of the skeleton (Randoin & Causseret, 1945).
487 Experimental specimens were fed nutritionally balanced pellets (15% protein) intended for pig

Commenté [RC1]: Much better explained that way 😊

488 breeding to ensure consistent growth and bone formation. In their natural habitat, food
489 availability, seasonal, and geographical variations are major factors influencing food selection
490 by wild boars (Ballari, & Barrios-García, 2014). Thus, the diet factor possibly
491 also contributes to the high variability observed in wild (free-ranging) individuals.
492 Furthermore, it is likely that there are differences in substrates between the forests and areas in
493 which these boars lived; this may have important implications for the autopod (Kappelman,
494 1988). Indeed, the substrate factor seems potentially important for the stall-reared group where
495 the flat ground, was covered with moss and straw mats, whereas the more irregular natural
496 terrain of wild boars (like control group) implies variable and multidirectional soil reaction
497 forces (Hanot et al., 2017). The group of pens were raised on a flat terrain covered with grass
498 and a few trees.

499 The overview of the different studies related to the DOMEXP project
500 show that bone plasticity associated with domestication
501 varies between bones. The most surprising
502 result of the present study is that bone plasticity seems to be that wild boar calcaneus' external
503 morphology is more variable than its microanatomy, although the latter is considered to be more
504 plastic than bone external morphology (Dumont et al., 2013; Kivell, 2016; Vlachopoulos et al.,
505 2017)

506 . Since the results of the
507 study show that boars living in different contexts can have the same
508 microanatomical pattern, calcaneal microanatomy cannot be used to infer a captive
509 lifestyle. However, a strong diversity is noted in the microanatomy between wild and Mesolithic
510 specimens. A better understanding of the factors that regulate calcaneal variability
511 would possibly allow inferences related to habitat (type of soil, open or closed environment)
512 or diet.

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518 specimens. Preprint version X of this article has been peer-reviewed and recommended by Peer
519 Community In Archaeology (https://XXX link to the article).

Commenté [RC2]: Comment of Ignacio A. Lazagabaster :
unless those individuals belong to the same population, right?

Commenté [RC3R2]: Yes, but it is still difficult to say that
micranatomy works well in a homogeneous population,
because we have only observed slight differences between
individuals on a single population.

a mis en forme : Anglais (Royaume-Uni)

a mis en forme : Surlignage

a mis en forme : Anglais (Royaume-Uni)

Code de champ modifié

521 **Conflict of interest disclosure**

522 The authors declare they have no conflict of interest relating to the content of this article.

523 Alexandra Houssaye is recommender of PCI *Paleontology*.

Commenté [RC4]: If required

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