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1 The architecture of cancellous bone in the hindlimb of moa

2 (Aves: Dinornithiformes), with implications for stance and gait

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10 The extinct, flightless moa of New Zealand included some of the largest birds to have existed, and possessed

11 many distinguishing pelvic and hindlimb osteological features. These features may have influenced stance and

12 gait in moa compared to extant birds. One means of assessing locomotor biomechanics, particularly for extinct

13 species, is quantitative analysis of the architecture of cancellous bone, since this architecture is adapted to suit

14 its mechanical environment with high sensitivity. This study investigated the three-dimensional architecture of

15 cancellous bone in the femur, tibiotarsus and fibula of three moa species: Dinornis robustus, Pachyornis

16 elephantopus and Megalapteryx didinus. Using computed tomographic X-ray scanning and previously

17 developed fabric analysis techniques, the spatial variation in cancellous bone fabric patterns in moa was found

18 to be largely comparable to that previously reported for extant birds, particularly large species. Moa hence

19 likely used postures and kinematics similar to those employed by large extant bird species, but this

20 interpretation is tentative on account of relatively small sample sizes. A point of major difference between moa

21 and extant birds concerns the diaphyses; cancellous bone invades the medullary cavity in both groups, but the

22 invasion is far more extensive in moa. Combined with previous assessments of cortical geometry, this further

23 paints a picture of at least some moa species possessing very robust limb bones, for which a convincing

24 explanation remains to be determined.

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40 Running head: moa cancellous bone structure

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55 BECOMING extinct only relatively recently, the flightless moa (Aves: Palaeognathae:

56 Dinornithiformes) of New Zealand included some of the largest birds to have ever existed.

57 Across the nine currently recognized species (Worthy & Scofield 2012), body mass in moa

58 has been estimated to range from about 20 kg in Megalapteryx didinus Owen, 1883 to over a

59 quarter of a tonne in Dinornis robustus Owen, 1846 (Alexander 1983a, Anderson 1989,

60 Worthy & Holdaway, 2002, Murray & Vickers-Rich 2004, Brassey et al. 2013). Moa also

61 possessed a number of unique anatomical features of the pelvis and femur (Worthy &

62 Holdaway 2002), as well as unusually proportioned hindlimb bones, in terms of both

63 intersegmental proportions (Gatesy & Middleton, 1997) and whole-bone robusticity

64 (Alexander 1983a, Alexander 1983b, Doube et al. 2012, Brassey et al. 2013). Collectively,

65 these observations suggest that moa, particularly the larger species, may have stood and

66 moved in a manner different to extant birds.

67   Moa were unique among birds in completely lacking wings, with the fused

68 scapulocoracoid lacking even a glenoid (Worthy & Holdaway 2002, Worthy & Scofield

69 2012). They also possessed an acarinate sternum, and the pelvis of all species except

70 Megalapteryx didinus and Anomalopteryx didiformis (Owen, 1844) was very broad caudal

71 to the acetabulum. These features suggest that the whole-body centre of mass of moa may

72 have been more caudally positioned compared to extant birds, which would have hence

73 influenced hindlimb positioning, stance and gait (Alexander 1983a). Despite these oddities,

74 the articular surface morphology of the main bones of the moa hindlimb does not differ

75 appreciably from that of extant birds, implying minimal differences in limb articulation, and

76 therefore posture (Anderson 1989, Worthy & Holdaway 2002, Zinoviev 2013). The

77 hindlimb myology of moa is also inferred to be largely comparable to that of extant

78 palaeognathous birds, with few major differences (Bishop 2015), also suggestive of minimal

79 difference in locomotor behaviour compared to extant birds. A number of fossil moa

80 trackways (sequences of footprints) are known, but analyses to date suggest that these too

81 are comparable to those made by extant birds (Worthy & Holdaway 2002); in any case, they

82 do not reveal any insight to movements of more proximal limb segments (Thulborn 1990,

83 Hutchinson & Gatesy 2006).

84   To better clarify stance and gait in moa, and to resolve apparently conflicting lines of

85 evidence, one way forward is to study evidence of locomotor biomechanics recorded in limb

86 bone osteology. The spatial distribution of cortical bone at midshaft has been examined

87 previously (Alexander 1983b, Worthy 1989, Brassey et al. 2013), but inferences drawn from

88 such observations must be viewed with caution, since experimental evidence with modern

89 species indicates that cortical bone morphology does not always correlate with the nature of

90 bone loading (e.g., Thomason 1995, Demes et al. 2001, Demes et al. 1998, Main &

91 Biewener 2004, Pearson & Lieberman 2004, Lieberman et al. 2004, Demes 2007, Wallace et

92 al. 2014). In contrast, the architecture of cancellous bone ('spongy bone') does show a

93 strong correlation with loading conditions experienced in vivo (Kivell 2016). Cancellous

94 bone is sensitive to its mechanical environment, and is able to adapt its architecture to suit

95 this environment in a highly predictable fashion. For example, numerous studies have

96 demonstrated that increased loading magnitude leads to an increase in the volume fraction

97 occupied by bone material (Biewener et al. 1996, van der Meulen et al. 2006, Wang et al.

98 2012), whereas a change in loading direction leads to a reorientation of the dominant

99 direction of the comprising trabeculae (Radin et al. 1982, Goldstein et al. 1991, Mullender

100 & Huiskes 1995, Huiskes et al. 2000, Adachi et al. 2001, Ruimerman et al. 2005, Pontzer et

101 al. 2006, Polk et al. 2008, Volpato et al. 2008, Barak et al. 2011). The mechanobiological

102 processes underpinning these responses are likely founded upon achieving a uniform

103 distribution of bone tissue strain, averaged across time and loading conditions (Fyhrie &

104 Carter 1986, Boyle & Kim 2011, Christen et al. 2013).

105   Given how well cancellous bone adapts to its mechanical environment, it is of little

106   surprise that differences in locomotor behaviour among extant species are often manifest in

107   differences in cancellous bone architecture (Fajardo & Müller 2001, Ryan & Ketcham

108   2002b, 2005, Maga et al. 2006, Hébert et al. 2012, Ryan & Shaw 2012, Barak et al. 2013,

109   Su et al. 2013, Tsegai et al. 2013, Matarazzo 2015, Kivell 2016, Georgiou et al. 2018). Of

110   particular note is that the principal orientations of trabeculae (i.e. the cancellous bone fabric)

111   tends to be especially telling of differences in locomotor behaviour (Ryan & Ketcham

112   2002b, 2005, Maga et al. 2006, Hébert et al. 2012, Ryan & Shaw 2012, Barak et al. 2013,

113   Su et al. 2013, Tsegai et al. 2013, Matarazzo 2015, Amson et al. 2017, Bishop et al. 2018b).

114   The architecture of cancellous bone therefore has the potential to shed new insight on whole-

115   bone loading mechanics and locomotor behaviour in extinct species such as moa (Bishop et

116   al. 2018b).

