



HAL
open science

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

Romain Cottereau, Katia Ortiz, Yann Locatelli, Alexandra Houssaye, Thomas Cucchi

► To cite this version:

Romain Cottereau, Katia Ortiz, Yann Locatelli, Alexandra Houssaye, Thomas Cucchi. Can growth in captivity alter the calcaneal microanatomy of a wild ungulate?. 2022. hal-03859279v1

HAL Id: hal-03859279

<https://hal.science/hal-03859279v1>

Preprint submitted on 18 Nov 2022 (v1), last revised 6 Jan 2023 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Romain cottereau^{1,#}, Katia Ortiz^{2,3}, Yann²⁻⁴ Locatelli,
Alexandra Houssaye^{1,*}, Thomas Cucchi^{5,*}

¹ CNRS, UMR 7179 Mécanismes Adaptatifs et Evolution, Muséum d'Histoire Naturelle de Paris, France

² Réserve Zoologique de la Haute-Touche, Muséum National d'Histoire Naturelle, Obterre, France

³ Institut de Systématique, Evolution, Biodiversité, UMR 7205, Muséum National d'Histoire Naturelle CNRS UPMC EPHE, UA, Paris, France

⁴ Physiologie de la Reproduction et des Comportements, UMR 7247, INRAE CNRS Université de Tours IFCE, Nouzilly, France

⁵ UMR 7209 Archéozoologie, Archéobotanique : Sociétés, Pratiques et Environnements, Muséum d'Histoire Naturelle de Paris, France

*: co last authors

#: corresponding author

Abstract

Reduced mobility associated with captivity induces changes in biomechanical stress on the skeleton of domesticated animals. Due to bone plasticity, bone's morphology and internal structure 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 a wild ungulate's life will alter the internal structure of its limb bones and provide a proof of concept for 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 volume, the calcaneus of the

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

38

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

41

42 **Introduction**

43

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

56 Captive animals may grow in limited areas, which does not involve the same range of
57 movement and forces applied to their bones compared to free-ranging individuals. 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 changes 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 of bipedalism in hominids (Ruff, 2018) and to observe the
63 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.,

2013). Despite this, archaeozoologists have always considered that the first observable morphological changes occurred only late in the domestication process and could not perceive its initial stages since they would not involve genetic isolation or selective reproduction (Vigne et al., 2011; Colledge et al., 2016). To develop new phenotypic proxy of the first steps of animal domestication, an experimental project (DOMEXP) has been performed to test whether growth in captivity of a wild ungulate would leave a morpho-functional imprint distinguishable from its natural habitat norm of reaction. Hence, early process of cultural control (Hecker 1982) could be traced from bones anatomy long before the so-called “domestication syndrome”. To test this hypothesis, the DOMEXP project used a genetically homogeneous wild boar (*Sus scrofa*)

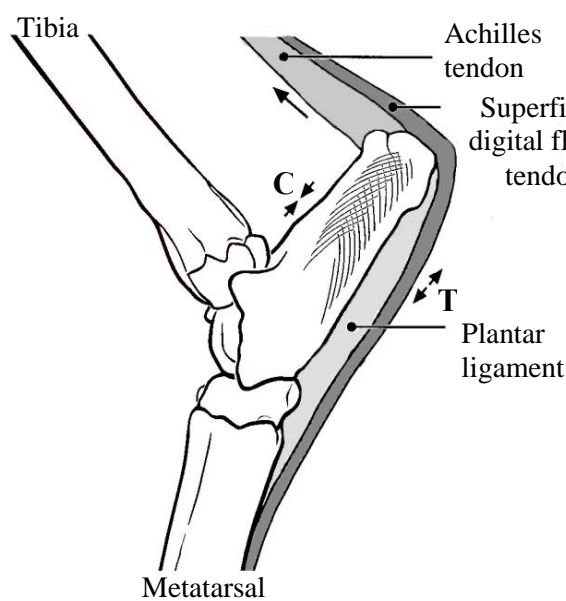


Fig. 1. Lateral view of the tarsal region of a skeletally mature artiodactyl, showing the calcaneus with other associated bones, ligaments and tendons. The large arrow in the dorsal direction indicates the orientation of the force exerted by the Achilles tendon during paw extension, loading the dorsal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

2020a). Microanatomical investigations have also revealed changes in the 3D topography of the cortical thickness in the humeral shaft (Harbers et al., 2020b). However, the impact of mobility reduction on the microanatomical features of the skeleton remained to be explored. For this reason, this study investigates the microanatomical variation of the calcaneus from the experimental specimens of the DOMEXP project. Tarsal bones like the calcaneum are particularly considered as more informative than others in investigating the mechanical forces that apply to individuals during locomotion (Lovejoy et al., 1999; Carter & Beaupré, 2001; Pearson & Lieberman, 2004). Proximally articulated to the fibula and distally to the cuboid

101 bone, the calcaneus is intensely stressed during locomotion in terrestrial tetrapods. It acts as a
102 lever arm for the ankle extensor muscles, and is subject to high tensile, flexural, and
103 compressive forces (Fig. 1; Hussain, 1975; Carrano, 1997; Bleefeld & Bock, 2002; Bassarova
104 et al., 2009; Barone, 2017). The calcaneus is therefore a particularly interesting object of study
105 to investigate differences in mobility between individuals (Harbers et al., 2020a). Moreover,
106 the high compactness of the calcaneus makes it resistant to taphonomic alterations, which gives
107 it a good general preservation in archaeological contexts (Binford, 1978).

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

114

115 **Material and method**

116

117 **Experimental protocol**

118 24 wild boars aged six months were sampled from a control population living in a fenced forest
119 of about 100,000 m² in northern France. These specimens were then divided into two equal
120 samples with the same sex ratio and reared until 24 month in two different contexts of reduced
121 mobility: (1) a stall of 100 m², where males and females were separated (each group had 45 m²)
122 and (2) a wooded pen of 3000 m². These two contexts represent respectively 99.9% and 97%
123 of range reduction compared to the control population, which prevents the average daily
124 distances measured in free boar populations (Palencia et al. 2019; Russo et al. 1997). The stall
125 presented no opportunity for foraging. In the wooded enclosure, this opportunity was limited
126 due to space limitations. Dry feed pellets suitable for feeding domestic pigs were provided to
127 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), five from the control population
132 (control) - 15 hunted wild boars from two Northern France forests: eight from the Chambord
133 forest and seven from the Compiègne forest (Natural habitat) and six archaeological specimens

134 from the Mesolithic contexts (Meso) of Noyen sur Seine (Mordant et al., 2013, Marinval-Vigne
135 et al., 1989) in Northern France (Table 1). The six archaeological specimens from Mesolithic
136 deposits have been accumulated by hunter-gatherer living in Western Europe before the
137 Neolithic dispersal via the Mediterranean and Danubian pathways, which introduced domestic
138 pigs from the Near East (Larson 2006) that interbred with local populations of wild boars
139 (Frantz et al. 2020). Two specimens are radiocarbon dated from the Middle Mesolithic (between
140 -8000 and -7300 cal. BP) and four specimens to the Final Mesolithic dated between -7000 and
141 -6200 cal. BP (Mordant et al., 2013). All the specimens bar the archaeological samples have
142 associated age and sex informations. Three of the archaeological calcanei belong to adult
143 individuals since their proximal epiphysis is fused which is known to happen around 2 years in
144 wild boars (Bridault et al., 2000).

145

146 Data acquisition

147 All the calcanei have been scanned with high-resolution microtomography (EasyTom 40-150
148 scanner, RX Solutions) at the MRI platform, hosted at ISEM, University of Montpellier (UMR
149 5554); reconstructions were then performed using X-Act (RX Solutions).

150

151 Virtual thin sections

152 For qualitative comparisons, three virtual sections (Fig. 2, Appendix 1,2 and 3) were made for
153 each calcaneus, following Barone (2017) for terminology and orientations. The bones were
154 oriented as follows: in dorsal view (Fig. 2a), bone's axis is vertical and the fibular trochlea is
155 oriented toward the observer, its dorsal part aligns with the contour of the bone's medial border;
156 in medial view (Fig. 2b) the *sustentaculum tali* is directed towards the observer, the observation
157 angle is fixed when the anterior edge of the fibular trochlea is no longer visible upon rotation
158 from the anterior view to the medial view. Sagittal sections (SS) run in dorsal view from the
159 distal end tip and the midpoint of the thickness at the proximal epiphysis base (Fig. 2). The
160 frontal sections (FS) extend from the distal end tip to the midpoint of the proximal epiphysis
161 base (see purple arrows on Fig. 2b). Cross-sections (TS) are taken perpendicular to the FS plane
162 at 1/3 of the total length of the bone (from the proximal epiphysis tip to the distal tip) (Fig. 2).
163 These sectional planes were chosen to depict large portions of the bone in order to analyze the
164 microanatomical structure (e.g., trabecular network, cortical thickness) while being easily
165 created with good reproducibility for all specimens. Virtual sections were created using
166 VGSTUDIO MAX, versions 2.2 (Volume Graphics Inc.).

