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Extending the Geometric Approach for Studying Biomechanical Motions

C. M. Martinez[1] and P. C. Wainwright
Department of Evolution and Ecology, University of California, Davis, CA 95616, USA

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[1] E-mail: cmimartinez@ucdavis.edu

Synopsis Whether it is swimming, walking, eating, or jumping, motions are a fundamental way in which organisms interact with their environment. Understanding how morphology contributes to motion is a primary focus of kinematic research and is necessary for gaining insights into the evolution of functional systems. However, an element that is largely missing from traditional analyses of motion is the spatial context in which they occur. We explore an application of geometric morphometrics (GM) for analyzing and comparing motions to evaluate the outputs of biomechanical linkage models. We focus on a common model for oral jaw mechanics of perciform fishes, the fourbar linkage, using GM to summarize motion as a trajectory of shape change. Two traits derived from trajectories capture the total *kinesis* generated by a linkage (trajectory length) and the *kinematic asynchrony* (*KA*) of its mobile components (trajectory nonlinearity). Oral jaw fourbar data from two subfamilies of Malagasy cichlids were used to generate form–function landscapes, describing broad features of kinematic diversity. Our results suggest that kinesis and KA have complex relationships with fourbar morphology, each displaying a pattern in which different shapes possess equivalent kinematic trait values, known as many-to-one mapping of form-to-function. Additionally, we highlight the observation that KA captures temporal differences in the activation of motion components, a feature of kinesis that has long been appreciated but was difficult to measure. The methods used here to study fourbar linkages can also be applied to more complex biomechanical models and broadly to motions of live organisms. We suggest that they provide a suitable alternative to traditional approaches for evaluating linkage function and kinematics.

Introduction

The ability of organisms to generate body movements, or kinesis, is necessary for many of life's major activities, including locomotion and feeding. Biomechanical research uses models of functional systems to explore how morphological traits contribute to dynamic changes that occur as an organism moves (e.g., Hutchinson 2004; Wilga and Lauder 2004; Westneat 2006). This explicit link between morphology and motion underscores a key tenet of functional morphology, that anatomical variation has mechanical implications that ultimately influences an organism's performance in some task (Dudley 2000; Shadwick and Lauder 2006). Therefore, the ability to evaluate mechanical and kinematic variation is vital to understanding the consequences of morphological diversity and evolution (Higham et al. 2016).

The rigid vertebrate skeleton has given rise to a number of lever and linkage-based morphological solutions to basic functional and performance needs, including frog legs (Emerson 1991), lizard jaws (Stayton 2006), bird skulls (Olsen and Westneat 2016), and many others. Several additional examples can be found within the fish feeding apparatus (Westneat 2004), which is composed of numerous mobile elements (Schaeffer and Rosen 1961; Liem 1980). To help understand this complexity, the anterior oral jaws of perciform fishes are often modeled with a fourbar linkage (Fig. 1A), describing the mechanics of a functional system responsible for generating anterior jaw protrusion during suction feeding (Westneat 1990; Muller 1996; Hulsey and García de León 2005; Westneat et al. 2005). The fourbar linkage does this by transmitting motion from a rotating

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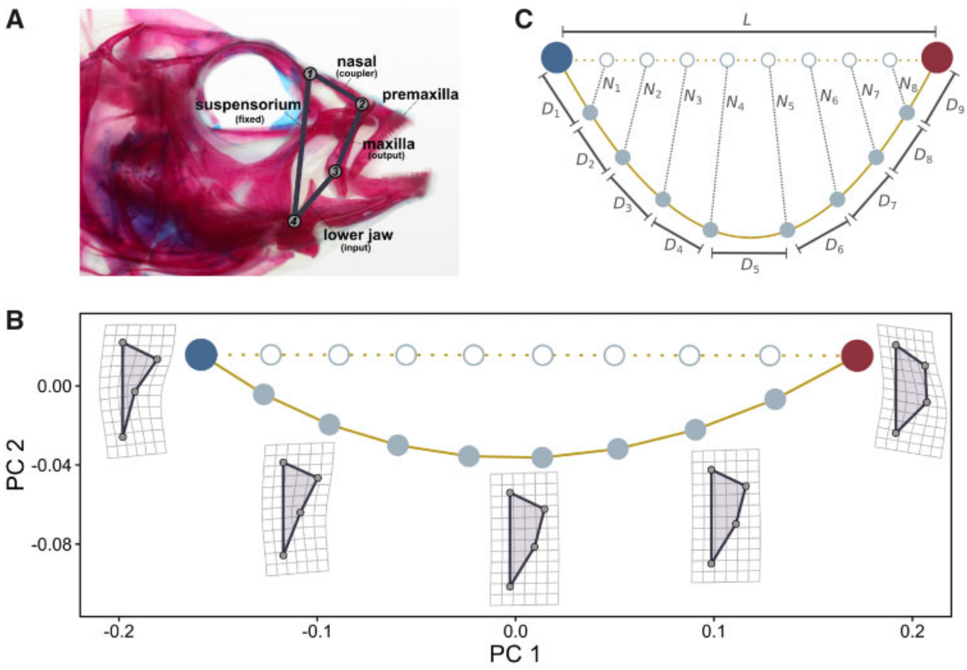

