



Functional and evolutionary consequences of cranial fenestration in birds

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Ostrich-like birds (Palaeognathae) show very little taxonomic diversity while their sister taxon (Neognathae) contains roughly 10,000 species. The main anatomical differences between the two taxa are in the crania. Palaeognaths lack an element in the bill called the lateral bar that is present in both ancestral theropods and modern neognaths, and have thin zones in the bones of the bill, and robust bony elements on the ventral surface of their crania. Here we use a combination of modeling and developmental experiments to investigate the processes that might have led to these differences. Engineering-based finite element analyses indicate that removing the lateral bars from a neognath increases mechanical stress in the upper bill and the ventral elements of the skull, regions that are either more robust or more flexible in palaeognaths. Surgically removing the lateral bar from neognath hatchlings led to similar changes. These results indicate that the lateral bar is load-bearing and suggest that this function was transferred to other bony elements when it was lost in palaeognaths. It is possible that the loss of the load-bearing lateral bar might have constrained diversification of skull morphology in palaeognaths and thus limited taxonomic diversity within the group.

KEY WORDS: Adaptive radiation, avian evolution, cranial morphology, fenestration, finite element modeling.

Modern birds are split into two groups, the Neognathae and the Palaeognathae (ratites and tinamous). The Neognathae consists of roughly 10,000 species that are morphologically and taxonomically very diverse. Conversely, the Palaeognathae consists of only about 60, very similar species (Livezey and Zusi 2007; Hackett et al. 2008; Phillips et al. 2010). Although this split is generally recognized, there is very little insight in the evolutionary processes that have led to this bifurcation. Although we cannot identify the

evolutionary forces that drove the bifurcation itself, we present evidence for the selective pressure that may have been at play and influenced further diversification by investigating the functional implications of characters that differ between the two groups.

Differences between the two groups seem obvious, as all the well-known ratites are flightless and most are very large. Their typical characters such as large legs, small wings, and loss of a keeled sternum are, however, not unique to ratites, as they are also

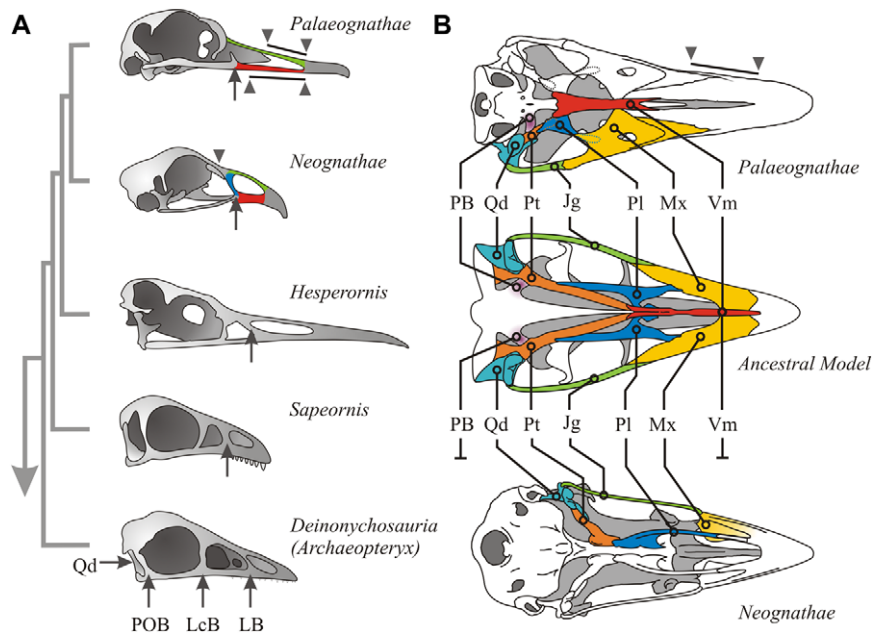


Figure 1. Palaeognaths differ from neognaths in the morphology of the ventral cranial elements and the absence of the lateral bar of the upper bill. (a) A simplified avian phylogeny (based on Xu et al. 2001; a more complete overview is given in Fig. S1) showing progressive reduction of lateral cranial elements. Basal in the avian lineage four bars are present: the quadrate (Qd), the postorbital bar (POB), the lacrimal bar (LcB), and the lateral bar of the upper bill (LB). The arrows in the derived exemplars indicate the lateral bar. The bill of birds consists of a single medial dorsal bar (green), and laterally symmetrical ventral (red) and lateral bars (blue). In neognaths, the quadrate and lateral bar remain intact, while in palaeognaths only the quadrate remains intact. Triangles and lines indicate flexible zones in the upper bill. Most neognaths have a single nasal-frontal hinge at the base of the skull; palaeognaths have long flexible zones along the upper bill. (b) The general differences between the skulls of palaeognaths (*Rhea americana*) and neognaths (*Gallus gallus*) in comparison to a general model of the ancestral state (based on *Archaeopteryx*; Elzanowski 2001). Palaeognaths have small and thick pterygoids (Pt, orange, dotted lines indicate dorsal extension of the bone), short and broad palatines (Pl, light blue, dotted lines indicate dorsal extension of the bone), thick short jugals (Jg, green), broad thin maxillae (Mx, yellow), a well-developed vomer (Vm, red) and extension of the cranial base (proc. basipterygoidei, PB, purple). Quadrates (Qd) are indicated in turquoise.

found in flightless neognathous birds. In addition, the majority of species in the Palaeognathae are the lesser known tinamous, which are roughly chicken sized, fly, and do not show the characters linked to flightlessness. The only truly discriminating morphological characters between the Palaeognathae and Neognathae that are not related to flightlessness are within the skull and have given rise to the name of the two taxa (*neo* = new, *palaeo* = old, *gnathae* = jaws; Huxley 1867; Gussekloo and Zweers 1999).

It is generally accepted that birds have their origin within the dinosaur maniraptoriformes lineage, and we can easily define an ancestral model for the skull (Fig. 1a). The ventral skull bones (pterygoids, palatines) in the ancestral model would have been robust, immobile, and with suture-like articulations. The pterygoids would be linked to cranial base via the processus basipterygoideus as is clearly observed in early theropod dinosaurs (Frazzetta and Kardong 2002). A vomer with connections to the maxillae would also have been present. The lateral aspect of the skull would contain a well-developed postnasal bar, mainly consisting of the maxilla, and at least a lacrimal and postorbital bar that connect the dorsal and ventral aspects of the skull (Xu et al. 2011; Fig. 1a).

Skulls of recent birds differ from this ancestral model in facial shortening, reduction of circumorbital bony elements, and general thinner bones, presumably because of the effects of neoteny (Bhullar et al. 2012, 2016), which has led two different morphologies in the Neognathae and Palaeognathae. The main cranial differences between these two major avian clades are in the morphology of the ventral skull bones (pterygoids, palatines) and the absence of the postnasal bar (Fig. 1).