167

168 **Calcaneus 3D mapping**

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

183

184 **Quantitative parameters**

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

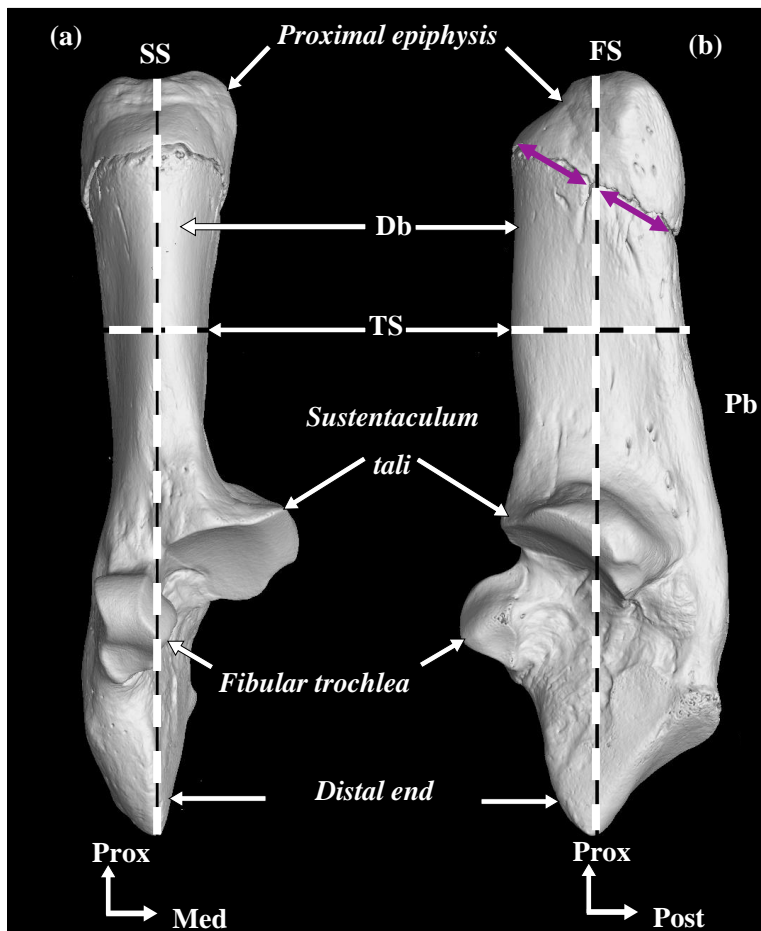


Fig. 2 Calcaneus of *Sus scrofa*, specimen 2017-578 illustrating the planes of the virtual sections: SS, Sagittal section; FS, Frontal section; TS, Transverse section. Db, Dorsal border; Pb, Plantar border. Purple arrows indicate the midpoint of proximal epiphysis base.

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 ($T_c = \text{trabecular bone tissue} * 100 / \text{trabecular volume}$).

207

208 (MeanT) and maximum (MaxT) cortical thicknesses that were obtained directly in Avizo 9.4
 209 using the 'distance' function were calculated 5) RMaxT and 6) RMeanT, relative maximum and
 210 mean thicknesses, by dividing MaxT and MeanT by a mean radius r , obtained from the whole
 211 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

213 **Statistical analysis**

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

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

236 MANOVA ("res.man" function) was also used to test the overall difference in microanatomical
237 variables between mobility contexts.

238

239 **Results**

240 **Qualitative descriptions**

241 *General microanatomical pattern*

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

247 medullary cavity, about 1cm (Pradat187; Fig. 3c,h) to 2 cm long (2013-1286; Fig. 3d,i) and 1
248 cm wide, at the level of the *Sustentaculum tali* (FigXa-j). The thickness of the trabeculae is
249 relatively homogeneous along the calcaneus, except around the medullary cavity, where they
250 are generally thicker (Fig. 3a-j). Also, the trabecular density is heterogeneous, with some
251 specimens having many trabeculae (2017-570; Fig. 3b,g,l) while others have twice as less but
252 thicker trabeculae (2013-1286; Fig. 3 d,I,n). Finally, the bone density in the distal part strongly
253 varies between individuals from compact (2017-570; Fig. 3b,g) to spongy (Calc2139; Fig.
254 3e,j).

255 *Sagittal sections*

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

268

269 *Frontal sections*

270 All individuals have a cortex that varies relatively little in thickness (about 2-3mm) on the
271 medial and lateral sides (Fig. 3f-e). In contrast, the cortical thickness in the proximal epiphysis
272 varies from thin (0.5 cm in specimen 2013-1286; Fig. 3i) to twice thicker (0.3 cm in specimen
273 2017-555; Fig. 3f). Similarly, the compactness and the cortical thickness of the *sustentaculum*
274 *tali* varies between individuals, some of which show compact bone and thick cortex (Pradat187;
275 Fig. 3h) while others show spongy bone and thin cortex (2013-1286; Fig. 3i).

276

277 *Transverse sections*

278 The transverse sections' shapes are generally oval and elongate (2017-555; Fig. 3k) but several
279 specimens present a rounder section (2017-570; Fig. 3l). The cortical thickness is fairly constant

280 across the sections, but some specimens show a cortical thickening at the plantar border (2013-
281 1286; Fig. 3n).

282

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

285

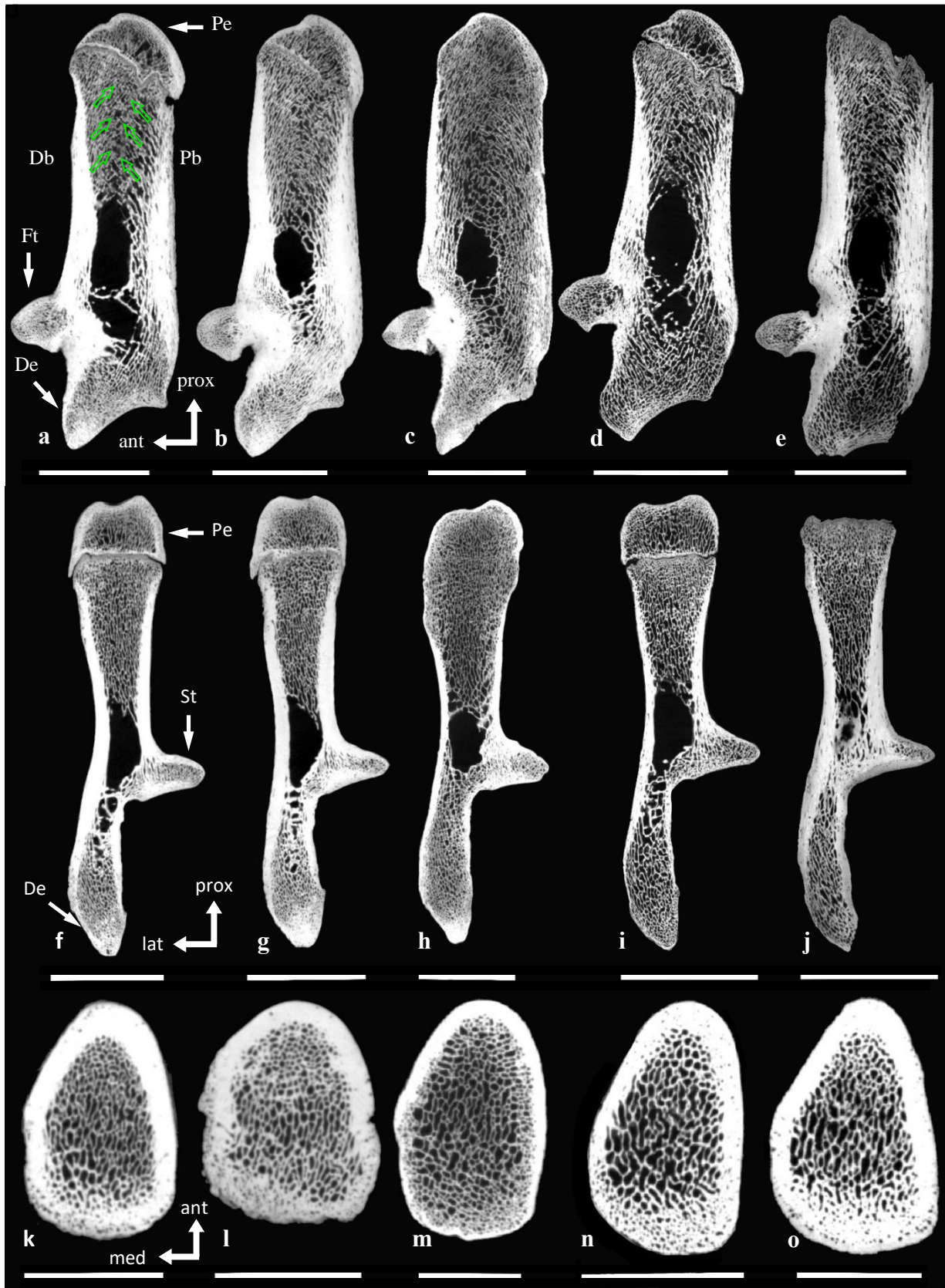


Fig. 3 Virtual thin sections of the calcaneus of boars, a,f,k, 2017-555 (Experimental stall); b,g,l 2017-570 (Experimental pen); c,h,m Pradat187 (Experimental control); d,i,n, 2013-1286 (Natural habitat); e,j,o, Calc2139 (Mesolithic). Db, Dorsal border; De, Distal end; Ft, Fibular trochlea; Pb, Plantar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

