Behavioral Correlates of Cranial Muscle Functional Morphology

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ABSTRACT

This issue of the *Anatomical Record* is the first of a two-volume set that focuses on new investigations into behavioral correlates of muscle functional morphology. Much of the research on functional morphology and adaptation to specific functional niches focuses on the shapes of hard-tissues—bones and teeth. Investigations into soft-tissue anatomy tend to be predominantly descriptive with only brief allusion to ontogenetic or evolutionary origins of structures. When muscles are included in analyses of functional systems, their function tends to be oversimplified—usually considered a simple force vector connecting two osteological points, with the force treated as a constant derived from some simple calculation of muscle size. The goal of these special issues is to present a series of studies that take a more elaborate look at how muscles can be viewed from a functional perspective in studies searching for morphological correlates of behavior. This first volume focuses on the behavioral correlates of cranial muscles—starting with a paper about the mimetic musculature of primates and ending with a series of papers on the masticatory muscles of many lineages of vertebrates. The next issue of the *Anatomical Record* (March 2018) includes our papers on the behavioral correlates of postcranial muscles. Taken together, we hope you agree that this series presents valuable insights into these form/function relationships using both traditional approaches+ and cutting-edge techniques. Anat Rec, 301:197–201, 2018. © 2018 Wiley Periodicals, Inc.

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This special issue of the *Anatomical Record* is the first of a two-volume set focused on new investigations into the behavioral correlates of muscle functional morphology. Most of the anatomical research published to date has primarily focused on describing specific muscles or muscle complexes. Often these papers are briefly contextualized within an evolutionary framework (particularly in comparative studies) or with reference to the ontogenetic development of the muscles. Additionally, most of these descriptive papers make reference to the function of the muscle. These past contributions highlight that

even the most didactic musculature description requires some contextualization as to how a muscle's anatomy became the way it is, from an evolutionary or ontogenetic perspective, and why it is the way it is; the description of form necessitates discussion of its function. It is this latter relationship that is at the heart of each of the papers in these two special issues—a deep dive into the form and function relationships of specific regions of myological anatomy.

Why study muscles? Functional morphologists have focused disproportionately on the anatomy of bones and teeth, in particular those of us who are ultimately interested in evaluating form and function relationships as they pertain to paleontological samples; with few exceptions, only hard tissues are preserved in the fossil record. However, even those of us interested primarily in extant taxa tend toward explorations of osteological or dental anatomy. This is perhaps because of the vast collections and ease of use of skeletal materials available at museums; it is much easier to get permission to study dry specimens than to destructively sample wet specimens for myological studies. Nevertheless, all of us anatomists know that, for the most part, trying to describe the function of a bone without a deep understanding of the muscles that pull on it is equivalent to describing the beam of a lever while ignoring the forces applied to the system. So, just as we can look at a pair of pliers and infer something about the hand that might grip it, osteologists and paleontologists have been making inferences about the function and evolution of bones based on assumptions about muscles. But, if we were to find a mysterious hinged tool that we knew nothing about, can we really look at its parts, study their lever ratios, and correctly guess what it was designed for? A detailed study of the forces that it requires to operate and the materials with which it interacts best is the only way that we can get close to the correct answer.

When the muscular components of a musculoskeletal system have been included in functional morphology analyses (e.g., in some of the great early functional morphology studies like those of Smith and Savage, 1956, 1959), they are often reduced to simpler concepts—i.e., we might look at an attachment area or a fossa volume, make an inference about the size of a muscle, and then use that estimate to further infer its potential force. Then, once that potential force has been inferred, it is assumed to be constant in the analysis, which, after all, is mostly focused on the osteology. In truth, and for the most part, this is probably good enough for many functional morphology questions. We can learn immense sums about the physical limits and the evolution of functional systems by, for instance, creating detailed Finite Element Models of bones and inputting overly simplified estimated muscle forces as if they were established constants. But, if the goal is to truly understand the functional morphology of the musculoskeletal system, and to be able to deduce the functional differences between disparate morphologies, a much more detailed understanding of the anatomy and functional morphology of muscles is necessary. That is, origin areas and fossa volumes can be reasonable estimators of muscle forces for some studies (though there are excellent studies that suggest otherwise! (e.g., Zumwalt, 2005, 2006), but understanding muscle fiber architecture, lengths, cross-sectional areas, and pennation will get us much closer to a better understanding of the system as a whole.

The papers in these two special issues attempt this focus on myology at a more elaborate level. Our collective goal is to present tools—modern methods and data driven analyses—and to discuss the functional morphology of muscles beyond oversimplified force constants along oversimplified vectors. Each paper also goes beyond analyses of muscle morphology and examines their correlations to specific functions—how limb muscles relate to locomotion and posture, how head muscles relate to social systems and masticatory abilities.