The ventral skull bones of neognaths are very slender and are connected with clear moveable joints. In palaeognaths, these elements are very robust and elements are connected with rigid sutures. In addition, the palaeognathous skull has a sturdy basipterygoid process and a well-developed vomer that connects with sutures to other ventral bones. These last two characters were also present in the ancestral condition, but are absent in neognathous birds (Fig. 1; McDowell 1948; Bock 1963; Gussekloo and Zweers 1999; Zusi and Livezey 2006). Remnants of the vomer might be present in neognathous birds, but then it is strongly reduced, not directly connected to the upper bill and can be considered vestigial.

The postnasal bar, or lateral bar of the upper bill (Zusi 1984), connects the upper and lower part of the upper bill just behind the nostril (nares) and consists in recent birds of a downward pointing process of the nasal bone (proc. maxillare of the os nasale) and an upward pointing process of the maxilla (proc. nasalis of the os maxillare; Zusi 1984). In neognathous birds, these two processi connect to form a full bar, but in palaeognaths they do not merge, although in tinamous they can be connected by non-ossified tissue resembling a tendon.

Both the ventral skull bones and the lateral bar can be linked to cranial kinesis, which is the ability to move a part of the skull relative to the braincase. Cranial kinesis is considered an evolutionary advantage associated with improved feeding behavior as a result of increased jaw closing speeds (Herrel et al. 2000), increased biting force (Zweers et al. 1997), or improved food handling (Kooloos 1989; Zweers and Gerritsen 1997). In birds, two main types of kinesis are distinguished based on which part of the skull can move (Zusi 1984). In prokinesis, the whole upper bill, including the lateral bars, rotates around a hinge between the cranium and the upper bill (nasal-frontal hinge, Fig. 1). Prokinesis is the most common condition among extant birds and is considered the ancestral morphology (Bock 1964) as it is also described in the Late Cretaceous Avialan *Hesperornis* (Bühler et al. 1988). The early ancestors of modern birds, however, most probably did not show kinesis, as they still possess characters that limit movement of the ventral skull elements (e.g., postorbital and ectopterygoid bones; Holliday and Witmer 2008). In rhynchokinesis, only the tip of the upper bill moves relative to the cranium, while the proximal part of the bill remains stationary and straight. Rhynchokinesis is mainly observed in probing shorebirds for which it is advantageous to have a long bill that displaces only a small amount of substrate whilst catching buried prey (Gerritsen 1988; Zweers and Gerritsen 1997).

The morphology of the lateral bar is of great importance in cranial kinesis, because rhynchokinesis is only possible when the ventral bar of the upper bill can slide forward while the dorsal bar remains stationary. For this sliding to be possible the two bars cannot be rigidly linked by the lateral bar, as is the case in prokinetic birds. In neognathous birds that exhibit rhynchokinesis the lateral bars are flexible and functionally decoupled from the upper bar to overcome this problem (Zusi 1984). In addition, rhynchokinetic birds have very distinct thin zones in the upper bill where bending can occur (Gerritsen 1988; Gussekloo and Bout 2005a).

In both types of kinesis, the forces inducing the bill movement are generated by muscles located behind the eye. The muscle forces are transferred to the upper bill via the ventral skull bones (pterygoids, palatines), making movement of these bones of great functional importance. Although the ventral elements and their articulations show differences between palaeognaths and neognaths,

their movement trajectories are very similar (Gussekloo et al. 2001).

Because palaeognaths and neognaths differ in characters related to cranial kinesis, it was hypothesized that the mechanism behind the diversification between the two taxa must have been induced by differences in feeding behaviors and cranial kinesis (Hofer 1954; Simonetta 1960; Bock 1963). This was mainly based on the incomplete lateral bar and the flexibility of the upper bill in osteological specimens of palaeognathous birds. Because the flexible upper bill and the decoupling of the dorsal and ventral bar are also present in neognathous rhynchokinesis, it was concluded that palaeognathous birds must also show rhynchokinesis. There are, however, some clear distinctions with the neognathous morphology, so the palaeognathous condition was considered a special form and was thus described as central or palaeognathous rhynchokinesis (Bock 1964; Zusi 1984). Based on the observations that important differences between the neognaths and palaeognaths could be linked to cranial kinesis, it was hypothesized that the specific palaeognathous morphology was the result of selection toward a feeding mechanism that included rhynchokinesis (Bock 1963; Zweers et al. 1997).

Analyses of the feeding behavior of Palaeognathae, however, fail to reveal a significant function for cranial kinesis, or improvements in feeding performance compared with the standard feeding behavior of neognathous birds (Gussekloo and Bout 2005a,b). Additional analyses of the upper bill morphology and the forces acting on the bill showed no adaptations that would facilitate kinesis (Gussekloo and Bout 2005a). This makes it seem unlikely that the unique palaeognathous cranial morphology is an adaptation to feeding or kinesis, or that a difference in feeding behavior has caused the split between neognaths and palaeognaths.

Incorrect links between morphological characters and kinesis have also been made in the analysis of the early ancestors of modern birds. As mentioned above early theropods could not have been kinetic because movement of the ventral skull elements is limited (Holliday and Witmer 2008). However, these species do possess synovial joints between the quadrate and the neurocranium, and between the pterygoids and basiptyergoid (Holliday and Witmer 2008). Holliday and Witmer (2008) hypothesize that these are not actual joints, as proposed before, but transition zones between enchondral and intramembranous bone growth zones, and that the activity of the retractor muscles protect these growth zones from high stresses. This implies that some characters previously linked to kinesis might actually be the effect of growth and development.

Here we propose an alternative hypothesis in which we explain the difference in cranial morphology between Palaeognathae and Neognathae based on the evolutionary trends observed in the transition from dinosaurs to birds, and linked to development. Recent studies show bird skulls are paedomorphic compared to

dinosaurs (Bhullar et al. 2012, 2016) and palaeognaths skulls are peramorphic compared to neognaths (Cubo and Arthur 2001; Gussekloo and Bout 2002; Zusi and Livezey 2006; Maxwell 2009; Gussekloo and Cubo 2013). This could indicate that a difference in the developmental pattern of the skull could have resulted in the observed cranial differences.

Changes that are observed in the transition from dinosaurs to birds are a progressive reduction in size of the lateral and ventral skull elements and the development of new moveable joints (Bhullar et al. 2016). The reduction of lateral elements of the skull results in the loss of the postorbital bar, the upper temporal bar, and the loss of the connection between the lacrimal bar and the jugal. In the ventral aspect of the skull new synovial joints and bending zones emerge (for example between the quadrate and pterygoid, and between the pterygoid and basipterygoid), and bone elements close to the pterygoid (ectopterygoid and epipterygoid) disappear (Figs. 1, S1; Holliday and Witmer 2008; Hu et al. 2010; Xu et al. 2011; Bhullar et al. 2016).