117   Cancellous bone architecture has recently been surveyed in the main limb bones of a

118   wide variety of extant ground-dwelling bird species (Bishop et al. 2018b). That study

119   identified patterns of fabric directionality which correlate to size-related changes in hip and

120   knee flexion during avian stance or gait, as well as a ubiquity of oblique trabeculae in the

121   femoral and tibial diaphyses that corresponds to strong torsional loading of these bones

122   during locomotion. These results provide a comparative framework upon which cancellous

123   bone architecture in moa hindlimb bones may be investigated, insofar as it relates to limb

124   bone loading and locomotor biomechanics. The present study aimed to investigate the three-

125   dimensional (3-D) architecture of cancellous bone in the hindlimb of three moa species,

126   Dinornis robustus, Pachyornis elephantopus (Owen, 1858) and Megalapteryx didinus. The

127   results of quantitative and qualitative analyses, when compared to similar results obtained

128   for extant birds, can help resolve questions concerning stance and gait in these species.

129   Given the size-related trends in posture and cancellous bone architecture reported previously

130 for extant birds by Bishop et al. (2018a,b), it is predicted that moa will exhibit architectural

131 patterns most comparable to large extant birds. In addition to providing insight into moa

132 locomotor biomechanics specifically, the results of this study can more broadly also shed

133 new light on the potential consequences of large body size for avian bipedalism.

134

Material and methods

136 The methodology employed in this study followed that outlined previously by Bishop et al.

137 (2018b), and so only a brief overview is given here. The species examined were chosen so as

138 to sample all three moa families as well as varying body sizes and proportions: the

139 dinornithid Dinornis robustus is large (up to 250 kg) and tall, the emeid Pachyornis

140 elephantopus is medium-sized (around 100 kg) and very graviportal, and the megalapterygid

141 Megalapteryx didinus is small (around 20 kg) and relatively gracile. The present study

142 focused on three main bones of the moa hindlimb, the femur, tibiotarsus and fibula; these

143 were the bones studied previously in extant birds, and collectively give a full picture of the

144 hip and knee joints. All specimens were obtained from the Natural History Collections of the

145 Canterbury Museum (Table 1); the large sizes of the specimens studied indicate that they

146 were from adult birds. The 3-D architecture of cancellous bone in the fossil specimens was

147 acquired through X-ray computed tomographic (CT) scanning, using a Siemens Somatom

148 Definition Flash dual energy scanner (Siemens AG, Germany); the scan settings used are

149 listed in Table 1. The resulting scans were processed using the software ImageJ 1.47

150 (http://imagej.nih.gov/ij/) and Mimics 17.0 (Materialize NV, Belgium), following protocol 2

151 of Bishop et al. (2018b). This was possible because of the excellent preservation of the

152 specimens, with little (if any) matrix inside the bones, affording good contrast between bone

153 and non-bone phases in the CT scans.

154

155   The processed and segmented CT scans were then subject to a number of architectural

156   analyses. The first was an analysis of cancellous bone fabric, where the 3-D fabric tensor

157   (Cowin, 1986) was calculated using the star volume distribution method (Cruz-Orive et al.

158   1992, Odgaard 1997, 2001), as implemented in the software Quant3D 2.3 (Ryan & Ketcham

159   2002a,b, Ketcham & Ryan 2004). When conducted for numerous volumes of interest

160   throughout a whole bone, this provides an assessment of how fabric direction (essentially,

161   trabecular orientation) varies spatially across the bone. In some specimens over 850

162   individual volumes of interest were analysed for a given bone. The second analysis

163   conducted concerned the results for the femoral head and medial femoral condyle, in

164   particular, the orientation of the primary fabric direction (the direction of strongest

165   trabecular alignment, equivalent to the first eigenvector of the fabric tensor) in these regions

166   of the bone. Here, the mean orientation of the primary fabric direction across each

167   anatomical region was calculated and referenced to an explicit femoral anatomical

168   coordinate system. Previously, these mean orientations were found to correlate with the

169   degree of hip and knee flexion in extant bipeds (Bishop et al. 2018b), and may therefore

170   provide insight into posture in extinct moa. These mean orientations, in terms of their

171   sagittal components, were also compared to body size, where the interarticular length of the

172   femur (i.e., less the trochanteric crest) was taken as a proxy for body size

173   The third and final analysis involved examination of gross morphological characteristics

174   of cancellous bone architecture in the diaphysis (shaft) of the femur and tibiotarsus. This

175   was undertaken through a categorical scoring study performed by five independent,

176   volunteer observers; these volunteers were the same as those used in the study of extant bird

177   bones (Bishop et al. 2018b), and were blind to the objectives of the current study. Using 3-D

178   isosurface renderings derived from the segmented CT scans, three features were scored: the

179   bulk spatial extent of cancellous bone in the diaphysis, the tendency of trabeculae to be

180   closely associated with other trabeculae, and the average orientation of trabeculae with

181   respect to the long-axis of the bone. Following scoring, the mean score across the five

182   observers was taken for each bone and for each morphological feature. The actual

183   architecture of cancellous bone in the diaphyses was also assessed by the authors through

184   visualization of the isosurface renderings.

185   Statistical comparison between moa and extant birds was conducted for the calculated

186   mean primary fabric directions for the femoral head and medial femoral condyle in both

187   groups. Setting an a priori significance level of $p = 0.05$, the 95% confidence interval of the

188   mean direction ('confidence cone') for both groups was then calculated using the software

189   StereoNet 9.5 (Allmendinger et al. 2013, Cardozo & Allmendinger 2013). If overlap

190   occurred between the mean direction of one group and the 95% confidence cone of the other

191   group, the mean directions of the two groups were not statistically significantly different; if

192   no overlap occurred between a mean direction of one group and the confidence cone of the

193   other group, the means were different (Butler 1992, Ryan & Ketcham 2005, Allmendinger et

194   al. 2013,). However, if there was overlap between the confidence cones of both groups, but

195   not between mean directions and confidence cones, an F-test was used to determine if the

196   difference in means was due to sampling error (i.e., inadequate sample size), rather than

197   legitimate differences between groups (Butler, 1992). In addition, comparisons of mean

198   orientation to body size were assessed using major axis regression in PAST 3.09 (Hammer

199   et al. 2001), with significance values calculated using a 100,000-replicate permutation test

200   of the slope (Legendre & Legendre 2012), as done previously.

