

1 **Evolutionary versatility of the avian neck**

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16

17 **Abstract**

18 Bird necks display unparalleled levels of morphological diversity compared to other
19 vertebrates, yet it is unclear what factors have structured this variation. Using 3D geometric
20 morphometrics and multivariate statistics we show that the avian cervical column is a
21 hierarchical morpho-functional appendage, with varying magnitudes of ecologically-driven
22 morphological variation at different scales of organisation. Contrary to expectations given the
23 widely-varying ecological functions of necks in different species, we find that regional
24 modularity of the avian neck is highly conserved, with an overall structural blueprint that is
25 significantly altered only by the most mechanically demanding ecological functions.
26 Nevertheless, the morphologies of vertebrae within subregions of the neck show more
27 prominent signals of adaptation to ecological pressures. We also find that both neck length
28 allometry and the nature of neck elongation in birds are different to other vertebrates. In
29 contrast to mammals, neck length scales isometrically with head mass and, contrary to
30 previous work, we show that neck elongation in birds is achieved predominantly by
31 increasing vertebral lengths rather than counts. Birds therefore possess a cervical spine that
32 may be unique in its versatility amongst extant vertebrates, one that, since the origin of flight,
33 has adapted to function as a surrogate forelimb in varied ecological niches.

34

35 **1. Introduction**

36 How, why and at what scale phenotypic variation arises in morphological structures
37 are amongst the most important questions in evolutionary biology (1). The avian neck is a
38 highly modular structure (2–9) that displays a wide array of morphological diversity. As the
39 forelimbs are dedicated to flight, the neck has adopted the role of aiding the beak in
40 environmental manipulation tasks (6,10-11). This phenotypic variation appears in many

41 forms throughout the avian neck; counts of cervical vertebrae vary between 10 and 26 (12-
42 13), as opposed to the count of seven found in almost all mammals (14–16). Avian vertebral
43 morphologies (3-10) and overall neck length (11) also display a wide diversity of form.
44 However, no previous body of work has quantitatively addressed the ecomorphological signal
45 in this variation, despite the clear functional significance and variability of the avian neck
46 (2,17-18).

47

48 Previous work on the vertebrate neck has provided insights into key innovations and other
49 traits that were pivotal to the diversification of major groups, such as neck elongation in
50 sauropod dinosaurs (19), neck retraction mechanisms in turtles (20) and patterns of vertebral
51 fusion across tetrapods (21). However, much previous investigation into the drivers of
52 phenotypic variation of cervical morphology has focused upon mammals (16,22–24), which
53 possess developmental and genetic restrictions on counts of cervical vertebrae (25-26),
54 potentially limiting the capacity for functional variation. In contrast, avian necks show great
55 capacity for evolutionary variation (3-15), but the effects of ecological adaptation and
56 intrinsic constraints on avian neck evolution have not been quantified. This represents a
57 major gap in understanding of phenotypic variation of the vertebrate neck and means that the
58 role that neck has played in the evolution of birds into one of the most taxonomically,
59 morphologically and ecologically diverse groups of vertebrates is therefore poorly
60 constrained.

61

62 A key unanswered question concerns the extent to which phenotypic variation of the avian
63 cervical column is driven by adaptive responses to extrinsic (ecological) factors, or by
64 intrinsic (scaling) constraints. This question has not been systematically addressed, due in

65 part to continuing disagreement about how the avian cervical column should be
66 compartmentalised into sub-regions (2,5,6,8–10,27) and subsequently compared across
67 species. Over the past 90 years the avian neck has been sub-divided into between 3 to 9
68 regions by different workers based on disparate methods, such as variation in joint motion (8-
69 9), qualitative comparative anatomy (3,6,27-28) and quantitative shape analysis techniques
70 (2,29). Crucially, in all these cases the link between regionalisation and the genetic and
71 development homology of the neck remains unclear. The boundaries between axial regions in
72 vertebrates are delineated by *Hox* gene expression limits (10,30). Recent work has shown that
73 these expression limits delineate five morphological regions within the cervical column of
74 *Gallus gallus domesticus* (10), raising the possibility that these five regions may be
75 homologous across all extant Aves. Issues concerning homology of individual vertebrae
76 among species with differing cervical counts could potentially be resolved using quantitative
77 information on morphological similarities to define regions, as a proxy of *Hox* gene
78 expression limits (10). However, the hypothesis that five cervical regions are present across
79 extant Aves has not been directly tested with a broad comparative sample.

80

81 Here, we apply a combination of 3D geometric morphometrics and phylogenetic comparative
82 methods to the cervical column of a diverse array of extant birds to investigate morphological
83 variation at multiple scales (whole-neck, regional and sub-regional) and its association with
84 key intrinsic (body size, neck length, head mass) and extrinsic (diet, locomotion) factors. Our
85 analysis recovers five morphological sub-regions, consistent with *Hox* gene expression limits
86 (10), in representative birds from all major extant taxonomic sub-groups, locomotor and
87 tropic ecologies. Analysis of this homologous 5-region structure highlights a highly scale-
88 dependent nature of phenotypic variation in the avian neck, with varying degrees of
89 ecological adaptation at macro to micro morphological scales. To our knowledge this is the

90 first quantitative demonstration of hierarchical ecologically-driven morphological
91 organisation of the vertebrate neck and suggests that similar assessments of anatomical
92 variation at different scales could provide important insight into diversity and adaptation in
93 the necks of other amniote groups, and indeed across the vertebrate skeleton.

