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TITLE: Relating neuromuscular control to functional anatomy of limb muscles in extant archosaurs

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JOURNAL: Journal of Morphology

PUBLISHER: Wiley

PUBLICATION DATE: 8 March 2019 (online)

DOI: <https://doi.org/10.1002/jmor.20973>

1 Relating neuromuscular control to functional anatomy of limb muscles in
2 extant archosaurs

3 Short title: Archosaur EMG patterns

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12

13 **Abstract**

14 Electromyography (EMG) is used to understand muscle activity patterns in animals. Understanding
15 how much variation exists in muscle activity patterns in homologous muscles across animal clades
16 during similar behaviours is important for evaluating the evolution of muscle functions and
17 neuromuscular control. We compared muscle activity across a range of archosaurian species and
18 appendicular muscles, including how these EMG patterns varied across ontogeny and phylogeny, to
19 reconstruct the evolutionary history of archosaurian muscle activation during locomotion. EMG
20 electrodes were implanted into the muscles of turkeys, pheasants, quail, guineafowl, emus (three
21 age classes), tinamous and juvenile Nile crocodiles across 13 different appendicular muscles.
22 Subjects walked and ran at a range of speeds both overground and on treadmills during EMG
23 recordings. Anatomically similar muscles such as the lateral gastrocnemius exhibited similar EMG
24 patterns at similar relative speeds across all birds. In the crocodiles, the EMG signals closely matched
25 previously published data for alligators. The timing of lateral gastrocnemius activation was relatively
26 later within a stride cycle for crocodiles compared to birds. This difference may relate to the
27 coordinated knee extension and ankle plantarflexion timing across the swing-stance transition in
28 Crocodylia, unlike in birds where there is knee flexion and ankle dorsiflexion across swing-stance. No
29 significant effects were found across the species for ontogeny, or between treadmill and overground

30 locomotion. Our findings strengthen the inference that some muscle EMG patterns remained
31 conservative throughout Archosauria: for example, digital flexors retained similar stance phase
32 activity and *M. pectoralis* remained an “anti-gravity” muscle. However, some avian hindlimb muscles
33 evolved divergent activations in tandem with morphofunctional changes such as bipedalism and
34 more crouched postures, especially *M. iliotrochantericus caudalis* switching from swing to stance
35 phase activity and *M. iliofibularis* adding a novel stance phase burst of activity.

36 Keywords: morphology, neural control, musculoskeletal system, evolution, locomotion.

37 **Research Highlights**

38 Crocodylians show appendicular muscle activity patterns linked to ancestral conservatism. Birds
39 show consistent differences from the ancestral state, which may have been inherited from
40 dinosaurian ancestors after the Triassic archosaurian divergence.

41

42 **Introduction**

43 Animals move using coordinated patterns of muscular activity stimulated by motor neurons. The
44 electrical signals associated with neuromuscular excitation and thence activation can be obtained
45 using electromyography (EMG). The relative amplitudes and timings of EMG signals can be used to
46 (qualitatively) approximate muscle force (Roberts and Gabaldón, 2008). Integrating EMG with
47 kinematic data and anatomical information facilitates interpretation of the individual function of
48 muscles (e.g. Roberts and Gabaldón, 2008; Carr et al., 2011).

49

50 Collecting EMG from non-human animals is difficult because surface EMG require ideal conditions
51 (thin skin, minimal skin motion, clean attachment sites, conductive gels, good adhesion, etc.) which
52 are not readily achieved in many animals. As such, the majority of EMG data in animals have been
53 collected by surgically implanted electrodes. Probably because of the difficulties inherent in

54 collecting EMG data from appendicular muscles during locomotion and ethical priorities to minimize
55 the number of invasive animal experiments, only a small range of non-mammalian amniote taxa
56 (and muscles) have been studied. These studies include birds (Jacobson and Hollyday, 1982; Gatesy,
57 1994, 1999; Daley and Biewener, 2003, 2011; Marsh et al., 2004; Daley et al., 2009; Ellerby and
58 Marsh, 2010; Gordon et al., 2015), but also alligators (Gatesy, 1991, 1994, 1997; Reilly et al., 2005),
59 caiman (Gatesy, 1994), turtles (Rivera et al., 2011; Rivera and Blob, 2013), and lizards (Jenkins and
60 Goslow, 1983; Reilly, 1995; Higham and Jayne, 2004; Foster and Higham, 2014, 2017). The majority
61 of these works have focussed on using these species as models for specific mechanistic questions.
62 However, a few studies have attempted to understand muscle activation patterns with the explicit
63 goal of reconstructing the evolutionary diversification of limb motor function (Gatesy and Dial, 1993,
64 1996; Gatesy, 1994, 1999; Rivera and Blob, 2013).

65

66 Better understanding of the relationships between morphology and muscle activity will enable
67 prediction of function for animals (whether extant or extinct) for which no data exist. Such
68 predictions are particularly important for lineages characterized by major changes in functional
69 disparity, such as the Archosauria (“ruling reptiles”; Crocodylia, birds/Aves, and all descendants of
70 their most recent common ancestor). For such clades there is a need to better predict function from
71 form. Archosauria is a clade that diversified first during Triassic period ~250 Mya, evolving a wide
72 variety of forms including small- and large-bodied, sprawling/erect-limbed, quadrupedal/bipedal,
73 aquatic/amphibious/terrestrial and flightless/flying. EMG data from extant archosaurs, particularly
74 the timings of activation during the stride cycle, have been used to infer locomotor changes across
75 Archosauria as a whole (Gatesy, 1994, 1999; Gatesy and Dial, 1996; Hutchinson and Gatesy, 2000).
76 The taxonomic sampling of these EMG data remains somewhat sparse, prompting the question of
77 whether variation in EMG patterns within extant Crocodylia or Aves might alter inferences of
78 neuromotor evolution in Archosauria.

79

80 Available hindlimb EMG data for birds are largely restricted to guineafowl (e.g. Gatesy, 1999; Marsh
81 et al., 2004; Higham et al., 2008; Daley et al., 2009; Ellerby and Marsh, 2010; Carr et al., 2011; Daley
82 and Biewener, 2011; Gordon et al., 2015) and domestic chickens (e.g. Jacobson and Hollyday, 1982;
83 Bradley and Bekoff, 1992), although some data exist for wild turkeys (Roberts and Gabaldón, 2008),
84 mallard ducks (*Anas platyrhynchos*) (Biewener and Corning, 2001) and pigeons (Gatesy and Dial,
85 1993, 1996). These data have revealed some consistent patterns of muscle activity such as co-
86 activation of muscle pairs (e.g. M. flexor cruris lateralis pars pelvica (hip extension and knee flexion)
87 and M. gastrocnemius pars lateralis (knee flexion and ankle extension) (Ellerby and Marsh, 2010).
88 Almost all avian taxa for which hindlimb EMG data currently exist belong to the clade Galliformes
89 (except mallard ducks – Anseriformes; and pigeons – Columbiformes), a useful model system
90 because they are relatively terrestrial and athletic compared to many other species belonging to the
91 speciose avian clade Neognathae. Hence, almost all present understanding of neuromuscular control
92 of hindlimb function in crown-clade Aves is based upon the assumption that Galliformes represents
93 the typical pattern for all or most birds. This assumption seems reasonable, but it deserves further
94 testing with more data from some of the 9000+ species of extant Aves.

95

96 Importantly, there have been no hindlimb EMG studies of the sister group of Neognathae, the
97 Palaeognathae. The palaeognaths include highly specialized, terrestrial, long-limbed (cursorial) forms
98 such as ostriches, emus, rheas, cassowaries and kiwis, but also the tinamous. Tinamous are of
99 particular interest because they are more similar to “ancestral avian” morphology compared to
100 other paleognaths, with small body size and retained flight capability, and are perhaps even more
101 plesiomorphic in locomotor function than many galliforms (Yonezawa et al., 2017). Prior studies
102 have simulated hindlimb muscle activities in larger palaeognaths (emus (Goetz et al., 2008) and
103 ostriches (Rankin et al., 2016)), which would benefit from further data for experimental validation.

104 Here, we present hindlimb EMG data during walking and running from two palaeognath species
105 (elegant-crested tinamous - *Eudromia elegans*, and emus - *Dromaius novaehollandiae*), and four
106 galliform species (helmeted guineafowl - *Numida meleagris*, wild American turkey - *Meleagris*
107 *gallopavo*, common pheasant – *Phasianus colchicus* and bobwhite quail - *Colinus virginianus*). We
108 aim to test whether morphologically conserved hindlimb muscles function similarly across a broad
109 range of Aves indicated by activation during the same phases of the stride cycles at similar speeds.

110

111 Birds span the range of ontogenetic strategies from altricial to precocial, with precocial birds
112 appearing to be miniatures of their adult form. In chickens (precocial), the neural pathways that
113 drive locomotion appear to arise before hatching, enabling them to walk within hours of hatching
114 (Bekoff, 1976; Bekoff et al., 1987; Bradley et al., 2014). There may also be developmental changes in
115 neural control and muscle activity within birds as they grow (e.g. Tobalske et al., 2017), similar to
116 that seen in certain turtle species (Blob et al., 2008). We measured ontogenetic variation of post-
117 hatching neuromuscular control within emus from young birds (< 4kg) to adults (>30kg), for
118 comparison with existing data on ontogenetic scaling of limb muscles (Lamas et al., 2014) and
119 ontogenetic changes of EMG patterns in chickens post-hatching (Bekoff, 1976; Bekoff et al., 1987;
120 Bradley et al., 2014).

121

122 Finally, we broaden our study's perspective to cover extant Archosauria by including novel EMG data
123 from Nile crocodiles (*Crocodylus niloticus*). Similarly to birds, all knowledge of appendicular
124 neuromuscular control in Crocodylia is based on the less diverse subclade Alligatoroidea (Gatesy,
125 1991, 1994, 1997; Reilly et al., 2005); almost exclusively *Alligator mississippiensis*. By adding EMG
126 measurements from a representative taxon within the more diverse clade Crocodyloidea, we will
127 test if similar EMG patterns hold for Crocodylia as a whole, or even more broadly within Archosauria,
128 Sauria or Tetrapoda (*vide* Gatesy, 1994, 1999). Furthermore, work on birds (domestic chickens)

129 comparing overground and treadmill locomotion show minor differences in EMG patterns (Jacobson
130 and Hollyday, 1982), so we test whether this is the case in crocodylians too.

