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Structure, ontogeny and evolution of the patellar tendon in emus (*Dromaius novaehollandiae*) and other palaeognath birds

The patella (kneecap) exhibits multiple evolutionary origins in birds, mammals, and lizards, and is thought to increase the mechanical advantage of the knee extensor muscles. Despite appreciable interest in the specialized anatomy and locomotion of palaeognathous birds (ratites and relatives), the structure, ontogeny and evolution of the patella in these species remains poorly characterized. Within Palaeognathae, the patella has been reported to be either present, absent, or fused with other bones, but it is unclear how much of this variation is real, erroneous or ontogenetic. Clarification of the patella's form in palaeognaths would provide insight into the early evolution of the patella in birds, in addition to the specialized locomotion of these species. Findings would also provide new character data of use in resolving the controversial evolutionary relationships of palaeognaths. In this study, we examined the gross and histological anatomy of the emu patellar tendon across several age groups from five weeks to 18 months. We combined these results with our observations and those of others regarding the patella in palaeognaths and their outgroups (both extant and extinct), to reconstruct the evolution of the patella in birds. We found no evidence of an ossified patella in emus, but noted its tendon to have a highly unusual morphology comprising large volumes of adipose tissue contained within a collagenous meshwork. The emu patellar tendon also included increasing amounts of a cartilage-like tissue throughout ontogeny. We speculate that the unusual morphology of the patellar tendon in emus results from assimilation of a peri-articular fat pad, and metaplastic formation of cartilage, both potentially as adaptations to increasing tendon load. We corroborate previous observations of a 'double patella' in ostriches, but in contrast to some assertions, we find independent (i.e. unfused) ossified patellae in kiwis and tinamous. Our reconstructions suggest a single evolutionary origin of the patella in birds and that the ancestral patella is likely to have been a composite structure comprising a small ossified portion, lost by some species (e.g. emus,

moa) but expanded in others (e.g. ostriches).

- 1 <u>Title:</u> Structure, ontogeny and evolution of the patellar tendon in emus (*Dromaius*
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Introduction

- 17 The patella (kneecap) is a sesamoid bone which develops within the main extensor tendon of
- the knee. Although the avian patella evolved independently from those of both lizards and
- mammals (Dye 1987; Sarin et al. 1999), it is unclear when and in what form the avian patella
- 20 first evolved, and whether it might also have evolved repeatedly within avian species. The
- 21 phylogenetic position of Palaeognathae (ratites and their relatives; emus, ostriches, tinamous,
- 22 kiwis and kin) as a relatively basal clade of birds sister group to the enormously diverse
- Neognathae, comprising all other crown clade birds means that they are an important
- 24 outgroup for exploring the early evolution of traits, such as the patella, in birds.
- 25 The configuration of the main knee extensor tendon in palaeognathous birds is a subject of
- some ambiguity. Some species are reported to have a patella (Deeming 1999; Picasso 2010),
- 27 whereas in others the equivalent structure is said to be absent (Shanthi et al. 2007); available
- data and literature indicate several contradictions. The patella's potential to alter limb
- 29 mechanics means that elucidation of this tendon's configuration could provide insight into the
- evolution of cursoriality and large body size in ratites, and whether flight has been lost
- 31 multiple times in palaeognaths. Skeletal development has long been used as a source of data
- for phylogenetic analyses (Maisano 2002; Sánchez-Villagra 2002; Maxwell & Larsson 2009),
- and additionally the little-studied patella may provide another morphological characters of
- use in resolving the unclear and highly controversial evolutionary relationships and history of
- palaeognathous birds (Cracraft et al. 2004; Harshman et al. 2008; Bourdon et al. 2009;
- 36 Johnston 2011; Smith et al. 2013; Mitchell et al. 2014).
- 37 The incomplete understanding and imprecision regarding form, function, ontogeny and
- 38 phylogeny of patellae in palaeognaths is typical of most, if not all, sesamoid bones. The
- 39 frequent perception of patellar structures as unimportant (Pearson & Davin 1921a; Kaiser
- 40 2007; Vickaryous & Olson 2007) is probably the reason that few studies have examined this
- anatomical region in detail, particularly in birds. As such, the configuration of the patella
- 42 (and its tendon) in palaeognaths is a confused issue. Within the clade, ratites have been
- described to lack the patella by some (Thompson 1890; Fowler 1991; Stewart 1994). In
- contrast, others note the prominence of the patella in these species (de Vriese 1909), and an
- extensive review of bird anatomical characteristics compiled by Livezey and Zusi (2006) lists
- 46 it as present in all ratites.
- 47 Gadow (1880, 1885) writes (though does not illustrate) that ratites do in fact have patellae,
- but that they give the impression of absence through fusion of the patella to the tibial crest.
- 49 Superficially this seems supported by other studies, which note fusion of the patella in kiwis
- and extinct moa (Beale 1985; Beale 1991; Sales 2005; Turvey & Holdaway 2005). However,
- Beale (1985) expressed doubt over his identification of the patella in kiwis, noting that such
- fusion would be unusual. Indeed, Beale's published radiographs confirm his doubts by
- showing that the form and position of the 'patella' structure is more consistent with that of
- 54 the cranial cnemial crest of the tibia (also called the crista cnemialis cranialis, proximal tibial
- ossification centre, or tibial epiphysis).