Initially, we invited authors based on their participation in a symposium that we held at the 2016 International Congress of Vertebrate Morphology (Hartstone-Rose and Marchi, 2016). The group grew from there as we made particular efforts to ensure participation across several axes of diversity; our roster of authors span all career stages (Professors, postdocs, graduate students, and undergraduates), a multitude of countries, and colleagues who focus on a wide variety of anatomical regions and phylogenetic groups. In the end, although we are proud of the diversity that we achieved, there is a clear skew toward papers that focus on primates and a strong anatomical region bias: roughly half of the papers focus on cranial muscles—the subject of this first issue. The second issue (coming out in the following month, March 2018) focuses on functional correlates of postcranial myology and will present an overview article coauthored by our other guest editor, Damiano Marchi (University of Pisa). As we (Hartstone-Rose and Santana) predominantly study cranial architecture, we have taken on the task of summarizing the wonderful contributions of our colleagues to the current issue.

Almost all of the papers in this first issue focus on the masticatory muscles. The notable exception to this trend is the first paper, by Anne Burrows (Burrows, 2018), which focuses on mimetic musculature. For many years, Burrows has led explorations of the behavioral correlates of the muscles of facial expression in primates. Her body of work is remarkable for several reasons. For one, unlike most of the muscles of the musculoskeleton, mimetic muscles, in general, anchor to bone only on one end. Thus, their function is fundamentally different than most other muscles; while all muscles function to bring one element toward another (e.g., most skeletal muscles bring bone toward bone), the muscles that Burrows studies move soft tissue structures toward bony processes or toward each other. Some have linear vectors (e.g., the zygomaticus mm.) while others are sphincters (e.g., the orbicularis oculi and oris mm.). Some function to orient important sensory structures (e.g., those that move the pinnae or whiskers), and others seem to function primarily in inter-individual visual communication. Mimetic muscles are also particularly remarkable because of their small size. Burrows has previously published papers on the mimetic muscles of some of the smallest primates in the world, and each of these muscles is literally paper thin! This requires a rather unique dissection approach: while most of us remove the skin and connective tissue to access the underlying muscles in which we are interested, Burrows removes the mimetic musculature along with the facial skin (the primatologist version of Hannibal Lecter), creating "face

masks" that have the intact mimetic musculature. Her previous work has focused primarily on smaller species, and she has now expanded her sample to include an impressive diversity of primate taxa that span nearly the entire body size range of the order. In her paper, she concludes that phylogeny influences the presence and absence of specific muscles more than any other factor. Upon accounting for phylogeny, body size is also associated with the number of facial muscles. Interestingly, Burrows found only a smaller influence of behavioral correlates (e.g., nocturnality/diurnality and social systems) in explaining the variation of the mimetic musculature across primates.

The rest of the papers in this first issue focus on masticatory muscles. There is a deep history of endeavors like these (e.g., Allen, 1880; Edgeworth, 1935), including previous studies by many of those of us involved in these issues (e.g., Herrel et al., 1996; Santana et al., 2010; Perry et al., 2011; Hartstone-Rose et al., 2012), but we are excited to put on collective display some of the latest ventures into this subject—particularly those being performed by some outstanding newcomers to the field. The first of these is led by Jessica Arbour and Hernán López-Fernández (Arbour and López-Fernández, 2018), who are leading experts on the ecomorphology and evolution of Neotropical cichlids. In their contribution, they studied the adductor mandibulae muscle complex across several Neotropical cichlid species to examine the functional and behavioral correlates of this most substantial jaw-closing muscle. Through a series of comparative analyses, they found that the size of this muscle complex was influenced more by geometric constraints (i.e., the shape of the skull and competition for space with the eye and subdivisions within the muscle itself) than with any behavioral specialization. Thus, the authors conclude that there is a tight covariation between cranial morphology and myology, but no substantial influence of feeding behavior.

The next paper in the issue (St. Clair et al., 2018) was contributed by Elizabeth St. Clair and colleagues, and focuses on the cranial and myological masticatory anatomy of phalangiraform marsupials. This paper includes original illustrations by coauthor Nicholas Reback, which are noteworthy both because of their value as an accurate anatomical record and because of their aesthetic beauty. The authors have predominantly studied primate masticatory anatomy and, in this paper, turn toward a fascinating group of marsupials that has been compared to primates due to many ecological and behavioral similarities. Chief among these are parallels in dietary specializations. Like primates, phalangiraforms span the dietary spectrum from folivory to frugivory, with several taxa specializing in insectivory and even gummivory. The authors evaluate the question of whether this lineage of marsupials is functionally adapted to these specific diets in the same way that ecologically equivalent primates are. Not surprisingly, they found a mix of congruencies: while the gummivorous sugar-glider has long jaw muscle fibers like its primate counterparts—specialized for dietary behaviors requiring large gape—the frugivorous and folivorous marsupials do not look much like their primate counterparts. This highlights that there are multiple ways for the masticatory apparatus to process leaves and fruits and these

two lineages, separated by tens of millions of years, have evolved different solutions to the same problems.