Fig. 1 (**A**) An oral jaw fourbar linkage is shown on a cleared and stained Malagasy cichlid, *Paratilapia polleni*. Links are labeled by their associated skeletal features, with mechanical descriptions provided in parentheses. The premaxilla, which protrudes by way of movement of the fourbar linkage, is also labeled for reference. Linkage joints (1–4) are used as fixed landmarks for geometric morphometrics. (**B**) The motion generated from 30 degrees of lower jaw input rotation in *P. polleni* is summarized on principle components (PCs) 1 and 2. As the motion progresses from start to end configurations (filled dots from left to right), shape change in the fourbar linkage traces a trajectory through morphospace (solid yellow line). Deformation grids of select shapes relative to mean shape are included for visualization along the trajectory. Shapes estimated along a linear trajectory (open dots on dotted yellow line) display the minimum amount of shape change possible between start and end shapes. (**C**) Functional metrics were estimated from fourbar shape trajectories. The total amount of shape change, kinesis, was the sum of Procrustes distances between motion shapes (D_1–D_9). Trajectory nonlinearity, or kinematic asynchrony (KA), was calculated as the maximum Procrustes distance of motion trajectory points from linear (N_1–N_8), standardized by the linear distance between start and end shapes (*L*). While shown here in two dimensions, traits were measured on motion shapes in their full dimensionality. This figure is available in color in the online version of the manuscript.

lower jaw to the maxilla, which then drives anterior displacement of the premaxilla. Accordingly, one of the common functional metrics of oral jaw fourbars, kinematic transmission (KT), is the ratio of output rotation of the maxilla to input rotation of the lower jaw (Westneat et al. 2005). In some fishes, KT has been shown to correlate with feeding ecology (Wainwright et al. 2004), purportedly capturing a major functional tradeoff of jaw systems relating to the transmission of force through the jaws versus speed (Westneat 1995). At the same time, KT has been a point of contention among biomechanists. One of the primary areas of disagreement surrounds the observation that different fourbar shapes can achieve equivalent values of KT, known as a many-to-one mapping (MTOM) of form-to-function (Alfaro et al. 2004, 2005; Wainwright et al. 2005). An argument against MTOM is that because KT is a ratio, the same value can be achieved by an infinite number of fourbar configurations (Cooper and Westneat 2009). It is therefore difficult to separate MTOM as a genuine mechanical feature of fourbar linkages versus a mere artifact of calculation. While there is merit to the concern about KT, the fourbar linkage itself remains a valuable biomechanical model, with evidence for its ecological (Westneat 1995) and evolutionary relevance (Anderson and Patek 2015; Muñoz et al. 2018).

We present a geometric approach for understanding the functional properties of fourbar linkages, recently used to study feeding motions in live cichlid fishes (Martinez et al. 2018), as a potential alternative to KT. The method relies on geometric morphometrics (GM), a landmark-based method for shape analysis that has been widely used and developed over the last several decades (Adams et al. 2004). Currently, GM is one of the most popular methods for quantifying and comparing morphological variation, having overwhelmingly been applied to static (non-moving) morphologies. However, a growing number of studies have focused on shape variation in dynamic systems, like deformations of structures under loading stress (O'Higgins et al. 2011; Stansfield et al. 2018) or motions generated by functional systems (Adams and Cerney 2007; Coelho et al. 2017). When studying motions, morphometric landmarks are placed on functional structures, tracking their movements through the completion of an activity. For "goal-oriented" motions (Adams and Cerney 2007) in which the start and end shapes are different, like the movement of a fourbar linkage over a set angle of input rotation, the various stages of the motion trace a trajectory through morphospace (Fig. 1B). While there are a limited number of studies evaluating multivariate shape trajectories, many of them follow the general approach implemented in the *geomorph* package in R (e.g., Adams and Cerney 2007; Adams and Collyer 2009; Collyer and Adams 2013; Powder et al. 2015). In these studies, trajectories are assessed by their size (trajectory length), shape, and orientation. The latter two are based on pairwise comparisons of trajectory characteristics and do not provide traits for the trajectories themselves. In order to directly link features of shape trajectories to tangible kinematic properties, we aimed to calculate traits that could be measured for each motion generated by an oral jaw fourbar linkage.

The fourbar linkage offers an ideal study system in which to showcase the GM approach for motion analysis, as it contains only two mobile landmarks (labeled 2 and 3 in Fig. 1A), greatly simplifying the interpretation of kinematics relative to more complex functional systems. The traits derived from shape trajectories can be used as tools for summarizing motions and provide convenient metrics to quantify kinematic diversity and compare form–function relationships. We focus on two previously defined functional traits, one describing the size of a trajectory and another capturing the curvature of its path through shape space (Martinez et al. 2018). The first, *kinesis*, is the total trajectory length, which describes the amount of shape change generated by the system during motion (Fig. 1C). The second, *kinematic asynchrony* (*KA*), describes the degree of nonlinearity of a trajectory. To understand what it means for a shape trajectory to be nonlinear, we first consider a linear trajectory. The shortest distance between two shapes, here start and end configurations of a motion, is a straight line through morphospace (Fig. 1, dotted yellow lines). Shapes sampled along this line (Fig. 1, open gray dots) create a linear trajectory on which the manner of shape change is invariable from start to end. Shape change along a linear trajectory is also synchronous in that all morphometric landmarks move proportionately to one another (i.e., relative landmark velocities vary linearly to each other through a motion) and each only moves in a single direction. In contrast, if any (or all) landmarks display disproportionate movement relative to each other or a change in their direction, their movements are asynchronous and the nature of shape change shifts during the motion, causing a nonlinear path through morphospace. By definition, this deviation from a linear shape trajectory results in a comparatively longer overall trajectory or greater kinesis. More linear trajectories are associated with relatively more synchronous landmark movements (lower KA) than those with deeper, nonlinear trajectory profiles (higher KA). We note that the name and calculation of KA used here differs from the trajectory nonlinearity trait used in Martinez et al. (2018), which was called "kinematic efficiency," for reasons outlined in the "Materials and methods" section. Both metrics capture the degree to which the elements of a motion are asynchronous through time, but we feel that the term KA provides a more inclusive description of the underlying process.