- 56 The cranial cremial crest is a "traction epiphysis" (Hogg 1980; Hutchinson 2002) an
- 57 initially separate intratendinous ossification that fuses with the tibia and so can easily be
- 58 misidentified as a patellar sesamoid. The true patella remains independent throughout life in
- 59 most birds and is normally located well proximal to the cranial cnemial crest, within the
- patellar sulcus of the femur. In contrast, the development of the cranial cnemial crest and its
- fusion to the tibia (remaining entirely separate from the patella) is well documented in
- 62 embryological/ontogenetic studies (Hogg 1980; Pourlis & Antonopoulos 2013). It seems that
- kiwis do possess a true independent patella in the expected location, as illustrated in a
- myological study of adult kiwis (McGowan 1979), but misidentification seems to be a
- prominent problem, with several other studies likewise mistakenly identifying the cranial
- cnemial crest as the patella in birds.
- 67 The potential for misidentification of the patella (or cranial cnemial crest) makes it difficult to
- evaluate statements when there are no clear descriptions or accompanying evidence.
- 69 Ostriches (*Struthio*) are well documented to have both a true (unfused) patella and a second
- more distal ossification, distinct from the first; a so-called double patella (Haughton 1864;
- Macalister 1864; Thompson 1890; de Vriese 1909; Deeming 1999; Gangl et al. 2004). For
- the other Palaeognathae, however, there are fewer definitive data explicitly exploring patella
- presence and form, and where they do exist these data lack clear illustrations. For example,
- some authors refer to a patella in greater rheas (*Rhea americana*) (Brinkmann 2010; Picasso
- 75 2010), whilst other earlier literature surprisingly suggests that this species possesses a double
- patella as in ostriches (de Vriese 1909). A patella is also referred to in dwarf cassowaries
- 77 (Casuarius bennetti) (Brinkmann 2010) but has not been found in a limited study of southern
- 78 cassowaries (Casuarius casuarius) (Biggs & Zoo 2013). Volant tinamous are said to have
- only a fibrocartilaginous structure in lieu of an ossified patella (Parker 1864). In extinct
- 80 elephant birds (Aepyorninithidae), patellar presence has been inferred from muscle scars on
- the cnemial crest (Livezey & Zusi 2006). However, the latter evidence is inconclusive
- because the patella itself would not articulate to such scars, but rather do so via a patellar
- tendon, and so the scars cannot by themselves establish the presence or absence of a patellar
- sesamoid bone. An independent patella was mentioned in just one specimen of moa
- 85 (Dinornithiformes) described by Owen (1883). Thus confusion, misidentification and
- specimen rarity all combine to make ascertaining the status of the patella in palaeognath
- 87 species difficult.
- Like almost all members of its clade, the presence and configuration of the patella and its
- 89 tendon in emus (*Dromaius novaehollandiae*) is also unclear. Myological studies have not
- explored the question (Patak & Baldwin 1998), and whilst one study lists patellar presence
- early in ontogeny (Maxwell & Larsson 2009), another has described the patella to be absent
- 92 in adult emu specimens (Shanthi et al. 2007). The former finding, however, is again a cranial
- cnemial crest rather than a patella (Maxwell pers. comm, 2013). In this study, we first
- 94 describe the gross and microscopic anatomy of the emu patellar tendon throughout ontogeny,
- 95 in order to better understand the patellar phenotype in this species. We then put these data
- 96 into the context of the evolution of Palaeognathae as a whole, using other published data and
- our own novel observations to infer the evolution of the patella in this clade. In doing so we

aim to clarify the status of the patella and its tendon amongst palaeognath species, infer how many times the patella has evolved and its temporal origin(s) within birds, and reconstruct patterns of evolutionary change (i.e. patellar loss or expansion) in this lineage.

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Materials and Methods

- A note on terminology: the triceps femoris muscle group is equivalent to the mammalian quadriceps femoris group, and in birds comprises Mm. iliotibialis (3 main heads), femorotibialis (3 heads), and ambiens (2 heads in ostriches; 1 in other palaeognaths). The
- common tendon of these extensor muscles is called the patellar tendon, aponeurosis, or sometimes ligament. Compositionally, this structure has been shown to be closer to tendon
- than ligament (Bland & Ashhurst 1997; Livezey & Zusi 2006), and it is homologous with the
- tendon that contains the patella in ratite and other birds, and so herein will be referred to as
- the patellar tendon, even if a patellar sesamoid ossification is absent.
- We harvested the right patellar tendon from nine emu (*Dromaius novaehollandiae*) cadavers,
- which were euthanized as part of another study and stored frozen (-20°C). The emus were
- from a UK farm population (Leicestershire Emus and Rheas, Leicestershire). Four were five
- weeks old (mean bodyweight 4.41kg ± standard deviation of 0.94kg), one was six months
- 115 (19.3kg), one was 12 months (30kg), and three were 18 months (mean 40.2kg ± 2.25 kg). All
- lacked any obvious musculoskeletal or gait pathologies that might complicate our
- interpretations. We also collected the left patellar tendon and patellae from an adult (71.3kg)
- ostrich (Struthio camelus) and the right patellar tendon and patella from an adult (1.8kg)
- guinea fowl (Numida meleagris), for comparative purposes, and checked the gross
- morphology of these against other museum specimens and the literature to ensure they were
- representative of these two species.
- The patellar tendons were dissected out of the thawed cadavers, fixed in 10% neutral buffered
- formalin and radiographed for evidence of gross mineralisation using a high powered ceiling
- mounted X-ray generator (Polydoros, Siemens Medical, Erlangen, Germany) in combination
- with a computerised radiography system (FCR Profect CS, Fujifilm, Bedfordshire, UK),
- using settings of 60kV and 32mAs.
- All specimens were sectioned longitudinally along the midline and, size permitting, most
- were further sectioned in longitudinal and transverse directions. The tendon sections were
- dehydrated and embedded in paraffin wax blocks for histological examination under light
- microscopy. Microtome sections were cut between 4-6µm, and stained with routine
- Haematoxylin and Eosin as well as SafraninO/Fast green stain for cartilage and von Kossa for
- calcium salts (von Kossa 1901). Ostrich sections were additionally stained with Masson's
- trichrome to highlight features of bone micro-anatomy.
- We reconstructed evolutionary patterns in Mesquite software (Maddison & Maddison 2011),
- using the phylogeny of Livezey and Zusi (Livezey & Zusi 2007) for the main topology of
- Ornithurae (crown group birds and their closest fossil outgroups). However, we adopted the

