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**Evolution of Craniofacial Architecture and Lateralization in
Perissodini Scale-Eaters**

A Capstone Project Submitted in Partial Fulfillment of the Requirements of
the Renée Crown University Honors Program at Syracuse University

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Abstract:

To understand the evolution of craniofacial asymmetry within the Perissodini tribe, a group of scale-eating fish found in Lake Tanganyika, we performed shape analysis on their craniofacial architecture, and applied a simple lever model to measure asymmetry in lower-jaw mechanics. We found that craniofacial asymmetries were the consequence of sided differences in size and shape of craniofacial elements, and that sided differences in shape predict the lateralization of force and speed of the lower jaw in derived species. Quantitative Trait Locus analysis in Lake Malawi cichlids identified a single locus of major effect for jaw laterality, the first genetic marker for handedness in an antisymmetric trait. Studying the evolution of laterality in *Perissodus* and ultimately identifying the genetic factors that contribute to the asymmetric development of skeletal structures will shed light on the evolutionary and clinical consequences of vertebrate laterality.

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Advice to Future Honors Students:

1. Daily, ask yourself, “Why does this matter?”
2. “Whatever advice you give, be brief.” -Horace

“It was the Law of the Sea, they said. Civilization ends at the waterline. Beyond that, we all enter the food chain, and not always right at the top.”

-Hunter S. Thompson

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Capstone Project Body:

Introduction:

The science of understanding how genes define the growth and patterning of bodies is a blossoming one. Over the last two decades, thanks to both increased computational power and advances in genomic technologies, scientists have begun to tease apart how bodies organize and construct themselves (Carroll *et al.* 2005). The field of evolutionary and developmental biology, informally known as evo-devo, has led to a fundamental restructuring of our understanding of the evolution of morphology. It is now evident that many of the mechanisms that define body organization are widely conserved across taxa, elevating both traditional and non-traditional model organisms as increasingly tractable avenues of research for understanding the human condition (Carroll *et al.* 2005). Scientists now look to organisms with unique and varying morphologies to ask deep biological questions, often bridging the fields of evolutionary theory, ecology, and human health.

Breaking Symmetry

Most multicellular animals exhibit one of two forms of symmetry: radial, in which multiple planes of symmetry can be drawn across an organism, or bilateral, where a single plane of symmetry, the saggital plane, bisects an organism into mirrored halves (Carroll *et al.* 2005). Both adult echinoderms (e.g., sea star, sea cucumber) and cnidarians (e.g., jelly fish, sea anemones) are defined by radial symmetry. Bilateral symmetry is a synapomorphy of the bilateria, a

taxonomic group that encompasses most animal phyla, specifically triploblastic organisms, those creatures with three germ layers (Tree of Life Project 2002).

Despite its utility as a diagnostic character, symmetry's ubiquity ought not be overestimated; asymmetries are remarkably frequent among animals, and of three types. The first type is fluctuating asymmetry, where the breaking of symmetry is a consequence of developmental "noise," and lacks a strict genetic basis. Fluctuating asymmetry is non-adaptive, but rather a consequence of environmental stresses and developmental instability. Asymmetries of this nature tend to be normally distributed with symmetry as the mean (Leary and Allendorf 1989). The second type of asymmetry is directional asymmetry, where asymmetries are biased to one side of the saggital plane and are genetically determined; this includes many adaptive asymmetries. A third classification of asymmetry is antisymmetry, where evolved asymmetries show no bias towards one side of the saggital plane at a population level, and both left and right handed morphs exist; left versus right handedness of antisymmetrical traits is believed to be random (Palmer 1996, Palmer 2005). For antisymmetry, the nature of the asymmetry (i.e., which traits are affected) is genetically determined, but the side in which the trait manifests itself is purported to be environmentally determined.

A myriad of directional asymmetries have evolved among various vertebrate lineages. Owls have evolved asymmetrical ears, which differ in size and placement on the skull, making them more effective auditory predators (Norberg 1977). The eyes of flatfish migrate over the midline of the body during development such that as adults both eyes are on the same side and they can lay

on the benthic substrate and predate upon passing fish (Hubbs and Hubbs 1945). Lateralization of behavior allows us to partition specific tasks to one side of our bodies (e.g., handwriting) (Corballis 2009). The list of examples is a long one, and serves to illustrate the point that evolved asymmetries allow organisms to attain evolutionary optima potentially beyond what can be obtained through symmetry.

It would seem intuitive that whenever breaking symmetry is adaptive, asymmetries will evolve. Among the bilateria, however, it is apparent that while certain traits have the potential to evolve directional asymmetries, others may not. For example, experimenters have repeatedly tried to artificially select for directional asymmetry in wings of *Drosophila melanogaster* without success (Tuinstra *et al.* 1990). The capacity of an organism to reach optimal asymmetries therefore seems to be trait and taxon specific (Cooke 2004).

Nature is replete with examples of craniofacial asymmetries [e.g., narwhals (Ness 1967), owls (Norberg 1977), flatfish (Hubbs and Hubbs 1945) and fruit bats (Juste and Ibañez 1992)], and the evolutionary potential for craniofacial asymmetry seems to include many vertebrate lineages. Moreover, the prevalence of laterality in nature may be more common than originally thought. For example, the Lake Tanganyikan cichlid *Neolamprologus moori* was assumed to possess symmetrical jaws, but appears to be asymmetrical and inherit jaw handedness according to Mendelian genetics (Hori *et al.* 2007).

While developmental biologists have begun to pinpoint some of the genes involved in defining the left-right body axis (e.g., *nodal*, *lft1*, *pitx1*), and

regulating the development of normally asymmetric structures (e.g., gut, heart, brain), the evolution of asymmetry in normally paired structures remains mysterious. Our ignorance regarding the mechanisms involved in asymmetrical growth and patterning of bodies is perhaps most strikingly illustrated by the many human birth defects characterized by asymmetric craniofacial malformations (e.g., hemifacial microsomia, Treacher-Collins syndrome, hemihypertrophy). Craniofacial defects account for 70% of human birth defects, many of which are characterized by asymmetries in either soft tissues or the skeleton (Hall 1999). Because we only poorly understand how and why asymmetries arise developmentally, early screening and preventative treatments for many of these conditions are yet unavailable. By studying natural systems with evolved craniofacial asymmetries, we hope be able to better understand birth defects that result in homologous conditions.

