PROJECT FINAL REPORT

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1 PUBLISHABLE SUMMARY

1.1 Executive summary

A lot of attention has been paid to the morphological variations of the craniofacial skeleton and its adaptation to diet, but the study of the mandible and its relation to feeding behavior lags behind. Whilst a combination of factors, not necessarily mutually exclusive, have been proposed as determinants of variation in mandible morphology (sexual dimorphism, facial orientation, dentition and phylogenetic constraints), most focus has been put on adaptation to mechanical loading during chewing. Chewing is a highly modulated behavior during which the food particles are processed using the postcanine dentition till the size and texture of the bolus permit a safe swallow. Previous studies on primates have shown that variations with regards to the material, structural, and physical properties of the processed food particles, coupled with the location of the bite force influence the activation patterns and the force modulation of the muscles of mastication. The electromyographic activity of the muscles of mastication and in particular the peak amplitude and timing affect the jaw kinematics and in particular the variance in the duration of gape cycles, gape-cycle phases and displacement of the lower jaw not only between chewing cycles but also within and between feeding sequences. But what is the effect of jaw kinematics and muscle activity on the skeletal morphology of the mandible? This study uses an interdisciplinary approach to combine state of the art experimental, computer simulation and analytical techniques to investigate the function of the feeding apparatus during intra-oral food processing. Preliminary results show a high correlation between jaw kinematics, the activation patterns of the muscles of mastication, force modulation and the stress and strain magnitudes and orientations when consuming food particles of varying toughness. This correlation is affected by the bite location, chewing rates and different gapes.

The results of our study offer a better understanding of the functionality of the feeding apparatus during intra-oral food processing and shed light to important biological questions regarding the sensitivity of the feeding variables to the toughness of the food items consumed and the location of the bite, hitherto obscured. The incorporation of our unique experimental data into computer simulations ensured the most precise, complete and validated study on the mechanical determinants of the mandibular morphology in response to diet and will become a landmark reference for future feeding biomechanics studies.

1.2 Project context and objectives

The functional morphology of the vertebrate craniofacial form is an area of intensive study and investigation in an attempt to better comprehend how and to what extent the mechanical factor influences growth, morphology and evolution of the anatomical structures. Since the masticatory apparatus is a dominant element of the craniofacial area, it has been extensively hypothesised that evolutionary changes in the structural interactions within the masticatory apparatus may influence vertebrate craniofacial form. Such variations are regarded as adaptive responses to occlusal biomechanical forces during mastication and incision (e.g. Daegling & McGraw 2007; Hylander 1984, 1985; Hylander & Crompton 1986; Hylander & Johnson 1994; Panagiotopoulou & Cobb 2011; Strait et al. 2009; Vinyard et al. 2005, 2006), jaw opening and closing (Koolstra & van Eijden 1995; van Eijden et al. 1997) and the provision of an adequate gape in particularly the non-human primates (Herring & Herring 1974; Smith 1984).

A lot of attention has been paid to the variations of the craniofacial skeleton in relation to dietary ecology but the study of the mandible and its relation to feeding lags behind (Ross et al. 2012). This is due to lack of combined experimental and computer simulation data that can highlight the movement of the mandible; the activity of the muscles of mastication during feeding and their effect on mandibular loading. Increased mandibular loading has been related to increased mandibular strains (e.g. Ross & Hylander 1996; Ross et al. 2011) and due to these findings it is generally assumed that different dietary categories account for specific mandibular morphologies. This assumption, nevertheless does not suggest evidence of a strong relationship between mandibular morphology and diet (Ross et al. 2012). For example, it is widely accepted that an increased depth in the mandibular corpora relates to consumption of a folivorous diet as per Old World monkeys (Ravosa 1990, 1996) but this is not the case for platyrrhines, which follow a folivorous diet but have reduced in depth and increased in width mandibular corpora (Bouvier 1986). Why mandibular morphological predictions between species do not match specific biomechanical predictions? Is it because major focus is given on the material and physical properties of the food items rather on variations in feeding behaviours? Previous studies have supported the hypothesis that feeding behaviours such as large-gape ingestive variations affect the size of the jaw elevator muscles and the mandibular robusticity (Taylor & Vinyard 2009; Norconk et al. 2009). It has been also proposed that variations in the food manipulation/preparation affect mandibular shape (Daegling 1992) but for specific anatomical areas and with regards to a restricted number of species (Vinyard et al. 2011).