The next paper in the issue (Ginot et al., 2018), submitted by Samuel Ginot and an excellent team of coauthors, meshes an impressive dataset of *in vivo* bite forces and anatomical data from 75 animals spanning 14 rodent species. Although one of us (Santana) collected similar data on bats, the other (Hartstone-Rose) is jealous of the ability to study bite forces and anatomy in the same individuals across such a wide sample—something seldom if ever possible for many lineages of mammals. This research is important not only for the value of being able to integrate *in vivo* performance and anatomical data, but also because of its intra- and interspecific breadth within Rodentia. Ginot et al. study the correlation of their measured bite forces and those predicted by their calculations derived from dissection data in unprecedented detail, find good agreement between their *in vivo* and estimated bite forces, and identify myological correlates of bite forces across rodent species. Consistent with other published studies, the investigators find that the form/function patterns are much harder to deduce within species. They conclude that intraspecific variation unrelated to feeding functions could be masking clear morphological signals.

Next, we include one of our own papers (Santana, 2018): a comparative study of the masticatory muscles of bats. In line with much of her previous work, Santana conducts a detailed analysis of the correlates of cranial morphology across a broad sample of dietarily diverse species. What is new and exciting about this and much of our current work (e.g., the cover illustration) is the use of Diffusible Iodine-based Contrast-Enhanced Computed Tomography (diceCT) scanning to quantify jaw muscle morphology. Traditional approaches to CT are nearly useless for myological research; bone is highly radio-opaque but soft tissue is not, thus while CT scanning of bone results in clear images, soft tissues disappear or exhibit a nearly uniform gray-scale. However, contrast agents (in this case, iodine) bind to specific proteins and lipids, which create variation in their X-ray opacity. Thus, when iodine-stained specimens are CT-scanned, soft tissues like muscles can be readily imaged and differentiated. This is particularly important for the study of jaw muscle morphology in bats; most bat species are small, and this makes the study of their muscles using traditional methods exceedingly difficult. Although contrast-enhanced approaches have been used sporadically for quite some time, there is a clear renaissance in the application of diceCT to myological research. Many of us are using this method to conduct "digital dissections"—visualizing complex, soft-tissue, morphology for the first time in three-dimensional space. However, there is still much validation that needs to take place—especially to establish the extent to which digital and traditional gross dissections yield equivalent results. In this study, Santana does that and also evaluates interspecific variation in gross muscle architecture across a diverse set of bat species.

The next paper (Curtis and Santana, 2018), led by Abigail Curtis and Santana, is an evaluation of one specific muscle in the masticatory apparatus: the digastric. Although there are dozens of papers on masticatory muscle architecture (almost a dozen in this issue alone!), these focus almost exclusively on muscles that close the

jaw (i.e., the masseter, temporalis and, to a slightly lesser extent, the medial pterygoid). However, these three muscle groups are not the only "muscles of mastication" —the primary mandibular *abductor*, the digastric, is a substantial muscle that exhibits anatomical variation across mammals. Yet, this muscle has received surprisingly very little attention in relation to its behavioral correlates. Curtis and Santana's sample spans a dietary radiation of bats with different demands on the force and speed of jaw abduction. Through comparative analyses of data generated via dissections and three-dimensional models, they find significant dietary signals on the morphology and function of the digastric, to the extent that some species' diet can be predicted based on digastric functional morphology alone. This emphasizes the need to explore the anatomy, function, and evolution of this muscle in greater detail across mammals.

The next paper (Fabre et al., 2018) in the issue, submitted by Anne-Claire Fabre and colleagues (including Hartstone-Rose), examines the relationship between masticatory muscles and skull shape in strepsirrhine primates. Many of us try to correlate muscle architecture with osteology, often with the hope of being able to reconstruct masticatory muscle abilities in fossil species (see Perry paper in this volume). However, in this Fabre-led investigation, the team explores the question from the opposite side: not, what can the bones tell us about the muscles, but to what extent do the muscles affect the shape of the bones? Fabre's big contribution in this paper is in her very detailed use of three-dimensional scans of the strepsirrhine skulls for complex analyses. These are used to consider not only the functional signals in the myological data but also how these signals relate to phylogenetic trends. In the end, Fabre and colleagues find that muscle architecture has a stronger impact on cranial shape than mandibular shape, and, to no one's surprise, aye-aye's are weird!