94

95 **2. Methods**

96 **(a) Specimen digitisation and assessment of regionalisation**

97

98 Three-dimensional digital models were created for every cervical vertebrae (except the atlas,
99 C1) for 54 specimens (48 distinct species, Table S2; ESM2.xlsx) of extant birds, from
100 medical and microCT scans using Avizo 7.1 (Visualisation Science Group, supplementary
101 information). To characterise vertebral morphology we used the combination of 15
102 morphological landmarks and qualitative characters shown previously to delineate
103 morphological regions that are consistent with *Hox* gene expression limits in *Gallus gallus*
104 *domesticus* (10) (Fig S1, Table S1). For each individual bird, landmarked vertebrae were
105 subjected to a generalised Procrustes analysis to remove the effects of size and rotation using
106 MorphoJ. A suite of qualitative characters were recorded for each species, which recorded
107 vertebral shape change along the length of the cervical column that was not accounted for by
108 the landmark scheme (10). The Procrustes coordinates were then combined with the
109 qualitative characters of that species to produce a Principle Coordinates Analysis (PCO) plot
110 and cluster dendrogram via a Gower single-linkage algorithm in PAST 3.0 (31).
111 Regionalisation was then assessed based on groups of vertebrae that clustered together on the
112 dendrogram which displayed smaller distance measures with each other than to other
113 vertebrae, along with bootstrap values for that node. When support values were low candidate
114 homologies based on similarity of form were assessed from the PCO plots (10).

115

116 **(b) Explanatory variables**

117 Head mass was quantified digitally by using an α -shape fitting algorithm (32) on 3D models
118 of CT scanned skulls of all 38 species. α -shapes were fitted to skulls using an in-house
119 modified version of the ‘alphavol’ package in MatLab, which calculates the volume of the
120 computed α -shape. Head mass was estimated by multiplying the α -shape volume by the
121 weighted mean density of soft tissues within the skull (approximated to the density of water,
122 997 kg m^3) and normalised by taking the head mass as a percentage of total body mass. Body
123 masses were weighed directly when possible. When not possible, body masses were
124 estimated using scaling equations based on femoral length, minimal circumference of the
125 femoral shaft and humeral articulation facet on the coracoid (33), and an average was taken.
126 Neck length was measured digitally as the summed length of each individual cervical
127 vertebrae of each bird. Neck length was normalised by neck length/(body mass^{0.33}). Diets
128 were assigned to birds based on data from multiple volumes of ‘Handbook of the Birds of the
129 World’(34–44), locomotor mode was collated from the literature (45–47).

130

131 **(c) Phenotypic trajectory analysis**

132 A dataset consisting of the mean vertebral shape for each of the cervical regions for all birds
133 in the study was created from the results of the PCO and cluster analysis. This dataset was
134 then subject to an initial generalised Procrustes analysis and PCA within the R package
135 ‘geomorph’ (48). Phenotypic trajectory analysis (PTA) (49) was used to quantify ecological
136 and phylogenetic effects on shape across the entire cervical column. In this instance PTA
137 plots a trajectory through shape space for a specific group within a factor (flightless birds
138 within locomotor ecology, for example) by connecting the mean shape for each cervical

139 region for that specific group with the mean shape of the next region (from region 1 to region
140 2, then from region 2 to region 3 etc.) until all cervical regions are connected and form a
141 trajectory that represents the shape change across the entire cervical column.

142

143 **(d) Procrustes Distance Generalised Least-squares modelling**

144 Procrustes Distance phylogenetic Generalised Least-squares (D-PGLS) was used to model
145 relationships between mean regional vertebral shape and extrinsic factors (dietary ecology,
146 locomotory ecology, head mass, body mass, and neck length). This, and other phylogenetic
147 comparative methods, used a distribution of supertree topologies from previous analyses (50).
148 There is no AIC framework for D-PGLS. Therefore, models were compared based on
149 rankings of residual R^2 . The best model was determined to be the model with the lowest
150 residual R^2 whereby all factors included in the model had a significant P -value (< 0.05).
151 Redundancies among pairs of variables were evaluated by consideration of their R^2 and p -
152 values in models that contained both variables together.

153

154 **(e) Neck length allometry**

155 Data for head mass, neck length and body mass could be collected from 38 out of 52
156 specimens used throughout the previous sections. Phylogenetic generalised least squares
157 (pGLS) regression (51) was used to model scaling relationships between neck length, head
158 mass, and body mass in a phylogenetic framework in R using ‘nlme’ and ‘ape’ packages.
159 Pagel’s λ (52) was used with a freely varying parameter to assess the impact of phylogeny on
160 statistical models and to scale them accordingly. Models were compared based upon rankings
161 of AICc scores.

162

163 **(f) Phylogenetic ANOVA of region lengths and counts**

164 D-PGLS (in the R package ‘geomorph’ (48)) was used to assess the correlation between
165 regional counts of cervical vertebrae and intrinsic (size) and extrinsic factors (diet,
166 locomotion). The coefficients of these relationships were examined to assess the effect of
167 each factor on region size in each region. Region length was measured digitally as the
168 summed length of each individual cervical vertebrae for each of the five regions for each
169 specimen. D-PGLS was used to model relationships between region length (coded as a
170 multivariate factor) and extrinsic factors in a similar manner to methods presented in
171 ‘Correlates of intra-regional morphological variation across Aves’. The coefficients of these
172 models were used to observe the effect of each factor on region length for each region.
173 Models were compared based on rankings of residual R^2 .

174

175 **3. Results**

176 **(a) Conservatism in avian cervical regionalisation**

177 Five cervical regions can be identified across all species in the dataset using Principle
178 Coordinates Analysis (PCO) and cluster analysis (Fig 1a, see Methods, Table S1). Regions 2-
179 5 all display considerable variation in both vertebral counts and region lengths (Fig 1a). PCO
180 morphospace occupation of each region was conserved for each individual bird studied, with
181 each region occupying a distinct area of morphospace in all species, with regions 3-5
182 displaying some overlap (Fig 1b, d). Region 1 (always consisting of just C2) occupies the
183 most distinct region of morphospace when all birds are considered (Fig 1d) and regions 2 and
184 5 also occupy distinct areas of morphospace, albeit to a lesser extent. The large overlap in
185 morphospace occupation indicates that the third and fourth regions are more morphologically

186 similar to each other (Fig 1d). Comparisons of mean region shape data (PCA plots, Fig 1d)
187 across species reveals that a common pattern of shape change along the cervical column
188 exists among all extant birds and that each cervical region has identifiable features of
189 vertebral anatomy (Fig 1c).