All these changes led to a lighter, more mobile facial part of the skull, and it can be assumed that these changes played an important role in the development of kinesis in birds (Holliday and Witmer 2008; Bhullar et al. 2016). However, thinning and reduction of bony elements also leads to large differences in stress distribution, as has been shown previously in lizards (Moazen et al. 2009). This is important because different skull elements reduce in the transition from the ancestral state to either the current palaeognathous or neognathous morphology. First, in palaeognaths we do not observe the strong reduction of the vomer, as we do in neognaths, and the pterygoid-palatine joint that is clearly synovial in neognaths is sutured in palaeognaths. Second, palaeognaths, just like their non-avian dinosaur ancestors, still possess a sturdy basipterygoid process, while a homologous structure is completely absent in neognaths. Finally, the lateral (postnasal) bar, which is reduced in thickness in neognaths compared to the ancestral model, is, as a bony structure, completely absent in palaeognaths.

We assume that this reduction of lateral elements is a main factor in the further evolution of the avian skull, as the loss of these elements probably has a large impact on the stress distribution within the skull. We hypothesize that the loss of the lateral bar will increase mechanical stresses in other parts of the skull, and that this shift in stress distribution may have led to further morphological change. We used finite element (FE) models to focus on mechanical stresses because high mechanical stresses can lead to bone fracture, which, especially in the skull, could be lethal. Changes in mechanical stresses also lead to bone-remodeling, which can result in thicker (stronger) or thinner (more flexible) bones (Klein-Nulend et al. 2013). It is therefore plausible that stress avoidance is a strong selective force that might have led to adaptations in both avian groups. These kinds of stress avoiding

adaptations have earlier been shown using FE models, for example, in the bills of Darwin finches feeding on hard seeds (Soons et al. 2010, 2012, 2015), and previously FE studies showed that the development of a rhamphotheca resulted in better stress-distribution in the skull of maniraptoriform dinosaurs (Lautenschlager et al. 2013).

To evaluate the effect of the absence of the lateral bar we used chickens (*Gallus gallus domesticus*), a basal neognath (Hackett et al. 2008). Although the chicken is a modern and derived species that does not resemble the ancestral condition, we used this species because these animals can easily be kept under laboratory conditions, and our main question focused on the effects of lateral bar removal only, and less on the actual ancestral conditions.

Using an FE model of a chicken skull, we analyzed the mechanical stress distribution in the intact skull, and then reran the analysis with the lateral bars removed. Comparing the same model with and without the lateral bars gave us insight into the mechanical significance of the lateral bars without introducing additional variation. This is similar to a previous study done on lepidosaurs, where a change in elements in the FE model showed that bone reduction leads to decreased skull robustness (Moazen et al. 2009). The FE method has proven to be useful because it has been shown to be a good predictor of in vivo strain distributions in ostriches and Darwin's finches (Rayfield 2011; Soons et al. 2012; Cuff et al. 2015). We did two separate analyses. One simulated static biting, which can be directly linked to advantages in feeding, and a second simulated cranial kinesis. The second analysis was important because the palaeognathous morphology has been linked to a special form of cranial kinesis. By simulating elevation and depression of the upper bill, we gained insight into the effect of cranial kinesis on stresses in the skull in both the palaeognathous and neognathous conditions.

In conjunction with the FE models, we also tested the effect of a missing lateral bar in vivo. We tested whether removal of the lateral bar in newly hatched chickens resulted in morphological changes when the animals were full grown. Based on bone remodeling under mechanical loading, one would expect morphological changes in the regions of increased stress predicted by the models (Klein-Nulend et al. 2013).

Methods

FINITE ELEMENT ANALYSES

The FE models were constructed by assembling a stack of 812 serial computed tomography (CT) images of a skeletonized chicken skull (76 μm pixel size and 83 μm interslice spacing) using Mimics Software (Materialise, Plymouth, MI). We extracted each bony element as a separate water tight stereolithography (STL) file. The lower jaw was excluded for all our analyses. We edited

the STLs in Geomagic Studio (3D Systems, Rock Hill, SC) to repair scanning artifacts and minor imperfections in the bone surfaces and to define the extensive regions of trabecular bone within the skull. We used Geomagic Studio to bend the nasal-frontal hinge and repositioned all of the bones so that the beak was in a closed, resting position. From the primary model we created two models using Geomagic Studio, one to simulate static biting and one to simulate cranial kinesis (see below). The STLs of the two models were then imported back into Mimics for solid meshing. The initial model was about 1.2 million elements, which we reduced to about 765,000 elements to decrease calculation times. We found no apparent difference between the initial large model and the reduced model, even in the thinner areas of the lateral bars where larger elements would be most likely to negatively influence the results (Fig. S2). The four-noded, tetrahedral, solid elements models were imported into ANSYS APDL version 13.0 (Canonsburg, PA), where we manually selected elements to represent the nasal-frontal hinge. Cortical bone was assigned a Young's modulus (E) of 13.5 GPa based on data from ostrich and chicken (Reed and Brown 2001). Because data on trabecular bone of birds were not available, we used data of mammals (Ding et al. 2005) and assumed that the ratio between the Young's modulus of cortical and trabecular bone (cortical bone eight times stiffer) was the same in the two taxa, resulting in a Young's modulus of $E = 13.5/8 = 1.7$ GPa. Data about the nasal-frontal hinge were also not available in the literature, and it is unclear whether the flexibility is the result of differences in thickness of the bones in the hinge area, or whether there is a difference in Young's modulus. We modeled the hinge with a Young's modulus of 0.4 GPa that rendered the hinge significantly more flexible than the surrounding cortical bone. We also tested the model with a hinge stiffness equal to cortical bone ($E = 13.5$ GPa) and found no large differences in the amplitude and distribution of stresses in our areas of interest (Fig. S3). A sensitivity test with arbitrary Young's moduli (cortical bone: $E = 2.5$ GPa, trabecular bone $E = 2.2$ GPa, nasal-frontal hinge $E = 0.4$ GPa) showed no apparent deviations in the location of peak stresses, indicating that variations in mechanical properties do not have a large influence on our results (data not shown). This confirmed our expectations because a change in material properties would mainly affect the amplitude of the peak stresses and not the location of the peak stresses. A summary of the model properties is given in Table S1. We removed the lateral bars from the model to create the model used to simulate the beak without the lateral bars and then created two sets of models: one to simulate static biting and one to simulate cranial kinesis.

In the model for static biting (Fig. S4a), we removed the caudal part of the neurocranium so we could constrain the quadrate from all movements except rotation along its natural rotational axis. The remaining caudal part of the neurocranium was constrained from all movements. A backward (caudal) force of

10 N was applied on the quadrates to simulate the retraction force of the jaw musculature and the tip of the bill was constrained to mimic reaction forces of a food item. The applied force of 10 N was chosen randomly and does not reflect maximum bite or muscle force. Because we are focusing on differences between models and materials are isotropic, any force will predict the locations and relative amplitudes of stress. This is also true for the kinetic model, which described the results of movement and also predicted locations but not absolute amplitudes of stresses in the skull.