286 3D mapping of the cortical thickness

287 The 3D mappings of the cortical thickness and its variation across each bone, are quite similar
288 between specimens (Appendix 1). In agreement with the observation of the virtual sections,
289 there is a fairly extensive area with greater cortical thickness at the plantar border of the
290 calcaneus (Pb; Fig. 4A). Although generally less extensive, thickenings of the cortex can also
291 be noted on the dorsal margin (Db) and on the dorsal base of the *sustentaculum tali* (Sb; Fig.
292 4B). There is little thickening of the cortex on the proximal end (Pe). Finally, there is no lateral
293 or medial thickening noted.

294 Twenty-five of the 47 specimens show strong cortical thickening at the plantar margin (Pb;
295 Appendix 1), like in 2017-568 (Fig. 4A); this is less visible in the 22 others, like Pradat 185 and
296 2013-1264 (Fig. 4D; Fig. 4G). Twenty-three specimens have thick cortical bone at the dorsal
297 margin (Db; Table 1; Appendix 1), like in Pradat 185 and 2017-568 boars (Fig. 4B and Fig.
298 4E), whereas the others do not show such thickening like the calcaneus of boar 2013-1264 (Fig.
299 4H). Twenty-eight specimens show a relative thickening at the base of the sustentaculum (Sb;
300 Table 1), this widening is particularly noticeable in boar 2013-1264 (Fig. 4H) and absent in
301 others like Pradat 185 (Fig. 4E). A slight recurrent cortical thickening on the distal end is
302 observed in part of the specimens like 2017-568 (Fig. 4a). Twenty-seven specimens have a
303 slightly thicker cortex on the proximal end (Pe) such as 2017-568 (Fig. 4C), 15 show no or very
304 little thickening, such as Pradat185 and 2013-1264, respectively (Fig. 4F and Fig. 4I). Four of
305 the proximal ends of the Mesolithic specimens were not found because they were not fused to
306 the rest of the bone.

307

308

309

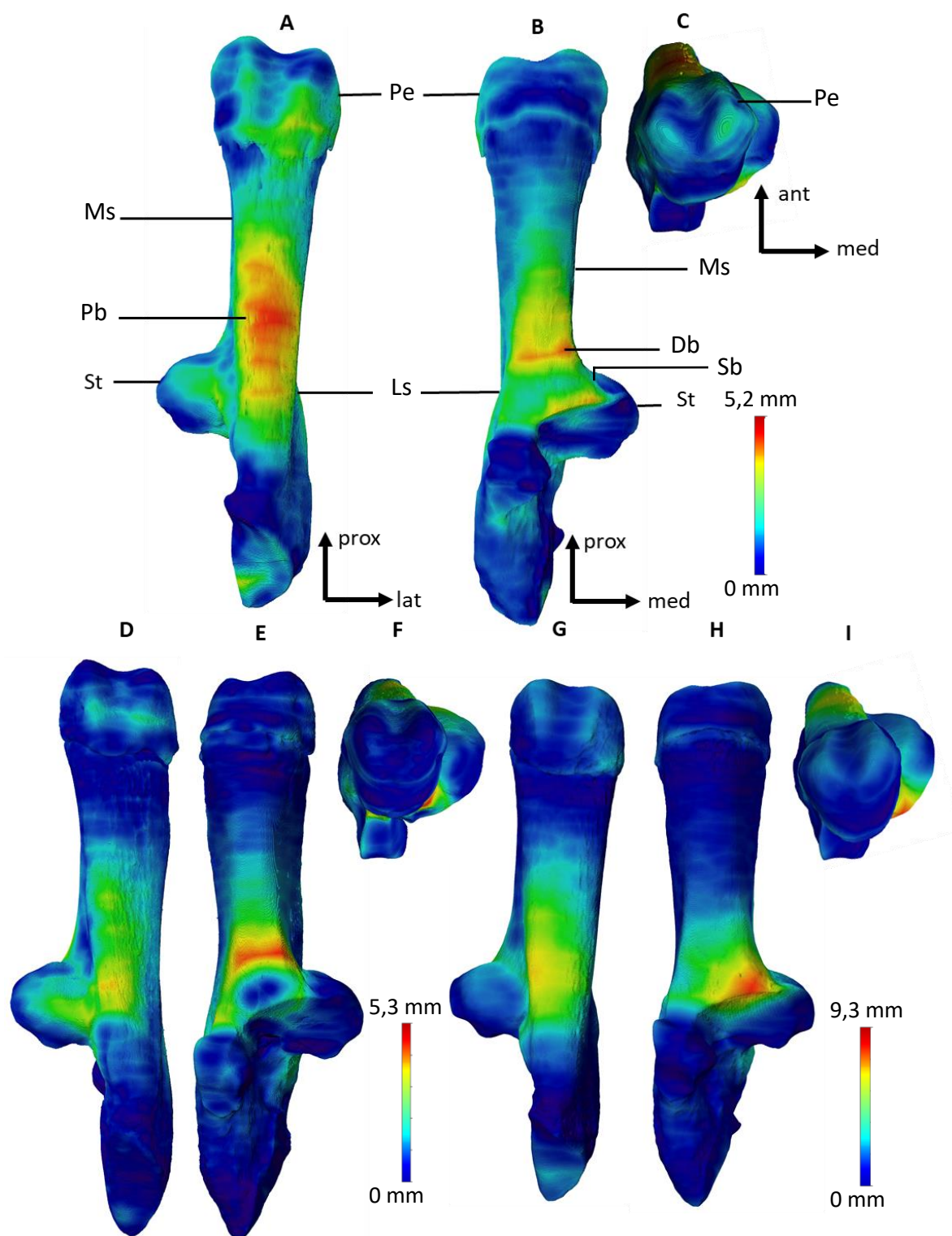


Fig. 4 3D mappings of the *Sus scrofa* calcaneus relative cortical thickness. A-C specimen 2017-568; D-F Pradat 185; G-I 2013-1264. Anatomical abbreviations: Db, dorsal border; Ls, lateral side; Ms, medial side; Pb, plantar border; Pe, proximal epiphysis; Sb, Sustentaculum base; St, *Sustentaculum tali*.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

310

311 Quantitative analyses

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

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

316 Variables C, %Trab, RMeanT, RMaxT, weight, and TC did not differ between males and
 317 females. Only whole calcaneus volume (WBV) variation differs with sex (Kruskal Wallis:
 318 $p<0.01$) with males larger than females (Fig. 6). Mesolithic specimens (sex unknown) have
 319 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		$r=0,08$ $p=0,65$	$r=-0,16$ $p=0,35$	$r=0,02$ $p=0,93$	$r=0,30$ $p=0,08$	$r=0,26$ $p=0,14$	$r=0,34$ $p=0,05$	$r=0,31$ $p=0,07$