131

132 Overall, we aim to use this extensive dataset on muscle activity patterns to revisit the questions
133 raised by Gatesy (1994, 1999) about how much diversity exists in the neuromuscular control of
134 locomotion among archosaurs. This comparative dataset will have intrinsic value in applications to
135 other archosaurs; both extant and extinct (e.g. Hutchinson and Gatesy, 2000; Rankin et al., 2016).

136

137 **Methods**

138 All species, numbers of individuals used, ontogenetic stage, sexes and body masses are listed in
139 Table 1.

140 Ethics

141 EMG data collection with Nile crocodiles and Elegant-crested tinamous, and the guineafowl and
142 pheasant procedures, were conducted at the RVC Structure and Motion Laboratory under two
143 different project licences approved by the college's Ethics and Welfare committee and granted by
144 the Home Office (United Kingdom). Bobwhite quail and wild turkey data were collected at the
145 Concord Field Station of Harvard University, following procedures licensed and approved by the
146 Harvard Institutional Animal Care and Use Committee in accordance with the guidelines of the
147 National Institutes of Health and the regulations of the United States Department of Agriculture.

148 Surgical procedures

149 For all species bipolar EMG electrodes were constructed of two strands of 0.004 inch diameter
150 platinum pure TC grade (100896) insulated by heavy poly-nylon (HPN) (California Fine Wire
151 Company, CA, USA) soldered to a connector. The free ends of the electrodes had a staggered 1mm

152 exposed wire region spaced 1.5mm apart. The electrodes were implanted under surgical anaesthesia
153 appropriate for that species (see details below). Surgeries involved: 1) making skin incisions over the
154 locations of electrode placement, 2) intramuscular implantation of fine-wire bipolar electrodes, 3)
155 subcutaneous tunnelling of electrodes to a connector on the dorsum or proximal hindlimb, 4)
156 closure of incisions and 5) peri- and post-operative administration of analgesia. The recorded
157 muscles (and their in-text abbreviations) are in Tables 2 and 3.

158

159 *Emu*

160 Six emus were anaesthetised either using mask inhalation of 5% isoflurane for the chicks, or using
161 intramuscular injections of xylazine (3mg/kg) and ketamine (15mg/kg) to the left caudolateral shank
162 muscles for the juveniles and adults. After inductions, the birds were intubated with an endotracheal
163 tube and maintained at an adequate surgical anaesthetic plane with a variable concentration of
164 inhaled isoflurane. Breathing, heart rate and body temperature were monitored throughout surgery.
165 The feathers in the surgical field were clipped and incisions were made for electrode implantation.
166 The EMG electrodes were successfully implanted into M. Iliotrochantericus caudalis (ITC), M.
167 iliotibialis lateralis pars postacetabularis (ILPO), M. iliofibularis (ILFB) and M. gastrocnemialis pars
168 lateralis (GL) (Figure 1). All wires exited via a skin incision caudal to the femoral trochanteric crest of
169 the right pelvic limb. After surgery, animals were rested in their habitual pen and administered non-
170 steroidal anti-inflammatories (meloxicam 1.5mg/kg, three times a day) until data collection was
171 completed. Birds were assessed for discomfort before and throughout data collection; which started
172 24 hours post-surgery; studies were postponed or interrupted if the animals appeared distressed or
173 lame.

174

175 *Other birds*

176 The guineafowl, pheasant, quail and turkey all underwent surgical procedures that have been
177 described previously (Daley and Biewener, 2003, 2011; Daley et al., 2009), with the birds
178 anaesthetised using isoflurane delivered via a mask. The tinamous followed a similar method (see
179 supplementary information for a more detailed protocol), although general anaesthesia was induced
180 using intramuscular injection of 0.075 mg/kg Ketamine (Ketamidor, Chanelle UK) and 22mg/kg
181 medetomidine (Sedastart, Animalcare UK) into the right pectoral muscle, and maintained using
182 inhaled sevoflurane using a non-cuffed endotracheal tube throughout the remainder of the
183 procedure. The surgical field was plucked of feathers and sterilised, and incisions were made over
184 the target muscles. The EMG electrodes were then implanted into the target muscles, while
185 connected to a micro-connector placed on the bird's back. The electrode leads were passed
186 subcutaneously from a 1-2cm incision over the synsacrum to the larger primary incision (4-5cm) over
187 the right lateral shank. Bipolar electrodes were constructed of 0.1mm diameter silver fine-wire
188 (California Fine Wire, Inc., Grover Beach, USA) with 0.5-1.0mm bared tips, and 5-8mm spacing.
189 Electrodes were emplaced using a 23 gauge hypodermic needle, and secured to the muscle using 5-0
190 silk suture; then skin incisions were closed using 3-0 silk. The birds were given analgesia every 12
191 hours and antibiotics every 24 hours. Experimental recordings took place over the next 1–3 days for
192 most birds, but the tinamous were given six days to recover due to their potential sensitivity to
193 stress (pers. obs.) perhaps due to their relatively small hearts (Altimiras et al., 2017).

194

195 For all birds, the *M. gastrocnemius pars lateralis* (GL) was successfully implanted. Additionally, the
196 *M. flexor perforatus digiti IV* (DFIV) was implanted in both the guineafowl and turkey. Some birds
197 also had implantations into uniquely measured muscles: in the turkey *M. flexor cruris lateralis pars*
198 *pelvica* (FCLP), in the guineafowl *M. femorotibialis lateralis* (FMTL) and in tinamous *M. fibularis*
199 *longus* (FL). The basic anatomical positions of all of these muscles are shown in Figure 1, and their
200 approximate actions listed in Table 2.

201

202 *Nile Crocodiles*

203 The anaesthetic procedure is covered in detail in Monticelli et al., (2019), but is briefly outlined here.

204 General anaesthesia was induced using a combination of medetomidine (Sedastart, Animalcare Ltd.,

205 York, UK; 0.2 mg kg⁻¹) and ketamine (Ketamidor, Chanelle, Berkshire, UK; 10 mg kg⁻¹) intramuscularly

206 in the left triceps brachii muscle. After anaesthetic induction, the crocodiles were intubated using an

207 uncuffed endotracheal tube and anaesthesia was maintained using sevoflurane (SevoFlo, Zoetis,

208 Belgium) in oxygen. Intramuscular meloxicam (Metacam, Boehringer Ingelheim, DE; 0.2 mg/kg) was

209 administered in the perioperative period. Active warming was provided by either HotDog®

210 (Augustine Surgical, Eden Prairie, MN, USA) or Bair Hugger® (3M, Maplewood, MN, USA) systems.

211

212 Five incisions, 1-2cm long, were made over the right ilium, proximolateral aspect of the tail, cranial

213 thigh, and caudal and cranial aspects of the lateral shank to enable visualisation and intramuscular

214 implantation for the four hindlimb implants. A further six incisions were made to access four

215 forelimb muscles, with incisions at the scapula, cranial and caudal aspects of the upper arm, medial

216 aspect of the lower arm, lateral aspect of the thorax, and ventral aspect of the thorax. Through these

217 incisions, the muscles were implanted. After post-mortem, the muscles from which data were

218 collected were confirmed to be *M. transversus perinei* (TP), *M. iliotibialis 2* (IT2), *M. gastrocnemius*219 *externus* (GE), *M. flexor digitorum longus* (FDL) of the hindlimb and *M. pectoralis* (PEC) of the

220 forelimb (Figure 1, Table 3).

221

222 The EMG electrode connector was anchored by suturing to two scutes near the dorsal-most incision

223 (iliac or scapular). Each pair of electrode wires was then subcutaneously tunnelled to their respective

224 insertion sites. Tunnelling was achieved subcutaneously using a section of size 3 (internal diameter)

225 uncuffed PVC endotracheal tubing and a looped guide wire. The electrodes were implanted using
226 the sew-through method and secured with two simple-interrupted sutures using 3-0 vicryl to
227 prevent both translation and rotation of the wires post-surgery. The excess wiring was pulled back
228 through to the dorsal incisions where it was coiled and tucked back into the incision site. Each
229 incision was then flushed with lidocaine and then closed using everted mattress stitches to prevent
230 wound contamination in the water within the enclosures. The anaesthesia was discontinued and
231 atipamezole (1 mg kg^{-1}) (Sedastop, Animalcare, UK) was administered intramuscularly in the left M.
232 triceps brachii, and repeated after 30 minutes in case of residual sedation. The crocodiles were then
233 given at least two days to recover in their enclosures before any data were collected

234

235 Experimental protocol

236 The tinamous were placed on a Starkerhund treadmill (Terraglione di Vigodarzere, Italy) within a box
237 with transparent acrylic sides to allow visualisation of the footfalls, and which had an opening for the
238 EMG wires. Trials ranged from 30s to 60s, with at least a 60s break between trials. The treadmill
239 speed varied from 0.1 ms^{-1} to 0.45 ms^{-1} ; faster speeds were not safely achievable with the birds. The
240 trials were initiated using a trigger system that created a short light flash that could be seen by the
241 two Hero 3+ GoPro cameras (San Mateo, CA, USA) recording at 60Hz which were used to capture the
242 footfall patterns of the animals during locomotion. Trials were maximally 60s long, although usually
243 far shorter, with at least 60s recovery between trials. The birds were in the experimental area for a
244 maximum of 1hr before being returned to their enclosure. A total of 64 trials were collected for the
245 two individuals, with the resulting data summarised in Table 5.

246

247

248 Emu experimental trials were conducted overground in a corridor of ~90cm width enclosed by wire
249 netting for the younger individuals, and metal fencing for the adults. Due to the wired EMG
250 implants, cable length limited the maximum length of the runway. Cable length was ~5m for the
251 youngest birds and 9m for the two older groups. All wires were tethered along a sliding pulley
252 system (suspended >1m off the ground) which kept the implant cables from dragging on the floor
253 and interfering with gait. The floor of the runway was instrumented with eight Kistler forceplates
254 (0.6x0.9m; model 9287B, Hook, Hampshire, UK), which were used to obtain timings of footfalls. The
255 emus were also marked with polystyrene hemispheres covered with infrared-reflective tape
256 (Scotchlite 8850; 3M, Manchester, UK) (1cm diameter for the youngest, 2cm diameter for the older
257 individuals) for joint motion analysis for another study (Lamas, 2015), which included two dorsal
258 midline body markers used here for obtaining locomotor velocities via a Qualisys Oqus 500 six-
259 camera system recording at 250Hz (Qualisys AB, Göteborg, Sweden). Across the six individuals, 405
260 trials were completed, and the resulting trials are listed in Table 6.