Here, we explore an application of GM for kinematic analysis of linkage models. We focus on oral jaw fourbar linkages of Malagasy cichlids, a group containing 30 putative species and two of four cichlid subfamilies, Etroplinae and Ptychochrominae. Previous work in this system showed that disparate patterns of jaw shape variation in each of the subfamilies follow features of the fourbar-KT form–function landscape, suggesting a potential role of MTOM in the morphological diversity of the group (Martinez and Sparks 2017). Our primary interest was to introduce a set of methods for studying form and function in biomechanical models that is broadly generalizable and incorporates aspects of kinematic diversity not captured with KT. This includes further development of the conceptual framework for kinematic/functional traits based on GM shape trajectories (kinesis and KA). We were particularly eager to strengthen our understanding of the drivers of trajectory nonlinearity (i.e., KA), as we do not believe that its role as a source of kinesis has been explicitly accounted for in previous kinematic work with animals. We were also interested in understanding relationships between traits, particularly the trajectory-based traits versus KT. We achieved this visually by plotting form–function landscapes for each trait and also statistically comparing their degrees of association. We initially predicted that KA and KT would be most similar as they are both measures of the relative movements of different parts of the linkage to each other, the former based on patterns of landmark displacements and the latter on angular changes of links. In addition, we explored how morphological variation of individual links comprising fourbar shapes contribute to overall change in the three functional metrics, expecting that the links would differentially impact each of them. We also discuss whether the findings of this study provided supporting evidence for MTOM of fourbar linkage systems.

Materials and methods

Specimens and data acquisition

We used jaw morphology data for Malagasy cichlids, originally obtained by Martinez and Sparks (2017). 176 specimens from 30 species were included, representing all 29 valid Malagasy cichlids plus one undescribed species, *Paretroplus n.* sp. "*Anjingo.*" Most specimens came from ichthyology collections at the American Museum of Natural History and the University of Michigan Museum of Zoology. Using a combination of X-rays and photographs of cleared and stained fishes, we gathered empirical data for oral jaw fourbar linkages. The linkage was defined by skeletal elements of the anterior jaw (Westneat 1995), and while different features have been used to assess fourbar shapes, we follow an anatomical definition used for Neotropical cichlids by Hulsey and García de León (2005). Lengths of fourbar links (Fig. 1A) were measured between the following joints, 1) the ligamentous connection of the posterior end of the nasal to the neurocranium, 2) the intersection of the anterior nasal and the head of the maxilla, 3) the ligamentous connection between the distal end of the maxilla and the dentary, and 4) the quadrate–articular joint. In order to standardize their positions, fourbars were divided into two triangles split along a diagonal connecting joints 1 and 3 above, and trigonometry was used to solve for the shape at which the lower jaw link was at 15 degrees relative to the fixed link (Hulsey and García de León 2005). Vertices of the resulting, standardized linkages were used as fixed landmarks for GM. After shape alignment in the *geomorph* package (Adams and Otarola-Castillo 2013) in R (R Core Team 2018), we calculated the average shapes of species. In taking the average shape, linkages did not all maintain a 15-degree angle between the fixed link and lower jaw link. This is important as we know functional outputs of linkages to vary depending on their starting position. Accordingly, we reset mean linkage shapes to the standardized orientation prior to simulation and functional calculations.

Simulating the linkage model

Given some degree of rotation to the input link (here, the lower jaw), the fourbar linkage moves in a deterministic manner, with one-degree of freedom. We used the *linkR* package (Olsen 2016; Olsen and Westneat 2016) in R to simulate fourbar movements over 30 degrees of lower jaw rotation, recording linkage shapes at 10 equidistant points during the course of rotation (Fig. 1B). While starting positions of fourbar linkages and amounts of lower jaw rotation during feeding likely vary across Malagasy cichlids, our approach was designed to compare mechanical properties of linkages in a standardized manner that was consistent with previous treatments of the system (Alfaro et al. 2004, 2005; Hulsey and García de León 2005; Wainwright et al. 2005). The resulting motion shapes trace a trajectory through morphospace (Adams and Cerney 2007), with characteristics that were used for calculations of linkage function.

Linkage functional metrics

Two metrics were used to describe variation in geometric shape trajectories (Fig. 1C). The first, kinesis, was the total length of trajectories and was measured as the sum of Procrustes distances between successive shapes, representing motion of the linkage during 30 degrees of rotation of the lower jaw link. Procrustes distance between two shapes is the square root of the sum of squared distances between their landmark coordinates. The other metric, KA, was associated with the degree of relative trajectory nonlinearity. To evaluate KA, we first estimated theoretical fourbar shapes along a linear trajectory between the start and end shapes (Fig. 1B), following methods by Martinez et al. (2018). To do this, we first calculated cumulative trajectory lengths at each shape along the actual motion trajectory and expressed them as proportions of total trajectory length. These values provided a collection of proportions, or "spacing regime," that informed the relative locations that shapes would be estimated along a corresponding linear trajectory. Next, the coordinates of the end shape were subtracted from that of the starting shape, the result of which was then multiplied by each element of the spacing regime, and finally added back to the start shape. This created eight theoretical shapes along a linear trajectory between start and end shapes that had proportionally similar spacing as shapes on the actual motion trajectory. To calculate a trajectory's divergence from linear as a univariate functional trait, we took the maximum Procrustes distance between corresponding linear and actual shape trajectories and scaled it by dividing by the Procrustes distance between start and end shapes. As mentioned in the "Introduction" section, this method differs slightly from Martinez et al. (2018), where a multivariate nonlinearity trait contained Procrustes deviations for *all* motion shapes (and not just the maximum value). In this study, a univariate nonlinearity metric was appropriate for the desired interpretability outcomes. Additionally, Martinez et al. (2018) originally named their trajectory nonlinearity trait "kinematic efficiency," referring to the relative conservation of kinesis in more linear trajectories. However, we feel that asynchrony more accurately and inclusively describes the process underlying nonlinearity of shape trajectories, and use it going forward.