320 *Patterns of calcanei microanatomical variations and mobility contexts*

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

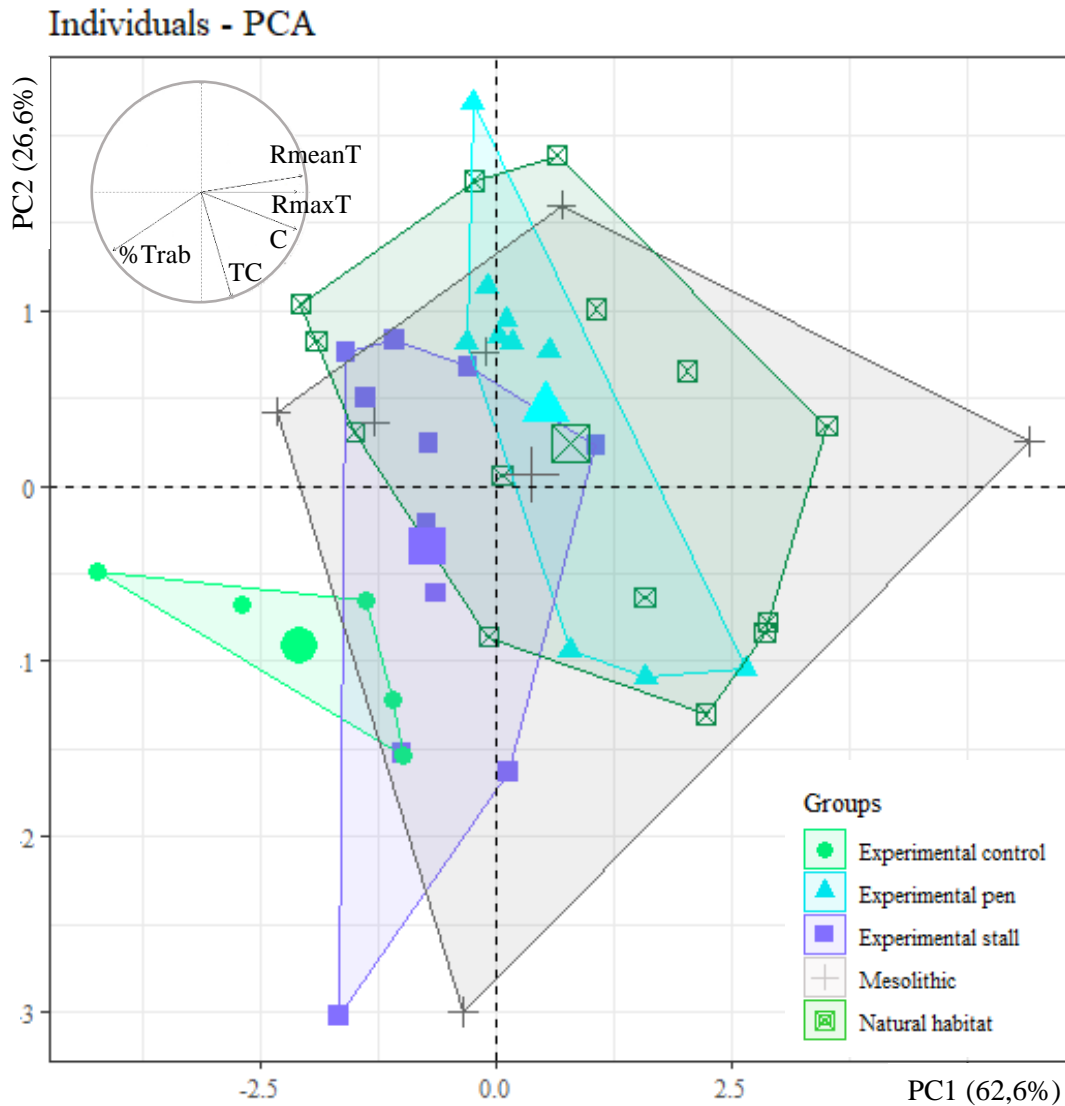


Fig. 5 Distribution of the 46 specimens and their associated context groups on the first two axes of the PCA computed on: WBV, total bone volume; RMeanT, relative mean bone thickness; RMaxT, relative maximum bone thickness; C, compactness ratio; TC, trabecular compactness ratio; %Trab, percentage of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

333 between wild boars from the Natural habitat and the Experimental control groups (Dunn test:
 334 $p < 0.01$) and between Experimental control and Experimental pen groups (Dunn's test: $p < 0.01$).
 335 RMaxT is significantly different between Experimental control and Mesolithic wild boars
 336 (Dunn's test: $p < 0.01$). %Trab is significant different between Experimental control and natural
 337 habitat (Dunn's test: $p < 0.01$) and between Experimental pen and control (Dunn's test: $p < 0.01$).
 338 Thus, the pen-reared boars have a higher average cortex thickness (RMeanT) than the control
 339 group, for which, conversely, it is the percentage of trabecular bone (%Trab) that is higher (Fig.

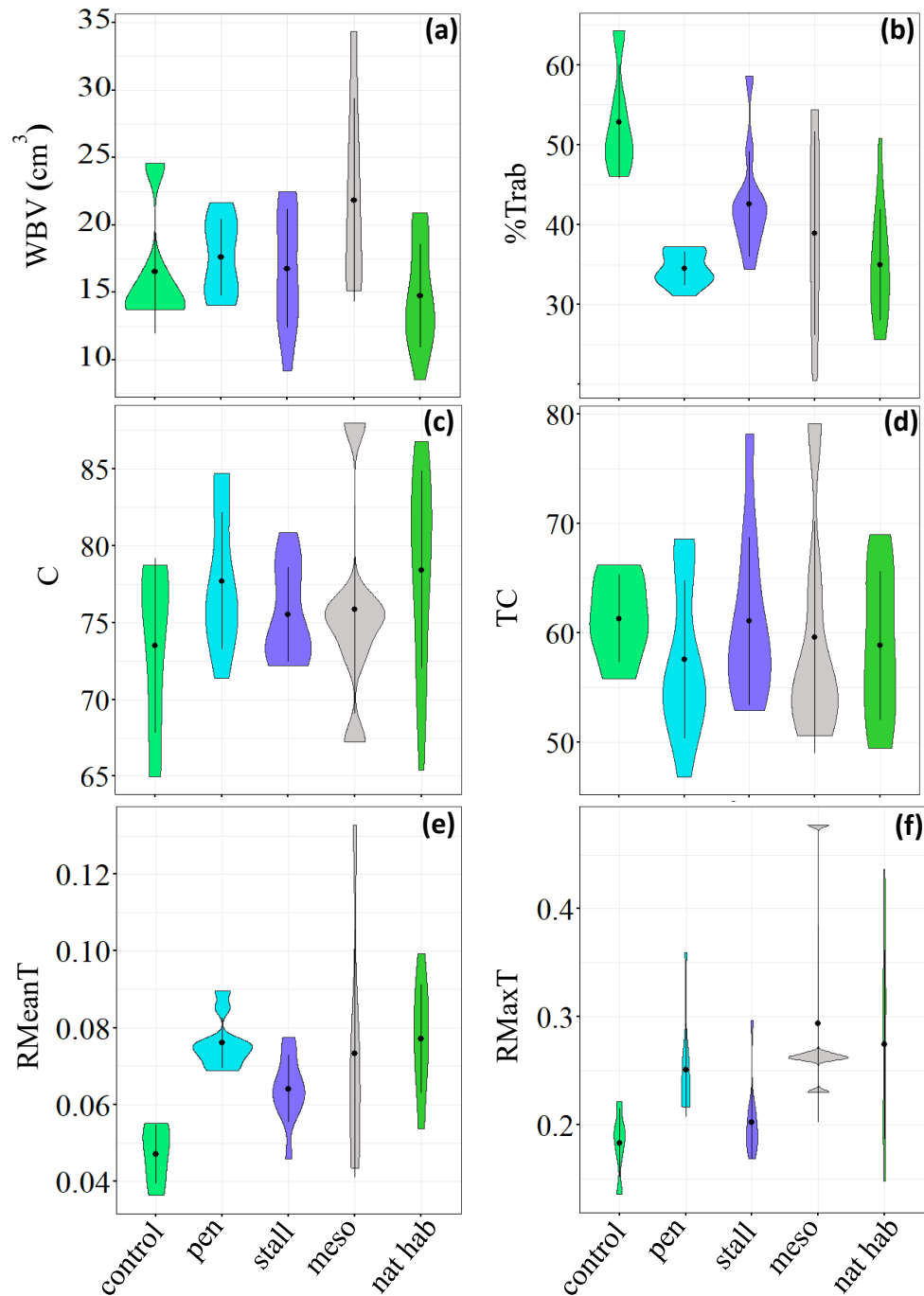


Fig. 6 Calcaneus microanatomical variation in *Sus scrofa* from experimental populations and Mesolithic contexts. WBV, whole bone volume; RMeanT, relative mean cortical thickness; RMaxT, relative maximum cortical thickness; C, compactness ratio; TC, trabecular compactness ratio; %Trab, percentage of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

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

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

349

350 **Discussion**

351

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

353 The qualitative description of the 3D maps and virtual thin sections identified a strong cortical
354 thickening on the plantar and dorsal borders (Pb and Db; Fig. 4). These observations are
355 consistent with the main constraints identified in the calcaneus of artiodactyls with significant
356 compression, bending, and tension forces on the plantar and dorsal borders (Lanyon 1973; Fig.
357 1). Thickenings of the plantar and dorsal margins have also been identified for wild deers
358 (Skedros et al., 2001) and pasture-raised domestic sheeps (Skedros et al., 2007). Moreover, the
359 anisotropic properties of the boar specimens trabeculae to follow an antero-posterior
360 orientations (outline arrows Fig. 3a) are congruent with the distribution of constraints
361 mentioned above, as internal bone structure organized itself to better respond to stress (Wolff,
362 1986; Ruff et al., 2006; Van der Meulen et al., 2006).

