

Simultaneous integration and modularity underlie the exceptional body shape diversification of characiform fishes

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Abstract

Evolutionary biology has long striven to understand why some lineages diversify exceptionally while others do not. Most studies have focused on how extrinsic factors can promote differences in diversification dynamics, but a clade's intrinsic modularity and integration can also catalyze or restrict its evolution. Here, we integrate geometric morphometrics, phylogenetic comparative methods and visualizations of covariance to infer the presence of distinct modules in the body plan of Characiformes, an ecomorphologically diverse fish radiation. Strong covariances reveal a cranial module, and more subtle patterns support a statistically significant subdivision of the postcranium into anterior (precaudal) and posterior (caudal) modules. We uncover substantial covariation among cranial and postcranial landmarks, indicating body-wide evolutionary integration as lineages transition between compressiform and fusiform body shapes. A novel method of matrix subdivision reveals that within- and among-module covariation contributes substantially to the overall eigenstructure of characiform morphospace, and that both phenomena led to biologically important divergence among characiform lineages. Functional integration between the cranium and post-crural skeleton appears to have allowed lineages to optimize the aspect ratio of their bodies for locomotion, while the capacity for independent change in the head, body and tail likely eased adaptation to diverse dietary and hydrological regimes. These results reinforce a growing consensus that modularity and integration synergize to promote diversification.

Keywords: geometric morphometrics, phylogenetic comparative methods, convergence, macroevolution

Introduction

Evolutionary biology has long sought to understand why some radiations evolve exceptional morphological diversity, while others do not (Claramunt, 2010; Foote, 1997; Sidlauskas, 2007; Simpson, 1944). Extrinsic factors such as the steepness and dynamism of the underlying adaptive landscape (Cooney et al., 2017), absence of stabilizing selection (Collar et al., 2009; Hansen, 1997), or the elimination of competitive exclusion (Ricklefs, 2010) can promote phenotypic diversification, as can the intrinsic evolution of key innovations in hyperdiverse clades (Donoghue, 2005; Hulsey et al., 2006; Martin & Wainwright, 2011; Schlüter, 2000). A growing number of studies show that the anatomical covariation produced by genetic, developmental, or functional links among structures (Cheverud, 1996; Olsen & Miller, 1958) can also promote or restrict change at macroevolutionary scales (Evans et al., 2021; Felice et al., 2018; Goswami & Polly, 2010; Goswami et al., 2014; Klingenberg, 2014). Thus, certain intrinsic patterns of covariation may facilitate exceptional radiations of form.

Integration and modularity are closely linked intrinsic factors that can limit or promote the potential for organisms to vary. Morphological integration is strong covariation among biological structures (Klingenberg, 2014; Mayr, 1982; Olsen

& Miller, 1958), such as that which can occur when morphological units combine to perform a function. The clear patterns of coevolution among the suite of cranial structures that eels used for suction feeding (Collar et al., 2014) exemplify this kind of functional integration, as do the tight covariations of shape change that exist throughout the mammalian lower jaw despite the disparate developmental origins of its subregions (Zelditch et al., 2009). Variational and evolutionary integration can also emerge at the population or lineage scale (Zelditch & Goswami, 2021) when structures are linked at the genetic level through pleiotropy (Cheverud, 1988; Wagner, 1996) or are controlled by the same developmental pathway (Futuyma et al., 1993; Kuratani et al., 1999, 2016; Wake, 1991). Conversely, modularity exists when more than one highly integrated region exists within an animal's body (Wagner & Altenberg, 1996), as is the case for the oral-nasal, molar, and basicranial regions of the therian mammal skull (Goswami, 2006), six different regions of the skull of pleuronectiform fishes (Evans et al., 2021), and 10 units of the caecilian skull (Bardua et al., 2019). Anatomists typically recognize the existence of such modules by identifying independent traits or regions of the body that covary more strongly among themselves than with those in other modules

(Klingenberg, 2014; Wagner, 1996; Wagner & Altenberg, 1996). Such patterns of variational or evolutionary modularity result from an interplay between selection and underlying functional or developmental modularity (Zelditch & Goswami, 2021). In essence, modularity at the organismal level structures the genotype-phenotype map and allows traits and regions of the body to evolve independently (Wagner & Altenberg, 1996), though the complex interplay between population-level genetic, developmental and functional integration can still lead to correlated evolution among structures in different modules (Evans et al., 2021; Zelditch et al., 2009). Over macroevolutionary timescales the intensity of intrinsic integration and modularity at the genetic, developmental, functional, and variational scales can influence profoundly the types and variety of morphologies that a radiation will explore, largely by restricting the direction of possible evolutionary change or influencing the rate of morphological evolution (Felice et al., 2018; Goswami & Polly, 2010).

Functional, developmental, and variational modularity have been thought to promote diversification by allowing structures in different modules to respond separately to natural selection, or simply to drift independently (Jablonski, 2017; Klingenberg, 2004, 2005; Mayr, 1982; Olsen & Miller, 1958; Wagner & Altenberg, 1996). For instance, the functional and developmental decoupling of the oral and pharyngeal jaws in cichlid fishes allowed the oral set to adapt to a various modes of food acquisition while the pharyngeal apparatus optimized for shredding, crushing, or grinding (Hulsey et al., 2006; Liem, 1973). That modular design permitted unparalleled trophic diversification by allowing each set of jaws to respond independently to different ecological pressures (Hulsey et al., 2006). Developmental or functional modularity at the organismal level can lead to evolutionary modularity among diverging lineages by reducing pleiotropy and preventing selection on one part of an organism from reversing or slowing adaptation in other modules (Welch & Waxman, 2003), thereby by allowing different modules to evolve at different rates (Denton & Adams, 2015; Larouche et al., 2018) and potentially increase the overall rate of phenotypic change. Mantis shrimp provide one good example of faster evolution in clades with higher evolutionary modularity: the raptorial appendage in “non-smashing” lineages exhibits more evolutionary modularity and a 10-fold faster rate of evolution than in the less modular “smashing” lineages (Claverie & Patek, 2013). Overall, because modularity at the organismal or populational level can divide traits into distinct evolutionary modules, modularity can increase the rate and potential for phenotypic macroevolution (Jablonski, 2017; Larouche et al., 2018, 2022; Marroig et al., 2009). Therefore, highly modular lineages may be more likely to diversify than lineages with few modules or those lacking distinct modules entirely.

Conversely, strong functional, developmental, or variational integration has been thought to constrain morphological diversification by limiting the movement of integrated phenotypes across an adaptive landscape (Olsen & Miller, 1958; Wake, 1991 p. 199; Klingenberg, 2004). Over deep time, such a phenomenon could manifest as high evolutionary integration in morphologically conservative clades, and low evolutionary integration in highly disparate ones. However, it has been long recognized that constraints can promote evolution in specific directions (Gould, 1989; Klingenberg, 2005), and that integration at the organismal or population

level may catalyze phenotypic evolution under certain conditions (Evans et al., 2021; Felice et al., 2018; Goswami et al., 2014; Randau et al., 2019). Specifically, variational integration within a population resulting from strong developmental or functional integration at the individual level can enhance phenotypic macroevolution by providing an adaptive line of least resistance (Futuyma et al., 1993; Klingenberg, 2005; Schlüter, 1996; Stebbins, 1974) along which lineages can evolve novel morphologies readily within a relatively constrained region of trait space, provided that the line aligns with a direction of selection (Goswami et al., 2014; Randau et al., 2019; Villmoare, 2013). Such strong integration can produce a pattern described recently as “a fly in a tube” (Felice et al., 2018) in which most diversification occurs along a single long morphospace axis, and the tight covariance among traits allows evolving lineages to access the ends of the tube more readily than they would if evolution in every direction were equally easy. Simulations have confirmed that integration can lead to higher variance and enhanced responses to selection and thereby promote morphological evolution (Goswami et al., 2014). Similar empirical results have been obtained across disparate vertebrate clades (Evans et al., 2017a; Marroig et al., 2009; Randau et al., 2019), though not every study finds a clear link between evolutionary integration and evolutionary rate (Bardua et al., 2019). Even so, under the right conditions integration can clearly facilitate morphological diversification within a radiation, not restrict it (Felice et al., 2018).

At least two different intrinsic patterns of covariation can augment a clade’s ability to diversify. High modularity and low integration can promote diversification by reducing constraint and maximizing the dimensionality of the morphospace open for exploration. Conversely, low modularity and high integration create a fast track through morphospace that can enhance a clade’s ability to reach particular extreme morphologies and may lead to high variance along the integrated axis. Morphospaces typifying the first scenario should have many important eigenvectors and a relatively spherical occupancy, while the second scenario implies concentration of most morphological variation along one or two eigenvectors and an occupancy well described by the fly-in-a tube metaphor of Felice et al. (2018).