For the kinesis experiment, the quadrate and pterygoids were removed from the initial model in which the beak was closed because it was impossible to model their movement accurately (Fig. S4b). Displacements (Table S2) were taken from *in vivo* data of feeding in chickens (van den Heuvel 1992; van den Heuvel and Berkhoudt 1998) and predictions for the movement of the jugals, palatines, and maxillae based on 3D-Roentgenphotogrammetry of osteological specimens (Gussekloo et al. 2001). Rather than displacing single nodes on each jugal and palatine bone, we displaced groups of nodes at the bases of these bones to ensure that entire bones were translated along the prescribed vectors. Using the "upgeom" command in ANSYS, which takes displacements from a previous analysis and updates the geometry of the FE model to the deformed configuration, we created a model in which the beak was initially open and then applied displacements in reverse to close it. While all of the meshes were within the quality tolerance of Mimics, ANSYS reported a very low percentage ($<0.17\%$) of elements with high aspect ratios in each model. Upon inspection of results files, none of these poorly formed elements appear to have contributed to singularities or localized stress concentrations.

In each of the analyses, we constrained a large patch of nodes on the back of the skull to prevent translation and rotation in all directions. Although they did not mimic natural constraints, they prevented rigid body motion and did not affect stress in the areas of interest for our analyses (i.e., the cranial base and beak). Element stresses are represented as von Mises stress, a derived value based on all components of the stress tensor that is related to the proportion of strain energy density that distorts the material. von Mises stress is a good predictor of ductile failure of materials, like bone (Nalla et al. 2003, 2005).

IN VIVO EXPERIMENT

The experiment was conducted at Leiden University under approval of the animal experiment ethics committee. Twenty fertilized eggs of white leghorns were obtained from a commercial breeder. The eggs were kept in an incubator for 21 days and then transferred to a breeding cage for hatching. Three days after hatching the lateral bars of ten animals (experimental group) were surgically removed under full anesthesia. The control group was also put under anesthesia but no surgery was performed, because it could have caused damage to the superficial and not yet ossified

Table 1. In vivo experiment data.

Char.	Description	Std. coeff.	Rank	Intact skulls Mean \pm SEM	Lateral bar removed Mean \pm SEM	Diff. %
A	Skull width at the quadrate-jugal articulation (standard)	—	—	—	—	—
B	Distance between most distal points of the <i>proc. orbitalis quadrati</i>	4.832	1	0.3912 \pm 0.0221	0.3916 \pm 0.0378	+0.1
C	Width at pterygoids at quadrate-ptyergoid articulation	-1.417	6	0.5735 \pm 0.0272	0.5728 \pm 0.0217	-0.1
D	Width at most rostral part of pterygoids at the pterygoid-palatine connection	0.821	10	0.1702 \pm 0.0209	0.1708 \pm 0.0171	+0.4
E	Maximal width of the right pterygoid in the transversal plane	1.199	8	0.0947 \pm 0.0091	0.0913 \pm 0.0133	-3.6
H	Distance between the <i>anguli caudolaterales</i> of the palatal wings (pars lateralis)	2.430	2	0.2627 \pm 0.0148	0.2548 \pm 0.0216	-3.0
I	Maximal distance between the lateral margins of the palatal wings at their rostral endings	-0.897	9	0.2757 \pm 0.0105	0.2776 \pm 0.0118	+0.7
K	Width between the palates at position "T"	2.099	3	0.0630 \pm 0.0134	0.0597 \pm 0.0146	-5.3
L	Width at most caudal part of the palatines at the pterygoid-palatine connection	0.499	13	0.1226 \pm 0.0106	0.1213 \pm 0.0094	-1.0
M	Width between the connection of the <i>proc. palatinus</i> and <i>proc. jugularis</i> of the <i>os maxillare</i>	-0.579	11	0.4075 \pm 0.0185	0.4198 \pm 0.0184	+3.0
N	Width of the <i>r. parasphenoidale</i> inc. <i>proc. Basispterygoidei</i> when present	0.420	15	0.1083 \pm 0.0101	0.1127 \pm 0.0128	+4.0
O	Distance <i>Foramen magnum</i> to measurement "N"	-1.352	7	0.5113 \pm 0.0267	0.5117 \pm 0.0376	+0.1
P	Distance <i>Foramen magnum</i> to the most caudal part of an element of the PPC connecting or crossing the <i>r. parasphenoidale</i>	-1.535	5	0.5807 \pm 0.0236	0.5953 \pm 0.0148	+2.5
Q	Maximum length of the palatine	-0.504	12	0.8058 \pm 0.0412	0.8213 \pm 0.0363	+1.9
R	Width at palatine-maxillae articulation	-1.592	4	0.2854 \pm 0.0136	0.2921 \pm 0.0166	+2.3
S	Internal width at palatine-maxillae articulation	0.438	14	0.1530 \pm 0.0221	0.1577 \pm 0.0135	+3.1

Description of the characters and the results of the discriminant analysis presented by the standardized canonical discriminant function coefficients (std. coeff.) and standardized measurement (mean and error of mean) per group (intact vs. lateral bar removed). The difference (Diff.) between the two groups is indicated as percentage. Characters have previously been used for comparison between palaeognathous and neognathous birds (Gussekloo and Zweers 1999). Characters F and G from that comparison are specific for palaeognathous birds and therefore omitted here.

lateral bars of the upper bill. Animals were marked with ink on the wings for identification. All 20 chicks were kept together in an indoor chicken pen with water and food pellets available ad libitum. The animals were weighed at regular intervals to confirm normal growth. After about 76 days (average 75.8 ± 2.7 days), the animals were euthanized and the intact heads were stored in 6% formaldehyde. We scanned the heads with a Micro CT scanner

(Skyscan 1076) with a voxel size of 11.82 μm . The reconstructed stack images were used to determine 3D-coordinates of morphological landmarks, from which we measured characters that have been used to discriminate between Palaeognathae and Neognathae (Gussekloo and Zweers 1999; description of characters in Table 1 and Fig. S5). Because of the multivariate nature of the characters and the fact that small differences were expected between groups,