190

191 **(b) Inter-regional cervical morphology correlates only with specialised extrinsic factors**

192 Phenotypic Trajectory Analysis allows pairwise comparisons of inter-regional vertebral
193 morphology for both ecological factors (diet, locomotion) and taxonomic groups (53,54) (Fig
194 2). Diet has little correlation with shape variation across the entire cervical column, with only
195 carnivores and insectivores recovered as significantly different from each other in trajectory
196 direction and shape ($P = 0.025$ and $P = 0.025$ respectively, Fig 2a-c). Carnivores have a
197 relatively enlarged, more upright neural spine of region 1 (Fig 2d), whereas regions 2 and 3
198 are similar between carnivores and insectivores (Fig 2d, e). Insectivores have a shallower
199 neural spine in region 4 when compared to carnivores (Fig 2d, e), whilst carnivores display
200 more variation in centrum length and height between regions 3 and 4 (Fig 2d, e). This pattern
201 is also observed between regions 4 and 5 of insectivores (Fig 2d, e).

202

203 Only two locomotor groups (soaring and continual flapping flight) showed statistically
204 significant differences in the PTA, and this difference was restricted to trajectory direction (P
205 = 0.045, Fig 2b, 2f, 2g). Continual flappers have a shorter neural spine than soaring birds and
206 display less inter-regional variation between regions 3 and 4 (Fig 2f, g). Some features of
207 regional morphological variation are specific to comparisons of soarers and continual
208 flappers, with the angle of orientation of the prezygopohyseal articular facet changing to a
209 greater degree between all 5 regions, as well as the cranocaudal enlargement of the neural

210 spine of region 1 in soarers (Fig 2f, g). Taxonomic groupings displayed no significant
211 differences between all three trajectory descriptors (Fig 2f, g).

212

213 **(c) Finer-scale (intra-regional) cervical morphology correlates with extrinsic factors**

214 We used D-PGLS to model the effect of both intrinsic (body mass, neck length, head mass)
215 and extrinsic (diet, locomotion) factors on the mean shape of each individual cervical region
216 while accounting for shared evolutionary history (Table S3, Tables S6-10). Models were then
217 compared based upon minimal residual- R^2 rankings (see Methods for more information).
218 Here we summarise the significant findings for each cervical region. More detailed
219 description of these results can be found in the supplementary information.

220

221 Vertebral shape in regions 1 and 2 are best explained by a combination of neck length and
222 intermittent bounding (Table S3, S6). In region 1 with intermittent bounding having
223 considerably greater R^2 than neck length, while this is reversed in region 2. In region 1,
224 intermittent bounding is associated with a tall neural arch and a cranially shifted centrum (Fig
225 S3). Increases in neck length are associated with an elongation of the centrum and a flattening
226 of the neural spine in region 2 (Fig S2a, b). Intermittent bounding is associated with a
227 heightened neural spine and a flattened centrum in region 2 (Fig S3). Vertebral shape in
228 region 3 is best explained by a model containing neck length, flightlessness and carnivory
229 (Table S3, S8). Within this model, flightlessness displayed the highest value of R^2 and this
230 ecology is associated with an elongated centrum and an increased height of the neural spine
231 (Fig S3). Increases in neck length are associated with an elongation of the centrum and a
232 flattening of the neural spine (Fig S2a, b). Vertebral shape variation in region 4 is best
233 explained by a model that contains neck length, flightlessness, intermittent bounding and

234 carnivory (Table S3, S9). Within this model, carnivory possesses the highest R^2 value (Table
235 S3, S9). Increases in neck length are associated with a deepening and elongation of the
236 centrum as well as an increase in neural spine height (Fig S2a, b), while carnivory is
237 associated with a shortened centrum and an increase in neural spine width and height in
238 region 4 (Fig 2d). The highest-ranking model (Table S3, S10) that explains vertebral shape in
239 region 5 contains body mass, intermittent bounding and carnivory. Carnivory displays the
240 highest value of R^2 within this model (Table S3, S10). Increases in body mass are associated
241 with an increase in robusticity and height of the neural spine and centra (Fig S2a, b).
242 Carnivory is associated with a dramatic increase to the width and height of the neural spine,
243 which is also angled more cranially compared to the species average (Fig 2c, d).

244

245 **(d) Isometric scaling of neck length in birds but with considerably variability**

246 pGLS models recover statistically significant isometric relationships between neck length and
247 body mass (Fig 3a) and head mass (Fig 3b) in our sample of birds, but with considerable
248 scatter in the data (Fig 3, Tables S4, S11). Model comparisons reveal that neck length
249 variation is best explained by a model that contains head mass only ($AICc = -6.90$, Table S4).
250 The coefficient of head mass in this model indicates isometric scaling of head mass with neck
251 length (coefficient = 0.319, CI = 0.066, Tables S4, S11; Fig 3b), and Pagel's λ indicates
252 strong phylogenetic signal ($\lambda = 1.009$, Tables S4, S11). A model that also includes
253 insectivory is also relatively well-supported ($AICc = -5.02$, Tables S4, S11) and has a
254 marginally-significant coefficient indicating that insectivorous birds have a generally longer
255 neck length than other birds in the study (coefficient = 0.141, SE = 0.068). The relationship
256 between neck length and body mass becomes non-significant when included in a model with
257 head mass. The same is true for flightlessness (Tables S4, S11).

