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2   Intraspecific scaling of the minimum metabolic cost of transport in Leghorn chickens

3   (*Gallus gallus domesticus*): links with limb kinematics, morphometrics and posture

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35 **ABSTRACT**

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37 The minimum metabolic cost of transport (CoT_{min}; J kg^{-1} m^{-1}) scales negatively with

38 increasing body mass ($\propto M_b^{-1/3}$) across species from a wide range of taxa associated

39 with marked differences in body plan. At the intraspecific level, or between closely

40 related species, however, CoT_{min} does not always scale with M_b. Similarity in

41 physiology, dynamics of movement, skeletal geometry and posture between closely

42 related individuals is thought to be responsible for this phenomenon, despite the fact

43 that energetic, kinematic and morphometric data are rarely collected together. We

44 examined the relationship between these integrated components of locomotion in

45 Leghorn chickens (*Gallus gallus domesticus*) selectively bred for large and bantam

46 (miniature) varieties. Interspecific allometry predicts a CoT_{min} ~16 % greater in

47 bantams compared to the larger variety. However, despite 38 % and 23 % differences

48 in M_b and leg length, respectively, both varieties shared an identical walking CoT_{min},

49 independent of speed and equal to the allometric prediction derived from interspecific

50 data for the larger variety. Furthermore, both varieties moved with dynamic similarity

51 and shared geometrically similar appendicular and axial skeletons. Hip height,

52 however, did not scale geometrically and the smaller variety had more erect limbs,

53 contrary to interspecific scaling trends. The lower than predicted CoT_{min} in bantams

54 for their M_b was associated with both the more erect posture and a lower cost per

55 stride (J kg^{-1} stride^{-1}). Therefore, our findings are consistent with the notion that a

56 more erect limb is associated with a lower CoT_{min} and with the previous assumption

57 that similarity in skeletal shape, inherently linked to walking dynamics, is associated

58 with similarity in $CoT_{min.}$

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INTRODUCTION

Body size has a significant influence on the morphology and metabolism of animals (Schmidt-Nielsen, 1975; Schmidt-Nielsen, 1984; Biewener, 1989). In animals that locomote terrestrially the absolute amount of metabolic energy required to move a given distance, increases with increasing body size, but not in direct proportion (slope <1) (Bruinzeel et al., 1999; Halsey and White, 2012). In relative terms, the mass-specific energy per unit distance [the cost of transport (CoT; $J\ kg^{-1}\ m^{-1}$)] is lower in larger species than in smaller ones. Often, at optimal self-selected speeds within a gait, animals incur a minimum cost of transport (CoT_{min}) and it seems reasonable to expect natural selection to favour strategies that minimise the CoT_{min}. For example, if the movement requirements of animals were similar, they would be expected to share optimum limb dynamics, and similar morphological proportions to allow it (Alexander and Jayes, 1983). The evolutionary allometry of CoT_{min} with body mass (M_b, kg) is widely reported. For example, across more than 90 species of mammals and birds (7 g - 260 kg), $CoT_{min} = 10.7M_b^{-0.32}$ (Taylor et al., 1982). Adding amphibians, reptiles and invertebrates (< 1 g) to this data set yielded a similar result ($CoT_{min} = 10.8M_b^{-0.32}$ (Full and Tu, 1991)) and African elephants (*Loxodonta africana,* $M_b = 1542$ kg) fall within the 95% CIs of this equation (Langman et al., 1995). The scaling exponent, however, is known to differ between walking and running (Margaria et al., 1963; Minetti et al., 1999; Rubenson et al., 2004; Rubenson et al., 2007; Maloiy et al., 2009; Nudds et al., 2011; Watson et al., 2011), and also between small crouched- and large upright-postured vertebrates (Reilly et al., 2007; Nudds et al., 2009). Furthermore, there is overlooked variation in CoT_{min} at a given M_b, associated with variation in body form (Full et al., 1990). The general trend of decreasing CoT_{min} with M_b, however, holds for over three orders of magnitude. Where outliers exist, their relatively more or less economical CoT_{min} compared to other species of the same ,M_b is attributed to adaptations associated with activity patterns (Watson et al., 2011), dominant locomotor mode (Dawson and Taylor, 1973; Fish et al., 2000; Griffin and Kram, 2000; Fish et al., 2001; Nudds et al., 2010), ecological niche (Bruinzeel et al., 1999), climate (Yousef et al., 1989; Maloiy et al., 2009) or having a protective shell (Baudinette et al., 2000; Zani and Kram, 2008). Ultimately, the reasons underlying the allometry of CoT_{min} with M_b and the factors that determine the CoT are not yet fully understood (Cavagna et al., 1977; Fedak et al., 1982;

101 Heglund et al., 1982a; Heglund et al., 1982b; Heglund and Taylor, 1988; Kram and

102 Taylor, 1990; Roberts et al., 1998; Pontzer, 2005, 2007).

103 Between disparate species, musculoskeletal morphology and shape vary with

104 size (Schmidt-Nielsen, 1975; Schmidt-Nielsen, 1984; Biewener, 1989; Reilly et al.,

105 2007), speed requirements (Garland, 1983), climate (Janis and Wilhelm, 1993),

106 ecological niche (Bruinzeel et al., 1999) and locomotor mode (Griffin and Kram,

