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Authors

Hulsey, C Darrin
Alfaro, Michael E
Zheng, Jimmy
et al.

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Author for correspondence:

C. Darrin Hulsey
 e-mail: darrin.hulsey@uni-konstanz.de

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Pleiotropic jaw morphology links the evolution of mechanical modularity and functional feeding convergence in Lake Malawi cichlids

C. Darrin Hulsey¹, Michael E. Alfaro², Jimmy Zheng², Axel Meyer¹ and Roi Holzman³

¹Department of Biology, Universität Konstanz, Universitätsstraße 10, 78457 Konstanz, Germany

²Department of Ecology & Evolutionary Biology, University of California-Los Angeles, 610 Charles E. Young Drive East, Los Angeles, CA 90095-7246, USA

³Department of Zoology, Tel Aviv University and the Inter-University Institute for Marine Sciences, PO Box 469, Eilat 88103, Israel

CDH, 0000-0002-9653-6728; RH, 0000-0002-2334-2551

Complexity in how mechanistic variation translates into ecological novelty could be critical to organismal diversification. For instance, when multiple distinct morphologies can generate the same mechanical or functional phenotype, this could mitigate trade-offs and/or provide alternative ways to meet the same ecological challenge. To investigate how this type of complexity shapes diversity in a classic adaptive radiation, we tested several evolutionary consequences of the anterior jaw four-bar linkage for Lake Malawi cichlid trophic diversification. Using a novel phylogenetic framework, we demonstrated that different mechanical outputs of the same four jaw elements are evolutionarily associated with both jaw protrusion distance and jaw protrusion angle. However, these two functional aspects of jaw protrusion have evolved independently. Additionally, although four-bar morphology showed little evidence for attraction to optima, there was substantial evidence of adaptive peaks for emergent four-bar linkage mechanics and jaw protrusion abilities among Malawi feeding guilds. Finally, we highlighted a clear case of two cichlid species that have independently evolved to graze algae in less than 2 Myr and have converged on similar jaw protrusion abilities as well as four-bar linkage mechanics, but have evolved these similarities via non-convergent four-bar morphologies.

1. Introduction

Phenotypic complexity could fundamentally influence how organisms diversify. Because most biological traits are assembled from an integrated hierarchy of phenotypes (e.g. genes, morphology and biomechanics), the complexities linking these levels of organization could commonly impact adaptive divergence [1–4]. For instance, structural phenotypes ranging from proteins to musculoskeletal systems often consist of nonlinear combinations of underlying parts that can frequently become morphologically distinct but still result in the evolution of surprisingly convergent mechanical traits, physiological attributes or ecological specializations [5–8]. This type of convergence at a more emergent level of biological organization via distinct non-convergent underlying mechanisms is often referred to as many-to-one mapping and could be common for complex structural phenotypes [8]. Yet, structures that contribute to equivalence in one emergent function might often pleiotropically, or simultaneously influence, a number of other emergent biological functions [9]. Interestingly, complexity in these many-to-one systems has been suggested to readily facilitate evolutionary decoupling of emergent mechanical and functional attributes during adaptive radiation, despite paradoxically having pleiotropic

effects on these same traits [10–13]. To simultaneously evaluate several of these predictions using a model many-to-one mapped system in a classic adaptive radiation, we examined how the anterior jaw four-bar linkage influences the evolution of linkage mechanics, jaw function and trophic convergence in Lake Malawi cichlid fishes.

Modelling components of the fish skull as discrete mechanical systems provides powerful insight into how morphological differences translate into ecological specialization [10–13]. Although simple lever-like systems are integral to the trophic apparatus of all jawed vertebrates [14,15], more complex mechanical systems such as four-bar linkages often characterize the many mobile elements in the teleost feeding apparatus (figure 1). These four-bar linkages are well suited for examining form-function evolution because the individual phenotypes parameterizing these models, while often composed of a large number of musculoskeletal structures, can be reduced to only four linear elements [15,16]. Nevertheless, several emergent mechanical properties can be precisely calculated from quantifying four-bar morphology and its movement during feeding [11,14]. For instance, the anterior jaw four-bar linkage has been used to highlight the evolutionary relationship between greater lower jaw-induced rotation of the maxilla, or higher kinematic transmission (KT), and more extensive jaw protrusion distance in several fish groups [14,17,18]. Understanding if this mechanism drives Malawi cichlid jaw protrusion distance is ecologically relevant because greater jaw protrusion is frequently favoured when feeding on highly elusive prey, whereas reduced jaw protrusion is often associated with cichlids obtaining attached resources from the substrate [18–20].

The morphology of the anterior jaw four-bar linkage has also been suggested, but never shown empirically, to have pleiotropic-like effects on other feeding functions [10,11]. We hypothesized that because the nasal and maxilla bones compose two elements of the four-bar linkage and should guide the upper jaw during jaw protrusion [21], the four-bar linkage could dictate divergence in another functional trait: jaw protrusion angle. Based on four-bar morphology, it is easy to imagine that the nasal bone's angular orientation following mouth opening (α_N ; figure 1) could determine cichlid jaw protrusion angle. Protrusion angle could also be as crucial during cichlid feeding as protrusion distance, because the orientation of a fish's mouth is often associated with exploitation of trophic resources from different substrates [22,23]. For instance, terminal mouths protrude in the same direction as the fish's body thereby enhancing the ability to rapidly close the distance to a prey item in open water [21,24–26]. Conversely, benthic-feeding species that feed from algae-covered rocks or by excavating sandy substrates often have subterminal mouths [27,28]. Transitions between feeding benthically or feeding in the water column represent major trophic shifts that have occurred repeatedly in Lake Malawi cichlids [29,30]. Both jaw protrusion distance and jaw protrusion angle could be predicted from four-bar linkage mechanics and could be functionally critical to replicated trophic specialization in Malawi cichlids.