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

378 Regarding the articular surfaces, the *sustentaculum tali* is a strong protuberance on the medial
379 side forming an articular surface with the talus, the fibular trochlea is an articular surface for
380 the malleolar bone and the end of the distal part articulate with the cuboid bone (Barone, 2017),
381 recurrent slight cortical thickening is also observed in those regions.

382 Overall, the microanatomy seems to reflect the tension and compression forces with a strong
383 cortical thickening on Pb and Db as well as the anisotropy of the trabeculae. The areas of contact
384 with other bones are also represented with important bone density on the fibular trochlea, the
385 *sustentaculum tali* and the end of the distal part.

386

387 2. Intra individual variation in calcaneal Microanatomy

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

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

412 2001) and sheep (Skedros et al., 2007), where a thickening of the cortex is observed with size
413 in relation to the medullary zone. Conversely, while it is surprising that age does not correlate
414 with whole bone volume (WBV; Table2), this shows that age and volume do not follow a linear
415 relationship or that intraspecific variability in calcaneus size between specimens exceeds the
416 effect of growth. However, our sample does not adequately test the relationship between the
417 variables and ontogeny because of the large proportion of individuals of the same age (25
418 months).

419

420 3.Change in mobility regime and calcaneal Microanatomy

421 Despite the lack of directly observable influence of the mobility context over the calcaneal
422 microanatomy, we found quantitative effect of mobility differences in the cortical thickness
423 (RmeanT and RmaxT), trabecular percentage (%Trab) and in the overall variation patterns
424 (PCA), indicating that difference in mobility context influences the microanatomical
425 characteristics of the calcaneus, although not in a strongly discriminatory pattern. However, we
426 didn't find the expected microanatomical proximity of wild boars living in their natural habitat
427 and their dissimilarity from wild boars kept in captivity, as seen in previous studies on the
428 calcaneus 3D externalshape and form (Harbers et al.2020). The variations in relative mean
429 cortical thickness (RMeanT) illustrates the general trend of variations related to the mobility
430 regime (Fig. 6). While we expected to observe similar average thicknesses between animals
431 that had similar mobility conditions (e.g. Natural habitat and Experimental control), we found
432 that control individuals have a much lower cortical thickness than wild individuals from natural
433 habitat. Thus, modern and Mesolithic wild boars hunted in their natural habitat display similar
434 microanatomy with the wild boars which grew in a very small living space (enclosure of 4m2
435 in a hangar of 100m2.). The most divergent microanatomy from the wild boar norm of reaction
436 have been observed in the control populations from the wild boar farm of Urcier, which have a
437 much lower cortical thickness than wild boars in their natural habitat. All these results suggest
438 that the microanatomy of the *Sus scrofa* calcaneus does not strongly reflect the mobility regime,
439 contradicting the strong microanatomical signal associated with locomotor restriction
440 evidenced in battery chickens, including osteoporosis related to inactivity (Rath et al., 2000).
441 However, the locomotor restrictions of these reared animals are generally greater than those
442 imposed on the animals of this study.

443

444 4. Greater microanatomical variability in modern and ancient wild boars hunted in 445 their natural habitat

446 The six Mesolithic calcanei from the archaeological site of Noyen-sur-Seine show similarity
447 for all parameters and variables with the other groups (Fig. 6). Thus, the Mesolithic individuals
448 share the same microanatomy with their modern relative. We also found greater microanatomical
449 variability in wild boars populations both modern and Mesolithic. External factors were much
450 less controlled than for the DOMEXP groups, thus resulting in more elements to affect bone
451 plasticity. In addition, the animals from Compiègne and Chambord (Natural habitat) and
452 NoyenEns2 and NoyenEns3 (archaeology) are different populations so they have greater
453 genetic variability in comparison to the DOMEXP group. Furthermore, the Mesolithic
454 specimens belong to individuals from multiple generations separated by several centuries. This
455 genetic variability is much a substantial factor that has a greater impact on the observed
456 phenotype than the intrapopulation variability related to motricity in this study. Consequently,
457 when several populations are included in the same group (Natural habitat and Mesolithic), their
458 variability exceeds that observed between the same population placed in different locomotor
459 contexts (stall, pen, control). Thus, the microanatomy of the calcaneus appears to be more
460 affected by population differences than by the locomotor context in which the animals grew.

461 In addition to this explanation, the wider locomotor regime of wild boars in their natural habitat
462 would foster greater ecophenotypic variation compared to captive specimens with reduced
463 mobility and more stereotypical locomotor behaviour. On the one hand, wild boars in nature
464 have a locomotor repertoire that must respond to several problems that are not encountered in
465 captivity, such as foraging or escape (Spitz & Janeau, 1990, 1995). Their daily travel is
466 generally less than 10km but it could be up to 80 km in one night (Keuling et al., 2009), peak
467 speeds of 40 km/h and high jumps up to 1.5 m have also been observed (Baskin & Danell,
468 2003). On the other hand, there is no study describing the skeletal repercussion of stereotypic
469 behaviours. However, because these types of behaviours performed to compensate for lack of
470 activity are induced during significant psychological depressions in individuals (Rushen, 1993;
471 Andre, 2007), other experimental approaches involving living specimens are not desirable.

472 quantifying

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

477 by wild boars (Ballari, & Barrios-García, 2014), Thus, the diet factor possibly also contributes
478 to the high variability observed in wild (free-ranging) individuals. Furthermore, it is likely that
479 there are differences in substrates between the forests and areas in which these boars lived; this
480 may have important implications for the autopod (Kappelman, 1988). Indeed, the substrate
481 factor seems potentially important for the stall-reared group where the flat ground, was covered
482 with moss and straw mats, whereas the more irregular natural terrain of wild boars (like control
483 group) implies variable and multidirectional soil reaction forces (Hanot et al., 2017). The group
484 of pens were raised on a flat terrain covered with grass and a few trees.

485 The overview of the different studies related to the DOMEXP project show that bone plasticity
486 associated with domestication varies between bones. The most surprising result of the present
487 study is that the bone plasticity of the external morphology of wild boar calcaneus' is more
488 variable than its microanatomy, although the latter is considered to be more plastic than bone
489 external morphology (Dumont et al., 2013; Kivell, 2016; Vlachopoulos et al., 2017). Since the
490 results of the study show that boars living in different contexts can have the same
491 microanatomical pattern, calcaneal microanatomy cannot be used to infer a captive lifestyle.
492 However, a strong diversity is noted in the microanatomy between wild and Mesolithic
493 specimens. A better understanding of the factors that regulate calcaneal variability would
494 possibly allow inferences related to habitat (type of soil, open or closed environment) or diet.

495

496 **Acknowledgements**

497 We thank the reviewers Ignacio Aguilar Lazagabaster and Max Price as well as our
498 recommender Nimrod Marom for their helpful and positive comments that have helped improve
499 this work. Thank to Florian Bogaert, who helped us to realize the segmentation of a part of
500 specimens. Preprint version **X** of this article has been peer-reviewed and recommended by Peer
501 Community In Archaeology (<https://XXX> link to the article).

502

503 **Conflict of interest disclosure**

504 The authors declare they have no conflict of interest relating to the content of this article.
505 Alexandra Houssaye is recommender of PCI Paleontology.

506

507 **Funding**

508 This research has been funded by the project Emergence SU-19-3-EMRG-02. This
509 research has also benefited from financial supports of the Muséum national d'Histoire naturelle
510 (Paris) and the CNRS INEE (Institut écologie et environnement).

511 This research was funded by ANR through the DOMEXP project (ANR\u000213-JSH3-0003-
512 01), the LabEx ANR-10-LABX-0003-BCDiv, in the programme 'Investissements d'avenir'
513 ANR-11-IDEX-0004-02, programme Emergence SU-19-3-EMRG-02.

514

515 **Bibliography**

516

517 Agarwal, S.C., 2016. Bone morphologies and histories: Life course approaches in bioarchaeology.
518 American Journal of Physical Anthropology. Am. J. Phys. Anthropol., 159: 130-149. URL
519 <https://onlinelibrary.wiley.com/doi/full/10.1002/ajpa.22905>

520

521 Andre, J.P., 2007. An introduction to normal and pathological behaviour in psittacine. Bulletin de
522 l'Académie Vétérinaire de France, 2007 vol.160, N°3 <https://doi.org/10.4267/2042/47885>

523

524 Barone, R., 2000. Anatomie comparée des mammifères domestiques Tome 2 -Arthologie et myologie-
525 (4eéd.). Association centrale d'entraide vétérinaire.