Lack of experimental data on feeding behaviour have obscured our understanding on how vertebrates manipulate and eat their food and have resulted in the generation of simplified finite element models that cannot tackle the mandibular form-function relationship. In the context of this scientific gap in feeding biomechanics and anatomy, MACACA used an interdisciplinary approach to apply the most detailed experimental *in vivo* data to 250 finite element (FE) models to study feeding behaviour during a complete chewing cycle. Data involve muscle activation patterns, jaw optico-kinematics when manipulating and chewing food particles with various size, physical and material properties. This unique experimental dataset coupled with FE models tackled currently obscured questions in the field of feeding biomechanics:

Questions:

I) The activation patterns of the muscles of mastication are sensitive to the location of the bite force and the material properties of the food items consumed. But to what extent does this sensitivity affect stress patterns and strain magnitudes of the mandibular bone?

II) Intra-oral food processing can be achieved through different bite positions and under different chewing frequencies and rates. However, is the mandible optimised for its loading regime? Can it better resist infrequent and greater in magnitude chewing cycles or reduced in magnitude and more frequent chewing cycles?

III) How does the feeding behaviour affect mandibular morphology? Is the deep corpus a functional adaptation of a folivorous or hard food diet?

To answer the abovementioned questions MACACA examined three major objectives:

Objective 1: *The effect of the activation patterns of the muscles of mastication (which reflect different bite locations, gapes and food material properties) on the deformation of the mandible using finite element analysis (FEA).*

FEA is a numerical technique for solving the function of independent variables and their partial derivatives using linear ordinary differential equations. With FEA a continuum structural system is divided into discrete sub-regions of finite size, the elements. Elements are systems with linear algebraic equations that model their response to loads and are bonded with each other at their vertices, called nodes. Each node has a displacement vector which represents 3 degrees of freedom corresponding to displacements in the x, y, z directions. FEA has been widely used to study deformations on the mandible and the accuracy of the FE models is reliant on a number of variables, not least the precision of muscle activation patters assigned to the FE models.

The subject-specific FEA analysis was based on the cadaver mandible of the same individual to the one used in the *in vivo* experiment. The monkey was euthanized for reasons unrelated to this project. The two main steps involved in FEA are: model creation and model solution. The creation phase involved the assignment of the model's geometry, transformation of the geometric model into a set of discrete finite elements and the assignment of its material properties, loading and constraints. The FE mesh file was created from the volume data of computed tomography (CT) and magnetic resonance images (MRI) on the cadaveric head of the *M. mulatta*. The CT and MRI data were processed using Mimics v13 image processing software and only the mandible was used for further analysis. The mandibular model was the converted into a virtual mesh file of sub-regions of fine size (elements). Precision of the size and type of the elements used was tested using a series of sensitivity analyses with regards to mesh density. The mandible mesh file was assigned heterogeneous and anisotropic material properties values that were experimentally measured using the ultrasound wave technique in collaboration of Prof. Dechow (Department of Biomedical Sciences, Baylor School of Dentistry, Dallas, USA). The mesh file with the anisotropic and heterogeneous material properties was then exported to Abaqus v6.9 FEA software (Dassault Systems Simulia Corp., Providence, RI, USA) for loading and solution. A series of 250 FE models of the *M. mulatta* mandible were generated to simulate static and dynamic incision and mastication of 15 different food items that varied in toughness and stiffness. The muscle forces assigned to the FE models simulated a complete chewing cycle at different bite locations and gapes and combined electromyographics (EMG) of the superficial and deep masseters, medial pterygoids, deep anterior and posterior temporalis and the digastric muscles for both the working (chewing) and the balancing (non chewing side) (previously collected at the Department of Organismal Biology and Anatomy, University of Chicago, USA in collaboration with Prof. Ross) and Physiological Cross Section Area (PCSA) data measured by Dr. Taylor, Department of Evolutionary Anthropology, Duke University, USA. FE models were solved using a sparse, Gauss elimination method to solve a large system $KU^{1/4}$ F, where K is the stiffness matrix, F is the external force with constraints and U is the final displacements.