The Perissodini Tribe

Lake Tanganyika is one of the oldest East African great lakes, between 9 and 12 million years old (Cohen *et al.* 1992), and it is home to an enormous diversity of fishes, including many endemic species, which exhibit a wide variety of feeding strategies (Fryer and Iles 1972). The Perissodini clade is unique in the lake as being the only group to have evolved lepidophagous predation, or scale eating. Asymmetry was first identified in this group in 1976 (Liem and Stewart). Since then, craniofacial asymmetries have been noted for every *Perissodus* species (*P. eccentricus*, *P. microlepis*, *P. elaviae*, *P. hecqui*, *P. multidentatus*, *P.*

paradoxus and *P. straeleni*) (Liem and Stewart 1976; Hori 1991, 1993; Takahashi and Hori 1994, 1998). Asymmetrical species are dimorphic; populations are composed of both 'lefty' and 'righty' individuals (Liem and Stewart 1976, Hori 1993). In 'lefty' individuals, the mouth is angled off to the right, and the left side of the skull is longer; correspondingly, the mouths of 'righty' individuals bend leftward (Hori 2007).

Most of the research on this clade has focused on one of the more derived species, *Perissodus microlepis*. Asymmetry in this species is attributed to asymmetry in the length of a joint between the jaw and suspensorium (Liem and Stewart 1976). It has been shown that the left and right morphs of *P. microlepis* are maintained through a frequency-dependent selection mechanism, where the minority morph experiences a higher fitness than the majority morph as a consequence of preferential prey avoidance of the more abundant morph (Hori 1993). The relative frequency of each morph fluctuates around a mean of 0.5, and the presence of both morphs appears to be an evolutionary stable state (Hori 1993). This system is a commonly cited example of antisymmetry, given that there appears to be no species level bias in handedness (Palmer 2005). Interestingly, while antisymmetry is assumed to be environmentally determined (Palmer 2005), Hori (1993, 2007) has suggested a genetic basis for this trait, distinguishing it from other examples of antisymmetry.

Lepidophagy, or scale-feeding, is a surprisingly common feeding strategy among fishes. Scales provide an excellent source of sustenance because they are reliably abundant, high in calcium phosphate and covered in a protein rich mucus

layer (*sensu* Sazima, Janovetz 2005). Scale-eating has evolved independently in at least five of seven marine fish families (*sensu* Sazima, Janovetz 2005), and at least three times among the East African Great Lakes (Lake Tanganyika, Lake Malawi and Lake Victoria) (Kocher *et al.* 1993). Of the Africa scale-eaters, the Lake Tanganyikan genus *Perissodus* exhibits the greatest degree of specialization, and claims the greatest number species (Fryer and Iles 1972). Perissodini scale eaters are believed to have evolved from generalist deep water carnivorous predators that have expanded into shallower habitats (Takahashi 2007). Scales compose roughly 90% of the diets for each of the five most derived species in this clade, with the exception of *P. straeleni*, for which scales constitute roughly 60% (Takahashi *et al.* 2007).

Scale eaters exhibit highly specialized dental morphologies, predictive of their feeding behavior. The broad pointy teeth of *P. microlepis* facilitate the removal of scales from prey by wrenching them off as they rotate their bodies, mouths pressed against their victims, whereas the laterally sharp-edged teeth of *P. straeleni* and *P. paradoxus* scrape scales off of prey as they slide down the length of the prey's body upon striking (Takahashi *et al.* 2007). Body shapes too are predictive of behavior, and these also vary among *Perissodus* species. *P. straeleni*'s deep body allows it to maneuver quickly as it pursues prey from close range, while *P. microlepis*' torpedo like body allows it to quickly approach prey from greater distances. The narrow body of *P. microlepis* also facilitates rotation around the long axis of the body once it has latched on to its prey (Takahashi *et al.* 2007).

Craniofacial Biomechanics

Teleost skeletal craniofacial structures are highly complex, with upwards of twenty independently moving skeletal elements moving in three dimensions (Wainwright *et al.* 2005, Hulsey *et al.* 2005). Numerous biomechanical models have been developed to understand the functional consequences of form on feeding mechanisms (Westneat, 1990). These models provide a means of testing hypotheses regarding the relationship between morphology and behavior (Westneat 2003).

Applying a simple lever model to the lower jaw and comparing sided differences in the mechanical properties of lower jaw opening and closing, we intend to evaluate whether *Perissodus* species exhibit asymmetries in shape that affect feeding performance. Looking for sided differences in biomechanics will allow us to better describe the nature of the jaw asymmetries within this clade. Describing trends in functional asymmetries may also illuminate selective pressures that have impacted feeding performance.

The genetic basis of laterality

A great deal of debate has surrounded the heritability of left versus right handedness in antisymmetric systems. Palmer (1996, 2005) has repeatedly claimed that almost without exception, the handedness of antisymmetrical traits is not inherited. Hori (1993, 2007) has directly contradicted these claims citing inheritance patterns from crosses of the two *P. microlepis* morphs. These studies,

however, have been called into question given the tendency of *P. microlepis* mothers to “brood swap” or accidentally raise the fry of other females while mouth brooding. How Perissodini asymmetries relate to traditional definitions of directional asymmetry or antisymmetry remains to be resolved. We investigated whether handedness is genetically determined among cichlids by performing quantitative trait loci (QTL) analysis on jaw laterality in a hybrid cross between Lake Malawi cichlids. The identification of a locus of major effect that defines laterality would substantiate Hori’s claims, and would be a critical first step in characterizing the genetic basis of evolved antisymmetries.

The aim of this research was to describe the evolution of craniofacial architecture and asymmetry in the Perissodini tribe: what are the anatomical underpinnings of this asymmetry, how have they changed as species evolved to specialize on scale eating, how do these changes in form affect jaw performance, and is laterality genetically determined in this group? Studying the evolution of laterality in the Perissodini and ultimately identifying the genetic factors that contribute to the asymmetric development of skeletal structures will shed light on the evolutionary and clinical consequences of vertebrate laterality.

Methods:

Morphometric Analysis

Seven of the nine species in the Perissodini tribe were included in this analysis [*Perissodus straeleni* (n=9), *Perissodus microlepis* (n=9), *Perissodus*

paradoxus (n=5), *Perissodus elaviae* (n=2), *Perissodus multidentatus* (n=2), *Perissodus hecqui* (n=5), and *Haplotaxadon microlepis* (n=4)]. Specimens came from the personal collection of Dr. R. Craig Albertson of Syracuse University, the University of Michigan at Ann Arbor, Cornell University, and the Royal Museum for Central Africa in Belgium.

The left and right sides of each specimen's skull were dissected to remove skin and connective tissues, exposing a set of 16 landmarks, points that characterize the kinematics and geometry of the skull (Figure 1). These landmarks include muscle origins and insertions, ligamentous origins and insertions, pivot points and lever arms. Dissected specimens were photographed using an Olympus SP-570. Landmarks were digitized using tpsDig, a computational package designed for shape analysis (Rohlf 2003a).