For comparison, we also estimated the commonly used metric of linkage function, KT. For each species' mean fourbar shape, we calculated KT as the ratio of output rotation of the maxillary link to input rotation of the lower jaw link. As noted above, the value of KT is sensitive to starting position of the linkage configuration. For this reason, the input–output ratios were calculated for each of nine transitions between the 10 recorded linkage shapes and the mean value was used for KT.

Mapping form–function landscapes

In order to visualize how functional traits vary with linkage shapes, we generated form–function landscapes. First, we estimated 1200 linkage shapes arranged in a 30 × 40 grid across principle components 1 and 2 of the Malagasy cichlid fourbar morphospace (Supplementary Fig. S1). To do this, we combined and modified existing R code used for predicting shapes within a morphospace (Olsen 2017) and the "plotTangentSpace" function in the package *geomorph*. Before we calculated functional metrics, we again reset each of the theoretical linkage shapes so that their lower jaw links were at 15 degrees to the fixed link. We then plotted landscapes for each of the functional traits (kinesis, KA, and KT) onto the background of PC plots of the fourbar morphospace. Additionally, we were interested in identifying features of form–function landscapes that exist beyond the morphospace of our study species. For this, we estimated fourbar morphologies for PC 1 and 2 scores outside of the range observed in Malagasy cichlids (Supplementary Fig. S2). The estimated linkage morphologies across this expanded morphospace were reset, as before, prior to motion simulation and trait calculations.

Functional relationships and morphological drivers

We evaluated relationships between functional traits measured on the 1200 estimated fourbar shapes across Malagasy cichlid morphospace. For this, we used nonparametric rank correlations, which can accommodate monotonic data that do not meet the assumption of linearity (Sokal and Rohlf 1995). We also examined which components of oral jaw fourbars were the strongest drivers of function with ordinary least-squares regression of functional traits on the lengths of individual links.

Results

Oral jaw fourbar shape variation

Shape variation of Malagasy cichlid oral jaw fourbar linkages was largely accounted for by the first two principle component axes, PCs 1 and 2, which contained 94.6% of total variation (Fig. 2). PC 1 was associated with variation in the relative length of the nasal link as well as dorso-ventral deflection of the nasal and maxillary links. PC 2, in contrast, was dominated by the level of antero-posterior projection of the landmark (i.e., linkage joint) between the nasal and maxillary links. Consistent with the results of Martinez et al. (2018), cichlid subfamilies differed in their primary directions of fourbar shape change. Ptychochromines varied broadly across the range of PC 1 scores, while etroplines displayed considerable variation on both PCs 1 and 2 (Fig. 2).

Form–function landscapes

Form–function landscapes for kinematic traits provided context to patterns of morphological diversity. Kinesis generally increased with increasing PC 1 scores, but the distribution was not uniform, with the largest range of values at high PC 2 scores (Fig. 2A). Fourbar shape variation of ptychochromine cichlids resulted in high variance in mobility (crossing several kinesis contours), where variation in etroplines occurred in a direction through morphospace that minimized variation in kinesis. This disparity in kinesis variance is clearly reflected in density plots grouped by subfamily (Fig. 2B). The landscape for KA was markedly different from that of kinesis. Contours of equivalent KA were much less uniform across the space, with a region of rapidly increasing asynchrony (top right of Fig. 2) adjacent to a conspicuously large region with intermediate KA and minimal functional change. Despite differences between the landscapes of trajectory traits, both KA and kinesis had maximum values in the same location (top right corners of Fig. 2A, C). Additionally, the distribution of cichlid fourbar shapes was such