Yet empirical studies often display a morphospace occupancy intermediate to these expectations, as seen in highly disparate groups such as birds (Felice & Goswami, 2018), acanthomorph fishes (Conith et al., 2020; Hulsey et al., 2006; Larouche et al., 2018), and multiple mammal radiations (Goswami, 2006; Goswami & Polly, 2010; Randau et al., 2019). While a single axis in these morphospaces typically indexes substantially more variation than any other, functionally meaningful evolution in many perpendicular directions still occurs. Do these clades simultaneously reap the benefits of integrated evolution without losing the flexibility afforded by modularity?

In a scenario of simultaneous integration and modularity, we predict to find at least one substantial axis of covariation between two or more modules alongside other major axes of independent within-module variation. Integration among modules would allow rapid change of the overall bauplan along a primary morphospace axis while regional change within each independent module would allow the fine tuning needed to expand away from that central axis and explore myriad variations of body parts. The resulting path of lineages through morphospace could resemble a signpost

pointing the way to many destinations (Figure 1) more than a fly in a narrow tube. Evolution along an integrated morphospace axis would create the signpost's central shaft, while subsequent motion of various lineages toward outlying regions of morphospace would evoke radial arrows pointing the way to diverse destinations.

Characiformes, a non-acanthomorph freshwater fish order with around 2000 described species, provides an excellent opportunity to reconstruct the patterns of covariation, evolutionary integration, and evolutionary modularity intrinsic to an exceptionally variable clade. Modern characiform lineages demonstrate diverse body shapes, many of which have no clear analogue in other actinopterygian fishes (Burns & Sidlauskas, 2019). These include fishes with backwards facing jaws (Géry, 1977; Myers & de Carvalho, 1959) and a lineage with an enlarged pectoral girdle hypothesized to aid in flying (Weitzman, 1954; Wiest, 1995). Other characiform lineages have evolved body shapes convergent on other ecologically similar characiforms or on fishes outside the radiation entirely. These include fusiform bodies arising in migratory species (Burns & Bloom, 2020; Géry, 1977), large pectoral fins evolving in rheophilic lineages (Lujan & Conway, 2015), and sagittiform bodies with large heads characterizing pelagic ambush predators (Burns & Sidlauskas, 2019).

Though characiform diversity has been long recognized (Géry, 1977; Regan, 1911), only recently have the data needed to reconstruct their history of diversification become available. Burns and Sidlauskas (2019) inferred the first comprehensive timetree for the radiation and quantified body shape diversity among over 200 characiform species in six broad ecological classes. That study reconstructed a morphospace with a primary axis of diversification summarizing 47% of the shape variation, but three other substantial axes

totaling another 38% and an overall morphospace occupancy intermediate between the sphere predicted by unconstrained evolution under high modularity and the long tube implied by high integration and substantial constraint. The clade's evolutionary history included numerous shifts in body shape, with many of these shifts occurring early and corresponding to simultaneous shifts in dietary ecology. Except for piscivory, dietary convergence rarely triggered whole body morphological convergence, suggesting that lineages of this clade can freely evolve along various evolutionary axes when adapting to the same trophic resources. That frequent evolution of unrepeated morphologies led Burns and Sidlauskas (2019) to infer a substantial role for evolutionary contingency in shaping the history of the radiation. It also makes the radiation a prime candidate for exploration of how modularity and integration might interact to promote exceptional diversification.

To date, empirical investigations of modularity or integration in characiform fishes have been limited to only a few studies (Boyle & Herrel, 2018; Ornelas-García et al., 2017). Only one of these examined modularity across the entire body, and that study included just two lake-dwelling species (Ornelas-García et al., 2017). That study found *Astyanax aeneus* and *A. caballeroi* to possess functionally distinct head and body modules and hypothesized that this functional division may have facilitated the divergence of feeding ecologies in the piscivorous *A. caballeroi* and the omnivorous *A. aeneus*. However, the presence of multiple body modules has never been tested in any other characiforms and patterns of within and among-module integration across the entire order are entirely unknown.

Here, we ask (1) whether the characiform body plan includes multiple modules and if so, (2) whether covariance among those modules indicates substantial integration among them. We also (3) visualize the major axes of morphological variation attributable to integration among modules and to independent evolution within modules and 4) use a novel method of covariance matrix subdivision to estimate the relative importance of integration and modularity to the diversification of Characiformes. By answering these questions, we reveal the intrinsic combination of modularity and integration underlying one of the world's most impressive ecomorphological radiations of fishes.

Materials and methods

Taxon sampling, morphometric analysis, and phylogenetic tree

Sampling, data collection, and morphospace construction followed Burns and Sidlauskas (2019). We radiographed every specimen in the same lateral orientation and only radiographed specimens that were unbent with closed mouths to avoid preservation artifacts and ensure that lateral rotation did not influence downstream analyses. When sampling multiple individuals of the same species, we ensured that all individuals fell within the same compact region of morphospace. One to ten specimens (average = 7.1 ± 2.3), dependent on availability, from the 129 species that match a tip on the phylogeny were used. Landmarks were digitized in tpsDig v 2.17 (Rohlf, 2015). Landmarks (Figure 2) were rotated, scaled, and aligned using a least-squares Generalized Procrustes Analysis in the R package geomorph (Bakker et al., 2021). Principal components analysis (PCA) in geomorph on the Procrustes coordinates identified the primary axes of shape variation

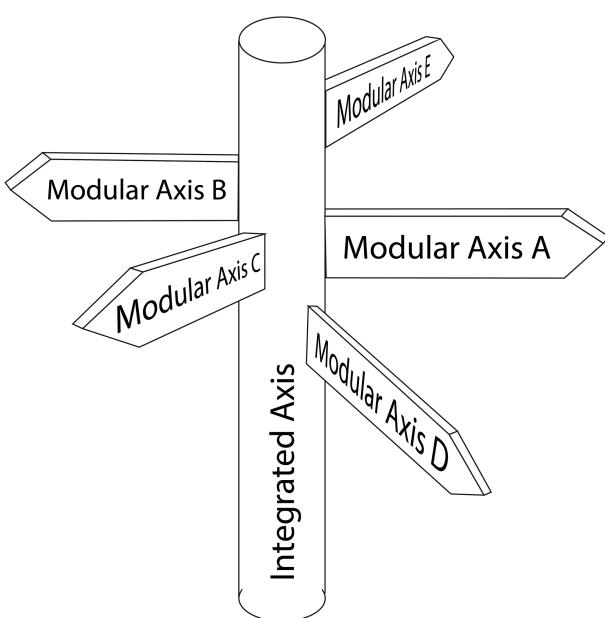


Figure 1. Schematic of hypothetical morphospace structure resembling a signpost in a system in which traits are both integrated and modular. The signpost's central shaft represents evolution along an integrated morphospace axis, while radial arrows pointing away from that axis represent motion of various lineages toward outlying regions of morphospace by modifying anatomy within distinct modules.



Figure 2. Landmark configuration used in subsequent tests of modularity. (1) anterior limit of premaxilla; (2) posterior limit of premaxilla; (3) anterior limit of orbit; (4) posterior limit of orbit; (5) ventral limit of orbit; (6) dorsal limit of orbit; (7) dorsal margin of neurocranium at vertical through center of orbit; (8) posterodorsal tip of supraoccipital; (9) dorsal-most portion of the base of the first dorsal-fin ray pterygiophore; (10) dorsal-most portion of the base of the last dorsal-fin ray pterygiophore; (11) base of the body dorsal of the fourth most posterior; (12) compound vertebral centrum at posterior of vertebral column; (13) posterodorsal limit of hypurals; (14) ventrodorsal limit of hypurals; (15) dorsal-most portion of the base of the of last anal-fin ray pterygiophore; (16) dorsal-most portion of the base of the first anal-fin ray pterygiophore; (17) pelvic-fin origin; (18) pectoral-fin origin; (19) ventral limit of joint between contralateral cleithra; (20) anterior tip of dentary; (21) joint between basioccipital and first vertebra of Weberian apparatus; (22) anterior limit of fifth vertebra (first vertebra not incorporated into Weberian apparatus and first bearing full sized pleural ribs); (23) vertebrae centroid posterior to first dorsal-fin ray pterygiophore; (24) vertebrae posterior to first anal-fin ray pterygiophore.

among species in the dataset. The phylogenetic comparative methods used require representation of each species by a single morphological data point. Since numerous individuals were measured for most species, we calculated the position of the centroid for each species in morphospace and used those coordinates in subsequent analyses.

