Supplementary material

Material and methods

(a) CT and MRI scanning

CT scans of skulls from five moa species were performed by Pacific Radiology, Wellington (New Zealand), on a General Electric Discovery CT750 HD scanner, at 80 kV and 40 µA, and reconstructed as axial 0.3mm slices. The jaw adductor muscles of mummified *M. didinus* (NMNZ S400) were MRI scanned by Pacific Radiology, Lower Hutt (New Zealand) using a Siemens Magnetom Avanto 1.5 Tesla scanner with a Siemens 12 channel head matrix coil and B17 software. Performance per axis details were: maximum amplitude 33 mT/m, minimum rise time 264 microseconds from 0-33 mT/m, maximum slew rate 125 T/m/s. T1 and T2 weighted turbo spin echo sequences were obtained. All moa specimens are housed at the Museum of New Zealand Te Papa Tongarewa, Wellington (NMNZ). Skulls of *C. casuarius* and *D. novaehollandiae* were from the Canterbury Museum, Christchurch (CM) and South Australian Museum, Adelaide (SAM) respectively.

(b) Muscle force estimation

Axial reconstructed MRI slices from NMNZ S400 were labelled manually in Amira 5.2.1 (Visage Imaging) to segment out identifiable muscle remnants. Muscle reconstruction on axial scan slices was completed in Amira 5.2.1 by drawing temporary guide beams between the origin and insertion of each muscle, and then manually entering muscle segmentation around these guides, revised as necessary by reviewing the surface models.

Maximum jaw muscle cross-sectional areas for *M. didinus* were determined using 3-matic (version 8.0). The cranium, mandible and one of the muscle subdivisions were imported into 3-matic as three separate layers. Small inclusions or holes in the cranium and mandible were closed using a wrapping tool to generate a ‘watertight’ model. To ensure adequate overlap between the skull and muscle origin and insertion regions, a resulting offset between 1-3mm was set as a wrapping parameter to increase the outer contour of the cranium and mandible mesh. The Boolean intersection tool was used to combine overlapping regions (i.e. areas of contact) between the cranium and muscle origin of each subdivision to form a separate entity layer. This layer contained two surfaces that can be individually selected; one with the cranium surface that was in direct contact with the muscle and the other was the exterior surface of the muscle. An endless flat plane, known as a datum plane, was created across the surface of the muscle origin exterior. The surface of the muscle exterior was selected while creating the datum plane, and the ‘fit plane’ option chosen so that the plane position and orientation is the best fit to the curved surface of the muscle exterior. The datum plane is visualised in the software as four quadrants. The point intersecting the four divisions was projected orthogonally to the muscle surface to create a centroid on the exterior muscle surface. This procedure was repeated for the mandible and muscle insertion for the same muscle subdivision. The line of action of each muscle subdivision was determined by joining the muscle origin and insertion centroids. Sketches (i.e. flat planes) perpendicular to the line of action were created at 0.2 mm intervals along the entire muscle. Each sketch was transformed into an ‘attached curve’, a three-dimensional entity consisting of segments that wrap around the triangle edges of the muscle surface mesh. The attached curves were used to form the muscle contours, from which the surface area could be calculated. The maximum muscle cross-sectional area was the surface area of the largest curve (table S1).

Muscle origin and insertion areas and lines of action of muscle forces (figure 2) were used for each FEM, using MRI data of NMNZ S400 jaw muscles superimposed on NMNZ S28206 as a guide. There may be minor differences in muscle origins between the moa and extant Australian ratites [1], however these would have little influence on the distribution of stress except around actual origin sites and we do not consider these regions in our analyses. A total of 40 truss elements were positioned on each side of the FEM skull following lines of action for jaw muscles. The number of truss elements allocated to each muscle subdivision was proportional to the muscle volume taken from the mummified moa, NMNZ S400 (table S1). To minimise artefacts and achieve more uniform distribution of forces at muscle origin and insertion areas, surface bricks surrounding the node for each truss element were tessellated using a network of stiff beam elements [2].

(c) Finite Element Model (FEM) assembly

<u>Intrinsic (unilateral bite) load case</u>

Global x, y, z rotation and translation restraints were applied to a single node embedded within a network of tessellated beams at the designated bite point of the premaxilla (upper bill) and dentrary (lower bill) regions of the models' surface. This set of restraints simulates the cranium being isolated from the body and fixed at the point of attachment of the head to the neck (the restraint at the occipital condyles). The restraints at the bill (superior premaxilla and inferior dentary) simulate the beak biting an infinitely hard twig/rock.

Together these restraints fix the cranium in space (occipital restraint) and at the bill. This enables an analysis examining the stress created in the crania and mandible bones by the jaw muscles to be performed by pre-tensioning beam elements that replicate the cross sectional area and vectors of the muscles (as obtained from the MRI of the *M. didinus* specimen, see figure 2 and table S1).