Compromises, or trade-offs, between multiple functions should influence the evolution of complex organismal systems [31,32]. For instance, if the anterior jaw four-bar linkage commonly determines both the distance as well as the angle of jaw protrusion, adaptive change in jaw protrusion distance might require jaw protrusion angle to be

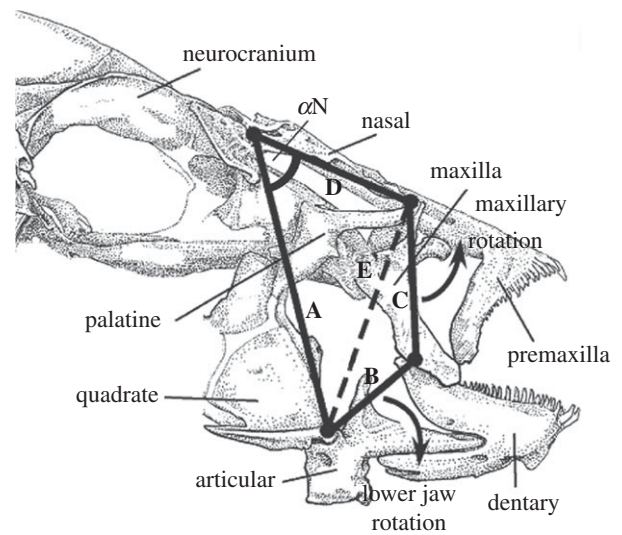


Figure 1. The morphology and mechanics of the cichlid anterior jaw four-bar linkage. The morphological elements of the anterior jaw linkage: input link (A), output link (B), coupler link (C) and fixed link (D) are shown with heavy black lines and the diagonal (E) is depicted with a dashed line. Arrows highlight the directionality of movement of the lower jaw and maxilla during jaw opening that determines kinematic transmission (KT) and the angle of the nasal (α_N).

altered in non-optimal ways. Because this type of trade-off should hinder the response to selection, the extent to which a system's shared emergent functions can change independently could influence how organisms diversify [3,11,12,33–38]. These types of trade-offs are often predicted to be especially prevalent when functional properties share an underlying morphological basis [10–12]. Yet, in many-to-one mapped systems, anatomical configurations that determine one mechanical property can sometimes be optimized without compromising other mechanical properties, thereby mitigating evolutionary trade-offs [9–12,26]. Interestingly, the ability of cichlid individuals to match the demands of obtaining particular prey types by independently modulating the distance and the angle that the oral jaws are protruded, or 'modularity multiplicity', has long been hypothesized to be a key factor in the evolutionary success of cichlids [39,40]. Despite any shared morphological underpinnings or associated mechanical trade-offs, jaw protrusion distance and jaw protrusion angle could have evolved largely independently on a macro-evolutionary level during the exceptional diversification of Lake Malawi cichlids.

The repeated evolution of functionally equivalent solutions to ecologically relevant tasks is one of the defining characteristics of adaptive radiation [8,41]. For instance, replicate lineages within iconic groups such as the *Anolis* lizards, Hawaiian honey creepers and East African cichlids have all independently evolved phenotypic similarities that allow them to exploit convergent ecological niches [27,42–44]. However, most of these examples come from allopatric lineages that have diverged on different islands or within separate lakes. Perhaps the most amazing examples of convergence involve situations where lineages have evolved similar adaptations rapidly, in parallel, and in the same geographical location [45,46]. For instance, the two most species-rich clades of cichlids in Malawi, the primarily rock-dwelling mbuna and largely sand-dwelling non-mbuna, have been suggested to have repeatedly evolved in less