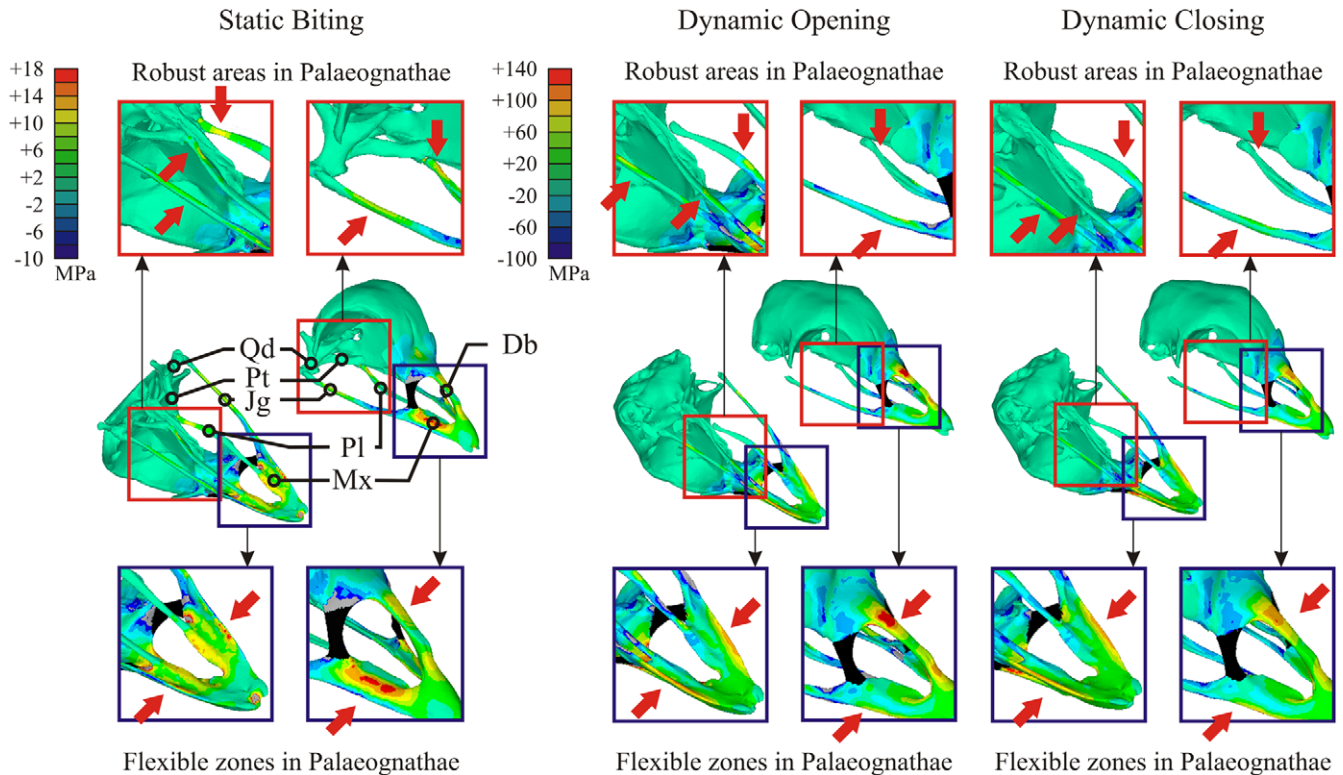


Figure 2. Removal of the lateral bar from the neognathous skull results in higher stresses at positions that are either more robust or more flexible in palaeognaths. Differences in von Mises stress distribution (in MPa) between intact models and models with the lateral bar (in black) removed. Positive values (warm colors) indicate higher stresses in the model with the lateral bar removed, negative values (cool colours) indicate lower stresses. Gray indicates values that fall outside the range of the stress values illustrated here. Top row shows how increased stresses coincide with areas that are more robust in Palaeognathae indicated by red arrows: Quadrates (Qd), Jugals (Jg), Pterygoids (Pt), and Palatines (Pl). Bottom row shows how increased stresses coincide with areas that are highly flexible zone in Palaeognathae indicated by red arrows: maxilla (Mx) and dorsal bar of the upper bill (Db).

a discriminant analysis was performed to determine which characters contributed the most to the difference between the groups.

Results

FE MODEL STATIC BITING

Simulation of static biting in the intact neognathous FE model resulted in the highest stresses at the caudal edge of the lateral bar, at both ends of the palatinum, and in the jugal bar one third from the caudal end (Fig. S6). With the lateral bar removed, stresses increased in the maxillae, jugals, pterygoids, and pterygoid-palatine articulations (Fig. 2). These areas of high stress (jugals, pterygoids, and the pterygoid-palatine articulation) coincide with areas that are more robust in Palaeognathae (Fig. 1; Gussekloo and Zweers 1999). Stress reductions were observed in the posterior parts of the jugals and palatines and near the nasal-frontal hinge.

FE MODEL UPPER BILL OPENING/CLOSING

During bill opening with the lateral bars intact, high stresses were present along the lateral bars and in the region of the rotation

point at the base of the upper bill (nasal-frontal hinge), while during closing stresses were relatively low across the entire facial skeleton (Fig. S7). During both opening and closing, the removal of the lateral bars resulted in a forward shift of stresses from the area near the nasal-frontal hinge (decreased stress) to the dorsal bar of the upper bill (increased stress). An increase in stress was also seen along the ventral region of the maxilla (Fig. 2). Both the ventral side of the maxilla and the dorsal bar have elongated flexible zones in palaeognaths (Gussekloo and Bout 2005a, Fig. 1). During kinesis without the lateral bar, stress also increased in the jugal bars and palatines (Fig. 2), which are more robust in Palaeognathae (Fig. 1; Gussekloo and Zweers 1999). At the posterior part of the palatines and jugals we see a decrease of stress after removal of the lateral bar.

In addition to evaluating the distribution of stress, we also determined reaction forces in the palatines and jugals, which are indicative for the amount of force necessary to move the upper bill. The reaction forces along both bones were reduced as a result of removing the lateral bar (Table 2). This illustrates that the absence of lateral bars makes the skull less stiff and easier to

Table 2. Reaction forces (in Newton) predicted in each of the four kinetic models.

Kinetic models	Reaction forces (N)				
	Right		Left		Bill Tip
	Jugal	Palatine	Jugal	Palatine	
Opening					
Intact	11.89	22.42	12.05	21.56	N/a
Lateral bars removed	11.29	15.84	10.92	15.33	N/a
Closing					
Intact	20.13	58.35	20.46	62.08	72.82
Lateral bars removed	15.57	49.12	16.4	49.84	55.74

deform. Reaction forces at the bill tip during bill closing, which are a proxy for bite force, were reduced by 24% when the lateral bars were removed (Table 2).

IN VIVO EXPERIMENT

There were no significant differences in overall growth rates between the experimental and control animals (Fig. S8). Overall differences between the control group and experimental group were small but noticeable when looking at skull reconstructions (Fig. 3). A difference was found between the discriminant score means of the experimental and control groups (t -test, $t = 9.07$, $df = 17$, $p < 0.001$). The discriminant scores are an overall description of morphology and include 83% of the variation found in the characters used the discriminant analysis (Fig. 4). A strong canonical correlation (0.910) and low Wilks' Lambda value (0.171, $\chi^2 = 16.76$, $df = 15$, $p = \text{N.S.}$) indicated that the groups could be discriminated (Fig. 4). Because we were interested in identifying the features that contribute to the difference between groups, and not in predicting group-membership, we used the absolute standardized canonical discriminant function coefficients to identify morphological characters that differed between the experimental and control groups (Std. Coeff, Table 1). When the lateral bars were removed, several changes occurred in the cranial morphology. The orbital process of the quadrates was shorter (character B inversed), which means a less effective elevation of the upper bill due to a reduced torque for the elevator muscles, but which is also a character of palaeognaths (Gusseklloo and Zweers 1999). The pterygoids became more oriented in an anterior–posterior direction (increase of characters B, E, and P, and a decrease of character C) but also became thinner (character E). A more anterior–posterior oriented pterygoid is also observed in palaeognaths although it is often shorter than observed here (see Bock 1963). As in palaeognaths, the parasphenoidale was thicker (character N) after removal of the lateral bars, but no clear development of a palaeognath-like basiptyergoid

process was observed. The palatines also became relatively longer (increase in character Q), probably due to elongation on the posterior side, resulting in more overlap with the pterygoids. Near the articulation with the pterygoids the palatines became smaller (decrease in characters H and L), but more posterior they became broader (increase in character I, decrease in character K) and more oriented to the lateral side of the skull (increase in character R,S). The more lateral position is also reflected in the broadening of the upper bill as a whole which is reflected in the increase of the distances between the anterior articulation of the jugals (character M). Broad bills and lateral attachments of the palatines to the upper bill are characters found in many palaeognaths, and might help mitigate the high stresses that occur in the ventral bar of the upper bill after removal of the lateral bar.