'Lefty' and 'righty' individuals were grouped in this analysis, and tests of laterality focused on differences between the longer and shorter sides of the skull. The sides of each individual were characterized as either 'towards' or 'away' depending on whether when photographed, the specimen's mouth was bending 'towards' or 'away' from the camera. The left side of a 'lefty' morph would be categorized as 'towards,' as would the right side of a 'righty' morph; the corresponding opposite sides of each morph were characterized as "away."

Using tpsRelw (Rohlf 2003b), partial warps were derived from these landmarks; these are descriptors of the geometric shape variation among various configurations of homologous landmarks. As eigenvectors of the bending energy matrix, they describe the deformation of a grid necessary to yield overlapping

points (i.e., landmarks) between a mean consensus configuration and each specimen when graphed in a Cartesian plane. This morphometric analysis accounts for size and orientation variation among specimens and photographs, focusing purely on the geometry of the various skeletal elements and their arrangement. Partial warps were then subjected to principal components analysis (formally referred to as relative warps analysis) to identify major axes of shape variation among all samples. This allows for mean shapes of elements in each fish's feeding system to be derived in multidimensional space, making cross-species comparisons relatively simple. A series of ANOVAs were used to compare general craniofacial shapes of each species along the relative warp axes responsible for the majority of shape variation. Relative warp analysis was used to characterize changes in craniofacial morphology among species and also to test for asymmetry within species.

Biomechanics & Laterality

The kinematics of the lower jaw was modeled as a first order lever (Figure 2). In this model, the fulcrum is a joint made by the quadrate and the articular; this is the point about which the lower jaw rotates. The out-lever is the distance from the foremost tip of the jaw to the fulcrum. The opening in-lever is the distance from the attachment point of the interopercular ligament that connects the interopercle and the retroarticular process of the lower jaw to the fulcrum. The closing in-lever is the distance from the insertion point of the A2 abductor mandibulae muscle on the ascending arm of the lower jaw to the fulcrum

(Wainwright *et al.* 2005). From these lengths, mechanical advantage (MA) was calculated. MA is the ratio of in-lever to out-lever length, and can be used to represent the relative speed and force of a lever (Westneat 2003). A high MA is predictive of fish with powerful bites, species which likely which consume sedentary prey, while a low MA is predictive of a fast, weaker bites, likely a species which pursues evasive prey. This model is a simplistic one, failing to take into account the angle of muscle and ligament insertion, and as such underestimates the speed of lever movement in most fishes (Westneat 2003). Given the laterally compressed nature of most Lake Tanganyika cichlid bodies, these models provide reasonable approximation for the kinematics of the oral jaw.

Specimens of *P. straeleni* (n=8), *P. microlepis* (n=7), *P. paradoxus* (n=4), and *P. hecqui* (n=3) were cleared and bones were stained with Alizarin red using a method adopted from Potthoff (1984). Photographs were taken of each side of the lower-jaw. Landmarks, pivot points and lever arms of the lower-jaw, were placed using TPS, and MAs were calculated. ANOVAs were used to test for asymmetry in MA within species.

Quantitative genetics and Laterality

We used quantitative genetics to identify whether left versus right handedness of the oral jaw is genetically determined in Lake Malawi cichlids. A mapping population of two Lake Malawi species, *Labeotropheus fuelleborni* (“LF”) and *Metriaclima zebra* (“MZ”) were crossed, and 173 F2 progeny were genotyped for roughly 200 genetic markers, including both anonymous

microsatellite markers and known genes. A genetic linkage map was constructed and quantitative trait locus analysis (QTL) was performed following methods previously described (Albertson *et al.* 2003, 2005). Twenty four linkage groups were identified, which likely correspond to the 24 chromosomes that Malawi cichlids possess. Thus, our QTL analysis is surveying most of the genome. Quantitative trait locus analysis correlates genotype with phenotype, identifying regions of the genome that are statistically associated with the traits of interest. In our analyses, the trait surveyed was left versus right handedness characterized by asymmetry in length of the retroarticular process of the lower jaw.

Results

Relative warp analysis of the seven *Perissodini* species reduced the majority of the variation to two relative warp axes (Figure 3A). Relative warp 1 (39.87%) described variation in skull length, whereas relative warp 2 (24.22%) was defined by variation in the angle of the mouth. *H. microlepis* differed significantly from all other species along the second relative warp axis, and appeared to be affecting axis definition disproportionately. A second relative warp analysis was run excluding *H. microlepis* (Figure 3B). Again, relative warp 1 (50.88%) described shifts in skull length, but this time relative warp 2 (17.00%) characterized differences in eye size. Mean craniofacial shapes were generated for each species from these analyses; shapes for *Perissodus* species were derived from the relative warp analysis of six species and the mean shape for *H.*

microlepis was derived from the relative warp analysis of all seven species (Figure 4).

Relative warp analysis comparing the ‘towards’ and ‘away’ sides of each species found a significant lateralization of shape in *P. straeleni* (Figure 5). While asymmetries were noted in all species with the exception of *H. microlepis*, no other species exhibited shape asymmetries sufficiently integrated into the entire craniofacial morphology so as to be separated by this analysis.

When only considering the lower jaw, both *P. straeleni* and *P. microlepis* showed significant differences in the lever mechanics of ‘towards’ and ‘away’ sides, reflecting an asymmetry in the biomechanics, and predicting a lateralization of force and speed in the oral jaw (Table 1). Asymmetries in feeding mechanics were not observed for either *P. paradoxus* or *P. hecqui*.

QTL analysis of asymmetry in the length of the retroarticular process of the lower jaw found a single locus of major effect corresponding to left versus right directionality of lower jaw asymmetries (Figure 6). Markers significantly associated with this trait were located on linkage group 19 and include GM294, UNH2105, and UNH2111.

Discussion

Within the Perissodini tribe, shifts in craniofacial morphology correspond to differences in the ecology of each species. Variation among species was reduced to shifts in skull shape that are indicative of evolutionary and ecological transitions within this group. Divergence between the genera *Perissodus* and

Haplotaxadon seems to have involved a change in the rotation of the mouth. Analysis of the six *Perissodus* species shows an evolutionary trajectory along the second relative warp axis corresponding to a reduction in eye size. As ancestral predators began to pursue new quarry in shallower waters, eyes reduced in size, adapting to higher light environments. Recently evolved scale-eaters tend to occupy shallow rocky habitats, with eyes that are proportionally much smaller than more ancestral species. A second shift occurred as the *Perissodus* genus continued to specialize in scale eating. The two most derived species, *P. microlepis* and *P. straeleni*, have diverged in skull length. Skull length directly relates to a fish's foraging strategy, reflecting predatory behavior and pursuit strategy. The short skull length and deep body of *P. straeleni* allows it to attack from short distances, relying on maneuverability to capture prey, while the longer skulls and shallow bodies of *P. microlepis* facilitate attacks from greater distances, employing speed to successfully capture prey. Alternatively, the shorted head of *P. straeleni* might also relate to the more diversified diet of this species (Takashi et al., 2007).