526

527 Barone, R., 2017. Anatomie comparée des mammifères domestiques Tome 1 - ostéologie – (5e éd.).
528 Vigot.

529

530 Baskin, L., Danell K., 2003 Ecology of ungulates: a handbook of species in Eastern Europe and
531 Northern and Central Asia. Heidelberg, Germany:Springer Science & Business Media.

532

533 Bassarova, M., Janis, C.M., Archer, M., 2009. The Calcaneum—On the Heels of Marsupial
534 Locomotion. J Mammal Evol 16, 1–23. <https://doi.org/10.1007/s10914-008-9093-7>

535

536 Bénévent, M., Bressot, C., 1968. Guide pour la dissection et l'identification des principaux muscles de
537 la carcasse chez le mouton. Annales de biologie animale, biochimie, biophysique 8, 147–193.

538

539 Binford, L.R., 1978. Nunamiut: ethnoarchaeology. New York, NY, Academic Press.

540

541 Bleefeld, A., Bock, W., 2002. Unique anatomy of lagomorph calcaneus. Acta Palaeontologica
542 Polonica, 47, 181–183

543

544 Carmeliet, G., Bouillon, R., 2001. Space flight: a challenge for normal bone homeostasis. Crit. Rev.
545 Eukar. Gene 11, 1–3.

546

547 Carrano, M.T., 1997. Morphological indicators of foot posture in mammals: a statistical and
548 biomechanical analysis. Zool. J. Linn. Soc. 121, 77–104. doi:10.1111/j.1096-
549 3642.1997.tb00148.x

550

551 Carter, D.R., Beaupré, G.S., 2001. Skeletal Function and Form. Mechanobiology of Skeletal
552 Development, Aging and Regeneration. Cambridge University Press, Cambridge.

553

- 554 Cowin, S.C., Hegedus, D.H., 1976. Bone remodeling I : a theory of adaptive elasticity. *J. Elasticity* 6,
555 313–326.
556
- 557 Cubo, J., Ponton, F., Laurin, M., De Margerie, E., Castanet, J., 2005. Phylogenetic Signal in Bone
558 Microstructure of Sauropsids. *Systematic Biology* 54, 562–574.
559 <https://doi.org/10.1080/10635150591003461>
560
- 561 Cucchi, T., Domont, A., Harbers, H., Leduc, C., Guidez, A., Bridault, A., Hongo, H., Price, M., Peters,
562 J., Briois, F., Guilaine, J., Vigne, J.D., 2021. Bones geometric morphometrics illustrate 10th
563 millennium cal. BP domestication of autochthonous Cypriot wild boar (*Sus scrofa circeus* nov.
564 ssp). *Sci Rep* 11, 11435. <https://doi.org/10.1038/s41598-021-90933-w>
565
- 566 Curran, S.C., 2012. Expanding ecomorphological methods: geometric morphometric analysis of
567 Cervidae post-crania. *J. Archaeol. Sci.* 39, 181-184
568
- 569 David, C.C., Jacobs, D.J., 2014. Principal Component Analysis: a method for determining the essential
570 dynamics of proteins. In: Livesay DR (ed) *Protein Dynamics: Methods and Protocols*. Humana
571 Press, Totowa, pp 193–226
572
- 573 Divé, J., Eisenmann, V., 1991. Identification and discrimination of first phalanges from Pleistocene
574 and modern *Equus*, wild and domestic. In: Meadow, R., Uerpmann, H.-P. (Eds.), 1991. *Equids in*
575 *the Ancient World*, vol. II. Ludwig Reichert Verlag, Wiesbaden, pp. 278-333
576
- 577 Djukic, K., Milovanovic, P., Hahn, M., Busse, B., Amling, M., Djuric, M., 2015. Bone
578 microarchitecture at muscle attachment sites: The relationship between macroscopic scores of
579 entheses and their cortical and trabecular microstructural design. *American Journal of Physical*
580 *Anthropology* 157, 81–93. <https://doi.org/10.1002/ajpa.22691>
581
- 582 Du, T.Y., Standen, E.M., 2020. Terrestrial acclimation and exercise lead to bone functional response
583 in *Polypterus senegalus* pectoral fins. *Journal of Experimental Biology* 223.
584 <https://doi.org/10.1242/jeb.217554>
585
- 586 Dumont, M., Laurin, M., Jacques, F., Pellé, E., Dabin, W., de Buffrénil, V., 2013. Inner
587 architecture of vertebral centra in terrestrial and aquatic mammals: a two-dimensional
588 comparative study. *Journal of Morphology* 274, 570–584.
589
- 590 Eisenmann, V., 1986. Comparative osteology of modern and fossil horses, half-asses, and asses. In:
591 Meadow, R.H., Uerpmann, H.-P. (eds.), *Equids in the Ancient World*. Ludwig Reichert Verlag,
592 Wiesbaden, pp. 68-116.
593
- 594 Eisenmann, V., Beckouche, S., 1986. Identification and discrimination of metapodials from Pleistocene
595 and modern *Equus*, wild and domestic. In: Meadow, R.H., Uerpmann, H.-P. (Eds.), *Equids in the*
596 *Ancient World*. Ludwig Reichert Verlag, Wiesbaden, pp. 117-163.
597
- 598 Eisenmann, V., 1995. What metapodial morphometry has to say about some Miocene Hipparions. In:
599 Vrba, E.S., Denton, G.H., Partridge, T.C., Burckle, L.H. (Eds.), *Paleoclimate and Evolution, with*
600 *Emphasis on Human Origins*. Yale University Press, New Haver, pp. 148-164.
601
- 602 Frost, H.M., 1983. A determinant of bone architecture. The minimum effective strain. *Clin Orthop*
603 *Relat Res* 286–292.
604

- 605 Frost, H.M., 1994. Wolff's Law and bone's structural adaptations to mechanical usage: an overview
606 for clinicians. *The Angle Orthodontist* 64, 175–188. [https://doi.org/10.1043/0003-
607 3219\(1994\)064<0175:WLABSA>2.0.CO;2](https://doi.org/10.1043/0003-3219(1994)064<0175:WLABSA>2.0.CO;2)
608
- 609 Fyhrie, D.P., Carter, D.R., 1986. A unifying principle relating stress to trabecular bone morphology.
610 *Journal of Orthopaedic Research* 4, 304–317. <https://doi.org/10.1002/jor.1100040307>
611
- 612 Hall, B. K., 1983. Epigenetic control in development and evolution, p. 353–379. In: *Development and*
613 *Evolution*. B. C. Goodwin, N. Holder, and C. C. Wylie (eds.). Cambridge University Press,
614 Cambridge.
615
- 616 Hall, B. K., 1989. Genetic and epigenetic control of vertebrate embryonic development. *Netherlands*
617 *Journal of Zoology* 40:352–361.
618
- 619 Hall, R.L., Shereff, M.J., 1993 Anatomy of the calcaneus. *Clin. Orthop. Relat. Res.* 290, 27–35.
620
- 621 Hall, B.K., 2005. *Bones And Cartilage: Developmental And Evolutionary Biology*. Academic Press
622 Inc, Australia ; San Diego, Calif.
623
- 624 Hanot, P., Herrel, A., Claude, G., Cornette, R., 2017. Morphological integration in the appendicular
625 skeleton of two domestic taxa: The horse and donkey. *Proceedings of the Royal Society B: Biological Sciences* 284, 20171241. <https://doi.org/10.1098/rspb.2017.1241>
626
627
- 628 Harbers, H., Neaux, D., Ortiz, K., Blanc, B., Laurens, F., Baly, I., Callou, C., Schafberg, R., Haruda,
629 A., Lecompte, F., Casabianca, F., Studer, J., Renaud, S., Cornette, R., Locatelli, Y., Vigne, J.D.,
630 Herrel, A., Cucchi, T., 2020a. The mark of captivity: plastic responses in the ankle bone of a wild
631 ungulate (*Sus scrofa*). *R Soc Open Sci* 7:192039. <https://doi.org/10.1098/rsos.192039>
632
- 633 Harbers, H., Zanolli, C., Cazenave, M., Theil, J.-C., Ortiz, K., Blanc, B., Locatelli, Y., Schafberg, R.,
634 Lecompte, F., Baly, I., Laurens, F., Callou, C., Herrel, A., Puymérail, L., Cucchi, T., 2020b.
635 Investigating the impact of captivity and domestication on limb bone cortical morphology: an
636 experimental approach using a wild boar model. *Scientific Reports* 10, 19070.
637 <https://doi.org/10.1038/s41598-020-75496-6>
638
- 639 Hegedus, D.H., Cowin, S.C., 1976. Bone remodeling II: small strain adaptive elasticity. *J. Elasticity* 6,
640 337–352
641
- 642 Houssaye, A., Taverne, M., Cornette, R., 2018. 3D quantitative comparative analysis of long bone
643 diaphysis variations in microanatomy and cross-sectional geometry. *J Anat* 232:836–849
644
- 645 Huiskes, R., Ruimerman, R., van Lenthe, G.H., Janssen, J.D., 2000. Effects of mechanical forces on
646 maintenance and adaptation of form in trabecular bone. *Nature* 405, 704–706.
647 <https://doi.org/10.1038/35015116>
648
- 649 Hussain, S., 1975. Evolutionary and Functional Anatomy of the Pelvic Limb in Fossil and Recent
650 Equidae (Perissodactyla, Mammalia). *Anatomia, Histologia, Embryologia: Journal of Veterinary*
651 *Medicine Series C*, 4, 193–222. doi:10.1111/j.1439-0264.1975.tb00637.x
652
- 653 Kappelman, J., 1988. Morphology and locomotor adaptations of the bovid femur in relation to habitat.
654 *Journal of Morphology*, 198, 119– 130. <https://doi.org/10.1002/jmor.1051980111>
655
- 656 Kelly, S.A., Czech, P.P., Wight, J.T., Blank, K.M., Garland, T., 2006. Experimental evolution and
657 phenotypic plasticity of hindlimb bones in high-activity house mice. *Journal of Morphology* 267,
658 360–374. <https://doi.org/10.1002/jmor.10407>