- alternative phylogeny for crown group birds (Neornithes/Aves) proposed by others (Hackett
- et al. 2008; Harshman et al. 2008; Phillips et al. 2010; Smith et al. 2013; Baker et al. 2014;
- Mitchell et al. 2014) which places tinamous within ratites and ostriches as occupying the
- most basal phylogenetic position within Palaeognathae. Alternate topologies were also
- examined in a sensitivity analysis of our conclusions, by comparing the evolutionary patterns
- of the patella resulting from the enforcement of different phylogenetic frameworks. We
- specifically examined the topologies of Lee et al. (1997), Cooper et al. (2001), Bourdon et al.
- 144 (2009) and Johnston (2011).
- On this basis, we scored the patella as: absent (0), a small flake of bone (1), a rounded nodule
- 146 (2), a subrectangular block with strong articular surfaces for the patellar sulcus of the femur
- 147 (3) or a craniocaudally expanded triangular crest, rising high above the patellar sulcus (4).
- 148 These five graded scores were assumed to be ordered because they form a continuous series
- 149 (Table 1). Due to easy confusion between the patella and cranial cnemial crest, we ensured
- that the patella was only counted as present when there was documented visual evidence of it,
- rather than if its presence was solely supported by in-text statements (unless these were
- extremely clear and detailed). This meta-analysis would ideally be from multiple specimens,
- and an absent score in juvenile specimens was treated more as uncertainty, rather than as
- definitive evidence of absence.
 - To gain further data for this meta-analysis, we also examined avian osteological collections
- held by the Natural History Museum at Tring (NHMUK), University Museum of Zoology
- 157 Cambridge (UMZC), and the Grant Museum at the University College of London (LDUCZ).

159 **Results**

- 160 Patella tendon morphology in ageing emus reveals the lack of an ossified patella
- Proximally, the emu patellar tendon in all ages (5 weeks to 18 months) is attached to the
- triceps femoris muscles via an abrupt and particularly distinctive junction (Fig. 1A). It is off-
- white and fairly firm, particularly proximally, close to its muscle junction. Other muscles
- overlie and are attached to the patellar tendon superficially, but are not ideally positioned, or
- sufficiently strongly connected, to contribute as much force along their line of action, as is
- the triceps femoris. The patellar tendon sits within the patellar groove on the distal femur,
- bordered by the lateral (larger) and medial (smaller) femoral condyles. At a position
- approximately one-third along its length, whilst it is still within the patellar groove, the
- tendon partially splits to form superficial and deep portions (Fig. 1A and B). The superficial
- portion bridges the femorotibial joint and inserts onto the lateral and cranial cnemial crests of
- the tibiotarsus, whilst the deep portion blends with a grossly fatty structure within the patellar
- sulcus of the femur, and is also attached to the cartilaginous femorotibial menisci (Fig. 1B);
- mostly to the medial meniscus, but also to cranial and medial parts of the lateral meniscus.
- Examination of transverse cross-sections of the patella in emus of all ages (five weeks to 18
- months) revealed that splitting of the distal patellar tendon creates a superficial roughly
- triangular portion and deep, rounded portion (Fig. 1C).

- 177 Radiography revealed no evidence of any gross mineralisation in the patellar tendons of 18
- month old emus; there was no patellar sesamoid (Fig. 1D-G). This lack of any discernible
- mineralisation/ossification was confirmed microscopically, by the absence of calcium salts
- using von Kossa staining in birds of this age (data not shown).
- 181 Cartilage-like tissue develops within a predominantly adipose patella tendon during emu
- 182 ontogeny
- Histological examination of the emu patellar tendon revealed a basic structure maintained
- across all the ages examined, with some variations throughout ontogeny. Histology
- confirmed the presence of an abrupt junction between the patellar tendon and the triceps
- 186 femoris muscles proximally. In all ages, the collagen fibres here have a dense and
- predominantly transverse orientation, which become more longitudinally oriented as they
- penetrate into the tendon distally (Fig. 2A). Surprisingly, the body of the patella tendon is
- overwhelmingly comprised of adipocytes, with collagen fibre bundles running throughout in
- mixed orientations resulting in a meshwork-like appearance. In some sections, a blood vessel
- is seen in the proximal deep region of the tendon, within the adipocyte-rich tissue (Fig. 2B).
- The body of adipose/collagenous tissue is bounded (both on superficial and deep surfaces) by
- 193 continuous, longitudinally-oriented and more classically tendon-like dense collagen bundles
 - (Fig. 2C). Cells in the collagen fibre bundles are typically slender and fusiform with elongate
- nuclei, resembling tenocytes (Fig. 2D).
- 196 Throughout all observed ontogenetic stages, the tendon's surface is covered with flattened or
 - polygonal cells in a layer 2-3 cells thick; likely synovial-lining cells. Blood vessels are visible
- in the synovial sub-intima and deeper fascia, particularly laterally (apparently away from the
- point of tendon contact with bone). Synovial villi are visible on the deep surface in some
- sections (Fig. 2E).
- Where it splits into superficial and deep portions distally, the surfaces of the tendon have a
- lining layer of cells that oppose each other (Fig. 2F). The synovial layer is continuous over
- the tendon split but does not interdigitate with it.
- 204 Histological sections from the five week old emu patellar tendons are generally more cellular
- overall compared to the older (six, 12 and 18 month old) emus. A further difference between
- 206 the age groups is the appearance of an apparent third tissue type, in addition to the adipose
- and collagenous tissues already described. In the five week old emu patellar tendons, this
- 208 tissue is basophilic with H&E staining and highly vacuolated or vesicular (Fig. 3A). This
- 209 material is most abundant in the deep, proximal region of the tendon and also close to the
- 210 tendon split laterally. There are lesser amounts along the deep surface distally, and around the
- dense collagen bundles running along the proximal (triceps) and superficial muscle-tendon
- 212 junctions. The substance is also present in minimal amounts within the body of the tendon,
- especially in the deepest third, where it seems to follow collagen bundles as they travel
- 214 through the adipose tissue. The cells associated with this material have plump and sometimes
- 215 finely stippled nuclei, in contrast to the compressed nuclei of adipocytes or the elongate
- 216 nuclei of tenocytes.