Relative warp analysis of *P. straeleni*, one of the two most derived species in the clade, found the shapes of the 'towards' and 'away' sides of the head to be significantly different from one another. Being both the most derived species in the clade and also the only species in which we observed sided asymmetry in shape, suggests that asymmetries have become increasingly elaborated in this clade, from sided differences in size to the lateralization of shape.

The lateralization of force and speed in *P. microlepis* and *P. straeleni* further supports this point. Asymmetries first manifested themselves within this group as sided differences in size. Asymmetries were elaborated upon to include differences in the shape of craniofacial elements that have a direct impact on feeding biomechanics. In *P. straeleni*, asymmetry has become further elaborated, becoming incorporated into the total shape of the craniofacial structure, and is represented by a more global asymmetry in shape among sides, whether these differences reflect a further elaboration of the lateralization of mechanics would be a fruitful direction of future research.

Asymmetry of the retroarticular process of the lower jaw, which defines part of the biomechanical asymmetries we observed in *P. microlepis* and *P. straeleni*, was used to characterize and map laterality in the *Metriaclima zebra* and *Labeotropheus feulleborni* hybrid cross using QTL analysis. The fact that this asymmetry, a trait directly related to foraging, was more elaborated in derived species suggests that it has been a basis of selection within this group, and critical in defining craniofacial laterality. The identification of a locus of major effect related to defining left versus right handedness confirms the hypothesized inherent genetic basis for handedness in African cichlids (Hori 2007). This finding is the first of its kind; a genetic locus for left versus handedness has never before been identified.

How craniofacial asymmetries in the Perissodini tribe relate to traditional definitions of directional asymmetry or antisymmetry has yet to be resolved. It has been argued repeatedly that the handedness (left versus right) of anti-

symmetric traits are not genetically determined, but rather a consequence of environmental factors that define sidedness (Palmer 1996, Palmer 2005). In an extensive survey of antisymmetrical traits, Palmer (2005) concluded that only in one case is there sufficiently robust evidence to attribute a genetic basis for the left versus right definition of asymmetrical traits. The case he cites involves directionality of style bending in *Heteranthera* flowers, which appears to be determined by a one gene, two allele system (Jesson and Barrett 2002). While Hori (1993) suggested that handedness in *P. microlepis* is heritable, some have questioned this claim by noting that young fish can be raised by non-parental individuals in a process known as brood farming, making it difficult to infer heritability in non-experimental settings. Our identification of a locus that defines laterality in handedness in our QTL analysis is the first of its kind, and perhaps the most robust example of a genetic basis for an antisymmetric trait.

Research is ongoing to confirm that this locus is also associated with handedness in the Perissodini tribe. Observed asymmetries in larval fish, supports the idea that this trait is genetically inherited in *Perissodus microlepis* (personal observations). The genes associated with this locus also remains to be confirmed, though preliminary evidence from comparative genomics implicates the gene *Wnt11* as a potential candidate. A blast analysis of the stickleback and zebrafish genomes shows *Wnt11* to be adjacent to the QTL marker UNH2105. WNTs are involved in the development of the craniofacial skeleton (Geetha-Loganathan *et al.* 2009), and recently WNT signalling has been implicated in regulating cardiac asymmetry (Lin and Xu 2009).

If in fact the handedness of Perissodini scale-eaters is determined by the same genetic mechanism as was identified in the hybrid cross of Lake Malawi cichlids, this would support the idea that evolved asymmetries arise from cooption of latent laterality of the jaw. The left-right axis is defined early in vertebrate development, and several zebrafish mutants have been identified that exhibit craniofacial asymmetries similar to those exhibited by Perissodini scale eaters (Albertson and Yelick, 2005; 2007; Albertson, unpublished data). Identifying the genes involved in the development of laterality and evolution of asymmetry in the Perissodini other systems will facilitate an understanding of left-right axis definition during growth and patterning of craniofacial structures.

By further integrating morphological analyses to identify evolutionary trends, specifically in a trait that appears to have been selected upon in the accentuation of craniofacial asymmetry, with quantitative genetics we will be able to develop a more complete picture of the evolution of this unique morphology. Understanding the genetics of asymmetric development of skeletal structures will also light will be shed on a number of human birth defects characterized by asymmetric craniofacial malformations. The Perissodini present a unique opportunity to address a range of questions fundamental to biology, and make many important contributions to the fields of evolutionary theory, ecology, and human health.

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Appendices:

Table 1: ANOVAs comparing sided differences in the lower jaw identified asymmetries in feeding the biomechanics of *P. microlepis* and *P. straeleni*. Lateralization of force and speed is a consequence of differences in the shape of the left and right halves of the lower jaw.

Species	MA _{out}						MA _{close}					
	Source	SS	df	MS	F	P-value	Source	SS	df	MS	F	P-value
<i>P. straeleni</i>	Between	0.0797	1	0.0797	124.74	< 0.0001	Between	0.2328	1	0.2328	79.12	< 0.0001
	Within	0.0102	16	0.0006			Within	0.0471	16	0.0029		
<i>P. microlepis</i>	Between	0.0848	1	0.0848	5.36	0.0362	Between	0.0342	1	0.0342	8.34	0.0119
	Within	0.2215	14	0.0158			Within	0.0574	14	0.0041		
<i>P. paradoxus</i>	Between	0.0010	1	0.0010	0.23	0.6428	Between	0.0145	1	0.0145	3.27	0.1083
	Within	0.0340	8	0.0042			Within	0.0355	8	0.0044		
<i>P. hecqui</i>	Between	0.0001	1	0.0001	0.11	0.7494	Between	0.0014	1	0.0014	2.01	0.2059
	Within	0.0046	6	0.0008			Within	0.0041	6	0.0007		