201   The results of the categorical scoring analyses were also compared to body size, where

202   the interarticular length of the relevant bone was taken as a proxy for body size, and

203   compared to the patterns observed for extant birds by Bishop et al. (2018b). Comparisons

204   used major axis regression as implemented above. The reliability of the scorers in the

205 analysis of diaphyseal cancellous bone was previously assessed (Bishop et al. 2018b), and

206 found to be moderate to good across the three features examined. Nevertheless, the results

207 presented here should still be viewed tentatively, pending the assessments being undertaken

208 by a greater number of scorers for more specimens.

209

Results

211 Summary

212 A brief overview of the key observations are first presented here, before being presented in

213 full for each bone. The femur of each species displays architectural patterns broadly

214 comparable to that observed in extant birds, particularly larger species, including in regards

215 to relatively limited anterior and posterior inclination of the primary fabric direction in the

216 head and medial condyle, respectively. The very broad distal femora of D. robustus and P.

217 elephantopus are associated with a radiating pattern in the coronal plane, which is not

218 known in extant birds of any size. Cancellous bone architecture in the tibiotarsus and fibula

219 of moa shows strong resemblance to that observed in extant birds, including a highly

220 anisotropic pattern in the distal tibiotarsus. As with extant birds, the femoral and tibial

221 diaphyses of moa possess abundant and markedly oblique trabeculae, although in moa the

222 abundance is conspicuously greater.

223 On a nomenclatural note, the term 'proximodistal' is always used here in reference to the

224 proximodistal axis of the whole bone under consideration, regardless of the specific

225 anatomical region concerned.

226

227 Femur

228 Cancellous bone architecture in the moa proximal femur displays a pattern of spatial

229 variation in fabric directions which is quite comparable to that previously reported for extant

230 birds (Bishop et al. 2018b). None of the bones studied were invaded by pneumatopores to

231 any significant extent, and thus the influence that pneumatization may have on cancellous

232 bone architecture in extant birds (Bishop et al. 2018b) was not of concern here. The primary

233 fabric direction (u_1) in the femoral head and neck is oriented largely proximodistally, with a

234 variable amount of anteromedial inclination (Fig. 1A, B). Under the facies antitrochanterica,

235 the anterior inclination diminishes while the medial inclination can become stronger or

236 weaker (Fig. 1C, D); sometimes a slight lateral inclination is also possible. The same general

237 pattern continues anteriorly toward the trochanteric crest, although it tends to be less

238 strongly organized in M. didinus compared to D. robustus (Fig. 1E, F); also, in D. robustus,

239 u_1 takes on a gentle anterior inclination once again (Fig. 1G, H).

240 [Fig. 1]

241

242 In the distal femur, u_1 is also oriented in a mostly proximodistal fashion. A double-

243 arcuate pattern, parallel to the sagittal plane, was noted previously for the central metaphysis

244 of large extant species of birds (Bishop et al. 2018b), but this is not particularly well-

245 developed in moa (Fig. 2A). Indeed, such a pattern is veritably absent in M. didinus. On the

246 other hand, a well-developed 'radiating' pattern is evident in the coronal plane in D.

247 robustus and P. elephantopus, whereby the orientation of u_1 is directed towards the medial

248 condyle medially and directed towards the lateral condyle laterally, sweeping across the

249 metaphysis (Fig. 2B). This pattern is not well developed in extant birds; its occurrence in D.

250 robustus and P. elephantopus may reflect the great mediolateral breadth of the distal end of

251 the femur relative to the shaft, flaring out to the sides more. The architectural patterns in the

252 medial and lateral condyles are more comparable to the pattern in extant birds (Fig. 2C–F).

253 Within both condyles, the orientation of u_1 is largely subparallel to the sagittal plane, and it

254 usually has a marked posterior inclination, particularly in the medial condyle. As with large

255   extant birds (Bishop et al. 2018b), u_1 sweeps a wide arc in the sagittal plane (usually well in

256   excess of 100°), such that in the posterior extremities of the condyles, it can be

257   perpendicular to the proximodistal axis of the bone. Moreover, in the anterior parts of the

258   condyles, u_1 is often anterodistally directed. As observed previously for extant birds, as well

259   as humans and extinct, non-avian theropod dinosaurs (Bishop et al. 2018b), the secondary

260   fabric direction (u_2) forms a 'butterfly pattern' in the plane that passes through the centres of

261   both condyles (Fig. 2G). Here, two 'tracts' (one in each condyle) arc from the anterior aspect

262   of their respective condyle back towards the posterior aspect.

263   [Fig. 2]

264

265   In large extant birds, it was previously observed that a transect through the metaphysis

266   from the ends of the femur towards the diaphysis often saw a noticeable increase in the

267   'disorganization' of the orientations of u_1. That is, the vectors took on a more oblique

268   orientation and the change in direction across the bone was no longer gradual (Bishop et al.

269   2018b). Such a change was apparent in both ends of the femora of M. didinus, as well as the

270   proximal femur of D. robustus and P. elephantopus (Fig. 3), but it was not particularly

271   evident in the distal femur of the latter two species. This may be in part due to the obliquity

272   already present in the distal femur of D. robustus and P. elephantopus, resulting from the

273   'radiating' pattern in the coronal plane noted above.

274   [Fig. 3]

275   Results of the calculations of mean primary fabric direction are presented in Fig. 4.

276   Consistent with the qualitative observations noted above, the mean orientation of u_1 in the

277   femoral head is anteromedially directed (moving proximally) for all six moa specimens (Fig.

278   4A). This direction is similar to the general pattern observed for extant birds. Indeed, the

279   mean direction of both groups falls within the 95% confidence cone of the mean of the other

280   group, indicating no significant difference between group means ($p > 0.05$). The mean

281   orientation of u_1 in the medial femoral condyle is posteriorly directed (moving distally) for

282   all six moa specimens (Fig. 4A), although some had a slight medial inclination whereas

283   others had a slight lateral inclination. Unlike the femoral head, the mean direction across

284   moa as a whole is different from the mean direction of extant birds as a whole ($p < 0.05$; Fig.