261

262 The turkey (2 individuals, 5 trials), quail (2 individuals, 6 trials), guineafowl (2 individuals, 6 trials),
263 and pheasant (1 individual, 1 trial) all ran on a custom-built treadmill, with a slatted black rubber-
264 coated steel belt with a 55.8x172.7 cm running surface. The treadmill speeds were selected to
265 achieve an approximately similar dimensionless speed (see below) of 1.25 across species (Table 5).
266 Dimensionless speed is the square root of the Froude number (Alexander and Jayes, 1983):

267
$$u = \frac{v}{\sqrt{(g \cdot l)}}$$

268 where u is dimensionless speed, v is velocity (ms^{-1}), g is acceleration due to gravity (9.81ms^{-2}) and l
269 is standing (or mid-stance) hip height (in metres). Dimensionless speed usually assumes geometric
270 similarity (Alexander and Jayes, 1983); however, dimensionless speed holds reasonably well across
271 animals that use similar locomotor modes even if not strictly geometrically similar (see Daley and

272 Birn-Jeffery, 2018) and references therein for a thorough review). The turkey, quail and quineafowl
273 were recorded using a Photron camera (Photron Europe Ltd., West Wycombe, UK) at 125Hz, whilst
274 the pheasant was recorded using Qualisys cameras (as per the emus above) at 125Hz.

275

276 The crocodiles were captured from their enclosures and their mouths were taped to prevent injury
277 to themselves or handlers, or damaging their EMG wires. The crocodiles were then either placed on
278 a Starkerhund treadmill (within an acrylic-sided enclosure to prevent the animals escaping), or on a
279 custom-made wooden runway (0.38x0.40x2.44m). Both the treadmill enclosure and custom wooden
280 runway had openings in the roof to allow the wires to exit to be connected to the EMG amplifiers.

281 The crocodiles were motivated to walk by stimulating the tail with a broom as needed. The hardware
282 otherwise was the same as that used with the tinamou. Trials were maximally 60s long, although
283 usually far shorter, with at least 60s recovery between trials. Across four individuals, a total of 160
284 trials were collected, with details of collected data in Table 4.

285

286 EMG recordings

287 Each of the sockets on the animals was connected via lightweight shielded cables to GRASS pre-
288 amplifiers (P511, Natus Neurology Inc., Pleasanton, CA, USA). EMG signals remained at a constant
289 amplification throughout data collection with a low-pass (10Hz for most birds; 30Hz for emus,
290 tinamous and crocodiles) and a high-pass (10kHz) filter. The EMG signals were sampled at 2500Hz
291 (emu) or 5000Hz (all other species). Signals were amplified between 1000 and 10000 times, but this
292 varied between and within data collection sessions and individuals as required to obtain visible
293 signals.

294

295 Data processing

296 Footfall events (foot on and off times) were manually recorded from the videos for the crocodiles,
297 tinamous, guineafowl, quail, turkey and pheasant for each trial. The emu footfall timing pattern was
298 determined by analysing the forceplate data, with foot on and off timings linked to the force traces
299 (recorded at 1000Hz; automatically filtered using a low-pass filter at 100Hz; threshold for foot on/off
300 events = 1 % body weight). Custom scripts in Matlab software (MathWorks, Natick, MA, USA) were
301 used for all post-processing.

302

303 The tinamous and crocodiles on the treadmill moved at three different speeds, the lowest being
304 driven by an electric drill turning the drive wheel, the other two (approximately 0.5, 0.7mph) driven
305 by the treadmill motor. The belt speed was calibrated from a video based on the movement of a
306 mark on the treadmill belt relative to a point at a known distance on the treadmill frame. For the
307 crocodiles on the runway, locomotor speed was measured by tracking the shoulder scutes (which
308 had the least lateral movement relative to direction of movement) across 20cm, using a dorsally
309 placed camera atop the runway. Emu speeds were calculated by tracking the cranial dorsal body
310 marker in 3D space, then extracting the horizontal displacements and dividing by the time elapsed.

311

312 Data were then cut into individual steps based on footfall timings extracted from video or forceplate
313 data from each species, described above. Each EMG sequence was filtered and then rectified. The
314 data were bandpass-filtered with a low-pass cutoff between 50-90 Hz and a 12-order Butterworth
315 filter applied, although the emu data underwent a 0.5 Hz low-pass filter to remove an underlying
316 noise waveform before being filtered as the other data (see Figure 2 for representative filtered EMG
317 signals).

318

319 In species with multiple speeds, dimensionless speed was used for comparisons. As the emu trials
320 spanned the largest range of dimensionless speeds, they were grouped into $0.2u$ bins from $0.3-1.5u$.
321 These bins covered all of the ontogenetic stages of the emu and overlapped with the recorded speed
322 range of other studied bird species except for the tinamou, which never reached a dimensionless
323 speed greater than 0.3 . The crocodiles' speeds were also normalised to dimensionless speed where l
324 is total hindlimb length instead of hip height due to the variety of postures (from sprawling to
325 upright) that they used. For each species, the rectified sequences for each grouping (Table 2,3) were
326 scaled to the same length with "foot-on" being 0 and 100 (equivalent to a full stride). The average
327 and 95% confidence intervals for each of these groupings were then calculated using custom Matlab
328 code. EMG onset was deemed to be where there were peaks beyond 10% of the baseline.

329

330 For one muscle (*M. gastrocnemius lateralis/externus*; GL/GE) there were sufficient data for statistical
331 tests of gross similarity of EMG signals across Aves and Crocodylia. An inter-species cross-correlation
332 analysis was carried out upon the average rectified EMG sequences for comparable speeds of $1.1-$
333 $1.3u$ (or maximum speeds for tinamous and crocodiles), using custom Matlab scripts. Due to the
334 small sample sizes in terms of both numbers of individuals and number of trials, no additional
335 statistics were carried out.

336

337 **Results**

338 **Birds**

339 Emus

340 There were no major differences between the age groups in terms of muscle activation timings
341 within the stride cycles. However, the baby emus may have had a slightly broader range of

342 activations for each muscle group relative to the older individuals. With so few individuals, it is
343 difficult to resolve whether this difference related to individual variance or age (Figure 3).

344

345 *ITC* (Figure 3, S1)

346 The *M. iliopsoas* was active from late swing through to mid-stance. However, at
347 slower relative speeds (below $1.1u$), the late swing and early stance activations were at lower levels,
348 and the peak activation occurred during mid-stance. At the fastest speeds, the activation peaked in
349 early stance.

350

351 *ILPO* (Figure 3, S2)

352 The *M. iliopsoas* had variable activation with speed. At slower speeds
353 ($<0.7u$), the EMG signals occurred at a fairly consistent level of activity from the end of swing
354 through late-middle stance phase. At faster relative speeds ($0.7-0.9u$), the EMG signal peaked
355 during mid-stance, with lower activity around late swing and later stance. At the fastest speeds ($0.9-$
356 1.1 and $1.1-1.3u$), there was a discontinuity between the signal in late swing and the large peak at
357 mid-stance; consistent with two bursts of activity.

358

359 *ILFB* (Figure 3, S3)

360 The *M. iliofibularis* displayed peak activation during early stance, but with activity persisting from
361 late swing to mid-stance.

362

363 *GL* (Figure 3, S4)

364 The M. gastrocnemius pars lateralis of emus, like the ITC and ILFB, was active from late swing
365 through mid-stance phase at most speeds. In the youngest emus, EMG activity extended through
366 stance and into early swing at u from 0.3-0.5. At higher values of u , the initial muscle activations for
367 all ages became increasingly earlier, so activation occurred more consistently during late swing and
368 ended earlier in stance, with higher relative activations and a smaller range as a proportion of total
369 stride time.

370

371 Tinamous (Figure 4, S5)

372 *GL*

373 Overall, the M. gastrocnemius pars lateralis EMG signals were similar across the small range in
374 speeds, with activity beginning in late swing and continuing through early stance phase, with a
375 reduced mid-late stance signal at faster speeds.

376

377 *FL*

378 From the one trial of 7 strides at 0.1ms^{-1} ($0.06u$ - a very slow walk), the M. fibularis longus showed
379 low level EMG activity from foot on through to late-middle stance phase.

380

381 Galliform birds

382 *FCLP*

383 The M. flexor cruris lateralis pars pelvica was only measured in turkeys, and was active through
384 stance phase, with a peak in mid-stance (Figure 5, S6).

385

386 *FMTL*

387 The M. femorotibialis lateralis was only measured in the guineafowl, and was active from late swing
388 through to late stance phase (Figure 6, S7).

389

390 *GL*

391 Across the quail, pheasants, guineafowl and turkey, the lateral gastrocnemius showed a similar
392 overall pattern of activity, with the primary burst of muscle activity occurring from late swing to
393 early mid-stance phase, with peak activity early in stance (Figure 7A-D, S8).

394

395 *DFIV*

396 The M. flexor perforatus digiti IV was measured in turkeys and guineafowl, and showed similar
397 activity from late swing through early stance phase; as in the GL (Figures 5-6, S6-S7).

398

399

400 **Crocodiles** (Figure 8, S9)

401 *PEC*

402 The M. pectoralis for the Nile crocodiles showed low-level activation through mid-stance phase, with
403 maximal activation in late stance. Unlike the TP (below), the pattern was not shifted earlier in the
404 cycle at faster speeds; instead, there was relatively shorter period of activation. For relatively similar
405 speeds, there was no apparent difference between EMG signals for animals on treadmill or runways.

406

407 *TP*

408 The *M. transversus perinei* was active through early to mid-stance phase, with peak activity from 20-
409 50% of the stride cycle. At faster speeds, the TP became active earlier, including late swing phase
410 (Figure 8).

411

412 *IT2*

413 The *M. iliotibialis 2* was active throughout most of the stance phase, with the greatest signals around
414 30% of total stride cycle.

415

416 *GE*

417 At 0.35-0.45ms⁻¹ walking speeds, the *M. gastrocnemius externus* was active during mid-late stance,
418 becoming active into early swing phase at faster speeds.

419

420 Our cross-correlation analysis of mean EMG timings for the GE of Crocodylia and GL of Aves showed
421 that these were most similar for Aves, and distinct for Crocodylia (Table 7). Interestingly, the
422 maximal correlations of timings for Crocodylia were more similar to those of Palaeognathae and
423 quails, whereas guineafowl and turkey were most similar to each other; with pheasant values in
424 between these. However, these similarities in maximal correlations were not so evident in the
425 offsets of EMG timings, which were ~18-27% of a stride earlier in swing (start-signal) and stance
426 (end-signal) for all Aves vs. Crocodylia.