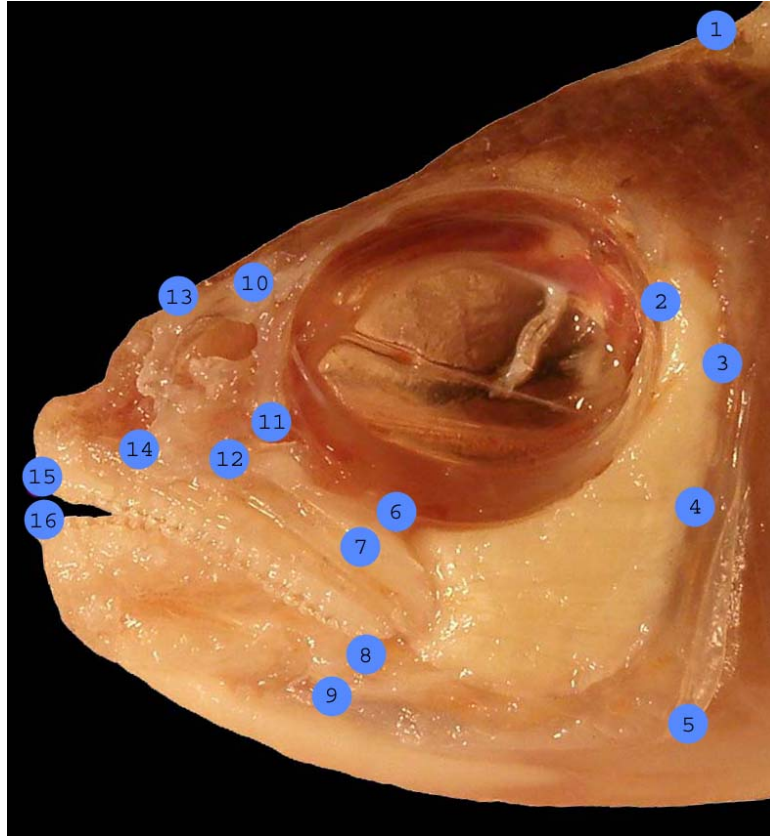


Figure 1: Craniofacial landmarks used in morphometric analysis characterize the shape and kinematics of the skull.

1. Dorsal-most tip of the supraoccipital crest on the neurocranium
2. Most posterior-ventral point of the eye socket
3. Most dorsal point on the origin of the A1 division of the *adductor mandibulae* on the preopercular
4. Most dorsal pint on the origin of the A2 division of the *adductor mandibulae* on the preopercular
5. Vento-posterior corner of the pre-opercular bone
6. Insertion of the A2 division of the *adductor mandibulae* on the maxilla
7. Maxillary-articular joint (lower point of maxillary rotation; tip of the closing in-lever in the lever model of the lower jaw)
8. Articular-quadrata joint (lower jaw joint; pivot point in the lever model of the lower jaw)
9. Insertion of the interopercular ligament on the articular (tip of the opening in-lever in the lever model of the lower jaw)
10. Joint between the nasal bone and the neurocranium
11. Most anterior-ventral point of the eye socket
12. Insertion of the A1 division of the *adductor mandibulae* on the maxilla
13. Posterior most tip of the ascending process of the premaxilla
14. Maxillary-palatine joint (upper point of maxillary rotation)
15. Anterior most tip of the anterior most tooth on the premaxilla
16. Anterior most tip of the anterior most tooth on the dentary

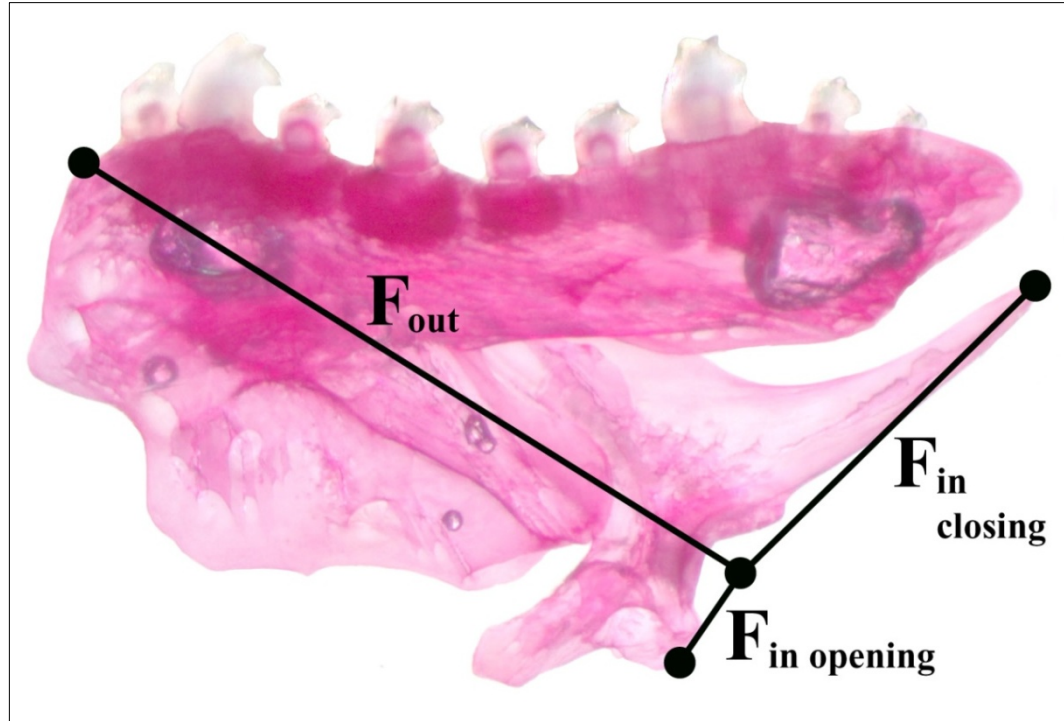


Figure 2: A first order lever model was used to characterize the biomechanics of the lower jaw. The fulcrum about which the jaw rotates is quadrate-articular joint. The out-lever is the distance from the foremost tip of the jaw to the fulcrum. The opening in-lever is the distance from the attachment point of the interopercular ligament which connects the interopercle and the posteroventral process of the lower jaw to the fulcrum. The closing in-lever is the distance from the insertion point of the A2 abductor mandibulae muscle on the posterodorsal most portion of the lower jaw to the fulcrum (Wainwright *et al.* 2005). Mechanical advantage was calculated as the ratio of in-lever to out-lever length.