107 2000; Abourachid, 2001; Nudds et al., 2010). Within species or between closely

108 related species, however, variation in shape is reduced, meaning insight can be gained

109 into the factors that dictate the CoT and how it scales with M_b independent of shape

110 (Griffin et al., 2004; Day and Jayne, 2007; Langman et al., 2012). For example,

111 miniature Arabian and draft horses (*Equus ferus caballus*) showed no difference in

112 CoT_{min} when trotting, despite spanning 8- and 2-fold differences in M_b and leg length,

113 respectively (Griffin et al., 2004). Similarly, there was little difference in walking

114 CoT_{min} within camels (*Camelus dromedaries*, M_b = 240-580 kg) (Yousef et al., 1989;

115 Maloiy et al., 2009) or donkeys (*Equus asinus*, M_b = 170-583 kg) (Yousef et al., 1972;

116 Maloiy et al., 2009), or between adult Asian (*Elephas maximus*) and sub-adult African

117 elephants (M_b = 1435-3545 kg) (Langman et al., 1995; Langman et al., 2012). It is

118 assumed that similarity in CoT_{min} across individuals of differing body masses is due

119 to them being geometrically, posturally and physiologically similar and locomoting

120 with dynamically similar gaits (Griffin et al., 2004; Langman et al., 2012).

121 Surprisingly, despite this explanation being widespread in the literature, there is no

122 empirical evidence linking CoT_{min} across a size range with similar limb kinematics

123 and skeletal proportions for a walking gait (the only gait over which dynamic

124 similarity can be investigated (Alexander and Jayes, 1983)). In humans, the only

125 bipedal species to have been examined across a size range (children-adults), walking

126 CoT_{min} scaled in a similar manner to that found across species (i.e., α $M_b^{-1/3}$) (Weyand

127 et al., 2010), which is contrary to findings from within quadruped investigations

128 where CoT_{min} was similar across sizes. To fully understand these results it is

129 necessary to expand the available data for bipeds and to investigate the relationships

130 between the CoT, M_b, limb-kinematics and skeletal proportions.

131 Domestic Leghorn chickens (*Gallus gallus domesticus*) are selectively bred

132 for large and bantam (miniature) varieties providing an opportunity to investigate how

133 size influences CoT_{min} independent of shape in an avian species. Rubenson et al.

134 (2007) derived an interspecific scaling equation of walking CoT_{min} against M_b

135   $[\text{CoT}_{\text{min}} = 17.80 \ (\pm 2.98) \ M_{\text{b}}^{-0.471 \ (\pm 0.032)}]$ using minimum measured values of the net

136   cost of transport (CoT_{net}; the amount of energy required to move 1 kg over 1 m minus

137   maintenance and postural costs) for a range of birds and mammals (0.29 – 1542 kg).

138   The aim of this study was to investigate whether large (n= 5; mean $M_{\text{b}} \pm$ s.e.m = 1.92

139   $\pm$ 0.13 kg; range: 1.62 – 2.19 kg) and bantam (n=9; mean $M_{\text{b}} \pm$ s.e.m = 1.39 $\pm$ 0.03

140   kg; range = 1.29 – 1.54 kg) Leghorns would show a 16 % difference in CoT_{min} as

141   predicted by the Rubenson et al. (2007) equation, and to compare their CoT_{min} to

142   animals of a similar M_{b}. Importantly, we simultaneously determined whether the two

143   varieties of Leghorn walked in a dynamically similar way and were geometrically and

144   posturally similar to gain insight into the links between these integrated components

145   of terrestrial locomotion.

146

147   **RESULTS**

148

149   **Scaling of morphological measurements**

150   Mean linear dimensions measured from large and bantam Leghorns are presented in

151   Table 1. The skeletal measurements of the bantams were, on average, ~83 % of those

152   of the larger variety. Predicted hind limb dimensions (Table 1) for the bantams, based

153   on the percentage difference in sternum length between the two varieties, all fell

154   within the range predicted from the large variety data (mean $\pm$ s.e.m), indicating that

155   the axial and appendicular skeletons of the two varieties were geometrically similar.

156   Independent samples t-tests (equal variances assumed unless otherwise stated)

157   showed that, represented as a proportion of total skeletal leg length (l_{skel} = femur +

158   tibiotarsus + tarsometatarsus lengths), the femur (0.28 in both varieties) was not

159   significantly different (equal variances not assumed: Levene's test, $F = 13.71$, p

160   $=0.003$) between varieties ($t = 1.00$, $df = 4$, $p = 0.374$). Similarly, the tibiotarsus ($t =$

161   0.07, $df = 12$, $p = 0.948$) and tarsometatarsus lengths ($t = -1.26$, $df = 12$, $p = 0.233$)

162   were the same proportion of total leg length in both varieties (0.42 and 0.30,

163   respectively). Femur width, as a proportion of femur length was also similar ($t = 1.63$,

164   $df = 12$, $p = 0.128$) between both varieties (femur: 0.11 and 0.10 in bantam and large

165   Leghorns, respectively). Similarly, the tibiotarsus width:length ratio (0.07 in both

166   varieties) did not differ (equal variances not assumed: Levene's test, $F = 5.25$, $p =$

167   0.041) between varieties ($t = 1.07$, $df = 5.70$, $p = 0.326$) and nor did the

168   tarsometatarsus width/length ratio, which was 0.10 in both ($t = 0.00$; $df = 12$, $p =$

169   1.00). The two varieties therefore shared similar hind limb skeletal proportions.