427

428

429 **Discussion**

430 We have presented a compilation of the largest dataset of electromyographic data for archosaurs to
431 date, including the first for emus and tinamous, thus adding palaeognathous birds to the existing
432 literature for birds and Nile crocodiles to the published data for Crocodylia. Below, we consider our
433 avian data first, then crocodylian EMG data, then all data in the broader context of archosaurian
434 neuromuscular evolution.

435

436 Published hindlimb EMG data from birds to date are primarily from guineafowl (*Numida*) and
437 domestic chickens (Jacobson and Hollyday, 1982; Gatesy, 1999; Ellerby and Marsh, 2010), as well as
438 wild turkeys (Roberts and Gabaldón, 2008), although some important data also exist for pigeons
439 (Gatesy and Dial, 1993, 1996). The guineafowl data presented here compare well with previously
440 published guineafowl data, both in terms of patterns and timings for the GL and DFIV muscles;
441 bolstering confidence in our results (Gatesy, 1999; Daley and Biewener, 2003; Gordon et al., 2015).

442

443 Prior to this study, there were no palaeognath EMG data, and the only relevant data were derived
444 from a musculoskeletal simulation studies of emus (Goetz et al., 2008) and ostriches (Rankin et al.,
445 2016), which predicted muscle activations for walking and running. Goetz et al. (2008) only depicted
446 muscle forces rather than activations, and solely for the stance phase—but for 40 muscles or
447 subdivisions thereof. Rankin et al. (2016) reported activation timings (e.g. see their figure 3 for 16
448 muscles) that generally match the additional palaeognath EMG data we present here, with the
449 exception of the ITC(p) which consistently had a secondary activation during swing phase in the
450 simulations. Whilst some small peaks were evident in our ITC data for the adult emus around foot-
451 off at slower speeds, these are likely a result of noise, and no peaks indicative of secondary
452 activations in mid-swing were found for running emus ($u > 1.0$) (Figure 3, S1). This discrepancy
453 between the simulated muscle activations and EMG data was also found when compared to
454 guineafowl data (Daley et al., 2009). Furthermore, the simulated muscle activations may be

455 misleading because these rely on Hill-type muscle models that presume basic relationships between
456 neural excitation, muscle activation and force, whereas in reality these relationships may be more
457 complex (e.g. Askew and Marsh, 1998; Perreault et al., 2003; Millard et al., 2013).

458

459 Did we observe ontogenetic variation within our emu data? Whilst variation in EMG signals through
460 ontogeny has been found *in ovo* (e.g. Watson and Bekoff, 1990), very little research has been done
461 on post-hatching birds (see Jacobson and Hollyday, 1982; Muir et al., 1996) as neural controls
462 appear to establish early within embryos. However, in chukar partridges (*Alectoris chukar*) there is
463 variation between young and adult birds in EMG patterns for pectoralis muscle EMG signals during
464 wing-assisted incline running, with younger birds having longer periods of activation of their muscles
465 relative to the adults (Tobalske et al., 2017). We found a similar pattern of longer activation times in
466 younger emus' leg muscles, although the variation between the different ages was small. These
467 similarities are in contrast to turtles. Juvenile turtles can undergo significant changes in muscle
468 activity patterns through ontogeny, usually decreasing the number of activations for muscles during
469 the stride cycle from two to one but sometimes profoundly changing the timing of muscle activation.
470 For example, across the ontogeny of juveniles to adults the femorotibialis muscle's activation
471 transitions from recovery (swing) to thrust (stance) phase in swimming (Blob et al., 2008).

472

473 The M. gastrocnemius pars lateralis (GL) has the EMG patterns that are most widely studied across
474 avian species, and serves as a useful as a reference muscle because it retains similar anatomy (origin
475 on the lateral distal femoral condyle and insertion on the tarsometatarsus) and locomotor function
476 (primarily ankle extension but also knee flexion) in terrestrial gait across species. Here we found that
477 the GL's EMG activity patterns of the palaeognathous birds were almost exactly the same as those
478 for the neognaths during walk/run behaviours (Figure 7, Table 7). Activity began in late swing phase
479 and continued throughout much of stance, but at faster speeds became increasingly concentrated in

480 early stance phase, in all birds studied to date. We suggest that across cursorial, ground birds as a
481 whole, muscle activation patterns are likely to be conserved for morphologically and functionally
482 similar muscles. More broadly, even GL activations during walking in the more aquatically specialized
483 mallard duck (*Anas platyrhynchos*) are similar to the species reported here (Biewener and Corning,
484 2001). Thus GL muscle activation patterns appear to be generally conserved across Aves and
485 correspond to the expected functional demands inferred from anatomical origins, insertions, joint
486 mobility and moment arms. However, there may be differences in EMG timing of the GL during the
487 stance phase that correlate with differences in limb posture (e.g. Gatesy and Biewener, 1991; Daley
488 and Biewener, 2011; Gordon et al., 2015; Daley and Birn-Jeffery, 2018) and anatomical variations; or
489 perhaps even across regions of the GL.

490

491 As in the previous study of chickens (Jacobson and Hollyday, 1982), regardless of whether the
492 crocodiles were moving within a runway or on a treadmill, their muscle activation patterns were
493 very similar, although the range of speeds assessed was very low, approximately at their natural
494 walking speed (0.1ms^{-1}). Previously published data for Crocodylia derive entirely from the American
495 alligator (*Alligator mississippiensis*) and spectacled caiman (*Caiman crocodilus*) hindlimb (Gatesy,
496 1994, 1997; Reilly and Blob, 2003); in both cases for the Alligatoidea lineage. Here, we provide
497 comparable data for the Crocodyloidea lineage (and the first forelimb PEC muscle EMG for
498 Crocodylia). The M. iliotibialis 2 (IT2) of Nile crocodiles had a relatively larger EMG signal than seen
499 in alligators but it occurred with the same timing around mid-stance (Gatesy, 1997). Whilst no
500 filtered signals are published for the M. gastrocnemius externus (GE), summarized timings (Reilly et
501 al., 2005) match very well with the signals seen in the Nile crocodile data at 0.345ms^{-1} presented
502 here (Table 7), despite being from different duty factors (0.6 here vs. 0.7 in Reilly et al., (2005)).

503

504 The *M. transversus perinei* (TP) overlies the *M. caudofemoralis longus* (CFL) in Crocodylia (Romer,
505 1923; Frey, 1982; Cong, 1998) and has similar EMG signals in terms of patterns and timings (Gatesy,
506 1997). Its activity has not been measured before, to our knowledge, in Reptilia. An explanation for
507 this similar activity may be that the TP was contracting simultaneously with the CFL. The two
508 muscles also have muscle fibres that run perpendicular to each other (TP dorsoventrally, CFL
509 craniocaudally). Perhaps the TP performs some function of locomotor relevance, limiting bulging of
510 the distal CFL belly near where it narrows into its insertion, or acts similarly to the *M. caudofemoralis*
511 *brevis*, which seems to help to change the moment arm of the CFL in lizards (Herrel et al., 2008).
512 However, the TP is very thin and whilst not visibly implanted within the CFL, the electrodes may have
513 been picking up “cross-talk” signals from this much larger muscle. Regardless, these novel data from
514 Nile crocodiles have intrinsic value for understanding function of the CFL and dynamics of the tail-to-
515 thigh region; and our EMG data are unlike those of the homologous *M. caudofemoralis* (*pars*
516 *caudalis*) of birds which is only (variably) active during late stance at fast speeds (Gatesy and Dial,
517 1993; Gatesy, 1999).

518

519 There are no other published forelimb EMG data for Crocodylia; however, some data exist for turtles
520 (Rivera and Blob, 2010), which may form the sister clade to archosaurs (Hedges and Poling, 1999;
521 Field et al., 2014 but see Gauthier et al., 1988; Lyson et al., 2012), and the perhaps more distantly
522 related Savannah monitor lizard (*Varanus exanthematicus*) (Jenkins and Goslow, 1983). The *M.*
523 *pectoralis* data from the literature are variable, with turtles showing activity from early to late stance
524 phase (Rivera and Blob, 2010, 2013). In contrast, in the monitor lizard, cranial portions of the PEC
525 were active predominantly in swing phase, whilst the middle of the PEC was active at low levels
526 during stance phase. In our Nile crocodile subject, the electrode was inserted into the cranial (i.e.
527 major sternal) portion of the PEC, but had activity through mid-to-late stance. The differences
528 between the species might relate to differences in the role of the pectoralis in resisting

529 glenohumeral abduction imposed by ground reaction forces, whether due to the “high-walking”
530 postures in crocodiles or the added mass of the shell in turtles.

531

532 What, then, do our EMG data indicate about the evolution of muscle activity in the clade
533 Archosauria? There are scarce overlapping and ideally comparable data, major differences in
534 locomotor biomechanics and some issues with muscle homology (Rowe, 1986; Gatesy, 1994) that
535 are cause for caution. On the other hand, there are clearly corresponding patterns of muscle activity
536 that also match similarities in limb dynamics and/or muscle anatomy for Archosauria (and even
537 other tetrapods) (Figure 9). For example, the large, ventral M. pectoralis would be expected to be an
538 antigravity muscle from the anatomy and indeed both Crocodylia (represented by our new data for
539 *Crocodylus niloticus*) and Aves activate M. pectoralis during their major antigravity functions (i.e.
540 stance phase for the former; downstroke of flight for the latter) (Gatesy and Dial, 1993, 1996;
541 Goslow et al., 2000) as expected for this large, ventrally disposed muscle. This antigravity activity is
542 broadly shared with turtles and Squamata (*Varanus exanthematicus*: caudal PEC region), consistent
543 with some “neuromotor conservation” at least across the broader amniote clade Sauria (Jenkins and
544 Goslow, 1983; Schoenfuss et al., 2010; Rivera et al., 2011; Rivera and Blob, 2013). This apparent
545 conserved activity would support the inference that quadrupedal archosaurs used their PEC muscles
546 to support themselves during locomotion, much as their M. adductor femoris muscles countered
547 abduction of the hindlimbs (Hutchinson and Gatesy, 2000). However, the swing phase activity of the
548 cranial region of PEC in *Varanus* is cause for caution, as it does not follow the expectation of a
549 ventrally located muscle acting simply as an anti-gravity adductor, and does not match the activity of
550 the comparable region in *Crocodylus*.