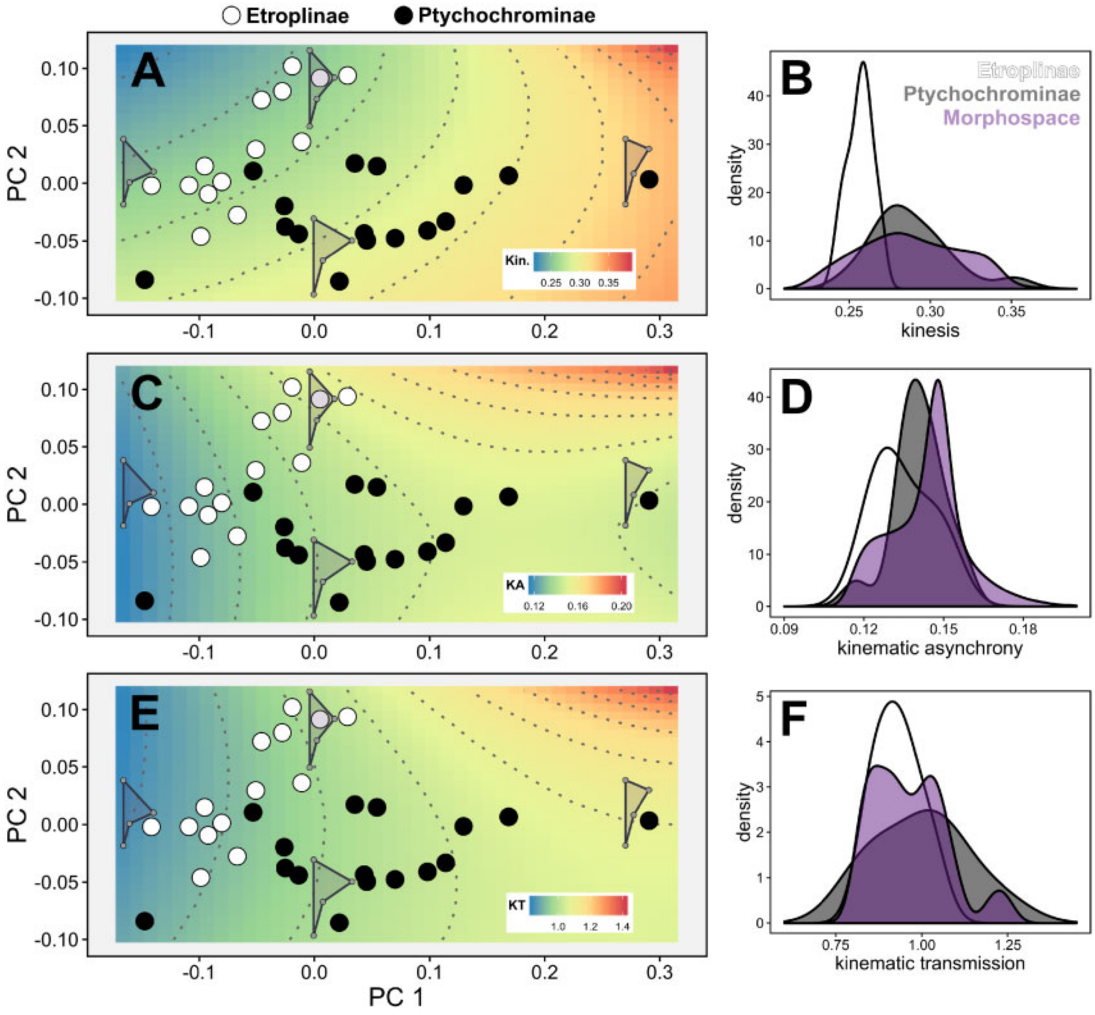

Fig. 2 Paired form–function landscapes and density plots for three functional traits of fourbar linkages, kinesis (**A** and **B**), kinematic asynchrony (KA) (**C** and **D**), and kinematic transmission (KT) (**E** and **F**). Each landscape is estimated across the morphospace of oral jaw fourbar starting shapes in Malagasy cichlids, represented here by PCs 1 and 2 (74.4% and 20.1% of total shape variation, respectively). Observations are colored by cichlid subfamily, which include Ptychochrominae (black dots) and Etroplinae (white dots). Trait values are represented by plot background color and dotted contour lines show directions of equivalent function across the space. Fourbar shapes at PC extremes are provided on the plots. Density plots show the relative distributions of trait values within cichlid subfamilies and for the hypothetical fourbar shapes estimated across the morphospace. This figure is available in color in the online version of the manuscript.

that the two subfamilies had much more broadly overlapping distributions for KA (Fig. 2D) than they did for kinesis (Fig. 2B). Lastly, the form–function landscape for KT was superficially similar (but not identical) to that of KA, suggesting that the two metrics, estimated by different approaches, potentially describe similar aspects of kinematic diversity. The two landscapes contained similarly located regions of intermediate functionality and widely divergent contour lines.

The extrapolation of fourbar shapes and associated trajectory traits beyond the morphospace of Malagasy cichlids revealed additional features of form–function landscapes. In this expanded space, there is an area where fourbar linkage morphologies could not undergo 30 degrees of rotation and functional values could not be estimated (upper right of panels in Fig. 3). This represents a biomechanical constraint on fourbar function, as we have defined it. In the expanded landscape, kinesis increases diagonally (from top left to bottom right) across Fig. 3A. The result suggests that shape variation along both PC 1 and PC 2 strongly influence overall linkage mobility, a pattern that was not as clear in the original landscape (Fig. 2A). The expansion of the KA landscape revealed that a large, functionally stable region with intermediate levels of KA was flanked along PC 1 by two distinct areas of low asynchrony and along PC 2 by areas of high asynchrony (Fig. 3B). Interestingly, KA increases rapidly along

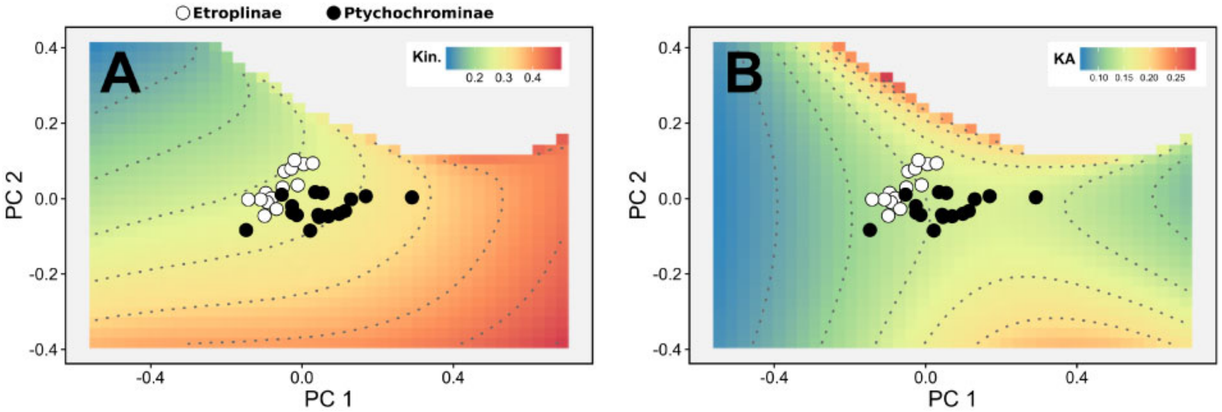

Fig. 3 Form–function landscapes of trajectory-based traits mapped onto an expanded and theoretical fourbar morphospace. Shapes and functions are estimated outside of the original PC 1 and 2 ranges for Malagasy cichlid species (black and white dots). Functional values for kinesis (**A**) and KA (**B**) are mapped onto the background of the plots, with contour lines showing the distribution of equivalent function for each metric. Note that in the expanded morphospace, a number of fourbar shapes could not undergo the full rotational input needed to estimate trait values (upper right of plots). This figure is available in color in the online version of the manuscript.

much of the boundary between functional and non-functional linkages, suggesting that at some point asynchrony begins to restrict the range of fourbar motion.