258

259 **(e) Mechanisms of avian neck elongation**

260 D-PGLS was used to model the relationships between region lengths and regional vertebral
261 counts with intrinsic scaling factors and extrinsic ecological parameters. Model comparisons
262 were used to assess which models best explained variation in the data. Region lengths are
263 best explained by a combination of neck length and soaring (residual $R^2 = 0.5676$, Tables S5,
264 S12). Within this model, neck length has more explanatory power than soaring does (R^2_{neck}
265 $\text{length} = 0.3031$, $R^2_{\text{soaring}} = 0.1374$, Tables S5, S12). The coefficients of the individual regions
266 from the region lengths \sim neck length model reveal that neck elongation is primarily achieved
267 by increases to the lengths of vertebrae in regions 2-4, and especially by increases in region 3
268 (region 3 coefficient = 8.6715, Tables S5, S12). Soaring birds appear to display a strong
269 decrease in the lengths of vertebrae in region 3, which is accounted for by a sharp increase of
270 lengths in region 4 (region 3 coefficient = -6.9142, region 4 coefficient = 6.4572, Tables S5,
271 S12). Models that contain body mass and head mass separately are significant but are less
272 supported than models containing neck length (residual $R^2_{\text{body mass}} = 0.8272$, residual R^2_{head}
273 $\text{mass} = 0.8318$, Tables S5, S12).

274

275 Region counts were best explained by soaring alone (residual $R^2 = 0.8884$, Tables S5, S13).
276 Coefficients reveal that vertebral counts are decreased in the third region of soaring birds and
277 this is accounted for by an increase in counts to region 4 (region 3 coefficient = -2.1939,
278 region 4 coefficient = 2.1990, Tables S5, S13). Frugivory also has a significant relationship
279 with regional counts of vertebrae but this model is less supported, and when frugivory is
280 combined with soaring in a single model, frugivory becomes redundant ($P = 0.07$, Tables S5,
281 S13). No intrinsic scaling factors had a significant correlation with regional vertebral counts

282 ($P = > 0.05$, Tables S5, S13), this indicates that neck elongation in Aves is not achieved by
283 additions to vertebral counts.

284

285 **4. Discussion**

286 Our analyses highlight that the avian cervical column is a hierarchical morpho-
287 functional appendage, with varying magnitudes of phenotypic variation at different scales.
288 We find that patterns of shape variation across the entire neck as well as vertebral counts are
289 not matched by high levels of variation in overall construction and regional modularity of
290 neck. The phylogenetically broad and ecologically diverse sample of birds studied here all
291 show five homologous regions (Fig 1), characterised by a similar pattern of shape change
292 between all regions (Fig 2 a-b) and few significant correlations between overall neck length,
293 region lengths and ecology (Tables S4, S5, Fig S2). Only mechanically demanding ecological
294 behaviours appear to be associated with statistically significant modifications to this universal
295 structural and morphological blueprint (Fig 2 c-g). Our results also reveal, contrary to
296 previous expectations (5,6,55,56), that lengthening of vertebrae rather than cervicalisation
297 (the addition of vertebrae to the neck) drives neck elongation in birds, and that neck length
298 scales isometrically with both body and head size (Fig 3) with little ecological signal (Fig S2,
299 Table S4). In spite of this overall conservation of neck architecture, our analyses of intra-
300 regional osteological variation indicate that intrinsic and particularly extrinsic factors do exert
301 significant adaptive morphological changes (Fig S2, Table S3), representing finer-scale
302 modifications to the generalised avian cervical system.

303

304 **(a) The avian neck: a hierarchical morpho-functional structure**

305 Our finding that birds of diverse taxonomic affinity, and varied locomotor and dietary
306 ecology, share the same five cervical regions (Fig 1) suggests that regional organisation may
307 be homologous across all extant Aves. In contrast, crocodylians and basal non-avian dinosaurs
308 have been shown to possess only four cervical regions (11), and new analysis of two non-
309 avian theropods (an allosauroid and a dromaeosaurid, Fig S4) also recovers four regions in
310 these taxa. This new data suggests that evolution of five cervical regions may be an avian-
311 specific synapomorphy, however further work investigating the regionalisation of the
312 theropod cervical column is needed to confirm this. The timing and selective pressures
313 behind the evolution of a fifth cervical region in birds remains unknown, but it is tempting to
314 speculate that increased regional differentiation in the neck may be causatively associated
315 with expanded locomotor and dietary ecology in birds compared to their non-avian theropod
316 ancestors. Indeed, previous work suggests that a shift away from carnivory may have
317 facilitated shifts in cervical count in herbivorous theropods (57). Alternatively, selective
318 pressure exerted on the cervical system by the evolution of flight, as the neck took over from
319 the forelimbs as the primary appendage for environmental manipulation, may have driven the
320 evolution of the five-region system seen in modern birds.

321

322 PTA demonstrates that inter-regional morphology across this five-region cervical system is
323 highly conserved across the majority of dietary and locomotor modes used by extant birds
324 (Fig 2). The neck of vertebrates primarily supports the head; providing it with a degree of
325 movement and allowing the head to partake in a multitude of functional tasks (feeding,
326 vigilance, conspecific interaction etc.) (58,59). In this regard the head and neck may act
327 together to allow the head to be utilised as a ‘hand’ in order to interact with the environment
328 in the stead of forelimbs that are primarily adapted for flight. Results from the PTA (Fig 2)
329 lend credence to this hypothesis as patterns of morphological variation across the entire

330 cervical spine as a whole are conserved across the majority of species studied, suggesting that
331 these patterns may be adapted for providing the neck with generalised kinematics. Birds share
332 patterns of cervical kinematics for many activities and the conservative nature of
333 regionalisation and inter-regional variation found herein provides the morphological evidence
334 the avian neck, generally, may be adapted to the ‘economics of continuous movement’ than
335 to any specific ecology or behaviour (4,6,55,56). Alternatively, the retainment of consistent
336 overall morphological blueprint across most ecological groups may represent constraints
337 imposed a conserved pattern of *Hox* gene expression, although that modifications have
338 evolved in response to mechanically demanding neck functions (Fig 2) suggests it is most
339 likely a product of both genetic and functional influences.