551

552 Our hindlimb EMG data for Crocodylia and Aves indicate broadly similar stance phase activity for
553 GE/GL in both groups of extant archosaurs; although unsurprising, this is nonetheless consistent

554 with a conserved antigravity function that would be expected possibly throughout Tetrapoda (e.g.
555 turtles (Schoenfuss et al., 2010), lizards (Reilly 1995, 1998), humans (Dietz et al., 1979), felids
556 (Rasmussen et al., 1978), and salamanders (FPC = medial gastrocnemius)(Ashley-Ross, 1995)). This
557 apparent conservatism also seems to apply to the digital flexors (FDL in the crocodile and DFIV in the
558 guineafowl; the latter homologous to a part of the former (Hutchinson, 2002)), which are also ankle
559 extensors (plantarflexors) with antigravity functions and thus show timings similar to those seen in
560 the GE/GL. Similarly, the IT2/ILPO (here represented by new data for *Crocodylus* and *Dromaius*) are
561 homologous muscles for Archosauria (Hutchinson, 2001) and exhibit stance phase activity (earlier in
562 stance/late in swing) in this study and related literature cited above, although our emus had an extra
563 potential burst that may be apomorphic. These data are most parsimoniously interpreted as
564 homologous muscle activity that was ancestral for Archosauria.

565

566 There was one potentially interesting difference in GE/GL timing we observed for Crocodylia vs.
567 Aves: the homologous GE/GL muscles are active only in stance phase in Crocodylia, whereas activity
568 starts in late swing phase in Aves (Table 7, Figure 7). Considering the grossly similar anatomy of
569 these muscles in extant archosaurs, published differences in knee and ankle joint kinematics suggest
570 one possible explanation: that the earlier onset of EMG activity in the avian GL is related to
571 maintaining synchronized knee flexion and ankle dorsiflexion across the swing-stance transition (e.g.
572 (Higham and Nelson, 2008)), unlike in Crocodylia where knee extension and ankle plantarflexion
573 occur from late swing to early stance phase (Gatesy, 1991, 1997; Reilly et al., 2005). This is simply
574 one reasonable speculation that deserves testing against alternatives such as plantigrade foot
575 posture in future studies, but we are unaware of it being previously proffered as an explanation.

576

577 Other EMG data for archosaurian hindlimb muscles are not feasible to compare directly within our
578 dataset. The CFL muscle surely maintained stance phase activity in early archosaurs (Gatesy, 1990).

579 Here, the overlying TP muscle of *Crocodylus* was sampled rather than the underlying CFL muscle, but
580 the TP has barely been studied in the clade Sauria and deserves further analysis in the context of
581 limb function. The ITC muscle of Aves is homologous with M. iliofemoralis of Crocodylia (Rowe,
582 1986). Our palaeognath EMG data strengthen the hypothesis that there was a switch from swing to
583 stance phase activity of the ITC within the clade Dinosauromorpha, perhaps concurrent with the
584 origin of bipedalism and increased need for hip abductor-based support (rather than adductors)
585 during stance phase (Hutchinson and Gatesy, 2000). Likewise, the late swing and stance phase
586 activity of the ILFB for emus and other birds, vs. mainly swing phase activity in *Alligator* (Gatesy,
587 1997; Reilly et al., 2005) and *Varanus* (Jayne et al., 1990), support the conclusion that this muscle
588 added a prominent stance phase burst at some point after the divergence of the
589 dinosauromorph/avian lineage from Archosauria, albeit apparently maintaining a swing phase burst
590 in most birds (Gatesy, 1999). Stance phase activity of the FL in our one tinamou subject and trial,
591 with other avian data (Gatesy, 1994, 1999), support the inference that this activity is ancestral for
592 Aves. Then, considering similar activity of the FL in the lizard *Sceloporus* (Reilly, 1995), perhaps the FL
593 has conserved stance phase (and perhaps late swing phase) activity across at least Sauria (like the
594 GE/GL) (Reilly, 1998); although EMG data for the FL are lacking in Crocodylia.

595

596 While our data, and synthesis of data from the literature, suggest “conservatism” in muscle
597 activation patterns across Archosauria, Sauria or even more broadly, an explanation for such
598 patterns in terrestrial locomotion remains lacking. The vertebrate sensorimotor control system is
599 plastic and adaptable to allow versatile function; yet retains similar elements of spinal central
600 pattern generators and segmentally arranged reflexes across vertebrates (Grillner and Wallén,
601 1985). Hardwired neural circuitry does not require conserved activation patterns if that circuitry
602 itself enables adaptive feedback (e.g. central pattern generators are well known to be
603 entrained/regulated by sensory feedback and descending control). However, our data here, and

604 from other recent studies; e.g. turtles (Rivera et al., 2011; Rivera and Blob, 2013); suggest that many
605 patterns of activation broadly are conserved. One potential reason is that the shared functional
606 demands of terrestrial locomotion across all animals (e.g. supporting body weight), combined with
607 shared substrates of actuation and control (muscle and nervous system intrinsic physical properties),
608 mean that most terrestrial animals use similar mechanisms for economic movement, which is
609 reflected in conserved function in muscles that have grossly similar morphology. However, other
610 perspectives have cited reasons to be wary (Smith, 1994; Alfaro and Herrel, 2001).

611

612 Yet regardless of the cause(s) of a lack of change of motor patterns, such homology is valuable to
613 evolutionary biomechanists. Here, we have added to other perspectives on the evolution of
614 appendicular muscle control in archosaurs (Gatesy, 1990, 1999; Gatesy and Dial, 1996; Hutchinson
615 and Gatesy, 2000; Reilly et al., 2005) by showing how a forelimb muscle (PEC) and several hindlimb
616 muscles (IT2/ILPO, ILFB, GE/GL, FDL/DFIV, potentially FL) have maintained similar motor patterns in
617 extant Archosauria, although the avian GL has modified its timing (Figure 9). These similarities were
618 somewhat expected from relatively conservative muscle morphology, and supported prior studies
619 (Gatesy, 1994, 1999), yet the GL timing difference might be less expected. This overall similarity of
620 muscle activations bolsters their usage in validating computer simulations, or otherwise inferring
621 locomotor function, for taxa without available EMG data, whether they are extant archosaurs (Goetz
622 et al., 2008; Rankin et al., 2016) or extinct, as long as the muscles are known or can be inferred
623 (Witmer, 1995). However, the evidence for changes of (or additions to) the motor patterns of avian
624 muscles such as the morphologically transformed ITC and more morphologically conservative ILFB is
625 cause for caution (neuromotor conservation demands to be tested, not assumed (Gatesy, 1994,
626 1999; Smith, 1994; Goslow et al., 2000; Rivera et al., 2011; Rivera and Blob, 2013)), and cause for
627 assembling datasets from more varied taxa and behaviours.

628

629 **Contributions**

630 JRH and MAD conceived the study. ARC, VRA, KBM, LPL and MAD all planned and performed
631 surgeries on the animals, assisted by CA, PM and LP. ARC, VRA, KBM, LPL, MAD and JRH all carried
632 out experiments. ARC conducted the data analysis assisted by MAD. ARC wrote the manuscript aided
633 by JRH and MAD. All authors contributed to reviewing the manuscript and approved the final draft.

634

635 **Acknowledgements**

636 Enrico Eberhard, Peter Bishop and Jorn Cheney all provided advice on Matlab code. We thank La
637 Ferme aux Crocodiles (Pierrelatte, France) for provision of the Nile crocodile subjects. We appreciate
638 the support of the Biological Services Unit at RVC for animal care. We thank Russell Main, Emily
639 Sparkes, Sandra Shefelbine and Heather Paxton for help with the experimental data collection for
640 emus, and Jeffery Rankin and James Usherwood for input on that study. Thanks to Alison Tarbell and
641 Sheridan Golding for assistance in quail, turkey and pheasant data collection. This study was
642 supported by funding from The Royal Veterinary College and the European Research Council (ERC)
643 under the European Union's Horizon 2020 research and innovation programme (grant agreement
644 #695517). Finally thanks to the anonymous reviewers whose comments greatly improved the
645 manuscript.