- 659
660 Keuling, O., Stier, N., Roth, M., 2009. Commuting, shifting or remaining?: Different spatial utilisation
661 patterns of wild boar *Sus scrofa* L. in forest and field crops during summer. *Mamm. Biol.* 74,
662 145–152. (doi:10.1016/j.mambio.2008.05.007)
663
664 Kivell, T.L., 2016. A review of trabecular bone functional adaptation: what have we learned from
665 trabecular analyses in extant hominoids and what can we apply to fossils? *Journal of Anatomy*
666 228, 569–594. <https://doi.org/10.1111/joa.12446>
667
668 Krølner, B., Toft, B., 1983. Vertebral Bone Loss: An Unheeded Side Effect of Therapeutic Bed Rest.
669 *Clinical Science* 64, 537–540. <https://doi.org/10.1042/cs0640537>
670
671 Lang, T., LeBlanc, A., Evans, H., Lu, Y., Genant, H., Yu, A., 2004. Cortical and Trabecular Bone
672 Mineral Loss From the Spine and Hip in Long-Duration Spaceflight. *Journal of Bone and Mineral*
673 *Research* 19, 1006–1012. <https://doi.org/10.1359/JBMR.040307>
674
675 Lanyon, L.E., 1973. Analysis of surface bone strain in the calcaneus of sheep during normal
676 locomotion: Strain analysis of the calcaneus. *Journal of Biomechanics* 6, 41–49.
677 [https://doi.org/10.1016/0021-9290\(73\)90036-5](https://doi.org/10.1016/0021-9290(73)90036-5)
678
679 Lanyon, L.E., 1992. Control of bone architecture by functional load bearing. *Journal of Bone and*
680 *Mineral Research* 7, S369–S375. <https://doi.org/10.1002/jbmr.5650071403>
681
682 Laros, G.S., Tipton, C.M., Cooper, R.R., Stimmel, P., Davis, R., Matthes, R.D., 1971. Influence of
683 Physical Activity on Ligament Insertions in the Knees of Dogs. *JBJS* 53, 275–286.
684
685 Lieberman, D.E., Devlin, M.J., Pearson, O.M., 2001. Articular area responses to mechanical loading:
686 effects of exercise, age, and skeletal location. *Am. J. Phys. Anthropol.* 116, 266–277.
687
688 Lovejoy, C.O., Cohn, M.J., White, T.D., 1999. Morphological analysis of the mammalian postcranium:
689 a developmental perspective. *Proc. Nat. Acad. Sci. U.S. A.* 96, 13247e13252
690
691 Maïmoun, L., Sultan, C., 2011. Effects of physical activity on bone remodeling. *Metabolism* 60, 373–
692 388. <https://doi.org/10.1016/j.metabol.2010.03.001>
693
694 Maïmoun, L., Coste, O., Philibert, P., Briot, K., Mura, T., Galtier, F., Mariano-Goulart, D., Paris, F.,
695 Sultan, C., 2013. Peripubertal female athletes in high-impact sports show improved bone mass
696 acquisition and bone geometry. *Metabolism* 62, 1088–1098.
697 <https://doi.org/10.1016/j.metabol.2012.11.010>
698
699 Marcus R., 2002. Chapter 86 - Mechanisms of Exercise Effects on Bone. In *Principles of bone biology*
700 (second edition) (eds JP Bilezikian, LG Raisz, GA Rodan), pp. 1477–1488. San Diego, CA:
701 Academic Press
702
703 Marinval-Vigne, M.C., Mordant, D., Auboire, G., Augereau, A., Bailon, S., Dauphin, C., Delibrias, G.,
704 Krier, V., Leclerc, A.S., Leroyer, C., Marinval, P., Mordant, C., Rodriguez, P., Vilette, P., Vigne,
705 J.-D., 1989. Noyen-sur-Seince, site stratifié en milieu fluvial: Une étude multidisciplinaire
706 intégrée. *Bulletin de la Société préhistorique française* 86, 370–379.
707
708 Martin, R.B., 2000. Toward a unifying theory of bone remodeling. *Bone* 26, 1–6.
709 [https://doi.org/10.1016/S8756-3282\(99\)00241-0](https://doi.org/10.1016/S8756-3282(99)00241-0)
710
711 Modlesky, C.M., Majumdar, S., Dudley, G.A., 2008. Trabecular bone microarchitecture in female
712 collegiate gymnasts. *Osteoporos Int* 19, 1011–1018. <https://doi.org/10.1007/s00198-007-0522-x>

- 713
714 Mordant, D., Boris, V., Vigne, J.D., 2013. Noyen-sur-Seine, vingt-cinq ans après. - Société
715 Préhistorique française, Séance 2, p. 37-50.
716
- 717 Neaux, D., Blanc, B., Ortiz, K., Locatelli, Y., Laurens, F., Baly, I., Callou, C., Lecompte, F., Cornette,
718 R., Sansalone, G., Haruda, A., Schafberg, R., Vigne, J.D., Debat, V., Herrel, A., Cucchi, T., 2021.
719 How Changes in Functional Demands Associated with Captivity Affect the Skull Shape of a Wild
720 Boar (*Sus scrofa*). *Evol Biol* 48, 27–40. <https://doi.org/10.1007/s11692-020-09521-x>
721
- 722 Newman, S., Leeson, S., 1999. The effect of dietary supplementation with 1,25-
723 dihydroxycholecalciferol or vitamin C on the characteristics of the tibia of older laying hens.
724 *Poultry Science* 78, 85–90. <https://doi.org/10.1093/ps/78.1.85>
725
- 726 Noyes, M.D., Frank, DELUCAS, J., Torvik, P., 1974. Biomechanics of Anterior Cruciate Ligament
727 Failure: An Analysis of Strain-Rate Sensitivity and Mechanisms of Failure in Primates. *The*
728 *Journal of bone and joint surgery. American volume* 56, 236–53.
729 <https://doi.org/10.2106/00004623-197456020-00002>
730
- 731 Palencia, P., Vicente, J., Barroso, P., Barasona, J., Soriguer, R.C. and Acevedo, P., 2019. Estimating
732 day range from camera-trap data: the animals' behaviour as a key parameter. *J Zool*, 309: 182-
733 190. <https://doi.org/10.1111/jzo.12710>
734
- 735 Parfitt, A.M., 2002. Targeted and nontargeted bone remodeling: Relationship to basic multicellular unit
736 origination and progression. *Bone* 30, 5–7.
737
- 738 Pearson, O.M., Lieberman, D.E., 2004. The aging of Wolff's 'law': Ontogeny and responses to
739 mechanical loading in cortical bone. *Am. J. Phys. Anthropol.* 125, 63–99.
740 (doi:10.1002/ajpa.20155)
741
- 742 Pelletier, M., Kotiaho, A., Niinimäki, S., Salmi, A.-K., 2020. Identifying early stages of reindeer
743 domestication in the archaeological record: a 3D morphological investigation on forelimb bones
744 of modern populations from Fennoscandia. *Archaeol Anthropol Sci* 12, 169.
745 <https://doi.org/10.1007/s12520-020-01123-0>
746
- 747 Pinhasi, R., Stock, J.T., 2011. *Human Bioarchaeology of the Transition to Agriculture*. John Wiley &
748 Sons.
749
- 750 R Core Team. 2017 *R: A language and environment for statistical computing*. Vienna, Austria: R
751 Foundation for Statistical Computing. <https://www.R-project.org>.
752
- 753 Randoin, L., Causeret, J., 1945. Experiments on the effect of the phosphorus, calcium, magnesium and
754 vitamin D contents of the diet on bone development in the white rat. *Bull: Soc. sci. Hyg. aliment.*
755 33, 134–143.
756
- 757 Rath, N.C., Huff, G.R., Huff, W.E., Balog, J.M., 2000. Factors Regulating Bone Maturity and Strength
758 in Poultry1. *Poultry Science* 79, 1024–1032. <https://doi.org/10.1093/ps/79.7.1024>
759
- 760 Robling, A.G., Castillo, A.B., Turner, C.H., 2006. Biomechanical and molecular regulation of bone
761 remodeling. *Annual Review of Biomedical Engineering*, 8, 455-498.
762 <https://doi.org/10.1146/annurev.bioeng.8.061505.095721>
763
- 764 Rossel, S., Marshall, F., Peters, J., Pilgram, T., Adams, M.D., O'Connor, D., 2008. Domestication of
765 the donkey: timing, processes, and indicators. *Proc. Natl. Acad. Sci. U. S. A.* 105 (10), 3715e3720.
766