340

341 Within dietary categories, only carnivores and insectivores differ from each other in their
342 macro-level (inter-regional) morphology (Fig 2 d-e). That these two particular ecologies are
343 associated with larger-scale modular changes to the neck is consistent with the fact that they
344 require ‘extreme’ and also juxtaposing mechanical demands: carnivory requires relatively
345 slow but forceful retraction to tear flesh from prey (18) (a force or work based system
346 retraction), while insectivory involves relatively high velocity protraction or extension of the
347 neck to help capture escaping prey (60) (a velocity or power based system of extension).
348 Carnivorous birds have significant association with cervical morphology across both inter-
349 (Fig 2) and intra-regional (Fig S2) analyses, (Table S3), and this effect is concentrated on
350 regions 4 and 5 of the cervical spine (Table S3). Large retraction forces required during the
351 ‘pull’ phase of feeding, as flesh is torn from the prey (18,61,62), are generated by muscles of
352 the *M. longus colli dorsalis* complex (62,63) which attach to the neural spines of vertebrae in
353 regions 4 and 5. The increased height of the neural spines of vertebrae in these regions (Fig
354 2d) may serve to increase the moment arm and attachment area of these muscles to power

355 carnivorous cervical kinematics, as well as increasing stiffness at the base of the neck (64,65)
356 , to provide stability during the ‘pull’ feeding phase. Such adaptations to enhance the force
357 and torque generating capacity of retractors muscles are likely to be relatively redundant in
358 insectivores, and indeed may actually be disadvantageous to both rapid neck extension and,
359 in some taxa, rapid flight (66). Recent work has suggested variation exists in the neck
360 musculature of vultures (17) and it would therefore be interesting to examine finer-scale
361 adaptations in cervical system within groups such as carnivores (e.g. to assess potential
362 adaptive responses to different prey types and modes of carnivory, such as predation versus
363 scavenging).

364

365 Within locomotor categories, only soaring and continual flapping birds differ from each other
366 in their macro-level (inter-regional) morphology (Fig 2 f-g). These two groups differ only in
367 trajectory direction with the PTA analysis, suggesting their inter-regional disparity is less
368 than seen between carnivores and insectivores, which differ in both trajectory direction and
369 shape (Fig 2). Vision must be stabilised during flight and this is achieved by oscillating
370 movements of the neck and head that counteract each wingbeat (67,68). Soaring birds flap
371 less during flight than continual flappers, and our results show they display more
372 morphological differentiation (Fig 2f) compared to the all-birds model (Fig 2c) than continual
373 flappers do (Fig 2g). This may be an indication that compensatory movements of the neck
374 that stabilise vision during flight may constrain vertebral morphology to an extent.

375

376 While at the macro-scale we find a conserved pattern of modularity and inter-regional
377 morphology (Figs 1-2), it is clear that phenotypic variation is present in the avian neck at the
378 intra-regional scale and this diversity is correlated with intrinsic and, uniquely, extrinsic

379 ecological factors. Indeed, our statistical analyses suggest that variation in intra-regional
380 morphology correlates more widely with extrinsic than intrinsic factors (Table S3), which (to
381 our knowledge) represents the first quantitative demonstration of ecologically associated
382 morphological variation in the avian cervical column.

383

384 Neck length (intrinsic) and carnivory, flightlessness and intermittent bounding (extrinsic) are
385 consistency correlated with intra-regional morphological variability in the neck (Table S3,
386 Fig 2d, Fig S3), suggesting adaptive responses in osteology to these factors. These extrinsic
387 ecological parameters appear to have the strongest correlation with vertebral morphological
388 variation in regions 1 (intermittent bounding), 3 (flightlessness), and 4 and 5 (carnivory).
389 Neck length shows the single strongest correlation in region 2. As in the case of carnivory,
390 cases in vertebral morphology associated with intermittent bounding and flightlessness also
391 appear to represent logical adaptations to the mechanical demands placed on the neck in these
392 behaviours. During flight birds must stabilise their gaze in order to safely and efficiently
393 navigate their flight path (69). Intermittent bounding involves an active flapping phase
394 followed by a passive phase whereby the wings are folded and the bird follows a ballistic
395 trajectory, thus vertical oscillations are introduced into the flight path (70). To maintain a
396 stable gaze the head must be able to counteract these movements, and do so via dorsal head
397 flexors such as *M. complexus*, *M. rectus capitis dorsalis* and *M. longus colli dorsalis pars*
398 *cranialis* (67,68). The neural spines of vertebrae in cervical regions 1 to 3 are heightened in
399 intermittent bounders, and as many of these dorsal head flexors attach to this feature of
400 vertebral anatomy (71), this may represent an adaptive response to counteracting the
401 oscillations during bounding flight. Terrestrial locomotion requires the neck to stabilise
402 vision whilst traversing variable terrain and at fluctuating speeds, and as such a combination
403 of passive ligament support and active muscle force activation across the entire neck is

404 required (72,73). Flightless birds possess robust vertebral morphologies across the entire
405 cervical spine and this may be an adaptation to providing a larger attachment area for
406 multiple neck- and head-supporting to provide adequate vision stabilisation during terrestrial
407 locomotion. This hypothesis is supported in part by previous work finding that multiple
408 species of flightless birds possess enlarged cervical muscles across the entire neck (6,74).
409 Flightless birds are also not constrained by selective pressures for weight reductions
410 necessary for flight and this may at least partly explain their more robust cervical
411 musculoskeletal system.