For comparative analyses, we used the fossil time-calibrated phylogeny from [Burns and Sidlauskas \(2019\)](#), which includes many of the species for which morphological data were available. This phylogeny was based on a four-gene dataset that included 129 species, representing members of 19 of 24 currently recognized families. The phylogeny covers the body shape spectrum observed in Characiformes, from deep bodied serrasalmids to sagittiform members of Acestrorhynchidae. There has been considerable debate over the monophyly of Characiformes with some studies supporting the sister relationship between the nominal characiform suborders Characoidei and Citharinoidei ([Arcila et al., 2017; Betancur-R et al., 2019; Fink & Fink, 1981](#)), while others have found a paraphyletic relationship between them ([Chakrabarty et al., 2017; Melo et al., 2022; Nakatani et al., 2011](#)) due to the reconstruction of Siluriformes (catfishes) as sister to Characoidei. The [Burns and Sidlauskas \(2019\)](#) phylogeny found a sister relationship between Characoidei and Citharinoidei, and thus our primary analysis includes members of both suborders. However, we also ran an analysis excluding members of the smaller suborder Citharinoidei, which contains only about 5% of the species richness of the order ([Géry, 1977; Melo et al., 2022](#)). That additional

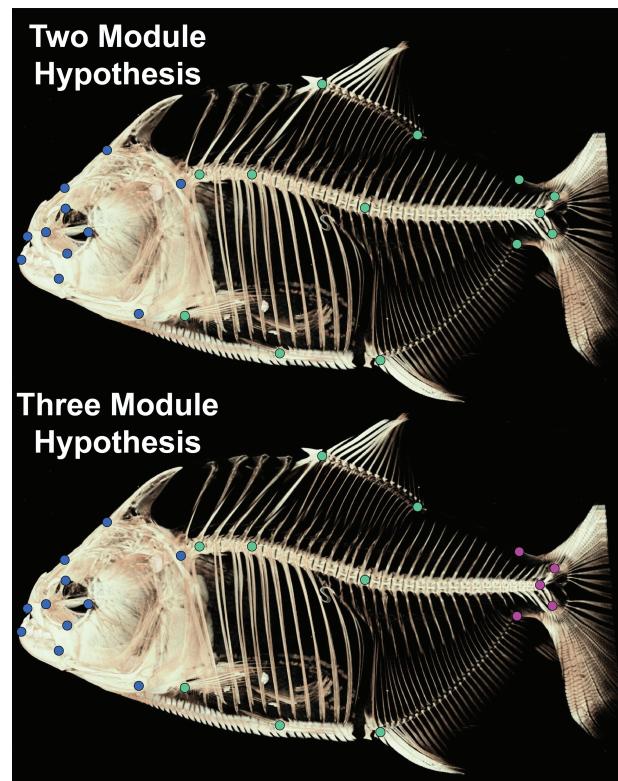


Figure 3. Landmark schematic of three two hypothesized subdivisions of 24 landmarks into modules. Cranial module comprising 11 landmarks; precaudal module comprising 7 landmarks; and caudal module comprising 4 landmarks. Null hypothesis is full integration, two-module hypothesis is independent evolution of a cranial module and postcranial module, and three-module hypothesis reflects independent evolution of cranial, precaudal, and caudal modules. Landmark configuration follows [Burns and Sidlauskas \(2019\)](#).

analysis provides a test of whether our results are robust to the current uncertainty surrounding characiform monophyly.

Among fishes, body size can influence body shape profoundly ([Price et al., 2019](#)) and studies sometimes require allometric correction to remove the evolutionary influence of size on shape. We tested for a relationship between centroid size and body shape using the function procD.pgl in the R package geomorph ([Baken et al., 2021](#)) to determine whether evolutionary allometry influenced patterns of body shape among Characiformes. We found no significant relationship between size and shape in our dataset ($R^2 = 0.008, p = .35$) and therefore opted to not size-correct our dataset.

Inferring modularity

We used the covariance among the aligned coordinates to evaluate the support for three hypotheses of modularity ([Figure 3](#)): (1) a null hypothesis of complete covariation between all regions of the body; (2) a two-module hypothesis with independent cranial and postcranial modules, ([Figure 3](#), top); and (three) a three-module hypothesis with landmarks divided into cranial, precaudal, and caudal modules ([Figure 3](#), bottom). [Ornelas-García et al. \(2017\)](#) found support for the second hypothesis in their recent analysis of two characiform species, while the analysis of [Aguilar-Medrano et al. \(2016\)](#) supported the three-module hypothesis in Pomacentridae, a family of tropical reef fishes. The configuration of landmarks assigned to the cranial module in hypotheses two and three

can help predict feeding kinematics (Norton, 1991) and can also affect locomotory efficiency through drag reduction and lift generation (Bushnell & Moore, 1991; Larouche et al., 2015; Lighthill, 1993). The postcranial landmarks (whether partitioned into one or two modules) primarily influence locomotion. The potential precaudal module describes variation in the trunk, and positions of the paired, dorsal, and anal fins, which affect the fish's stability and influence how it produces thrust (Drucker & Lauder, 2001, 2005; Harris, 1938; Standen, 2008). Variation in the potential caudal module influences the fish's ability to accelerate and produce thrust (Gibb et al., 1999; Webb, 1982, 1984).

To infer the number of modules present in the characiform body plan, we assessed the fit and strength of the different modular hypotheses using an effect size measure (Z_{CR}) derived from the covariance ratio (Adams & Collyer, 2019). The Z_{CR} statistic was calculated to determine whether the characiform body plan was composed of one, two, or three modules using the compare.CR function in the R package geomorph (Baken et al., 2021). The statistical significance of each test was assessed through 10,000 permutations. In strict terms, this test estimates evolutionary modularity and not functional modularity because it compares trait covariances among lineages in a phylogenetic context, rather than estimating functional or developmental links within an organism. However, landmark configurations in all three potential modules have clear functional implications (see above) and thus a conclusion of distinct evolutionary modules would imply the likely presence of underlying functional modules.

Because the CR ratio test has the potential to return statistically significant estimates of modularity resulting solely from covariances introduced during the Procrustes fit (Cardini, 2019), we also used a non-statistical visualization to ground-truth the results and verify that any statistically significant results also reflected clear patterns in the dataset. To that end, we visualized the pattern of pairwise landmark coordinate covariance in heatmap grids, with coordinates grouped along the axes according to their membership in each of the a priori hypothesized modules. The intensity of color reflects the magnitude of covariance between each pair of coordinates, and the vertical or horizontal lines indicate the divisions among the putative modules. In such a figure, strongly supported modules should stand out as including clusters of higher-than-average covariance near the diagonal of the matrix.

We did not perform GPA separately on each module, because this would produce arbitrary inter-module covariances (unless orientation of separate modules were fixed, which would mean also introducing an additional arbitrary assignment of how modules should be spatially aligned), which could be unrelated to any anatomical covariances between landmarks not found within the same modules. This is not an issue for statistical tests based on cross-products between modules but would limit simultaneous interpretation of all covariances within a covariance matrix.

Because the head region contained more landmarks than the other two potential modules, we considered whether variation in the density of landmark sampling could substantially influence our conclusions about modularity. We tested the log-linear association between interlandmark distance and the major eigenvalue (strength of covariance) for the portions of the covariance matrix covered by landmark pairs. The relationship between distance and eigenvalue was clearly not linear, but evaluation of linear model assumptions with residual

diagnostic plots from a linear model using the natural log of variables confirmed that the log-linear association was appropriate. We found no appreciable relationship ($R^2 = 0.0006$; $p = .694$), suggesting that any supported modular hypothesis would not result merely from disparities in the proximity and density of landmarks.

Assessing and visualizing patterns of integration

We used two-block partial least squares (PLS) analysis (Rohlf & Corti, 2000) to estimate whether distinct body shape modules nevertheless evolved in a significantly correlated fashion, which would imply substantial evolutionary integration among landmarks in different modules despite strong covariances within the modules. If so, we estimated the intensity of integration and characterized the patterns of shape covariation between the recovered modules. We evaluated statistical significance in a phylogenetic context following Adams and Felice (2014) using the function phylo.integration in the R package geomorph (Baken et al., 2021). The strength of covariation between each pair of modules was calculated as the correlation coefficient (rPLS) among species means on the first PLS axis of each module. The significance of each PLS correlation was assessed statistically through 10,000 permutations. Potential differences in the strength of evolutionary integration among pairs of modules under the three-module hypothesis were assessed with two-sample Z-tests using geomorph's compare.pls function. Because this "within a configuration" method of superimposing landmarks in a global Procrustes fit and then subdividing them during the test of integration has been shown to sometimes yield spurious results (Cardini, 2019), we also conducted a parallel analysis in which we performed separate Generalized Procrustes Analysis on the raw coordinates for each putative module and then calculated correlation coefficients between the Procrustes shape variables for each separate module.

To visualize integration, we performed a two-block PLS analysis of all specimens and plotted the first PLS axes of one module against that of another module. The shape changes along these axes were visualized using the picknplot function in geomorph (Baken et al., 2021) to select the minima and maxima of the axes. As with the CR test, this implementation estimates patterns of evolutionary integration among traits in distinct lineages and does not test directly for functional or developmental integration. However, because the latter can lead to the former, the presence of clear evolutionary integration among modules can help support a hypothesis that underlying functional or developmental linkages also exist.