170   The ratio h_{hip}:l_{skel}, a measure of posture (Gatesy and Biewener 1991), was on

171   average ~5 % greater in the bantam compared to the large variety (0.79 ± 0.02 and

172   0.74 ± 0.01, respectively), but was not statistically different between varieties ($t =$

173   1.96, $df = 12$, $p = 0.074$). The predicted h_{hip} for the bantams (Table 1), however, fell

174   outside of the range predicted from the large varieties hip height data, being

175   approximately 1 cm shorter than measured. Bantam h_{hip} was 0.87 times that of the

176   larger birds, which was a greater fraction than found for the skeletal element

177   measurements. Therefore, the bantams adopted a more erect posture compared to the

178   large variety.

179

180   **Walking kinematics**

181   Duty factor (DF) decreased linearly with speed (U, m s^{-1}) and neither the slope nor

182   intercept of this relationship differed between varieties (Figure 1a; Table 2). Stride

183   frequency (f_{stride}, Hz) increased at the same rate with U in both varieties, but was 0.37

184   Hz greater in the bantam variety across all U (Figure 1b; Table 2). Similarly, the

185   incremental increase in stride length (l_{stride}, m) with U was the same in both size

186   groups, whilst l_{stride} was longer by 0.09 m across all U in the large variety (Figure 1c;

187   Table 2). The duration of the swing phase of the limb (t_{swing}, s) decreased curvilinearly

188   with U at the same rate in both groups, but was 0.05 s longer in the large variety

189   across all U (Figure 1d; Table 2). Stance phase duration (t_{stance}, s) also decreased

190   curvilinearly with U and at the same rate in both size groups. t_{stance} was, however, 0.08

191   s longer in the large variety across all U (Figure 1d, Table 2). Therefore, each

192   parameter responded to increasing U the same way in both varieties and differences in

193   their absolute values (related to size) were fixed across all speeds.

194

195   **Metabolic power and cost of transport**

196   The positive relationship between mass-specific metabolic power (P_{met}, W kg^{-1}) and

197   walking U (Figure 2a) was similar (both the slopes and intercepts) for both varieties

198   (Table 1). Calculating CoT$_{min}$ as the slope of this relationship (slope method)

199   therefore gives 16.20 J kg^{-1} m^{-1} in each variety. During quiet standing, resting

200   metabolic rate (RMR, W kg^{-1}) did not differ (Figure 2a, Table 2) between bantam and

201   large Leghorns (7.24 ± 0.42 W kg^{-1} and 7.21 ± 0.48 W kg^{-1} respectively), indicating

202 that they shared the same mass-specific energetic cost of general maintenance and
203 maintaining their posture combined. Therefore, the relationship between net mass-
204 specific metabolic power (net-P_{met}, W kg^{-1}: the metabolic rate required for locomotion
205 exceeding that required for standing quietly) and U (Figure 2a) was also similar for
206 the two size groups (Table 2).

207 Total cost of transport (CoT$_{tot}$, J kg^{-1} m^{-1}) decreased curvilinearly with U
208 indicating that the highest walking speeds of the birds were most metabolically
209 optimal. Net cost of transport (CoT$_{net}$, J kg^{-1} m^{-1}; net-P_{met}/U), however, was not
210 correlated with U and fell within a similar range for both size groups (bantam: 9.44 -
211 16.10 J kg^{-1} m^{-1}; large: 9.72 – 15.33 J kg^{-1} m^{-1}) (Figure 2b; Table 2). Calculating
212 CoT$_{min}$ as the minimum measured CoT$_{net}$ (subtraction method), taken as the mean of
213 all CoT$_{net}$ values across all speeds and both varieties, gives 13.04 J kg^{-1} m^{-1}. Predicted
214 walking CoT$_{min}$ values for large and bantam Leghorns based on (Rubenson et al.,
215 2007) were 13.09 and 15.24 J kg^{-1} m^{-1}, respectively. Both varieties therefore shared a
216 CoT$_{min}$ closer to that predicted for the larger variety, contrary to the 16 % difference
217 predicted. This corresponds to the bantams having a CoT$_{min}$ ~14 % lower than
218 predicted for their M_b, which fell within the 95 % C.Is of Rubenson et al's (2007)
219 equation. The cost per stride (J kg^{-1} stride^{-1}) and its rate of increase with U were lower
220 in bantams compared to in the larger variety (Fig 2c; Table 2).

221

222 **DISCUSSION**
223 Across species, CoT$_{min}$ is reported to scale hypoallometrically with M_b (Taylor et al.,
224 1970; Fedak et al., 1974; Taylor et al., 1982; Kram and Taylor, 1990; Full and Tu,
225 1991; Langman et al., 1995; Roberts et al., 1998). However, we find that bantam and
226 large varieties of Leghorn chickens have identical CoT$_{min}$ despite the smallest and
227 largest individuals differing 1.7-fold in M_b and 1.35-fold in leg length. An
228 independence of CoT$_{min}$ from body size was previously reported within large
229 quadrupedal species (>90 kg) spanning 1.5- to 8-fold ranges in M_b and up to 2-fold
230 ranges in leg length (Griffin et al., 2004; Maloiy et al., 2009; Langman et al., 2012).
231 The present data represent the first evidence of a lack of correlation between M_b and
232 CoT$_{min}$ within an avian species. No effect of M_b or leg length suggests that size itself
233 does not influence the CoT, but rather, some other factor, perhaps correlated with
234 body size, may be responsible.