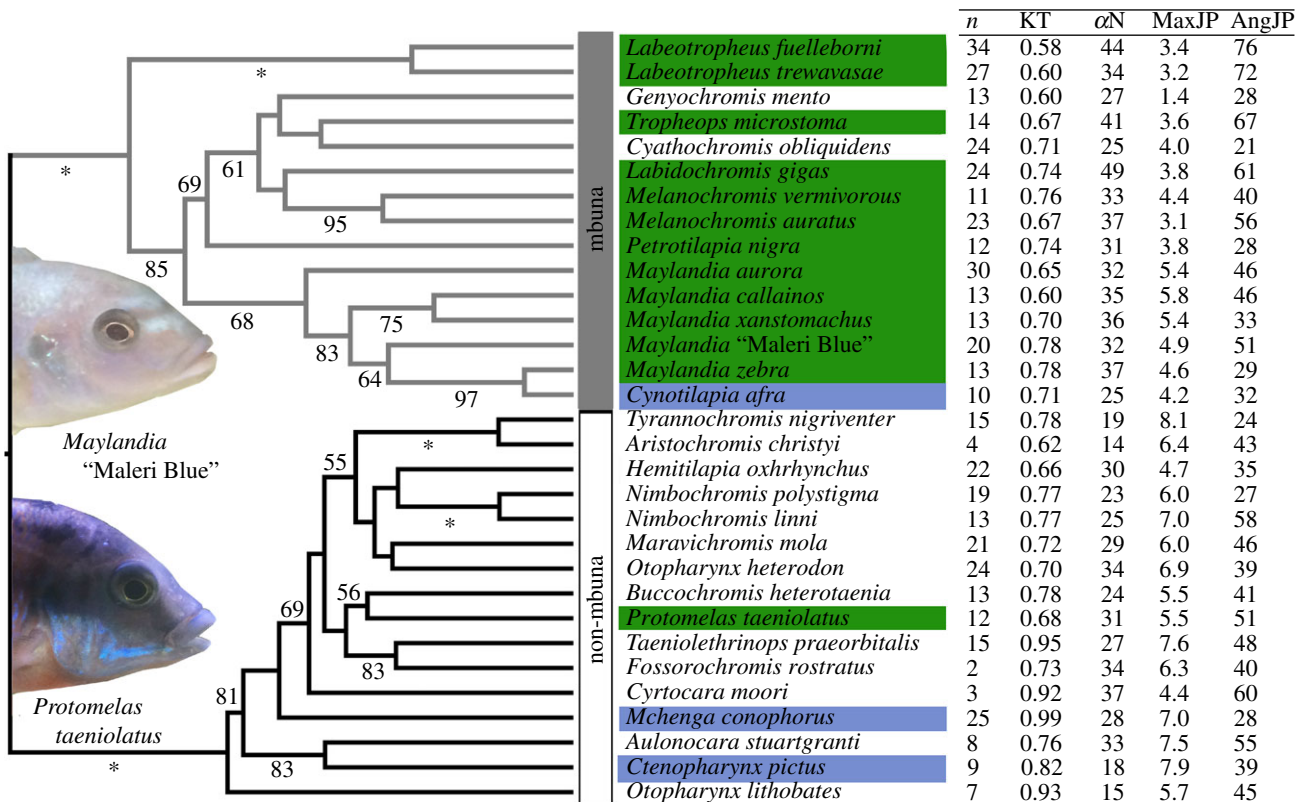


Figure 2. The ultra-conserved element (UCE) inferred consensus phylogeny for the 31 species of Malawi cichlids. Species highlighted in green feed primarily from algae-covered rocky outcrops and species highlighted in blue feed in the water column on plankton. The early split separating the two major clades of mbuna (grey) and non-mbuna (black) is highlighted. The numbers behind the nodes represent the percentage of times that node was recovered in replicate tree searches and an asterisk (*) represents 100% bootstrap support. The species values of KT and α N were calculated from linkage measurements generated previously [3]. Also, the numbers (*n*) of individuals examined for the jaw protrusion measurements are given. Additionally, the species average maximum jaw protrusion distance (MaxJP) adjusted as a percentage of standard length (SL) and average jaw protrusion angle (AngJP) are shown.

than 2 Myr to feed from a diversity of similar ecological substrates [47,48]. Both groups have members that exploit plankton in the open water and that feed from the algal mats that cover rocky outcrops found throughout the Lake [30,49]. Yet, because closely related lineages like these inhabiting the same location could often simply inherit or exchange adaptive alleles, it is often difficult to know if putatively convergent lineages evolved similar functional abilities completely *de novo* or simply assimilated the same ancestral alleles that generate adaptive similarities [45,50–53]. Yet, if the underlying morphological basis of functional convergence is many-to-one mapped and thereby generated through highly divergent morphologies, this would provide more definitive evidence that the mechanistic basis of convergent phenotypes arose independently even in the same geographical setting.

Using an integrative approach, we examined the evolution of the anterior jaw four-bar linkage and its trophic consequences in Lake Malawi cichlids. To generate a more robust framework for tracing phenotypic evolution in Malawi cichlids, we first reconstructed a phylogenetic hypothesis using approximately 1000 loci. Then, we asked whether morphologies (the links in the four-bar linkage) pleiotropically map to the mechanical properties (KT and α N), and how these properties map to functional abilities (protrusion distance and protrusion angle) that should be relevant to feeding from different substrates. We then tested whether the two functional abilities coevolve or generally evolve independently during Malawi cichlid macroevolution. Finally, to examine an underappreciated way that many-to-one mapping can contribute to recent adaptive radiation, we

tested the hypothesis that (i) across the entire radiation as well as (ii) within particular species pairs that feeding specialization in Malawi cichlids can show evidence of convergence in oral jaw function and linkage mechanics without convergence in underlying anterior jaw morphology.

2. Material and methods

(a) Ultra-conserved element isolation and phylogenetic reconstruction

We collected specimens for phylogenetic and morphological analyses from several locations in Lake Malawi. For phylogenetic analyses, we combined published ultra-conserved element (UCE) sequence data [19,29,54,55] with data produced for four additional species (GenBank BioProject: PRJNA344532). UCE sequence data were generated according to the protocols outlined in Hulsey *et al.* ([40]; electronic supplementary material, Methods S1). Our resulting 1024 single nucleotide polymorphisms (SNPs) were analysed with the SNP_{PHYLO} pipeline [56] to generate a maximum-likelihood phylogeny that was then passed through PHANGORN [57] to produce 1000 bootstrap replicate trees to assess node confidence.