Identifying axes of independent evolution within modules

The well-established method of partial least squares offers a tractable way to identify the axis of greatest covariation between any pair of modules (Bookstein et al., 2003; Mitteroecker & Bookstein, 2007). It does not, however, offer a mechanism for locating axes within each module that covary minimally with those in other modules, nor are we aware of any existing method that can identify such axes. As such we wrote a custom script that finds the residuals from a linear regression using the coordinates of the landmarks in each putative module as predictors of the remaining landmarks. The principal vector of those residuals represents an axis of within-module morphological variation that is oriented orthogonally (or nearly so) to the axis of greatest evolutionary

integration between that module and the rest of the landmark configuration. This axis therefore indexes an important axis of independent morphological variation within the module. On each such axis, we used geomorph's picknplot routine to identify and visualize the extremes of morphological variation.

Inferring the relative importance of integration and modularity

The analysis of any dataset bearing the simultaneous signals of modularity and integration inevitably raises the question of which of these patterns represents the dominant signal. Does most of the divergence among species stem from independent variation within each of the modules, do species diversify primarily along an integrated axis resulting from simultaneous, dependent change in two or more body regions, or are both signals about equally strong? To find out, we partitioned the overall covariance matrix into two separate matrices: a modularity matrix representing landmark covariation within the modules identified in the earlier analysis, and an integration matrix representing landmark covariation among those modules. The modularity matrix includes all the off-diagonal elements representing covariances between landmarks in the same module, plus the diagonal trace representing the variance at each landmark. All other off-diagonal entries were set to zero. The integration matrix included the covariances among landmarks in separate modules, plus the diagonal trace of variances. The remaining off-diagonal elements in this submatrix were also set to zero. Because the trace of each submatrix was unchanged from the full matrix, both had the same sum of eigenvalues as the original. The eigenstructures of the two new matrices, however, have the potential to differ substantially from each other, from the original complete matrix, and from a null model representing independent variation among all landmarks.

If either the modularity or the integration submatrices have an eigenstructure closely matching that of the original matrix (as determined by vector by vector correlations, or the Krzanowski (1979) average squared vector correlation), then that match indicates a dominant signal of either integration or modularity in the original dataset. If neither submatrix's eigenstructure closely matches the original, then either both patterns are inherent in the data, or the dataset's covariance patterns are indistinguishable from the null model. Scree plots can visualize all these alternatives, with the key insights arising from examination of the eigenvectors of the integration, modularity and total matrices with eigenvalues that exceed the variance predicted by the null by more than a threshold value (here chosen to be 10%). If neither the modularity nor the integration submatrices closely approximate the eigenstructure of the original, and if both contain vectors that index more covariance than would be expected by chance, then that pattern would support a conclusion that the high-variance eigenvectors in both submatrices represent biologically important axes of diversification. In other words, one could conclude that the clade had diversified along integrated and modular axes, and that both phenomena were important in that history of diversification.

Results

Patterns of evolutionary modularity

The three-module hypotheses differed significantly from the null hypothesis of no modularity when all species were

included in the analysis ($Z_{CR\ 3\ mod} = -3.13; p = .001$) and when analyses included only Characoidei ($Z_{CR\ 3\ mod} = -2.69; p = .01$). Comparison of the two-module hypothesis against the null skirted the edge of statistical significance ($Z_{CR\ 2\ mod} = -1.96; p = .05$), and exclusion of Citharinoidei did not change that conclusion ($Z_{CR\ 2\ mod} = -1.81; p = .06$). The overall strength of modularity did not differ significantly between the two- and three-module hypotheses whether comparing across all species ($p = .28$) or Characoidei alone ($p = .4$).

The modularity heatmap (Figure 4) reveals exceptionally strong covariance within the head module (box at upper left), and a more diffuse pattern in the postcranium. Results are similar when only including species in the suborder Characoidei (Supplementary Figure S1). While the visualization reveals some notable pairwise covariances among postcranial landmarks, these are lesser than the most extreme values in the head module and in some cases more muted than the covariances among landmarks in separate nominal modules (see section on Signal of Integration below).

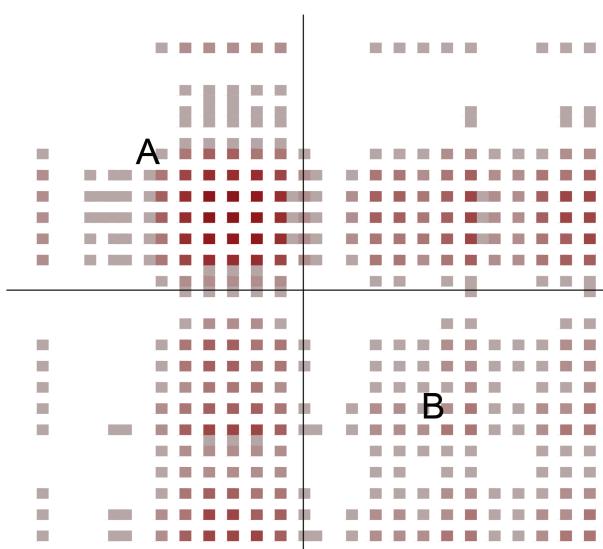
Overall, both approaches support division of the characiform body plan into cranial and postcranial modules, with the cranial module being particularly distinct. The statistical CR ratio test supports further separation of the postcranial region into two modules, though such subdivision is not readily apparent in the heatmap. While the central body and caudal region may represent biologically real modules, covariance within them is less intense than within the cranium. We conclude that the evidence rules out the single module hypothesis, and prefers the three-module hypothesis, which receives the strongest statistical support. However, the evidence supporting three modules versus two remains somewhat equivocal, and both hypotheses remain plausible.

Signal of evolutionary integration

Partial least squares analyses indicated significant integration among all three putative modules in the characiform body plan (Figure 5; Table 1). Similar results obtained when using only species from suborder Characoidei (Table 1; Supplementary Figure S2), when assuming subdivision into just two modules (Table 1; Supplementary Figure S3) and when performing separate Procrustes fits for each module before performing the correlations (Supplementary Table 1; Supplementary Figures S4 and S5). The strongest among-module covariation under either modular hypothesis indexes concerted change in body depth and aspect ratio (Figure 6; Supplementary Figures S6 and S7). Under the assumption of three modules, this covariation is most pronounced between the cranial and abdominal modules ($r\text{-PLS}_{hd-abd} = 0.92; p < .001$) but extends to the caudal module ($r\text{-PLS}_{hd-caud} = 0.72; p < .001$; $r\text{-PLS}_{abd-caud} = 0.68; p < .001$). That region shortens and deepens as the cranial and abdominal shape becomes deeper and elongates as the other modules become longer. Under the three-module hypothesis, the strength of integration was roughly equal across all pairwise comparisons with no significant difference in the strength of integration across comparisons (Table 2). Excluding members of Citharinoidei from the analysis did not fundamentally alter these conclusions (Table 2).

The heatmap visualizations (Figure 4) confirm the integration inferred statistically. Several cranial and postcranial landmark pairs (deep red squares at upper right or lower left) covary almost as strongly as do the landmarks within the cranial module (deep red squares near upper left). This visualization, combined with

Two Module Hypothesis



Three Module Hypothesis

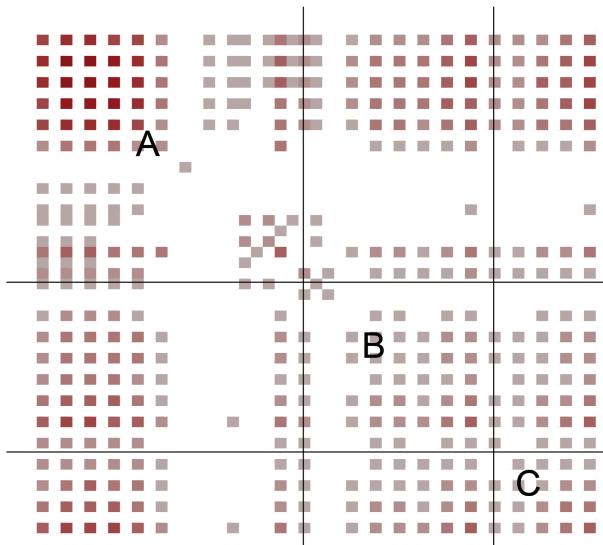


Figure 4. Visualization pattern of pairwise landmark coordinate covariance in heatmap grids. The intensity of color reflects the magnitude of covariance between each pair of coordinates, and the vertical or horizontal lines indicate the divisions among the putative modules, labeled by letters; two-module hypothesis: A = cranial, B = postcranial; three-module hypothesis: A = cranial, B = pre-caudal, C = caudal. Large covariances in lettered boxes along the diagonal support the reality of the module containing those landmarks, with particularly strong evidence here for the existence of the cranial module. The large covariances in off-diagonal boxes indicate evidence for among-module integration. Note that sorting matrices by modules does not guarantee that covariances are located in the same positions in both plots.

the strong statistical signal that persists across “among configuration” and “within configuration” superimposition approaches, confirms the biologically important evolutionary integration among the modules of the characiform body plan.