235   The simultaneous collection of kinematics and morphological data here allow

236   us to investigate further previous hypotheses on what is driving the interspecific

237   CoT_{min} versus M_b relationship. Larger species perform the same amount of mass-

238   specific mechanical work as those of smaller species, whilst using less mass-specific

239   metabolic energy during terrestrial locomotion (Fedak et al., 1982; Heglund et al.,

240   1982a; Heglund et al., 1982b; Alexander, 2005). How this is possible is not fully

241   understood. It is generally accepted that M_b has no independent influence over CoT

242   (Pontzer et al 2005; 2007). Leg length, however, is often discussed as the

243   morphological factor explaining the allometry of CoT_{min} (Kram and Taylor 1990;

244   Schmidt 1984; Pedley 1977; Biewener 2003; Alexander 2002) since longer legs allow

245   longer t_{stance} for the muscles to apply force through recruiting slower, less

246   metabolically expensive muscle fibres (metabolic rate is inversely proportional to

247   t_{stance} during which the muscles apply force) (Kram and Taylor, 1990). In addition,

248   longer limbs allow lower f_{stride}, requiring fewer muscle contractions. In the present

249   study, however, the different sized birds shared the same mass-specific CoT_{min},

250   despite the bantams having shorter limbs, shorter t_{stance} and higher f_{stide} compared to

251   the larger variety. Using the maximum height of the limb as a strut (effective limb

252   length, h_{hip}) as the indicator of size has been shown to better predict CoT_{min} across

253   species (h_{hip}, $r^2=0.98$), than the sum of the skeletal element lengths (L_{skel}, $r^2=0.78$)

254   (Steudel and Beattie, 1995; Pontzer, 2007). Over a small size scale of analysis,

255   however, it has been demonstrated that between-individual differences in limb

256   arrangement [e.g. limb excursion angle], the cost of swinging the limb and the

257   coefficient of converting metabolic energy into muscle force 'k' (which were not

258   measured in this study) prevent a clear relationship between h_{hip} and CoT_{min} [Pontzer

259   2005; Pontzer 2007b]. In agreement with Pontzer's [Pontzer 2005; Pontzer 2007b]

260   findings, despite the greater absolute h_{hip} of the larger variety, compared to the

261   bantams, they did not have a lower CoT_{min} in comparison. It may be that variation in

262   limb excursion angle [i.e. the difference in posture], rather than h_{hip}, dominated

263   variation in CoT_{min}. Indeed, by using a model to predict the rate of force production

264   associated with both supporting body weight and swinging the limb as a function of

265   all of these parameters, Pontzer (2007a) found this was a better predictor of metabolic

266   rate than contact time, limb length or M_b at both inter- and intra-specific levels.

267   Equally, the shared CoT_{min} of the two varieties may be due to their identical

268 appendicular and axial skeletal geometry, consistent with previous assumption in

269 intraspecific analyses (Langman et al., 2012).

270   Another potential explanatory factor is limb posture (linked to effective limb

271 length). Across vertebrates, the limb bone lengths scale positively and almost

272 geometrically with M_b, but become increasingly more aligned with one another and

273 less crouched (Biewener, 1989). A prominent step-change exists in the scaling of both

274 CoT_{min} and the mechanical cost of transport (E_{mech}; J kg^{-1} m^{-1}) across species

275 associated with crouched postures in those <1 kg and upright postures in those >1 kg,

276 making their efficiency of transport (CoT_{min}/E_{mech}) approximately 7% and 26%,

277 respectively (Reilly et al., 2007; Nudds et al., 2009). Unlike larger species with more

278 upright posture, small crouched-postured (non-cursorial) species do not benefit from

279 elastic energy savings, nor pendular mechanisms (Reilly et al., 2007). Furthermore, a

280 more vertical limb decreases the muscular force required to support a unit of body

281 weight and improves the mechanical advantage of the muscles (Biewener, 1989). The

282 change in posture with increasing size means that muscle stress is nearly independent

283 of M_b across species (rather than $\propto M_b^{1/3}$). Griffin et al (2004) suggested that between

284 closely related individuals, consistent limb posture might account for consistent

285 CoT_{min} across a range of body sizes since muscle stress would in this case scale

286 geometrically ($\propto M_b^{1/3}$). The volume of active muscle would therefore increase with

287 size and counter any metabolic savings associated with having longer legs (Griffin et

288 al., 2004). However, in the present study the shared CoT_{min} of the chicken groups did

289 not correspond to similar posture. When comparing the posture of the two size groups

290 as $h_{hip}:l_{skel}$, the limbs were 5% more erect in the variety selected for smaller size. The

291 shared CoT_{min} in this case is perhaps better explained by the posture and lower cost

292 per stride of the bantams. Across avian species, h_{hip} represents a greater proportion of

293 l_{skel} with increasing M_b One potential explanation for why we find the opposite to

294 what would be expected, as well as the lower cost per stride in the bantams, may be

295 that the two varieties differ in their derived muscle properties or architecture due to

296 selective breeding.