Functional relationships and morphological drivers

Functional metrics were all positively related to each other (Fig. 4). Kendall's rank correlations confirmed a slightly stronger relationship between KT and KA ($\tau = 0.77$, $P < 0.001$) than for KT and kinesis ($\tau = 0.63$, $P < 0.001$). Comparison of the two trajectory-based traits, KA and kinesis, revealed a positive but asymptotic relationship ($\tau = 0.43$, $P < 0.001$). In addition, functional traits were variably associated with the lengths of individual links of oral jaw fourbar systems. Not surprisingly, kinesis was most strongly associated with variation in the lengths of the three mobile links (Supplementary Fig. S3A–D), showing positive relationships with the lower jaw (slope $= 1.66$; $R^2 = 0.92$; $P < 0.001$) and maxilla (slope $= 0.86$; $R^2 = 0.66$; $P < 0.001$), but a negative relationship with the nasal (slope $= -2.82$; $R^2 = 0.69$; $P < 0.001$). KA was most strongly driven by a positive relationship with lower jaw length (slope $= 2.26$; $R^2 = 0.34$; $P < 0.001$) and a negative relationship with nasal length (slope $= -6.63$; $R^2 = 0.76$; $P < 0.001$) but had weaker relationships with the two remaining links (Supplementary Fig. S3E–H). KT displayed similar relationships with fourbar links to KA (Supplementary Fig. S3I–L).

Discussion

Geometric analysis of motion expresses movement as a trajectory of shape change, the characteristics of which provide information about overall mobility (kinesis) and the relative asynchrony of movements made by mobile elements (KA). Our work suggests that of the two trajectory-based functional traits, KA was most closely associated with KT. Fourbars that have high transmission of motion also have the most asynchronous movement (Fig. 4). This was apparent from the general similarities of form–function landscapes for KA and KT (Fig. 2C, E) and their associations with the lengths of individual links (Supplementary Fig. S3). The relationship between the two traits makes sense, as the relative input-to-output movements of morphological components that define KT (Westneat 1990) are in their own way a form of rotational asynchrony. Therefore, KA (like KT) is a mechanical feature of fourbar morphological diversity that is likely relevant to feeding ecology in perciform fishes (Wainwright et al. 2004; Westneat et al. 2005).

Another outcome of this work is the revelation that there are two factors determining the total kinesis of a functional system: the spatial displacement of its mobile components based on morphology and the relative asynchrony of the components' movements over time. The former is straightforward; comparatively larger moving parts, when put in motion, produce greater kinesis. For example, on a similarly sized head, a larger jaw creates more movement simply by opening than a smaller jaw does. The influence of asynchrony on

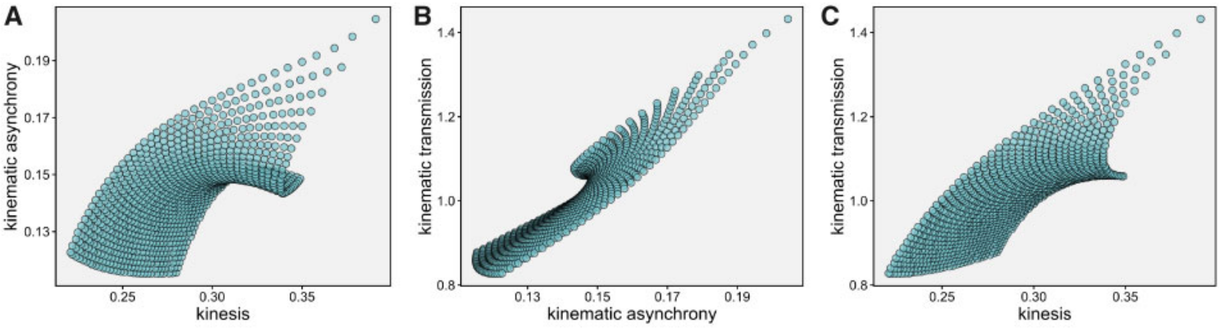

Fig. 4 (**A**) Functional traits derived from fourbar shape trajectories, KA and kinesis, plotted against each other and (**B** and **C**) in relation to KT. Points represent trait values estimated from theoretical shapes across the entire Malagasy cichlid fourbar morphospace.

overall kinesis is more involved and discussed in detail in the following section. However, while it was not surprising that the relationship between kinesis and KA was generally positive, the apparently asymptotic nature of their association (at least over the range of fourbar morphologies examined) was not expected and warrants further consideration.