(b) Trophic phenotypes

For the oral jaw functional trait values, we measured both maximum jaw protrusion distance and jaw protrusion angle on 2–30 anesthetized individuals per species immediately following collection from Lake Malawi (figure 2). Maximum protrusion distance was determined using two measurements: (i) ‘jaw

closed' and (ii) 'jaw open'. Jaw closed was measured as the distance from the premaxilla's anterior tip to the most posterior point of the eye when the jaws were closed. Jaw open was determined as the distance from the tip of the premaxilla to the posterior point on the eye when the jaws were maximally extended. We then subtracted jaw closed from jaw open and size-standardized these measurements by standard length (SL). This size-standardization allowed us to comparatively assess how maximum jaw protrusion might differ functionally among these species during prey capture. To measure jaw protrusion angle, we placed specimens upon waterproof graph paper. Then, we centred the pupil where two graph lines intersected and positioned the specimen so that one line ran through the body axis to the centre of the caudal peduncle. The point that the front of the head intersected the line running through the body axis was then marked. Critically, this point approximates the internal anatomical point at which the nasal attaches onto the suspensorium which could be important for the angle of jaw protrusion (see below). We then rotated the lower jaws with forceps and marked the anterior tip of the premaxilla when the upper jaw was maximally protruded. Using a protractor, we determined the jaw protrusion angle (in degrees) with respect to the body axis.

The four morphological measurements of the anterior jaw four-bar linkage (figure 1) were taken from a previous study [3]. Briefly, we cleared and stained a subset, approximately three specimens per species, of wild-caught individuals and measured the links of the four-bar when the jaws were in a closed resting position. The distance from where the nasal attaches to the neurocranium down to the quadrate-articular joint was measured as the immobile fixed link (A). The lower jaw rotates on this fixed link and serves as the input link (B) that transmits motion into the system. The lower jaw link is measured from where the articular rotates on the quadrate to the maxilla's ligamentous attachment on the dentary. We then determined the distance between the maxilla's dentary attachment and the ligamentous connection of the nasal on the maxilla (C). This functions as the output link in the four-bar linkage. The final link measured was the nasal (D) that is often modelled as the anterior jaw four-bar linkage coupler link [9–14]. For all comparisons, these four morphological elements were size-standardized as a percentage of SL.

For the determination of four-bar linkage mechanics, an initial resting angle of 15° was assumed between the lower jaw and the fixed link [18] thereby mathematically defining the diagonal (E). Defining this angle also allows the physical location of the four links to be exactly defined [14]. All mechanical values were then calculated following an input of 30° lower jaw rotation. The KT coefficient in the four-bar was calculated as the ratio of degrees of maxillary link output rotation for a given lower jaw input rotation and is determined through a series of trigonometric equations [18]. Following the modelled 30° of lower jaw rotation, we also inferred the angle between the nasal and the fixed link (αN) with the same equations used to calculate KT from the movement of the four-bar. Angles are non-dimensional so they were not standardized by SL.

(c) Pleiotropic morphology and modularity of jaw protrusion

Because species are not evolutionarily independent [58], we performed several phylogenetic-independent contrast (PIC) correlations among our morphological, mechanical and functional variables. We performed all comparative trait analyses on 100 randomly chosen trees from the SNP_{PHYLO} analyses [59] which we subsequently rendered ultrametric using the R function 'chronopl' implemented in APE [59]. Using these trees, we first determined whether evolutionary increases in KT were

correlated with evolutionary increases in protrusion distance. We also determined whether changes in αN was correlated with changes in protrusion angle. The correlations of contrasts between KT and αN were also examined. Finally, we determined whether changes in the two functional abilities, protrusion distance and protrusion angle, were evolutionarily correlated.

(d) Convergence at multiple levels

To test for Malawi-wide convergence in oral jaw function (protrusion angle and protrusion distance), mechanics (KT and αN) and anterior jaw morphology (four-bar link lengths), we first evaluated the evidence for stabilizing peaks of phenotypic evolution associated with feeding regimes that most clearly independently evolved in Malawi. We divided all species into the feeding guilds: (i) planktivore, (ii) algivore, and (iii) other (figure 2). Although the 'other' category includes substantial trophic diversity, we wanted to focus our analyses on convergent feeding guilds shared between the mbuna and non-mbuna clades that often represents an early and phylogenetically well-supported split in the Malawi cichlids. Although the inclusion of this diverse other trophic guild as a single category could influence our inferences, our reasoning was that the range of phenotypes included in this grouping should provide a robust null model for convergence in planktivorous and/or algivorous lineages from the two major clades.

Using the above trophic categories, we tested four models of trait evolution for each level of phenotypic organization (function, mechanics and morphology). We first used the 100 random ultrametric trees generated above and the command 'make.simmap' [60] to trace the evolution of the three feeding modes. We then implemented the command 'Ouwie' [61] to estimate the support for four competing models of trait evolution: (i) a single rate Brownian motion model (BM1), (ii) a multiple rate Brownian model (BMS), (iii) a single peak Ornstein-Uhlenbeck model (OU1), and (iv) a multiple peak OU model (OUM). Under BM1, traits evolved with a single rate parameter across the tree, regardless of the trophic regime. Under BMS, traits evolved with a different rate parameter fitted to each of the trophic regimes. Under OU1, traits evolved towards a single optimum, with a single rate and attraction parameter, regardless of the trophic regime. Finally, under OUM, traits evolved towards trophic regime dependent optima, with a single rate and attraction parameter. While more complex OU models exist that allow multiple rate and attraction parameters, our data lacked sufficient power to fit these parameters [61]. The four evolutionary models (BM1, BMS, OU1 and OUM) were fitted to individual traits at each level (function, mechanics and morphology) of phenotypic organization (electronic supplementary material, Methods S2). Convergent traits should generally fit more OU patterns of evolution and exhibit peaks of phenotypic association across similar feeding regimes [62], while many-to-one mapped components might be expected to evolve more consistently via Brownian motion.