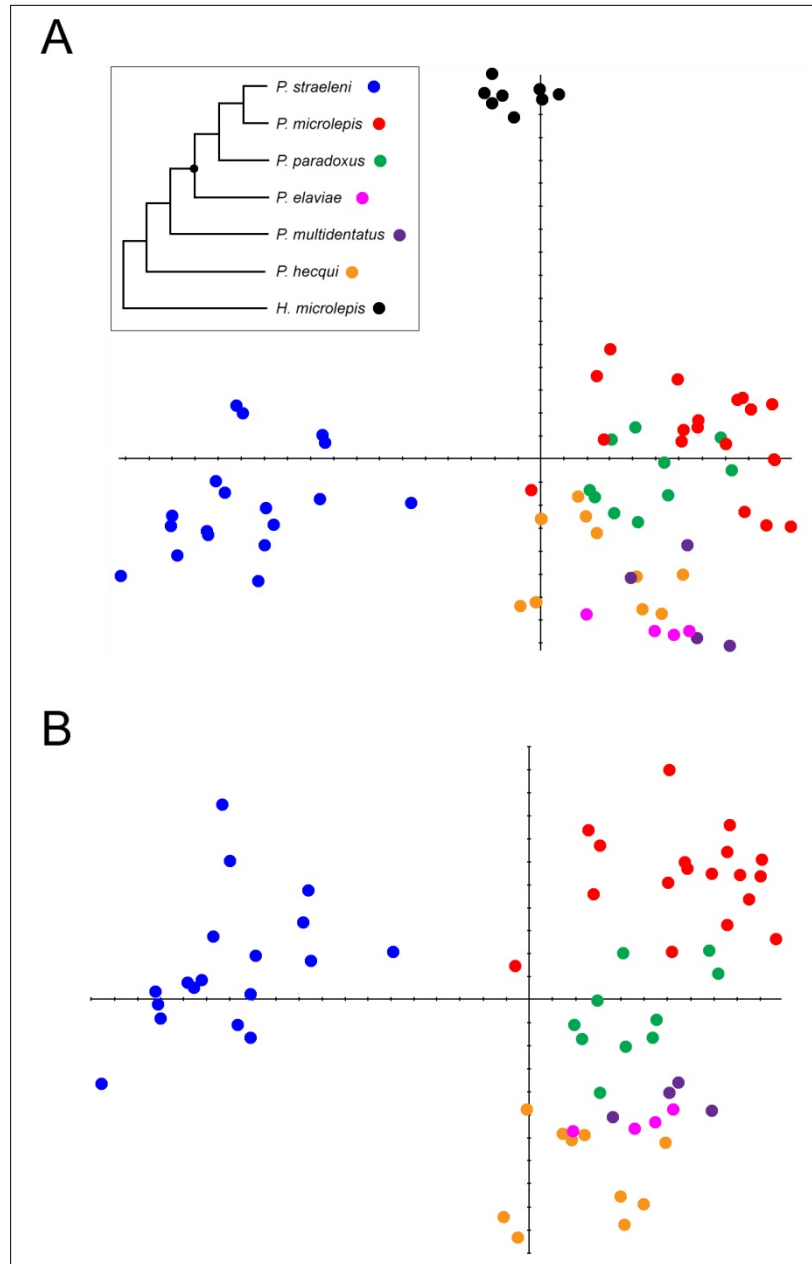


Figure 3: Relative warp analysis of the craniofacial morphologies of seven (A) and six (B) species of the Perissodini tribe. A: RW1 accounts for 39.87% of the variation in shape among species and represents variation in skull length. RW2 accounts for 24.22% of shape variation among species and reflects differences among species in the angle of the mouth. B: RW1 accounts for 50.88% of the variation in shape among species and represents variation in skull length. RW2 reflects variation in eye size for 17.00% of shape variation.

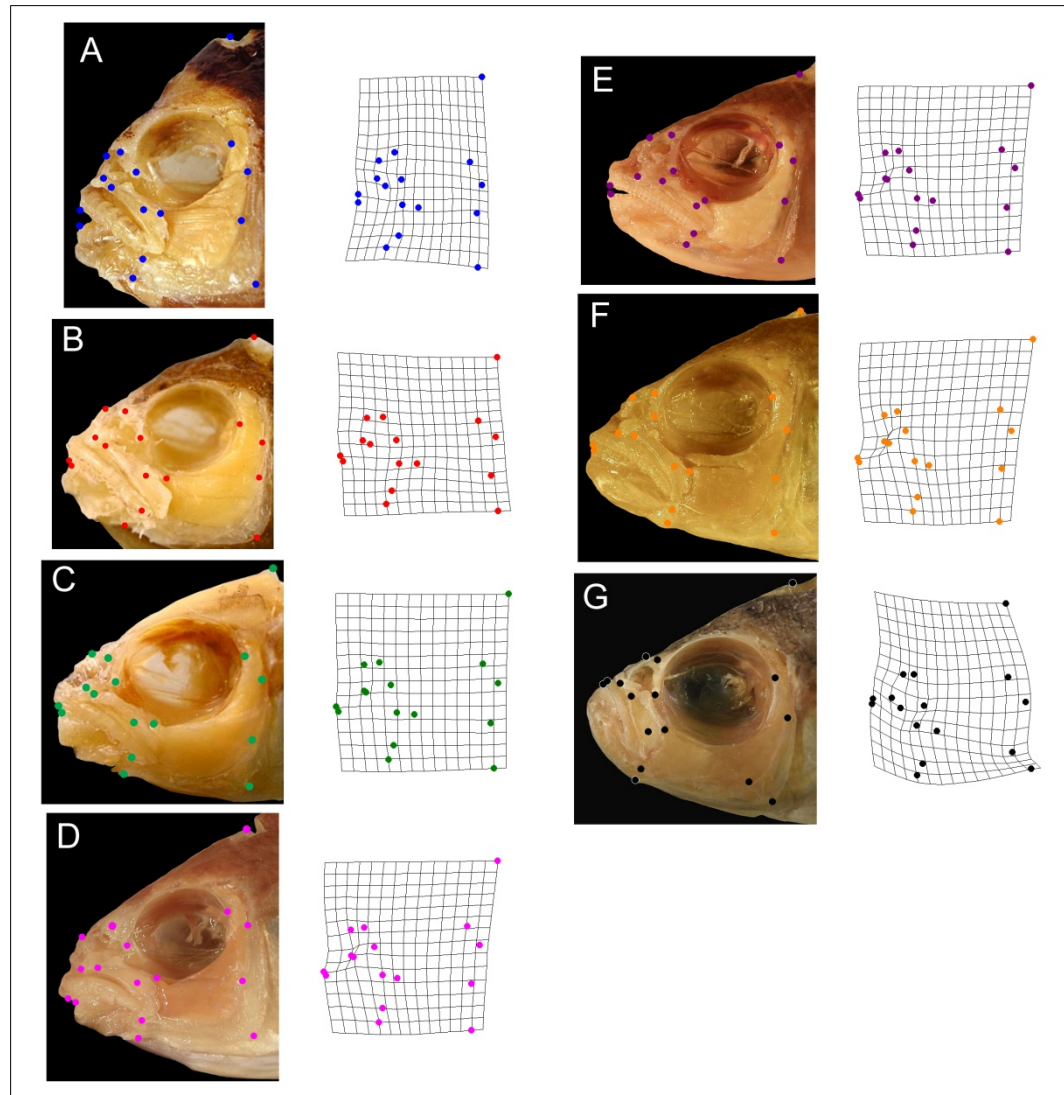


Figure 4: Representative individuals of the seven species included relative warp analyses and their associated warps (A- *P. straeleni*; B- *P. microlepis*; C- *P. paradoxus*; D- *P. elaviae*; E- *P. multidentatus*; F- *P. hecqui*; G- *H. microlepis*). *Perissodus* warps were derived from the relative warp analysis of six species. The mean shape for *H. microlepis* was derived from the relative warp analysis of all seven species.