412

413 **(b) The unique nature of avian neck elongation, its constraints and variability**

414 Avian neck length scales isometrically with head mass and body mass (Fig 3, Table S4). This
415 differs from other groups of vertebrates (e.g. negative allometry in mammals (24)), as was
416 also reported in other recent work (11). Here we show that increased vertebral length is the
417 primary mechanism by which neck elongation occurs (Table S5), rather than through the
418 addition of vertebrae as might be supposed given the high levels of variation in cervical
419 vertebrae number across extant birds. In other groups of vertebrates (mammals) it is the
420 weight of the head that is the predominant constraint upon neck length, as head mass scales at
421 a faster rate than the cross-sectional area of the neck which must resist the stress of the
422 weight of the head (75–79). Our findings suggest that this constraint appears to be removed in
423 birds, as both head mass and neck length scale isometrically with body mass and with each
424 other (Table S4, Fig S2). Head mass is reduced in birds due to the negative scaling of brain
425 and eye size with body mass (80,81), the reduction of jaw musculature (as food processing
426 occurs in the gizzard (82)) and the widespread pneumatisation of the skull (83).
427 Morphological adaptations of vertebrae may also contribute to the release of constraint in

428 neck length. Vertebrae in the mid portion of the neck display multiple adaptations to
429 increased intervertebral flexion in response to increasing neck length and these features allow
430 the neck to achieve the ‘S’ shaped curvature seen across Aves (5). This curvature allows the
431 mass of the head to be held closer to the centre of mass, and in tandem with the lightweight
432 head, this combination of craniocervical traits allows for a variety of head shapes and sizes to
433 be supported by an elongated neck, overcoming the constraint of head mass that is present in
434 many other vertebrates. Some additional discussion of the weak association between neck
435 length and ecology is presented in the supplementary information.

436

437 While isometric scaling of the neck with respect to body and head mass is recovered as
438 statistically significant (Table S4), there is clearly considerable variation in the data and this
439 is only partially explained by the intrinsic and extrinsic variables assessed here (Fig 3, Tables
440 S4, S11). Our analyses consistently recover strong phylogenetic signals in regression models,
441 suggesting that phylogenetic history may explain a sizeable portion of observed variation in
442 cervical morphology. Variables not considered in this study may also explain at least some of
443 the variability in our data. For example, a recent study recovered a relationship between neck
444 length and leg length in birds, and suggested this relationship was a product of the need to
445 maintain a neck length capable of allowing the head to reach the ground (11). It is perhaps
446 likely that variation in relative neck lengths we observe here (Fig. 3) are reflective of the
447 multiple intrinsic and extrinsic selective factors acting upon the avian neck, which must
448 function as a multi-purpose surrogate arm (see previous section).

449

450 Cervicalisation (increases to the number of cervical vertebrae) was previously thought to be
451 responsible for neck elongation in birds (5,6,55,56). This hypothesis can now empirically be

452 rejected as there is no significant relationship between cervicalisation and neck length (Table
453 S5). Alternatively, our data suggests that vertebral elongation is the primary mechanism by
454 which neck elongation occurs, specifically it is increases in the length of vertebrae in all
455 regions except region 1 that are the epicentres of neck elongation across Aves (Table S5).
456 This is in contrast to the more localised method by which mammalian neck elongation
457 occurs, as it is vertebrae from just the middle portion of the neck length that lengthen (24).
458 Our results therefore suggest that birds are not only unique in showing morphological
459 responses to extrinsic ecological factors at multiple hierarchical levels, but also in their
460 patterns of neck length allometry and elongation. Amalgamated, these results suggest that
461 birds possess a cervical spine which is unique in its construction and elongation amongst
462 vertebrates, one that has adapted to the burden of becoming a surrogate forelimb as well
463 specialising under ecological pressures.