The same methods used in this study to evaluate form and function of fourbar linkages are generalizable to a wide variety of kinematic data. In addition to treating functional systems as integrated units, we believe that the flexibility of the approach is one of its largest assets. For example, the same kinematic traits can be extracted from simulated biomechanical data as well as kinematic data from organisms moving in real time, and in both cases, those traits can be interpreted in the same manner. In fact, the relatively simple motions of fourbar linkages, with only two mobile landmarks, facilitated a more complete understanding of the relationship between trajectory length (kinesis) and nonlinearity (KA), and provided valuable context to patterns observed in live fishes examined in Martinez et al. (2018). In that study, which focused on feeding motions in African rift lake cichlids, the authors found that some species with high-kinesis feeding motions also had among the most linear (low KA) shape trajectories, the opposite of the pattern observed here (Fig. 4). Based on insights gained from the current study, the apparent discrepancy seems to be explained by disproportionate effects of morphological variation in cichlid jaw size on the two kinematic traits. As noted above, the relative size of mobile parts determines the length of a trajectory's linear baseline (*L* in Fig. 1C), and this length is also the denominator of KA. If, for example, relative jaw size increased, as seen in many piscivorous cichlids, the linear baseline would lengthen, reflecting a larger shape change between start and end configurations (closed mouth and full gape, respectively). If there was not also a comparably large increase in the asynchrony of motion components so that the level of trajectory nonlinearity matched the change in baseline length, the overall trajectory would stretch in the direction of the increasing baseline and would appear more linear. Despite the high degree of morphological variation present in rift lake cichlids, they are all comprised of the same basic cranial components and the sequence of movements within the feeding apparatus does not change drastically. The implication is that while changes in static morphology can readily increase the linear baseline of a motion trajectory (also increasing kinesis), unless there is a fundamental change to the underlying functional system, variation in KA may be limited. In the case of rift lake cichlids, the variation in kinesis that can be attributed to KA was likely drowned out by the effect of morphological change in jaw size. An intriguing possibility from this realization is that for morphologies with smaller capacities to create kinesis (shorter linear baseline), variation in KA would have a disproportionately larger impact on total kinesis. If confirmed, this pattern could have important implications for adaptive evolution of jaw systems along axes of trophic diversity, like transitions from suction-feeding to biting forms (e.g., Alfaro et al. 2001).

The role of asynchrony in kinematics

Each of the motion trajectories evaluated from oral jaw fourbar morphologies in this study (including 30 from Malagasy cichlid species, plus 1200 hypothetical shapes across the fourbar morphospace) displayed some level of nonlinearity, and therefore asynchrony of landmark movements across their respective motions. But how does a fourbar motion trajectory achieve nonlinearity? We can get a picture of this by examining the relative movements of the two mobile landmarks of the fourbar linkage (Fig. 5). For

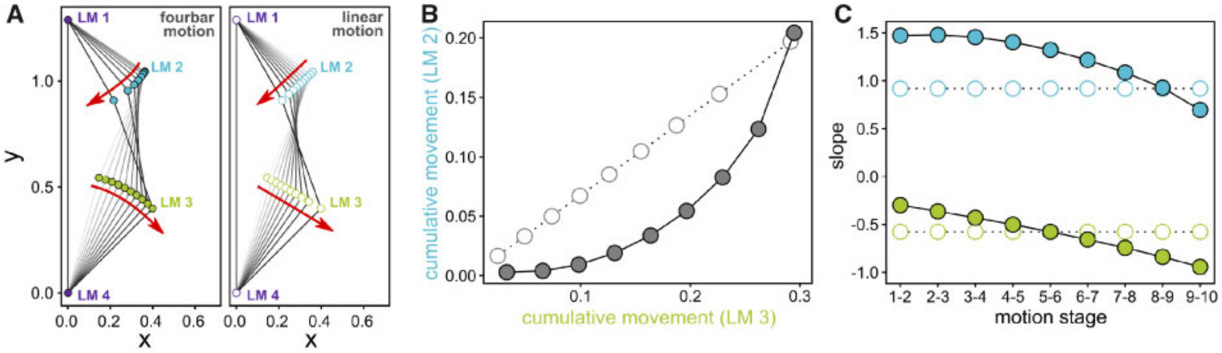

Fig. 5 (**A**) Simulated movement of a fourbar linkage with 30 degrees of lower jaw input rotation (left, filled dots) and associated motion along a hypothetical linear trajectory (right, open dots). For the same start and end shapes as the actual motion, landmark movements in a linear motion follow paths that minimize their total distances traveled, but do not represent true linkage movements (link lengths change during the motion). The motion shown is for the linkage shape with maximum KA across the Malagasy cichlid morphospace. The two immobile landmarks (LMs) are shown in purple, LM 2 is blue, and LM 3 is green. Red arrows indicate the primary directions of landmark movement (note, LMs for linear trajectories both move in straight lines). (**B**) Cumulative distances traveled by mobile landmarks at each motion stage are plotted to show how movement is accumulated in the actual fourbar motion (filled dots and solid line) versus the linear version (open dots and dotted line). Colors of axis labels correspond to landmark colors. For the actual motion, LM 2 moves slow at first relative to LM 3, but then accelerates. These asynchronous movements generate nonlinearity of the shape trajectory compared with the linear motion, where both LMs start slower and accelerate proportionately toward the end. (**C**) For each mobile landmark, slopes are measured between subsequent motion stages, representing the direction of landmark movement. Color and fill patterns correspond to landmarks. Directions of movement vary for the actual fourbar motion (filled dots), but not for the linear motion (open dots). This figure is available in color in the online version of the manuscript.

motions displaying trajectory nonlinearity (KA), the distance traveled by landmarks accumulates unevenly across the motion (solid curved lines in Fig. 5B), versus proportionately for a linear trajectory (dotted straight lines in Fig. 5B). For a moving organism, this means that different parts of a functional system are differentially activated through time. An example of this is the well-documented anterior-to-posterior expansion of the buccal cavity of fishes, where peak activation and acceleration of the hyoid occurs later in a suction feeding strike than initial expansion of the anterior jaw (Bishop et al. 2008; Day et al. 2015). In addition, landmarks on a nonlinear trajectory change their direction of movement during a motion, versus maintaining the same direction for a linear trajectory (Fig. 5C). The change of direction means that landmarks themselves are not traveling in straight lines from their start to end locations and are therefore moving farther than they otherwise would have. When considered across all landmarks, this accounts for the additional overall kinesis (trajectory length) that increasing nonlinearity creates.