285   4A), but it cannot be discounted that this difference was due to inadequate sampling of the

286   underlying populations ($F_{2,52} = 1.819$, $p = 0.172$). In terms of the amount of sagittal

287   inclination of u_1 with respect to femoral length, moa are seen to follow and reinforce the

288   patterns observed in extant birds. Previously, the amount of anterior inclination of u_1 in the

289   femoral head of extant birds was not able to be demonstrated to correlate significantly with

290   femur length (Bishop et al. 2018b). However, the inclusion of data from the moa specimens

291   examined here produces a statistically significant relationship, with larger birds tending to

292   exhibit lower anterior inclination ($p = 0.01687$; Fig. 4B). In a similar fashion to the femoral

293   head, the inclusion of data for the medial femoral condyle of moa reinforces the tendency for

294   larger birds to exhibit a lower degree of posterior inclination of u_1 in this region of the femur

295   ($p = 00062$; Fig. 4C).

296   [Fig. 4]

297

298   Tibiotarsus

299   As with the femur, the spatial pattern of cancellous bone fabric in the tibiotarsus of moa is

300   quite comparable to that previously reported for extant birds (Bishop et al. 2018b). The

301   orientation of u_1 in the proximal tibia is overall proximodistal, but superimposed upon this

302   are marked inclinations that vary throughout the bone. It has an anteroproximal inclination

303   in the cnemial crests, essentially following the anterior margins of the crests (Fig. 5A, B),

304   whereas under the condyles it has a marked posterior inclination, projecting up to 30°

305 posterior of the proximodistal axis (Fig. 5C–F). Additionally, under the lateral condyle,

306 there is also a strong lateral inclination (Fig. 5E), which as in extant birds can sometimes

307 exceed the amount of posterior inclination. Another feature often present in moa is a double-

308 arcuate pattern in u_1 in the sagittal plane of the proximal metaphysis, whereby the individual

309 fabric vectors are largely contained within the sagittal plane. This feature was not always

310 present in extant birds (Bishop et al. 2018b), nor all moa specimens examined here, but is

311 well developed in D. robustus (Fig. 5G). Here, one tract arcs from the posterior metaphysis

312 anteriorly towards the cnemial crests, whereas the other arcs from the anterior metaphysis

313 posteriorly towards the articular condyles. A small quantity of cancellous bone is present

314 under the fibular crest of moa, but was usually not abundant enough to permit quantitative

315 fabric analysis. This is on account of both the relatively low projection of the crest from the

316 diaphysis in these species, and the fact that the crest comprised a large proportion of

317 relatively high-porosity cortical bone. Similar to the femur of moa, a transect through the

318 metaphysis from the proximal end of the tibia towards the diaphysis sometimes reveals

319 increased 'disorganization' of the orientations of u_1 (Fig. 5H).

320 [Fig. 5]

321   In the distal tibiotarsus, moa exhibit the same characteristic pattern previously reported

322 for birds (Bishop et al. 2018b). Here, u_1 is largely oriented proximodistally and parallel to

323 the sagittal plane, whereas u_2 is largely oriented anteroposteriorly and parallel to the sagittal

324 plane (Fig. 6A–E). Within the condyles themselves, u_1 and u_2 can become 'rotated' within

325 the sagittal plane to a variable degree. As in extant birds, this pattern reflects a highly

326 anisotropic arrangement of plate-like trabeculae, parallel to the sagittal plane, as is evident in

327 3-D renderings of the segmented CT scans (Fig. 6F). The 'disorganization' of u_1 vectors

328 often observed for the femur and proximal tibiotarsus of moa (above) was not observed in

329 the distal tibiotarsus and any specimens examined.

330   [Fig. 6]

331

332   Fibula

333   Cancellous bone architecture in the moa fibula demonstrates the same spatial patterns as

334   observed in extant birds (Bishop et al. 2018b). The orientation of u_1 is consistent throughout

335   the fibular head, being oriented proximodistally and subparallel to the local bone margin,

336   with a gentle posterior inclination of up to 20° form the long-axis of the bone (Fig. 7). This

337   posterior inclination tends to be greater in the more posterior parts of the fibular head. A

338   small quantity of cancellous bone extends distally from the head, maintaining the same

339   general fabric orientation as observed in the head. Some cancellous bone often occurs under

340   the iliofibularis tubercle on the fibular shaft, but it was not extensive enough for quantitative

341   analysis; rather, cortical thickness in this part of the bone is increased. Cancellous bone is

342   virtually absent distal to the tubercle, where the bone rapidly diminishes to a thin splint of

343   low-porosity cortical bone.

344   [Fig. 7]

345

346   Diaphyses

347   The results of the categorical analyses of diaphyseal cancellous bone architecture are

348   presented in Fig. 8 and Table 2. As in large extant bird species, the femoral and tibial

349   diaphyses of moa contain considerable quantities of cancellous bone, in which individual

350   trabeculae are obliquely oriented relative to the bone long-axis, by about 45°. However, the

351   patterns they exhibit are sometimes exaggerated compared to extant birds. The femoral

352   diaphysis of all three moa species examined has a higher quantity of cancellous bone than

353   would be predicted for their size, based on extant birds (higher 'extent scores', Fig. 8A); the

354   data for moa consistently plot above the regression line derived previously for extant birds,

355 were it extrapolated to their size (dashed line in Fig. 8A). The same result occurs for the

356 tibial diaphysis in D. robustus and P. elephantopus, although the result is mixed for M.

357 didinus (Fig. 8B, dashed line). Trabeculae in the femoral diaphysis consequently tend to be

358 more strongly associated with each other: they are more closely packed together with high

359 'association scores' (Fig. 8C). The pattern of association in the tibial diaphysis is more in

360 line with what would be expected based on extant birds (Fig. 8D). Similarly, the average

361 orientation of trabeculae in the femoral and tibial diaphyses is comparable to what would be

362 expected for birds of their size (comparable 'orientation scores', Fig. 8E,F), although it must

363 be acknowledged that the sample size for tibiae in extant birds was not large.

364 [Fig. 8]

365 [Table 2]

366   Three-dimensional visualization of the segmented CT scans further highlights the

367 aberrant nature of diaphyseal cancellous bone in moa in comparison to extant birds (Fig. 9,

368 Supplementary Movie S1). The medullary cavity is much reduced in volume, as a result of

369 extensive encroachment by cancellous bone into the diaphysis. Individual trabeculae can

370 also often be quite massive, being both long (> 20 mm) and thick (> 1 mm), and sometimes

371 plate-like as well. Despite this, moa display the same whole-bone gross architectural pattern

372 observed in the diaphyses of extant birds; in the femur especially, the typically oblique

373 trabeculae form conjugate helices that spiral about the length of the diaphysis. The condition

374 observed in moa may therefore be seen as quite similar to that of extant birds, just that it is

375 greatly exaggerated through a greater quantity of bone material per unit volume.