464

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481

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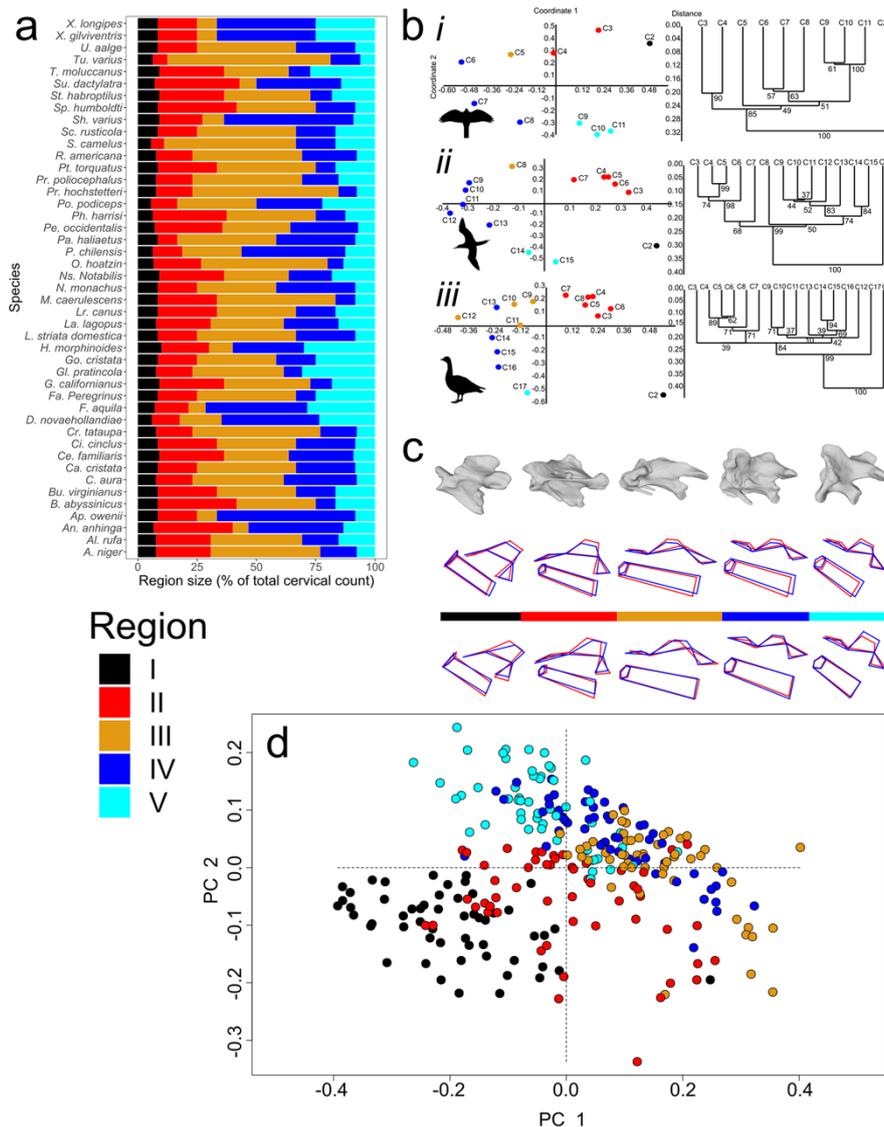
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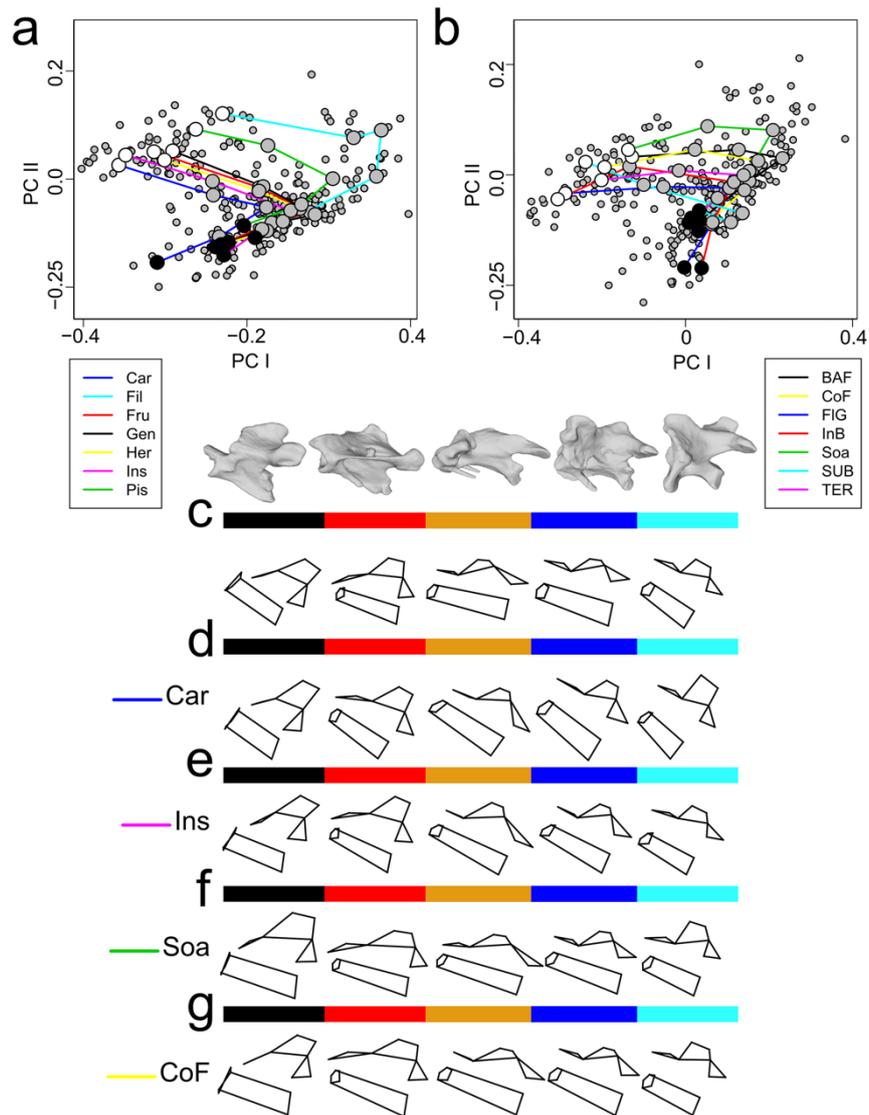
674

675 **Figure 1.** Conservatism in avian cervical regionalisation. A) Region size (as a normalised
 676 measure of percentage of total cervical vertebrae) variation for all birds studied. Colours
 677 denote region number. All extant birds have five cervical regions. Regions 1 and 5 are stable
 678 in their number of vertebrae, whereas regions 3 and 4 display the largest variations in
 679 vertebrae per region. B) Principle Coordinate graphs (left – most show proportions of
 680 variance explained by each axis on the axis labels) and cluster analysis charts (right)
 681 depicting the delineations between cervical regions in 3 taxa; i) *Hieraaetus morphnoides*, ii)
 682 *Sula dactylatra*, iii) *Branta leucopsis*. Colours on Principle Coordinate graphs denote cervical
 683 regions. Numerical values underneath cluster branches denote bootstrap support after 1000
 684 replicates. Despite changes to total number of cervical vertebrae and ecology, all birds
 685 display 5 cervical regions when PCO and cluster analyses are used together to designate
 686 regions. C) upper; shape change across PC1 for all 5 regions (the colour bar indicates region
 687 number, cranial regions are on the left), with CT images of vertebrae from each region above
 688 (species: *Alectoris rufa*). Red outline denotes mean shape, blue outline displays the maximum
 689 shape change across PC1. C lower; Shape change across PC2 for all 5 regions (colour
 690 notations are as in B). Region 1 is defined by an antero-caudally restricted centrum length, a

691 deepened centrum, a tall neural spine, and small prezygopophyses with cranially facing
692 articular facets. Region 2 retains the enlarged neural spine but displays an elongated, thinner
693 centrum and larger more cranially positioned prezygopophyses. Region 3 displays the
694 smallest neural spine of all 5 regions, as well as the most elongate centrum, with the articular
695 facets of the prezygopophyses facing dorsocranially, whilst the facet of the postzygapophyses
696 are oriented ventrocaudally. Neural spine height increases slightly within region 4, whilst the
697 centrum is shorter and deeper than in region 3, and the articular facets of the
698 prezygapophyses are more dorsally oriented in region 4. Region 5 displays a larger neural
699 spine still, with a shorter and deeper centrum, the articular facet of the prezygapophyses face
700 more cranially than in region 4. D) PCA of mean regional vertebral shape for all birds. For all
701 birds, shape change along the first principal component involves a variation in the height of
702 the neural spine, rotation of both pre- and postzygophyseal articulation facets, and a
703 cranocaudal variation in the length of the centrum.