When different parts of a functional system can move independently of each other, there is the possibility that an organism can behaviorally modulate the relative timing that components are activated (Olsen et al. 2019), causing KA. For example, prey type will often influence the nature of movements for different components of feeding strikes (Liem 1980; Holzman et al. 2012). This is not the case for rigid biomechanical systems, like fourbar linkages, where a change in any one angle causes deterministic change in all others. In fact, for the fourbar morphologies evaluated in this study to achieve fully linear shape trajectories, they had to violate a basic principal of a fourbar linkage by changing their link lengths (second panel of Fig. 5A). This means that the pattern of KA is a built-in characteristic of fourbar linkage shape.

The use of KA in studies of linkage systems and other biomechanical motions may provide a useful extension of existing tools. Interestingly, KA is already a commonly observed pattern in traditional kinematic studies that rely on collections of independently measured kinematic variables (e.g., cranial elevation, premaxillary protrusion, hyoid depression, etc.) with peak activations that are often staggered through time (Gillis and Lauder 1995; Wilga and Motta 2000; Camp et al. 2018). However, it is not clear from the structure of those data that the total amount of kinesis or mobility in any one variable is partially due to its interaction with others. It is only when these variables are treated as a spatially-explicit integrated system, as morphometric shape data does, that the emergent impact of KA on overall kinesis is fully realized.

One area of research that KA may be particular well-suited to address is the branch of kinematics concerned with motion coordination and integration. Using the methods outlined in our study, it is possible to simultaneously evaluate multiple functional systems and to use the framework for KA to evaluate interactions within and among functional units. For example, a study including movements of both oral jaw and pectoral fins would provide insights on locomotor aspects of feeding in relation to cranial activity. Of course, the same approach can also be applied to any research system focusing on coordinated or synchronized movements of disparate motion components (e.g., Montuelle et al. 2012; Olsen et al. 2019). Additionally, the kinematic relationships captured by this method may be particularly relevant to studies on motor control and the neurological basis of motion (Todorov and Jordan 2002; Biewener and Daley 2007).

MTOM of fourbar form-to-function

Form–function landscapes for kinematic traits (Fig. 2) provided evidence in support of MTOM in the oral jaw fourbar linkage, based on characteristics of these systems outlined in previous work (Alfaro et al. 2004, 2005). Each landscape displayed continuous contours, representing directions through morphospace over which shape change does not result in variation in the kinematic trait in question. This was the case whether a trait was measured as a ratio (KA and KT) or a sum (kinesis). The results mean that kinematically similar fourbar shapes are not randomly and disjointedly distributed across morphospace, as might be expected if functional equivalence arose simply as a chance occurrence due to the redundancy of ratio data. Moreover, there is some indication that the form–function landscape has played an important role in the evolution of Malagasy cichlid oral jaws, where the two subfamilies occupy directions through morphospace that differentially limit (Etroplinae) and maximize (Ptychochrominae) variation in kinesis (Fig. 2A, B). The ability to visualize form–function relationships beyond the morphospace of Malagasy cichlids (Fig. 3) additionally provides information on the functional consequences of morphological evolution for more trophically diverse groups of cichlids (e.g., African rift lake radiations) that almost certainly possess morphologies occurring outside of the range observed for species in this study.

The functionally equivalent contours caused by MTOM were irregularly distributed within each of the form–function landscapes examined (Fig. 2), which is indicative of variation in mechanical sensitivity, or the amount of mechanical/functional change per unit of morphological change. When contours are close together, fourbar shape change will result in greater functional variation compared with the same degree of morphological change where contours are more widely dispersed. This means that the functional consequences of morphological change differ depending on where a species is located in morphospace. The implications of this are quite important, as it suggests that the ability to respond to selection on jaw functional traits may be predicated on the topology of the form–function landscape. It also means that for a given population with continuous morphological variation, one direction of shape change may change the value of a functional trait while another will allow for morphological exploration with no consequences for the trait in question. It is not surprising, then, that previous work on fourbar linkages in different organismal and functional systems has shown that mechanical sensitivity has repeatedly and independently influenced rates of morphological evolution (Anderson and Patek 2015; Muñoz et al. 2017, 2018). Future research simulating morphological evolution within the context of the form–function landscape (e.g., Polly et al. 2016) will provide an opportunity to test specific predictions of MTOM.

Conclusion

Over 25 years ago, Rohlf and Marcus (1993) dubbed the new (at the time) field of landmark-based GM a "revolution in morphometrics." Their argument was that by considering anatomical spatial structure, GM provided an additional feature of morphological information that had been previously missing from traditional linear-based methods. That feature was shape itself, which could be evaluated as the item of comparison, versus a collection of measurements with no reference to relative location. A similar analogy can be made for geometric trajectory-based kinematics versus those evaluating motions as a series of disparate kinematic traits. Shape trajectories contain relevant information about motions in the spatial and temporal contexts that naturally occur, also taking into account how interactions between different components contribute to overall kinesis of the integrated functional system. Whether geometric analyses of motions will result in significant advances in the fields of biomechanics and kinematics is yet to be seen, but there is certainly great potential for further development of the methods for kinematic applications and for any other process in which morphologies transform over time.

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Supplementary data

Supplementary data are available at *ICB* online.

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