Discussion

Our FE results clearly show that the removal of the lateral bar from a neognathous skull increases stress in the dorsal and ventral bars of the upper bill and in the pterygoid-palatine complex, locations where the palaeognathous morphology is either more robust (the pterygoids, palatines, and jugals) or thinner and more flexible (the dorsal and ventral bars of the upper bill) than neognathous morphology (Gusseklloo and Zweers 1999; Gussekloo and Bout 2005a). These results from the FE models were supported by our in vivo experiment, in which removal of the lateral bars induced changes in morphology in locations where high stresses were predicted by the FE model and where we see differences between neognath and palaeognath birds.

Both the robustness of the ventral skull elements and the flexibility in the upper bill can be adaptations to mitigating high stresses, because there are two different mechanisms to achieve this. On the one hand, stress can be dealt with by strengthening a structure and limiting its deformation. On the other hand, stress can also be dispersed by not opposing the stress, but, in contrast, facilitating deformation of the structure. The more robust morphology, as found in the ventral skull elements of the Palaeognathae, is an example of the first where the strengthened bones can counteract the additional stress that would otherwise result from the absence of the lateral bar. The thin and broad maxillae of the palaeognaths are an example of the second mechanism, where the bones will endure lower stress levels because the stress can be more easily dispersed during bending than in thicker, less flexible bars. In the maxillary region bending instead of reinforcement is possible because in this region it does not interfere with normal behaviors such as feeding (Gusseklloo and Bout 2005b), and it ensures a lightweight construction that will not fracture under normal behavioral loading conditions.

Of special interest is the area of the pterygoid-palatine articulation, because in the static loading experiments it was found

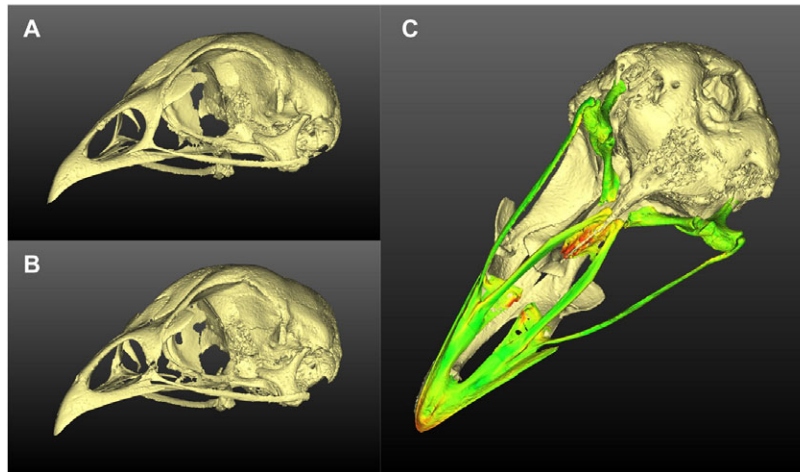


Figure 3. Examples of the cranial morphology after the in-vivo experiment. (a) Cranio-lateral view of a smoothed reconstruction of the animal from the control group with the highest discriminant score. (b) Cranio-lateral view of a smoothed reconstruction of the animal from the experimental group with the lowest discriminant score. (c) Ventral view of the skull showing differences in the morphology between the two animals (warmer colors indicate larger differences).

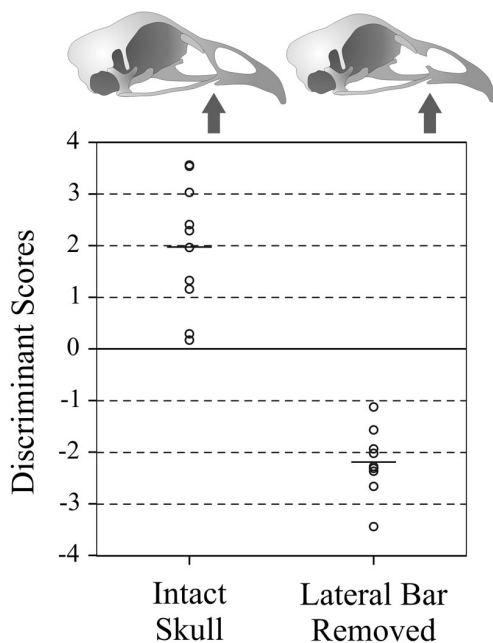


Figure 4. Removal of the lateral bar in growing chicken resulted in an altered morphology as demonstrated in the clear difference in discriminant scores of animals from the in vivo experiment. Left animals with intact skulls (control group) and right animals with lateral bars removed (experimental group). Circles indicate individual values, the long horizontal dash indicates the mean value per group. Differences between groups are significant (t -test, $t = 9.07$, $df = 17$, $p < 0.001$).

that the posterior part of the palatines will endure increased stress, while the in vivo experiment showed a decrease of width of this part of the palatines and the pterygoids. We do, however, see a

change in configuration of the articulation in the animals where the lateral bar was experimentally removed. In those animals, the pterygoid and palatine had a more straight, anterior–posterior orientation that might transfer the forces more efficiently onto other elements. In addition, the slight elongation of the palatines might indicate a larger overlap and thus a stronger link between the pterygoids and palatines. It is noteworthy that the pterygo-palatine joint in the chicken is mainly loaded in tension and compression, and bending is of less importance. Additionally, in neognaths the joint is a movable synovial joint while in palaeognaths it is a linked suture. Therefore, it is also possible that in chickens the joint could be reinforced by other factors, such as increased bone mineral density or elastic structures in the joints, rather than by gross morphological changes. We could not investigate these possibilities with our current dataset, but a more detailed analysis of this joint would be of great interest.

Analysis of the forces in the skull showed that removal of the lateral bar reduces the stiffness of the moving parts of the skull and reduces bite force. It may be assumed that, in palaeognaths, this decrease of stiffness is overcome by reinforcing the ventral side of the skull. The retention of both the vomer and the large basipterygoid process, as well as the reduction of movement in the joints most likely contribute to this increase in stiffness. The reduction in bite-force is in agreement with previous predictions that Palaeognathae have relatively lower biting forces compared to other birds (Gussekkloo and Bout 2005a).