- In the patellar tendon of the six month old emu, the basophilic vesicular tissue is still present,
- 218 distributed near to the proximal and superficial muscle junctions, near to the deep surface
- 219 proximally and also laterally prior to the tendon split. None is visible in any appreciable
- 220 quantity in the tendon distally. This material has taken up more H&E stain and is less
- vesicular and more homogenous than in the five week old emus. Certain collagen fibres near
- 222 to this material also appear slightly more basophilic (Fig. 3B).
- Much like the in the six month old emu, the vesicular material in the 12 month emu patellar
- tendon takes up more H&E stain and looks less vesicular and more homogenous. In addition,
- there are some areas where rounded cells sit within chondrocyte-like lacunae, giving the
- 226 tissue a chondroid (cartilage-like) appearance. The tissue has a similar distribution as in the
- six month old emu.
- In the 18 month old emus, the basophilic chondroid and vesicular tissue is most extensive.
- 229 This chondroid tissue is abundant along the proximal muscle junction, interspersed between
- collagen bundles and often contains large clusters of adipocytes (Fig. 3C). Rounded,
- chondroid cells occupy lacunae within the basophilic tissue, and also within nearby collagen
- fibre bundles (Fig. 3D). In both the five week and 18 month old patellar tendons,
- 233 SafraninO/fast green stains the vesicular and chondroid tissue intensely red, differentiating it
- from nearby collagen fibre bundles (Fig. 4A-4D).
- 235 Patellar tendon sections from the ostrich and adult guineafowl are, by comparison, more
- conventional with the patellar tendon comprising predominantly dense collagenous bundles
- 237 (5A) and containing ossified patellar sesamoids (one in guineafowl and two in ostriches) (Fig.
- 5B-D). Similar to the emu, the patellar tendon of the ostrich includes regions of cartilage-like
- 239 tissue, especially between and deep to the ossified sesamoids. It also contains areas with
- 240 apparently discrete strata of adipose tissue, unlike the diffuse and abundant incorporation of
- fat within the emu patellar tendons. Both the proximal and distal ostrich patellae are almost
- 242 entirely trabecular bone with fatty and cellular marrow, with regions of well differentiated
- 243 cortical bone, mostly superficially, containing secondary osteons running along the bones'
- longitudinal axes. There are small areas of calcified cartilage at some edges of the bones, but
- 245 no articular cartilage lining the deep surface as in the guineafowl. The guineafowl patella has
- an outer compact bone cortex with some longitudinal secondary osteons, inner trabecular
- structure with mostly fatty marrow, and a thick articular cartilage pad lining its deep surface.
- 248 The patella morphology in other palaeognaths and outgroups
- 249 Based upon many extremely well-preserved complete skeletons, particularly from Cretaceous
- 250 fossil deposits in China and Mongolia, a patella is clearly absent in extinct birds that are basal
- to Hesperornithiformes (Table 1) (Hou 1997; Clarke & Norell 2002; Clarke et al. 2006; Zhou
- et al. 2008; Wang et al. 2013). Although a patella may have originated earlier than in the
- common ancestor of Hesperornithiformes and Neornithes (see below), the fossil record of
- 254 many ornithurine taxa argues strongly against this possibility. Hou (1997) speculated that a
- 255 sliver of bone in a *Confuciusornis* specimen was a patella, but its disarticulated position
- renders its identification uncertain, and the absence of a patella in dozens of other

- 257 Confuciusornithidae casts additional doubt on this identification, which could be pathology or
- another unusual ossification.
- In our studies of museum specimens, we have observed a true osseous patella in multiple
- 260 tinamou and kiwi specimens. Where present in these taxa, the patella is a small flake-like
- bone enclosed in the patellar tendon, situated in the patellar groove of the femur (Fig. 6). We
- also observed the double patella in ostriches evident in over 12 specimens from diverse
- 263 collections and well documented in the literature (Haughton 1864; Macalister 1864;
- Thompson 1890; de Vriese 1909; Deeming 1999; Gangl et al. 2004). In contrast, we did not
- 265 find evidence of patellae in other palaeognath specimens (emus, rheas, cassowaries, moa, and
- elephant birds).
- Our reconstructions of the evolution of the patella in birds show it to have evolved only once
- in this lineage, in the common ancestor of Neornithes and Hesperornithiformes (Fig. 7).
- Other trees (not shown) using variations on the two main topologies presented give similar
- 270 results. No matter how the relationships within Palaeognathae are organised (based on the
- 271 most detailed recent analyses), our phylogenetic optimizations always show a small flake of
- bone as plesiomorphic for Neornithes (ancestral for the Hesperornithiformes + Neornithes
- clade); a condition shared by the common ancestor of Neognathae. However, patterns of
 - patellar evolution within Palaeognathae itself can vary considerably depending on tree
- 275 topology.

77 Discussion

- 278 The patellar tendon of emus has a highly unusual morphology, and lacks any evidence of a
- 279 patellar sesamoid ossification. Unlike the typical tendon of most tetrapod vertebrates, in
- 280 which the microanatomy consists predominantly of dense, parallel collagenous bundles
- 281 (Khan et al. 1999; see also Fig. 5A), the patellar tendon of emus is composed almost entirely
- of adipocytes contained in a collagen bundle meshwork. Additionally, in older emus there are
- patchy regions of cartilage-like tissue that are most prominent near the proximal muscle-
- tendon junction, which corresponds to the location of the patellar sesamoid in most other
- birds, including the proximal patella in ostriches. This appears to represent a vestigial, totally
- 286 unossified sesamoid remnant.
- As it first appears, the basophilic tissue seen in the tendons resembles tendon/ligament
- 288 changes described elsewhere in the literature as mucoid degeneration, myxoid degeneration,
- chondroid metaplasia, and/or fibrocartilage metaplasia (Khan et al. 1999; Vigorita 1999;
- 290 Hashimoto et al. 2002; Buck et al. 2009). Terminology varies, but characteristically the
- tendon cells become rounded and chondroid in appearance and produce a stainable,
- sometimes granulomatous matrix (Järvinen et al. 1997; Buck et al. 2009). This type of tendon
- change is usually reported in the context of tendon pathology, particularly in cases where the
- duration of symptoms is longstanding (Hashimoto et al. 2002). Chondroid cells can also be
- seen at tendon/ligament junctions with bone (Vigorita 1999).