- 767 Roux, W., 1881. Der Kampf der Teile des Organismus. Ein Beitrag zur Vervollständigung der
768 mechanischen Zweckmäßigkeitstheorie. Wilhelm Engelmann, Leipzig
769
- 770 Ruff, C., B. Holt, and E. Trinkaus., 2006. Who's afraid of the big bad Wolff?: "Wolff's Law" and
771 bone functional adaptation. *American Journal of Physical Anthropology* 129:484–498
772
- 773 Ruff, C. B., Puymeraul, L., Macchiarelli, R., Sipla, J., Ciochon, R. L., 2015. Structure and composition
774 of the Trinil femora: Functional and taxonomic implications. *Journal of Human Evolution*, 80,
775 147-158. <https://doi.org/10.1016/j.jhevol.2014.12.004>
776
- 777 Ruff, C.B., 2018 Chapitre 6 Biomechanical analyses of archeological human skeletons. Dans M.A.
778 Katzenberg, & A.L. Grauer, *Biological Anthropology of the Human Skeleton*. John Wiley &
779 Sons.
780
- 781 Ruimerman, R., Hilbers, P., van Rietbergen, B., Huiskes, R. 2005. A theoretical framework for strain-
782 related trabecular bone maintenance and adaptation. *Journal of Biomechanics*, 38, 931-941.
783 <https://doi.org/10.1016/j.jbiomech.2004.03.037>
784
- 785 Rushen, J., 1993. The "coping" hypothesis of stereotypic behaviour. *Animal Behaviour*, 45(3), 613–
786 615. <https://doi.org/10.1006/anbe.1993.1071>
787
- 788 Russo, L., Massei, G., Genov, P.V., 1997. Daily home range and activity of wild boar in a
789 Mediterranean area free from hunting. *Ethology Ecology & Evolution* 9, 287–294.
790 <https://doi.org/10.1080/08927014.1997.9522888>
791
- 792 Schriefer, J.L., Warden, S.J., Saxon, L.K., Robling, A.G., Turner, C.H., 2005. Cellular accommodation
793 and the response of bone to mechanical loading. *Journal of Biomechanics* 38, 1838–1845.
794 <https://doi.org/10.1016/j.jbiomech.2004.08.017>
795
- 796 Shackelford, L., Marshall, F., Peters, J., 2013. Identifying donkey domestication through changes in
797 cross-sectional geometry of long bones. *Journal of Archaeological Science* 40, 4170–4179.
798 <https://doi.org/10.1016/j.jas.2013.06.006>
799
- 800 Skedros, J.G., Mason, M.W., Bloebaum, R.D., 2001. Modeling and remodeling in a developing
801 artiodactyl calcaneus: A model for evaluating Frost's Mechanostat hypothesis and its corollaries.
802 *The Anatomical Record* 263, 167–185. <https://doi.org/10.1002/ar.1094>
803
- 804 Skedros, J.G., Sorenson, S.M., Hunt, K.J., Holyoak, J.D., 2007. Ontogenetic structural and material
805 variations in ovine calcanei: A model for interpreting bone adaptation. *The Anatomical Record*
806 290, 284–300. <https://doi.org/10.1002/ar.20423>
807
- 808 Spitz, F., Janeau, G., 1990. Spatial strategies: an attempt to classify daily movements of wild boar. *Acta*
809 *Theriol.* 35, 129–149. doi:10.4098/AT.arch.90-14
810
- 811 Spitz, F., Janeau, G., 1995 Daily selection of habitat in wild boar (*Sus scrofa*). *J. Zool.* 237, 423–434.
812
- 813 Su, S.C., Skedros, J.G., Bachus, K.N., Bloebaum, R.D., 1999. Loading conditions and cortical bone
814 construction of an artiodactyl calcaneus. *Journal of Experimental Biology* 202, 3239–3254.
815 <https://doi.org/10.1242/jzeb.202.22.3239>
816
- 817 Taylor, D., 1997. Bone maintenance and remodeling: A control system based on fatigue damage.
818 *Journal of Orthopaedic Research* 15, 601–606. <https://doi.org/10.1002/jor.1100150417>
819

- 820 Trinkaus, E., Churchill, S.E., Ruff, C.B., 1994. Postcranial robusticity in Homo. II: Humeral bilateral
821 asymmetry and bone plasticity. *American Journal of Physical Anthropology* 93, 1–34.
822 <https://doi.org/10.1002/ajpa.1330930102>
823
- 824 Turner, C.H., Owan, I., Takano, Y., 1995. Mechanotransduction in bone : Role of strain rate. *American*
825 *Journal of Physiology-Endocrinology and Metabolism*, 269(3), E438-E442.
826 <https://doi.org/10.1152/ajpendo.1995.269.3.E438>
827
- 828 Turner, C.H., 1998. Three rules for bone adaptation to mechanical stimuli. *Bone*, 23, 399-407.
829 [https://doi.org/10.1016/S8756-3282\(98\)00118-5](https://doi.org/10.1016/S8756-3282(98)00118-5)
830
- 831
- 832 Turner, C.H., 1999. Toward a mathematical description of bone biology: the principle of cellular
833 accommodation. *Calcif Tissue Int* 65, 466–471. <https://doi.org/10.1007/s002239900734>
834
- 835 Van der Meulen, M.C.H., Morgan, T.G., Yang, X., Baldini, T.H., Myers, E.R., Wright, T.M., Bostrom,
836 M.P.G., 2006. Cancellous bone adaptation to in vivo loading in a rabbit model. *Bone* 38, 871–
837 877. <https://doi.org/10.1016/j.bone.2005.11.026>
838
- 839 Vlachopoulos, D., Barker, A.R., Ubago-Guisado, E., Fatouros, I.G., Knapp, K.M., Williams, C.A.,
840 Gracia-Marco, L., 2017. Longitudinal Adaptations of Bone Mass, Geometry, and Metabolism in
841 Adolescent Male Athletes: The PRO-BONE Study. *Journal of Bone and Mineral Research* 32,
842 2269–2277.
843
- 844 Vigne, J.D., Carrere, I., Briois, F., Guilaine, J., 2011. The early process of mammal domestication in
845 the Near East: New evidence from the Pre-Neolithic and Pre-Pottery Neolithic in Cyprus. *Current*
846 *Anthropology* 52, S255–S271.
847
- 848
- 849 Wolff, J., 1986. "The Law of Bone Remodeling" (translation of the German 1892 edition by P. Maquet
850 and R. Furlong). Berlin Heidelberg New York: Springer
851
- 852 Woo, S.L., Kuei, S.C., Amiel, D., Gomez, M.A., Hayes, W.C., White, F.C., Akeson, W.H., 1981. The
853 effect of prolonged physical training on the properties of long bone: a study of Wolff's Law. *J*
854 *Bone Joint Surg Am* 63, 780–787.
855
- 856 Zanker, C.L., Swaine, I.L., 2000. Responses of bone turnover markers to repeated endurance running
857 in humans under conditions of energy balance or energy restriction. *Eur J Appl Physiol* 83, 434–
858 440. <https://doi.org/10.1007/s004210000293>