To simulate the stresses generated in both the cranium and the mandible accurately the temperomandibular joint (TMJ) was simulated in the FEM. The anatomical joint axis of the TMJ was calculated through analysis of the condyle (mandible) and cotyle (cranium) articular joint surface morphology [3] in the following fashion. A node was created in the FEM at the centre point of rotation for both Left Hand Side (LHS) and Right Hand Side (RHS) TMJs. These nodes were connected inferiorly, via rigid links, to the beams covering the mandibular TMJ condyle surfaces (created from tessellation of the mandibular TMJ surface). Two rigid links (one each for LHS and RHS) with an absolute length of 3mm each were also extruded laterally from the created nodes (the centre point of rotation for the cotyle facets) along the calculated TMJ axis. Two stiff (Young's modulus = 200.00 GPa, Poisson's ratio = 0.25, diameter = 5mm) beams (one each for LHS and RHS), each with an absolute length of 3mm, were then extruded laterally from the end of the two links, again along the calculated TMJ axis. The lateral ends of these beams were un-restrained for rotation around their long axis (the calculated TMJ). These lateral beam ends then were connected superiorly, via rigid links, to the beams covering the cranial TMJ cotylar surfaces (created from tessellation of the cotylar TMJ surface). When the FE model is solved with the pretensioned muscle beams as the load on the mandible originating from the constrained cranium, the configuration of beams and links allows the mandible to rotate around the calculated TMJ, whilst being fixed distally at point on the dentary where the bite is simulated. This simulates the micro displacement, and consequential stress generated, in the cranium and mandible during a bite.

Extrinsic load cases

For the extrinsic load cases a different approach was taken. The muscle beams were not pre-tensioned, so did not contribute to the load case except in that they connected the cranium to the mandible. The unilateral bill restraint was also not used. Instead, a new restraint was created at the tip of the bill for all specimens. The restraints formed an H-frame comprised of rigid links attached to the bill using protocols published by and see Wroe *et al.* [4]. The H-frame was constructed by joining the tessellated (beams) covering the inferior (dentary) and superior (pre-maxilla) bones of the bill with vertical rigid links on both sides of the skull. These two rigid links were split into four (0.5 distance split) and joined at their half-way length with a rigid link positioned horizontally to complete the 'H-shaped' frame. This horizontal link was also split in half, with the central node being the point of loading for the extrinsic cases. The force (N) applied to the models at this centre node was calculated as 10 times the specimen's estimated body mass (see table S3) for a lateral shake, pullback and dorsoventral pull. As in previous studies the magnitude of the load applied is varied in the specimens so that both the skulls' morphology and the relative size of the specimen are taken into account for the comparative analysis [2].

(d) VM stress

VM stress was measured for all tet4 elements surrounding each landmark (between four to nine elements surround a landmark), and values were averaged to determine the distribution of stress anteriorly and posteriorly [2, 5]. Stress values measured along the mid-sagittal plane (N=10) and the mandible (N=10) were examined separately using a Principal Component Analysis (PCA) for each loading case (following [1]). PCA is a commonly used ordination technique that involves a rotation of the original variables to create a variance-maximised coordinate system, in which each Principal Component (PC) axis represents a successively smaller proportion of variance in the sample. PC1 and PC2 were plotted for each loading case to identify similarities between species based on stress values. Each PC can be interpreted based on the estimated component weights for each variable. Loading plots were included to determine the contribution of each landmark to variability of PC1 and PC2. Statistical analyses were performed using PAST software (version 3.05). Kruskal-Wallis tests were used to compare VM stress values between species [6], and significance values were corrected for multiple comparisons using Bonferroni correction, which modifies the □ parameter used to assess significance [7].

Blomberg's *K* statistic was used to test for phylogenetic signal in stress in each loading case based on the three PC axes. Blomberg's *K* statistic uses a permutation approach to assess the variance of phylogenetically independent contrasts relative to tip reshuffling randomisation, and assesses the strength of phylogenetic signal by dividing observed mean squared error of tip data by expected mean squared error, based on the variance-covariance matrix of trait data from the phylogenetic framework under the assumption of Brownian motion (BM). BM reflects a random walk in which variance in the distribution of measured traits is directly proportional to branch length.

Results

(a) Body mass estimate

The taxon *E. curtus* now incorporates all specimens formerly called *E. geranoides,* and the largest specimen from Pyramid Valley was reported to have a mass of 115 kg (95% CI 69-191) based on femur circumference [8]. The *E. curtus* specimen incorporated in our study has one of the largest predicted body masses (190.2 kg) among individuals recovered to date.

This specimen was from the Pleistocene, a period where all *E. curtus* individuals were around 50% larger than those in the mid to late Holocene [8].