376 [Fig. 9]

377

378

379 Discussion

380 This study investigated the 3-D architecture of cancellous bone in the femur, tibiotarsus and

381 fibula of three species of moa, and comparisons were made to previously reported

382 observations for these bones in a variety of extant species of ground-dwelling bird (Bishop

383 et al. 2018b). Overall, it was found that the spatial variation in cancellous bone fabric

384 patterns was quite comparable between moa and extant birds, for all three bones

385 investigated. Where notable differences were found to exist between the two groups, these

386 can probably be attributed to either the unique osteology or generally large size of moa. For

387 example, the 'radiating pattern' of the primary fabric direction (u_1) in the coronal plane of

388 the distal femur of D. robustus and P. elephantopus (Fig. 2B) is probably a consequence of

389 the strong mediolateral flaring of the distal end of the bone relative to the shaft in these large

390 (and in the case of the latter, robust) species. Previously, it was shown that extant birds can

391 exhibit significant intra- and interspecific variation in cancellous bone architectural patterns

392 (Bishop et al. 2018b), and some results of the present study affirm this (Figs 4, 8). The

393 currently available sample sizes do not permit proper exploration of how such variation may

394 relate to differences in limb segment proportions, bone robusticity, ecology or other factors,

395 but this topic is well deserved of future study, and may provide further insight into moa

396 biology.

397

398 That the fabric patterns of moa are qualitatively quite comparable to those reported for

399 extant birds, particularly large species, implies that moa limb bones experienced similar

400 loading regimes to those experienced by extant bird limb bones. This is further suggested by

401 the results of quantitative analysis of mean primary fabric directions in the femoral head and

402 medial femoral condyle (Fig. 4). Here, moa have clarified and reinforced the tendency for

403 larger birds to exhibit a lower degree of anterior inclination of the mean direction of u_1 in

404 the femoral head, and a lower degree of posterior inclination of the mean direction of u_1 in

405   the medial femoral condyle. This pattern parallels the tendency for larger birds to use a more

406   upright limb posture with more extended hip and knee joints, which likely causes

407   reorientation of joint contact forces to be more parallel to the long-axis of the femur (Bishop

408   et al. 2018a,b). In this regard, moa are simply following the same size-related biomechanical

409   trends observed in extant birds. The results therefore collectively suggest that moa used limb

410   postures and locomotor kinematics similar to those of large extant bird species, an

411   interpretation also suggested by bony articular surface morphology (Anderson 1989, Worthy

412   & Holdaway 2002, Zinoviev 2013). In light of the small sample sizes used for each species

413   and bone in the present study, this interpretation should be viewed as tentative, pending a

414   more comprehensive study that would facilitate more rigorous analysis. Nevertheless, if

415   locomotor biomechanics in moa did not appreciably differ from that of large extant birds,

416   this suggests that maximal locomotor performance – such as speed and agility capability –

417   of large moa species may have been relatively poorer compared to extant birds (but see

418   Hutchinson, 2004). This inference is based upon general principles derived from extant

419   terrestrial vertebrates: in the absence of major locomotor specialization or innovation,

420   relative locomotor performance declines with increasing body size (Garland 1983, Biewener

421   1989, Gatesy & Biewener 1991, Hutchinson et al. 2003, Sellers & Manning 2007, Clemente

422   et al. 2009, Dick & Clemente 2017).

423

424   One striking difference between moa and extant birds that does not seem explicable by large

425   body size is the nature of cancellous bone in the femoral and tibial diaphyses. The medullary

426   cavity in both groups of bird is encroached by cancellous bone from the proximal and distal

427   ends of the bone, but it is generally much more 'filled' in moa compared to what would be

428   expected for their size (Figs 8, 9, Supplementary Movie S1). Additionally, individual

429   trabeculae in moa are often quite long and thick. This observation parallels those made for

430   external diaphyseal (cortical) dimensions in moa limb bones: the diaphyses of moa,

431   particularly emeids (e.g., P. elephantopus), are more robust than what would be expected for

432   birds of their size (Alexander 1983a,b, Doube et al. 2012, Brassey et al. 2013). Hence, the

433   limb bones of moa are robust both inside and out. One hypothesis proposed to explain this is

434   that, since moa lacked any natural predators, they could afford to evolve more robust limb

435   bones that were more resistant to fracture, yet being heavier were more cumbersome to

436   move around (Alexander 1983b, 1985). This is incorrect, however, as abundant fossil

437   evidence indicates that moa were preyed upon by the extinct giant eagle Harpagornis

438   moorei Haast, 1872 (Worthy & Holdaway 1996, 2002). Increased bone robusticity could

439   alternatively be a mechanical adaptation to increased unpredictability of loads, providing a

440   higher factor of safety against accidental and extreme, but rare, loading regimes (Currey &

441   Alexander 1985, Blob et al. 2014). Yet, many of the more robust moa species lived in low-

442   relief, sparsely forested environments (Worthy & Holdaway 2002), where locomotor-

443   induced bone loading would be expected to be relatively predictable (Brassey et al. 2013).

444

445   Given that mechanical hypotheses have so far proven inadequate in explaining moa limb

446   bone robusticity, the possibility must be considered that one or more other, non-mechanical,

447   reasons exist. Moa had a unique suite of life history traits among birds, and it is possible that

448   this may have influenced limb bone robusticity. They had a strongly K-selected reproductive

449   strategy, with prolonged juvenile growth phases before reaching maturity (taking several

450   years, possibly even over a decade), and potentially living for a very long time thereafter

451   (Turvey & Holdaway 2005, Turvey et al. 2005). At least some species also exhibited

452   extreme reversed sexual size dimorphism, with females up to 280% larger than males

453   (Bunce et al. 2003, Huynen et al. 2003). Coupled with their large size, this raises the

454   possibility of heterochrony – in particular, hypermorphosis – playing an important role in

455   shaping the adult morphology of these birds (Churchill 1998, McNamara 2012). For a given

456   bone, the present study sampled two large individuals for each species, and for D. robustus

457   and P. elephantopus at least it is known that only adult females were sampled here (Allentoft

458   et al. 2010). In addition to allometric effects and heterochrony, it may also be the case that

459   repeated deposition and resorption of medullary bone during each reproductive cycle over

460   many years may have had an effect on the morphologies observed here. Yet another

461   possibility is that strong seasonality in temperature, food availability or food quality in

462   Pleistocene–Holocene New Zealand (Anderson 1989, Worthy & Holdaway 2002) may have

463   resulted in moa undergoing marked annual fluctuations in body mass, with subsequent

464   effects on bone loading magnitudes and in turn bone adaptation. Currently these hypotheses

465   are purely speculative, yet by using a combination of CT and histological approaches, future

466   work should be able to investigate the above possibilities. This will require greatly expanded

467   sample sizes than that used here, incorporating material from both males and females, from a

468   wide range of ontogenetic stages and from a wide range of geographic localities.