Axes of independent evolution within each module

In each of the three putative modules, the most important axis of independent variation indexed shape change with

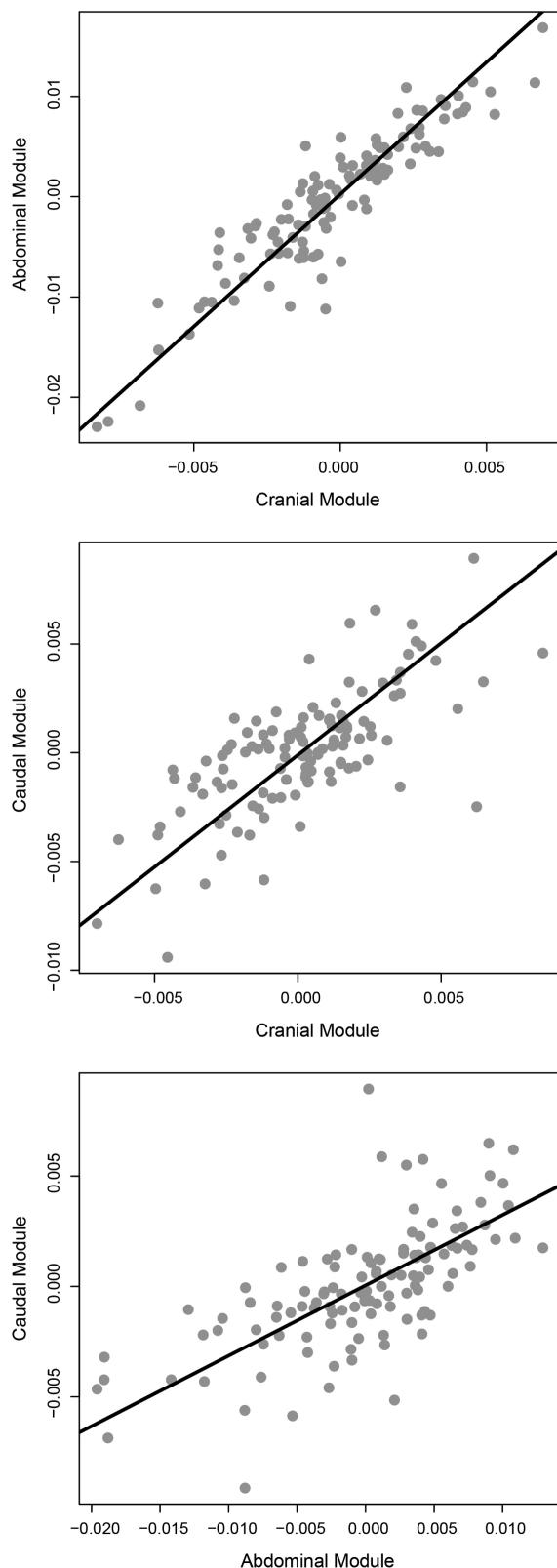


Figure 5. Morphological integration as described by a two-block PLS analysis of each module for all specimens. The more integrated specimens are distributed along the central axis of the scatter.

clear biological interpretability that is likely related to feeding and locomotion (Figure 7; Supplementary Figures S8 and S9). In the cranial module, this axis describes mainly the size and orientation of the mouth, which ranges from small and

Table 1. Results of phylogenetic PLS analysis between the different modules using both a single and separate General Procrustes Alignment for the two- and three-module hypothesis with statistical significance calculated from 10,000 permutations.

Single General Procrustes Alignment			Separate General Procrustes Alignment		
Two-module hypothesis			Two-module hypothesis		
	Cranium	Post-cranial body	Cranium	Post-cranial body	
All species					
Cranium	–		–		
Post-cranial body	0.92*	–	0.87*	–	
Characoidei					
Cranium	–		–		
Post-cranial body	0.92*	–	0.86*	–	
Three Module Hypothesis			Three Module Hypothesis		
	Cranium	Abdominal	Caudal	Cranium	Abdominal
All species					
Cranium	–			–	
Abdominal	0.92*	–		0.83*	–
Caudal	0.72*	0.68*	–	0.69*	0.59*
Characoidei					
Cranium	–			–	
Abdominal	0.92*	–		0.83*	–
Caudal	0.72*	0.68*	–	0.68*	0.58*

Note. Matrices of subsequent pairwise differences represent the PLS score. An asterisk represents significance at $p < .05$.

upturned to quite large and terminal. Independent variation in the abdominal region largely involves the length of the abdominal cavity and the position of fin insertions, with one extreme describing species with long cavities and relatively short dorsal fin bases. In these species, the dorsal fin is placed posteriorly on the body such that the dorsal-fin origin falls behind the pelvic-fin insertion, and the dorsal-fin insertion lies almost directly dorsal to the anal-fin origin. This placement of fins typifies fishes with reduced drag reduction, fast acceleration, and lunging predation strategies (Webb, 1984). The opposite extreme on this axis describes morphologies with short abdominal cavities, longer dorsal-fin bases, and a more anterior placement of the dorsal fin. In these species, the dorsal-fin origin lies directly dorsal to the pelvic-fin origin, and the dorsal-fin insertion lies substantially anterior to the anal-fin origin. Fishes with these fin placements tend to be steady state swimmers with high maneuverability in structurally complex habitats (Webb, 1984). Independent variation in the caudal module involves primarily an anterior-posterior shift in the position of landmark 15, marking the anal-fin insertion (and thus indexing the length of the anal fin). This axis also describes some change in the shape of the hypural plate, such that species with shorter anal fins have slightly more elongate caudal-fin bases, a morphology that can increase swim performance by reducing the drag associated with lateral movement of the tail.

Under the two-module hypothesis, the dominant axis of independent evolution in the cranial region remains largely unchanged from the results under the three-module hypothesis. In the unified postcranial module, the independent change represents primarily the length of the abdominal cavity and the position of fin insertions, with one extreme describing species with long cavities and relatively short dorsal and

anal fin bases. In these species, the dorsal fin is placed posteriorly on the body with the dorsal-fin origin falls behind the pelvic-fin insertion, and the dorsal-fin insertion lies almost directly dorsal to the anal-fin origin. The opposite extreme on this axis describes morphologies with short abdominal cavities, longer anal-fin bases, and a more anterior placement of the dorsal fin.

Eigenvalue analysis

The first two eigenvectors of the modularity and integration submatrices index substantially more variation than would be expected under the null model of complete independence (Figure 8), indicating that both phenomena contribute substantially to characiform diversification. Because neither the eigenstructure of the modularity-alone submatrix nor that of the integration-alone submatrix closely resembles the total eigenstructure (in which a single eigenvector describes most of the variation in the dataset), neither phenomenon dominates. While the first eigenvector of the integration submatrix does explain slightly more variance than that of the modularity submatrix under the three-module hypothesis, that order reverses under the two-module hypothesis, and in all cases the scree plots of the submatrices are broadly similar. Exclusion of Citharinoidei does not change these fundamental conclusions (Supplementary Figure S10). Overall, these results indicate that both phenomena influenced the clade's evolutionary radiation.