Finally, to test the idea that convergent feeding modes could show convergence in function or mechanics without convergence in underlying morphology for two convergent species, we focused on pairs of trophic specialists that are present in both the Malawi mbuna and non-mbuna clades. First, we examined if planktivores in the non-mbuna showed evidence of convergence with the planktivore *Cynotilapia afra* that is nested within the primarily algivorous mbuna. Then to examine convergence in algivores, we focused on one of the most unambiguous examples of Lake Malawi feeding convergence, the species *Protomelas taeniolatus*. This species is nested within the primarily sand-dwelling non-mbuna (figure 2), but has colonized rocky reefs and independently evolved an algivorous feeding habit that characterizes many mbuna. To isolate putative pairs of convergent species, we first generated a phylofunctionalspace [63] of

Table 1. Correlations among four-bar linkage jaw mechanics and jaw protrusion functions. (A correlation matrix of the phylogenetically independent contrasts between the mechanical (KT and αN) as well as putative functional (protrusion distance and angle) attributes of the anterior jaw four-bar linkage. The mean correlation coefficient \pm the standard error, $r \pm$ s.e., of the two traits from 100 randomly chosen topologies is shown above the diagonal and the median \pm median absolute deviation for p -values of the relationship between the traits is given below the diagonal. Correlations having a median p -value of 0.05 or below are highlighted in italics.)

	mechanics		function	
	KT	αN	distance	angle
KT	—	0.21 \pm 0.14	0.36 \pm 0.13	-0.02 \pm 0.16
αN	0.31 \pm 0.19	—	0.30 \pm 0.12	-0.49 \pm 0.10
distance	0.05 \pm 0.05	0.11 \pm 0.09	—	-0.12 \pm 0.11
angle	0.69 \pm 0.17	<0.01 \pm 0.01	0.51 \pm 0.18	—

the jaw protrusion distance and angle. We used this to determine which species of non-mbuna planktivore showed the most similarity to *Cyn. afra* and which species of algivorous mbuna showed the most similarity in jaw protrusion abilities to *P. taeniolatus*. For comparison to the functional similarities, we also generated a phylogenetic space of the mechanical divergence in the Malawi cichlids and the morphological divergence as represented by the first two phylogenetic principal component analyses (PCA) scores [60] of each species.

Then, to determine quantitatively if these two pairs of species showed exceptional convergence in protrusion distance and angle, we calculated two measurements of convergent evolution [64]. The first metric implemented was $C1 = 1.0 - (D_{tip}/D_{max})$, where D_{tip} is the Euclidean distance between trait values of convergent tips in phenotypic space and D_{max} is the maximum Euclidean distance between any pair of taxa. This metric represents the proportion of the maximum distance between two lineages that has been 'closed' by subsequent evolution, and thus ranges from 0.0 to 1.0. A value of 1.0 indicates two lineages have evolved to be indistinguishable, whereas a value of 0.0 indicates lineages are as different as any two lineages have ever been in the radiation. Another metric can be defined that does not use ratios and more linearly accounts for the magnitude of change: $C2 = D_{max} - D_{tip}$. Again, larger values of this metric indicate greater convergence. These two metrics were calculated in the R program 'convevol' [64] across the 100 trees. Focusing on these two sets of species, we asked if their protrusion abilities or underlying mechanics showed exceptional similarity given the divergence across the Lake Malawi radiation. When examining convergence in multiple traits simultaneously, such as the length of the four links, this program effectively generates a phylogenetic PCA-like reconstruction, in this case in three-dimensions, for the four morphological trait values [64]. So, to test if the four-bar morphology for these species was exceptionally similar, we determined if the lengths of the four links in the convevol generated multi-variate space were significantly more similar than expected by chance.

3. Results

(a) Convergence in diet

Our phylogenetic reconstructions highlighted that ecological convergence has occurred multiple times between the two major clades of Malawi cichlids (figure 2). As has been found previously [19,30,54,55], the primarily rock-dwelling mbuna formed a monophyletic clade. The species in the mbuna genera *Genyochromis*, *Maylandia*, *Cyathochromis*, *Petrotilapia*, *Melanochromis*, *Tropheops* and *Labidochromis* form a monophyletic

clade with moderate support (85% bootstrap support) that is sister to the genus *Labeotropheus*. The planktivorous *Cyn. afra* was inferred to be nested within the *Maylandia* species. *Maylandia callainos* and *Maylandia xanostomachus* were recovered most frequently as sister, and *Maylandia zebra* and *Cyn. afra* had strongest affinities with *Maylandia* 'Maleri Blue.' We also recovered a large clade of non-mbuna, or largely sand-dwelling cichlids, containing species in the genera *Nimbochromis*, *Placidochromis*, *Taeniolethrinops*, *Aristochromis* and *Tyrannochromis* with strong support (100% bootstrap support). This clade also included several planktivorous species such as *Ctenopharynx pictus* and *Mchenga conophoros*. Although its exact position was unresolved, the algivorous species *P. taeniolatus* was strongly nested within the non-mbuna group of primarily sand-dwelling, non-herbivorous species.