469

Conclusion

471   The 3-D architecture of cancellous bone in the femur, tibiotarsus and fibula of three extinct

472   moa species is on the whole comparable to that of extant ground-dwelling birds, especially

473   larger species. Notwithstanding the small sample size of the present study, this would

474   suggest much similarity in posture and locomotor biomechanics between moa and extant

475   birds, which could be further clarified through computational modelling of whole-body

476   centre of mass location (e.g., Henderson 1999, Hutchinson et al. 2007, Bates et al. 2009) or

477   muscle moment arms (e.g., Hutchinson et al. 2005, Bates & Schachner 2012, Maidment et

478   al. 2014). By incorporating greater samples sizes and more species than that used in the

479   current study, future work may be able to refine the interpretations made here. Additionally,

480 such future studies could explicitly address the potential for phylogenetic influences on

481 cancellous bone architecture, which was not examined in the present study. Previously, it

482 was noted that comparably-sized small palaeognaths and neognaths showed similar

483 architectural patterns, suggesting minimal phylogenetic influence (Bishop et al. 2018b), but

484 whether this holds true at larger body size remains to be determined. One salient difference

485 between the cancellous bone architecture of moa and extant birds is the markedly greater

486 extent of cancellous bone in the diaphyses of the femur and tibiotarsus. The reason for the

487 great robusticity of moa limb bones, inside and out, may not be mechanical in nature, but

488 rather related to one or more aspects of their unique life history or environment.

489

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505 History Collections of the Canterbury Museum. Additionally, 3-D models of each bone

506 studied can be viewed at www.faunatoolkit.com. The authors declare no conflict of interest.

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754   Figure captions

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756   Fig. 1. The main architectural features of cancellous bone in the proximal femur of moa. A,

757   B, vector field of u_1 in the femoral head and inferior neck of D. robustus (CM Av8488)

758   plotted on a translucent rendering of the external bony geometry, in anterior (A) and medial

759   (B) views. C, D, vector field of u_1 under the facies antitrochanterica of P. elephantopus (CM

760   Av 8716), in posterior (C) and lateral (D) views. E, F, vector field of u_1 in the trochanteric

761   crest of M. didinus (CM Av8507J), in anterior (E) and lateral (F) views. G, H, vector field

762   of u_1 in the trochanteric crest of D. robustus (CM Av 8488), in anterior (G) and lateral (H)

763   views. In this and all subsequent illustrations of fabric vector fields, all images are of bones

764   from the right side of the body.

765

766   Fig. 2. The main architectural features of cancellous bone in the distal femur of moa. A,

767   vector field of u_1 in the central metaphysis of D. robustus (CM Av 8422), in a 3-D slice,

768   parallel to the sagittal plane and between the condyles, shown in lateral view. Schematic

769   inset illustrates the modestly developed double-arcuate pattern. B, vector field of u_1 in the

770   central metaphysis of P. elephantopus (CM Av15029), in a 3-D slice, parallel to the coronal

771   plane, shown in anterior view. Schematic inset illustrates the 'radiating' pattern. C, D, vector

772   field of u_1 in the medial condyle of P. elephantopus (CM Av8716), shown in anterior (C)

773   and medial (D) views. E, F, vector field of u_1 in the lateral condyle of D. robustus (CM

774   Av8422), shown in anterior (E) and lateral (F) views. G, vector field of u_2 in a 3-D slice

775 through the middle of the condyles in M. didinus (CM Av8507O), shown in distal view.

776 Schematic inset illustrates the 'butterfly' pattern.

777

778 Fig. 3. Examples of increased disorganization or obliquity in the vector field of u_1 in the

779 femoral metaphyses. A, proximal femoral metaphysis of D. robustus (CM Av8422). B,

780 distal femoral metaphysis of M. didinus (CM Av8507J). Regions of increased

781 disorganization are indicated by the braces.

782

783 Fig. 4. The mean orientation of u_1 in the femoral head and medial femoral condyle of moa

784 and extant birds, referenced in an explicit anatomical coordinate system. A, the mean

785 directions as plotted on an equal-angle stereoplot (using StereoNet 9.5; Allmendinger et al.

786 2013, Cardozo and Allmendinger, 2013). The data for the femoral head are plotted with a

787 northern hemisphere projection, and are shown as filled symbols; the data for the medial

788 femoral condyle are plotted with a southern hemisphere projection, and are shown as hollow

789 symbols. Grey symbols are extant birds as reported previously (Bishop et al. 2018b), black

790 symbols are moa; for moa, squares represent D. robustus, triangles represent P.

791 elephantopus and diamonds represent M. didinus. For each dataset, the large circle symbol

792 indicates the mean direction across the group, and the surrounding dotted line indicates the

793 95% confidence cone about the mean. B, comparison of the anterior inclination of u_1 in the

794 sagittal plane of the femoral head versus femur length in moa and extant birds. C,

795 comparison of the posterior inclination of u_1 in the sagittal plane of the medial femoral

796 condyle versus femur length in moa and extant birds. In both B and C, the data for extant

797 birds are as reported previously (Bishop et al. 2018b) and are shown in grey, whereas the

798 data for moa are shown in black, with the same symbols as for A. Major axis regressions are

799 plotted and associated statistics are shown.

800

801 Fig. 5. The main architectural features of cancellous bone in the proximal tibiotarsus of moa.

802 A, B, vector field of u_1 in the cranial and lateral cnemial crests of D. robustus (CM Av8488)

803 shown in anterior (A) and medial (B) views. C, D, vector field of u_1 under the medial

804 condyle of M. didinus (CM Av8513T) shown in posterior (C) and medial (D) views. E, F,

805 vector field of u_1 under the lateral condyle of P. elephantopus (CM Av8383) shown in

806 posterior (E) and lateral (F) views. G, vector field of u_1 in a 3-D slice through the middle of

807 the proximal metaphysis, cnemial crests and condyles of D. robustus (CM Av8422), parallel

808 to the sagittal plane and shown in medial view. Schematic inset illustrates the well-

809 developed double-arcuate pattern present. H, vector field of u_1 throughout the entire

810 proximal tibia of P. elephantopus (CM Av8716), shown in medial view, illustrating

811 increased obliquity and disorganization of vectors in the distal metaphysis and transition to

812 the diaphysis (region with braces).