These findings also open the possibility that differences in cranial mechanics could have played a role in the evolution of diversity in Neognathae and Palaeognathae. Within dinosaurs, the ancestral cranial morphology of modern birds evolved through

neoteny (Bhullar et al. 2012), in which initially both a lateral bar and a vomer were retained. It appears that palaeognathous birds lost the lateral bar in a continuation of the trend toward cranial fenestration that was present in the ancestors of birds. Our results show the disappearance of the lateral bar induces increased stresses in the bill and ventral elements of the skull. The palaeognathous skull morphology adapted such that it could withstand the increased stresses. The retention of the vomer and the basiptyergoid process ensures a distribution of the stress over a large area, and the elements essential for the transport of forces to the upper bill (i.e., pterygoids, palatines) are robust to withstand these high stresses. In chickens, it has recently been shown that a more robust, or ancestral, morphology of the palate can be obtained by inhibiting only a single developmental pathway, indicating that this requires very small changes in development (Bhullar et al. 2015). Remarkably, the same study showed that this inhibition resulted in a more rounded rostrum, a condition also observed in palaeognaths.

The dorsal and ventral bars of the bill itself became more flexible to mitigate increased bending stresses. As proposed earlier, the whole system could be further stabilized by retractor muscles that pull the ventral skull bones to the cranial base, especially to the well-developed basiptyergoid process (Gussekkloo and Bout 2005a; Holliday and Witmer 2008).

It is possible that the variety of morphological rearrangements of these elements is limited by the necessity to provide structural support which, in turn, could limit the range of shapes that the palaeognathous skull can adopt. We also show that the loss of the lateral bar is associated with reduced bite force, potentially limiting the potential food sources available to palaeognaths.

In contrast, the lateral bar remains intact in the basal neognaths, reducing the stress on the ventral elements of the skull while total bite force is unaffected. Thus, while the load-bearing requirements of the palaeognath skull could have placed limits on their morphological diversity, further modifications in the skull of neognathous birds, such as the loss of the vomer, increased mobility, and strengthening of the upper bill may have been possible due to the presence of the load-bearing lateral bar. Examples of these modifications may include active cranial kinesis for improved food handling, improved force transmission, and rigid bills for crushing hard food items. As is well known from Galapagos finches, morphological changes associated with feeding can lead to rapid adaptive radiation (Grant and Grant 2003).

If the palaeognathous skull is constrained by its particular load-bearing requirements, and the neognathous skull is not, the difference in cranial morphology may explain the spectacular adaptive radiation seen in Neognathae and the relative dearth of species in Palaeognathae.

AUTHOR CONTRIBUTIONS

SG designed the experiments, wrote most of the paper and supervised the project. DP made the FE-models. JW and IW performed the CT-scans, reconstruction and segmentation for the in-vivo experiment. MB, IG and ED performed FE-Analyses. SG did the in vivo experiment, and together with RH the morphometric analysis of it. ED contributed to the writing, all authors have read and approved the final manuscript.

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DATA ARCHIVING

The doi for our data is <http://dx.doi.org/10.5061/dryad.q9474>.

LITERATURE CITED

- Bhullar, B.-A. S., J. Marugan-Lobon, F. Racimo, G. S. Bever, T. B. Rowe, M. A. Norell, and A. Abzhanov. 2012. Birds have paedomorphic dinosaur skulls. *Nature* 487:223–226.
- Bhullar, B.-A. S., Z. S. Morris, E. M. Sefton, A. Tok, M. Tokita, B. Namkoong, J. Camacho, D. A. Burnham, and A. Abzhanov. 2015. A molecular mechanism for the origin of a key evolutionary innovation, the bird beak and palate, revealed by an integrative approach to major transitions in vertebrate history. *Evolution* 69:1665–1677.
- Bhullar, B.-A. S., M. Hanson, M. Fabbri, A. Pritchard, G. S. Bever, and E. Hoffman. 2016. How to make a bird skull: major transitions in the evolution of the avian cranium, paedomorphosis, and the beak as a surrogate hand. *Integr. Comp. Biol.* 56:389–403.
- Bock, W. J. 1963. The cranial evidence for ratite affinities. Pp. 39–54 in C. G. Sibley, J. J. Hickey, and M. B. Hickey, eds. *Proceedings of the XIIIth International Ornithological Congress*. American Ornithologists' Union, Baton Rouge, LA.
- . 1964. Kinetics of the avian skull. *J. Morphol.* 114:1–42.
- Bühler, P., L. D. Martin, and L. M. Witmer. 1988. Cranial kinesis in the Late Cretaceous birds *Hesperornis* and *Paraesperornis*. *Auk* 105:111–122.
- Cubo, J., and W. Arthur. 2001. Patterns of correlated character evolution in flightless birds: a phylogenetic approach. *Evol. Ecol.* 14:693–702.
- Cuff, A. R., J. A. Bright, and E. J. Rayfield. 2015. Validation experiments on finite element models of an ostrich (*Struthio camelus*) cranium. *PeerJ* 3:e1294. Available at <https://doi.org/10.7717/peerj.1294>.
- Ding, M., C. C. Danielsen, and I. Hvid. 2005. Effects of hyaluronan on three-dimensional microarchitecture of subchondral bone tissues in guinea pig primary osteoarthritis. *Bone* 36:489–501.
- Elzanowski, A. 2001. A novel reconstruction of the skull of *Archaeopteryx*. *Neth. J. Zool.* 51:207–215.
- Frazzetta, T. H., and K. V. Kardong. 2002. Biomechanics (communication arising): prey attack by a large theropod dinosaur. *Nature* 416:387–388.
- Gerritsen, A. F. C. 1988. Feeding techniques and the anatomy of the bill in sandpipers (*Calidris*). Univ. of Leiden, Leiden, the Netherlands.
- Grant, B. R., and P. R. Grant. 2003. What Darwin's finches can teach us about the evolutionary origin and regulation of biodiversity. *Bioscience* 53:965–975.
- Gussekkloo, S. W. S., and R. G. Bout. 2002. Non-neoteny origin of the palaeognathous (Aves) pterygoid-palate complex. *Can. J. Zool.* 80:1491–1497.