Next comes the contribution by Hartstone-Rose et al. (2018) examining the muscle fiber architecture across a broad sample of primates. This paper follows similar work that Hartstone-Rose published with Jonathan Perry on strepsirrhines and felids. Although several colleagues have published data on the fiber architecture of some of the masticatory muscles (mostly portions of masseter) of some species of monkeys and apes, this study has the widest taxonomic breadth of any study to date, and includes calculations of cross-sectional areas and fascicle lengths for all of the mandibular adductors. Importantly, the results from this study refute previous scaling patterns that were hard to explain and find that, indeed, fiber architecture seems to conform to expectations based on dietary specialization. The authors achieved these results through analyses of fiber architecture data, which were generated using the technique of chemically breaking down the muscles; other studies measure fiber lengths by physically slicing between them.

One such study is the next paper in the issue, submitted by Taylor et al. (2018), in which they examine similar questions of how diet correlates with masticatory muscle architecture in the durophagous sooty mangabey. While many studies of masticatory architecture sacrifice intraspecific depth of analysis for interspecific breadth (e.g., the two papers that we lead in this issue), Taylor et al. use an admirable sample of their target species

and relatively large samples of a few other species for comparative purposes. Sooty mangabeys (a species also included in the Hartstone-Rose et al. paper) are remarkable in their documented ability to masticate extremely hard and stiff seeds. Contrary to expectations (and the Hartstone-Rose et al. paper), Taylor et al. conclude that there are no morphological signals in the muscles that would explain the powerful bite forces exhibited by this species. Together, these two papers give a fascinating example of how inter- and intraspecific analyses, and analyses based on different methods, can yield surprisingly different results.

The next paper in the series, by Perry (2018), is an exploration of how we can apply myological findings to the fossil record. Perry has spent over a decade studying the masticatory muscles of strepsirrhines. Following a recent paper in which he and colleagues used these analyses to reconstruct the masticatory abilities of adapids, he is now presenting his inferences of the diets of the giant extinct lemurs from Madagascar. Extant lemurs are among the most fascinating radiations of living mammals. The group includes the smallest primates in the world (the mouse lemurs), most of the nocturnal primates (including the largest nocturnal primate), and the most bizarre of all primates, the aye-aye. It also includes species of impressive dietary specialization: frugivores, gummivores, insectivores, and folivores—including a lineage of small lemurs that consume bamboo—one of the most obdurate foods consumed by any mammal. As impressive as the extant lemurs are, their extinct relatives are even more remarkable: almost all of them were large—several species as large as apes. One lineage was specialized for below-branch suspension, with adaptations as dramatic as those seen in sloths. Some species had masticatory anatomy that is unlike that of any living lemur. In this paper, Perry demonstrates that most species were highly folivorous and several were hard-object specialists. Perry's addition of myological reconstructions takes these findings to new levels of conclusiveness.

The final two papers in this first issue of our two-part series by Dickinson et al. (2018), Orsbon et al. (2018), and their colleagues are more examples of the technological leading-edge of muscle analysis. In the paper led by Dickinson, they employ diceCT but use an algorithmic approach to calculate fascicle lengths and pennation angles across a much larger number of fibers than implemented in previous techniques. They compare their results with those assessed using the muscle slicing technique (i.e., that used by Taylor et al. in this volume) and confirm the validity of the digital approach. Dickinson et al.'s method is probably the most reproducible, likely the most objective, and could become the most automated approach to muscle fiber analysis. It is also clearly the least destructive; it will essentially allow us to study muscle fiber architecture in digital three-dimensional space without even directly accessing the muscles.

Orsbon et al. are likewise at the leading edge of technology. In their paper, they combine diceCT approaches with analyses of data collected using X-Ray Reconstruction of Moving Morphology (XROMM)—a technique in which X-ray is employed to observe the skeletal anatomy of an animal while it is performing an activity. In their paper, Orsbon et al. use these two technological approaches to evaluate the chewing and swallowing

muscles in a macaque. They create remarkable three-dimensional kinetic visualizations of the cranium, mandible, and hyoid and the muscles that move them and the tongue to elucidate these biomechanical processes at a level of detail never before achieved. Together, Orsbon and Dickinson—two senior graduate students and rising stars in our field—represent the future of muscle functional morphology analysis.

These are exciting times to be researchers interested in muscle functional morphology. Traditional techniques are still yielding fascinating results and modern techniques, being developed and driven by new generations of anatomists, are opening up whole new realms of research explorations. This first set of cranial muscle papers has exceeded our expectations, and we hope that you agree that they are an impressive addition to our growing literature. The second set of papers, on behavioral correlates of postcranial musculature, is also an exciting piece. We look forward to sharing it with you in the next issue.

ACKNOWLEDGMENTS

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