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296 Similarly, the presence of fat within tendon is associated with age-related degeneration (Hashimoto et al. 2002). Accumulation of adipocytes in a tendon, variably called 297 tendolipomatosis, lipoid degeneration, or fatty infiltration, is well documented (Hashimoto et 298 al. 2002; Gagliano et al. 2013), though the aetiology is unclear. The adipocytes occupy spaces 299 300 between the collagen fibres and may amass to such a point that they weaken and disrupt the 301 fibres (Józsa et al. 1984; Hashimoto et al. 2002). However, it seems unlikely that the appearance of the patellar tendon in these emus is due to an underlying pathology. The 302 303 appearance is present from a young age and consistent between individuals, suggesting that it represents a 'normal' phenotype for this tendon in emus. Tendinopathies also predispose the 304 tendon to rupture (Sharma & Maffulli 2005), and there are no reports of patellar tendon 305 rupture in emus. Rather than tendinopathy, this emu patellar tendon composition is more 306 likely indication of a limited ability for tendon remodelling (in this case physiologically 307 rather than pathologically) (Khan et al. 1999). It may also reflect the strange evolutionary 308 history of emus (and other ratites) which seemingly involves loss of the patella, possibly during the convergent evolution of flightlessness (e.g., Harshman et al. 2008; Johnston 2011; Smith et al. 2013; Baker et al. 2014).

Tendon is known to modify its structure in response to external mechanical load, and this is a more plausible explanation for the unusual appearance of the emu patella tendon. The presence of a cartilage-like tissue in the adult emus is consistent with fibrocartilage formation observed in tendons subject to bending or compression (Vogel & Peters 2005). This hypothesis is supported by the apparent ageing-related differentiation of this tissue. In the youngest emus, corresponding regions exhibit basophilic vesicular material and rounded cells with plump nuclei; Safranin O-positive staining supports the presence of high proteoglycan content in this tissue. The evidence suggests that the basophilic vesicular material may be the extra-fibrillar matrix of the tendon which contains proteoglycan and has a vacuolated appearance when newly formed (Maffulli et al. 2005). The vesicular appearance might also result if this tissue derives from metaplastic adipocytes.

- In older emus, this apparent extra-fibrillar matrix becomes more basophilic and less vesicular.
- 324 Some of the rounded cells are surrounded by a lacunar space, giving the impression of
- 325 chondrocytes. Nearby collagen bundles are also more basophilic-staining and contain
- 326 chondrocyte-like cells, suggesting they are either transforming or being replaced by this
- 327 tissue. In the oldest emus, this tissue is highly reminiscent of cartilage and Safranin O
- 328 staining indicates it has high proteoglycan content. Regardless, this tissue is far from ossified,
- and the absence of a documented patellar ossification in the literature on emu morphology at
- least circumstantially supports the inference that this absence represents the general condition
- 331 for emus.
- The appearance of so much fat tissue within what is supposed to be a tendon is less
- 333 straightforward to understand. A commonly presumed function of sesamoids is protective;
- reinforcing the tendon from high compressive forces as it bends around bone. Fat pads near
- joints are hypothesised to have a similar function in cushioning tendon and stress dissipation
- (Benjamin et al. 2006). We speculate that emu patellar tendon morphology might be the
- result of assimilation of a peri-articular fat pad. Through comparative dissections, we have

- observed that the deep portion of the emu patellar tendon corresponds in its location and
- attachments to the infrapatellar fat pad of other birds, lending some support to our fat pad
- assimilation hypothesis. If this hypothesis were correct, it would represent a novel solution
- for dealing with tendon compression. Other animals lacking ossified patellae have patellar
- tendon modifications (e.g. a fibrocartilaginous patelloid structure in marsupials (Reese et al.
- 343 2001)) which are thought to play a similar protective role. However, so far emus appear
- unique in possessing diffuse cartilaginous changes and incorporation of fat within the patellar
- 345 tendon.
- In attempting to complete our character matrix, which had ambiguous entries due to a lack of
- published data, we have observed osseous patellae in several tinamou and kiwi specimens.
- 348 Some previous studies note the patella to be a cartilage (or fibrocartilage) structure in kiwis
- and tinamous (Owen 1839; Parker 1864), which has led to it being considered absent as a
- skeletal feature by others (e.g. for tinamous in Livezey and Zusi, 2006). In other birds, the
- patella begins as a cartilaginous nodule and ossifies late relative to the other bones
- 352 (Lansdown 1970; Hogg 1980; Pourlis & Antonopoulos 2013), and considering our studies of
- museum specimens it seems likely that this is also the case for (at least some species of)
- kiwis and tinamous. It is also possible that in these species, the patella does not ossify in
- every mature individual (e.g., the apparent absence of a patella in Beale's (1985, 1991)
- studies of kiwis). Other sesamoid bones can exhibit variable presence between individuals
- 357 (e.g. dorsal carpal sesamoid (Hogg 1980)); however this would be unusual for the patella
- which, as far as research to date has shown, is a relatively constant feature in the species
- possessing it. Much larger sample sizes of mature individuals would be needed to answer this
- 360 question.
- Unfortunately, the lack of patellae in other palaeognath specimens cannot reliably be used to
- infer its absence. As a small, late-ossifying bone buried in soft tissue, accidental loss would
- also be a very plausible explanation for the lack of patellae in osteological museum
- specimens. For the species where patellar presence and form remains unclear, further study of
- 365 tissues in situ is necessary, or substantial evidence from well-preserved museum or fossil
- specimens with an unambiguous ossified patella *in situ*.
- The situation with moa specimens poses an excellent example. Although Owen (1883)
- 368 figured and described what he thought to be a patella in one *Dinornis* specimen, we consider
- this identification dubious. The absence of a patella in so many other moa skeletons
- examined in the NHMUK collection and in the literature, despite the preservation of gut
- 371 contents, scleral ossicles, tracheal rings and other fine details in the same specimens renders
- the argument that the patella simply was not preserved or found to be implausible.
- Unfortunately, Owen's single "patella" specimen has gone missing in the NHMUK
- 374 collections (S. Chapman, pers. comm., 2014). It is likely to have been a tarsal sesamoid or
- other bone rather than a patella, as the tarsal sesamoids are grossly similar in size and shape
- to a patella, are commonly present in moa skeletons (our observations numerous NHMUK
- 377 specimens of *Dinornis* and *Pachyornis*) and yet Owen did not describe a tarsal sesamoid.
- Regardless, future studies of the anatomy of diverse dinornithiform (and other Palaeognathae,
- including the key stem taxa called lithornithids) species should remain alert for the possibility