- . 2005a. Cranial kinesis in palaeognathous birds. *J. Exp. Biol.* 208:3409–3419.
- . 2005b. The kinematics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *J. Exp. Biol.* 208:3395–3407.
- Gussekloo, S. W. S., and J. Cubo. 2013. Flightlessness affects cranial morphology in birds. *Zoology* 116:75–84.
- Gussekloo, S. W. S., and G. A. Zweers. 1999. The paleognathous pterygoid palatinum complex. A true character? *Neth. J. Zool.* 49:29–43.
- Gussekloo, S. W. S., M. G. Vosselman, and R. G. Bout. 2001. Three-dimensional kinematics of skeletal elements in avian prokinetic and rhynchokinetic skulls determined by Roentgen stereophotogrammetry. *J. Exp. Biol.* 204:1735–1744.
- Hackett, S. J., R. T. Kimball, S. Reddy, R. C. K. Bowie, E. L. Braun, M. J. Braun, J. L. Chojnowski, W. A. Cox, K. L. Han, J. Harshman et al. 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* 320:1763–1768.
- Herrel, A., P. Aerts, and F. De Vree. 2000. Cranial kinesis in geckoes: functional implications. *J. Exp. Biol.* 203:1415–1423.
- Hofer, H. 1954. Neue Untersuchungen zur Kopf Morphologie. Pp. 104–137 in A. Portmann and E. Sutter, eds. *Acta XI congressus internationalis ornithologici*. Birkhäuser Verlag, Basel.
- Holliday, C. M., and L. M. Witmer. 2008. Cranial kinesis in dinosaurs: intracranial joints, protractor muscles, and their significance for cranial evolution and function in diapsids. *J. Vertebr. Paleontol.* 28:1073–1088.
- Hu, D., L. Li, L. Hou, and X. Xu. 2010. A new sapeornithid bird from China and its implication for early avian evolution. *Acta Geol. Sin.* 84:472–482.
- Huxley, T. H. 1867. On the classification of birds; and on the taxonomic value of the modifications of certain of the cranial bones observable in that class. *Proc. Zool. Soc.* 27:415–472.
- Klein-Nulend, J., A. D. Bakker, R. G. Bacabac, A. Vatsa, and S. Weinbaum. 2013. Mechanosensation and transduction in osteocytes. *Bone* 54:182–190.
- Kooloos, J. G. M., A. R. Kraaijeveld, G. E. J. Langenbach, and G. A. Zweers. 1989. Comparative mechanics of filter feeding in *Anas platyrhynchos*, *Anas clypeata* and *Aythya fuligula* (Aves, Anseriformes). *Zoomorphology* 108:269–290.
- Lautenschlager, S., L. M. Witmer, P. Altangerel, and E. J. Rayfield. 2013. Edentulism, beaks, and biomechanical innovations in the evolution of theropod dinosaurs. *Proc. Natl. Acad. Sci. USA* 110:20657–20662.
- Livezey, B. C., and R. L. Zusi. 2007. Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zool. J. Linn. Soc. Lond.* 149:1–95.
- Maxwell, E. E. 2009. Comparative ossification and development of the skull in palaeognathous birds (Aves: Palaeognathae). *Zool. J. Linn. Soc. Lond.* 156:184–200.
- McDowell, S. 1948. The bony palate of birds. Part I, the Paleognathae. *Auk* 65:520–549.
- Moazen, M., N. Curtis, P. O'Higgins, S. E. Evans, and M. J. Fagan. 2009. Biomechanical assessment of evolutionary changes in the lepidosaurian skull. *Proc. Natl. Acad. Sci. USA* 106:8273–8277.
- Nalla, R. K., J. H. Kinney, and R. O. Ritchie. 2003. Mechanistic fracture criteria for the failure of human cortical bone. *Nat. Mater.* 2:164–168.
- Nalla, R. K., J. S. Stolken, J. H. Kinney, and R. O. Ritchie. 2005. Fracture in human cortical bone: local fracture criteria and toughening mechanisms. *J. Biomech.* 38:1517–1525.
- Phillips, M. J., G. C. Gibb, E. A. Crimp, and D. Penny. 2010. Tinamous and moa flock together: mitochondrial genome sequence analysis reveals independent losses of flight among ratites. *Syst. Biol.* 59:90–107.
- Rayfield, E. J. 2011. Strain in the ostrich mandible during simulated pecking and validation of specimen-specific finite element models. *J. Anat.* 218:47–58.
- Reed, K. L., and T. D. Brown. 2001. Elastic modulus and strength of emu cortical bone. *Iowa Orthop. J.* 21:53–57.
- Simonetta, A. M. 1960. On the mechanical implications of the avian skull and their bearing on the evolution and classification of birds. *Q. Rev. Biol.* 35:206–220.
- Soons, J., A. Herrel, A. Genbrugge, P. Aerts, J. Podos, D. Adriaens, Y. de Witte, P. Jacobs, and J. Dirckx. 2010. Mechanical stress, fracture risk and beak evolution in Darwin's ground finches (*Geospiza*). *Philos. Trans. R Soc. Lond. B Biol. Sci.* 365:1093–1098.
- Soons, J., A. Herrel, A. Genbrugge, D. Adriaens, P. Aerts, and J. Dirckx. 2012. Multi-layered bird beaks: a finite-element approach towards the role of keratin in stress dissipation. *J. R. Soc. Interface* 9:1787–1796.
- Soons, J., A. Genbrugge, J. Podos, D. Adriaens, P. Aerts, J. Dirckx, and A. Herrel. 2015. Is beak morphology in Darwin's finches tuned to loading demands? *PLoS One* 10:e0129479.
- van den Heuvel, W. F. 1992. Kinetics of the skull in the chicken (*Gallus gallus domesticus*). *Neth. J. Zool.* 42:561–582.
- van den Heuvel, W. F., and H. Berkhoudt. 1998. Pecking in the chicken (*Gallus gallus domesticus*): motion analysis and stereotypy. *Neth. J. Zool.* 48:273–303.
- Xu, X., H. You, K. Du, and F. Han. 2011. An *Archaeopteryx*-like theropod from China and the origin of Avialae. *Nature* 475:465–470.
- Zusi, R. L. 1984. A functional and evolutionary analysis of rhynchokinesis in birds. *Smithsonian contributions to zoology* 395:1–40.
- Zusi, R. L., and B. C. Livezey. 2006. Variation in the os palatinum and its structural relation to the palatum osseum of birds (Aves). *Ann. Carnegie Mus.* 75:137–180.
- Zweers, G. A., and A. F. C. Gerritsen. 1997. Transitions from pecking to probing mechanisms in waders. *Neth. J. Zool.* 47:161–208.
- Zweers, G. A., J. C. Vanden Berge, and H. Berkhoudt. 1997. Evolutionary patterns of avian trophic diversification. *Zoology* 100:25–57.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Summary of model properties.

Table S2. The direction and distance of displacements applied to the jugal and palatine bones to simulate the opening and closing of the beak.

Figure S1. Phylogeny of birds, showing increased loss of lateral elements in the skull (cranial fenestration) toward modern birds (Neognathae and Palaeognathae).

Figure S2. Reduction of elements did not significantly alter the magnitude and distribution of the von Mises stresses.

Figure S3. Changing the Young's modulus of the nasal-frontal hinge did not significantly alter the magnitude and distribution of the von Mises stresses.

Figure S4. Overview of constraints, forces and displacements used in the FE-models.

Figure S5. Characters used in the in vivo experiment to describe morphology for the comparison between animals with the lateral bar removed and those with intact skulls.

Figure S6. von Mises stress distribution during static biting.

Figure S7. von Mises stress distribution during dynamic analysis.

Figure S8. Growth curves of control and experimental animals in the in vivo experiment.