(b) Trophic phenotypes

We recovered several striking examples where closely related species did not differ substantially in jaw protrusion distance, but exhibited either a terminal mouth or a highly subterminal mouth. For instance, *Ma. zebra* and *Labeotropheus fuellebornii* differ by less than 1.0% in standardized jaw protrusion but have jaw protrusion angles that differ by almost 50°. Similarly, the congeneric *Nimbochromis polystigma* and *Nimbochromis linni* differ by less than 1.0% in standardized jaw protrusion but have jaw protrusion angles that differ by 30°. Across the Malawi species, jaw protrusion distance varied widely, and ranged from 1.4% of SL in *Genyochromis mento* to 8.1% in *Tyrannochromis nigriventer*. Average jaw protrusion angle for all the species was 44°, and ranged from 21° in the terminally protruding *Cyathochromis obliquidens* to a high of 76° in the very subterminal mouth of *L. fuellebornii*. The average αN was 60°, and ranged from 76° in *Aristochromis christyji* to 38° in *Tropheops microstoma*. The KT values had a mean of 0.73 with a low of 0.56 in *L. fuellebornii* and a high of 1.00 in *Mc. conophoros*.

(c) Pleiotropic morphology and modularity of jaw protrusion

The two derived mechanical attributes of the four-bar linkage were related to separate functional aspects of jaw protrusion (table 1). The evolution of higher KT was associated with greater jaw protrusion distance (mean $PIC_r = 0.36$; median $PIC_p = 0.05$). Evolutionary change in αN was also correlated with the evolutionary change in jaw protrusion angle (mean

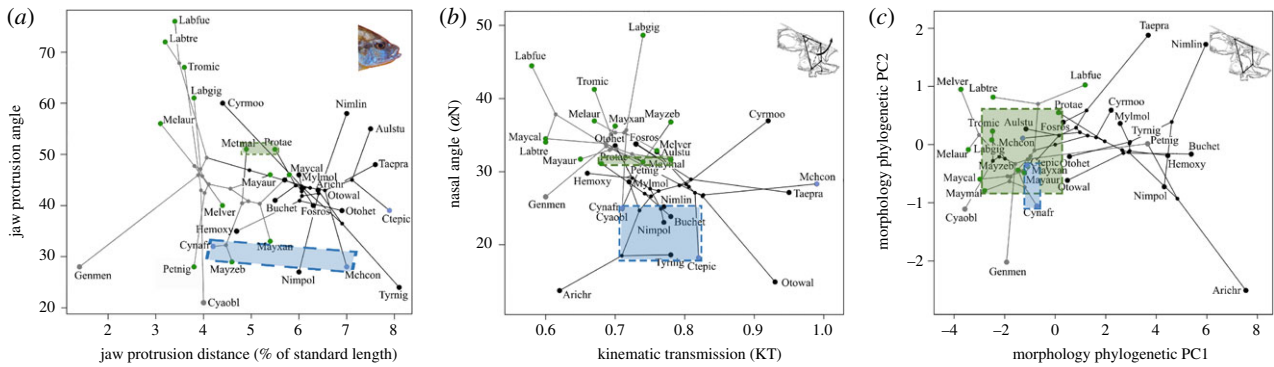


Figure 3. Cichlid anterior jaw phylogenetic divergence. A phylofunctional space (a) depicting maximum protrusion distance and protrusion angle in Malawi cichlids with respect to phylogenetic divergence highlights the substantial evolution in these traits. The phylomechanical space (b) visualizes cichlid diversification in KT and αN . The first two axes of the principal component analyses (PCA) from a phylogenetic PCA of the SL standardized four link lengths are projected in a phylomorphospace (c). In all panels, colours at the tips match highlighted substrate feeding specializations and species names are abbreviated with the first three letters of the genus and species depicted in the phylogeny (figure 2). The branches subtending the mbuna lineages are shown in grey while black demarcates non-mbuna. The translucent dashed blue box highlights the planktivores from the two major lineages that are closest in each phylogenetic space. The translucent dashed green box highlights the convergence between *Protomelas taeniolatus* and *Maylandia* ‘Maleri Blue’ that is substantial with respect to jaw function and mechanics, but is not apparent in anterior jaw morphology.

Table 2. Convergence at multiple levels. The independently evolved algae feeding Malawi cichlids *Protomelas taeniolatus* and *Maylandia* ‘Maleri Blue’. (The values of C1 and C2 are given for the jaw functions (protrusion distance and protrusion angle), mechanics (KT and αN) and morphology (fixed, nasal, maxilla and lower jaw link lengths) associated with the anterior jaw four-bar linkage. The values and their significance ($p < 0.05$ highlighted with italics) were estimated from 100 random post burn-in phylogenetic reconstructions. The mean of the point estimates and median of the p -values from these 100 phylogenetic replicates with \pm standard error or median absolute deviation, respectively, are given. These two species are convergent in their ecology, jaw protrusion abilities and mechanics without being convergent in their underlying anterior jaw morphology.)