- of the presence of a patella in some species or individuals, to test our assumption and Owen's
- identification and to refine understanding of the evolution of the patellar sesamoid in
- 382 Palaeognathae.
- 383 Sesamoid structures, such as the patella, are thought to increase the mechanical advantage of
- the muscles by increasing the tendon's moment arm around a joint. Sesamoids are also
- thought to protect the tendon from the considerable compressive forces it experiences
- bending against bony surfaces over joints. These two functions are the most frequently cited
- as to why sesamoids like the patella might have (repeatedly) evolved and been retained. The
- patella may confer further functional advantages, or develop for other reasons however,
- especially if the morphology of the patella when first evolved differs from its current one (i.e.
- it may have been exapted into new roles).
- 391 As the patella may have played an important role in the locomotor adaptation of
- 392 palaeognaths, elucidation and comparison of its configuration may also prove informative in
 - the question of whether ratite flightlessness was inherited from a single flightless ancestor
- 394 (synapomorphic) or whether flight has been convergently lost on multiple occasions in ratites
- 395 (e.g., Harshman et al. 2008; Johnston 2011; Smith et al. 2013; Baker et al. 2014; Mitchell et
- al. 2014). Though a conclusion cannot be made on the basis of this one piece of evidence,
- significant differences in ratite knee anatomy and patellar presence/absence are more
 - plausible in a scenario where there are repeated losses of flight and convergent evolution of
- 399 cursorial specializations.
- 400 Curiously, the loss(es) of an ossified patella in palaeognaths seems paralleled by other
- 401 reductions of accessory ossifications in the limbs: the tarsal sesamoid seems lost in most taxa
- 402 except some moa (see above; present in numerous NHMUK specimens of *Pachyornis* and
- 403 Dinornis), and ossifications of the lower limb tendons are likewise absent in almost all taxa—
- although we have noticed them in some tinamou specimens and Houde and Haubold (1987)
- described them in the stem ratite *Palaeotis*. Both were probably ancestrally present in the
- 406 most recent common ancestor of crown group birds (e.g., Hogg 1980; Hutchinson 2002),
- although even less is known about the tarsal sesamoid than the patella in birds. The
- 408 coincidence of these reductions of limb ossifications with the evolution of cursorial
- 409 morphology, flightlessness and large body size in Palaeognathae remains an intriguing
- 410 mystery.
- Our reconstructions of patellar evolution suggests that the patella has a single origin in the
- distant ancestor of modern birds (the clade containing Hesperornithiformes + Neornithes),
- rather than independent evolutions in Palaeognathae and Neognathae. Given our results, the
- 414 form of the ossified patella in their common ancestor is most likely to have been a small,
- 415 flattened flake of bone rather than a large lever-like structure as in later birds. As might be
- expected, the different tree topologies proposed for Palaeognathae result in different patterns
- of patellar evolution within this clade. However, we infer that within palaeognaths, the
- osseous patella has been lost at least once by emus and likely in moa too, possibly separately.
- In contrast, an ossified patella has been retained by some other palaeognaths and even
- 420 evolved into a larger structure (e.g. ostriches). Data from other species (cassowaries, rheas,

- and especially fossil taxa such as *Lithornis*, stem ostriches or hesperornithiforms, or less well
- preserved taxa such as *Ichthyornis*) as well as a robust palaeognath phylogeny would be of
- great value in more clearly tracing the evolution of the patella in this clade.

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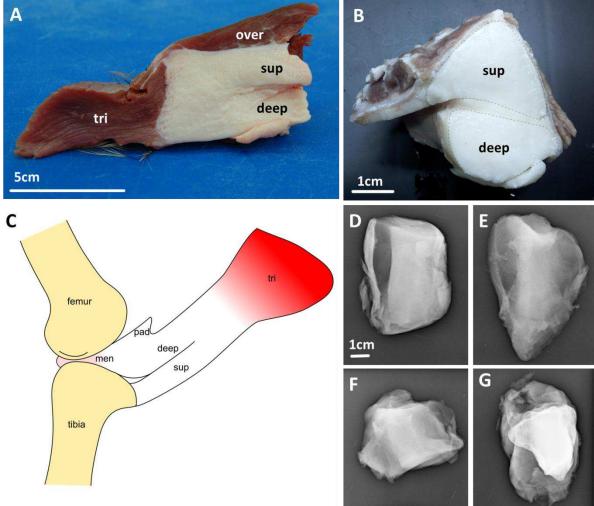
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Figures



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Figure 1: Gross patellar morphology. A; excised patellar tendon of a six month old emu in longitudinal cross section, showing the triceps femoris mucle group attaching proximally (tri), overlying muscles superficially (over), and the tendon partially splitting distally to form superficial (sup) and deep (deep) portions. Left of image = proximal. B; transverse cross section of the distal patellar tendon from an 18 month old emu, showing the triangular superficial portion (sup) and rounded deep portion (deep). Left of image = lateral. C; schematic drawing of the emu patellar tendon reflected from the femur. Distally the tendon splits into superficial (sup) and deep (deep) portions, with the former inserting onto the cranial tibial crest while the latter blends with a triangular fatty structure (pad) that attaches to the menisci (men). D-F; patellar tendon radiographs of the three oldest emus (18 months), showing no evidence of any mineralisation. Top of image = proximal. G; patellar tendon radiograph of a reindeer with similar dimensions as an adult emu tendon, demonstrating the appearance of a mineralised patellar sesamoid. Top of image = proximal.