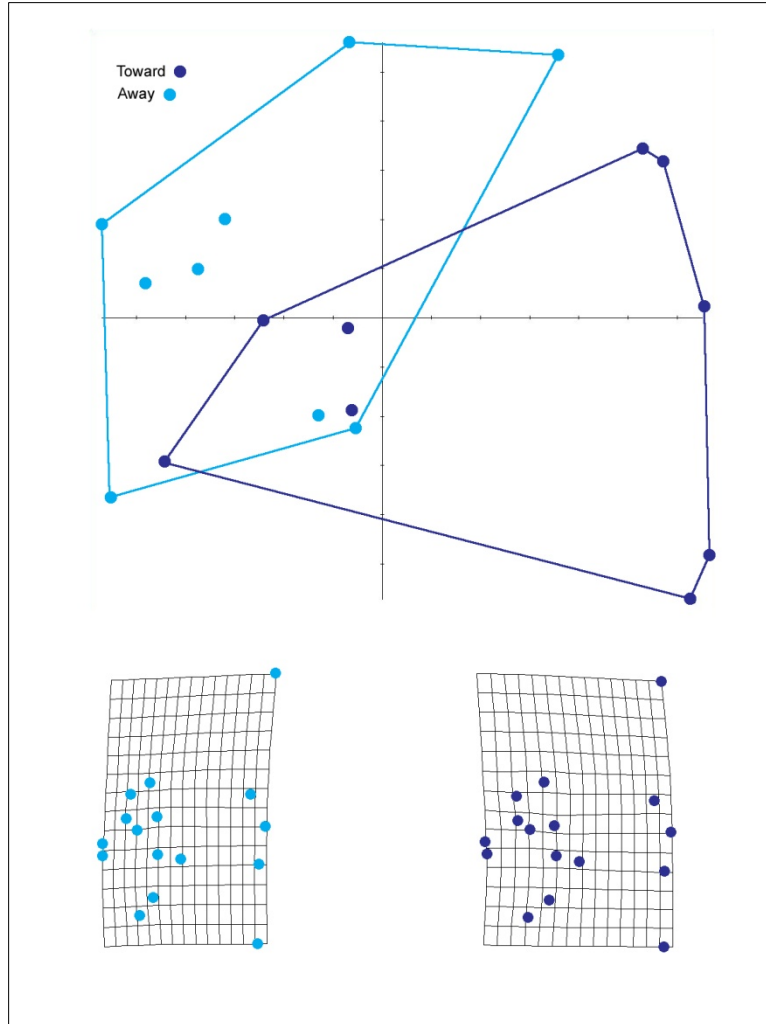


Figure 5: Relative warp analysis of *P. straeleni* craniofacial morphology, which indicates sided differences in shape of the skull. RW1 accounts for 32.13% of shape variation; RW2 accounts for 17.58% of shape variation. Sided differences in shape reflect a lengthening of the anterior portion of the skull.

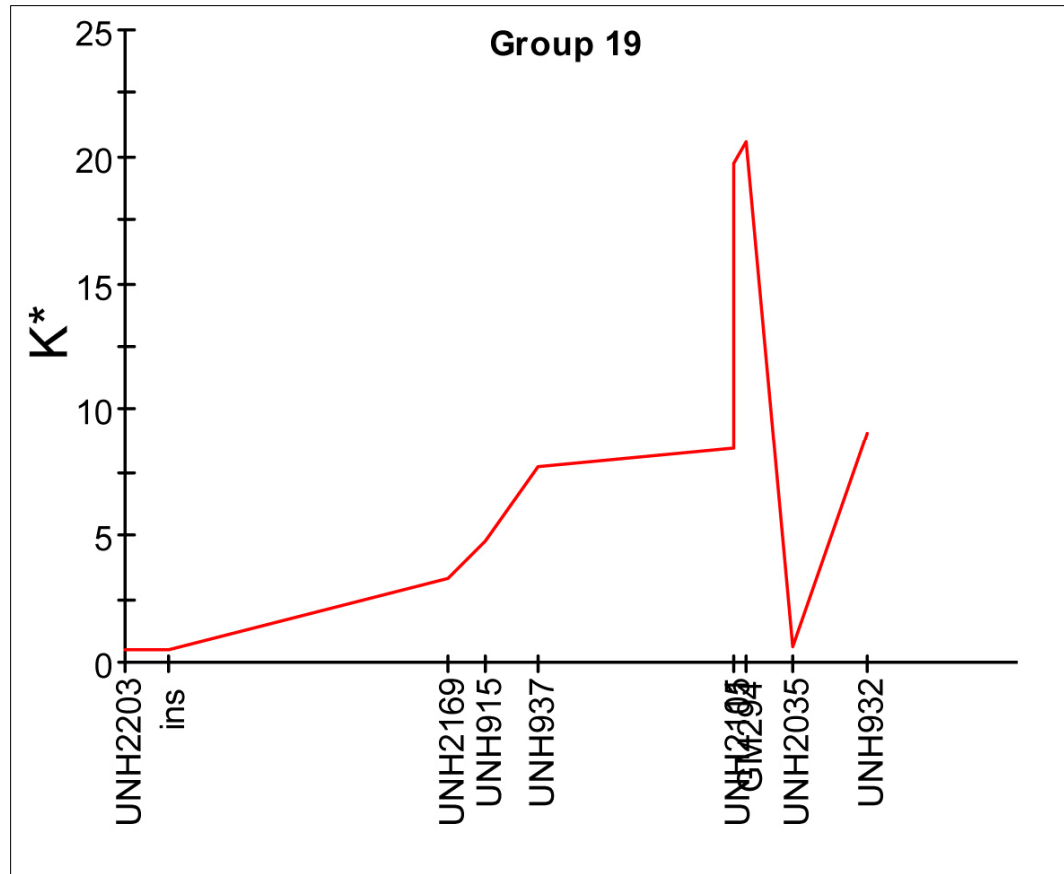


Figure 6: QTL analysis of a hybrid cross between the Lake Malawi cichlids *Metriaclima zebra* and *Labeotropheus feulleborni* identified a locus of major effect in defining left versus right directionality of the retro articular process of the lower jaw ($P > 0.00001$).