646

647 **Conflict of Interests**

648 None are declared.

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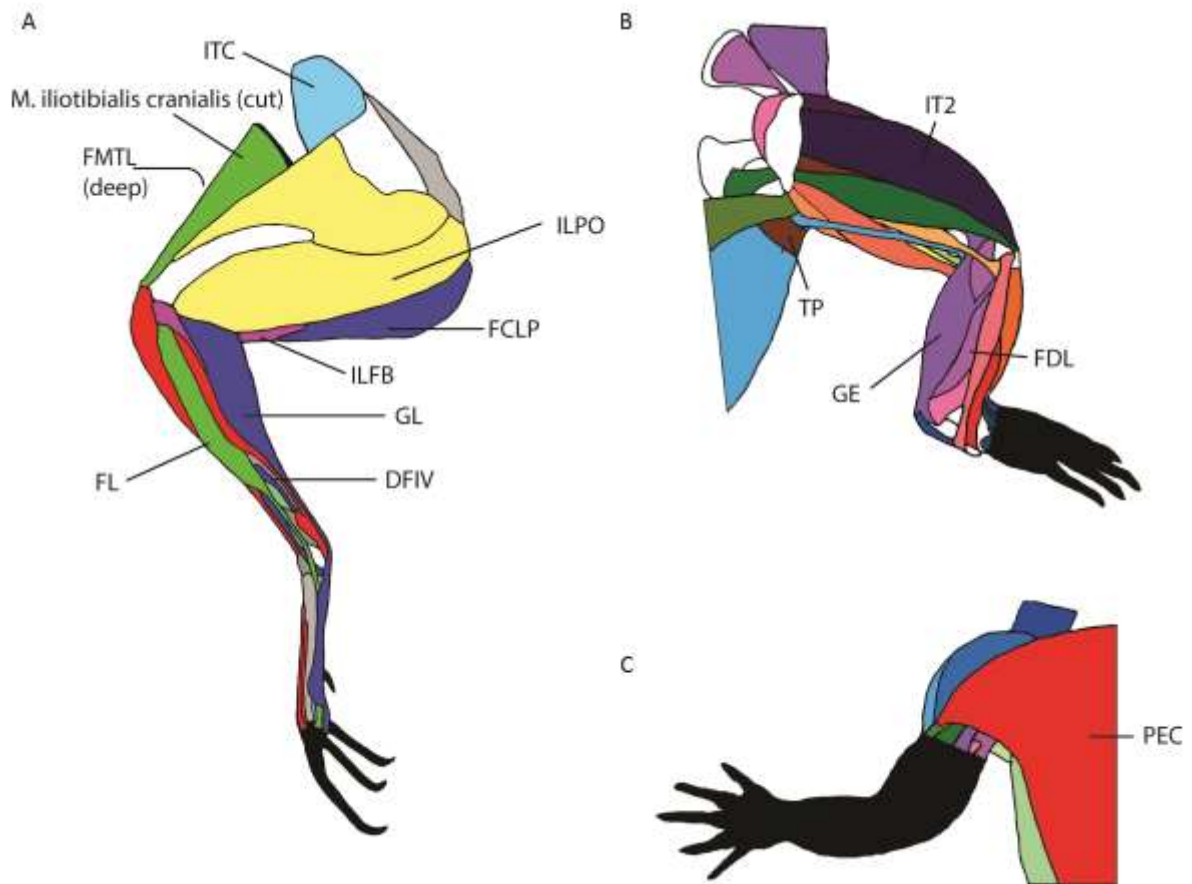
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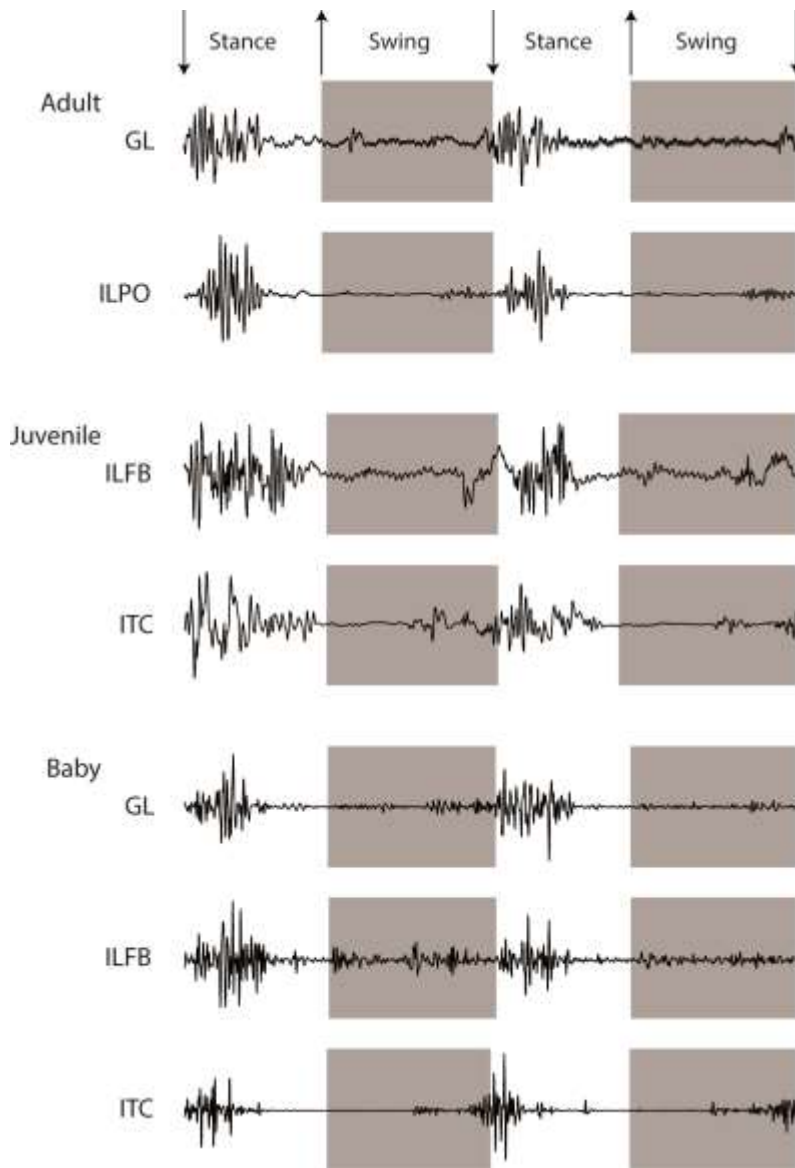
828 Figure 1. Avian and crocodile muscles from which EMG data were obtained . A) Avian hindlimb is
829 from a representative tinamou; figure modified from (Hudson et al., 1972). Muscle abbreviations
830 from Table 2. B) Crocodile hindlimb dorsal view, C) crocodile forelimb ventral view. Both right limbs,
831 modified from Allen et al., (2014). Muscle abbreviations in Table 3.



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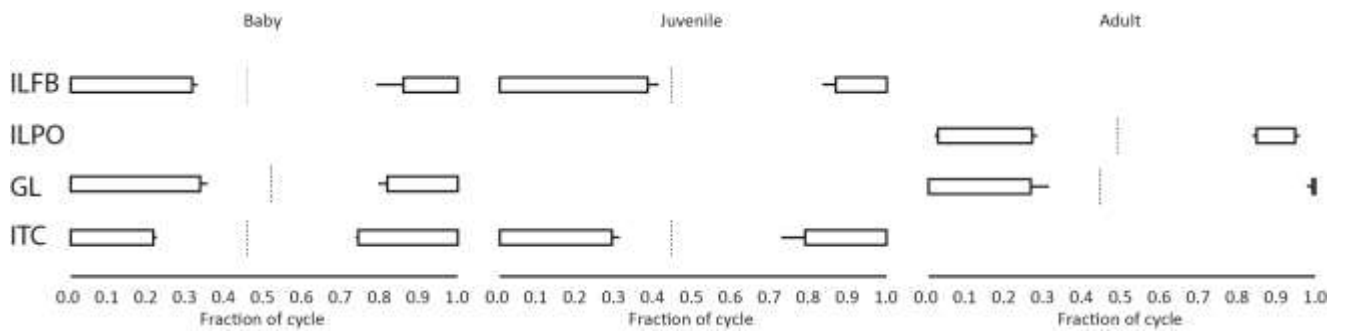
834 Figure 2. Filtered EMG signals from three emus at three ages, showing the signal variation at 1.1-1.3
835 dimensionless speed.



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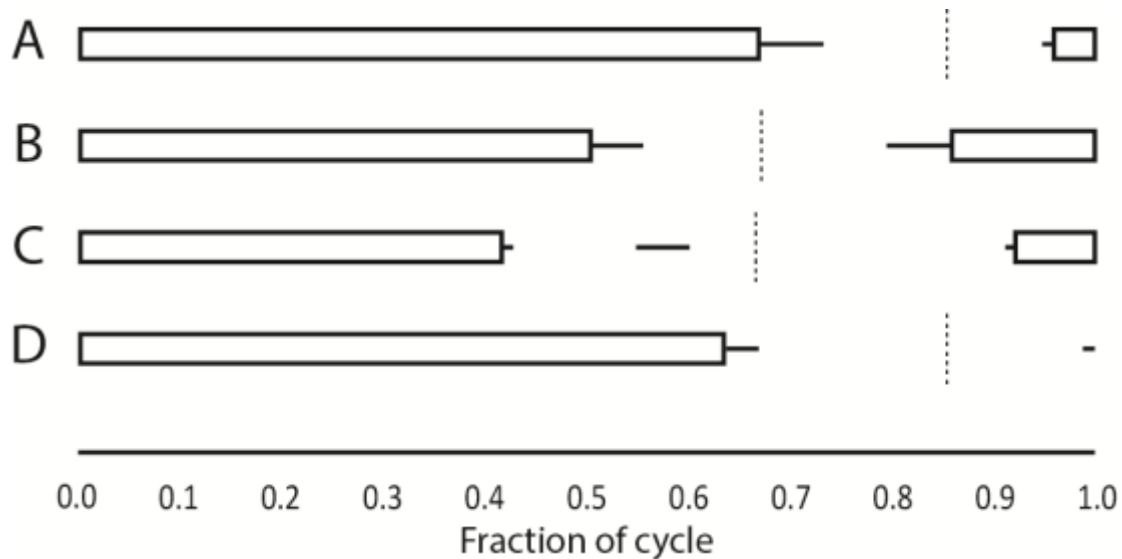
838 Figure 3. Timing of emu muscle activations at 1.1-1.3 dimensionless speed. Bars represent average
 839 periods of activity, with the lines representing 95% confidence intervals. Vertical dashed lines are
 840 foot-off events.



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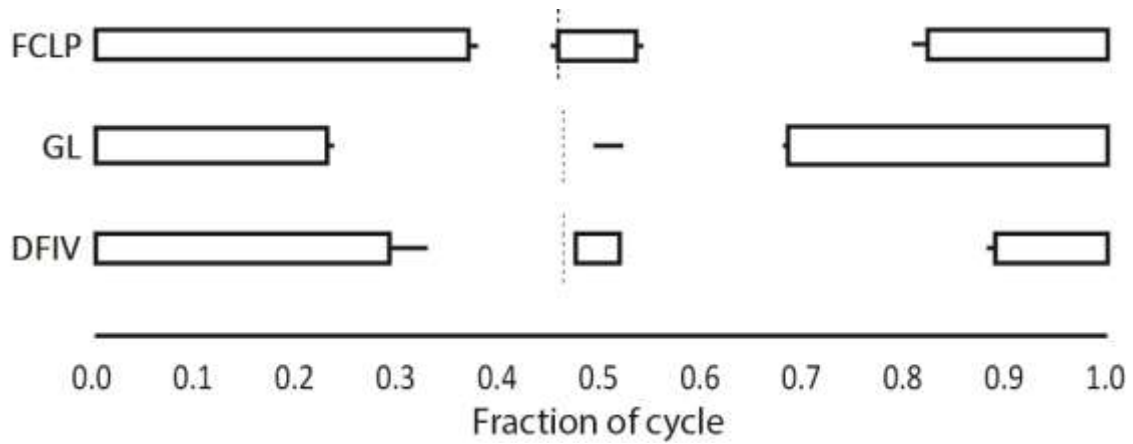
843 Figure 4. Timing of tinamou muscle activations for A-C) GL, and D) FL muscles. A,D) $u = 0.06$, B) $u =$
 844 0.23 , C) $u = 0.29$. Bars represent average period of activity, with the lines representing 95%
 845 confidence intervals. Vertical dashed lines are foot-off events.