Objective 2: *Validation of the FE models against experimental in vivo strain gauges data.*

FEA has considerable promise in the study of feeding biomechanics however it is a modelling technique and as such the validity of any FEA solution depends on the validity of the input data (e.g. geometry, material properties, loading conditions). The validity of the solution of the FE models used in the MACACA project were tested by comparing all 250 FE models against unpublished *in vivo* experimental strain gauges and bite force transduction data previously measured by bonding rosette strain gauges to medial and lateral aspects of the left mandibular corpus by Drs. Panagiotopoulou, Iriarte-Diaz and Prof. Ross at the Department of Organismal Biology and Anatomy, University of Chicago, USA. The scope of the validation study was to test whether the *in vivo* and the *in silico* mandibles deform in the same way under the same forces.

Objective 3*: Estimation of force modulation during a chewing cycle.*

Force modulation is the ability of mammals to adjust tooth occlusion, chewing movements and gapes, and masticatory forces in response to the size and the material properties of the food particles consumed. To estimate to what extent strain magnitudes and orientations in the corporal area are associated with loading and unloading times; chewing rates and frequencies; and the relationship between principal strains and Von Mises stresses magnitudes and different gapes, a series of bivariate correlations and multivariate statistical analysis of Cartesian coordinate data were conducted.

References: **Daegling** DJ, & McGraw WS 2007. Functional morphology of the mangabey mandibular corpus: relationship to dental specializations and feeding behavior. American Journal of Physical Anthropology, 134(1) 50–62. **Bouvier** M. 1986. Biomechanical scaling of mandibular dimensions in New World monkeys. International Journal of Primatology, 7 551–567. **Daegling** DJ 1992. Mandibular morphology and diet in the genus Cebus. International Journal of Primatology, 13 545–570. **Herring** SW, Herring SE. 1974.The superficial masseter and gape in mammals. American Naturalist 108 561-576. **Hylander** WL, Crompton AW. 1986. Jaw movements and patterns of mandibular bone strain during mastication in the monkey *Macaca fascicularis*. Archives of Oral Biology 31 (12) 841-848. **Hylander** WL, Johnson KR. 1994. Jaw muscle function and wishboning of the mandible during mastication in macaques and baboons. American Journal of Physical Anthropology 94 523–547. **Hylander** WL. 1984. Stress and strain in the mandibular symphysis of Primates: a test of competing hypotheses. American Journal of Physical Anthropology 64 1–46. **Hylander** WL. 1985. Mandibular function and biomechanical stress and scaling. American Zoology 25 315–330. **Koolstra** JH, van Eijden TMGJ. 1995. Biomechanical analysis of jaw closing movements. Journal of Dental Research 74 1564-1570. **Norconk** MA, Wright BW, Conklin-Brittain NL, & Vinyard CJ 2009. South American primates: Testing new theories in the study of primate behavior, ecology, and conservation. In A. Estrada PA, Garber JC, Bicca-Marques E, Heymann & K Strier (Eds.), Mechanical and nutritional properties of foods as factors in platyrrhine dietary adaptations (pp. 279–319). New York: Springer. **Panagiotopoulou** O, Cobb SN 2011. The mechanical significance of morphological variation in the macaque mandibular symphysis during mastication. American Journal of Physical Anthropology 146 253-261. **Ravosa** MJ 1990. Functional assessment of subfamily variation in maxillomandibular morphology among Old-World monkeys. American Journal of Physical Anthropology 82(2) 99–212. **Ravosa**, M. J. (1996). Jaw morphology and function in living and fossil Old World monkeys. International Journal of Primatology, 17(6), 909–932. **Ross**, C. F., & Hylander, W. L. (1996). In vivo and in vitro bone strain in the owl monkey circumorbital region and the function of the postorbital septum. American Journal of Physical Anthropology 101(2) 183–215. **Ross**, C. F., Berthaume, M. A., Dechow, P. C., Iriarte-Diaz, J., Porro, L. B., Richmond, B. G., et al. (2011). In vivo bone strain and finite-element modeling of the craniofacial haft in catarrhine primates. Journal of Anatomy 218(1) 112–148. **Ross** CF, Iriarte-Diaz J, Nunn CL. 2012. Innovative approaches to the relationship between diet and mandibular morphology in primates. International Journal of Primatology 33, 632-660. **Smith** RJ. 1984. Comparative functional morphology of maximum mandibular opening (gape) in primates. In: Chivers DJ, Wood BA, Bilsborough A, editors. Food Acquisition and Processing in Primates. New York: Plenum. p 231–255. **Strait** DS, Weber GW, Neubauer S, Chalk J, Richmond BG, Lucas PW, et al. (2009). The feeding biomechanics and dietary ecology of Australopithecus africanus. Proceedings of the National Academy of Sciences of the USA, 106(7), 2124–2129. **Taylor** AB & Vinyard CJ 2009. Jaw-muscle fiber architecture in tufted capuchins favors generating relatively large muscle forces without compromising jaw gape. Journal of Human Evolution 57(6) 710–720. **Van Eijden** TM, Korfage JA, Brugman P. 1997. Architecture of the human jaw-closing and jaw-opening muscles. Anatomical Record 248: 464-474. **Vinyard** CJ, Williams SH, Wall CE, Johnson KR, Hylander WL. 2005. Jaw-muscle electromyography during chewing in Belanger's treeshrews (Tupaia belangeri). Am J Phys Anthropol 127:26–45. **Vinyard** CJ, Wall CE, Williams SH, Johnson KR, Hylander WL. 2006. Masseter electromyography during chewing in ring-tailed lemurs (Lemur catta). Am J Phys Anthropol 130:85–95. **Vinyard** CJ, Taylor AB, Teaford MF, Glander KE, Ravosa MJ, Rossie JB, et al. (2011). Are we looking for loads in all the right places? New research directions for studying the masticatory apparatus of New World monkeys. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 294(12) 2140–2157.