The body mass estimated for the *P. australis* specimen (134.6 kg) was within the 95% confidence intervals for mean body mass of 116 kg (95% CI 86-157 kg) based on femur circumference [9, 10], yet was above the body mass range reported in the literature derived from convex hulls of the skeleton [33-68 kg; 11] and femur length [44-90kg; [44-90kg; 8]. Multiple body mass estimates have been reported for *D. robustus*, including 226-517 kg based on palaeognath-specific regressions [8], 155-245 kg based on the convex hull of their skeletons [11] and 76-242 kg for females and 34-85 kg for males based on femur length [12]. The body mass estimated for the *D. robustus* specimen included in this study came from a population of small individuals on the West Coast of South Island and its body mass estimate (83.0 kg) suggests that it was a male or small female, and so is much smaller than individuals typically associated with this species. The body mass estimated for *A. didiformis* and *M. didinus* specimens included in this study (47.4 kg and 26.8 kg) was within the body mass range for these species [range 26-64 kg and 16.9-34.3 kg respectfully, 8, 13, 14]. The body mass estimated for the *C. casuarius* specimen (33.6 kg) was within the species known body mass range (average body mass 31.7 kg for males; 45.8 kg for females) [15-17], while the *D. novaehollandiae* specimen had a mass (25.2 kg) slightly outside the species previously predicted body mass range [average body mass 31.5 kg males; 36.9 kg females, 15-17, 18, 19].

(b) Unilateral clip (intrinsic case)

Mean ‘brick’ stress was higher in the mandible than the cranium for all species during a unilateral clip (figure S4). All moa experienced the highest peak stresses along the mandible for a unilateral clip at the tomial margin (landmarks 2 and 3). All moa except *P. australis* experienced a second stress peak, located in the caudal part of the mandible (landmarks 7-9). Stress values along the mandible in both extant ratites were highest between landmarks 3 to 7 (figure S2).

The right side of the premaxilla, where force was applied, was the region of highest stress on the cranium for all species during a unilateral clip (figure 1). Stress measured along the mid-sagittal plane followed a similar distribution for all species (excepting *C. casuarius*), with peak stress located around the craniofacial hinge (landmarks 3 and 4) (figure S2). Compared to all moa species, *D. novaehollandiae* exhibited the lowest peak stress along the mid-sagittal plane, had the lowest PC1 score (denoting low stress at landmark 1) after *A. didiformis*, and highest PC2 score (denoting high stress at landmark 7 and 9-10; figure 1, S2).

(c) Pullback (extrinsic load)

Mid-sagittal plane peak stress for a pullback increased with body mass among moa species (table S3, figure S3). The only exception was *P. australis*, which exhibited lower peak stress along the mid-sagittal plane for a pullback than the smaller bodied individual of *D. robustus*. Peak stress in the extant *D. novaehollandiae* was similar to that of *P. australis*. *Euryapteryx curtus* had two points of peak stress along the mid-sagittal plane for a pullback (landmarks 1 and 4), whereas all other species only have one point of peak stress, between landmark 2 and 4. Stress along the mid-sagittal plane increased from the bill tip (landmark 1) to the nasal process of the premaxilla (landmarks 2-3) then decreased until the supraoccipital (landmark 10) for *P. australis*, *D. robustus*, *M. didinus* and *D. novaehollandiae.*

(d) Lateral shake (extrinsic load)

Stress distribution along the mid-sagittal plane for this load closely mirrored that found for a unilateral clip. *Pachyornis australis* and *A. didiformis* were the only species to show close PC scores for both PC1 and PC2 mid-sagittal stress, with intermediate levels of stress displayed in both species (figure S4). No pairwise differences were found for stress along the mid-sagittal plane during lateral shake before and after Bonferroni correction (table S5).

(e) Dorsoventral pull (extrinsic load)

All species experienced considerably higher mean 'brick' stress in the cranium than the mandible for a dorsoventral pull (figure S7). Stress along the mandible for a dorsoventral pull gradually decreased posteriorly from landmark 1, 2 or 3 in *D. robustus*, *E. curtus* and *A. didiformis*. *Megalapteryx didinus* experienced two points of peak stress along the mandible during a dorsoventral pull (one at landmark 1 and one at landmarks 6 to 8). *Dromaius novaehollandiae* experienced a similar distribution in stress along the mandible to *M. didinus*, while *C. casuarius* showed a gradual increase in stress from landmark 1 to 7 followed by a decrease to landmark 10. Despite these differences in the distribution of stress along the mandible between extant ratites, both species had similar PC1 and PC2 scores that were distinct from all moa (figure S5).

Along the mid-sagittal plane, *D. novaehollandiae* had the lowest PC1 score for a dorsoventral pull while all moa species grouped together along PC1. The lower PC1 and high PC2 scores for *D. novaehollandiae* denotes higher stress compared to species with higher PC1 and lower PC2 scores, as the landmark contributing most to PC1 (landmark 7) and PC2 (landmarks 1, 4 and 9) scores had a positive loading value (figure S5, S6).

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