Discussion

Characiform fishes have adapted their crania and postcrania independently, while also exhibiting integrated evolution among modules. Strong covariances indicate that all modules

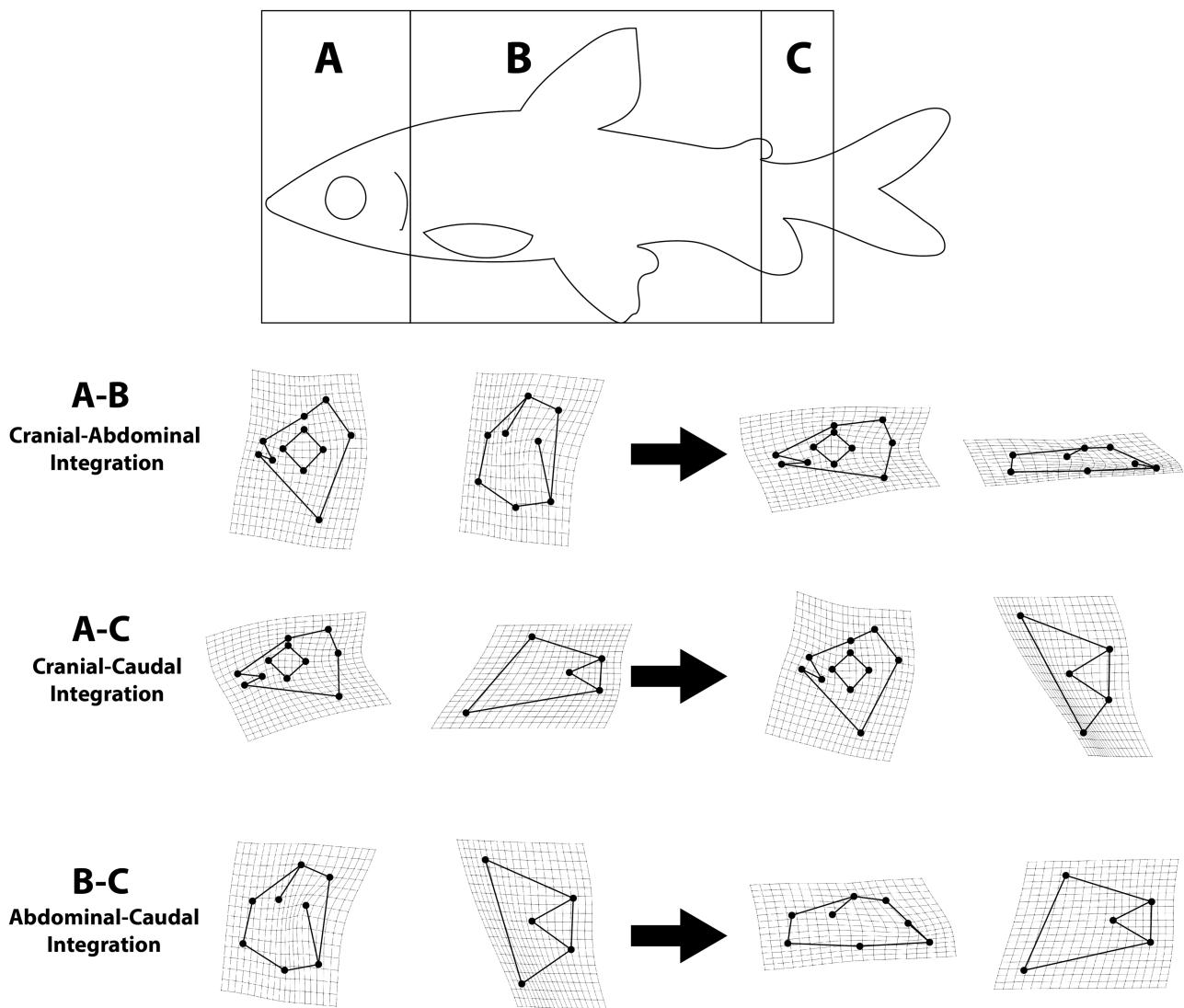


Figure 6. Warp grids depicting shape change within each module that is the result of integration.

Table 2. Results of two-sample Z-test analysis between the different module comparisons with statistical significance calculated from 10,000 permutations.

	Abdominal-caudal	Cranial-abdominal	Cranial-caudal
All species			
Abdominal-caudal	-	0.61	0.74
Cranial-abdominal	0.61	-	1.49
Cranial-caudal	0.74	1.49	-
Characoidei			
Abdominal-caudal	-	0.53	0.79
Cranial-abdominal	0.53	-	1.49
Cranial-caudal	0.79	1.49	-

Note. Matrices of subsequent pairwise differences represent the Z scores. An asterisk represents significance at $p < .05$.

evolved in unison to adjust the body's aspect ratio, thereby generating disparate body shapes ranging from compressiform to fusiform. Despite the presence of that strongly integrated axis, the clear patterns of evolutionary modularity

suggest that underlying developmental or functional modularity freed these fishes to adapt subregions of the bodies to fit disparate ecological niches. For example, different lineages altered the size and the orientation of the mouth, length of

the abdominal cavity, length and placement of the dorsal and anal fins, the elongation of the caudal peduncle, and shape of the hypural plate without triggering linked changes in other body regions. The combination of the fast track to different regions of morphospace afforded by integrated evolution of the whole body, and the freedom to explore localized variations on themes afforded by modularity appears to have catalyzed the evolution of exceptional phenotypic diversity in the order.

Characiformes have at least two body shape modules, and probably three

The body plan of Characiformes includes a strong cranial module and clear functional division between the cranium and all other regions of the body. Given the order's widely ranging body forms and propensity for morphological innovation (Burns & Sidlauskas, 2019), the presence of independent functional modules in the body plan is not surprising. Though our study obtained strongest statistical support for separation of the postcranial region into abdominal and caudal modules, that preference for three modules versus two is not ironclad, and there is a possibility that the apparent subdivision of the postcranial region in the CR test results from subtle covariances introduced during the Procrustes superimposition (Cardini, 2019). In support of this artifactual interpretation, we note that the module map visualization does not highlight a strong cluster of covarying landmark coordinates in the postcranial region while such a cluster is obvious within the cranium, and that Ornelas-García et al. (2017) recovered a unified postcranial region in their examination of two lacustrine species of *Astyanax*.

At the same time, the inference of module-specific axes of variation revealed biologically meaningful changes in all three modules, and several different lineages within Characiformes appear to have adapted a single body module to achieve distinctive morphologies, which likely aided them in annexing novel adaptive zones. For example, species of the family Gasteropelecidae have modified the pectoral girdle (functionally part of the post-cranial module) to enable burst speed swimming in slow water (Weitzman, 1954; Wiest, 1995). Detritivores and herbivores such as members of Prochilodontidae, Curimatidae, and Citharinidae have elongated their abdominal cavities to accommodate the incredible intestine lengths needed to extract key nutrients from an energy-poor diet (Burns, 2021). The fin-eating members of the African family Distichodontidae offer an excellent example of extreme modification to the caudal module, in which their hyperelongate caudal peduncles putatively assist the fast darts that they use to ambush other fishes and snip off their appendages (Lavoué et al., 2017; Matthes, 1961; Roberts, 1990). The clear functional interpretability of all three independent axes (Figure 7) and these examples of lineages that have specialized one region or another argue in favor of the reality of all three modules.

Ultimately, more studies with denser taxon sampling and more extensive landmark coverage may be needed to fully resolve whether the characiform body plan contains two or three distinct modules. The lack of a clear knot of strong covariances in the module map for the precaudal and the caudal landmarks (Figure 4) may result from the relative scarcity of landmarks in those modules. It is nevertheless clear from our data that the characiform cranium forms a distinct module that evolved independently from the postcranial regions,

which in turn implies the likely existence of pleiotropic, functional, or developmental links among cranial elements. Several studies of other fish clades have obtained similar patterns (Aguilar-Medrano et al., 2016; Larouche et al., 2015, 2018; Parsons et al., 2011), with most of those supporting the distinction of the cranium, and one of these studies recovering the same three modules that we identify herein (Aguilar-Medrano et al., 2016). However, the modularities of most fish radiations have not been explicitly examined. Several more datasets of similar scope will be needed before a test of the generality of any modular hypothesis will become possible.

Simultaneous modularity and integration underlie characiform body shape diversification

Traditionally, modularity has been thought to facilitate the evolution of novel phenotypes by allowing component parts to evolve and adapt independently of one another (Wagner & Altenberg, 1996). Modularity has been shown to increase the potential for many fish lineages to evolve novel morphologies (Collar et al., 2014; Denton & Adams, 2015; Evans et al., 2017b; Larouche et al., 2015). For instance, the three-module arrangement of damselfishes likely facilitated the evolution of a distinct morphology in *Amphiprion* clownfishes that combines the rounded cranium common to benthic species with the elongate trunk and caudal peduncle that otherwise typifies upper water column species (Aguilar-Medrano et al., 2016). Furthermore, the evolution of biting behavior in eels increased the modularity of the component parts of the trophic apparatus and led to higher morphological diversity than observed among the highly integrated suction-feeding species (Collar et al., 2014).

However, recent research has shown that integration among modules may enhance phenotypic evolution by providing an adaptive line of least resistance along which lineages can evolve novel morphologies within a relatively constrained region of trait space (Goswami et al., 2014; Randau et al., 2019; Villmoare, 2013). Results herein provide another example of integration and modularity acting in concert, in this case to shape the morphological diversification of characiforms.