297   The kinematic data indicate that with U, the two varieties shared identical

298 rates of change in all parameters, which would be expected to imply geometric,

299 postural and dynamic similarity. Each kinematic parameter differed between the two

300 varieties only by a fixed value across all speeds. The larger variety took longer strides

301   by 9 cm, less frequent strides by 0.37 Hz and had longer durations of both swing and

302   stance phases of the limb by 0.05 and 0.08 s, respectively. At a given absolute U, duty

303   factor is generally higher in larger species than smaller ones (Gatesy and Biewener,

304   1991); however, the duty factors of the chickens were not significantly different

305   between size groups. Similarly, 9 different felid species spanning a 46-fold range in

306   M_b were found to use similar duty factors at their preferred walking speed (Day and

307   Jayne, 2007). For what was previously an expectation (Griffin et al., 2004; Maloiy et

308   al., 2009; Langman et al., 2012), the present data offer the first empirical evidence of

309   a link between identical walking CoT_{min} in individuals of differing size and similar

310   limb dynamics and skeletal geometry. We can speculate that for a given skeletal

311   shape, regardless of M_b, walking CoT may be consistent. Some additional studies in

312   which shape was controlled for also support this idea. For example, adding back loads

313   up to 50% of M_b has negligible effect on the CoT in quadrupedal rats, dogs and horses

314   as well as bipedal humans, guinea fowl and other birds (Taylor et al., 1980; Ellerby

315   and Marsh, 2006; Tickle et al., 2010; Tickle et al., 2013). Furthermore, obese and thin

316   humans of the same height (likely to be similar in skeletal proportions) show no

317   difference in CoT_{min} (Browning et al., 2006).

318   In contrast to our findings, a comprehensive study of 48 humans spanning a 6-

319   fold range in M_b and 1.5- fold range in height concluded that CoT_{min} was $\propto M_b^{-1/3}$

320   (Weyand et al., 2010). This result, however, may be associated with ontogenetic

321   differences in shape, because the human subjects ranged from 5 to 32 years of age and

322   the data were intentionally separated into four size groups to reduce individual

323   variability (Weyand et al., 2010). Indeed, dividing the CoT by body height accounted

324   for the observed differences between the human size groups. Therefore, at any given

325   speed, all subjects incurred the same CoT to cover the same horizontal distance

326   relative to their own body height (Weyand et al., 2010). In one of the few invertebrate

327   species examined, small (2 g) ghost crabs (*Ocypode quadrata*) were found to have a

328   higher CoT than larger ones (47 g), despite their similar appearance in shape (Tullis

329   and Andrus, 2011). In the absence of detailed kinematic measurements, however, it is

330   not possible to conclude much from this result. It is, of course, possible that the link

331   we find here between energetics, kinematics and skeletal morphometrics may not be

332   characteristic of species with more than two legs.

333

CONCLUSIONS

Leghorn chickens selectively bred for large and bantam varieties shared the same walking CoT_{min} despite a 1.70- fold difference in M_b and 1.35- fold difference in total leg length between the smallest and largest individuals. These data represent the first evidence of CoT_{min} being independent of M_b within a small crouched-postured bipedal species. Our findings also provide the first evidence (for what was previously only assumed) of a link between this and similar walking dynamics and skeletal geometry. In contrast to interspecific trends, however, hip height did not scale geometrically between varieties and represented a greater proportion of total leg length in the bantam variety compared to the large. All birds shared a CoT_{min} closer to that predicted for the larger variety and the CoT_{min} of the bantams was approximately 14 % lower than predicted from their M_b. Our findings are therefore in agreement with the general consensus that for a given body size, CoT_{min} decreases with limb erectness. The lower than predicted CoT_{min} in the bantams was also associated with lower mass-specific energy requirements per stride, compared to the larger variety, which may be linked to differences in their derived muscle morphology/physiology. We emphasise the importance of intra- in addition to interspecific investigations as well as the combination of kinematics, morphometric and posture measurements towards gaining insight into the factors that dictate CoT.

MATERIALS AND METHODS

Study species

Adult (>16 week) male bantam ($n = 9$; mean $M_b \pm$ s.e.m = 1.39 ± 0.03 kg) and large ($n = 5$; mean $M_b \pm$ s.e.m = 1.92 ± 0.13 kg) Leghorn chickens were purchased from a local breeder and housed in the University of Manchester's animal unit. All housing was maintained on a 13h:11 h light-dark cycle, at 18-22 °C. Food and water were provided *ad libitum*, and the birds were not fasted prior to experiments. Birds were trained for one week to locomote on a motorised treadmill (T60 Tunturi®, Finland) prior to data collection. All experiments were carried out in accordance with the

368 Animals (Scientific procedures) Act (1986), were approved by the University of
369 Manchester Ethics Committee and performed under a UK Home Office Project
370 Licence held by Dr Codd (40/3549).