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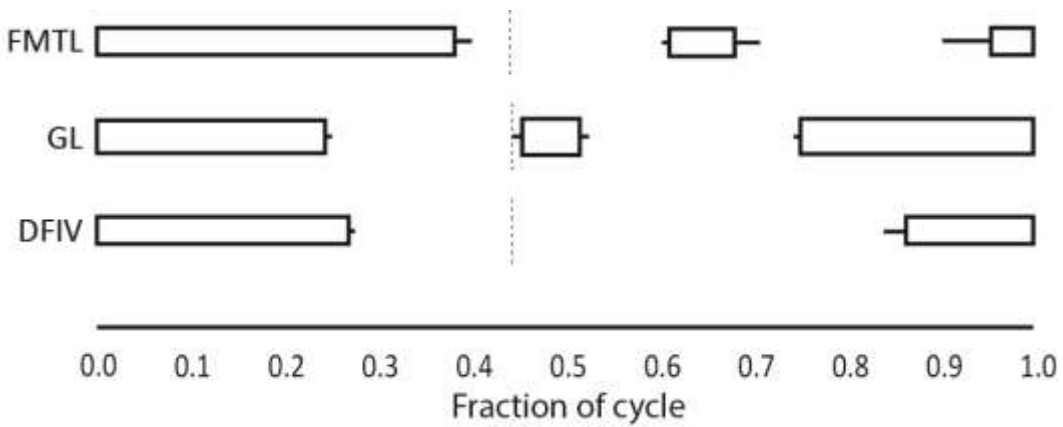
848 Figure 5. Turkey muscle activation timing at 1.2 dimensionless speed for FCLP, GL and DFIV muscles.
849 Bars represent average period of activation, with the lines representing 95% confidence intervals.
850 Vertical dashed lines are foot-off events.



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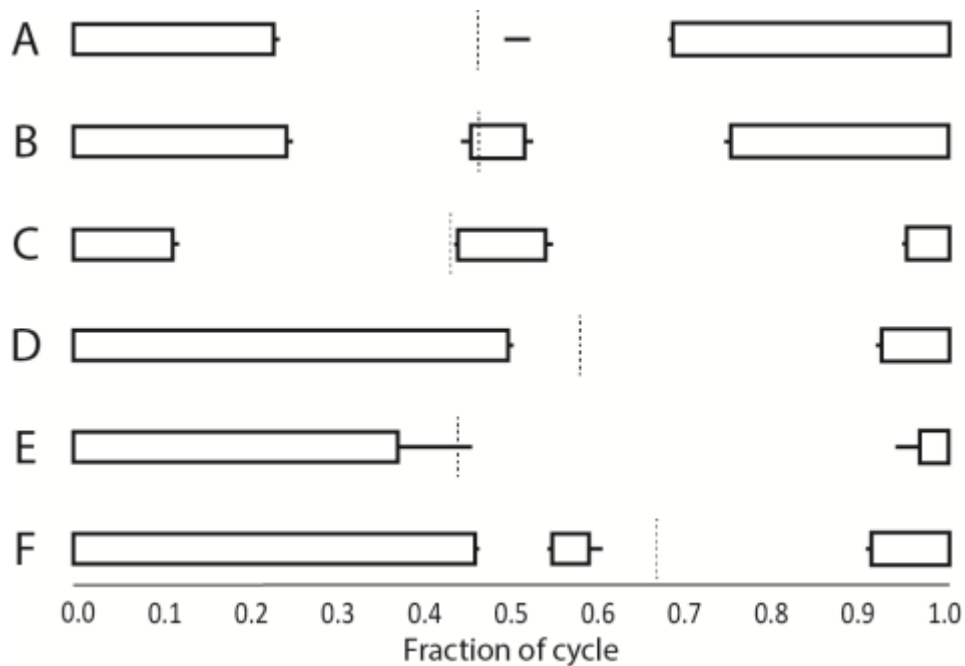
853 Figure 6. Timing of guineafowl muscle activity at 1.2 dimensionless speed for FMTL, GL and DFIV
854 muscles. Bars represent average activations, with the lines representing 95% confidence intervals.
855 Vertical dashed lines are foot-off events.



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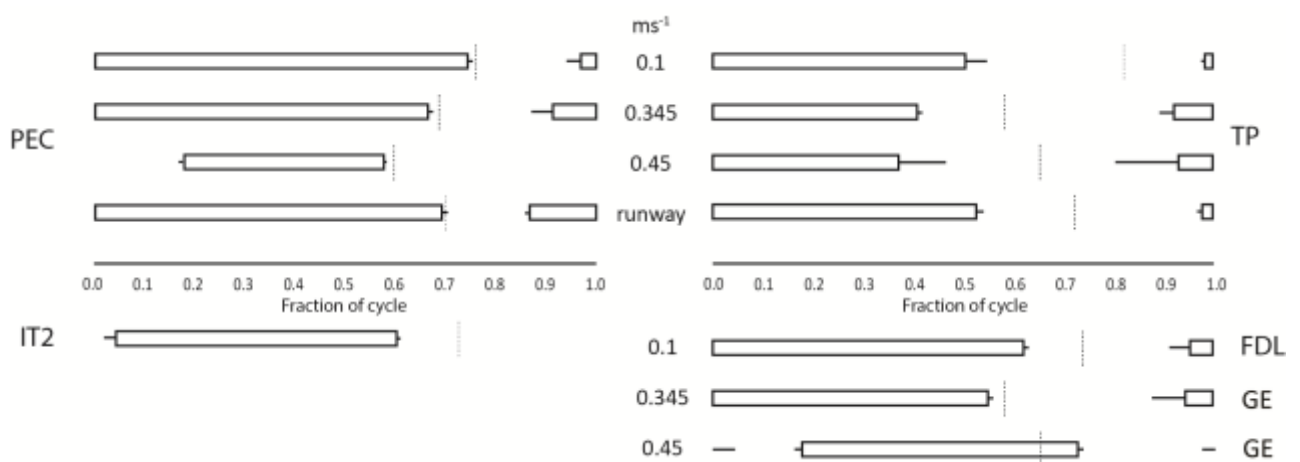
858 Figure 7. Activation timing for *M. gastrocnemius pars lateralis* (GL) across bird species at similar
 859 dimensionless speed (1.1-1.3 u). A) turkey, B) guinea fowl, C) pheasant, D) quail, E) emu, F) tinamou
 860 (0.29 u). Bars represent average period of activation, with the lines representing 95% confidence
 861 intervals. Vertical dashed lines are foot-off events.



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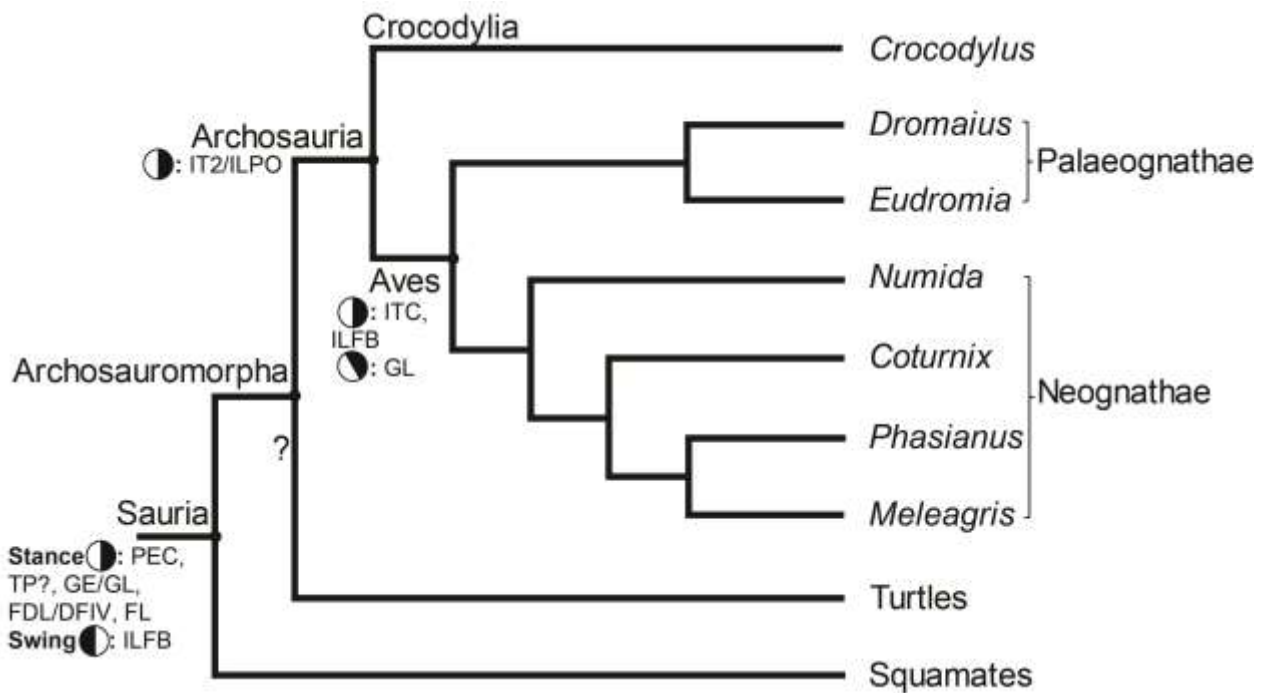
864 Figure 8. Timing of crocodile muscle activity. Bars represent average activations, with the lines
 865 representing 95% confidence intervals. Vertical dashed lines are foot-off events.



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868 Figure 9. Phylogeny of Sauria (based on Gauthier et al., 1988; Hedges and Poling, 1999; Field et al.,
 869 2014; Prum et al., 2015), with the most parsimonious reconstruction of the evolution of EMG
 870 patterns mapped onto it (following Gatesy 1994, 1999). Muscle abbreviations are in Tables 2 and 3.
 871 “?” for turtle stem reflects controversy over their precise phylogenetic position. Nodes for Sauria,
 872 Archosauria and Aves are annotated with key ancestral EMG patterns for muscles focused on in this
 873 study; simplified into “Stance” (circle filled on right half) for mainly stance phase activity (potentially
 874 with some late swing phase), “Swing” (circle filled on left half) for mainly swing phase activity, and a
 875 “Stance” circle rotated 30 degrees anticlockwise for the more pronounced earlier swing phase
 876 activity (and earlier stance phase end of activity) evident in the GL of Aves. Additional EMG data for
 877 ducks (Biewener and Corning, 2001) and pigeons (Gatesy and Dial, 1993, 1996) further bolster the
 878 results here for Aves but for simplicity are not shown.