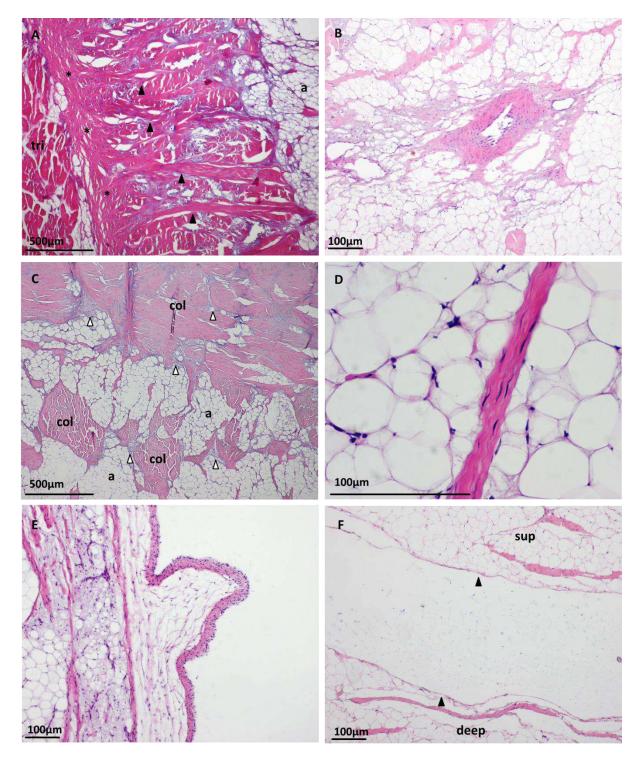


Figure 2: Histology of emu patellar tendon. A; the patellar tendon junction with the triceps muscles (tri) in an 18 month old emu. Collagen bundles appear to run transversely along the muscle junction (asterisks), before turning longitudinally (filled arrows) and penetrating into the adipocyte rich (a) body of the tendon. H&E. B; the patellar tendon of a 12 month old emu, with a blood vessel within the tendon body seen in the proximal deep region. H&E. C; the patellar tendon of an 18 month old emu. Collagen bundles (col) run along the superficial surface (top right of image), interspersed with basophilic vesicular and chondroid tissue (unfilled arrows). The body of the tendon is mostly composed of adipocytes (a) and collagen

bundles (c) in mixed orientations. Vesicular tissue, when present in the body of the tendon, is associated with collagen fibre bundles. H&E. D; slender tenocytes within the crimped collagen fibre bundles. E; patellar tendon of a five week old emu, displaying a synovial villus on the deep surface, close to the midline of the tendon. H&E. F; the patellar tendon of an 18 month old emu. The edges of the superficial (sup) and deep (deep) portions of the distal tendon have a lining layer of cells (filled arrows). H&E.

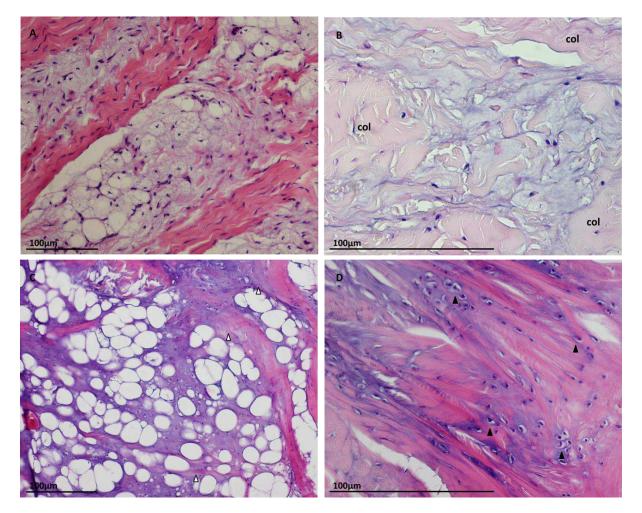


Figure 3: Differentiation of tissue from a vesicular to cartilage-like appearance with age. A; the patellar tendon of a five week old emu, showing the basophilic, vesicular material which is often seen in association with collagen fibre bundles. H&E. B; the patellar tendon of a six month old emu. The basophilic material has a more homogenous and less vesicular appearance than in the five week old emus. The nearby collagen fibre bundles (col) are more basophilic here than in other regions of the tendon. H&E. C; the patellar tendon from an 18 month old emu, near to the proximal muscle junction, showing the basophilic vesicular and cartilage-like tissue containing clusters of adipocytes. This tissue has the appearance of encroaching on nearby collagen fibre bundles (unfilled arrows). H&E. D; the patellar tendon of the same 18 month old emu, from another region near the proximal muscle junction. Chondroid cells (filled arrows) sit within the normally eosinophilic collagen fibre bundles, creating an intense localised basophilia. H&E.