371

372 **Respirometry**

373 An open flow respirometry system (all equipment Sable Systems International®, Las
374 Vegas, USA) was used to measure the birds' rates of oxygen consumption ($\dot{V}_{O_2}$, ml
375 min^{-1}) and carbon dioxide production ($\dot{V}_{CO_2}$, ml min^{-1}). Perspex® respirometry
376 chambers were built (bantam: 66 x 46.5 x 48 cm, large: 97.5 x 53.5 x 48 cm) and
377 mounted upon the treadmill. Air was pulled through the chambers using a FlowKit
378 500 at flow rates (FRs) of 150 L min^{-1} (bantam) and 250 L min^{-1} (large). Excurrent
379 airflow was sub-sampled (0.11 L min^{-1}) for gas-analysis. Water vapour pressure
380 (WVP) was measured using an RH-300 before the air was scrubbed of H_2O with
381 calcium chloride (2-6mm granular, Merck, Darmstadt, Germany) and passed through
382 a CO_2 analyser (CA-10A). The dry air was scrubbed of CO_2 using soda lime (2-5mm
383 granular, Sigma Aldrich, Steinheim Germany) and passed through a dual absolute and
384 differential O_2 analyser (Oxilla II). Ambient air (scrubbed of H_2O and CO_2 as before)
385 was simultaneously passed through a second O_2 channel on the Oxilla II at 0.11 L
386 min^{-1} by a pump (SS-3) to enable calculation of differential O_2 concentration (ΔO_2).
387 CO_2 traces were base-lined to calculate differential CO_2 concentration (ΔCO_2).
388 Voltage outputs were recorded using a UI2 interface and analysed using ExpeData® v
389 1.1.15 software. The accuracy of the respirometry set up (±5%) across all speeds was
390 determined using a N_2 dilution test (Fedak et al., 1981). Primary flow rates (FR) were
391 adjusted to dry-corrected flow rates (FR_c), to account for the H_2O scrubbed from air
392 samples prior to gas measurements using

393

394
$$FR_C = \frac{FR \cdot (BP - WVP)}{BP} \tag{1}$$

395

396 where BP is barometric pressure (measured with the Oxilla II) and WVP is water
397 vapour pressure (Lighton, 2008). $\dot{V}_{O_2}$ was calculated using (Lighton, 2008)

398
$$\dot{V}_{O_2} = \frac{FR_C (\Delta O_2)}{(1 - 0.2095)} \tag{2}$$

399

400 and $\dot{V}_{CO_2}$ using (Lighton, 2008)

401

402 $$\dot{V}_{CO_2} = \frac{(FR_C(\Delta CO_2) - (0.0004(\dot{V}_{O_2})))}{(1 - 0.0004)}$$ (3)

403   The birds were exercised over a range of randomised speeds (3 per day) up to

404 the maximum sustainable (bantam: 0.28-1.11 m s^{-1}, large: 0.28-1.39 m s^{-1}). Birds

405 were given a rest of a minimum of 5 min to stand quietly between each period of

406 exercise. RMRs were taken from the final rest period of each trial. Data were

407 collected from stable gas readings lasting >1 min. Only data from speeds at which

408 both varieties used a walking gait (0.28, 0.42, 0.56 and 0.69 m s^{-1}) were included in

409 analyses.

410

411 **Metabolic rate calculations**

412 Five values were calculated at each speed: (1) P_{met}, was converted from $\dot{V}_{O_2}$, using

413 respiratory exchange ratios (RERs: $\dot{V}_{CO_2} : \dot{V}_{O_2}$) and thermal equivalents taken from

414 Brody (1945); (2) net-P_{met} was calculated by subtracting RMR from locomotor P_{met}

415 (both from the same trial); (3) CoT$_{tot}$ was calculated as P_{met}/U; (4) CoT$_{net}$ was

416 calculated as net-P_{met}/U; and (5) the cost per stride was calculated as net-P_{met}/f_{stride}.

417   CoT$_{min}$ was calculated using two methods. First as the slope of the linear

418 relationship between P_{met} and U (slope method) and, second, as the minimum

419 measured CoT$_{net}$ (subtraction method). CoT$_{min}$ values calculated using the subtraction

420 method were compared with predictions for walking birds and mammals of a similar

421 M_b using equation 3 from Rubenson et al. (2007).

422

423 **Gait kinematics**

424 The birds were filmed (100 frames s^{-1}) at all speeds in lateral view using a video

425 camera (HDR-XR520VE, Sony, Japan). The left foot of each bird was tracked ($\approx$ 10

426 strides) at each speed using Tracker software (v. 4.05, Open Source Physics) in order

427 to quantify DF, f_{stride}, l_{stride} (U/f_{stride}), t_{stance} and t_{swing}. Fluctuations in the kinetic and

428 potential energy of the centre of mass (CoM) across a stride were determined through

429 frame-by-frame tracking of a marker positioned over the left hip joint of the birds

430   (indicative of hip height, h_{hip}). To ensure that the birds were using a walking gait at all

431   speeds analysed, the phase relationship between the horizontal kinetic energy (E_{kh})

432   and the sum of the potential and vertical kinetic energies ($E_p + E_{kv}$) of the CoM (h_{hip})

433   was determined. An out-of-phase relationship, indicating a walking gait, was found

434   for all speeds used in the analyses.