813

814 Fig. 6. The main architectural features of cancellous bone in the distal tibiotarsus of moa. A–

815 D, vector field of u_1 (A, C) and u_2 (B, D) in the distal tibiotarsus of P. elephantopus (CM

816 Av8383) in oblique anterolateral (A, B) and oblique anteromedial (C, D) views. E, vector

817 field of u_1 (black) and u_2 (white) in the condyles of P. elephantopus (CM Av8383) in

818 proximal view. Note how both u_1 and u_2 are aligned approximately parallel to the sagittal

819 plane. F, isosurface rendering of cancellous bone in the distal tibiotarsus of D. robustus (CM

820 Av8488), shown in oblique anterolateral view, with multiple cuts through the bone to

821 illustrate the 3-D architecture. Cut surfaces are coloured black to better illustrate the nature

822 of the cancellous bone architecture, in particular the plate-like nature of many trabeculae,

823 largely aligned parallel to the sagittal plane.

824

825 Fig. 7. The main architectural features of cancellous bone in the proximal fibula of moa, as

826 exemplified here by D. robustus (CM Av8490), which shows the vector field of u_1 in lateral

827 view.

828

829 Fig. 8. Size-dependent variation in the nature of diaphyseal cancellous bone architecture in

830 the femora and tibiae of moa compared to extant birds. A, B, the extent of cancellous bone

831 in the femur (A) and tibia (B); a higher score indicates greater extent. C, D, the degree of

832 association of trabeculae with one another in the femur (C) and tibia (D); a higher score

833 indicates that trabeculae tend to be more closely associated with other trabeculae of similar

834 character. E, F, the average orientation of trabeculae in the femur (E) and tibia (F); a higher

835 score indicates that trabeculae are at a higher angle to the bone's long axis. The data for

836 extant birds are as reported previously (Bishop et al. 2018b) and are shown in grey; data for

837 moa are shown in black, with the same symbols as for Fig. 4. The major axis regression

838 lines derived for the whole data set are also plotted as solid lines; regression statistics are

839 reported in Table 2. Additionally plotted in A and B are the major axis regressions derived

840 previously for just the extant bird sample (dashed lines); these demonstrate that moa

841 diaphyses almost ubiquitously have a greater quantity of cancellous bone than would be

842 predicted for their size based on extant birds.

843

844 Fig. 9. Cancellous bone in the diaphyses of moa, illustrated here with several examples

845 illustrating the variety of manifestations it can assume. A, femur of D. robustus (CM

846 Av8488); see also Supplementary Movie S1. B, proximal tibiotarsus of D. robustus (CM

847 Av8488). C, femur of P. elephantopus s (CM Av8716). D, proximal tibiotarsus of P.

848 elephantopus (CM Av8716). Note especially the abundant, oblique trabeculae throughout

849 the diaphysis of the femur. In all figures, proximal is towards the top of the page. In C, the

850 asterisk denotes a core produced during the extraction of cortical bone for genetic sampling

851 (Allentoft et al. 2010).

852

853 Supplementary material

854

855 Movie S1. Animated isosurface rendering of the femur of Dinornis robustus (CM Av8488),

856 to illustrate the architecture and extent of cancellous bone in the diaphysis. For scale, the

857 total length of the bone (vertical axis in the video) is 339 mm.

Tables

Table 1. The specimens investigated in this study, as well the settings used in acquiring the CT scan data for each specimen. All specimens are curated in the Natural History Collections of the Canterbury Museum.

Species	Specimen number	Element	CT scan settings				
			Peak tube voltage (kV)	Tube current (mA)	Exposure time (ms)	In-plane pixel resolution (mm)*	Slice thickness (mm)*
Dinornis robustus	CM Av8422	femur	80, 140	189	1,000	0.498	0.4
Dinornis robustus	CM Av8422	tibiotarsus	80, 140	199	1,000	0.551	0.4
Dinornis robustus	CM Av8488	femur	80, 140	221	1,000	0.553	0.6
Dinornis robustus	CM Av8488	tibiotarsus	80, 140	221	1,000	0.816	0.6
Dinornis robustus	CM Av8488	fibula	80, 140	199	1,000	0.551	0.4
Dinornis robustus	CM Av8490	fibula	80, 140	199	1,000	0.551	0.4
Pachyornis elephantopus	CM Av8383	tibiotarsus	80, 140	200	1,000	0.551	0.4
Pachyornis elephantopus	CM Av8383	fibula	80, 140	200	1,000	0.551	0.4
Pachyornis elephantopus	CM Av8716	femur	80, 140	180	1,000	0.498	0.4
Pachyornis elephantopus	CM Av8716	tibiotarsus	80, 140	201	1,000	0.551	0.4
Pachyornis elephantopus	CM Av8716	fibula	80, 140	200	1,000	0.551	0.4
Pachyornis elephantopus	CM Av15029	femur	80, 140	159	1,000	0.498	0.4
Megalapteryx didinus	CM Av8507J	femur	80, 140	201	1,000	0.551	0.4
Megalapteryx didinus	CM Av8507O	femur	80, 140	201	1,000	0.551	0.4
Megalapteryx didinus	CM Av8513P	tibiotarsus	80, 140	200	1,000	0.551	0.4
Megalapteryx didinus	CM Av8513T	tibiotarsus	80, 140	201	1,000	0.551	0.4
Megalapteryx didinus	CM Av8506E	fibula	80, 140	201	1,000	0.551	0.4
Megalapteryx didinus	CM Av8506N	fibula	80, 140	201	1,000	0.551	0.4

*The scans were processed according to protocol 2 of Bishop et al. (2018b), whereby the resulting images were of an isotropic voxel resolution equal to one third of the original in-plane pixel resolution.

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Table 2. Statistical results of categorical scoring analyses of cancellous bone architecture in moa and extant bird femora and tibiotarsi versus bone length (in mm).