	C1	p -value	C2	p -value
function	0.97 ± 0.02	0.01 ± 0.01	24.98 ± 10.55	0.04 ± 0.08
mechanics	0.95 ± 0.03	0.02 ± 0.02	12.05 ± 6.29	0.04 ± 0.06
morphology	0.40 ± 0.12	0.25 ± 0.12	2.40 ± 1.27	0.17 ± 0.15

$PICr = -0.49$; median $PICp < 0.01$). Conversely, KT was not correlated with protrusion angle (median $PICp = 0.69$), and αN was not correlated with protrusion distance (median $PICp = 0.11$). However, the two mechanical attributes, KT and αN , were not themselves correlated (median $PICp = 0.31$). Finally, although they are both related to anterior jaw mechanics inferred from the same configuration of four-bar morphological elements, protrusion angle and protrusion distance were not evolutionarily correlated (median $PICp = 0.51$).

(d) Convergence at multiple levels

A different pattern of evolution towards ecological associated optima emerged for each organizational level (electronic supplementary material, table S1). Although there was little support for an OU model for jaw protrusion distance (ΔAIC information criterion (AIC) = 1.2), an OU model was strongly supported over the Brownian motion models for jaw protrusion angle ($\Delta AIC > 4$). For both mechanical variables, KT and αN , there was also strong support for the OU model over the Brownian models providing substantial evidence for feeding regime convergence in mechanics. By contrast, for nasal length, there was some support ($\Delta AIC = 2.24$) for the OUM over the BM1 model, but the anterior jaw’s morphological links largely evolved via Brownian motion and showed little predictable differentiation associated with convergent feeding regimes.

The phylofunctional space (figure 3a) highlights Malawi cichlid divergence in both jaw protrusion angle and distance. There is substantial evolution in the mbuna along the jaw angle axis, but these species tended to have less jaw protrusion than non-mbuna. There also has been repeated convergence on similar jaw angles in many Malawi lineages that nevertheless differ substantially in jaw protrusion distance. For the planktivorous pair, there is little evidence of convergence in phylogenetic space. More quantitatively, *Cyn. afra* and the closest non-mbuna planktivore in both jaw protrusion abilities, *Mc. conophoros*, showed no evidence for convergence in jaw protrusion abilities (mean C1 = 0.25, median $p = 0.42$; mean C2 = 9.12, median $p = 0.45$). However, the phylofunctional space and phylomechanical space both suggested that the algivorous *P. taeniolatus* has evolved to the edge of the functional and mechanical spaces occupied by many mbuna who feed on algae (figure 3a,b), indicating this non-mbuna is probably convergent in jaw protrusion abilities with mbuna algae feeders.

The species closest to *P. taeniolatus* in the phylofunctional space was the herbivorous *Ma.* ‘Maleri Blue’ although the phylomorphospace (figure 3) suggested there might be a disconnect between different levels of biological organization. Both protrusion distance and protrusion angle for these two species did exhibit a surprisingly high level of convergence when compared to divergence in the Malawi radiation (table 2; mean C1 = 0.97, median $p < 0.01$).

Additionally, the two mechanical attributes, KT and αN , were also significantly more similar than one would expect (mean C1 = 0.95, median $p = 0.02$). However, the morphology of their four-bar linkages showed no evidence of convergence (mean C1 = 0.40, median $p = 0.25$). Although their lower jaw links seemed to be morphologically similar in length, the fixed link, nasal and maxilla were all relatively much longer in *P. taeniolatus*. Therefore, although the jaw protrusion variables seem to be determined by four-bar mechanics and these mechanics are determined by the anterior jaw morphology, the four-bar linkage is many-to-one mapped in these Malawi species that have convergently evolved specialization to graze from algae-covered rocks.

4. Discussion

Complexity in the mapping of the anterior jaw morphology to feeding specialization has played a central role in Lake Malawi cichlid trophic diversification. Two different mechanical attributes generated from the same anterior jaw linkage morphology, KT and αN , are associated with the evolution of two distinct feeding functions, protrusion distance and protrusion angle. Contrary to the idea that these two functions should show strong trade-offs, jaw protrusion distance and angle generally evolve independently in Lake Malawi cichlids. Furthermore, within Lake Malawi cichlids, evolutionary independent clades of algivores have converged in anterior jaw mechanics as well as jaw function, despite a lack of convergence in their underlying anterior jaw morphology.

Most morphological elements probably influence multiple aspects of musculoskeletal mechanics and their emergent functions. For instance, terrestrial vertebrate limbs must function during running, walking, jumping, turning and stopping [65,66]. However, it is rare to be able to make explicit quantitative predictions about multiple mechanical attributes of skeletal systems while linking these to clear functional outputs. Yet, as has been found for several groups of fishes [17,18], we found higher four-bar KT is evolutionarily correlated with more protrusible jaws (table 1). This association is an important axis of Malawi cichlid trophic diversity because their standardized jaw protrusion distance varies by almost 10-fold (figure 2). Also, as we predicted, αN is evolutionarily correlated with jaw protrusion angle. Effectively, when αN is smaller, Lake Malawi cichlids have an internal four-bar skeletal structure that leads to a more subterminal mouth and thereby greater jaw protrusion angle. Therefore, the morphological configuration of the same elements composing the four-bar linkage is pleiotropically influencing not only jaw protrusion distance but also jaw protrusion angle.