Written Capstone Summary:

I'm interested in evolution, specifically the evolution of morphology—how forms come to be. Studying the evolution of body design allows us to simultaneously ask questions of how species diversity relates to diversity in form, how form relates to function, and what the genes that underlie form are. My specific research, conducted in collaboration with Dr. R. Craig Albertson, has involved understanding the evolution of craniofacial asymmetry within the Perissodini tribe, a group of scale-eating fish found in Lake Tanganyika. Describing the architecture of Perissodini skulls and understanding how asymmetry evolved is a first step towards understanding the genetics of craniofacial asymmetries.

Most multicellular organisms exhibit symmetry. This symmetry is either radial, in which multiple planes of symmetry may be drawn across the organism (e.g., sea stars, jellyfish), or bilateral, where a single plane of symmetry, the sagittal plane, bisects the organism into mirrored halves. Vertebrates are bilaterally symmetric. The prevalence of superficially paired structures among vertebrates (e.g., eyes, ears, hands, and feet) can lead us to overestimate symmetry's ubiquity, and take for granted evolved asymmetries, which are exceedingly common.

Breaking symmetry can be incredibly advantageous from an evolutionary perspective. Owls have evolved asymmetrical ears, differing in size and placement on the skull, that make them more effective auditory predators. Our organs are arranged asymmetrically within our viscera to maximize available

space. Lateralization of behavior allows us to better perform certain tasks, though only with one side of our bodies (e.g., handwriting). The list of examples is long; deviation from the bilateral body plan is remarkably frequent. Despite this fact however, we don't yet fully understand how asymmetries evolve or the genetics behind asymmetry.

Our ignorance regarding the mechanisms involved in asymmetrical growth and patterning of bodies is perhaps most painfully illustrated by the many human birth defects characterized by asymmetric craniofacial malformations (e.g., hemifacial microsomia, Treacher-Collins syndrome, hemihypertrophy). Because we only poorly understand how and why asymmetries arise developmentally, early screening and preventative treatments for many of these conditions are yet unavailable. By studying natural systems with evolved craniofacial asymmetries, we hope be able to better understand birth defects that result in homologous conditions.

In Lake Tanganyika, one of East Africa's Great Lakes, reside several species of fish that make their living by feeding on the scales of other fishes. It is by their unique morphology that they are able to survive; their teeth are highly specialized for tearing or wrenching off the scales of their prey, and amazingly, they possess mouths that bend away from their midline, angled to either the left or right sides of their faces. Scale-eating is a surprisingly common feeding strategy, one which has repeatedly evolved among a number of fish lineages across the globe. What distinguishes this group, the Perissodini tribe, from other scale-eaters, is that it uses an asymmetrical skull to facilitate feeding. This asymmetry

was first noted in *Perissodus microlepis* by Leim and Stewart (1967), and is assumed to help them better hunt, allowing them to approach prey species from behind, presumably at an angle that increases successful prey strikes. In spite of its importance, the anatomy of this asymmetry has never fully been characterized.

Through this research, we sought to describe the evolution of this asymmetry: what are its anatomical underpinnings of this asymmetry, how have they changed as species evolved to specialize on scale eating, how do these changes in form affect jaw performance, and is laterality genetically defined in this group?

To tease apart the modifications undergone by the skull during this evolutionary trajectory, we obtained specimens for seven of the nine species in the Perissodini tribe (*Perissodus straeleni*, *Perissodus microlepis*, *Perissodus paradoxus*, *Perissodus elaviae*, *Perissodus multidentatus*, *Perissodus hecqui*, *Haplotaxadon microlepis*). Specimens used in our analysis came from museum collections and were preserved in alcohol. Each specimen was dissected, and skin and connective tissues were removed to expose points that would allow us to infer the mechanics of the jaw and describe the geometry of various elements of the skull. These landmarks, a collection of points that define the kinematics and geometry of the skull, include muscle origins and insertions, ligamentous origins and insertions and joints. Each side of the skull was photographed, and we compared the geometry of these landmarks in related species using a computer program designed for shape analysis. By comparing photographs of both sides of an individual's skull, we also measured asymmetry in the shape and size of

craniofacial elements. We also applied a simple lever model to quantify the mechanics of the lower jaw. Using mechanical advantage, a metric for lever systems, we were able to describe both the general trends in the evolved mechanical properties of the oral jaws within this group, and also describe implications of asymmetry on the mechanics of the oral jaw. Using quantitative trait locus (QTL) analysis, we also tested whether there's a genetic basis for left versus right handedness in jaw asymmetries of cichlids by analyzing genetic markers in a population of hybrid cichlids—the offspring of two different species mated in the laboratory. Comparing the direction of jaw asymmetry to markers in the genome, we can infer whether left versus right handedness is genetically defined.

The evolution of scale-eating in this group appears to have involved two fundamental shifts in skull shape. As ancestral predators began to pursue new quarry in shallower waters, eyes reduced in size, adapting to higher light environments. Recently evolved scale-eaters tend to occupy shallow rocky habitats, with eyes that are proportionally much smaller than more ancestral species. The second shift involved modifications to the length of the skull. Differences in skull length are a reflection of differences in the way that each species moves. Short and deep skulls, which tend to be associated with deep bodies, like that of *Perissodus straeleni* are better suited for maneuverability than for speed. Long and narrow skulls, with associated torpedo shaped bodies, like that of *Perissodus microlepis*, on the other hand, lend themselves more to speed than to dextrous maneuvers. By studying the skulls of these organisms, we gain

insight into their ecology. We can discover how their environment has influenced their morphology, and now their morphology relates to behavior.

We noted asymmetry in all members of the Perissodini tribe, with the exception of *H. microlepis*, the oldest species. Among older species, these asymmetries mostly involved differences in size of skull components, but in the most recently evolved scale-eaters *P. microlepis* and *P. straeleni*, the asymmetry was one of shape as well. These asymmetries in shape influence the mechanics of the lower jaw lever system, producing lateralization of force and speed in jaw mechanics.

Traditionally, it has been assumed that for dimorphic asymmetrical traits, the nature of the asymmetry (i.e., which traits are affected) is genetically determined, but the side in which the trait manifests itself is environmentally determined. Our QTL analysis identified a locus that defines left versus right handedness of the jaws in Lake Malawi cichlids. This finding is the first of its kind, and perhaps the most robust example of a genes defining laterality of a dimorphic asymmetrical trait. Based on the region of the genome identified by our analysis, we have identified WNT11 as a candidate gene for defining laterality, and ongoing research will seek to determine whether it is responsible for defining handedness in the Perissodini clade.

By studying the Perissodini scale-eaters, we have both broadened our understanding of the functional morphology of this group, and opened avenues for future research. Understanding the anatomy of the asymmetry, and identifying regions in the genome associated with the left versus right handedness allows us

to discover the genes involved in defining laterality. Research is ongoing in the Albertson lab to understand the genetics of craniofacial asymmetry.