Visualization of the strongest axes of covariance among putative modules using partial least squares analysis (Figures 5 and 6) revealed a clear signal of integration: a consistent pattern of coevolution in body depth in which all modules shift in lockstep. This evolutionary integration is not an artifact of the global Procrustes superimposition and persists in analysis using separate superimpositions of the landmarks in each module (Table 1). We interpret this axis of integrated body depth evolution as a possible adaptive line of least resistance in morphospace, though a full test of that conjecture would involve estimation of genetic as well as phenotypic covariances. Even in the absence of quantitative genetic information, it is clear that elongate and deep bodied lineages evolved repeatedly throughout the history of characiform diversification, and that variation in body depth loads heavily on the principal component axes describing the greatest shares of morphological variation in the order. That integration is most apparent in PC2 (see Burns & Sidlauskas, 2019, fig. 10), but body depth also varies along PC1, suggesting that the axis of greatest integration does not exactly parallel either principal component. Because of the strong integration among the modules, it appears that selection on the depth of any part of the body can trigger similar changes in the rest of

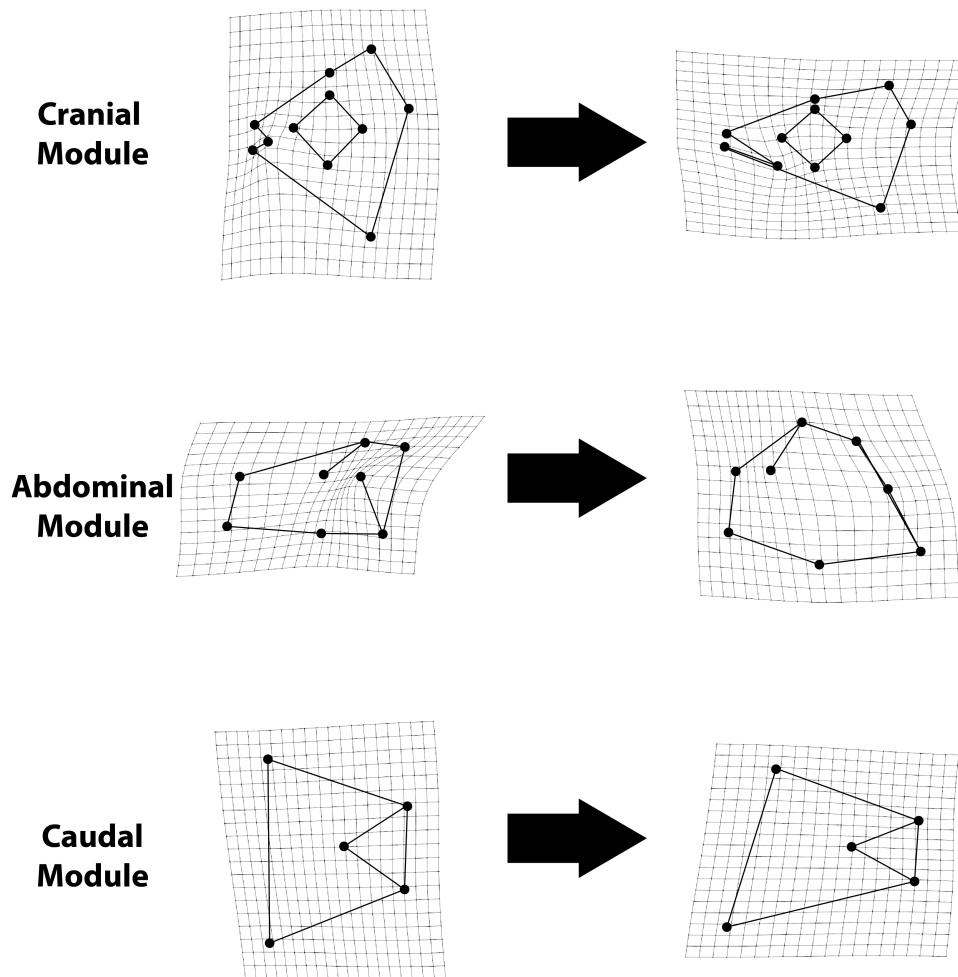


Figure 7. Warp grids depicting shape change within each module that are orthogonal to the axis of integration.

the body, offering an easy way for these fishes to adjust major hydrodynamic properties.

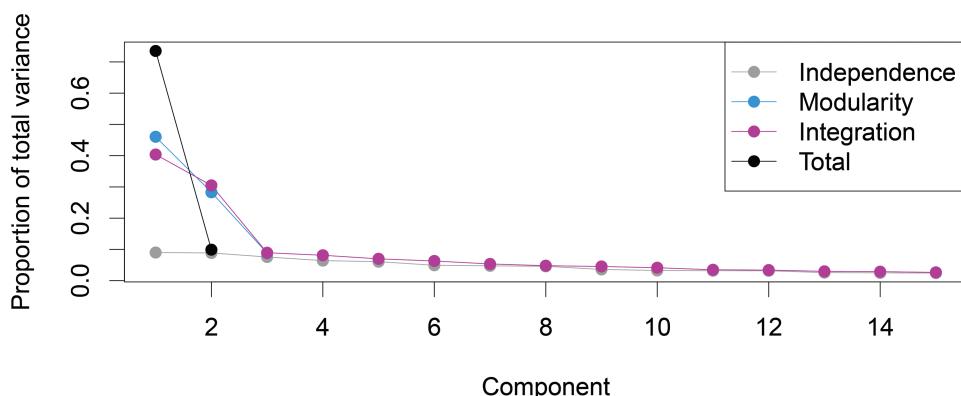
It is also clear that shape change along the axis of greatest integration does not explain all or even most of the morphological diversification within Characiformes. In the original study of these data (Burns & Sidlauskas, 2019), the principal component that most purely indexes variation in body depth is the second (23% of overall variance), not the first (47%), which also describes changes in fin position, fin length, and mouth orientation. In this study, visualizations of the dominant directions of change within each module revealed patterns having little to do with body depth, and that do not covary significantly with the integrated axis. Independent variation within single modules allowed characiforms to diversify their jaws to fit the needs of alternative hunting strategies, adjust the size of their abdominal cavities to accommodate larger and longer intestines in herbivorous and detritivorous taxa (Burns, 2021), or to alter the position and size of their fins without also triggering changes in other character systems. Such changes fine-tuned the overall body plan and optimized species to feed in different trophic niches or to inhabit different microhabitats.

Eigenanalysis of the characiform covariance matrix and comparison with data simulated under scenarios privileging modularity or integration confirms the conclusion of shared importance intuited above. The proportions of total variance

indexed by the empirical modularity and integration submatrices are about equal, suggesting that neither modularity nor integration alone set the stage for the order's morphological diversification. While the first eigenvector of the integration submatrix explains more slightly variance than does the first eigenvector of the modularity submatrix under the assumption of three modules (Figure 8), this is not a marked difference, and it disappears under the assumption of two modules. As such, there is no strong evidence that either modularity or integration dominated the spread of species throughout morphospace.

Overall, a mix of modular and integrated evolution shaped the morphological diversification of Characiformes. The resulting morphospace patterns are more reminiscent of the “signpost” model introduced above (Figure 1) than the “fly in a tube” of Felice et al. (2018), suggesting that intrinsic modularity has allowed substantial morphological evolution to occur away from the main axis of integrated change. Importantly, the integrated axis of body depth variation does not match PC1 precisely; the first three morphospace axes all index body depth to some degree, with the second doing so most clearly. That pattern implies a relatively short signpost most closely aligned with PC2, and elongate directional arrows leading to families and species occupying extreme values on the perpendicular axes. More work is needed to understand how integration and modularity at every level combine

Two Module Hypothesis



Three Module Hypothesis

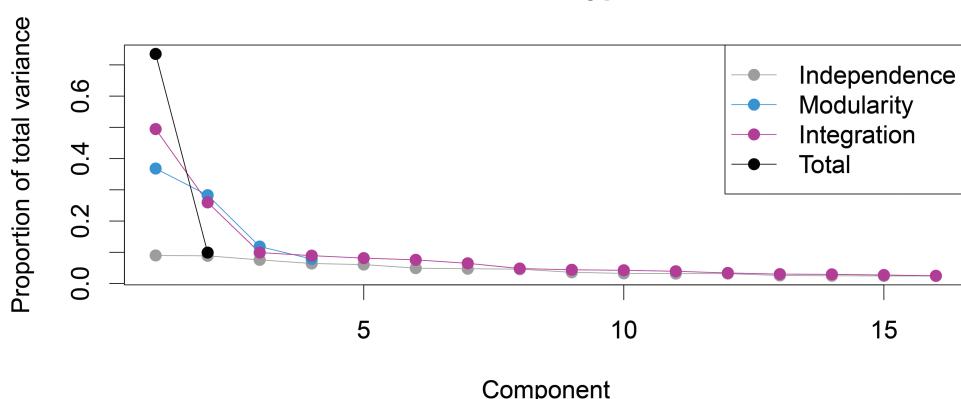


Figure 8. Scree plots visualizing the eigenvectors of the integration, modularity and total matrices with eigenvalues that exceed the variance predicted by the null. There are 44 possible (positive) eigenvalues; a limited number of relevant eigenvalues for either integration or modularity indicates strength of signal for these phenomena. Because neither the modularity nor the integration submatrices closely approximate the eigenstructure of the original (total) matrix, and because both contain vectors that index more covariance than would be expected by chance (independence), this pattern supports a conclusion that the high-variance eigenvectors in both submatrices represent biologically important axes of diversification.

to influence patterns of morphological novelty across diverse and spatiotemporally widespread vertebrate radiations, and to elucidate whether the pattern reconstructed here for Characiformes is typical, or extraordinary.