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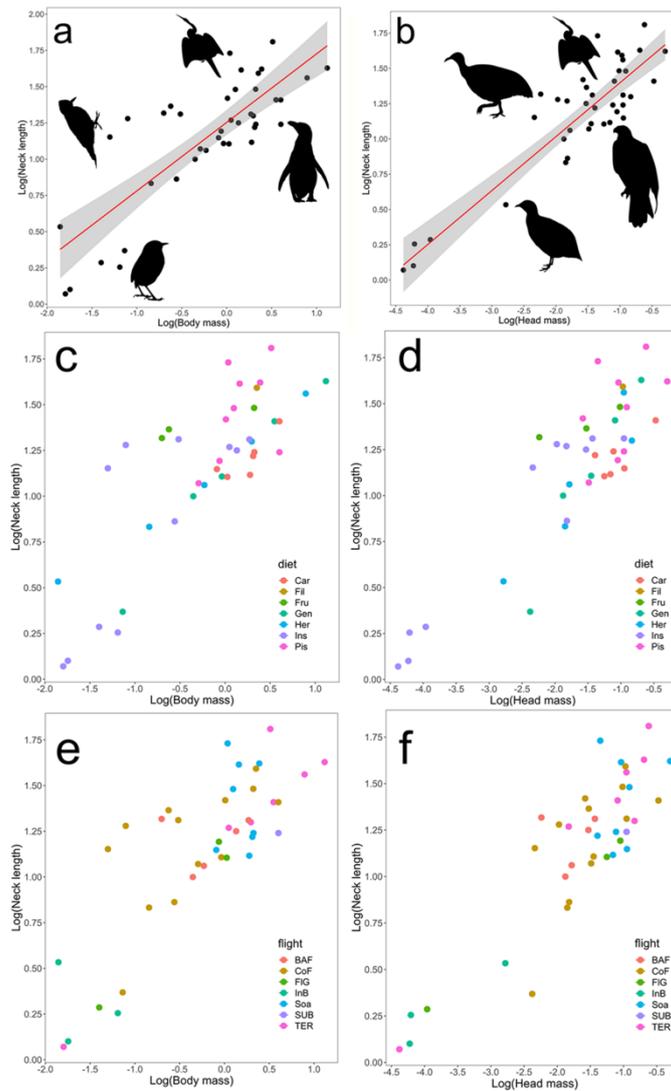
706

707 **Figure 2.** Inter-regional cervical morphology correlates only with extrinsic factors that have
 708 specialised cervical kinematics. A) Phenotypic trajectories of dietary ecologies. Colours
 709 denote diet. Car = carnivory, Fil = filter feeding, Fru = frugivore, Gen = generalist, Her =
 710 herbivore, Ins = insectivore, Pis = piscivore. PTA analyses shows that despite large
 711 differences in dietary ecology, the gross morphology of the entire cervical column does not
 712 change (except between ecologies that are extremely divergent, see table S5 below). Black
 713 circles represent the group mean region shape for region 1, white circles represent group
 714 mean region shape for region 5. Grey circles represent the group mean region shape for
 715 regions 2-4. B) Phenotypic trajectories of different flight styles. Colours denote flight. BAF =
 716 burst-adapted flight, CoF = continual flapping, FIG = flap gliding, InB = intermittent
 717 bounding, Soa = soaring, SUB = subaqueous, TER = terrestrial. As for dietary ecologies (Fig
 718 5 above), locomotory mode (flight style) has little impact on gross morphology across the
 719 entire cervical column (except in extremely divergent taxa, see table S5 above). C-G) Mean
 720 regional shape change across the cervical column with CT images of vertebrae from each
 721 region above (species: *Alectoris rufa*). Lateral view of each region mean vertebral shape,
 722 color indicates region, cranial regions are towards the left, caudal regions are to the right. C)

723 Mean region shapes for all birds, D) mean region shapes for carnivorous birds, E) mean
724 region shapes for insectivorous birds, F) mean region shapes for soaring birds, G) mean
725 region shapes for continual flapping birds. In ecologies that are extremely divergent, the
726 vertebral morphology across the entire cervical column changes.

727

728



729

730 **Figure 3.** Avian neck length scaling is predominantly isometric. Neck length scales with
 731 body mass (a) and head mass (b) according to isometry. Inset silhouettes (clockwise
 732 beginning at noon) a) *Anhinga anhinga*, *Spheniscus humboldti*, *Xenicus logipes*, *Sphyrapicus*
 733 *varius* and b) *Anhinga anhinga*, *Haliaeetus morphnoides*, *Crypturellus tautapa*, *Agelastes*
 734 *niger*. Extrinsic factors (c-d diet, e-f locomotor mode) have a limited effect on neck length
 735 scaling with body mass (c, e) and head mass (d, f). Flightless birds have a significantly
 736 different pattern of neck length scaling with both body mass and head mass, with both
 737 relationships displaying a negative allometric signal. Insectivorous birds also display a
 738 negative scaling relationship between neck length and head mass (f). Coloured lines denote
 739 ecology. Car = carnivorous, Fil = filter feeder, Fru = frugivore, Her = herbivore, Ins =
 740 insectivore, Pis = piscivore, BAF = burst adapted flight, CoF = continual flapping, FIG = flap
 741 gliding, Soa = soaring, SUB = subaqueous, TER = flightless.

742