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Table 1. Species used in the study

| Species | Common name | Ontogenetic stage | Number of individuals | Sex | Mass (kg) |
|---------------------------------|-------------------------|-----------------------------|-----------------------|---------|------------------------------|
| <i>Crocodylus niloticus</i> | Nile crocodile | Juvenile | 4 | F | 1.59-4.63 |
| <i>Dromaius novaehollandiae</i> | Emu | Baby, Juvenile, Adult | 2 2 2 | Unknown | 3.95-4.1, 17-18, 36-37 |
| <i>Eudromia elegans</i> | Elegant-crested tinamou | Adult | 2 | F,M | 0.555-0.616 |
| <i>Numida meleagris</i> | Guineafowl | Adult | 2 | F,M | 1.5, 1.7 |
| <i>Phasianus colchicus</i> | Common pheasant | Adult | 2 | M | 1.0, 1.1 |
| <i>Coturnix virginianus</i> | Bobwhite quail | Adult | 2 | F | 0.15, 0.17 |
| <i>Meleagris gallopavo</i> | Wild turkey | Adult | 2 | F | 5.2, 6.0 |

Table 2. Bird muscles that EMG data were obtained from, with previously reported anatomy and function. Nomenclature follows Vanden Berge and Zweers (1993) for Aves. “Actions” (presumed potential functions around joints crossed) are inferred from anatomy following Vanden Berge and Zweers (1993) and Lamas et al. (2014).

| Muscle | Origin | Insertion | Action |
|---|--|---|--|
| Iliotrochantericus caudalis (ITC) | Preacetabular ilium | Trochanteric crest of proximal femur | Hip flexor, abductor and internal rotator |
| Iliotibialis lateralis pars postacetabularis (ILPO) | Dorsal postacetabular ilium | Patella and cranial tibial crest of tibiotarsus | Hip extensor, abductor and external rotator; knee extensor |
| Iliofibularis (ILFB) | Postacetabular ilium | Fibula (M. iliofibularis tubercle) | Hip extensor, abductor and external rotator; knee flexor |
| Flexor cruris lateralis pars pelvica (FCLP) | Posterior rim of terminal iliac process | GE/GL and flexor cruris medialis tendon | Hip extensor, abductor and external rotator; knee flexor |
| Femorotibialis lateralis (FMTL) | Lateral surface of femur | Cranio-lateral surface of proximal tibiotarsus | Knee extensor |
| Gastrocnemius pars lateralis (GL) | Caudal side of lateral condyle of femur | Caudal surface of proximal (tarso)metatarsus (i.e. hypotarsus of birds) | Knee flexor; ankle extensor |
| Fibularis longus (FL) | Cranio-lateral surface of proximal tibiotarsus | Tarsus (with connections to digital flexor tendons) | Ankle extensor (and potentially digital flexor) |
| Flexor perforatus digiti IV (DFIV) | Lateral knee ligaments | Phalanx IV of Digit IV | Ankle extensor; digit IV flexor |

Table 3. Crocodile muscles that EMG data were obtained from, with previously reported anatomy and function. Nomenclature follows Meers (2003) and Allen et al. (2014) and references therein for Crocodylia. "Actions" (presumed potential functions around joints crossed) are inferred from anatomy.

| Muscle | Origin | Insertion | Action |
|--|---|--|---|
| Pectoralis (PEC) | Ventral surface of sternum, ribcage, surrounding area | Deltopectoral crest of humerus | Glenohumeral extensor, abductor and supinator |
| Transversus perinei (TP) | Ischium | Centra of caudal vertebrae 1+2 | Unknown: possibly shaping tail base |
| Iliotibialis 2 (IT2) | Dorsal ilium | Cranial side of proximal tibia | Hip abductor (also long-axis rotator and flexor/extensor?); knee extensor |
| Gastrocnemius externus (GE) | Caudal side of lateral condyle of femur | Caudal surface of proximal (tarso)metatarsus | Knee flexor; ankle extensor |
| Flexor digitorum longus – hindlimb (FDL) | Disto-lateral femoral condyle | Distal pes | Digital flexor; ankle extensor |

Table 4. EMG data summary for bird subjects.

| Species | Tinamou | | | | Turkey | | | Pheasant | Quail | Guineafowl | | |
|----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|-----------|-----------|
| Muscle | GL | | | FL | GL | FCLP | DFIV | GL | GL | GL | DFIV | FMTL |
| N individuals | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| Surface | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill |
| Speed (ms^{-1}) | 0.10 | 0.35 | 0.45 | 0.10 | 2.15 | 2.15 | 2.15 | 1.7 | 1.12 | 1.7 | 1.7 | 1.7 |
| u | 0.06 | 0.23 | 0.29 | 0.06 | 1.27 | 1.27 | 1.27 | 1.25 | 1.23 | 1.28 | 1.28 | 1.28 |
| # trials | 2 | 2 | 2 | 2 | 5 | 5 | 5 | 1 | 6 | 6 | 6 | 6 |
| # steps | 7 | 7 | 17 | 7 | 46 | 46 | 46 | 14 | 45 | 54 | 54 | 54 |

Table 5. EMG trial and data summary for Nile crocodile subjects.

| Species | Nile crocodile | | | | | | | | | | | |
|----------------------------|----------------|-----------|-----------|-----------|-----------|-----------|--------|-----------|-----------|-----------|-----------|-----------|
| Muscle | TP | | | | IT2 | FDL | PEC | | | | GL | |
| N individuals | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Surface | Runway | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill | Runway | Treadmill | Treadmill | Treadmill | Treadmill | Treadmill |
| Speed (ms^{-1}) | 0.10 | 0.10 | 0.35 | 0.45 | 0.10 | 0.10 | 0.10 | 0.10 | 0.35 | 0.45 | 0.35 | 0.45 |
| u | 0.07 | 0.07 | 0.26 | 0.33 | 0.07 | 0.07 | 0.07 | 0.07 | 0.26 | 0.33 | 0.26 | 0.33 |
| # trials | 5 | 9 | 2 | 2 | 2 | 5 | 5 | 4 | 2 | 2 | 2 | 2 |
| # steps | 14 | 25 | 8 | 7 | 6 | 16 | 14 | 19 | 8 | 9 | 8 | 7 |

Table 6. EMG trial and data summary for emu subjects across ontogeny.

| | Emu - adult | | | | | | | | | |
|---------------------------|-------------|----------|-----------|-----------|----------|----------|----------|-----------|-----------|----------|
| Muscle | GL | | | | | ILPO | | | | |
| N individuals | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| Surface | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway |
| Speed (ms ⁻¹) | 0.98-1.4 | 1.4-2.02 | 2.02-2.65 | 2.65-3.23 | 3.23-3.7 | 0.98-1.4 | 1.4-2.02 | 2.02-2.65 | 2.65-3.23 | 3.23-3.7 |
| <i>u</i> | 0.3-0.5 | 0.5-0.7 | 0.7-0.9 | 0.9-1.1 | 1.1-1.3 | 0.3-0.5 | 0.5-0.7 | 0.7-0.9 | 0.9-1.1 | 1.1-1.3 |
| # trials | 2 | 4 | 12 | 21 | 7 | 2 | 4 | 6 | 8 | 3 |
| # steps | 4 | 8 | 24 | 41 | 13 | 4 | 8 | 12 | 16 | 6 |

| | Emu - juvenile | | | | | | | |
|---------------------------|----------------|---------|----------|---------|---------|---------|---------|---------|
| Muscle | ILFB | | ITC | | | | | |
| N individuals | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Surface | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway |
| Speed (ms ⁻¹) | 2.9-3.3 | 3.3-4.0 | 1.19-1.3 | 1.4-1.9 | 1.9-2.4 | 2.4-2.9 | 2.9-3.3 | 3.3-4.0 |
| <i>u</i> | 1.1-1.3 | 1.3-1.5 | 0.3-0.5 | 0.5-0.7 | 0.7-0.9 | 0.9-1.1 | 1.1-1.3 | 1.3-1.5 |
| # trials | 10 | 9 | 2 | 1 | 5 | 7 | 10 | 9 |
| # steps | 18 | 16 | 4 | 2 | 11 | 15 | 18 | 16 |

| | Emu - baby | | | | | | | | | | | | | | | |
|---------------------------|------------|----------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|---------|---------|---------|---------|
| Muscle | GL | | | | | | ILFB | | | | ITC | | | | | |
| N individuals | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| Surface | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway | Runway |
| Speed (ms ⁻¹) | 0.6-0.92 | 0.92-1.3 | 1.3-1.7 | 1.7-2.0 | 2.0-2.4 | 2.4-2.7 | 1.3-1.7 | 1.7-2.0 | 2.0-2.4 | 2.4-2.7 | 0.6-0.92 | 0.92-1.3 | 1.3-1.7 | 1.7-2.0 | 2.0-2.4 | 2.4-2.7 |
| <i>u</i> | 0.3-0.5 | 0.5-0.7 | 0.7-0.9 | 0.9-1.1 | 1.1-1.3 | 1.3-1.5 | 0.7-0.9 | 0.9-1.1 | 1.1-1.3 | 1.3-1.5 | 0.3-0.5 | 0.5-0.7 | 0.7-0.9 | 0.9-1.1 | 1.1-1.3 | 1.3-1.5 |
| # trials | 2 | 3 | 11 | 8 | 19 | 10 | 7 | 8 | 9 | 1 | 2 | 1 | 9 | 2 | 10 | 9 |
| # steps | 6 | 10 | 43 | 34 | 81 | 43 | 26 | 34 | 40 | 4 | 6 | 3 | 31 | 9 | 41 | 39 |

Table 7. Cross-correlation analysis results for lateral gastrocnemius (GL/GE) EMG timings for all species at 1.1-1.3*u* or maximal speed obtained. “Max Cor” is the maximum correlation between the average signals; “offset” is the offset of the maximum correlations between the species (as fraction of a stride). “Croc” is Nile crocodile; “Guinea” is guineafowl.

| | Croc | Emu | Quail | Tinamou | Turkey | Guinea | Pheasant | |
|---------|-------|-------|-------|---------|--------|--------|----------|---------|
| Croc | 1.000 | 0.855 | 0.802 | 0.817 | 0.487 | 0.478 | 0.746 | Max Cor |
| | 0.000 | 0.178 | 0.230 | 0.228 | 0.266 | 0.230 | 0.183 | Offset |
| | | | | | | | | |
| Emu | | 1.000 | 0.940 | 0.941 | 0.728 | 0.699 | 0.834 | Max Cor |
| | | 0.000 | 0.000 | 0.000 | 0.014 | 0.020 | 0.000 | Offset |
| | | | | | | | | |
| Quail | | | 1.000 | 0.953 | 0.814 | 0.789 | 0.900 | Max Cor |
| | | | 0.000 | 0.000 | 0.004 | 0.012 | 0.000 | Offset |
| | | | | | | | | |
| Tinamou | | | | 1.000 | 0.759 | 0.753 | 0.869 | Max Cor |
| | | | | 0.000 | 0.000 | 0.000 | 0.000 | Offset |
| | | | | | | | | |
| Turkey | | | | | 1.000 | 0.970 | 0.788 | Max Cor |
| | | | | | 0.000 | 0.000 | 0.000 | Offset |
| | | | | | | | | |
| Guinea | | | | | | 1.000 | 0.782 | Max Cor |
| | | | | | | 0.000 | 0.000 | Offset |