The role of modularity and integration during ecomorphological convergence

The simultaneous integration and modularity inherent in the characiform body plan may explain why Burns and Sidlauskas (2019) found a general scarcity of shape convergence despite repeated ecological convergence. Though integration can promote a fast track through morphospace and increase the potential for lineages to reach the extremes of this integrated axis (Felice et al., 2018), modularity allows lineages to disperse away from that axis in various directions, particularly when lineages vary in their initial phenotype or genotype (Losos, 2011, 2017). With independent modules facilitating uncorrelated evolution in at least two and possibly three distinct body regions, the lack of intrinsic constraints may have allowed multiple phenotypic solutions to the same ecological pressure in characiforms. Even when multiple lineages independently increased or reduced the body depth (perhaps as adaptations to improve maneuverability or acceleration, respectively), there would be no intrinsic reason that the size

or orientation of their mouths, configuration of their body cavities or placement of their fins should necessarily converge. Indeed, the empirical evidence suggests that they rarely did.

For instance, certain members of Anostominae and Crenuchidae have converged ecologically on a benthic, rheophilic, invertivorous lifestyle (Lujan & Conway, 2015). These species occupy different regions of morphospace even though they share elongate, fusiform bodies (Burns & Sidlauskas, 2019), because anostomine genera such as *Synaptolaemus* have evolved a superior mouth (Géry, 1977; Myers & de Carvalho, 1959) that contrasts greatly with the subterminal mouth of benthic crenuchids. The variation in mouth position permits *Synaptolaemus* to adopt an unusual benthic feeding strategy in which they scrape invertebrates from the underside of large flat rocks (Lujan & Conway, 2015), while crenuchids feed on benthic invertebrates on the surface of sand or gravel (Buckup, 1993; Géry, 1977). These two lineages did not converge completely in ecology or morphology despite their similar habitats. The presence of a distinct cranial module allowed the position and shape of the jaw to evolve in opposite directions in *Synaptolaemus* and Crenuchidae even while the lineages adopted similar body depth profiles.

Even in a highly modular system, convergence can result from adaptation toward an optimum morphology

through natural selection (Harmon et al., 2005; Losos, 2011; Simpson, 1953), morphological constraints increasing redundant morphotypes (Losos, 2011; Smith et al., 1985), or a combination of the two (Sanger et al., 2012). However, in most cases, Burns and Sidlauskas (2019) found no evidence of a single adaptive optimum for each given feeding ecology. Rather, they determined that species with similar diets occupied disparate regions of morphospace. The lack of single adaptive optima for broad trophic categories suggests that strong selection or hard constraints did not force lineages into specific regions of morphospace. In the absence of such limiting forces, the high modularity of their bodies appears to have permitted characiform fishes to discover different anatomical solutions to similar ecologies at various points along the fusiform to compressiform continuum. In other words, there are lots of ways to build a fish that eats insects or plants, and their combination of integration and modularity seems to have allowed characiform fishes to discover many of those workable forms.

Piscivorous characiforms provide a prominent exception, in that several phylogenetically distant characiform lineages have converged on a sagittiform, large jawed morphology well suited for fast acceleration and lunge predation (Burns & Sidlauskas, 2019; Claverie & Wainwright, 2014; Friedman et al., 2021; Price et al., 2019). These fishes occupy a margin of morphospace near the slender end of the body shape continuum on PC2, and it is tempting to conclude that the ease of evolution along the axis of greatest integration led to this convergence. However, all characiforms exhibit similarly strong evolutionary integration among the three modules; the piscivores are not special in this regard. As such, integration alone cannot fully explain why piscivores converged in body shape, and characiforms possessing other ecologies did not.

In highly integrated systems, strong selection can lead to convergence when the direction of selection lies at a substantial angle to the axis of integration (Eble, 2004; Felice et al., 2018; Randau et al., 2019). In such cases, the architecture of the body's integration impedes the ability of the animals to respond to selection because the optimum morphology lies in a direction difficult to traverse in morphospace; the desired destination effectively lies off-road. However, this scenario does not seem to apply at all to piscivorous characiforms, which occupy an optimum situated squarely at one end of the integrated axis. When direction of selection parallels the axis of phenotypic or genetic least resistance rather than running orthogonal to it, theory predicts that such alignment should promote morphological diversification, not constrain it (Goswami et al., 2014; Schlüter, 1996).

Why then, did piscivorous characiform converge? The answer seems to lie in the existence of a very strong adaptive peak for predators situated at one end of the body shape continuum, with no corresponding optimum at the other. Integration increased the likelihood of lineages discovering the single peak, at which point selection against deeper bodies or smaller heads held them there. And indeed, we see very few instances of characiform fishes abandoning a predatory ecology once it evolved (Burns & Sidlauskas, 2019, Figure 4), presumably because a sagittiform (pike-like) body shape performs so well at fast-start strikes in the complex habitats that typify rivers running through densely forested regions (Domenici & Blake, 1997; Harper & Blake, 1991).

Selection restricting predatory morphologies to a more elongate body shape is not surprising because the shape of the axial skeleton and the cranium both clearly relate to a piscivorous niche. A more elongate head and axial skeleton reduce pressure drag while increasing speed and an elongate cranium can also help predators overcome gape limitation. All these morphologies assist a piscivorous lifestyle, regardless of habitat or specific feeding strategy (Lawrence, 1957; Webb, 1982, 1984; Werner, 1974). Thus, selection toward a single strong hydrodynamic optimum that happens to be easily reached by integrated changes to body shape led to the marked and frequent convergence among piscivorous members of Characiformes.

Conclusion

Though the exact role that integration and modularity play in the evolution of novel phenotypes is still strongly debated (Evans et al., 2021; Felice et al., 2018; Wagner & Altenberg, 1996), empirical studies have demonstrated that both can significantly impact trajectories of organismal diversification and the magnitude of the response to selection (Evans et al., 2017a; Felice & Goswami, 2018; Goswami et al., 2014; Watanabe et al., 2019). Here, we show that the characiform radiation bears significant signal of evolutionary modularity and evolutionary integration alike. We also suggest that these lineage-level patterns result from underlying functional modularity and integration that helped catalyze and shape exceptional phenotypic evolution. Integration allowed these fishes to repeatedly evolve deep- and shallow-bodied phenotypes along an apparent line of phenotypic least resistance, while modularity allowed the invasion of novel phenotypic trait spaces by unlinking change within the cranial and postcranial regions from change along the integrated axis. The resulting radiation spun out more than two thousand different species inhabiting diverse ecological niches and displaying such impressive variation in body form that most of the 24 characiform families occupy distinct regions of morphospace.

While it is becoming increasingly clear that integration and modularity can synergize to promote exceptional diversification, the patterns of covariation that characterize less diverse clades have not been well studied. Most investigations of the links between trait covariance and phenotypic evolution have focused on ecomorphologically diverse (Bardua et al., 2020; Denton & Adams, 2015; Sanger et al., 2012) or exceptional radiations (Evans et al., 2017a, 2021; Fabre et al., 2021; Randau et al., 2019). Our study continues that trend. Fascinating as they are, evolutionary patterns in iconic radiations might not typify the rest of the tree of life. Indeed, the few studies that have looked broadly at the role of trait covariance during phenotypic evolution in large clades of vertebrates have not recovered a consistent relationship (Felice & Goswami, 2018; Goswami et al., 2014; Watanabe et al., 2019). For the field of morphological macroevolution to move forward, new studies need to examine patterns of modularity and integration in phenotypically restricted radiations as well as in the highly variable groups that have captured most previous effort. Such studies will offer much deeper insight into the role that modularity and integration play in morphological evolution generally, and will reveal whether or not underlying patterns of covariance differ consistently among depauperate and exuberant branches of the tree-of-life.

Supplementary material

Supplementary material is available online at *Evolution* (<https://academic.oup.com/evolut/qpac070>)

Data availability

The data are deposited in Dryad (<https://doi.org/10.5061/dryad.hmgqnmk8>) and scripts are available on Zenodo (<https://doi.org/10.5281/zenodo.7487776>).

Author contributions

All authors conceived of the project and wrote the manuscript; M.L.C. generated novel scripts to visualize and analyze within-module variation, and M.D.B. performed all data collection and analyses.

Conflict of interest: The authors declare no conflict of interest. Editorial processing of this manuscript was conducted independently of B.L.S., who is an Associate Editor of *Evolution*.

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