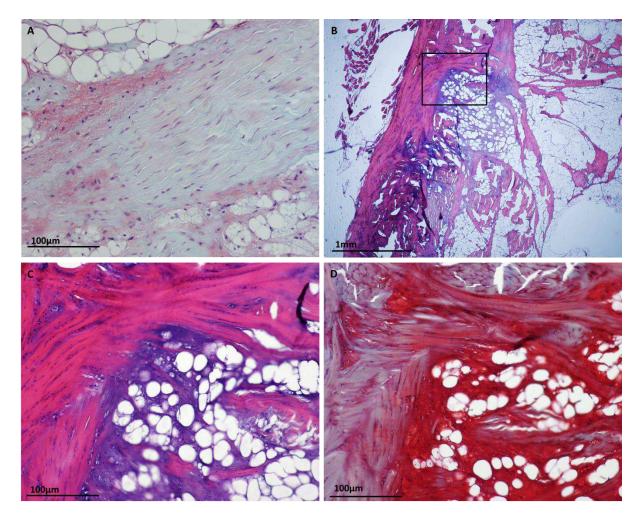


Figure 4: Appearance of the vesicular and cartilage-like tissue with Safranin O/fast green staining vs. H&E. A; the patellar tendon of a five week old emu, showing the vesicular material staining red with Safranin O/fast green, indicating proteoglycan content. Safranin O/fast green. B; the patellar tendon from an 18 month old emu, showing the deeply basophilic appearance of the chondroid tissue near the proximal muscle junction with the triceps tendon (left of image). The black box indicates the region magnified in C and D. H&E. C; magnified region of chondroid tissue in an 18 month old emu patellar tendon. H&E. D; the same magnified region showing the chondroid tissue staining intensely red. Safranin O/fast green.

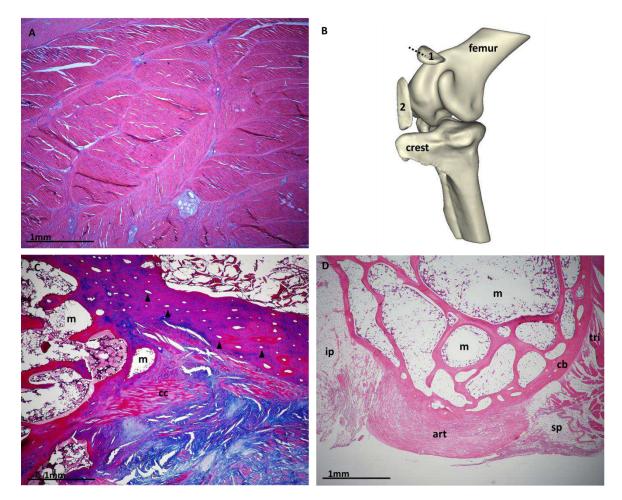


Figure 5: Configuration of ostrich and guineafowl patellae. A; part of a longitudinal section of the patellar tendon of an adult ostrich, predominantly composed of thick collagenous bundles with patchy regions of cartilage-like tissue and the occasional solitary adipocyte. H&E. B; 3D segmentation of computed tomography (CT) scan data from ostrich left knee, provided by Kyle Chadwick. The proximal patella (1) sits over the patellar sulcus of the femur, whilst the distal patella (2) overlies the large medial femoral condyle. The cranial enemial crest (crest) is fused to the tibia in the adult. The dotted line shows the cut through the proximal patella seen in Fig C. C; transverse section of the proximal patella of an adult ostrich, showing osteons (black arrows) in the superficial aspect of the bone, trabeculae and medullary cavity occupying the majority of the patella (m), and calcified cartilage (cc). Superficial = top right of image, medial = right of image. Masson's trichrome. D; Longitudinal section of an adult guinea fowl patella, showing the triceps muscle attaching proximally (tri), suprapatellar (sp) and infrapatellar (ip) fat pads, outer cortical bone (cb), inner trabeculated structure, and deep articular cartilage lining (art). Proximal = right of image, deep = bottom of image. H&E.

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Figure 6: Limb bones of a tinamou (Crypturellus obsoletus specimen NHMUK S/1972.1.23). Attached to the tibiotarsus (left) is a remnant of the patellar tendon containing a small osseous patella. When articulated, the patella sits within the patellar sulcus of the femur (right).

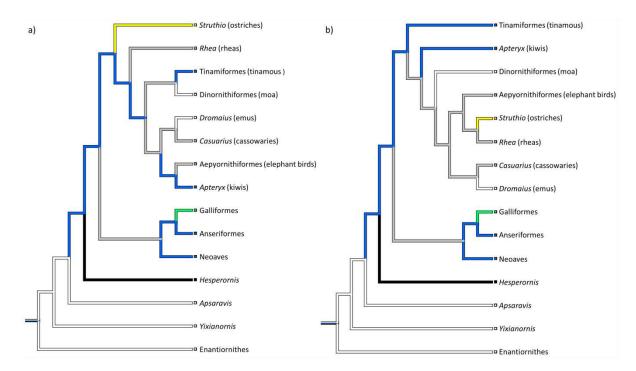


Figure 7: Phylogenetic trees constructed from the data of a) Hackett et al 2008, Harshman et al 2008, Phillips 2010, Smith 2013, and Mitchell et al 2014 and b) Livezey and Zusi 2007. When the evolution of the patella is reconstructed over these trees and those of other authors, the patella shows a single origin in birds (blue). Key: grey = ?/no data or ambiguous data; white = 0/absent; blue = 1/small flake of bone; green = 2/nodule of bone; yellow = 3/subrectangular block; black = 4/expanded triangular crest.

| Taxon | Patellar form (character state) |
|-----------------------------|---|
| Hesperornithiformes | Proximodistally elongate crest (4) |
| Neoaves | Small flake of bone (1) |
| Galloanserae | Rounded nodule (2) |
| Struthio (ostriches) | Large rectangular block with articular surfaces |
| | mediolaterally (3) |
| Rheiidae (rheas) | No data (?) |
| Tinamiformes (tinamous) | Small flake of bone (1) |
| Casuarius (cassowaries) | No data (?) |
| Dromaius (emus) | Absent (0) |
| Dinornithiformes (moas) | Absent (0) |
| Apteryx (kiwis) | Small flake of bone (1) |
| Aepyornithiformes (elephant | No data (?) |
| birds) | |

Table 1: Character state scores for patellar form in Palaeognathae, Neognathae and Hesperornithiformes, following the scores 0-4 described in the Methods. Basal outgroups to these taxa (e.g. *Apsaravis*, *Yixianornis*, *Yanornis*, Enantiornithes, other extinct birds) lack a patella (see text). Scores for taxa above the genus level were gauged by comparisons of multiple taxa within that clade and the polarity of characters within it (using published phylogenies as cited in the main text), where variation existed.