1.3 Main S&T results/foregrounds

MACACA has achieved all of its target objectives. All analyses has been completed and most of the results have been translated. Due to some technical difficulties with the FEA software and the extensive sensitivity analyses to ensure model accuracy and reliability the full map of the results has not yet been translated. All statistical and morphometric analyses are close to completion and will provide all the essential data for the publications. Planned paper submissions are as follows:

1. Panagiotopoulou O, Iriarte-Diaz J, Allen V, Ross CF. The effect of the material properties of food on feeding variables of a *M. mulatta* mandible during a complete chewing cycle. Current Biology
2. Panagiotopoulou O, Iriarte-Diaz J, Dechow P., A. Taylor, Ross CF. Determinants of the interactions between feeding behavior, food material properties and mandibular morphology during a complete chewing cycle using finite element analysis. Science
3. Panagiotopoulou O, Iriarte-Diaz J, Ross CF. The functional significance of morphological variation in the M. mulatta mandible using a combination of Geometric morphometrics and finite element analysis. PNAS

The preliminary results of this study showed that concentration of high principal strains and Von Mises stresses in the corporal and symphyseal area of the mandible are not products of food material properties but of feeding behaviour. The highest variations in the temporal and spatial motion of the mandible did not occur between chewing cycles under the consumption of food particles of different material properties but within chewing sequences, when the monkey was consuming the same food item. Nevertheless, food material properties had an effect on the lateral displacement of the mandible with high toughness food particles showing less displacement that low toughness food between but also within a chewing cycle. No increase in the duration of the chewing cycle was found but more data are needed to conclude that this is the case in Macaques.

Different bite locations and gapes coupled with the force modulation of muscle activity due to the different material properties of food items and chewing frequencies can equally generate high stresses and deformations on mandibular aspects. For example increased Von Mises stresses in the mandibular corpora of the working (chewing) side are high during biting of hard nuts on the molar teeth. Nevertheless, similar stresses are generated when chewing a fibrous leaf at higher frequencies.

As such, anthropologists need to be very careful when they try to infer the dietary ecology of fossil *taxa* based on morphological aspects of the mandible as such the increased depth of the corpus. Surely diet plays an important role in masticatory biomechanics, but feeding behaviour influences loading and deformation regimes and eventually affects morphology via adaptive remodelling. Lastly, food material properties had little effect on strain orientation in the mandibular corpus of the working side.