435

436   **Morphological measurements**

437   Keel length and the length and width (mid-shaft) of the right femur, tibiotarsus and

438   tarsometatarsus was measured from the birds used in the respirometry experiments

439   using digital vernier calipers (accuracy, ±0.01 mm). Geometric similarity in linear

440   dimensions between the two size groups was investigated by determining whether

441   their axial and appendicular dimensions scaled 1:1. The mean appendicular

442   dimensions of the bantams were predicted based on the ratio of their keel length: large

443   variety keel length. Skeletal element lengths were also compared as a percentage of

444   total leg length. The ratio of h_{hip} to total skeletal leg length (l_{skel} = femur + tibiotarsus

445   + tarsometatarsus lengths) was calculated and used as a means of comparing posture

446   between the two size groups, with a lower value indicating a more crouched posture.

447   Back height (h_{back}, m) was measured during the mid-stance as the distance from the

448   hind toe to the back at 90° to the direction of travel. Where birds (N=3) did not walk

449   with ease with a hip marker, the ratio h_{hip}: h_{back} (bantam: 0.80 ± 0.01, large: 0.77 ±

450   0.00) was used to estimate h_{hip}.

451

452   **Statistical analyses**

453   The slopes and the intercepts of the relationships between the dependent variables

454   (metabolic or kinematics measures) and U were investigated for differences between

455   chicken varieties using general linear models (GLMs). Models included variety as a

456   fixed factor, U as a covariate and the interaction term (variety x U). If the interaction

457   term was non-significant (indicating similar slopes between varieties), it was removed

458   from the model and the updated model was rerun (assuming parallel lines) in order to

459   test for differences in intercepts. Where the relationship between a dependent variable

460   and U was curvilinear the data were $\log_{10}$ transformed. All best-fit lines were taken

461   from coefficients-tables produced by the GLMs. Between-variety differences in hind-

462   limb skeletal element proportions (% total leg length) were investigated using

463   independent samples t-tests. Hind-limb proportion data were tested for equality of

464   variance using a Levene's test for equality of variance.

465

466   **List of abbreviations**

467   U speed

468   CoT_{tot} total cost of transport

469   CoT_{net} net cost of transport

470   CoT_{min} minimum cost of transport

471   DF   duty factor

472   f_{stride} stride frequency

473   l_{stride} stride length

474   net-P_{met} net metabolic power

475   P_{met} metabolic power

476   RMR   resting metabolic rate

477   t_{swing} swing duration

478   t_{stance} stance duration

479    $\dot{V}_{CO_2}$ rate of carbon dioxide production

480    $\dot{V}_{O_2}$ rate of oxygen consumption

481

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484   respirometry data collection.

485

486   **Competing interests**

487   The authors declare that they have no competing interests

488

489   **Authors' contributions**

490   The study was conceived and designed by JC and RN. KR collected and analysed all

491   data with assistance from RN and JC. All authors contributed to preparation of the

492   manuscript, approved and read the final submission.

493

494

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656 **Figure 1 Relationships between kinematics parameters and walking speed.** Red

657 filled circles and solid lines represent data for bantam Leghorns and open circles and

658 dashed lines represent data for large Leghorns.. The lines of best fit are (*a*) duty factor

659 = -0.18U + 0.79 (bantam) and = -0.18U +0.78 (large), (*b*) stride frequency = 1.51U +

660 0.83 (bantam) and = 1.51U + 0.46 (large), (*c*) stride length = 0.36U + 0.13 (bantam)

661 and = 0.36U + 0.23 (large) and (*d*) swing time = 0.16$U^{-0.22}$ (bantam) and = 0.21$U^{-0.22}$

662 (large), and stance time = 0.28$U^{-0.64}$ (bantam), and = 0.36$U^{-0.64}$ (large). Data points

663 are means ± s.d. (standard errors are not large enough to be seen).

664

665 **Figure 2 Relationships between mass-specific energetic parameters and walking**

666 **speed**. Data points and best-fit lines indicate the same as in Figure 1. The lines of best

667 fit are (*a*) P_{met}=16.20U + 6.93 (bantam), and =16.20U + 5.86 (large), and net-P_{met}

668 =16.00U - 0.88 (bantam), and =16.00U – 1.26 (large); (*b*) COT_{tot}= 22.39$U^{-0.50}$

669 (bantam) and = 19.95$U^{-0.50}$ (large), and COT_{net}= 4.77U + 11.89 (bantam), and =

670 4.77U +10.53 (large); and (*c*) cost per stride = 7.10U +2.42 (bantam) and =21.21U +

671 0.24 (large). Mass-specific resting (standing) metabolic rates are also included on

672 graphs (*a*) at 0 m s^{-1}. Data points are means ± s.e.m.