Despite being associated with the mechanics of the same anterior jaw elements, jaw protrusion distance and jaw protrusion angle evolve independently in Malawi cichlids (table 1). In Malawi, many closely related species have diverged to possess either a terminal mouth or a subterminal mouth but do not differ substantially in jaw protrusion distance [40,44,47]. For example, within the rock-dwelling mbuna that generally show limited jaw protrusion, some groups such as *Maylandia* spp. have fairly terminal mouths whereas other mbuna such as *Labeotropheus* have a highly subterminal mouth (figure 2). The diversity of feeding orientations associated with jaw

protrusion angle probably allows these species to specialize on different substrate locations when grazing algae [40,49]. Likewise, within the non-mbuna, there are species like *N. poly stigma* that possess a highly terminal mouth while its congener *N. linni* has quite similar jaw protrusion but a subterminal mouth [47]. Conversely, there are closely related Malawi species that differ in jaw protrusion distance but not jaw protrusion angle. This capacity for aspects of the cichlid feeding apparatus to change or evolve independently has repeatedly been invoked to explain their unparalleled trophic diversity [33–39]. For instance, what has been termed modularity multiplicity, or the ability of cichlid individuals feeding on different prey types to alter both the angle and amount of jaw protrusion, has long been suggested to be a key factor in their evolutionary success [38,39]. Based on our results, the intraspecific ability of cichlids to modulate their jaw protrusion abilities appears to also characterize macroevolutionary change among cichlid species. It would, therefore, be interesting to know if the angle and amount of jaw protrusion are commonly behaviourally and evolutionarily decoupled in other teleost fishes, or if the independence observed here is a particular attribute of highly successful groups like the Lake Malawi cichlids.

Although the evolution of surprisingly similar functional abilities and novel ecologies might be expected to occur in many-to-one mapped systems via divergent morphologies over long evolutionary timeframes [5,22,42], discordance in how levels of biological organization contribute to convergence has rarely been investigated in young, sympatric radiations. In general, across the entire Malawi radiation, different feeding guilds exhibited evidence of convergence onto peaks of anterior jaw functional and mechanical similarity while showing limited evidence of morphological convergence (electronic supplementary material, table S1). Additionally, the phylofunctionalspace (figure 3a) indicates there are clear cases of functional convergence among feeding guilds in the two major clades of Malawi cichlids. For instance, the algae grazing *P. taeniolatus* evolved towards phenotypes characterizing the mbuna and is very similar to the species *Ma. 'Maleri Blue'*. Not only is *P. taeniolatus* close to *Ma. 'Maleri Blue'* in functional space, but they are quantitatively more convergent than one would expect by chance for jaw protrusion traits (table 2). Furthermore, the mechanics of the four-bar linkage, KT and αN , are significantly more convergent for these two species than one would expect. However, the four-bar linkage morphologies of these two species were not convergent. In line with, but expanding, on the levels of phenotypes previously examined for the many-to-one mapping of morphology [9–13,67–69], components of the Malawi cichlid radiation are characterized by convergence in feeding ecology, function and jaw mechanics without underlying convergence in anterior jaw morphology. Many-to-one mapping of this trophic system in this very recent radiation is therefore mechanistically associated with convergence at three levels of emergent biological diversity without convergence in underlying morphology.

In cichlids and many other emerging model systems, we are at the cusp of understanding the complete genotype to phenotype map for many qualitatively convergent phenotypes. Yet, even large phylogenomic datasets as presented here have limited power to fully resolve whether traits have arisen repeatedly in very recent adaptive radiations like the Lake Malawi cichlids (figure 2; [54]). Additionally, this

mapping for many complex traits will probably not be straightforward [3,35]. The intricacy of this mapping will in part stem from genetic processes. We know the segregation of the same mutant alleles at similar loci, different mutations in similar loci, as well as different mutations in different loci can all readily produce convergent phenotypes [53,70]. For instance, in recently diverged stickleback fishes where the genetic bases of several convergent adaptations are well documented, parallel phenotypic change has resulted from the repeated fixation of either the same alleles or different mutations at the same locus [44,49,50]. Increasingly, our understanding of convergent evolution, especially in recent radiations, will rely less on phylogenetic reconstruction and more on the genetic dissection of traits. However, analyses of rapid ecological convergence should account not only for the genomic substrate of phenotypes but also the mechanical differences in traits that might have arisen from many-to-one morphological changes. More extensive exploration of how complexities in morphology map to mechanics, function

and ecology will continue to clarify the multi-faceted mechanisms underlying convergence during adaptive radiation.

Ethics. All fieldwork was approved by permits from the Malawi Parks Service in May 2010 to C.D.H. Permit no.1 as well as the University of Tennessee Animal Care and Use Committee. We complied with all relevant guidelines for field collections of teleost fishes.

Data accessibility. All genetic data for this study are available from GenBank BioProject PRJNA344532. The 100 random trees and phenotypes used for comparative analyses are available in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.6p6568r> [71].

Authors' contributions. C.D.H. and R.H. conceived of the study. J.Z. and M.E.A. generated data. J.Z. performed bioinformatic analyses and deposited data on GenBank. C.D.H., M.E.A., R.H. and A.M. wrote the paper.

Competing interests. We declare we have no competing interests.

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