Element	Feature	Slope	Intercept	r^2	p
	extent	0.005733	0.2395	0.5624	< 0.0001
Femur	association	0.002625	0.8782	0.3176	<0.0001
	orientation	-0.005919	6.4007	0.2680	0.0002
	extent	0.002449	-0.006592	0.5294	<0.0001
Tibiotarsus	association	0.000982	1.157	0.4563	0.0003
	orientation	-0.00288	5.991	0.2013	0.0054

Figure 1

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Figure 2

Figure 3

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Figure 4

Figure 5

Figure 6

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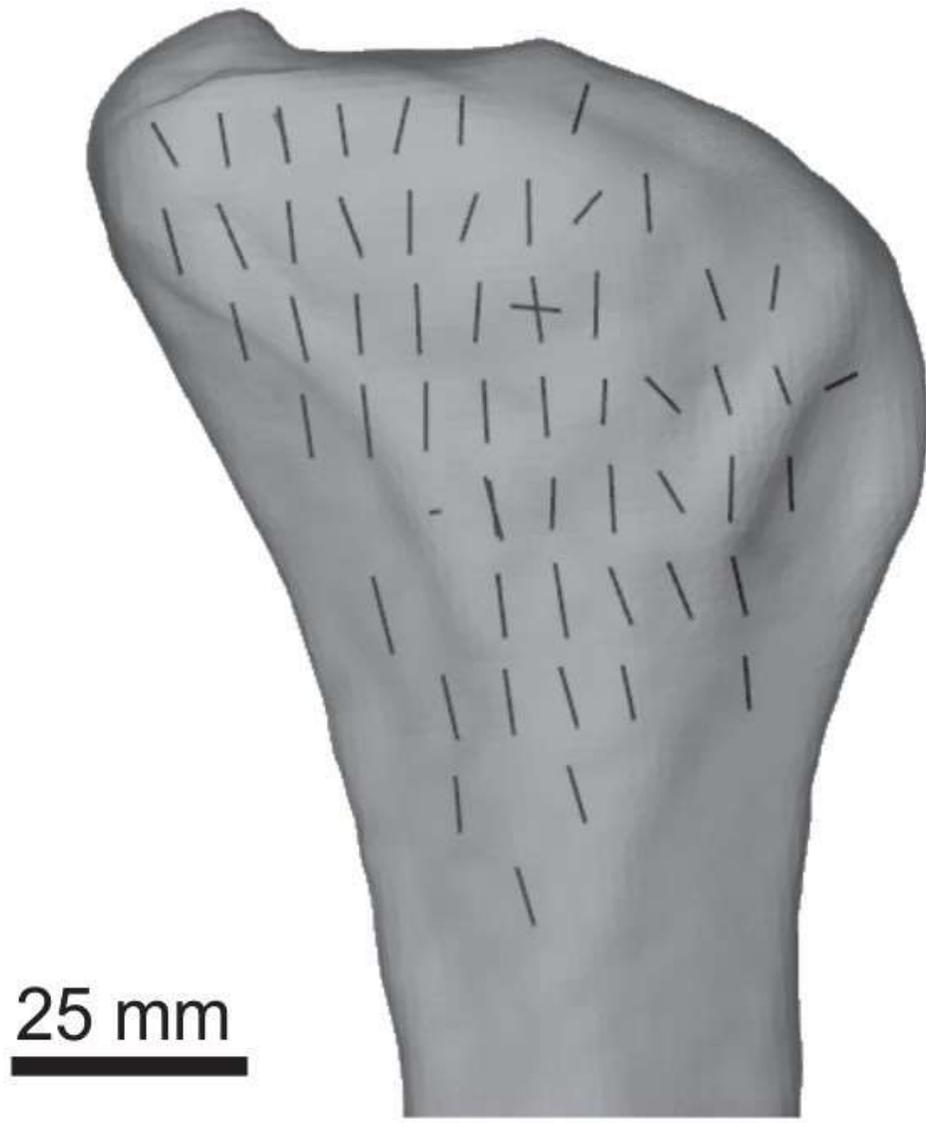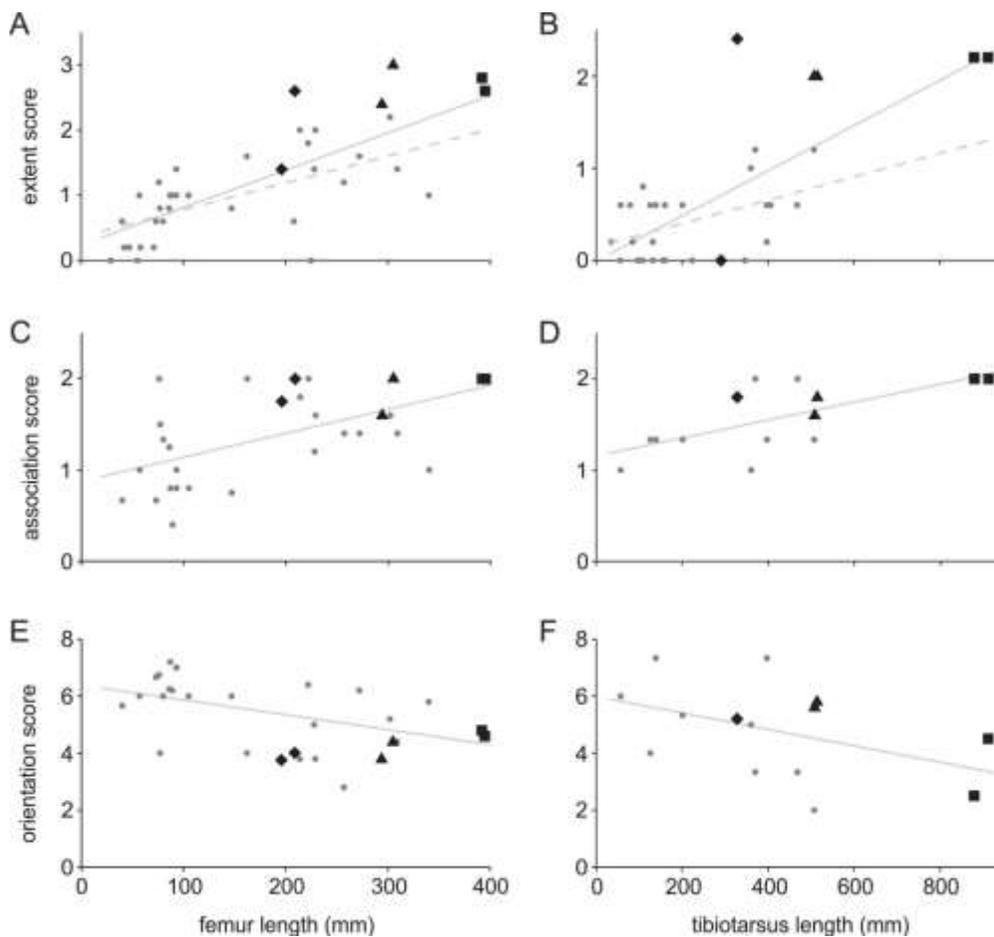
Figure 7

Figure 8

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Figure 9