673

674 **Table 1 Hind limb segment measurements and sternal keel lengths (mm) from the birds used**
675 **in experiments and geometric predictions for appendicular measurements of the bantams**

length/width	bantam (± s.e.m)	large (± s.e.m)	bantam prediction[b]
l_{keel}	90.00 (± 1.30) [a]	107.40 (± 5.29)	
l_{fem}	71.04 (± 0.83)	85.92 (± 1.74)	**70.71 – 73.64**
l_{tib}	107.21 (± 1.23)	129.29 (± 2.19)	**106.77 – 110.44**
l_{tars}	75.45 (± 1.19)	93.15 (± 2.36)	**76.27 – 80.23**
l_{skel}	253.70 (± 3.11)	308.37 (± 6.06)	**253.94 – 264.12**
w_{fem}	7.78 (± 0.11)	8.96 (± 0.24)	7.33 – 7.73
w_{tib}	7.18 (± 0.11)	8.62 (± 0.25)	**7.03 – 7.45**
w_{tars}	7.57 (± 0.15)	9.27 (± 0.22)	**7.60 – 7.97**
h_{hip} [c]	200.00 (± 3)	229.00 (± 6)	187.85 – 197.54

676 Bold values represent geometric predictions that were not significantly different from observed bantam
677 measurements
678 [a] N= 6 for bantam sternum measurements
679 [b] predicted value ranges for the bantams were calculated as (large linear dimension ± s.e.m) x (0.84)
680 based on the percentage difference in keel length between the varieties
681 [c] h_{hip} measurements are given to the nearest mm because the measurements were made in metres to the
682 nearest mm

683

684

685 **Table 2 Results of the GLMs that tested for differences in metabolic and kinematic measurements between chicken varieties**

parameter	covariate/ factor interaction[a]	GLM1			GLM2			n_p^2	Observed power	$r^{2\,c}$
		df^b	F	P	df^b	F	P			
DF	U	1,47	61.90	<0.001	1,48	59.43	<0.001	0.56	1.00	0.54
	variety	1,47	0.70	0.406	1,48	1.80	0.186	0.04	0.30	
	variety x U	1,47	0.17	0.199	*	*	*	*	*	
f_{stride} (Hz)	U	1,47	217.96	<0.001	1,48	231.94	<0.001	0.84	1.00	0.88
	variety	1,47	5.52	0.023	1,48	144.11	<0.001	0.77	1.00	
	variety x U	1,47	1.80	0.186	*	*	*	*	*	
l_{stride} (m)	U	1,47	242.10	<0.001	1,48	244.37	<0.001	0.85	1.00	0.89
	variety	1,47	8.42	0.006	1,48	172.30	<0.001	0.80	1.00	
	variety x U	1,47	1.20	0.228	*	*	*	*	*	
$\log_{10}t_{swing}$ (s)	$\log_{10}U$	1,47	17.14	<0.001	1,48	18.57	<0.001	0.29	0.99	0.65
	variety	1,47	13.22	0.001	1,48	78.62	<0.001	0.66	1.00	
	variety x $\log_{10}U$	1,47	0.02	0.877	*	*	*	*	*	
$\log_{10}t_{stance}$ (s)	$\log_{10}U$	1,47	339.11	<0.001	1,48	341.40	<0.001	0.88	1.00	0.90
	variety	1,47	10.38	0.002	1,48	117.64	<0.001	0.72	1.00	
	variety x $\log_{10}U$	1,47	1.48	0.230	*	*	*	*	*	
RMR (W kg^{-1})	variety	1,12	1.64	0.22	*	*	*	*	*	0.05
P_{met} (W kg^{-1})	U	1,50	52.61	<0.001	1,51	53.35	<0.001	0.51	1.00	0.50
	variety	1,50	1.59	0.214	1,51	2.31	0.135	0.04	0.32	
	variety x U	1,50	0.71	0.404	*	*	*	*	*	
Net-P_{met} (W kg^{-1})	U	1,50	52.94	<0.001	1,51	53.23	<0.001	0.51	1.00	0.49
	variety	1,50	1.08	0.303	1,51	0.29	0.591	0.00	0.08	
	variety x U	1,50	0.85	0.362	*	*	*	*	*	
$\log_{10}CoT_{tot}$ (J kg^{-1} m^{-1})	$\log_{10}U$	1,50	28.34	<0.001	1,51	33.43	<0.001	0.53	1.00	0.71
	variety	1,50	0.01	0.912	1,51	3.79	0.057	0.05	0.35	
	variety x $\log_{10}U$	1,50	0.93	0.338	*	*	*	*	*	
CoT_{net} (J kg^{-1} m^{-1})	U	1,50	1.82	0.184	1,51	1.08	0.304	0.02	0.18	0.00
	variety	1,50	2.35	0.132	1,51	0.87	0.355	0.02	0.16	
	variety x U	1,50	1.71	0.196	*	*	*	*	*	
Net cost per stride	U	1,46	18.07	<0.001				0.28	0.99	0.44
(J kg^{-1} stride^{-1})	variety	1,46	0.42	0.521				0.01	0.10	
	variety x U	1,46	4.49	0.039				0.09	0.55	

686

687   [a] speed (U, m s^{-1}) is a covariate, chicken variety is a fixed factor and variety x U the interaction term in the models

688   [b]df are represented as (df, error df)

689   [c] the adjusted r^2 values are reported for second GLM analyses

690   variables that did not have a significant effect on parameters were not included in second GLM analyses and are represented by an asterisk

691

(A) — y-axis: **W kg^{-1}**; labels: P_{met}, net-P_{met}

(B) — y-axis: **J kg^{-1} m^{-1}**; labels: CoT$_{tot}$, CoT$_{net}$

(C) — y-axis: **net J kg^{-1} stride^{-1}**

x-axis: U (m s^{-1})