All FEA models gave similar strain results to the experimentally measured strains, at least within the sampling area, and therefore are sufficient accurate to draw reliable conclusions from them. Our validation study is the most complete hitherto available and upon publication will make accessible a unique strain dataset during a complete chewing cycle when consuming food particles with a wide range of toughness and hardness. These results will pave the way for more accurate studies on the primate mandible because the current lack of data has forced scientists to (rather questionably) use *in vivo* strain data during molar biting to validate FE models that simulate premolar and or incisor biting. Lastly our preliminary results showed that changes in activation patterns of the muscles of mastication in response to bite locations, and food items influence the temporal and spatial component of the kinematics of the mandible during a chewing cycle and within a gape cycle.

1.4 Potential Impact and the main dissemination activities and exploitation of results.

The results of our study offer a better understanding of the functionality of the feeding apparatus during intra-oral food processing and shed light to important biological questions regarding the sensitivity of the feeding variables to the toughness of the food items consumed and the location of the bite, hitherto obscured. MACACA offers new insights into the nature of the stresses and strains experienced by the primate mandible under various feeding behaviours and enables us to comprehend the impact on the mandible of different activation patterns of the muscles of mastication, with regards to different bite locations. Finally a thorough understanding of the form-function relationship of the mandible during the processing of foods of various material properties will allow us to reassess safety factors and to better comprehend the reasons why many non-human primates in captivity develop pathologies in the jaw related to masticatory stresses; i.e. trauma, condylar wear, TMJ dislocations; and other modifications related to an inappropriate diet.

The necessity for the conduction of more detailed *in vivo* experimental studies for a variety of non-human primate species and the subsequent construction of more detailed FE models is the main discussion topic in every scientific symposium on FEA to date. To this end, the novelty of the MACACA project is that it constructs the most accurate and detailed models of the macaque mandible ever conducted by incorporating a range of highly detailed experimental *in vivo* data to a series of controlled FE models. Experimental data represent muscle activation patterns, bite force and strain data, and jaw kinematics during incisor, premolar and molar biting and also while consuming various in size and material properties food particles. The incorporation of our unique experimental data into computer simulations ensured the most precise, complete and validated study on the mechanical determinants of the mandibular morphology in response to diet and will become a landmark reference for future feeding biomechanics studies.

A better understanding of the functionality of the feeding system and its relation to the diet is essential for better comprehending the feeding systems of species and *taxa* for which feeding behavior cannot be measured in vivo due to experimental constraints (e.g., extant and fossil hominids). The latter is particularly important to understand the mechanics and evolution of the feeding system and its dietary adaptations and make valid assumptions when reconstructing the diet of hominins in relation to anatomical and morphological traits of the mandible and the craniofacial skeleton. MACACA can be compared with future projects on other mammals and primate species to reveal the mechanisms by which mammals facilitate an effective occlusion by optimizing jaw movements and forces with regards to the hardness of the food items consumed, and accomplish this without damaging their teeth. A thorough understanding of the functional consequences of a complete chewing cycle with regards to the anatomical variation of the masticatory apparatus and the material properties of the consumed food items will help us predict what masticatory strains and in what frequencies can be carried safely by different animals and consequently predict the effects of feeding on craniofacial health and thereby spin-off to commercial/medical applications.

MACACA provided a new synthesis of feeding biomechanics and mandibular morphology by building on collaborations with high profile scientists in the field of masticatory biomechanics. Such interactions transferred high quality research in the European community; built up on new collaborative projects to continue excellence in the field of masticatory biomechanics and enabled

OP to get a Lectureship in Anatomy, at the School of Biomedical Sciences, University of Queensland. MACACA assisted OP to received world-class experience in quantitative analysis, anatomy, phylogeny, 3D computer graphics and modelling and generated the **most accurate validated FE models** of the primate mandible, by incorporating the most detailed *in vivo* experimental EMG, optico-kinematics, bite force transduction and strain data, **into high precision computer simulation models**. MACACA has generated gigabytes of experimental, anatomical, and computer simulation data which have been collected and managed in accordance with the RVC policies on Good Research Practice and will be made accessible to the wider scientific and medical community, industry and health care professionals/policy makers after public release.

MACACA brought to the host institution (RVC) a novel expertise in skull/feeding biomechanics and assisted in broadening the Structure & Motion Lab's overall research and teaching capacity under the development of a joint research team on feeding biomechanics with other institutions. This project also enabled OP to involve an integration of experimental and finite element techniques to study the form-function and size relationships of musculoskeletal structures.